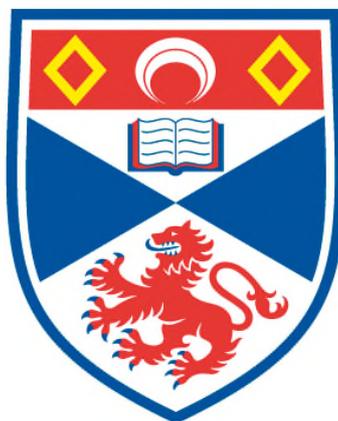


**N-HYDROXYGUANIDINES AND RELATED COMPOUNDS
AS NITRIC OXIDE DONORS**

Agnieszka Kulczynska

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



2009

**Full metadata for this item is available in
St Andrews Research Repository
at:**

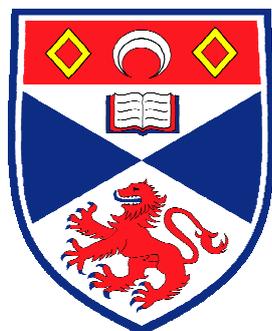
<http://research-repository.st-andrews.ac.uk/>

Please use this identifier to cite or link to this item:

<http://hdl.handle.net/10023/957>

This item is protected by original copyright

***N-HYDROXYGUANIDINES AND RELATED COMPOUNDS
AS NITRIC OXIDE DONORS***



**University
of
St Andrews**

School of Chemistry and
Centre for Biomolecular Sciences
Fife, Scotland

Agnieszka Kulczynska

December 2008

*Thesis submitted to the University of St Andrews in application
for the degree of Doctor of Philosophy*

Supervisor: Dr Nigel P. Botting

I, Agnieszka Kulczynska, hereby certify that this thesis, which is approximately 61,000 words in length, has been written by me, that it is a record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Date..... Signature of candidate.....

I was admitted as a research student in October 2005 and as a candidate for the degree of PhD in September 2006; the higher study for which this is a record was carried out in the University of St Andrews between 2005 and 2008.

Date..... Signature of candidate.....

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of PhD in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date..... Signature of supervisor.....

In submitting this thesis to the University of St Andrews we understand that we are giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. We also understand that the title and the abstract will be published, and the copy of the work may be made and supplied to any bona fide library or research worker, that my thesis will be electronically accessible for personal or research use unless exempt award of an embargo as requested below, and that the library has the right to migrate my thesis into new electronic forms as required to ensure continued access to the thesis. We have obtained any third-party copyright permissions that may be required in order to allow such access and migration, or have requested appropriate embargo.

Access to all part of printed copy but embargo of all part of electronic publication of thesis for a period of 1 year on the following ground: publication would preclude future publications.

Date..... Signature of candidate.....

Date..... Signature of supervisor.....

This thesis is dedicated to my parents:

Teresa and Krzysztof Kulczynski

Acknowledgments

Many people deserve to be mentioned in this part of my thesis. First of all I would like to thank my supervisor, Dr Nigel Botting, who guided me throughout my PhD studies. His support, never-ending optimism and encouragement helped me a lot during my research. Additionally, thanks to him I discovered a lot of good music (“The Dears”, “The Decemberists” and many other fantastic bands) as well as amazing aspects of Scottish Highlands.

I would like to express my deep gratitude to Dr Qing Zhang who was my lab mate and my good friend during these years. She shared her enormous knowledge with me and was always ready to help with either practical or theoretical issues of my PhD. It was a real pleasure to know her and work with her.

I would like to thank Prof. Ian Megson (UHI) and Dr Phil Milliken for the biological data as well as valuable discussions about rat aorta rings.

I also wish to thank Dr Tomas Lebl for his help with all aspects of X-ray and NMR studies. Thanks to him a lot of complex NMR techniques became less complex. In addition, he is mainly responsible for my passion to hiking along Scottish Highlands and weird and funny Czech movies.

Special thanks go to Prof. Alex Slawin who is the sunshine of the School of Chemistry and meeting her at the corridor is like the warmest moment in the day. Her positive thinking and the real fighter’s temper left none of my crystals unsolved.

I also would like to thank Melanja Smith, for her smile, optimism and polish chats and of course NMR assistance which was huge support for me over these past three years of my PhD studies.

I am grateful for all the assistance from the analytical, IT and technical staff of the School of Chemistry at the University of St Andrews. Thanks to Catherine Botting (MS), Caroline Horsborough (MS), Sylvia Williamson (Microanalysis), Derek Waddel (IT), Jim Bews (IT), Andrew Watson (orders), Marjory Parker (stores), Colin Millar (stores), Artur Czernik (stores), Colin Smith (glass blowing), Brian Walker (electronics), Bobby Cathcart (workshop).

I also want to thank Dr Fiona Gray, Dr Peter Kilian, Dr Iain Smellie, Dr David Richens, Arnot Williamson and Peter Pogorzelec for their support and help during demonstration.

I would like to thank all the members of the Botting research group I have had the pleasure of working alongside, many of whom became good friends; Gemma, Graham, Jo, Katie, Laura, Nawaf, Rahheem, Sarah, Su and Qing.

I also wish to thank all the amazing people who I met during my time in St Andrews. Especially, the first year basketball team: Deng, Gildas, Guillaume, Jana, Luke, Matthieu, Natalie, Nelly, Robert and Vincent for great games. Thanks to Philippa and Luke for all the amazing parties at their house. Merci beaucoup to Nelly and Nicolas for all the great time that we spent together. Thanks to Mayca for the bathrooms' talks, Annick for Absinthe times and Maria for the movie experience and the detox diet. I would like to thank Marzia for her support and friendship and Peter for the "Prison Break". I also thank Gina for her encouragement.

Thanks also go to Anna and Katie (my first year flat mates) for all the fantastic time which we spent together, the friendship and support.

Finally, I would like to say thank you to my husband Marcin for his endless love and great support during our time together in St Andrews.

Dziękuję wszystkim poznanym w St Andrews polskim znajomym: Ani, Asi, Arturowi, Ilonie, Irenie, Izie, Krzyškowi, Lilce, Łukaszowi, Michałowi, Monice i Piotrowi za wspólne spotkania i polskie klimaty.

Jestem ogromnie wdzięczna wszystkim moim bliskim przyjaciółom w Polsce: Kasi i Grzesiowi, Kumci i Piotrowi, Agacie, Gosi, Elwirze, Magdzie i Adamowi, Agnieszce i Pawłowi za duchowe wsparcie oraz wiarę we mnie i moje możliwości.

Bardzo dziękuję moim rodzicom Teresie i Krzysztofowi Kulczyńskim za bezinteresowną miłość i nieocenioną pomoc oraz mojemu rodzeństwu Dorocie i Danielowi za wsparcie i wspólne rozmowy.

Dziękuję również moim "nowym rodzicom" Irenie i Edwardowi Kosińskim za nieustającą wiarę we mnie.

Jednak największe podziękowania należą się mojemu mężowi Marcinowi, za niekończącą się miłość, oddanie i ogromne duchowe i fizyczne wsparcie.

Abbreviations

AIDS	Acquired immune deficiency syndrome
Akt/PKB	Serine/threonine protein kinase
ARF	Acute renal failure
BH ₄	Tetrahydrobiopterin
BHG	<i>N</i> -Butyl- <i>N</i> '-hydroxyguanidine
Boc	Di- <i>tert</i> -butyl dicarbonate
bp	Boiling point
BzCl	Benzoyl chloride
CaM	Calmodulin
Cbz	Carboxybenzyl
cGMP	Cyclic guanosine monophosphate
CNS	Central nervous system
COSY	Correlating coupled spins spectroscopy
CPHG	<i>N</i> -(4-Chlorophenyl)- <i>N</i> '-hydroxyguanidine
Cyt-P450	Cytochrome P450
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DETA	Diethylenetriamine
DIC	1,3-Diisopropylcarbodiimide
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EC ₅₀	Concentration of a compound where 50% of its effect is observed
EDRF	Endothelium-derived relaxing factor
EEDQ	<i>N</i> -Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
EI	Electron impact
eNOS	Endothelial nitric oxide synthase
ET	Electron transfer
EXSY	Exchange spectroscopy
FAD	Flavin adenine dinucleotide
FMN	Flavin adenine mononucleotide
Fmoc	<i>N</i> -9-Fluorenylmethoxycarbonyl

GC	Gas chromatography
GSH	Glutathione
GSNO	<i>S</i> -Nitroglutathione
GTN	Glyceryl trinitrate
Hb	Hemoglobin
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HR-ESI-MS	High resolution mass spectrometry
IC ₅₀	Concentration of a drug that is required for 50% inhibition
IHG	<i>N</i> -Isopropyl- <i>N</i> '-hydroxyguanidine
iNOS	Inducible nitric oxide synthase
IP ₃	1, 4, 5 - Inositoltriphosphate
IPK	Isolated perfused kidney
IR	Infrared spectroscopy
IRAG	IP ₃ receptor associated cGMP kinase substrate
ITU	Isothiourea
K _i	Inhibitor constant [M]
L-Arg	L-arginine
LC	Liquid chromatography
L-NIL	L-iminoethyl-lysine
L-NIO	L-iminoethyl-omithine
L-NMA	L- <i>N</i> ^G -methyl-arginine
L-NNA	L- <i>N</i> ^G -nitro-arginine
L-SETC	<i>S</i> -ethyl-L-thiocitrulline
L-SMTC	<i>S</i> -methyl-L-thiocitrulline
mp	Melting point
MPUP	1-(3-Methylsulfonyl-phenyl)-3-phenyl-1-urea
MS	Molecular sieves
MS	Mass spectrometry
NADPH	Nicotinamide adenine dinucleotide phosphate
NHA	<i>N</i> ^ω -hydroxy-L-arginine
NHG	<i>N</i> -hydroxyguanidines
NMM	<i>N</i> -Methylmorpholine
NMR	Nuclear magnetic resonance

nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser effect spectroscopy
NOS	Nitric oxide synthase
NSAID	Nonsteroidal anti-inflammatory drug
ODQ	1H-[1,2,4]Oxadiazole[4,3-a]quinoxalin-1-one
PKG (I & II)	Protein kinases
PR5	<i>N</i> -(2-Chloro-3,4-dimethoxybenzylideneamino)- <i>N</i> '-hydroxyguanidine
PS-HOBt	Polystyrene-supported-1-hydroxybenzotriazole
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SAHA	Suberoylanilide hydroxamic acid
SERCA	Sarco/endoplasmic reticulum Ca ²⁺ -ATPase
sGC	Guanylate cyclase
SIN-1	3-Morpholinosydnonimine
TBAB	Tetrabutylammonium bromide
TBAHS	Tetrabutylammonium hydrogensulfate
TCT	Cyanuric chloride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin layer chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
UV-VIS	Ultraviolet-visible spectroscopy
VT	Variable temperature
XO	Xanthine oxidase
γ-GT	γ-Glutamyl transpeptidase

Table of Contents

<i>Declaration</i>	<i>ii</i>
<i>Acknowledgments</i>	<i>iv</i>
<i>Abbreviations</i>	<i>vi</i>
<i>Table of Contents</i>	<i>x</i>
ABSTRACT	2
THEORETICAL BACKGROUND	4
1.1 INTRODUCTION	5
1.2 NITRIC OXIDE FORMATION	5
1.3 CHEMICAL REACTIVITY OF NITROGEN SPECIES	8
1.4 NITRIC OXIDE SYNTHASE INHIBITORS	9
1.4.1 L-ARGININE AND L-ARGININE DERIVATIVES	10
1.4.2 NON-AMINO ACID INHIBITORS	11
1.5 NITRIC OXIDE DONORS	14
1.5.1 N-HYDROXYGUANIDINES	16
1.5.2 HYDROXAMIC ACIDS	27
1.6 NITRIC OXIDE IN BIOLOGICAL SYSTEMS	31
1.6.1 NITRIC OXIDE DONORS IN CARDIOVASCULAR DISEASE	31
1.6.2 NITRIC OXIDE DONORS IN CANCER	34
1.6.3 NITRIC OXIDE IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM	34
1.6.4 OTHER BIOLOGICAL ROLES OF NITRIC OXIDE	35
1.7 NITRIC OXIDE – RELEASING PRO-DRUGS	36
1.7.1 NO DONORS/DRUG HYBRIDS	36
1.7.2 ENZYME-ACTIVATED NITRIC OXIDE DONORS	42
1.7.3 OTHER STRATEGIES	43
1.8 REFERENCES	45
N-HYDROXYGUANIDINES	50
2.1 INTRODUCTION	51
2.1.1 SYNTHESIS OF CYANAMIDES	53
2.1.2 SYNTHESIS OF N-ARYLALKYL-N ² -HYDROXYGUANIDINES	59
2.1.3 SYNTHESIS OF N-PHENYL-N ² -METHOXYGUANIDINE	68
2.1.4 SYNTHESIS OF N-ARYLALKYL-N ² -HYDROXYGUANIDINES BEARING NH ₂ GROUPS	69
2.2 BIOLOGICAL RESULTS	79
2.2.1 MYOGRAPHY STUDIES	79
2.3 CONCLUSIONS	90
2.4 REFERENCES	91

MONO- & DIMETHYL-N-HYDROXYGUANIDINES.....	92
3.1 INTRODUCTION.....	93
3.2 X-RAY CRYSTALLOGRAPHY AND NMR SPECTROSCOPY STUDIES.....	101
3.2.1 X-RAY STUDIES.....	101
3.2.2 NMR SPECTROSCOPY STUDIES.....	106
<i>Aminoiminomethanesulfonic Acids.....</i>	<i>106</i>
<i>N-Hydroxyguanidines.....</i>	<i>111</i>
3.3 CONCLUSIONS.....	124
3.4 REFERENCES.....	125
HYDROXAMIC ACIDS.....	126
4.1 INTRODUCTION.....	127
4.1.1 SYNTHESIS OF HYDROXAMIC ACIDS.....	127
4.2 BIOLOGICAL RESULTS.....	131
4.3 CONCLUSIONS.....	134
4.4 REFERENCES.....	135
NO-DONOR PRO-DRUGS ACTIVATED BY GLYCOSIDASES.....	136
5.1 INTRODUCTION.....	137
5.2 SYNTHESIS.....	138
5.2.1 ROUTE ONE.....	138
5.2.2 ROUTE TWO.....	143
5.3 CONCLUSIONS.....	147
5.4 REFERENCES.....	148
RENAL SELECTIVE NO-DONOR PRO-DRUG.....	150
6.1 INTRODUCTION.....	151
6.2 IMPROVED LINKER.....	153
6.2.1 IMPROVED LINKER DESIGN.....	153
6.2.2 IMPROVED LINKER SYNTHESIS.....	155
6.3 CONCLUSIONS.....	163
6.4 REFERENCES.....	164
SUMMARY AND FUTURE WORK.....	166
EXPERIMENTAL.....	170
7.1 GENERAL.....	171
7.2 REFERENCES.....	255
APPENDIX: X-RAY CRYSTALLOGRAPHIC DATA.....	258

Abstract

The design of new, improved NO-donor drugs is an important pharmacological objective due to the biological importance of nitric oxide. *N*-Hydroxyguanidines represent a useful class of NO donors where the mechanism of action is based on the biosynthetic pathway for NO.

Thirty new *N*-arylalkyl-*N'*-hydroxyguanidines were synthesized and their vasodilatation activity examined by myography in rat aortic rings. The observed relaxations were reversed by ODQ, which is an inhibitor of the guanylate cyclase, implying that this was an NO dependent vasodilatation. The most active compounds were also tested in the isolated perfused kidney (IPK) giving the vasodilatation properties. Preliminary results indicated that *N*-phenyl-*N'*-hydroxyguanidine showed the best pharmacological profile with $EC_{50} = 19.9 \mu\text{M}$ and *ca.* 100% reversibility with ODQ. A series of *N*-phenylalkyl-*N'*-hydroxyguanidines were synthesised. NO donor activity was found to be fairly constant up to three methylene groups, and then decreased. Substitutions in the benzene ring of *N*-phenylethyl-*N'*-hydroxyguanidine demonstrated that various electron-withdrawing and electron-donating groups in the *para* position did not significantly affect the NO donor activity of this series of analogues. The nitro and trifluoromethyl substituted compounds gave the best biological profiles. Additionally, a novel heterocyclic, *N*-furfuryl-*N'*-hydroxyguanidine possessed very promising vasodilatation properties. In general, almost all the *N*-arylalkyl-*N'*-hydroxyguanidines behaved as potent NO donors in the rat aorta assay.

In order to establish the influence of the free NH_2 group in the hydroxyguanidine functionality on the vasodilatation properties, *N,N*-dimethyl and *N*-methyl-*N'*-hydroxyguanidines were successfully synthesised. Unfortunately, they have not been tested yet in the biological assay. However, their NMR spectra showed some unusual features and their detailed analysis and X-ray data are presented herein.

In addition a series of hydroxamic acids was synthesised and the NO donor activity investigated using the same biological methodology. It was found that the 3-phenylpropionohydroxamic acid was the most potent compound with $EC_{50} = 6 \mu\text{M}$ and

ODQ = 96%. However, behavior in the IPK indicated that hydroxamic acids did not undergo the same biological pathway as in the rat aorta.

Two different types of enzyme-activated pro-drugs were designed using *N*-hydroxyguanidines as the NO donating molecule. Synthetic studies towards these targets were carried out using various synthetic approaches. The desired molecules have not yet been synthesised but the chemistry explored so far has indicated potentially more successful approaches that could be attempted.

THEORETICAL BACKGROUND

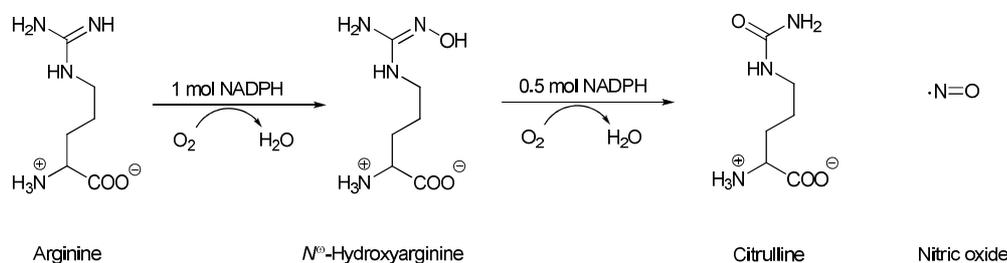
1.1 Introduction

Before 1987, nitric oxide (NO) was mostly mentioned as a molecule that participated in complex reactions which caused a “photochemical smog”. However, in 1987, two publications indicated that the sought-after “endothelium-derived relaxing factor” (EDRF) was in fact NO.¹

NO is small, diatomic, inorganic radical. Its physical and chemical properties have been well known for many years, but its biological activity was discovered about twenty years ago. It is known that a deficiency of endothelial NO causes the pathogenesis of vascular dysfunctions, including coronary artery disease, atherosclerosis, hypertension, diabetic vasculopathy and others. In addition, NO is involved in the inhibition of platelet aggregation, neurotransmission (Viagra reverses impotence by enhancing a NO-stimulated pathway) and immune regulation.²⁻⁵ Due to its instability, it is very difficult to introduce NO into biological systems. Therefore, the development of synthetic agents that possess the ability to release NO under biological conditions has become a very important issue. On the other hand, inappropriate production of nitric oxide can also lead to a series of diseases such as neurodegeneration, rheumatic disorders, septic shock and diabetes mellitus. The biochemical generation of NO is stimulated by nitric oxide synthase (NOS).^{3, 6, 7} As a result, many research groups have started working on potential NO donor drugs as well as on isoform-specific NOS inhibitors.

1.2 Nitric oxide formation

Since the importance of nitric oxide has been established in many physiological processes, an enormous number of studies have been carried out in order to understand its mechanism of action. In general, endogenous production of NO mainly proceeds *via* a two-step enzymatic reaction shown in Scheme 1.1.^{1, 3, 8} In the first step, L-arginine (L-Arg) is hydroxylated to give the stable intermediate *N*^ω-hydroxy-L-arginine (NHA) and 1 mol of NADPH and O₂ are utilized. In the next step, NHA is further oxidized to citrulline and NO, with the consumption of 0.5 mol of NADPH and 1 mol of O₂. An enzyme-bound heme derives electrons from NADPH and participates in oxygen activation in both steps.

Scheme 1.1³

The enzyme responsible for the production of NO is nitric oxide synthase (NOS). Three isoforms of nitric oxide synthase have been identified. They are divided into groups based on their location in the human body: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). The most important properties of the NOS are summarized in Table 1.1.

Table 1.1 Properties of NOS isoforms^{3,9}

<i>NOS</i>	<i>Locations</i>	<i>Characteristics</i>	<i>Major Biological Functions</i>
nNOS (NOS-I)	Brain, spinal cord, peripheral	Constitutive, Ca^{2+} dependent	Neuromediator
iNOS (NOS-II)	Macrophages, other tissues	Inducible, Ca^{2+} independent	Host defender, cytotoxic
eNOS (NOS-III)	Endothelium	Constitutive, Ca^{2+} dependent	Vasodilator tone modulator

Each of the isolated subtypes of NOS is associated with a different physiological function. Neuronal NOS generates nitric oxide as a neurotransmitter which is crucial for brain development, memory and learning. eNOS mediates endothelial production of NO and plays an important role in vascular biology. It controls smooth muscle relaxation and inhibits platelet aggregation. iNOS produces NO as a cytotoxic agent in activated macrophage cells. It helps the immune system to fight against microorganisms and tumor cells. The constitutive isozymes (nNOS and eNOS) generate relatively small amounts of NO and in order to be active they require the presence of Ca^{2+} and calmodulin. However, iNOS produces relatively large amounts of NO and already possesses bound Ca^{2+} and calmodulin.^{3,9-11}

Mammalian NOS are made up of an *N*-terminal oxygenase domain and *C*-terminal reductase domain. NOS monomers are very large (~133-161 kD per monomer). The oxygenase region binds iron protoporphyrin IX (heme), tetrahydrobiopterin (BH₄) and L-arginine, while the reductase region binds FMN, FAD and NADPH as shown in Figure 1.1. In order to possess activity, two NOS polypeptides must form a homodimer, however, recently it has been reported that they actually exist as tetramers of two NOS monomers connected by two calmodulins, which promote electron transfer (ET) between them.^{9, 10, 12}

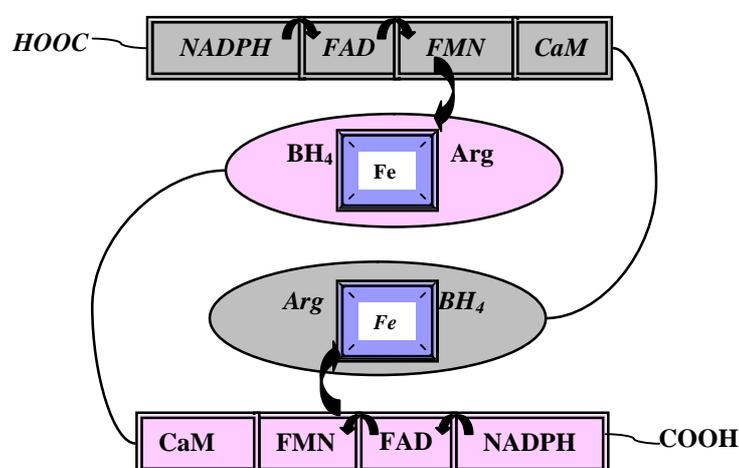


Figure 1.1 The structure of NOS dimer and the path of electron transfer¹²

By cloning the subtypes of NOS it has been revealed that they share only 50% primary sequence homology, possibly because they differ from each other in regulatory aspects. However, interspecies similarity of each isozyme is rather high (81-93% identical). Based on the crystal structures of the oxygenase domain of the murine iNOS monomer, murine and human iNOS dimers, human and bovine eNOS dimers and rat brain nNOS, it has been found that there is a high degree of similarity within the catalytic center between the NOS isoforms. However, employing a novel approach to examine the active site of the three NOS using various aryldiazenes indicated that the topologies of the active site of the three subtypes are actually different.¹⁰ The size of the active site decreases in the order nNOS > iNOS > eNOS. Even though the three isoforms of NOS possess a lot of similarities, the subtle structural differences may still allow the development of subtype-specific inhibitors and substrates.^{4, 10, 12}

The results of crystallographic studies indicated that the endogenous substrates (L-Arg and NHA) of NOS bind to the enzyme close to the heme by a network of hydrogen bonds.

1.3 Chemical reactivity of nitrogen species

Due to its structure nitric oxide can undergo various chemical transformations. NO can be oxidized and reduced leading to its conversion to various reactive nitrogen species (RNS). One of the most important RNS is peroxynitrite. It is a strong oxidizing and nitrating agent which causes molecular damage leading to disease-causing cellular dysfunction. Nitric oxide can react with molecular oxygen (O_2), superoxide anion (O_2^-) or transition metals (M) to produce RNS such as N_2O_3 , NO_2 , NO_2^- , NO_3^- , peroxynitrite ($OONO^-$), and metal-nitrosyl adducts (Figure 1.2). Since NO reacts with superoxide anion to give peroxynitrite anion, the production of this species is inevitably a source of hydroxyl (OH^\bullet) and nitrogen dioxide (NO_2^\bullet) radicals. These reactive species, when generated in high concentrations, can induce oxidative stress, and therefore be the source of toxicity.^{2,3}

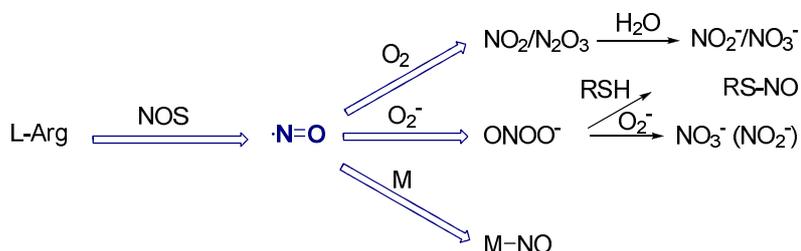


Figure 1.2 Chemical reactions of nitric oxide³

Nitrogen oxide can be rapidly oxidized, which leads to the nitrosonium ion (NO^+). This species reacts with nucleophiles such as alcohols (ROH), thiols (RSH) and secondary amines ($RR'NH$) to generate RO-NO, RS-NO or $RR'N-NO$, respectively (Figure 1.3).

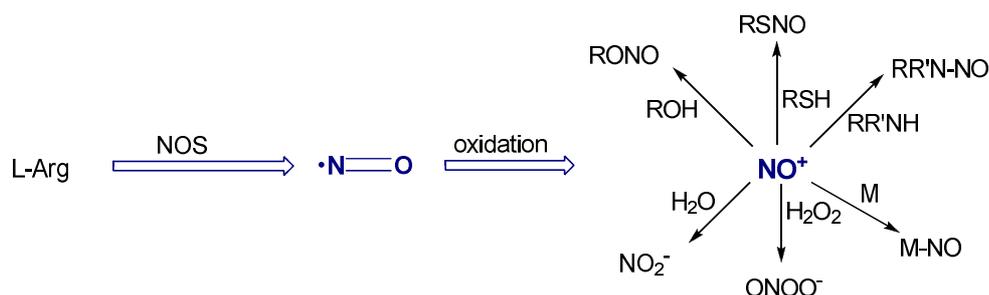


Figure 1.3 Oxidation of NO^3

NO can also undergo one-electron reduction to nitroxyl ion (NO^-), which is rapidly transformed to N_2O under physiological conditions. Other competing reactions are illustrated in Figure 1.4.

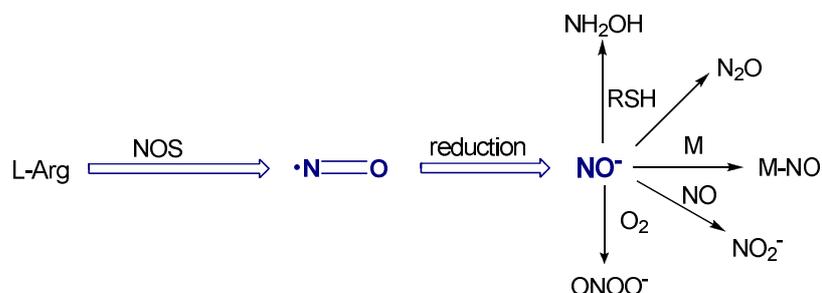


Figure 1.4 Reduction of NO^3

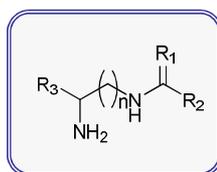
1.4 Nitric oxide synthase inhibitors

Despite the fact, that NO is a free radical it plays an important role in many physiological processes and its presence is crucial for the normal function of the human organism. However, overproduction of nitric oxide in some cases may cause deleterious effects. As mentioned before, iNOS produces a large amount of NO , which sometimes can lead to a variety of diseases, including sepsis, tissue damage, arthritis and inflammation. Therefore, selective iNOS inhibitors would be beneficial for the treatment of these pathological conditions.¹²⁻¹⁵ On the other hand, it has been reported that NO overproduction by nNOS is responsible for neurodegeneration during stroke, chronic headaches, Parkinson's disease, Alzheimer's disease, and Huntington's disease. Therefore, the development of potent nNOS inhibitors also would bring an enormous advantage to medicinal chemistry and pharmacology. However, as previously

described the differences between various isoforms of NOS are not very significant and the development of specific inhibitors is very demanding. In spite of this problem, several classes of compounds have been recognized as potential NOS inhibitors.^{9, 12, 13, 15}

1.4.1 L-Arginine and L-Arginine Derivatives

In general, derivatives of the endogenous substrate of NOS isoforms, L-arginine, can act as inhibitors or substrates.¹⁴ Many analogues of L-arginine have been synthesized and investigated as potential inhibitors of NOS. In fact, many derivatives of L-arginine are capable of inhibition of NOS. Generally, they are modified at one or both of the terminal guanidine nitrogens, as shown in Figure 1.5.³



<i>n</i>	<i>R</i> ₁	<i>R</i> ₂	<i>R</i> ₃	<i>name</i>
3	NCH ₃	NH ₂	COOH	L- ^{NG} -methyl-arginine (L-NMA) (1)
3	NNO ₂	NH ₂	COOH	L- ^{NG} -nitro-arginine (L-NNA) (2)
3	NNH ₂	NH ₂	COOH	L- ^{NG} -amino-arginine (3)
3	NCH ₂ CH ₃	NH ₂	COOH	L- ^{NG} -ethyl-arginine (4)
3	NCH ₂ CHCH ₂	NH ₂	COOH	L- ^{NG} -allyl-arginine (5)
3	NOCH ₃	NH ₂	COOH	L- ^{NG} -methoxy-arginine (6)
3	NH	CH ₃	COOH	L-iminoethyl-omithine (L-NIO) (7)
3	NH	SCH ₃	COOH	<i>S</i> -methyl-L-thiocitrulline (L-SMTC) (8)
3	NH	SCH ₂ CH ₃	COOH	<i>S</i> -ethyl-L-thiocitrulline (L-SETC) (9)
4	NH	CH ₃	COOH	L-iminoethyl-lysine (L-NIL) (10)
4	NH	CH ₃	CH(OH)CH(OH)	<i>N</i> -[5(<i>S</i>)-amino-6,7-dihydroxyheptyl]ethanimidamide (11)

Figure 1.5 Structure of L-arginine analogues and amino acid-based NOS inhibitors³

IC₅₀ and K_i values for these inhibitors against various NOS isoforms have been obtained. Most of these compounds are potent inhibitors however they show rather poor selectivity for NOS

subtypes. L-NMA (**1**) and L-NNA (**2**) are used as the prototypic inhibitors for *in vitro* and *in vivo* testing due to their chemical stability, water solubility and low toxicity. They were found to be more potent inhibitors of nNOS and eNOS than of iNOS. The first amino acid-based inhibitor selective for iNOS ($IC_{50} = 5 \mu\text{M}$) *vs.* eNOS ($IC_{50} = 138 \mu\text{M}$) was L-NIL (**10**). Further modifications of this compound, by replacing the carboxylic acid moiety with a vicinal glycol, led to the analogue (**11**) which was 700-fold more selective for iNOS than for eNOS. Alternatively, L-SMTC (**8**) and L-SETC (**9**) showed greater specificity for nNOS than iNOS or eNOS.^{3,13,14}

1.4.2 Non-amino Acid Inhibitors

In general, derivatives of arginine can be considered as analogues of guanidine. Therefore different amidine compounds (Figure 1.6) have been prepared and investigated as NOS inhibitors.

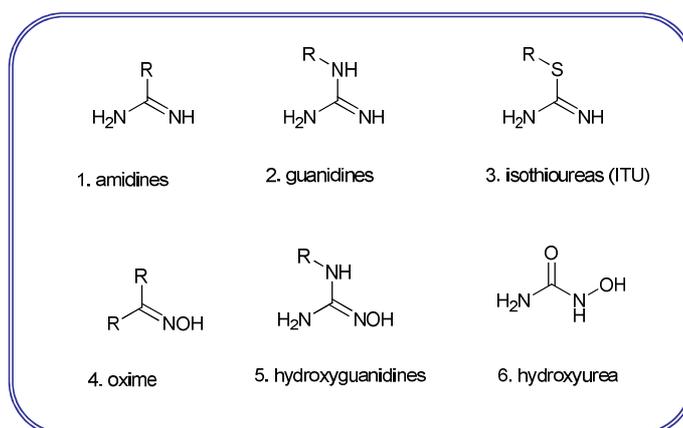


Figure 1.6 Structures of guanidine derivatives and similar analogues^{3,13}

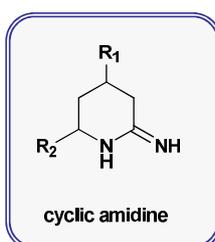
Guanidine itself is neither an inhibitor nor a substrate of NOS. However, methylguanidine and ethylguanidine possess the ability to inhibit NOS, but they are much less selective than the earlier described L-NMA. The only important molecule from this class of compounds is aminoguanidine (Fig. 1.6-2, $\text{R}=\text{NH}_2$). It has been reported that it plays a beneficial role in various experimental models of inflammation and septic shock.

The simple alkyl isothioureas (ITU) have been known to be potent inhibitors of the three human NOS subtypes since 2001. It has been found that substitution on the sulfur atom is

critical for activity. On the other hand, modification of the nitrogen atoms resulted in the loss of the inhibitory properties. Based on the reported $1/K_i$ values there is a borderline between the size of the alkyl substituent and the compound's activity. If the alkyl groups were too small ($R = H$), too large ($R = (CH_2)_3CH_3$) or too bulky ($R = C(CH_3)_3$), the inhibitor activity decreased significantly. The optimal length chain for *S*-alkyl-ITU is between methyl and propyl. Overall, the isopropyl group seemed to be the most beneficial alkyl group for the inhibitory potency.^{3, 12, 13}

The simplest amidines, namely formamidine and acetamidine have been found not to be NOS inhibitors. However, propionamidine and longer chain amidines revealed some activity towards NOS inhibition. The optimal length of the carbon chain for the alkyl amidine is between ethyl and butyl, which is similar to that for the *S*-alkyl-ITU.

On the other hand cyclic amidines are much more promising inhibitors of iNOS compared to L-NMA. Some of the synthesized and examined analogues of cyclic amidines are shown in Figure 1.7.³



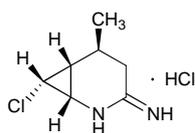
R_1	R_2	name
H	H	2-iminopiperidine (12)
CH ₃	H	4-methyl-2-iminopiperidine (13)
H	CH ₃	6-methyl-2-iminopiperidine (14)
CH ₃	CH ₃	4,6-dimethyl-2-iminopiperidine (15)
H	CH ₂ C ₆ H ₁₁	cyclohexymethyl-2-iminopiperidine (16)

Figure 1.7 Structure of cyclic amidines³

It has been reported that substitution of 2-iminopiperidine ring increased the inhibitory potency of the new derivatives towards iNOS. Recently, a novel cyclic amidine analogue (1*S*, 5*S*, 6*R*, 7*R*)-7-chloro-3-imino-5-methyl-2-azabicyclo[4.1.0]heptane hydrochloride has been synthesized (Scheme 1.2) and examined as a potent iNOS inhibitor. It was found that it revealed 10-fold

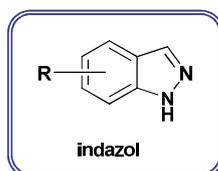
more selectivity for human iNOS over human eNOS. The preliminary pharmacological studies indicated that it can be considered as a potential therapeutic agent.

Scheme 1.2³



ONO-1714

Indazoles also act as NOS inhibitors. Several nitro derivatives have been synthesized (Figure 1.8). The biological studies showed that nitration at the 5, 6 or 7-position caused an increase in inhibitory potency. However, the presence of an amino substituent at the 5- or 6-position made the corresponding compounds less active as seen from the IC₅₀ values.^{3,13}



<i>Compound</i> <i>R</i>	<i>iNOS</i>	<i>IC</i> ₅₀ [<i>μM</i>]	
		<i>nNOS</i>	<i>eNOS</i>
H		177.8	
5-NO ₂		47.3	
6-NO ₂		31.7	
7-NO ₂	5.8	0.9	0.71
5-NH ₂		>1000	
6-NH ₂		>1000	

Figure 1.8 Inhibitory activities of indazole derivatives for various NOS subtypes³

1.5 Nitric oxide donors

Since, NO was identified as the EDRF, the number of compounds which can act as a potential NO donors has increased dramatically. One of the most well known is glyceryl trinitrate (GTN), which was used to treat angina pectoris long before its NO donating properties were discovered.^{1, 3-5, 8, 16-18} The diversity in the structures of NO donors indicates that their chemical reactivities and mechanisms of NO production are different. In general, NO is released by three distinct pathways. The first route involves spontaneous generation of NO through thermal or photochemical degradation, e.g. *S*-nitrosothiols, diazeniumdiolates, or oximes.^{3, 4, 8, 19-22} The second mechanism is based on chemical reactions with acids, alkali metals or thiols. NO is produced by this route from organic nitrates, nitrites and sydnonimines.^{3, 6, 16, 23, 24} The last pathway is an enzymatic oxidation of NO donors such as *N*-hydroxyguanidines, which require the presence of NOS or Cyt-P450 to release NO.^{3, 8, 10, 25-30} Major classes of current NO donors are summarized in Table 1.2, along with details of the pathway by which NO is generated from each class, if known. As seen in Table 1.2 some of the compounds are able to produce NO through more than one mechanism e.g. organic nitrates can also generate NO by enzymatic catalysis.

Table 1.2 Major classes of NO donors³

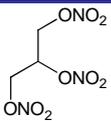
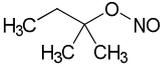
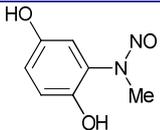
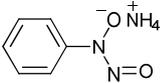
Chemical Class	Representative compounds	Pathway of NO Generation	
		Non-enzymatic	Enzymatic
Organic nitrate		Thiols	Cyt-P450, GST, etc.
Organic nitrite		Hydrolysis, transnitrosation, thiols, light, heat	Xanthine oxidase, etc
Metal-NO complex	$\text{Na}_2[\text{Fe}(\text{CN})_5(\text{NO})] \cdot 2\text{H}_2\text{O}$	Light, thiols reductants, nucleophiles	A membrane bound enzyme
<i>N</i> -Nitrosamine		OH ⁻ , light	Cyt-P450 related enzyme
<i>N</i> -Hydroxynitrosamine		Light, heat	peroxidases

Table 1.2 Major classes of NO donors³ (continuation)

Nitrosimine		Thiols, light	?
Nitrosothiol		Spontaneous, enhanced by thiols, light, metal ions	unknown enzymes
C-Nitroso compound		Light, heat	?
Diazetidine dioxide		Spontaneous, thiols	?
Furoxan & benzofuroxan		Thiols	Unknown enzymes
Oxatriazole-5-imine		Thiols	?
Syndonimine		Spontaneous, enhanced by light, oxidants, pH>5	Prodrugs require enzymatic hydrolysis
Oxime		Spontaneous, O ₂ /Fe ^{III} -porphyrine	Cyt-P450
Hydroxylamine		Autoxidation enhanced by metal ions	Catalase/H ₂ O ₂
N-Hydroxyguanidine		Oxidants	NOS, Cyt-P450
Hydroxyurea		H ₂ O ₂ /CuZn-SOD or ceruloplasmin, H ₂ O ₂ /Cu ²⁺ , heme-containing proteins	Peroxidase
Hydroxamic acid		?	Guanylate cyclase

For this project the classes of NO donors which contain a *N*-hydroxyl functionality are of major interest. These compounds require enzymatic oxidation to generate NO, either *via* NOS or some other oxidizing enzyme.

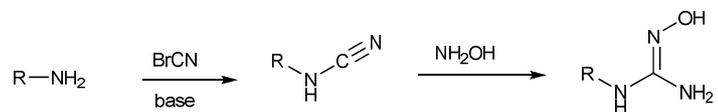
1.5.1 *N*-Hydroxyguanidines

Recently, it has been proven that NOS enzymes are able to generate nitric oxide *via* the oxidation of many non- α -amino acid monosubstituted *N*-hydroxyguanidines, including *N*-alkyl- and *N*-aryl-*N'*-hydroxyguanidines.^{3, 10, 30-33} Some of these compounds were shown to be as active as the endogenous substrate, *N*-hydroxyarginine. On the other hand, some proved to be selective for only one NOS isoform.^{10, 30} *N*-Hydroxyguanidines can be oxidized and generate nitric oxide, not only by NOS, but also by other oxidizing enzymes such as cytochromes P450, xanthine oxidase and NADPH oxidoreductase. They also undergo chemical oxidation with the production of NO or HNO (nitroxyl) depending on the oxidizing agent.^{3, 10} Due to the properties of *N*-hydroxyguanidines there has been a lot of interest in the development of new guanidine-based NO donor drugs.

Synthesis of *N*-Hydroxyguanidines

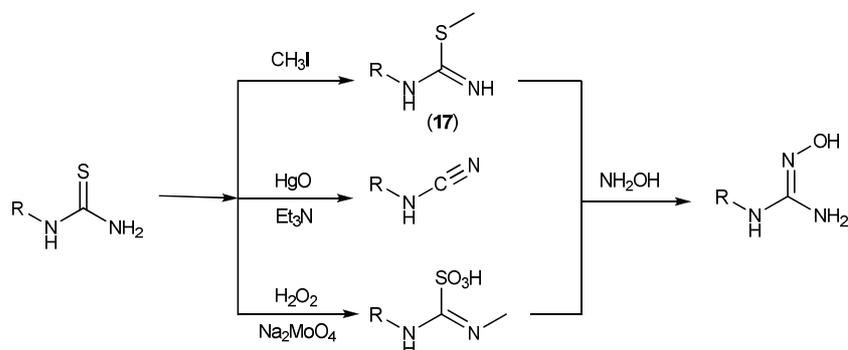
N-Hydroxyguanidines can be synthesized in various ways, depending on the structure of the final compound. Most techniques involve the reaction of electrophilic nitrogen rich species with hydroxylamine or its derivatives. The most popular and widely employed procedure involves cyanamides as key intermediates as shown in Scheme 1.3³⁰

Scheme 1.3³⁰



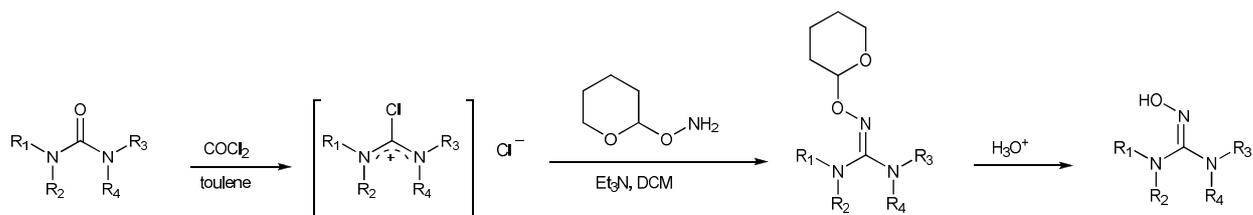
This method has been successfully utilized previously and was shown to be a reliable procedure for the preparation of NHA, homo-NHA and nor-NHA. However, only monosubstituted *N*-hydroxyguanidines can be synthesized using the above procedure.¹⁰

The second method employs the thioureas as starting materials as presented in Scheme 1.4.

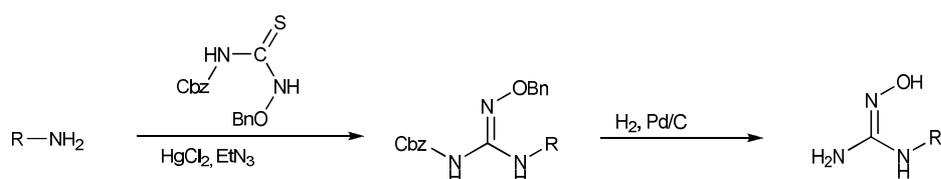
Scheme 1.4^{3, 10}

There are three methods for the conversion of thioureas to *N*-hydroxyguanidines. Firstly, the thiourea can be methylated to yield a reactive intermediate (17). The replacement of the thiomethyl group is then achieved by reaction with hydroxylamine, most of the time in the presence of silver nitrate or mercuric chloride.^{10, 34} Another method employs mercuric oxide which reacts with the thiourea to give a cyanamide.^{26, 35} This compound is then treated with hydroxylamine to give the final product. However, due to the high toxicity of the previously used reagents, the aminoiminomethanesulfonic acids have gained a lot of interest since their synthesis is reasonably mild. The key transformation involves activation of the sulfur in the thiourea through *S*-oxidation, followed by its displacement by hydroxylamine. A variety of oxidizing agents have been employed, among them a $\text{H}_2\text{O}_2/\text{Na}_2\text{MoO}_4$ mixture, peracetic acid and quaternary ammonium permanganate.³⁶⁻³⁹ The methods presented in Scheme 1.4 can be successfully used in the synthesis of disubstituted derivatives.

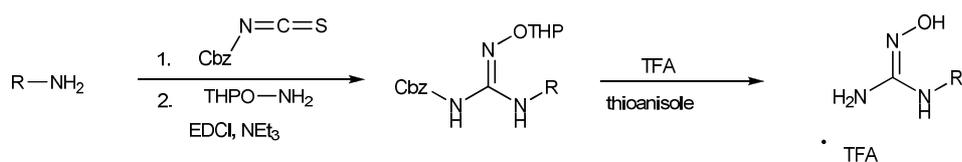
Ziman reported the synthesis of tri- and tetrasubstituted *N*-hydroxyguanidines using chloroformamidium chlorides, generated from ureas or thioureas, as reactive intermediates. These were reacted with THP protected hydroxylamine as shown in Scheme 1.5.⁴⁰

Scheme 1.5⁴⁰

Kalvins *et al.* recently developed a convenient reagent, 1-benzyloxy-3-benzyloxycarbonylthiourea, for *N*-hydroxyguanylation.⁴¹ This reagent can be treated with an amine to give *N*- and *O*-protected hydroxyguanidine derivatives as illustrated in Scheme 1.6. Catalytic hydrogenation is then used to remove protecting groups and give the final product.

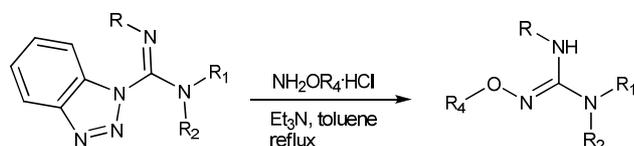
Scheme 1.6⁴¹

Recently, Marletta *et al.* reported a concise and general method for the preparation of *N*-hydroxyguanidines from primary amines (Scheme 1.7).⁴² It is a one-pot procedure followed by the deprotection under non-reducing conditions, which is a big advantage compared to Kalvins' method, because the *N*-hydroxygyanulation is possible in the presence of the reduction sensitive moieties. Both these recent methods can be employed to introduce the hydroxyguanidine functional group into relatively complex molecules.

Scheme 1.7⁴²

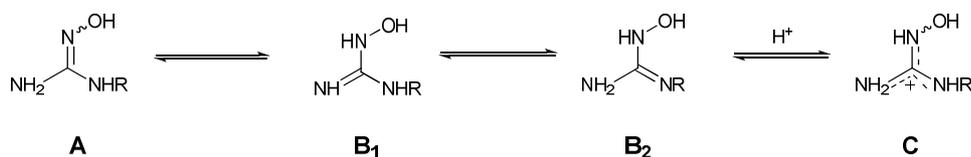
Yoshioka *et al.* published a procedure for the preparation of unsymmetrical *N*-hydroxyguanidines, using benzotriazole intermediates as presented in Scheme 1.8.⁴³ A variety of different *N*-hydroxyguanidines have been obtained using this approach in good yields. However, the major disadvantage of this method is the purification step in which basic alumina is used as a packing for the column chromatography, which is known to be a quite expensive material.

Scheme 1.8



Physical properties

Most *N*-hydroxyguanidines are stable at $-20\text{ }^{\circ}\text{C}$. Some of them are very hygroscopic. Therefore, special precautions must be taken in order to avoid their decomposition. They are also unstable with respect to base. In most cases they are synthesized as salts of a strong acid and stored under nitrogen. *N*-Hydroxylation of the guanidine moiety decreases the basicity. Thus for L-arginine the $\text{pK}_a=13.6$ while it is 8.1 for *N*-hydroxyarginine (NHA).^{3, 4, 10} The structure of NHA has been depicted in three different ways, as the oxime tautomer (**A**), the imine tautomer (**B₁** or **B₂**), and the protonated species (**C**) as shown in Scheme 1.9. According to Fukuto *et al.*, the structure (**C**), is the most likely form of NHA bound to NOS.^{10, 44}

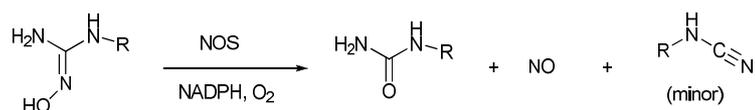
Scheme 1.9³

N-Hydroxyguanidines as NO donors

Production of NO from L-arginine is a result of the oxidation of its guanidine fragment by NOS. Therefore, it was anticipated that molecules which possess the guanidine or hydroxyguanidine moiety in their structure would act as NO donors *via* enzymatic action. Very few guanidine-containing compounds have been found to be substrates for NOS. However, endogenous NHA, *N*-hydroxy-L-homoarginine, *N*-aryl-*N'*-hydroxyguanidines, and some *N*-alkyl-*N'*-hydroxyguanidines have been shown to be substrates. The way these compounds are oxidized by NOS is similar to NHA, but the exact mechanism is not clear. There are several

proposed mechanisms, which involve various intermediates during the oxidation of *N*-hydroxyguanidines. However, the major products are ureas and NO, with cyanamides as a minor secondary metabolite (Scheme 1.10).^{3, 4, 10, 28, 29, 44-50}

Scheme 1.10³



This unexpectedly limited number of the exogenous substrates for NOS indicates that this enzyme needs very specific structural features. Structural studies showed that with attachment of a suitable hydrophobic group such as *n*-butyl or propyl to the *N*-hydroxyguanidine, a substrate for NOS could be obtained.¹⁰ The crystal structure of NHA bound to NOS indicated that the substrate binds close to the heme using a network of hydrogen bonds, in an almost identical manner to L-arginine.^{10, 51} However, an additional hydrogen bond is present between the NHA-hydroxy oxygen and the amido hydrogen of G365 as shown in Figure 1.9.

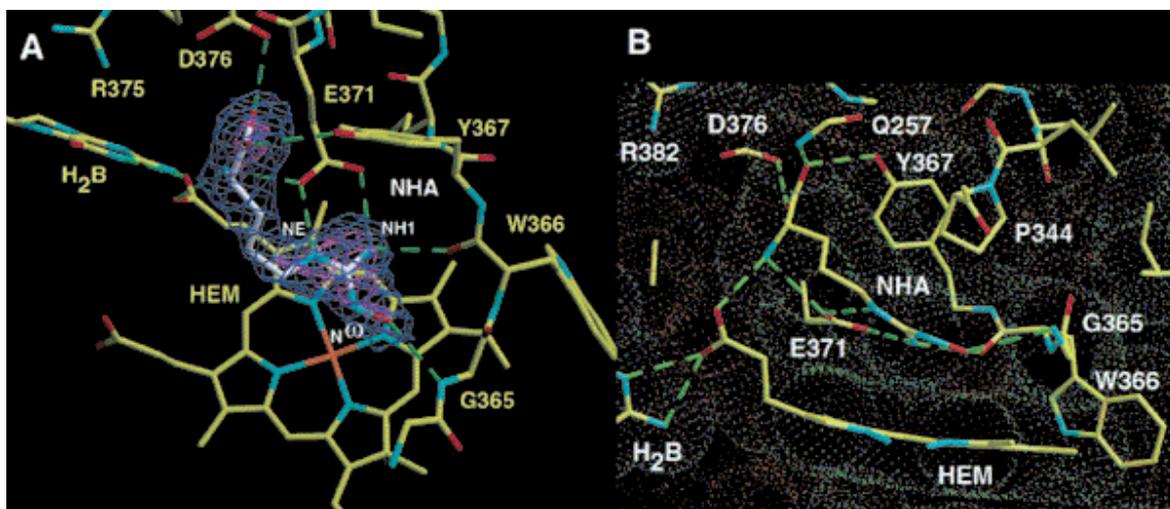


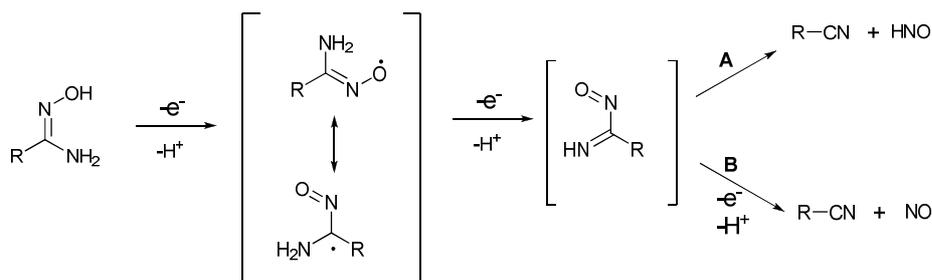
Figure 1.9 The crystal structure of NHA bound to NOS.⁵¹

The *N*-hydroxy group is tilted away from iron towards the protein backbone which indicates that NHA does not ligate Fe(III) heme. The NHA binding mode is almost identical for all three isoforms of NOS. The subtle variation is that the hydroxyguanidine moiety is planar in eNOS,

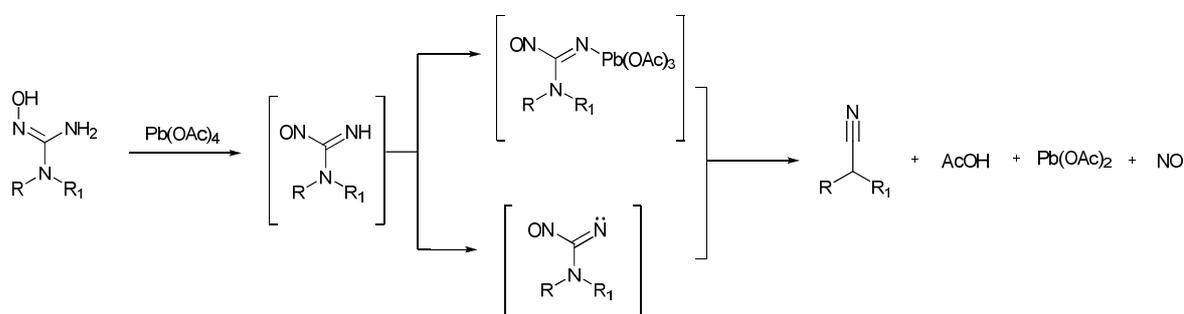
but not in iNOS or nNOS, although it is not clear why.^{3,51} Some crystal structures with different *N*-hydroxyguanidines bound to NOS have also been obtained and will be discussed later in this chapter.

However, as mentioned before, not only NOS is able to oxidize the *N*-hydroxyguanidines, but also the family of cytochrome P450 enzymes possess the ability to oxidize most of these compounds.⁴⁷ The result of this reaction is the formation of nitrogen oxides (NO/NO₂⁻/NO₃⁻), the ureas and also the corresponding cyanamides. The mechanism for this oxidation proposed by Mansuy *et al.* is shown in Scheme 1.11.⁵² It involves a one-electron oxidation of *N*-hydroxyguanidines, or amidoximes, with formation of an intermediate iminoxy radical, which is further oxidized to the nitrosoimine. Elimination of HNO produces RCN and HNO (route A) while further oxidation of the nitrosoimine produces RCN and NO (route B).

Scheme 1.11^{3, 4, 10}

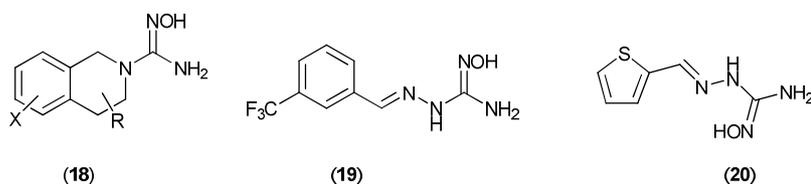


The chemical oxidation of *N*-hydroxyguanidines and their derivatives was studied by Fukuto *et al.*⁴⁵ Their studies indicated that various oxidants led to different products. It was found that lead tetraacetate and potassium ferricyanide/hydrogen peroxide generated NO (Scheme 1.12), while other oxidants (Fe-(III), PbO₂, Ag₂CO₃ and peracids) produced HNO. The organic product of the oxidation of *N*-hydroxyguanidines can be either the corresponding urea or the cyanamide, depending on the oxidants.^{3, 4, 10, 45}

Scheme 1.12³

Biological Activities and Applications

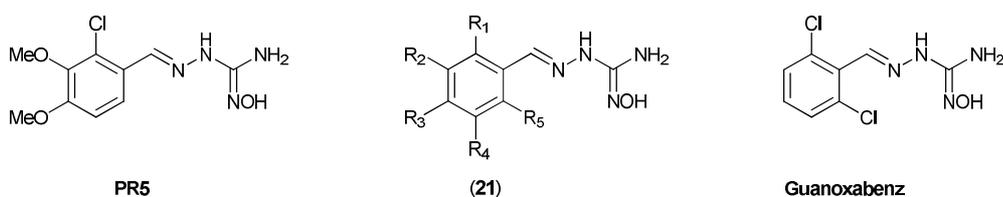
N-Hydroxyguanidines were reported as biologically interesting compounds long before the biosynthesis of NO was established. In 1973 DeGrazia *et al.* reported the synthesis of a series of 3,4-dihydro-2(1H)-isoquinolinecarboxamidoximes (**18**) and their evaluation for antihypertensive activity in two hypertensive rat models. The results indicated that *N*-hydroxyguanidines could be a potent, novel class of antihypertensive agents.²⁵

Scheme 1.13^{25, 27}

Ten years later, Khwaja *et al.* published the synthesis of a new series of *N*-hydroxyguanidines as shown in Scheme 1.13, with an aromatic (**19**) or heterocyclic ring (**20**).²⁷ Their anticancer and antiviral activities were tested using L1210 leukemia cells and chicken embryo fibroblasts infected with Rous sarcoma virus, respectively. Evaluated compounds exhibited cytotoxicity in leukemia cells and an anti-tumor activity *in vivo*.²⁷ In addition, Wikberg *et al.* studied *N*-hydroxyguanidines for their electron acceptor activity in the xanthine oxidase (XO) catalysed oxidation of xanthine. In their research they found that *N*-(2-chloro-3,4-dimethoxybenzylideneamino)-*N'*-hydroxyguanidine (PR5) was very effective as an alternative electron acceptor. PR5 possessed a significant protective effect against the induction of

myocardial necrosis and the arrhythmias in a heart ischemia and reperfusion model. However, the mechanism of action of PR5 remains elusive and requires further study.⁵³

Scheme 1.14^{53, 54}

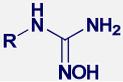


Recently, the same group synthesized a series of novel *N*-amino-*N'*-hydroxyguanidines (**21**) and also evaluated them for the electron acceptor activity in the XO-catalyzed oxidation of xanthine. It was found that compounds bearing nitro groups possessed the highest acceptor activity, but also three other derivatives showed reasonably good biological profiles.⁵⁴ These novel, XO electron accepting *N*-hydroxyguanidines can be potent therapeutic agents for the protection of tissues during conditions related to the ischemia-reperfusion. Additionally, Guanoxabenz (Scheme 1.14), which is a structurally related to the *N*-hydroxyguanidines tested by Wikberg *et al.*, has been used clinically as an antihypertensive agent.⁵⁴ Recently, NitroMed has claimed that NHA and other derivatives showed potential to be therapeutics for vasodilatation dysfunctions, hypoxia, cardiovascular disorders and even loss of memory.¹⁰

N-Alkyl-*N'*-Hydroxyguanidines

As most substrates of NOS are L-arginine or NHA derivatives, simple guanidines and isothiureas were tested as potential NOS substrates. However, it was found that *N*^ω-methyl-L-arginine, aminoguanidine and *S*-ethyl-isothiurea actually act as strong NOS inhibitors.³ Xian *et al.* synthesized and tested several *N*-alkyl-*N'*-hydroxyguanidines in order to examine their activity with NOS.⁵⁵ Table 1.3 is a summary of their findings.

Table 1.3 NO formation [nmol/min/mg protein] from oxidation of various *N*-alkyl-*N'*-hydroxyguanidines in the presence of NOS⁵⁵

	<i>iNOS</i>	<i>nNOS</i>	<i>eNOS</i>
R = ethyl	3 (3%)	6(1%)	4 (6%)
R = n-propyl	18 (15%)	418 (70%)	17 (24%)
R = iso-propyl	38 (32%)	450(76%)	27 (38%)
R = n-butyl	50 (42%)	380 (64%)	14 (20%)
R = sec-butyl	14 (12%)	107 (18%)	3 (4%)
R = iso-butyl	6 (5%)	14 (2%)	7 (10%)
R = tert-butyl	0 (0%)	0 (0%)	0 (0%)
R = n-pentyl	38 (32%)	185 (31%)	0 (0%)
R = iso-pentyl	29 (24%)	39 (6%)	0 (0%)
R = n-hexyl	3 (3%)	24(4%)	0 (0%)
R = cyclohexyl	4 (3%)	19 (3%)	0 (0%)

*The initial rate of NO synthesis was determined at 37 °C using a spectrophotometric oxyhemoglobin assay for NO. The concentration of substrate was 0.5 mM. The rates were also expressed as a percentage of those found for NHA.

The parent *N*-hydroxyguanidine is not a substrate of NOS. However, the introduction of small alkyl substituents produced new derivatives that are actually substrates for all NOS subtypes. When an ethyl group was attached to the *N*-hydroxyguanidine very low activity was detected. The elongation of the alkyl chain up to the four methylene groups increased the reactivity of the tested compounds. Further extension, e.g. to *n*-pentyl, caused the activity to start to decrease. On the other hand, bulky substituents such as *tert*-butyl or cyclohexyl destroyed activity completely. Therefore, the alkyl substituents can not be too small, too large or too bulky in order to maintain activity. In general, it has been found that simple exogenous compounds could be oxidized by NOS in a similar fashion to NHA.^{10, 11, 56}

The structure – activity relationships indicated that potential NOS substrates should possess:

- 1) a *N*-hydroxyguanidine functional group, which could anchor the substrate in the NOS active site;
- 2) a hydrophobic chain that interacts preferentially with the hydrophobic cavity in the NOS active site.

Additionally, Li *et al.* have published the crystal structures of rat nNOS complexed with *N*-butyl-*N'*-hydroxyguanidine (BHG) and *N*-isopropyl-*N'*-hydroxyguanidine (IHG), which are the most potent compounds among the *N*-alkyl-*N'*-hydroxyguanidines (Figure 1.10).⁵⁷

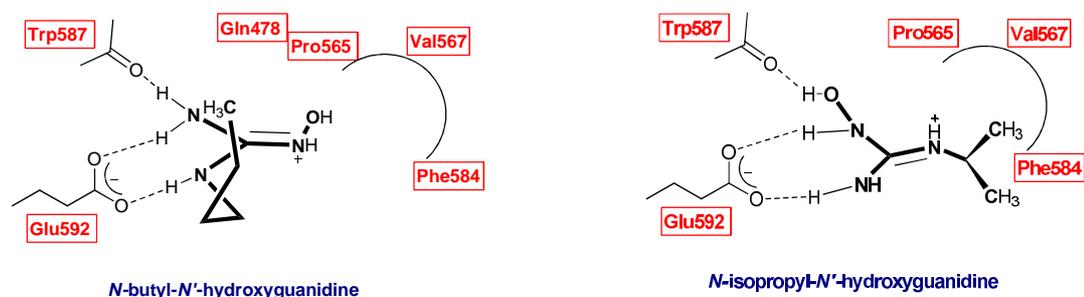


Figure 1.10 Schematic structures of BHG bound to nNOS *vs* IHG bound to nNOS.⁵⁷

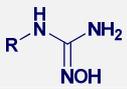
As presented above, the two compounds have quite different binding modes. BHG binds to the enzyme active site in a similar manner to the endogenous NHA. The only difference is in the position of the butyl group, which is tilted toward Gln478 and Val567 away from the binding site, while the methylene carbons of NHA are directed toward the active site. However, in the case of IHG, the isopropyl-substituted nitrogen occupied the space which is normally taken by the hydroxy-substituted terminal nitrogen. This surprising binding mode of IHG gives new details about the oxidation mechanism of *N*-hydroxyguanidines.^{10, 57} Studies done by Mansuy *et al.* with Val567 mutants of nNOS indicated that position of the hydrophobic isopropyl group of Val567 is closely related to the stability of the active site, the substrate recognition and the biosynthesis of the natural and exogenous substrate to NO.⁵⁸ Recently, the same group reported their findings relating to the relationship between the structure of *N*-hydroxyguanidines and their binding to iNOS.^{31, 59} They found that the binding affinity is maximal for the compounds bearing a butyl or *n*-pentyl chain, $K_d=20 \mu\text{M}$ and $100 \mu\text{M}$ respectively.⁶⁰ However, in the case of

an *n*-hexyl chain the binding affinity dropped significantly. In general, it was determined that the binding properties of the *N*-hydroxyguanidines were mainly dependent on the aromatic or aliphatic character of the tested compounds and the *N*-alkyl-*N'*-hydroxyguanidines showed significantly higher affinities for iNOS than the aryl derivatives. Mansuy *et al.* also claimed that *N*-(4-chlorophenyl)-*N'*-hydroxyguanidine was not a specific substrate for iNOS even though previously it was reported as an iNOS-specific substrate.⁶⁰

N-Aryl-*N'*-Hydroxyguanidines

Recently, Renodon-Cornière and co-workers synthesized several new *N*-aryl-*N'*-hydroxyguanidines, and related derivatives, as potential NOS substrates.^{30, 48} Results showed that nNOS, eNOS and iNOS possessed different affinities toward *N*-aryl-*N'*-hydroxyguanidines (Table 1.4).

Table 1.4 NO formation [nmol/min/mg protein] from the oxidation of monosubstituted *N*-aryl-*N'*-hydroxyguanidines catalyzed by eNOS, nNOS, iNOS*.³⁰

	<i>iNOS</i>	<i>nNOS</i>	<i>eNOS</i>
R = - C ₆ H ₅	20 ± 3	1.5 ± 0.5	< 0.2
R = - 4-F-C ₆ H ₄	41 ± 6	4.5 ± 1	< 0.2
R = - 4-Cl-C ₆ H ₄	13 ± 3	< 0.2	< 0.2
R = - 4-CH ₃ -C ₆ H ₄	17.5 ± 4	< 0.2	< 0.2
R = - 4-OH-C ₆ H ₄	16.5 ± 3	8.5 ± 1	< 0.2
R = - 4-CH ₃ O-C ₆ H ₄	8 ± 2	3.5 ± 1	< 0.2
R = - 3-NH ₂ -C ₆ H ₄	3.5 ± 1	2.5 ± 1	6.5 ± 2
R = - 4-NH ₂ -C ₆ H ₄	8 ± 2	4.5 ± 1	< 0.2
R = - 3-CH ₂ OH-C ₆ H ₄	6 ± 1	< 0.2	< 0.2

*The initial rate of NO synthesis was determined at 37 °C using spectrophotometric oxyhemoglobin assay for NO. The concentration of substrate was 20-30 nM.

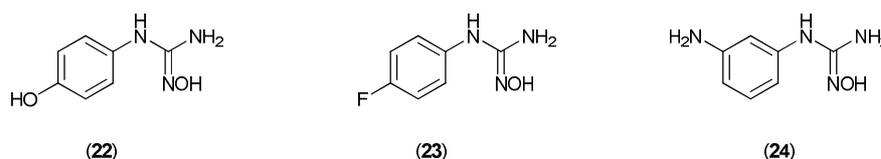
In general, it was found that there were more substrates for iNOS compared to eNOS and nNOS. In addition, the best substrates for each of the NOS isoforms differed significantly.

The testing of iNOS substrates led to general conclusions that:

- 1) introduction of a substituent in the *para* position of the phenyl ring leads to better activity than the introduction of the same substituent in the *ortho* or *meta* position;
- 2) smaller substituents give larger rates of NO formation;
- 3) the electron-donating substituents give higher rates of NO formation than electron-withdrawing groups.

Compound (22) (Scheme 1.15), bearing a *para*-hydroxy substituent, gave the highest rate of NO formation in the case of nNOS, whereas compounds (23) and (24) (Scheme 1.15) led to the highest rates of NO production in the case of iNOS and eNOS, respectively.³⁰

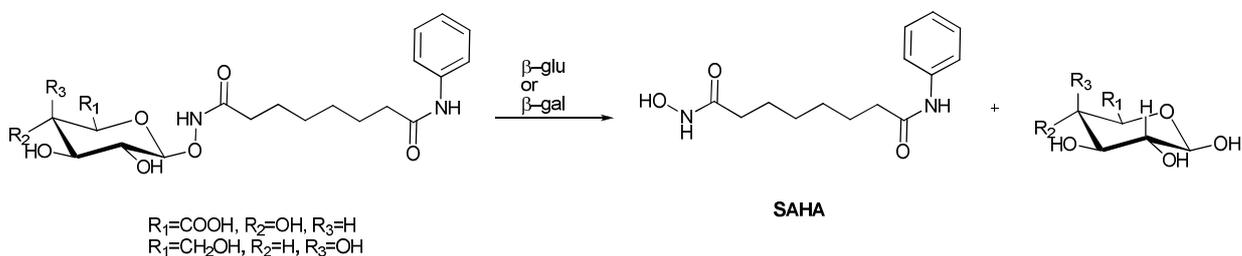
Scheme 1.15³⁰



Since some of the *N*-aryl-*N'*-hydroxyguanidines proved to be selective substrates, it was beneficial to obtain crystallographic data. Poulos *et al.* solved the crystal structure of bovine eNOS complexed with *N*-(4-chlorophenyl)-*N'*-hydroxyguanidine (CPHG). As expected the hydroxyguanidine functional group in CPHG adapts essentially the same configuration as *N*-hydroxyarginine (NHA).⁶¹

1.5.2 Hydroxamic Acids

Hydroxamic acids have been known since 1869, but until the 1980s they did not arouse a lot of interest. Hydroxamic acids are mainly recognized as a strong metal chelators and they are known to possess a wide spectrum of biological activities including enzyme-inhibitory properties.⁶²⁻⁶⁸ Recently, Papot *et al.* reported the synthesis and biological evaluation of the suberoylanilide hydroxamic acid (SAHA) β -glucuronide and β -galactoside as potential prodrugs for chemotherapy (Scheme 1.16).^{69, 70}

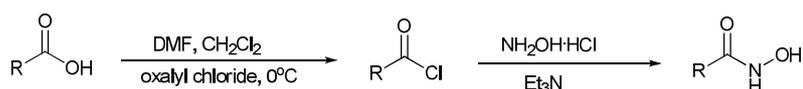
Scheme 1.16⁷⁰

SAHA demonstrated antitumour activity in several *in vivo* models and is currently progressing through phase III clinical trials; however it exhibits poor pharmacodynamic properties. Therefore, the non-toxic prodrug design was employed and SAHA coupled with sugar moieties, which were demonstrated elsewhere to be easily cleaved off by either β -glucuronidase or β -galactosidase enzymes once the prodrug reached the site of action. However, only the β -galactoside prodrug was active under the biological assay. Therefore it is a promising candidate for further *in vivo* evaluation.⁷⁰

In addition, some of the physiological roles of hydroxamic acids, such as their hypotensive effect (a known nitric oxide property) may be due to their ability to generate nitric oxide. However, there are still very few publications regarding this matter.

Synthesis of Hydroxamic Acids

Hydroxamic acids may be synthesized by treating esters, acid chlorides or acid anhydrides with hydroxylamine. The most common methods start from the corresponding carboxylic acids which are transformed into acid chlorides and then reacted with hydroxylamine hydrochloride (Scheme 1.17).^{62-64, 71-74}

Scheme 1.17⁷⁵

Recently, Devocelle and co-workers have published a useful method for the parallel synthesis of low molecular-weight hydroxamic acids from carboxylic acids and hydroxylamine using a solid state catalyst (Figure 1.11), namely a polymer-supported 1-hydroxybenzotriazole.⁷³

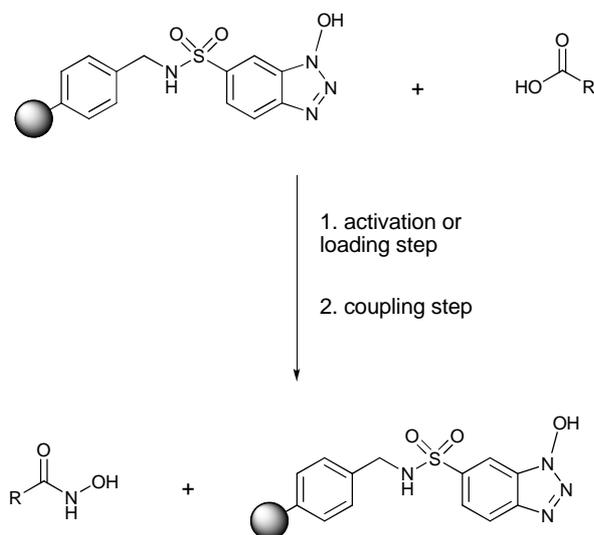
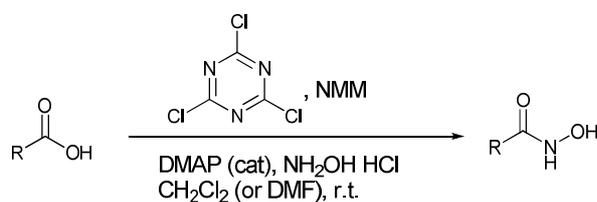


Figure 1.11 Parallel synthesis of low molecular-weight hydroxamic acids; reagents and conditions; 1. PS-HOBt (1 equiv.), carboxylic acid (1.5 equiv.), DIC (4.5 equiv.), DMAP (0.6 equiv.), CH₂Cl₂/DMF (1:1), 3h; 2. hydroxylamine (0.6 – 0.8 equiv.), THF, 5h.⁷³

The one-pot conversion of carboxylic acids to hydroxamic acids has also been recently reported by Giacomelli *et al.* (Scheme 1.18).⁷⁴

Scheme 1.18⁷⁴



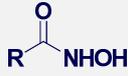
A suitable carboxylic acid is treated with 2,4,6-trichloro[1,3,5]-triazine (cyanuric chloride, TCT) (0.3 equiv.) and *N*-methylmorpholine (NMM) (1 equiv.) in CH₂Cl₂, and dimethylaminopyridine (DMAP) as a catalyst (0.1 equiv.), followed by hydroxylamine hydrochloride (1.1 equiv.) in dichloromethane. The corresponding hydroxamic acids are

obtained in pure form and in high yields. However, the reaction is not fast and requires from 6 to 12 h for completion.⁷⁴

Hydroxamic acids as NO donors

Koikov *et al.* synthesized a series of hydroxamic acids and investigated their ability to generate NO as a result of chemical oxidation using potassium ferricyanide as the oxidant.¹⁹ Among the tested hydroxamic acids, acetohydroxamic acid was shown to be the most effective NO donor. In general, it could be said that the incorporation of the pyridine ring inhibited the activity of suitable hydroxamic acids. However, there was no reasonable trend observed in the aromatic sub-series of hydroxamic acids as shown in Table 1.5. The preliminary biological results showed that about a two-fold activation of soluble guanylate cyclase from human platelets was observed for benzohydroxamic acid and 2-hydroxyphenylhydroxamic acid.¹⁹

Table 1.5 Yield of NO [%] from hydroxamic acids: oxidation (2×10^{-4} M, 4% EtOH/H₂O) by K₃[Fe(CN)₆] at pH 12.¹⁹

	% of NO
R = Me	22.9
R = Ph	9.0
R = 2-HOC ₆ H ₄	14.0
R = 2-MeOC ₆ H ₄	5.1
R = 4-HOC ₆ H ₄	6.5
R = 4-MeOC ₆ H ₄	16.4
R = 4-HOC ₆ H ₄ CH ₂	-
R = 2-Pyridine	0.0
R = 4-Pyridine	0.8
R = 2,6-disubstituted pyridyl	3.1

On the other hand, Marmion and co-workers have recently shown that hydroxamic acids are effective NO donors by forming highly stable ruthenium(II)-nitrosyls.^{67, 68, 76} It has been proven that the generated complex possesses the ability to cause vascular relaxation of rat aorta. Of the hydroxamic acids investigated, benzohydroxamic acid was shown to be the most effective. In general, the NO generated from hydroxamic acids was readily transferred to ruthenium(III).

Furthermore, hydroxamic acids can cause vascular relaxation in a rat aorta by the NO-mediated activation of enzyme guanylate cyclase.^{68, 76} Recently, the same group synthesized a series of Ru(III)-hydroxymato complexes and carried out a UV-VIS spectroscopic investigation in order to determine the NO-releasing properties of hydroxamic acids. They found out that NO release was the fastest from benzohydroxamic acids bearing an amino substituent in the *ortho* and *para* position. Their studies indicated that in the case of substituted aromatic hydroxamic acids the mesomeric as well as the inductive effect of the substituent on the phenyl ring played a crucial role in the rate of NO release. However, there is still very little known about the enzymatic mechanism of NO generation from the hydroxamic acids, although Marmion *et al.* observed that in order to cause smooth muscle relaxation, hydroxamic acids had to generate nitric oxide and activate guanylate cyclase.^{67, 68, 76}

1.6 Nitric oxide in biological systems

As discussed in previous sections both overproduction and deficiency of nitric oxide in the human body can be harmful. Since our work is focused on the development of novel NO donor pro-drugs the following chapter will consider mainly those disorders which are related to insufficient NO production.^{3, 16, 77}

1.6.1 Nitric Oxide Donors in Cardiovascular Disease

Nitric oxide plays a critical and diverse role in the cardiovascular system. Under healthy conditions, NO synthesized in the endothelium has a protective impact on a number of diseases. The pharmacological mechanisms of action of NO donors can be grouped into five categories: vasodilatation, decrease in myocardial oxygen consumption, improvement in hemodynamic performance, increase in myocardial blood flow and antithrombotic effects.^{1, 3-6, 18, 77-80}

Activation of eNOS in endothelial cells is mainly caused by shear stress (flow-dependent NO formation). This stimulates the vascular production of NO. Kojda *et al.* reported that eNOS is also activated by phosphorylation by serine/threonine protein kinase Akt/PKB.⁷⁸ In addition, recent studies indicated that receptor-mediated activation of eNOS by agonists such as acetylcholine is also related to phosphorylation by Akt.⁷⁸

NO activates guanylate cyclase (sGC) which catalyzes the formation of cyclic guanosine monophosphate (cGMP), an intracellular second messenger, followed by kinase-mediated signal transduction. Activation of sGC is related to the binding of NO to the heme fragment of the enzyme to form the nitrosyl – heme adduct of sGC (Figure 1.12).^{1, 81, 82}

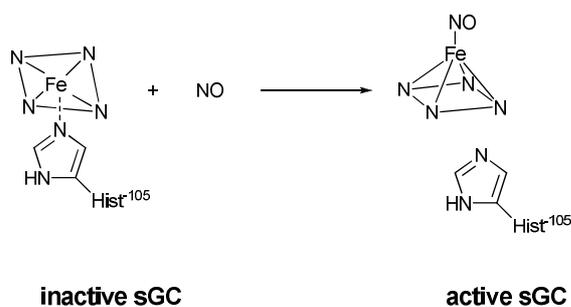


Figure 1.12 NO activation of cGC by binding to iron in the heme component.¹

The distortion of the heme iron out of the plane of the porphyrin ring causes the activation of sGC and therefore, formation of cGMP. Cyclic GMP induces two protein kinases (PKG I and PKG II). One of the protein kinases, (PKG I) is an important mediator in vasodilatation and the inhibition of platelet aggregation.^{78, 82, 83}

The most well understood activity of NO in the cardiovascular system is vasodilatation. Within the space of twenty years, studies have proved that endogenous NO synthesis is related to the regulation of vasomotion and blood pressure. It has been shown that pharmacological inhibition of NO production causes a rise in blood pressure in man. It is well known that diseases such as stroke and myocardial infraction are caused by the elevated blood pressure. Therefore, keeping the blood pressure at the proper level may be considered as part of the vasoprotective action of NO.⁷⁸

The mechanism underlying NO-induced vasodilatation has been intensively investigated. Recent findings indicate a major role for cGMP-dependent activation of PKG I which can phosphorylate various membrane proteins in the sarcoplasmic reticulum (Figure 1.13). Subsequently, phosphorylation of these proteins causes a decrease in the intracellular Ca^{2+} concentration.

Other more recent results show that activation of the PKG I phosphorylates a newly-found protein, the 1,4,5-inositoltrisphosphate (IP_3) receptor associated cGMP kinase substrate (IRAG).

Schlossmann *et al.* reported that phosphorylation of IRAG is a result of a strong inhibition of IP_3 -induced Ca^{2+} release from the sarcoplasmic reticulum.⁸⁴ However, the mechanism of the interaction between phosphorylated IRAG and the IP_3 receptor is not completely understood.⁷⁸

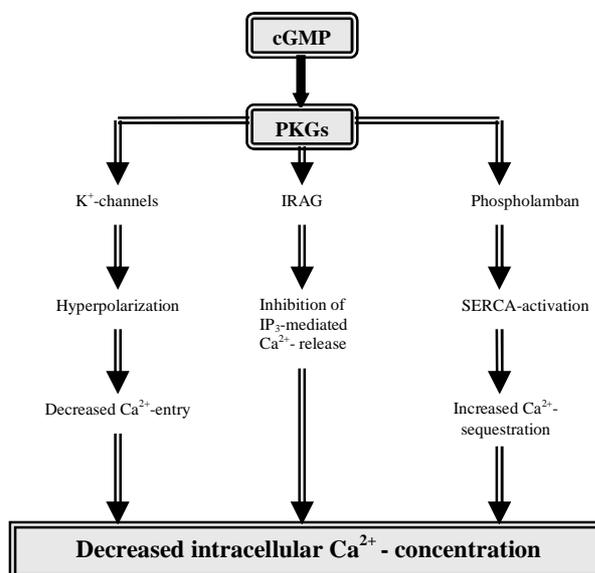


Figure 1.13 Mechanism of vasorelaxation induced by NO activation of cGMP⁷⁸

In addition, NO activates Ca^{2+} -dependent K^+ channels which causes a rise in the outward potassium current. The resulting hyperpolarization of the cell membrane evokes vasodilatation. Recent reports show that this NO action can be both independent and dependent on activation of PKG I.⁷⁸

Nitric oxide also participates in the inhibition of platelet aggregation. It has been reported that hydrophobic polymer materials that slowly release NO can find a wide application in medicine. Recent studies indicated that it is possible to design such a polymeric surface, which would mimic the inner surface of blood vessels by locally releasing NO. Platelets play a crucial role in haemostasis, whose proper functionality is controlled by nitric oxide. Constitutive or inducible generation of NO, results in the inhibition of platelet aggregation. This process may be induced by some aggregating factors or endothelial injury and increase of bleeding time. As discussed above the inhibition is based on the ability of NO to stimulate activation of soluble guanylate cyclase by binding to the heme moiety of the enzyme. Therefore, NO is an essential molecule in these diseases where a reduced vasodilatation is coupled to an impaired platelet functionality such as e.g. atherosclerosis and coronary artery diseases.^{1, 3, 18, 77, 78, 81}

1.6.2 Nitric Oxide Donors in Cancer

Nitric oxide's role in the cancer biology is complex and both, NO decrease and increase, anticancer strategies appear to be effective in preclinical models. The dual role of NO in carcinogenesis still remains unclear. NO generated by activated macrophages plays a crucial role in the defense mechanism against tumor cells thanks to its cytotoxic properties. On the other hand, some studies have indicated that elevated levels of NO are carcinogenic. Thus, far this dichotomy has challenged the development of novel and valuable anticancer therapeutics.⁸⁵⁻⁹⁰

Several publications reported the expression of iNOS by infected cells or within the tumor microenvironment. Studies indicated that iNOS activity depends on various factors such as: type of cancer and tumor stage. The most investigated are breast and colon cancer, but iNOS activity was also localized in the case of head and neck, lung, prostate, bladder and other types of cancer.⁸⁸

Several anti-cancer strategies have been reported, most of them are based on the NO-generating molecules.^{89,90}

1.6.3 Nitric Oxide in the Central and Peripheral Nervous System

Nitric oxide has been found to be a neurotransmitter and/or neuromodulator in both the central and peripheral nervous system. Its functionality is also controlled by a cGMP-dependent mechanism. NO production is catalyzed by the isoform nNOS, which is expressed in most brain regions. The CNS, which has the ability to synthesize NO, plays an important role in the activation of neuronal receptors for the major excitatory neurotransmitter glutamate. Ongoing studies have indicated that NO is involved in regulating feeding and drinking, male and female sex behavior, learning and memory and several other sensory pathways.^{1,3,6,13,15,79}

In the case of the peripheral nervous system, it is commonly accepted that the L-arginine-NO system is responsible for the non-adrenergic, non-cholinergic transmission in various anatomic sites. This kind of transmission is known as the nitrergic transmission. In addition, NO production occurs throughout the entire length of the nerve fibre.

On the other hand an overproduction of NO changes its role from the neuromodulator to a neurotoxic agent. NO is especially harmful under pathological conditions which involve the synthesis of reactive oxygen species (ROS) and ONOO⁻ formation. All neurodegenerative

disorders reveal a slow and gradually evolving death of selective neuronal populations. Therefore, Parkinson's disease, Huntington's disease, Alzheimer disease, amyotrophic lateral sclerosis, multiple sclerosis and ischemia are classified as neurodegenerative processes.^{3,6,13,15}

1.6.4 Other Biological Roles of Nitric Oxide

Recent findings postulated that a rise in the concentration of NO by human monocyte-derived macrophages is caused by the presence of the HIV envelope glycoprotein. In general, it was found that NO production was increased in patients who had AIDS. Most of the generated nitrogen species were detected as a nitrite and nitrate. Some of the reported results indicated that increasing formation of NO was related to the type of infection while some of them found that it was irrelevant. The contrasting effect of exogenous NO seems to be dependent on the type of NO donors, their releasing kinetics, and the dose used in the study.³

On the other hand, it has been known that NO possesses antiviral activity for several viruses. This includes murine poxvirus, herpes simplex virus, Epstein-Barr virus, coxsackievirus, and influenza virus. It has been suggested that many pathological consequences of NO proceed *via* its interaction with oxygen radicals, producing peroxynitrite. Based on the mechanism of the antiviral effects of NO, it has been indicated that NO may be a host response modulator rather than a simple antiviral agent. In addition, NO has been implicated in bone renewal in both healthy and diseased states. It mediates bone cell metabolism, where it regulates osteoblast and osteoclast functionality. Incorporation of NO or NO donors (GTN, 3-morpholinosydnonimine (SIN-1), S-nitrosothiols) into osteoclasts *in vitro* results in a reduction in bone resorption in comparison with NO synthase inhibitors which increase bone resorption. Recently, it has been reported that some of the non-enzymatic NO donors possess the ability to inhibit insulin release in isolated pancreatic islets. In other studies, L-arginine could stimulate glucose-induced insulin secretion from the pancreas of diabetic rats. In addition, sodium nitrite, sodium nitroprusside and S-nitroso-N-acetyl-DL-penicillamine increased insulin sensitivity through stimulation of NO production in the liver.³

In summary, it seems that it is much easier to develop new NO donors than actually introduce them as commonly used therapeutic agents - mainly, because it is very difficult to obtain tissue selectivity, controlled release and low toxicity of synthesized compounds. However,

some classes of the NO donors have found clinical applications as will be discussed in Section 1.7.

1.7 Nitric oxide – releasing pro-drugs

In general, the NO-releasing pro-drugs can be divided into two major classes. The first one includes the drug hybrids and the second one contains compounds which require enzymatic activation to release the active NO donor.^{3-6, 16, 79}

1.7.1 NO Donors/Drug Hybrids

One of the most recent innovations in the development of NO donor drugs is the combination of an NO donor fragment with another currently available drug. This approach has been designed to reduce the drug's toxicity on one hand and introduce NO-dependent biological activity on the other.

RSNO/Drug Hybrids

S-Nitrosothiols represent an important class of NO donor, which contains a single chemical bond between a thiol group (R-SH) and the NO fragment. The NO generation from *S*-nitrosothiols can be induced by various factors such as light, heat, transition metals and enzymes. Some of the *S*-nitrosothiols possessed tissue selectivity (GSNO is selective for arteries over veins). Additionally, they possess less stringent metabolic requirements which may be a reason that they do not induce tolerance with long-term use. *S*-Nitrosothiols are also potent antiplatelet agents. All these properties make them more potent NO donors compared to other classes of NO donor. Therefore, some pharmaceutical companies decided to modify currently available drugs with this functional group, mainly due to the fact that NO releasing drugs have proved to reduce the severity of mucosal injury.¹⁶ This activity may be due to NO and prostacyclin production. In Figure 1.14 the anti-inflammatory effect of NO-NSAIDs is presented to emphasize their activity in biological systems.

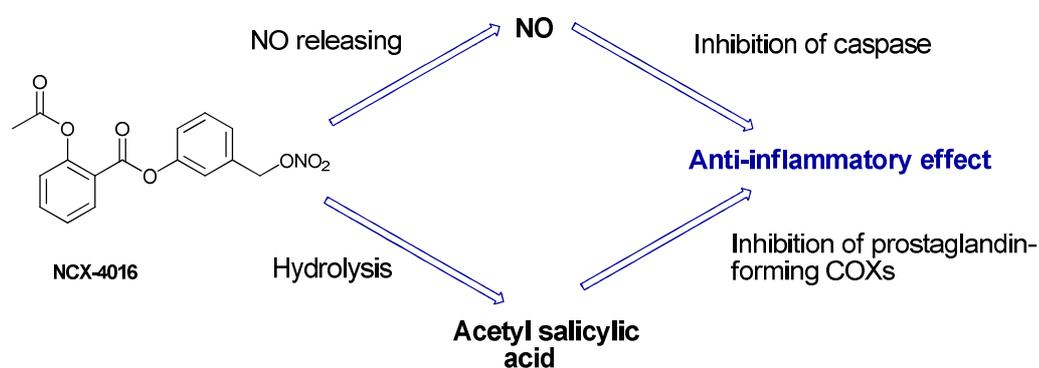
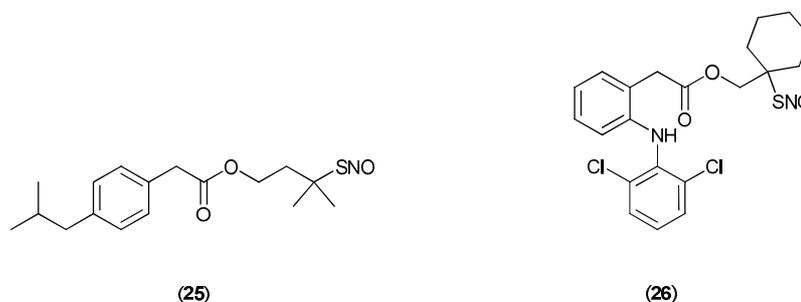


Figure 1.14 Anti-inflammatory mechanism of NO-NSAIDs³

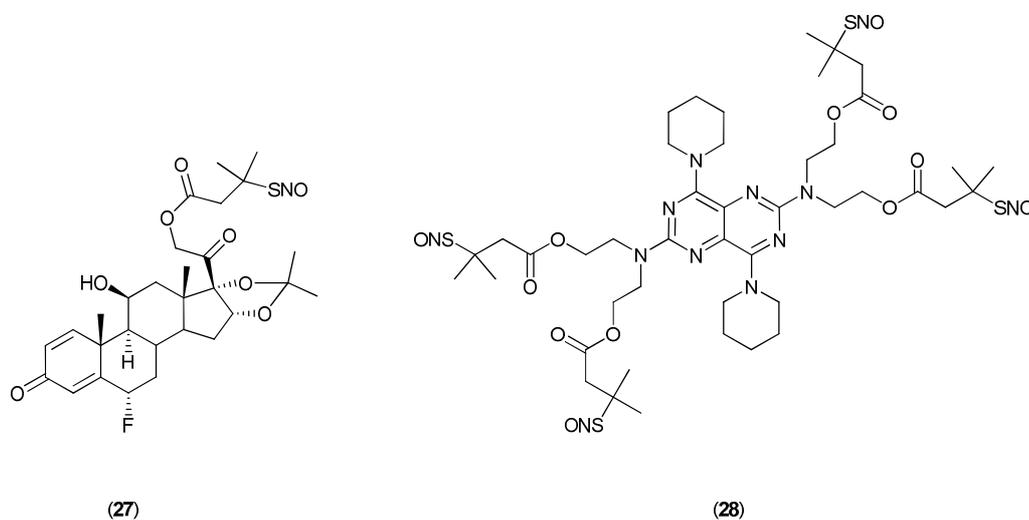
NitroMed has combined the *S*-NO moiety as the NO releasing fragment with ibuprofen (**25**), diclofenac (**26**) and other nonsteroidal anti-inflammatory drugs (NSAIDs–Scheme 1.19). These compounds constitute a unique class of NO-donating drugs with anti-inflammatory and analgesic properties but with an enhanced gastric safety profile. The major limitation for the long-term use of standard NSAIDs is their gastrointestinal toxicity.^{3, 6, 83, 91}

Scheme 1.19³



Indeed, the nitrosothiol esters of diclofenac revealed therapeutic potential as nonsteroidal anti-inflammatory agents with milder gastric effects.

NitroMed also attached an *S*-NO moiety to other bioactive molecules such as steroid and dopamine agonists as shown in Scheme 1.20.

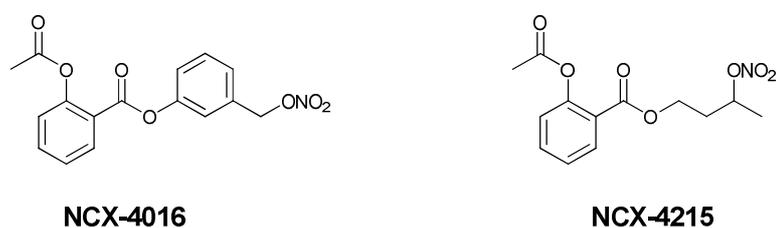
Scheme 1.20³

The steroid hybrid (27) was shown to be a more effective inhibitor of the immediate response in the ascaris-sensitive sheep assay, which indicated that (27) and its derivatives might be potent therapeutic agents in the treatment of asthma and other respiratory disorders. Compound (28) revealed an enhanced ability to relax the phenylephrine-induced contraction of humane corpus cavernosum tissue biopsy samples compared to dipyramidole.

Nitrosylated α -adrenergic receptor antagonists were also synthesized and examined as therapeutics for the treatment of impotence. It has been found out that they are more potent than their parent compounds (nitrosylated yohimibine and moxislyte).³

Nitrate/ Drug Hybrids

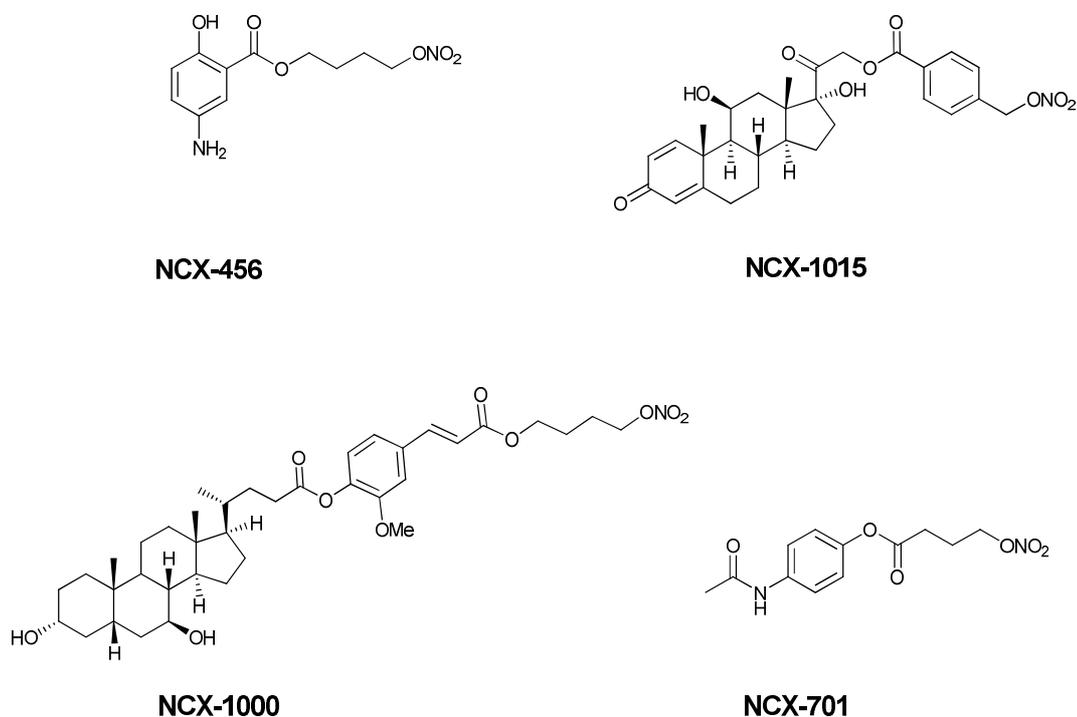
Nicox have designed several novel nitroxylated NSAID prodrugs, most of which are nitrate ester hybrids. Two aspirin hybrids were made 2-acetoxybenzoate-2-(2-nitroxymethyl)phenyl ester (NCX-4016) and 2-acetoxybenzoate-2-(2-nitroxy)butyl ester (NCX-4215) as illustrated in Scheme 1.21.^{3,87,91}

Scheme 1.21³

NCX-4016 and NCX-4215 were originally made to attempt to inhibit the gastric toxicity of aspirin. This activity may be due to NO and prostacyclin production.

It has been shown that NCX-4016 possesses higher effectiveness as an anti-inflammatory and antithrombotic agent than aspirin itself. Recently, Rigas *et al.* published that NCX-4016 is a novel drug for cancer prevention.^{87, 89} Interestingly, the same group reported that biologically active moiety of NO-aspirin is actually the spacer, which gives a reactive quinone methide.^{87, 92}

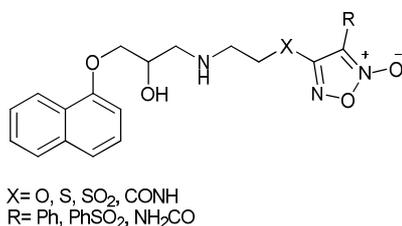
As well as NO-aspirin hybrids, NO-mesalamine (NCX-456 – Scheme 1.22) showed improved anti-inflammatory effects and can protect colonic epithelial cells from cytokine-induced apoptosis. NCX-1000 and NCX-1015 (Scheme 1.22) are steroid/NO bifunctional donors.⁹³ The addition of the NO-releasing fragment to NCX-1015 enhanced its anti-inflammatory activity, whilst NCX-1000 shared effective immunoregulatory and antiapoptotic activities.^{3,4,18}

Scheme 1.22³

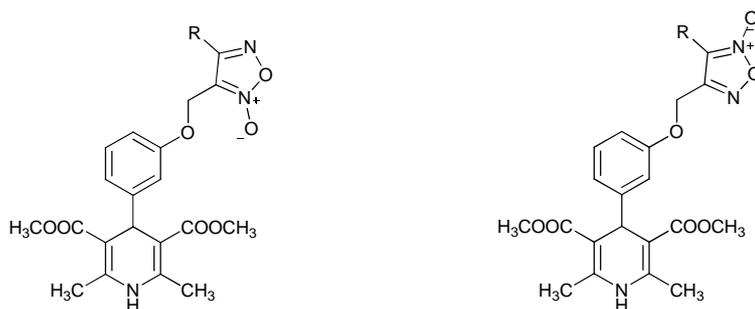
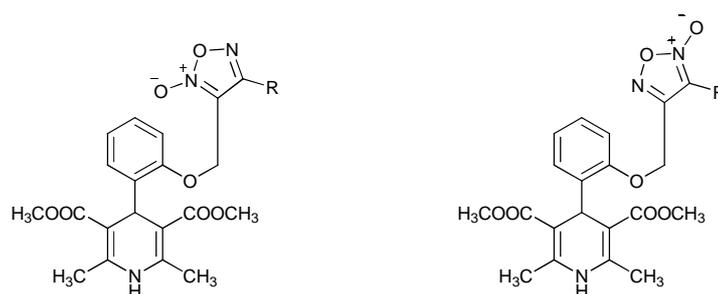
Herrero *et al.* reported that nitroacetamol (NCX-701) possessed better analgesic and anti-inflammatory properties than paracetamol in the case of the acute soft-tissue inflammation, monoarthritis and neuropathic pain models.⁹⁴ In addition, NCX-701 was safer to administrate than its parent compound from the gastric point of view.

Furoxan/Drug Hybrids

In order to obtain new vasodilators capable of showing both NO-dependent effects and the α_1 -antagonist activities, furoxan analogues of Prazosin were synthesized. The resulting bifunctional drugs possess various pharmacological properties. Furoxansulfonyl piperidine derivatives proved to be active for vasodilation and as α_1 -antagonists. Hybrids with mixed NO-dependent vasodilator and β -blocking activities (Scheme 1.23) have also been reported. Some of them displayed both activities, while others were only effective vasodilators or only β -blocking agents.¹⁶

Scheme 1.23³

De Stilo *et al.* have developed a series of novel 4-phenyl-1,4-dihydropyridines (Scheme 1.24) modified with a NO-releasing furoxan functionality.^{3, 95} These hybrid molecules show vasodilation by both NO dependent and calcium channel antagonist mechanisms.

Scheme 1.24³*meta series**ortho series*

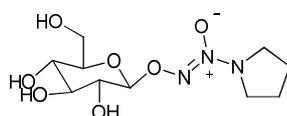
R = CH₃, CONH₂, CN

Recently, the synthesis of hybrid molecules was reported which combined the furoxan functionality with nicorandil (Scheme 1.25).³

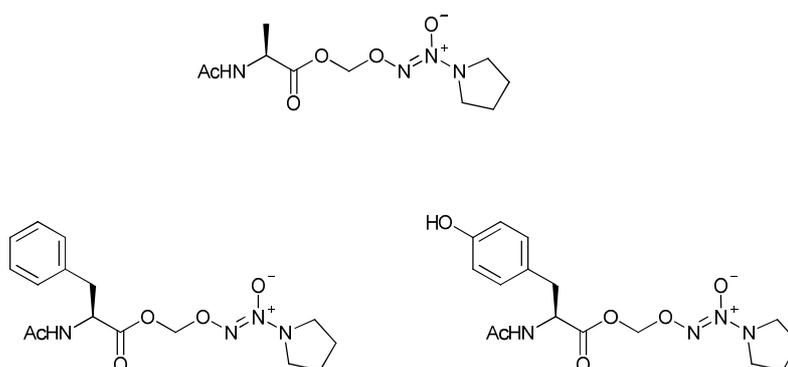
protect the liver from apoptotic cell death. It caused a rise in liver cGMP levels and protected rats from apoptosis and hepatotoxicity.

Most recently, Wang's group attached diazeniumdiolate functional groups to carbohydrate units, obtaining compounds as shown in Scheme 1.27. These prodrugs are more stable than their parent diazeniumdiolate salts and can produce NO upon activation by specific glycosidases.^{3, 20}

98-100

Scheme 1.27³

In addition Wang's group has also designed PSA (serine protease) substrate-nitric oxide donor pro-drugs as potential therapeutic agents against metastatic prostate cancer (Scheme 1.28).⁹⁹

Scheme 1.28⁹⁹

1.7.3 Other strategies

Recently, several research groups have been working on macromolecular NO donors. Meyerhoff *et al.* investigated water-soluble poly(ethylenimine)-based nitric oxide donors as

anti-thromobosis agents in hemodialysis systems.¹⁰¹ Additionally, Schoenfisch *et al.* synthesized *S*-nitrosothiol-modified dendrimers as a potent NO delivery vehicles. Novel dendrimers were capable of storing about 2 $\mu\text{mol NO/mg}$ when exposed to factors (light and copper) responsible for the decomposition of *S*-nitrosothiol, which makes them a promising tool for the site-selective delivery of NO.²²

Berke *et al.* developed water soluble iron nitrosyl complexes as NO donor prodrugs. They reported that depending on the character of the ligand, nitric oxide or nitroxyl donors were produced.²³ A similar approach was employed by Mascharak *et al.* and a set of ruthenium nitrosyl-dye conjugates have been synthesized and tested for NO release when exposed to visible light. Since, the complexes produced are photosensitive NO donors, they were easily tracked in the cellular environment. In addition, one of the conjugates was used to promote NO-induced apoptosis in MDA-MB-231 human breast cancer cells, which allowed for controlled delivery of NO to the site of action.¹⁰²

1.8 References

- 1 A. Butler and R. Nicholson, 'Life, death and nitric oxide', The Royal Society of Chemistry, Cambridge, 2003.
- 2 S. Pfeiffer, B. Mayer, and B. Hemmens, *Angew. Chem., Int. Ed.*, 1999, **38**, 1715.
- 3 P. G. Wang, Cai, T.B., Taniguchi N., 'Nitric Oxide Donors - For Pharmaceutical and Biological Applications', Willey-VCH Verlag GmbH & Co. KGaA, 2005.
- 4 P. G. Wang, M. Xian, X. Tang, X. Wu, Z. Wen, T. Cai, and A. J. Janczuk, *Chem. Rev.*, 2002, **102**, 1091.
- 5 C. Napoli and L. J. Ignarro, *Annu. Rev. Pharmacol. Toxicol.*, 2003, **43**, 97.
- 6 G. R. J. Thatcher, *Curr. Top. Med. Chem.*, 2005, **5**, 597.
- 7 P. Vetrovsky and G. Entlicher, *Collect. Czech. Chem. Commun.*, 1997, **62**, 1355.
- 8 Y. C. Hou, A. Janczuk, and P. G. Wang, *Curr. Pharm. Des.*, 1999, **5**, 417.
- 9 W. K. Alderton, C. E. Cooper, and R. G. Knowles, *Biochem. J.*, 2001, **357**, 593.
- 10 T. B. Cai, D. Lu, and P. G. Wang, *Curr. Top. Med. Chem.*, 2005, **5**, 721.
- 11 Q. Jia, T. Cai, M. Huang, H. Li, M. Xian, T. L. Poulos, and P. G. Wang, *J. Med. Chem.*, 2003, **46**, 2271.
- 12 E. S. Furfine, M. F. Harmon, J. Paith, R. G. Knowles, M. Salters, R. J. Kiff, C. Duffy, R. Hazelwood, J. Oplinger, and E. P. Garvey, *J. Biol. Chem.*, 1994, **269**, 26677.
- 13 E. P. Erdal, E. A. Litzinger, J. Seo, Y. Zhu, H. Ji, and R. B. Silverman, *Curr. Top. Med. Chem.*, 2005, **5**, 603.
- 14 N. M. Olken and M. A. Marletta, *J. Med. Chem.*, 1992, **35**, 1137.
- 15 F. X. Guix, I. Uribesalgo, M. Coma, and F. J. Munoz, *Progress in neurobiology*, 2005, **76**, 126.
- 16 M. R. Miller and I. L. Megson, *Br. J. Pharmacol.*, 2007, **151**, 305.
- 17 H. Katsumi, M. Nishikawa, and M. Hashida, *Cardiovasc. Hematol. Agents Med. Chem.*, 2007, **5**, 204.
- 18 L. J. Ignarro, C. Napoli, and J. Loscalzo, *Circ. Res.*, 2002, **90**, 21.
- 19 L. N. Koikov, N. V. Alekseeva, E. A. Lisitza, E. S. Krichevsky, N. B. Grigoriev, A. V. Danilov, I. S. Severina, N. V. Pyatakova, and V. G. Granik, *Mendeleev Commun.*, 1998, 165.
- 20 J. E. Saavedra, P. J. Shami, L. Y. Wang, K. M. Davies, M. N. Booth, M. L. Citro, and L. K. Keefer, *J. Med. Chem.*, 2000, **43**, 261.
- 21 S. I. Zavorin, J. D. Artz, A. Dumitrascu, A. Nicolescu, D. Scutaru, S. V. Smith, and G. R. J. Thatcher, *Org. Lett.*, 2001, **3**, 1113.
- 22 N. A. Stasko, T. H. Fischer, and M. H. Schoenfisch, *Biomacromolecules*, 2008, **9**, 834.
- 23 S. A. T. Dillinger, H. W. Schmalle, T. Fox, and H. Berke, *J. Chem. Soc., Dalton Trans.*, 2007, 3562.

- 24 M. J. Gorczynski, J. Huang, H. Lee, and S. B. King, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 2013.
- 25 D. M. Bailey, C. G. DeGrazia, H. E. Lape, R. Frering, D. Fort, and T. Skulan, *J. Med. Chem.*, 1973, **16**, 151.
- 26 S. C. Cherkofsky, 'Mono- and disubstituted hydroxyguanidines in the treatment of depression', Application: US, 1976.
- 27 A. W. Tai, E. J. Lien, M. M. C. Lai, and T. A. Khwaja, *J. Med. Chem.*, 1984, **27**, 236.
- 28 S. Dijols, C. Perollier, D. Lefevre-Groboillot, S. Pethe, R. Attias, J.-L. Boucher, D. J. Stuehr, and D. Mansuy, *J. Med. Chem.*, 2001, **44**, 3199.
- 29 C. Moali, J.-L. Boucher, A. Renodon-Corniere, D. J. Stuehr, and D. Mansuy, *Chem. Res. Toxicol.*, 2001, **14**, 202.
- 30 A. Renodon-Corniere, S. Dijols, C. Perollier, D. Lefevre-Groboillot, J. L. Boucher, R. Attias, M. A. Sari, D. Stuehr, and D. Mansuy, *J. Med. Chem.*, 2002, **45**, 944.
- 31 D. Lefevre-Groboillot, J. L. Boucher, D. J. Stuehr, and D. Mansuy, *FEBS J.*, 2005, **272**, 3172.
- 32 P. Beranova, K. Chalupsky, and G. Entlicher, *Collect. Czech. Chem. Commun.*, 2004, **69**, 499.
- 33 D. Lefevre-Groboillot, Y. Frapart, A. Desbois, J. L. Zimmermann, J. L. Boucher, A. C. F. Gorren, B. Mayer, D. J. Stuehr, and D. Mansuy, *Biochemistry*, 2003, **42**, 3858.
- 34 P. L. Feldman, *Tetrahedron Lett.*, 1991, **32**, 875.
- 35 V. D. Le and C.-H. Wong, *J. Org. Chem.*, 2000, **65**, 2399.
- 36 C. A. Maryanoff, R. C. Stanzione, J. N. Plampin, and J. E. Mills, *J. Org. Chem.*, 1986, **51**, 1882.
- 37 A. E. Miller and J. J. Bischoff, *Synthesis*, 1986, 777.
- 38 A. E. Miller, J. J. Bischoff, and K. Pae, *Chem. Res. Toxicol.*, 1988, **1**, 169.
- 39 A. R. Katritzky and B. V. Rogovoy, *ARKIVOC* 2005, 49.
- 40 S. D. Ziman, *J. Org. Chem.*, 1976, **41**, 3253.
- 41 A. Jirgensons, I. Kums, V. Kaus, and I. Kalvins, *Synth. Commun.*, 1997, **27**, 315.
- 42 N. I. Martin, J. J. Woodward, and M. A. Marletta, *Org. Lett.*, 2006, **8**, 4035.
- 43 A. R. Katritzky, N. M. Khashab, S. Bobrov, and M. Yoshioka, *J. Org. Chem.*, 2006, **71**, 6753.
- 44 D. J. Tantillo, J. M. Fukuto, B. M. Hoffman, R. B. Silverman, and K. N. Houk, *J. Am. Chem. Soc.*, 2000, **122**, 536.
- 45 J. M. Fukuto, D. J. Stuehr, P. L. Feldman, M. P. Bova, and P. Wong, *J. Med. Chem.*, 1993, **36**, 2666.
- 46 R. Zamora, A. Grzesiok, H. Weber, and M. Feelisch, *Biochem. J.*, 1995, **312**, 333.
- 47 A. Jousserandot, J.-L. Boucher, Y. Henry, B. Niklaus, B. Clement, and D. Mansuy, *Biochemistry*, 1998, **37**, 17179.
- 48 A. Renodon-Corniere, J.-L. Boucher, S. Dijols, D. J. Stuehr, and D. Mansuy, *Biochemistry*, 1999, **38**, 4663.

- 49 T. Cai, M. Xian, and P. G. Wang, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1507.
- 50 H. Kabasawa, M. Ikeno, M. Suzukawa, K. Higuchi, K. Yanagisawa, H. Seki, T. Tokunaga, and T. Ishikawa, *Helv. Chim. Acta*, 2002, **85**, 2636.
- 51 B. R. Crane, A. S. Arvai, S. Ghosh, E. D. Getzoff, D. J. Stuehr, and J. A. Tainer, *Biochemistry*, 2000, **39**, 4608.
- 52 D. Mansuy and J.-L. Boucher, *Free Radic. Biol. Med.*, 2004, **37**, 1105.
- 53 M. Dambrova, L. Baumane, A. Kiuru, I. Kalvinsh, and J. E. S. Wikberg, *Arch. Biochem. Biophys.*, 2000, **377**, 101.
- 54 P. Prusis, M. Dambrova, V. Andrianov, E. Rozhkov, V. Semenikhina, I. Piskunova, E. Ongwa, T. Lundstedt, I. Kalvinsh, and J. E. S. Wikberg, *J. Med. Chem.*, 2004, **47**, 3105.
- 55 M. Xian, N. Fujiwara, Z. Wen, T. Cai, S. Kazuma, A. J. Janczuk, X. Tang, V. V. Telyatnikov, Y. Zhang, X. Chen, Y. Miyamoto, N. Taniguchi, and P. G. Wang, *Bioorg. Med. Chem.*, 2002, **10**, 3049.
- 56 P. Beranova, K. Chalupsky, A. L. Kleschyov, C. Schott, J. L. Boucher, D. Mansuy, T. Munzel, B. Muller, and J. C. Stoclet, *Eur. J. Pharmacol.*, 2005, **516**, 260.
- 57 H. Li, H. Shimizu, M. Flinspach, J. Jamal, W. Yang, M. Xian, T. Cai, E. Z. Wen, Q. Jia, P. G. Wang, and T. L. Poulos, *Biochemistry*, 2002, **41**, 13868.
- 58 M. Moreau, H. Takahashi, M.-A. Sari, J. L. Boucher, I. Sagami, T. Shimizu, and D. Mansuy, *J. Inorg. Biochem.*, 2004, **98**, 1200.
- 59 D. Mansuy and P. Lafite, *J. Porphyrins Phtthalocyanines*, 2007, **11**, 258.
- 60 M. Moreau, J. L. Boucher, T. A. Mattioli, D. J. Stuehr, D. Mansuy, and J. Santolini, *Biochemistry*, 2006, **45**, 3988.
- 61 H. Li, C. S. Raman, P. Martasek, V. Kral, B. S. S. Masters, and T. L. Poulos, *J. Inorg. Biochem.*, 2000, **81**, 133.
- 62 M. A. Stolberg, W. A. Mosher, and T. Wagner-Jauregg, *J. Am. Chem. Soc.*, 1957, **79**, 2615.
- 63 J. B. Summers, H. Mazdiyasi, J. H. Holms, J. D. Ratajczyk, R. D. Dyer, and G. W. Carter, *J. Med. Chem.*, 1987, **30**, 574.
- 64 M. J. Miller, *Chem. Rev.*, 1989, **89**, 1563.
- 65 V. Tiwari and R. Pande, *Chem. Biol. Drug Des.*, 2006, **68**, 225.
- 66 M. Flipo, T. Beghyn, J. Charton, V. A. Leroux, B. P. Deprez, and R. F. Deprez-Poulain, *Bioorg. Med. Chem.*, 2007, **15**, 63.
- 67 D. Griffith, K. Krot, J. Comiskey, K. B. Nolan, and C. J. Marmion, *J. Chem. Soc., Dalton Trans.*, 2008, 137.
- 68 C. J. Marmion, D. Griffith, and K. B. Nolan, *Eur. J. Inorg. Chem.*, 2004, 3003.
- 69 M. Thomas, J. P. Gesson, and S. Papot, *J. Org. Chem.*, 2007, **72**, 4262.

- 70 M. Thomas, F. Rivault, I. Tranoy-Opalinski, J. Roche, J. P. Gesson, and S. Papot, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 983.
- 71 M. C. Pirrung and J. H. L. Chau, *J. Org. Chem.*, 1995, **60**, 8084.
- 72 M. A. Bailen, R. Chinchilla, D. J. Dodsworth, and C. Najera, *Tetrahedron Lett.*, 2001, **42**, 5013.
- 73 M. Devocelle, B. M. McLoughlin, C. T. Sharkey, D. J. Fitzgerald, and K. B. Nolan, *Org. Biomol. Chem.*, 2003, **1**, 850.
- 74 G. Giacomelli, A. Porcheddu, and M. Salaris, *Org. Lett.*, 2003, **5**, 2715.
- 75 A. R. Ritter and M. J. Miller, *J. Org. Chem.*, 1994, **59**, 4602.
- 76 C. J. Marmion, T. Murphy, K. B. Nolan, and J. R. Docherty, *Chem. Comm.*, 2000, 1153.
- 77 D. McGrowder, D. Ragoobirsingh, and P. Brown, *Int. J. Pharm.*, 2006, **2**, 366.
- 78 M. T. Gewaltig and G. Kojda, *Cardiovasc. Res.*, 2002, **55**, 250.
- 79 G. Cirino, *Dig. Liver Dis.*, 2003, **35**, S2.
- 80 A. G. Herman and S. Moncada, *Eur. Heart J.*, 2005, **26**, 1945.
- 81 B. E. Mann and R. Motterlini, *Chem. Comm.*, 2007, **41**, 4197.
- 82 F. Murad, *N. Engl. J. Med.*, 2006, **355**, 2003.
- 83 A. Martelli, S. Rapposelli, and V. Calderone, *Curr. Med. Chem.*, 2006, **13**, 609.
- 84 J. Schlossmann, A. Ammendola, K. Ashman, X. Zong, A. Huber, G. Neubauer, G. X. Wang, H. D. Allescher, M. Korth, M. Wilm, F. Hofmann, and P. Ruth, *Nature*, 2000, **404**, 197.
- 85 Q. Jia, A. J. Janczuk, T. Cai, M. Xian, Z. Wen, and P. G. Wang, *Expert Opin. Ther. Pat.*, 2002, **12**, 819.
- 86 T. B. Cai and P. G. Wang, *Expert Opin. Ther. Pat.*, 2004, **14**, 849.
- 87 K. Kashfi and B. Rigas, *Biochem. Soc. Trans.*, 2005, **33**, 701.
- 88 S. Mocellin, V. Bronte, and D. Nitti, *Med. Res. Rev.*, 2007, **27**, 317.
- 89 B. Rigas, *Biochem. Soc. Trans.*, 2007, **35**, 1364.
- 90 B. Bonavida, S. Baritaki, S. Huerta-Yepez, M. I. Vega, D. Chatterjee, and K. Yeung, *Nitric Oxide*, 2008, **19**, 152.
- 91 S. Fiorucci, L. Santucci, and E. Distrutti, *Dig. Liver Dis.*, 2007, **39**, 1043.
- 92 K. Kashfi and B. Rigas, *Biochem. Biophys. Res. Commun.*, 2007, **358**, 1096.
- 93 S. Fiorucci, E. Antonelli, V. Brancaleone, L. Sanpaolo, S. Orlandi, E. Distrutti, G. Acuto, C. Clerici, M. Baldoni, P. Del Soldato, and A. Morelli, *J. Hepatol.*, 2003, **39**, 932.
- 94 E. A. Romero-Sandoval, M. M. Curros-Criado, G. Gaitan, C. Molina, and J. F. Herrero, *CNS Drug Rev.*, 2007, **13**, 279.
- 95 A. Di Stilo, S. Visentin, C. Cena, A. M. Gasco, G. Ermondi, and A. Gasco, *J. Med. Chem.*, 1998, **41**, 5393.

- 96 J. E. Saavedra, T. R. Billiar, D. L. Williams, Y.-M. Kim, S. C. Watkins, and L. K. Keefer, *J. Med. Chem.*, 1997, **40**, 1947.
- 97 H. Chakrapani, B. M. Showalter, L. Kong, L. K. Keefer, and J. E. Saavedra, *Org. Lett.*, 2007, **9**, 3409.
- 98 X. Wu, X. Tang, M. Xian, and P. G. Wang, *Tetrahedron Lett.*, 2001, **42**, 3779.
- 99 X. Tang, M. Xian, M. Trikha, K. V. Honn, and P. G. Wang, *Tetrahedron Lett.*, 2001, **42**, 2625.
- 100 X. Tang, T. Cai, and P. G. Wang, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1687.
- 101 Z. Zhou, G. M. Annich, Y. Wu, and M. E. Meyerhoff, *Biomacromolecules*, 2006, **7**, 2565.
- 102 M. J. Rose, N. L. Fry, R. Marlow, L. Hinck, and P. K. Mascharak, *J. Am. Chem. Soc.*, 2008, **130**, 8834.

N-HYDROXYGUANIDINES

2.1 Introduction

The importance of NO in biological systems is fully discussed in the introduction. Recently, it has been proven that various *N*-hydroxyguanidines can act as NO donors and some of the current studies have investigated the importance of the structural and electronic factors which determine whether a specific molecule would be a good NO donor.¹ In addition, more detailed studies have been carried out to establish what determines their potency and selectivity as substrates of the three NOS isoforms.

The initial goal was to synthesize a series of new *N*-arylalkyl-*N'*-hydroxyguanidines and test their ability to release NO and cause smooth muscle relaxation in the isolated rat aorta. The most potent molecules were then tested in the isolated perfused kidney, since the ultimate goal is to find a selective drug for an acute renal failure. In addition, they were subjected to toxicological studies. An investigation of their structural and electronic factors will also be carried out in order to determine how they affect nitric oxide release. The next step will then focused on designing a NO donor prodrug based on the best parent molecule which will be then attached to a suitable carrier molecule to allow selective delivery of NO to the desired sites of action.

From the results of biological testing carried out by our collaborators at the University of Edinburgh, the most effective *N*-hydroxyguanidine in terms of NO donation so far was *N*-phenylethyl-*N'*-hydroxyguanidine (QZNO193, Figure 2.1) synthesized in the Botting group.²

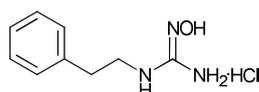
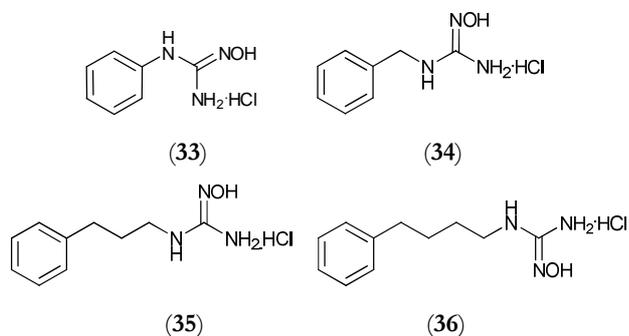


Figure 2.1 *N*-phenylethyl-*N'*-hydroxyguanidine (QZNO193)

This molecule was tested in the isolated rat aorta and revealed good vasorelaxation properties (see section 2.2). QZNO193 contained a hydrophobic nitrogen substituent, but was still water soluble due to the polar nature of the *N*-hydroxyguanidine functionality. The initial target was therefore to make similar *N*-hydroxyguanidines with longer alkyl chains and investigate the effect of chain extension on the biological activity. Therefore, four new analogues were required with 4, 3, 1 or 0 methylene groups between the nitrogen and benzene ring, as shown in Scheme 2.1.

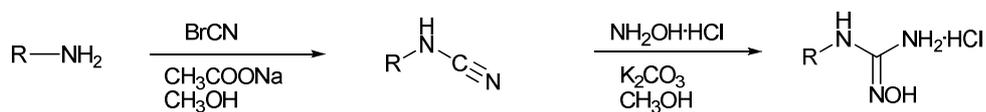
Scheme 2.1



As presented in the introduction a number of methods are available for the synthesis of *N*-hydroxyguanidines and the target compounds were synthesized using methods based on the published procedures which employed a cyanamide intermediate.¹ However, some modification had to be introduced in order to improve the purity of the final products.

The general procedure, which has been successfully used in the Botting group, is shown in Scheme 2.2. The first series of *N*-hydroxyguanidines was obtained from commercially available amines.

Scheme 2.2



R = Ph, Ph-CH₂, Ph-(CH₂)₃, Ph-(CH₂)₄

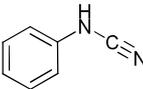
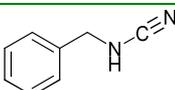
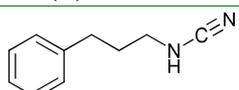
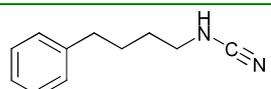
2.1.1 Synthesis of cyanamides

For N-arylalkyl-N'-hydroxyguanidines

As shown in the Scheme 2.2, most of the cyanamides were synthesized from the commercially available amines. The detailed procedure is described based on the 4-phenylbutylcyanamide. Reaction of the 4-phenylbutylamine with cyanogen bromide (1.05 equivalents) and sodium acetate in methanol gave the corresponding cyanamide (**37**). Analysis of the crude product by TLC indicated that there was still some of the unreacted amine. Therefore, the product was partitioned between CH_2Cl_2 and an aqueous solution of HCl (0.1 M) in attempt to remove the starting amine. However, this was not completely successful. Therefore, further purification was required by column chromatography.

4-Phenylbutylcyanamide (**37**) was obtained in 50% yield and characterized by ^1H and ^{13}C NMR spectroscopy, mass spectrometry and IR spectroscopy. The most clear-cut indications of the product structure are the presence of a weak signal at 116 ppm in the ^{13}C NMR spectrum and a sharp peak at 2215 cm^{-1} in the IR spectrum, which are both characteristic of a nitrile group ($\text{C}\equiv\text{N}$). The most indicative data for the synthesized cyanamides are presented in Table 2.1.

Table 2.1 Yields and characteristic spectral data for synthesized cyanamides

<i>Cyanamide</i>	<i>IR $\nu_{\text{max}}/\text{cm}^{-1}$ $\text{C}\equiv\text{N}$</i>	<i>$\delta^{13}\text{C}$ NMR [ppm] $\text{C}\equiv\text{N}$</i>	<i>yield [%]</i>
 (38)	2227	111.3	73
 (39)	2214	116.0	79
 (40)	2215	116.2	65
 (37)	2215	116.0	50

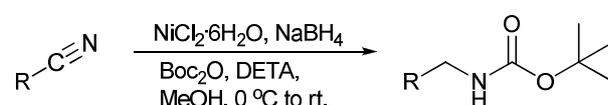
As seen in Table 2.1 all the cyanamides were synthesized in good yields. The phenylcyanamide data differed slightly compared to three other cyanamides. This is due to the electronic effect of the benzene ring directly attached to the cyanamide functional group. The electron withdrawing effect shifted the ^{13}C NMR resonance upfield by 5 ppm relative to the other derivatives. Also the $\text{C}\equiv\text{N}$ vibration in the IR spectrum is moved to higher wavenumber compared to three other analogues.

For derivatives of N-phenylethyl-N'-hydroxyguanidines and heterocyclic N-hydroxyguanidines

As mentioned previously N-phenylethyl-N'-hydroxyguanidine showed the best pharmacological profile. Therefore, various analogues with electron-donating and electron-withdrawing groups, mainly in *para* position of the benzene ring, were synthesized to probe further the NO donor activity.

For this series, the starting amines had to be obtained by reduction of commercially available nitriles. The most common procedures for reduction of nitriles to amines employ strong hydride donors, such as lithium aluminum hydride or catalytic hydrogenation.³ Recently, a new approach has been published for the catalytic reduction of nitriles.^{4, 5} Caddick *et al.* have synthesized a number of Boc protected amines *via* a mild catalytic process.⁵ This method greatly reduces the toxicity of the reduction due to its catalytic character. Nickel (II) chloride (NiCl_2) and an excess of sodium borohydride (NaBH_4) are used as an indirect reducing agent. However, there are a few more requirements which have to be met in order for the reaction to work. Namely, the starting nitrile needs to be present in the reaction mixture before formation of the metal boride. In addition, the nickel boride has to be prepared freshly before the reaction. The excess of sodium borohydride and an addition of the trapping agent (diethylenetriamine) are crucial in order to avoid the formation of dimers.⁵ The advantage of the method is that Boc protection reduces the water solubility of the synthesized amines and reduces their ability to complex to metals. This allows for their easier isolation and purification. In general, the reaction conditions are very mild and the reaction route is shown in Scheme 2.3.

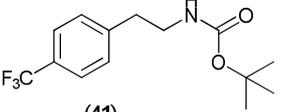
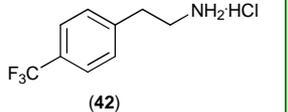
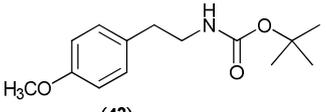
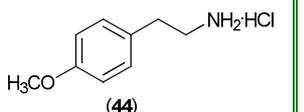
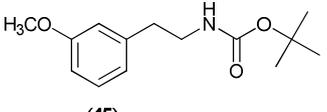
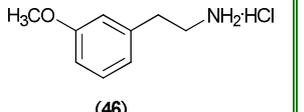
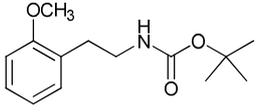
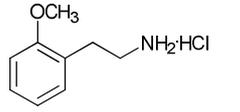
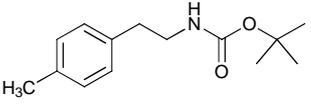
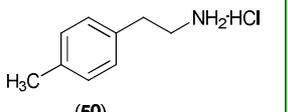
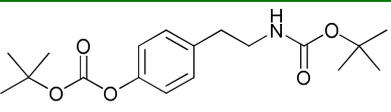
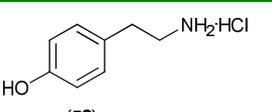
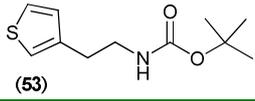
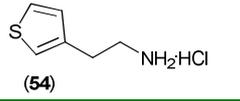
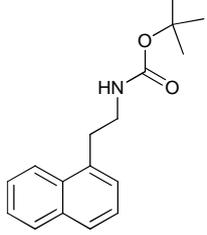
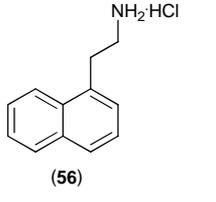
Scheme 2.3⁵



Even though the general procedure has been reported for *N*-Boc-benzylamine, various nitriles required longer reaction times to give products in reasonable yields. Therefore, an attempt was made to find the optimum requirements for the reduction of commercially available 4-(trifluoromethyl)phenylacetonitrile to the corresponding protected amine. By trying various reaction conditions a few main conclusions could be drawn. Firstly, the extension of the reaction time worsened the yield of the reaction. Secondly, going from 1 g of starting nitrile up to 4 g also negatively affected the overall yield of the reduction. This may be due to the fact that the sodium borohydride must be added in small portions because the reaction is exothermic and effervescent. In order to control the reaction temperature the sodium borohydride had to be added very slowly. The slower addition of a larger amount of borohydride extended the overall reaction time, which might affect the composition of the reaction mixture as well as temperature and result in a lower yield.

The synthesis of the Boc-protected 4-(trifluoromethyl)phenylamine (**41**) eventually resulted in a satisfactory 61% yield. The amine was characterized by ^1H , ^{19}F and ^{13}C NMR spectroscopy and elemental analysis. The presence of the *tert*-butyl protons (9H, 1.51 ppm) in the ^1H NMR spectrum confirmed the chemical structure. In addition, the characteristic peak (155.8 ppm) for the carbonyl group in the ^{13}C NMR spectrum was observed as well as a single peak (-62.8 ppm) for the CF_3 group in the ^{19}F NMR spectrum. The purity of the synthesized material was confirmed by elemental analysis, which pointed to nearly 100% purity. However, a major problem was met while trying to remove the protecting group. The first attempt employed 1M HCl in diethyl ether and reaction was carried out at 0 °C. In the second one, the temperature was increased to room temperature, but still the protected amine was recovered. Finally, the successful deprotection was achieved using 4M HCl in 1,4-dioxane at room temperature. The previous trials indicated that the strength of the acid and temperature were essential for favorable deprotection. The desired amine (**42**) was obtained in 90% yield and characterized by ^1H , ^{13}C NMR spectroscopy, MS and elemental analysis. The disappearance of the peak corresponding to *tert*-butyl group in the ^1H NMR spectrum confirmed successful deprotection. In addition, removal of the Boc group was indicated by the absence of the carbonyl carbon in the ^{13}C NMR spectrum. The 4-(trifluoromethyl)phenylamine (**42**) was almost 100% pure according to results of MS and elemental analysis (see experimental). The yields for the catalytic reduction of nitriles to Boc protected amines and for the deprotection to the amine are given in Table 2.2 for all the other derivatives.

Table 2.2 Yields for the catalytic reduction of nitriles together with yields from the deprotection step

<i>Boc-protected amine</i>	<i>yield [%]</i>	<i>amine</i>	<i>yield [%]</i>
 (41)	61	 (42)	90
 (43)	60	 (44)	98
 (45)	61	 (46)	86
 (47)	75	 (48)	75
 (49)	72	 (50)	100
 (51)	55	 (52)	71
 (53)	54	 (54)	87
 (55)	63	 (56)	94

The Boc protected amines were obtained in very good yields and high purity. The next step involved removal of the Boc group using 4M HCl in 1,4-dioxane and the final amines were synthesized in excellent yields.

Once, the required amines were obtained they were subjected to the reaction with cyanogen bromide in the presence of base (Scheme 2.2). Since the IR and ^{13}C NMR chemical shift are the most informative data for the $\text{C}\equiv\text{N}$ group they are tabulated for all synthesized cyanamides in Table 2.3.

Table 2.3 Yields of cyanamide formation together with the characteristic data for synthesized cyanamides

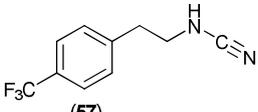
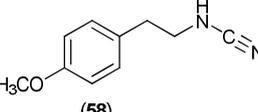
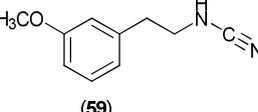
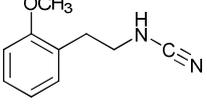
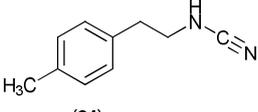
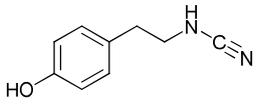
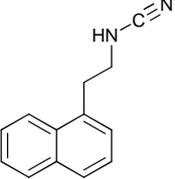
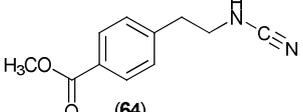
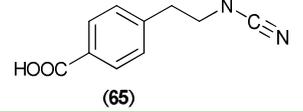
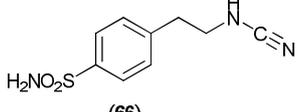
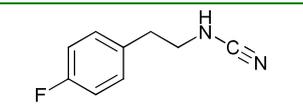
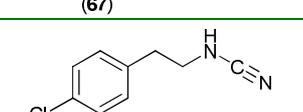
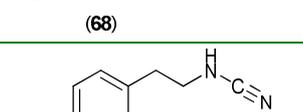
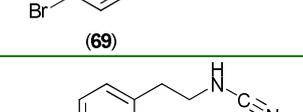
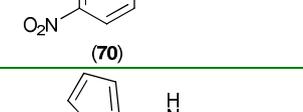
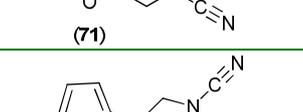
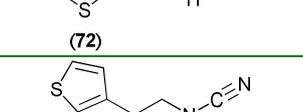
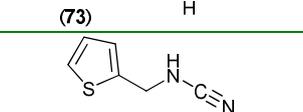
<i>cyanamide</i>	<i>IR $\nu_{\text{max}}/\text{cm}^{-1}$</i> <i>$\text{C}\equiv\text{N}$</i>	<i>$\delta^{13}\text{C}$ NMR [ppm]</i> <i>$\text{C}\equiv\text{N}$</i>	<i>yield [%]</i>
 (57)	2225	116.2	76
 (58)	2222	117.2	80
 (59)	2221	117.2	72
 (60)	2221	116.1	46
 (61)	2220	115.3	39
 (62)	2235	117.7	63
 (63)	2223	116.1	58
 (64)	2223	N/A	30

Table 2.3 Yields of cyanamide formation together with the characteristic data for synthesized cyanamides (continuation)

 (65)	2209	117.5	41
 (66)	2220	117.0	73
 (67)	2220	116.2	N/A
 (68)	N/A	115.6	N/A
 (69)	2221	116.2	56
 (70)	2220	116.9	47
 (71)	2227	115.2	78
 (72)	2222	115.8	78
 (73)	2221	N/A	40
 (75)	2224	115.9	75

As illustrated in Table 2.1, all the cyanamides were synthesized in moderate to good yields. Due to the presence of C≡N group, they tend to dimerize, therefore it was necessary to react them further as quickly as possible. Oligomerization was observed for the samples left in the fridge for long periods and masses corresponding to dimers and trimers were seen in the electrospray mass spectra. The cyanamides obtained were analyzed using ¹H and ¹³C NMR spectroscopy, mass spectrometry and IR spectroscopy. They all possessed a very sharp peak at

about 2220 cm^{-1} in the IR spectrum, as well as a weak signal in ^{13}C NMR spectrum for quaternary carbon of $\text{C}\equiv\text{N}$ group in the region of 115-117 ppm. In the case of 2-(4'-hydroxyphenyl)ethylcyanamide (**62**) the X-ray structure was obtained as shown in Figure 2.2.

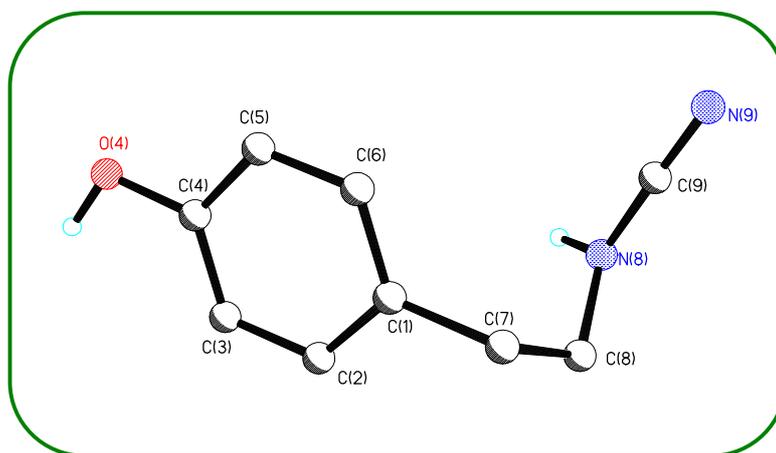


Figure 2.2 The X-ray crystal structure of 2-(4'-hydroxyphenyl)ethylcyanamide (**62**)

The X-ray structure confirmed the structure of 2-(4'-hydroxyphenyl)ethylcyanamide (**62**). The bond length between C(9) and N(9) is equal to 1.148 \AA , which is a correct value for the length of the triple bond for the nitrile. In addition, the bond angle of 178 degree for N(8)-C(9)-N(9) indicates that the bond has almost linear geometry which is in agreement with the properties of the triple bond.

2.1.2 Synthesis of *N*-Arylalkyl-*N*'-Hydroxyguanidines

The synthesized cyanamides were reacted with hydroxylamine hydrochloride in the presence of a catalytic amount of potassium carbonate as shown in Scheme 2.2. As previously, the detailed procedure is described based on the reaction of 4-phenylbutylcyanamide (**37**) with hydroxylamine hydrochloride.

Purified 4-phenylbutylcyanamide (**37**) was mixed with hydroxylamine hydrochloride in dry methanol in the presence of potassium carbonate as a catalyst and heated under reflux for 4 hours under an argon atmosphere. The elemental analysis of the crude product gave unsatisfactory results probably due to the presence of traces of hydroxylamine hydrochloride

(found: C 52.1, H 6.7, N 16.3%; calculated: C 54.2, H 7.4, N 17.2%). The purification included consecutive crystallizations from CH_2Cl_2 . The third crystallization gave the desired *N*-hydroxyguanidine (**36**) in 55% yield. The results of elemental analysis improved significantly indicating, that the synthesized compound was nearly 100% pure. In addition, the presence of the single peak in the mass spectrum at 208.1 ($\text{M}+\text{H}^+$) confirmed the structure of the synthesized product. *N*-(4-Phenylbutyl)-*N'*-hydroxyguanidine (**36**) was also characterized using ^1H and ^{13}C NMR spectroscopy. The structure of the compound was confirmed by the appearance of new peaks in the ^1H and ^{13}C NMR spectra compared to the starting cyanamide. For example, there are characteristic broad signals in the low field region of the spectrum, which indicate the presence of $-\text{OH}$, $-\text{NH}$ and $-\text{NH}_2$ groups. Based on the integration pattern in the ^1H NMR spectrum, the NH_2 protons were assigned at 7.66 ppm. In addition, one NH proton should be split into a triplet by the neighboring CH_2 protons, and a triplet at 7.92 ppm (1H) was identified as this NH . In addition two further peaks were observed at 9.76 and 10.34 ppm. In order to assign these peaks the 2D ^1H - ^{15}N HMBC as well as 2D ^1H - ^{13}C HMBC spectra were obtained. As shown in the Figure 2.3, the singlet at 9.76 ppm is correlated to the carbon at 158.2 ppm, which is characteristic for the quaternary carbon in the hydroxyguanidine functionality. However, the second signal at 10.34 is not correlated to any carbon.

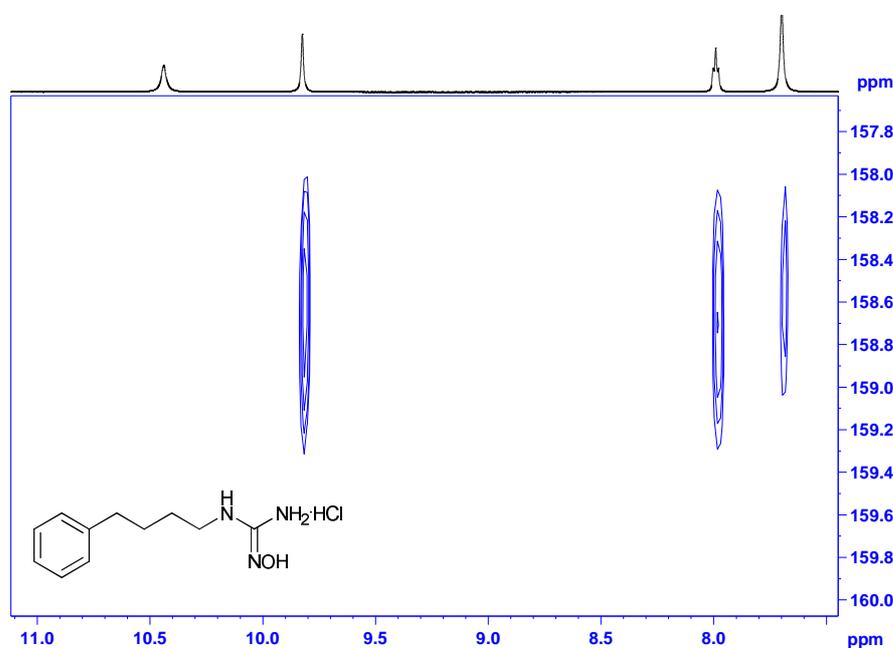


Figure 2.3 The expanded 2D ^1H - ^{13}C HMBC spectrum of *N*-(4-phenylbutyl)-*N'*-hydroxyguanidine (**36**)

Therefore, a 2D ^1H - ^{15}N HMBC experiment was performed to assign this final proton. Unfortunately, the spectrum only showed correlations between protons on NH and NH_2 groups (Figure 2.4), which confirmed the previous results, but did not help with assignment of the additional singlet in the downfield region of the ^1H NMR spectrum.

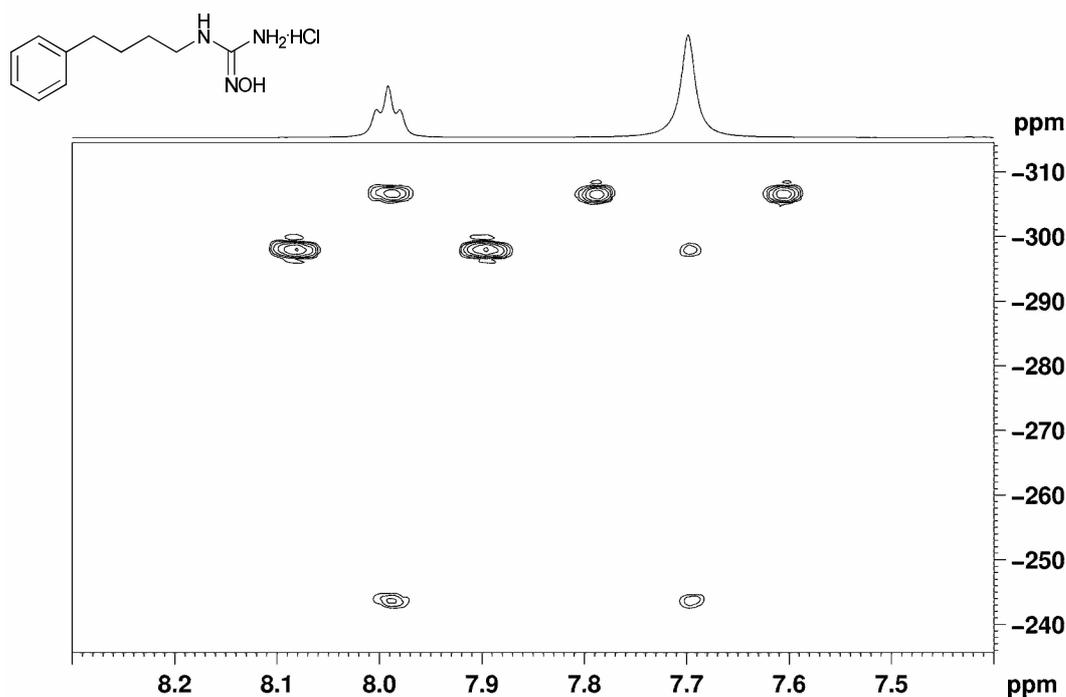
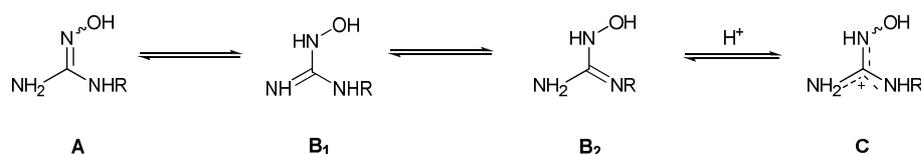


Figure 2.4 The expanded 2D ^1H - ^{15}N HMBC spectrum of *N*-(4-phenylbutyl)-*N'*-hydroxyguanidine (**36**)

Therefore, a 2D ^1H - ^{15}N HMBC experiment was performed to assign this final proton. Unfortunately, the spectrum only showed correlations between protons on NH and NH_2 groups, which confirmed the previous results, but did not help with assignment of the additional singlet in the downfield region of the ^1H NMR spectrum.

Previous studies by Fukoto *et al.* concluded that structure **C** is the most likely form of NHA bound to NOS (Scheme 2.4), and indicated that NHA exists as a protonated form in solution.^{6,7}

Scheme 2.4⁷



Our NMR results confirm this hypothesis, as four signals were observed in the ^1H NMR spectrum for NH and OH protons, as shown in Figure 2.5.

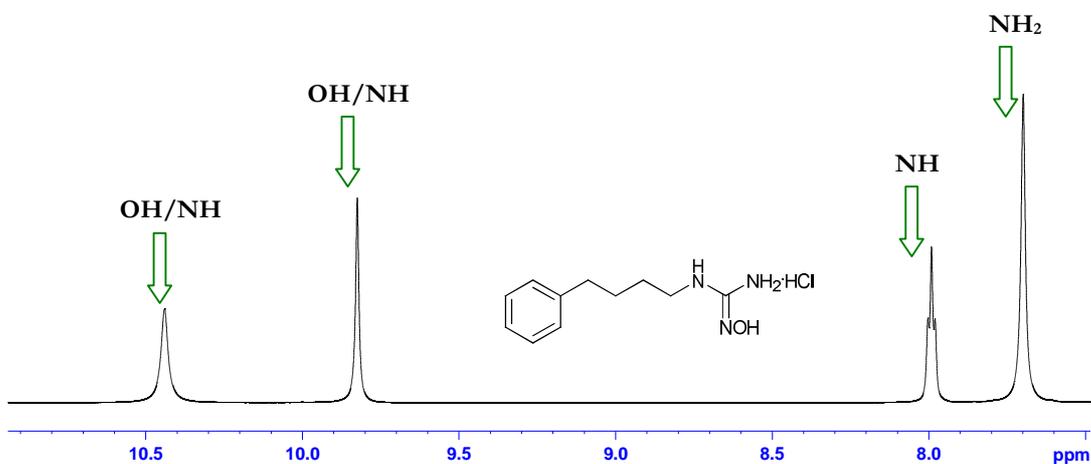


Figure 2.5 The expanded ^1H NMR spectrum of *N*-(4-phenylbutyl)-*N'*-hydroxyguanidine (**36**)

In general, most of the *N*-arylalkyl-*N'*-hydroxyguanidines were obtained in a similar manner to *N*-(4-phenylbutyl)-*N'*-hydroxyguanidine (**36**). Sometimes, depending on the polarity of the final compound, different solvents were used for crystallization (see experimental), therefore it is difficult to define one procedure for all the *N*-hydroxyguanidines. However, it is worth pointing out that in the ^1H NMR spectra for all of the synthesized *N*-hydroxyguanidines, a similar pattern was observed for active protons in the downfield region of the spectra. As illustrated in Figure 2.6, in all cases one additional signal was seen indicating the protonated form of these compounds. In the case of *N*-phenyl-*N'*-hydroxyguanidine hydrochloride (**33**), however the chemical shifts moved even further downfield in the ^1H NMR spectrum due to the direct influence of the benzene ring on the active protons of the hydroxyguanidine functionality. Namely, the additional signal is observed at about 11 ppm, not as in case of other hydroxyguanidines at about 10.5 ppm. The NH signal is shifted up to 10.14 ppm (for the other analogues the chemical shifts are about 8-8.5 ppm) and is no longer a triplet but singlet because of the absence of CH_2 protons. However, for the three other examples in Figure 2.6, the values of the chemical shifts of active protons do not differ that significantly and they all possess a similar pattern.

The yields and spectral data for the entire *N'*-arylalkyl-*N*-hydroxyguanidines synthesized are given in Table 2.4.

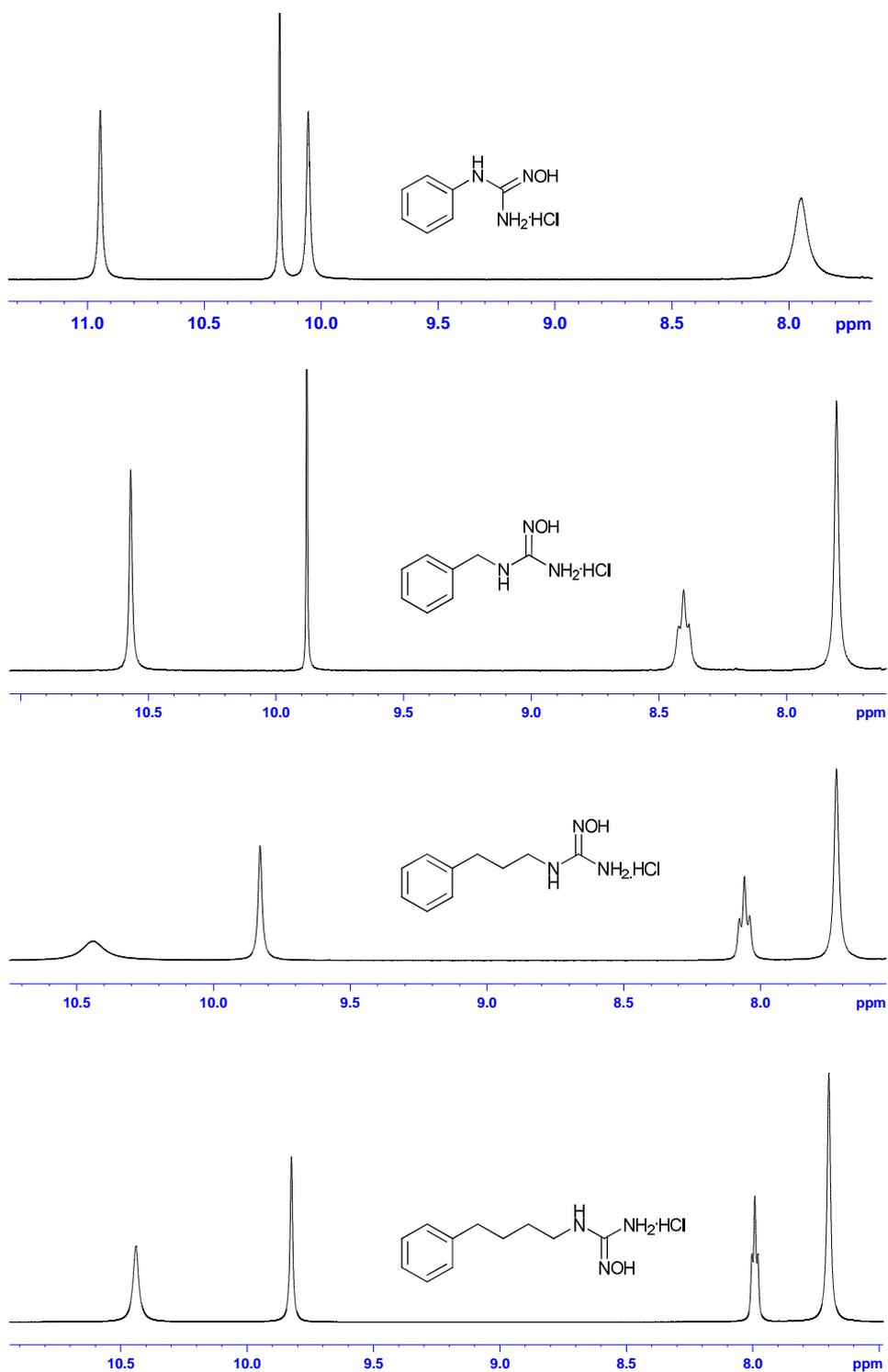
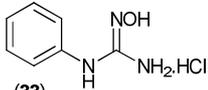
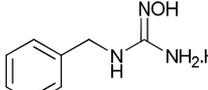
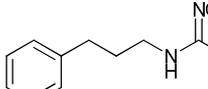
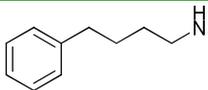
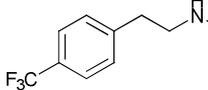
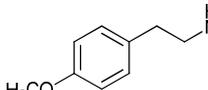
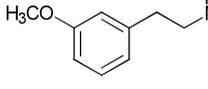
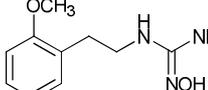
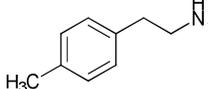
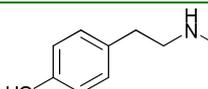
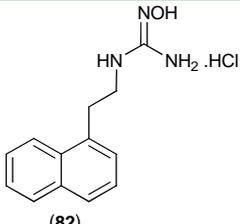
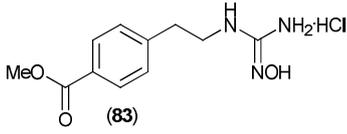
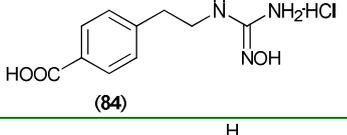
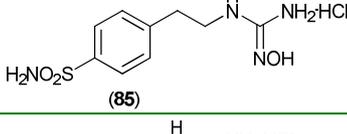
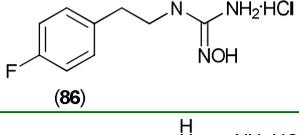
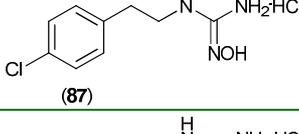
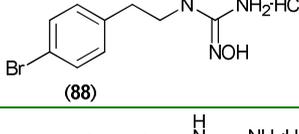
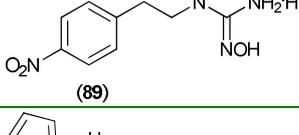
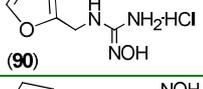
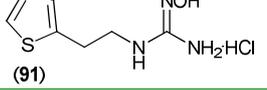
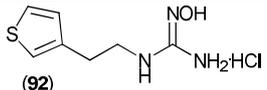
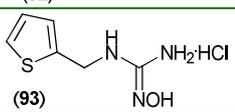


Figure 2.6 The expanded downfield region of the ^1H NMR spectra of selected *N*-hydroxyguanidines

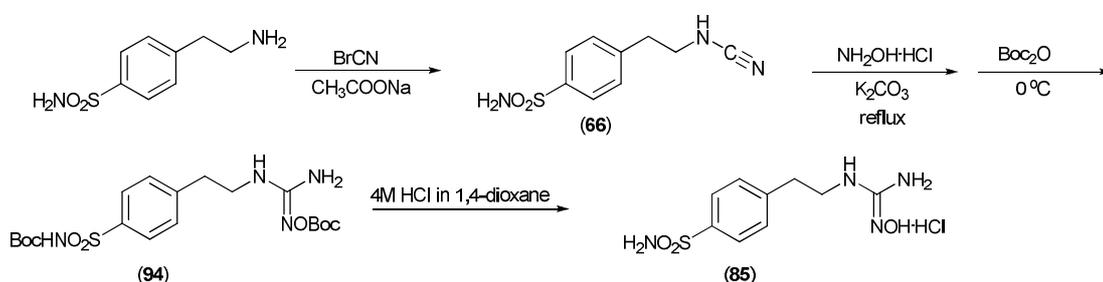
Table 2.4 Yields and characteristic spectral data for synthesized N-arylalkyl-N'-hydroxyguanidines

<i>N</i> -arylalkyl- <i>N'</i> -hydroxyguanidine	δ ¹ H NMR [ppm]			δ ¹³ C NMR [ppm]CNHOH	yield [%]
	NH ₂	NH	NH/OH		
 (33)	7.94	10.14	10.24, 10.95	156.7	47
 (34)	7.81	8.40	9.88, 10.57	158.3	45
 (35)	7.71	8.05	9.82, 10.43	158.6	58
 (36)	7.66	7.92	9.76, 10.34	158.2	55
 (76)	7.73	7.94	9.82, 10.43	158.1	62
 (77)	7.69	7.89	9.83, 10.45	158.1	58
 (78)	7.72	7.92	9.84, 10.29	158.5	53
 (79)	7.65	7.85	9.79, 10.38	158.2	46
 (80)	7.69	7.89	9.82, 10.36	158.6	30
 (81)	7.65	7.81	9.77, 10.38	158.1	40

 <p>(82)</p>	7.73	8.05	9.85, 10.46	158.1	79
 <p>(83)</p>	7.68	7.98	9.80, N/A	158.1	32
 <p>(84)</p>	7.71	7.94	9.81, 10.42	158.1	83
 <p>(85)</p>	7.69	7.87	9.75, 10.34	158.6	21
 <p>(86)</p>	7.71	7.94	9.83, 10.47	158.5	N/A
 <p>(87)</p>	7.72	7.95	9.85, 10.44	158.1	N/A
 <p>(88)</p>	7.70	7.91	9.81, 10.42	158.1	67
 <p>(89)</p>	7.44	7.99	9.85, 10.48	158.5	53
 <p>(90)</p>	7.86	8.29	9.91, 10.65	158.1	20
 <p>(91)</p>	7.75	8.00	9.89, 10.55	158.1	46
 <p>(92)</p>	7.74	7.97	9.86, 10.50	158.1	51
 <p>(93)</p>	7.88	8.41	9.92, 10.68	157.9	34

Most of the *N*-arylalkyl-*N'*-hydroxyguanidines were synthesized in good yields. However, in some cases the yields are moderate mainly due to the difficulties encountered during purification of the final compounds. Also, the compounds were to be tested in a biological assay and so high purity was of prime importance. For example, in the synthesis of *N*-2-[4'-(sulfamoyl)phenyl]ethyl-*N'*-hydroxyguanidine (**85**), an additional step had to be included in order to purify the product. Namely, the sulfonamide and hydroxyl groups were Boc protected as shown in Scheme 2.5.

Scheme 2.5



Protection of the hydrophilic groups allowed purification of the intermediate (**94**) using column chromatography. The Boc protected analogue was then easily deprotected using 4M HCl solution in 1,4-dioxane to give the desired *N*-hydroxyguanidine in a moderate 21% yield over three steps, but excellent purity.

In the case of some hydroxyguanidines repeated recrystallizations did not give satisfactory results. This was a problem with compounds with polar or easily oxidizable groups such as -OH, -COOH, and NO₂. The higher polarity made the whole molecule more hygroscopic, therefore difficult to purify, whilst the propensity for the oxidation resulted in low stability of the final compounds. Based on the published literature, Mansuy *et al.* had to face the same issues since in their experimental data there are no elemental analysis results for *N*-hydroxyguanidines with polar substituents.¹ In addition, solvent molecules were often included to improve the results of the elemental analysis. Attempts were made to crystallize these hydroxyguanidines but this only succeeded for *N*-2-(4'-hydroxyphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (**81**) and *N*-2-(3'-methoxyphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (**78**). The X-ray crystal structures are presented in Figure 2.7.

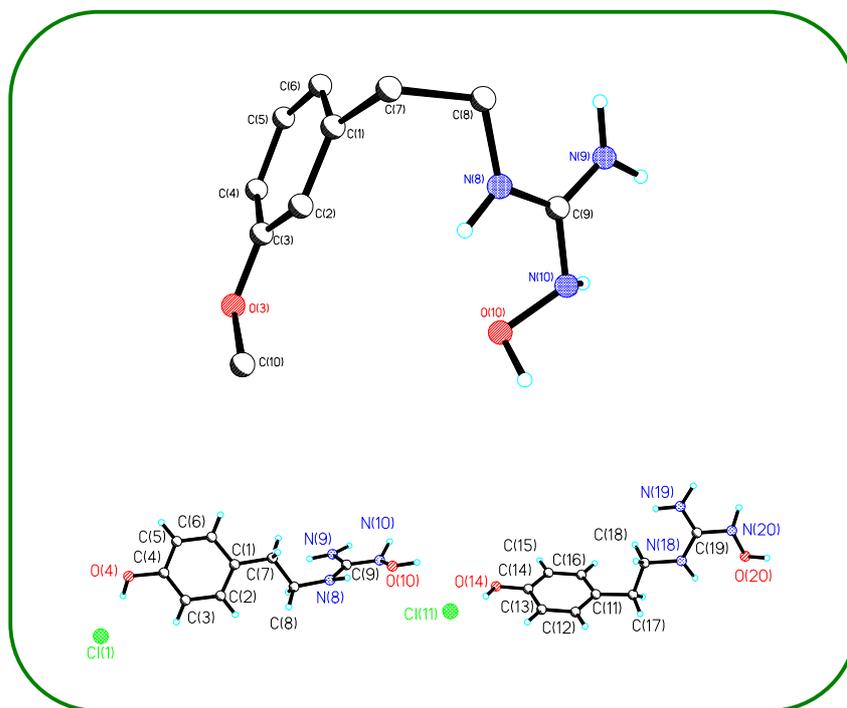


Figure 2.7 The X-ray crystal structures of *N*-2-(3'-methoxyphenyl)ethyl- (78) and *N*-2-(4'-hydroxyphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (81)

The X-ray crystal structures showed that the additional proton observed by NMR spectroscopy appears to be attached to the nitrogen of the NOH group, whilst in the solution it impossible to determine its position. However, the bond lengths for *N*-2-(3'-methoxyphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (78) are: N(8)-C(9)-1.333 Å, C(9)-N(9)-1.343 Å and C(9)-N(10)-1.375 Å, which indicate delocalization of the double bond along the N(8)-C(9)-N(9) fragment. The same is seen for 2-(4'-hydroxyphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (81), namely the bond lengths are N(8)-C(9)-1.321 Å, C(9)-N(9)-1.338 Å and C(9)-N(10)-1.356 Å, respectively. These findings are in agreement with results obtained by Fukoto *et al.* concerning the protonated state of NHA, as described earlier (Scheme 2.4).^{6,7}

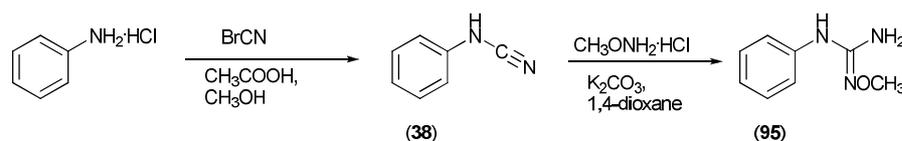
Twenty two *N*-arylalkyl-*N'*-hydroxyguanidines have thus been successfully synthesized; among them four novel heterocyclic analogues and fifteen new derivatives of *N*-phenylethyl-*N'*-hydroxyguanidine (QZNO193). Most of them were obtained in good yields (45-83%). In a few cases the yields are lower due to the purification problems. The purity of the synthesized

molecules was confirmed by the elemental analysis indicating that almost all hydroxyguanidines were obtained as nearly 100% pure compounds, which was crucial for biological testing.

2.1.3 Synthesis of *N*-phenyl-*N'*-methoxyguanidine

As described in the previous chapter, the hydroxyguanidine moiety is essential for NO generation. Therefore, by substitution on the oxygen of the NOH group one should get inactive control compound. A suitable compound was prepared by reacting the corresponding cyanamide with methoxylamine hydrochloride in the presence of base and heating under reflux for two days according to the Scheme 2.6.

Scheme 2.6

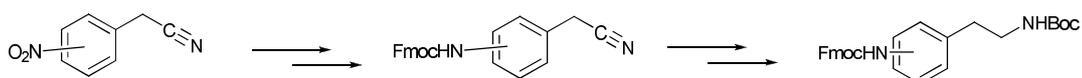


Despite the forcing reaction conditions *N*-phenyl-*N'*-methoxyguanidine (**95**) was obtained in a poor 7% yield. This might be caused by the fact that methoxylamine is much more volatile than hydroxylamine and perhaps an excess of this compound would be more effective. However, the main goal was to obtain a reasonable amount of pure product for biological testing and the optimisation of the reaction conditions was not necessary. *N*-Phenyl-*N'*-methoxyguanidine (**95**) was characterized by various analytical techniques. The most significant indication of the structure was the presence of a singlet at 3.57 ppm for the methyl group in the ^1H NMR spectrum as well as a shift of the quaternary carbon from 156 ppm ($\text{C}=\text{NOH}$) to 151 ppm ($\text{C}=\text{NOCH}_3$) in the ^{13}C NMR spectrum. In addition, the correctness of the structure and purity were confirmed by mass spectrometry and elemental analysis, respectively.

2.1.4 Synthesis of *N*-aryalkyl-*N'*-hydroxyguanidines bearing NH₂ groups

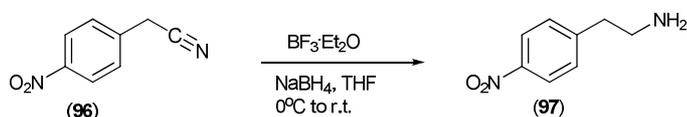
It was observed by Mansuy *et al.* that *N*-(3-aminophenyl)-*N'*-hydroxyguanidine was the only derivative, of those tabulated in Table 1.4, that was oxidized by eNOS with the formation of NO at a reasonable rate.¹ Therefore, it could be beneficial to synthesize the derivative of QZNO193 bearing the amino group in the *meta*-position of the phenyl ring, even though e-NOS may not be the only enzyme in our biological assay that causes the oxidation of *N*-aryalkyl-*N'*-hydroxyguanidines. In order to synthesize *N*-hydroxyguanidines which possess amino groups in their structure, either as a substituent of the phenyl ring or as part of the alkyl chain, a more sophisticated approach had to be employed. The proposed synthetic approach is presented in Scheme 2.7.

Scheme 2.7



The first route required selective reduction of a nitrile over an aromatic nitro group and then protection of the resulting amine using 9-fluorenylmethyl chloroformate to give Fmoc protection, stable under acidic conditions. A suitable procedure to selectively reduce a nitrile in the presence of a nitro had been already employed to synthesize *N*-2-(4'-nitrophenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (**89**) and is presented in Scheme 2.8.⁸

Scheme 2.8⁸



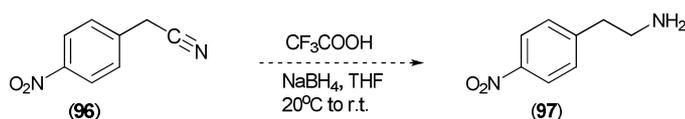
Even though it was possible to successfully reduce the nitrile (**96**) and leave the nitro group untouched, the yield of this transformation was very poor 11% (NaBH₄ – 1 equiv.; BF₃·Et₂O – 2 equiv.). Therefore, it was necessary to optimize this method, especially as the published procedure was very vague. Firstly, the reaction conditions were kept the same (see experimental

for (**96**)), except that a new batch of boron trifluoride diethyl etherate ($\text{BF}_3 \cdot \text{Et}_2\text{O}$) was used, since the previous $\text{BF}_3 \cdot \text{Et}_2\text{O}$ was quite old. Unfortunately, it did not improve the reaction. TLC monitoring indicated that, apart from the required product, a whole variety of different compounds had been formed. Since, the reduction of nitriles occurs in two steps there is a possibility that the intermediates, or even final amine can react with the starting nitrile. Next, the number of equivalents of sodium borohydride was increased from one to two. However, no improvement was observed and again a very complex mixture of products was formed.

It was then found that a similar approach had been employed by Su-Dong Cho *et al.* to reduce carboxylic acids, esters, amides and nitriles using $\text{NaBH}_4/\text{BF}_3 \cdot \text{Et}_2\text{O}$ as an inexpensive and highly versatile reducing system.⁹ The major modification compared to the previous procedure was to use a catalytic amount of boron trifluoride diethyl etherate and 1.5 equivalent of NaBH_4 together with heating the reaction mixture under reflux instead of stirring at room temperature. Unfortunately, this attempt was also not successful.

Itoch *et al.* reported the use of sodium acyloxyborohydride ($\text{NaBH}_3(\text{OCOR})$) as a novel reducing agent.¹⁰ They claimed a 71% yield for the reduction of the 4-nitrophenylacetonitrile (**96**) to the corresponding amine (**97**). Therefore, the same methodology was attempted in our case as shown in Scheme 2.9.

Scheme 2.9

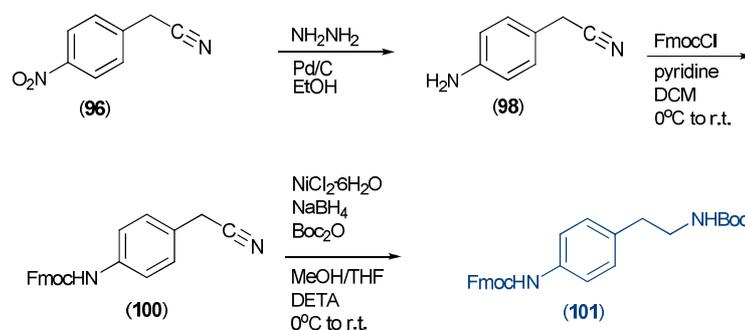


Several attempts were made in order to repeat the published procedure, but none of them gave satisfactory results, this time leaving the starting material untouched.

It has been also published that dimethylsulfide borane $[(\text{Me})_2\text{S}\cdot\text{BH}_3]$ is a valuable reagent for the reduction of functional groups. Unfortunately, attempts to reduce 4-nitrophenylacetonitrile (**96**) using $(\text{Me})_2\text{S}\cdot\text{BH}_3$ under reflux conditions failed and mainly starting material was recovered.^{11, 12} Then, it was decided to employ the nickel boride procedure but decrease the number of equivalents of NaBH_4 from seven to two. However, this time the reaction was not chemoselective and both groups were reduced at the same time.

Since for the overall synthetic route it was not essential which group was reduced first, the selective reduction of nitro group to the amine was examined next. Adger *et al.* reported the selective reduction of nitroarylalkylnitriles with hydrazine and a metal catalyst.¹³ The published procedure was employed to reduce 4-nitrophenylacetonitrile (**96**) using hydrazine hydrate ($\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$) and 10% Pd/C catalyst. The TLC monitoring indicated that this transformation was cleaner compared to the previous methods. However, the first attempt gave the corresponding hydroxylamine instead of the amine according to the ^1H NMR data, which suggested incomplete reduction of the nitro group. Therefore, the same methodology was employed changing the number of equivalents of $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$ from 1 to at least 2.5. This led to successful reduction of 4-nitrophenylacetonitrile (**96**) to 4-aminophenylacetonitrile (**98**). The resulting amine was then protected using 9-fluorenylmethyl chloroformate.¹⁴ Using our previously developed methodology, the nitrile was then reduced to the Boc-protected amine.⁵ This leaves the two functional groups with orthogonal protecting groups. The model synthetic route was first performed with 4-nitrophenylacetonitrile (**96**) as shown in Scheme 2.10, since the commercially available 3-nitrophenylacetonitrile (**99**) was expensive.

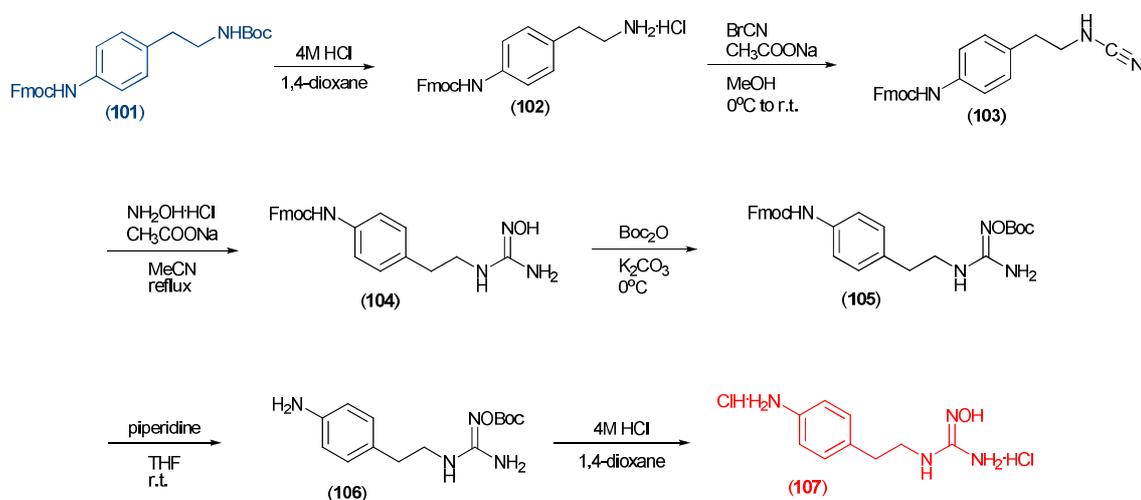
Scheme 2.10



As shown in Scheme 2.10, the nitro group was selectively reduced to the amine employing the hydrazine hydrate and a catalytic amount of palladium as a reducing agent. 4-Aminophenylacetonitrile hydrochloride (**98**) was synthesized in a very good yield (74%) and characterized by various analytical methods. The most indicative piece of data was the appearance of a broad singlet at 5.11 ppm in the ^1H NMR spectrum integrating for two protons which corresponded to the amino group. In addition, there was a significant shift of the aromatic protons from downfield to upfield of the ^1H NMR spectrum due to the change in the

electronic properties of the aromatic substituents from a strongly electron withdrawing to a strongly electron donating group. The next step involved protection of the amino group using 9-fluorenylmethyl chloroformate in the presence of pyridine to give the Fmoc protected phenylacetone nitrile (**100**) in a very good yield (77%). The product was analyzed by ^1H and ^{13}C NMR and IR spectroscopy, MS spectrometry and the purity was confirmed by the elemental analysis (see experimental). All the data proved the correctness of the final compound. The 4-(*N*-9-fluorenylmethoxycarbonylamino)phenylacetone nitrile (**100**) was then subjected to the reduction reaction using the same methodology as previously described. However, this time a mixture of anhydrous methanol and tetrahydrofuran had to be employed due to the solubility issues of the starting nitrile. The nickel hydride reduction of the nitrile group and consecutive Boc protection of the amine yielded the key intermediate (**101**), in 58 % yield over 3 steps, which could be reacted one of two ways.

Scheme 2.11



The first route involved the selective deprotection of the *tert*-butoxycarbonyl group using 4M HCl solution in 1,4-dioxane as shown in Scheme 2.10. The amine (**102**) was reacted as before with cyanogen bromide in the presence of base to give the corresponding cyanamide (**103**). The cyanamide (**103**) bearing the *N*-9-fluorenylmethoxycarbonyl (Fmoc) group caused some problems during work-up. Due to the presence of the Fmoc group (hydrophobic) and the cyanamide functionality (hydrophilic), the molecule possessed an amphiphilic character. It had a

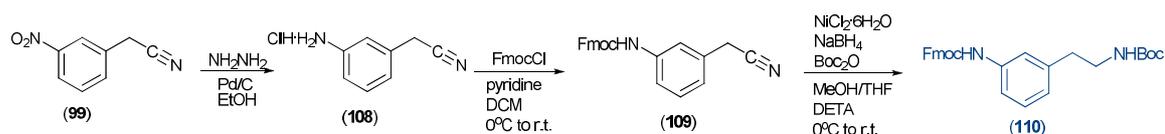
propensity to form emulsions. Therefore, the extraction time had to be longer than usual, but this caused the hydrolysis of the cyanamide to the corresponding urea. In order to avoid this problem, it was found that ethyl acetate was a better solvent for the extraction than dichloromethane. As in the case of all other cyanamides, this one also had a very sharp peak at 2218 cm^{-1} in the IR spectrum, as well as a weak signal in the ^{13}C NMR spectrum for the $\text{C}\equiv\text{N}$ group at 117.4 ppm .

2-(4'-*N*-9)-Fluorenylmethoxycarbonylaminophenyl)ethylcyanamide (**103**) was then reacted with hydroxylamine hydrochloride in dry methanol in the presence of a catalytic amount of potassium carbonate. However, due to the amphiphilic character and solubility problems of the starting cyanamides, after work-up mainly starting material was recovered. Therefore, the reaction conditions were slightly modified and 3 equivalents of hydroxylamine hydrochloride along with sodium acetate instead of potassium carbonate were employed. In addition, methanol was replaced by acetonitrile, in which the cyanamide was more soluble. The intermediate (**104**) was then treated with di-*tert*-butyl dicarbonate to protect the hydroxyguanidine functionality. The successful removal of the Fmoc group gave the Boc protected hydroxyguanidine (**106**) which was purified using column chromatography and reacted with 4M HCl solution in 1,4-dioxane to give *N*-2-(4'-aminophenyl)ethyl-*N*'-hydroxyguanidine dihydrochloride (**107**) in a reasonable 23% yield over 4 steps. The final compound was characterized by various analytical techniques such as the ^1H and ^{13}C NMR spectroscopy, IR spectroscopy and MS spectrometry. The presence of a single peak in the mass spectrum at 195 (M+H)^+ indicated the correct molecular mass. Additionally, HR-ESI-MS gave the mass as 195.1240 while $\text{C}_9\text{H}_{15}\text{N}_4\text{O}$ required 195.1246. However, both ^1H and ^{13}C NMR spectra of the product in $\text{d}_6\text{-DMSO}$ suggested that the synthesized molecule was actually a mixture of two species. Two sets of signals were present for the CH_2 protons, while the signals for the aromatic protons and active protons (NH , OH , NH_2) overlapped with each other. Additional experiments were performed to establish the character of the two species. Firstly, the sample was heated up to 373 K. The ^1H spectrum showed that increasing temperature caused broadening of peaks, but the coalescence was not observed. In addition, the 1D gs-NOESY spectrum, recorded at room temperature, did not show saturation transfer (EXSY peak) upon selective irradiation of the CH_2 resonance. These results implied that the two species existing in $\text{d}_6\text{-DMSO}$ were not interconvertible. The elemental analysis also did not give satisfactory results and consecutive recrystallizations did not improve the data. The closest result to the theoretical composition (%C 40.46, %H 6.04, %N 20.97) was the following

percentage: %C 41.92, %H 5.77, %N 20.06. Attempts made to crystallize *N*-2-(4'-aminophenyl)ethyl-*N'*-hydroxyguanidine dihydrochloride (**107**) were not successful.

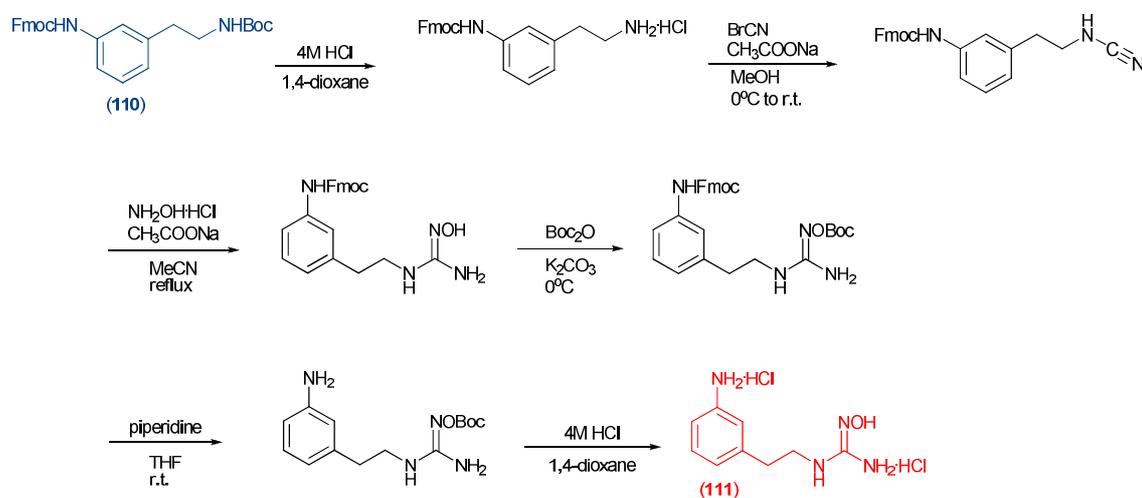
The same scheme of reactions was then performed starting from 3-nitrophenylacetonitrile (**99**) as shown in Scheme 2.12.

Scheme 2.12



As previously described the nitro group was selectively reduced to the amine, which was then Fmoc protected to yield 3-(*N*-9-fluorenylmethoxycarbonylamino)phenylacetonitrile (**109**). The nitrile (**109**) was then treated with sodium borohydride in the presence of nickel chloride and di-*tert*-butyl dicarbonate to give *N*-Boc-2-(3'-*N*-fmocaminophenyl)ethylamine (**110**) (57%) as the major product along with the Fmoc deprotected amine (19%) and the unreacted starting material (24%). The compound (**110**) was characterized by ^1H and ^{13}C NMR and IR spectroscopy, mass spectrometry and the purity confirmed by elemental analysis. The most clear-cut indication of the product structure was the presence of the singlet at 1.48 ppm in the ^1H NMR spectrum for nine *tert*-butyl protons. In addition, the elemental analysis indicated that obtained compound was nearly 100% pure (found: C 73.6, H 6.9, N 6.2%; calculated: C 73.3, H 6.6, N 6.1%). The di-protected amine (**110**) was then taken through the same reaction pathway, as for the *para* analogue, as illustrated in Scheme 2.13.

Scheme 2.13



Interestingly, *N*-2-(3'-aminophenyl)ethyl-*N'*-hydroxyguanidine dihydrochloride (**111**) showed the same pattern in the ¹H and ¹³C NMR spectra, suggesting the presence of two species. However, this time it was possible to crystallize the final compound and the X-ray data confirmed the absolute structure of the product as shown in Figure 2.8.

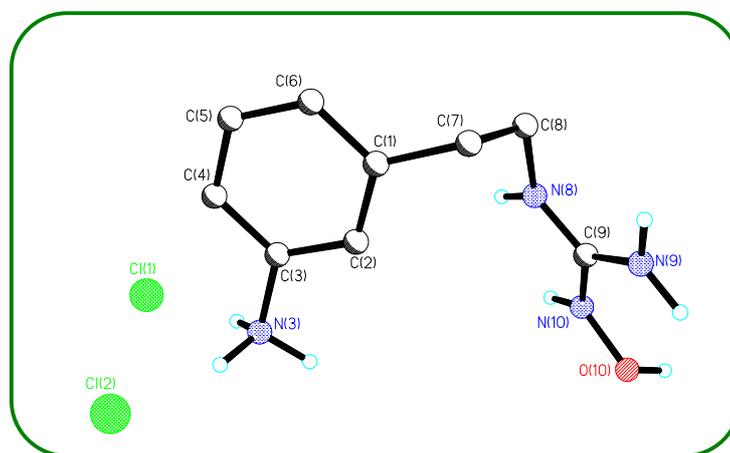


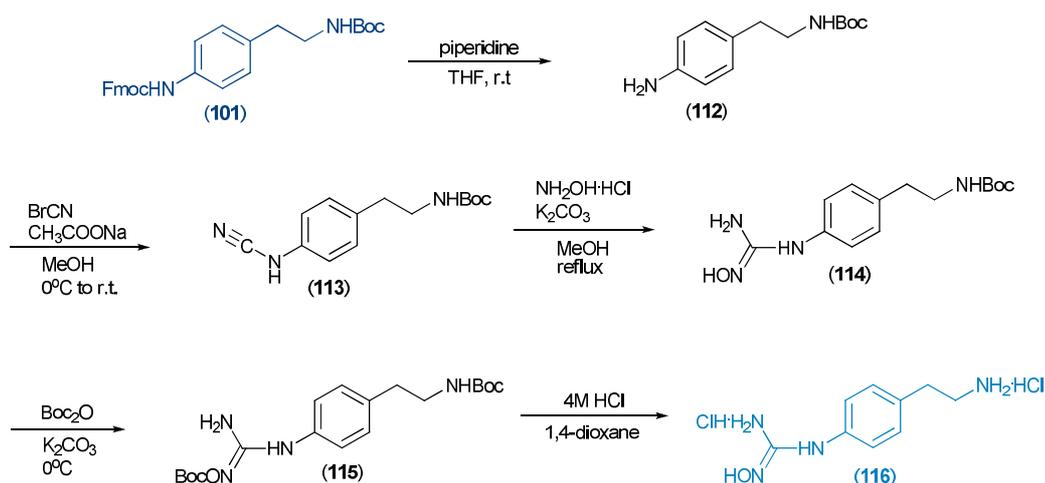
Figure 2.8 The X-ray crystal structure of *N*-2-(3'-aminophenyl)ethyl-*N'*-hydroxyguanidine dihydrochloride (**111**)

The obtained crystal structure indicated that in the solid state the molecule exists as the dihydrochloride salt. One proton appears to be attached to the amino group on the benzene ring which is confirmed also by the ¹H NMR spectroscopy, as the integration pattern indicated the presence of NH₃⁺ in the downfield part of the spectrum. The second proton is located as in the

case of all hydroxyguanidines on the nitrogen of NOH moiety. The bond lengths equal N(8)-C(9)-1.310 Å, C(9)-N(9)-1.311 Å and C(9)-N(10)-1.373 Å respectively which suggested that the double bond is actually delocalized through the N(8)-C(9)-N(9) fragment. Additionally, the presence of the single peak in the mass spectrum at $195 (M+H)^+$ indicated the correct molecular mass. HR-ESI-MS gave the mass of 195.1252 while $C_9H_{15}N_4O$ required 195.1246. However, the results of elemental analysis, as in the case of *para* analogue, were not satisfactory. Therefore, based on the X-ray data and 1H and ^{13}C NMR spectra one cannot identify two species present in the solution.

The second route firstly involved removal of the Fmoc group using piperidine in anhydrous THF as shown in Scheme 2.14.

Scheme 2.14



N-(*tert*-Butoxycarbonyl)-2-(4'-aminophenyl)ethylamine (112) was then subjected to the reaction with cyanogen bromide in the presence of sodium acetate to yield the corresponding cyanamide (113). The desired product was analyzed by various analytical methods. It possessed a very sharp peak at 2222 cm^{-1} in the IR spectrum, as well as a weak signal in the ^{13}C NMR spectrum for the $C\equiv N$ group at 111.2 ppm. The value of the chemical shift had slightly moved upfield due to the electronic properties of the benzene ring. The X-ray crystal structure confirmed the absolute structure of the final product as shown in Figure 2.9.

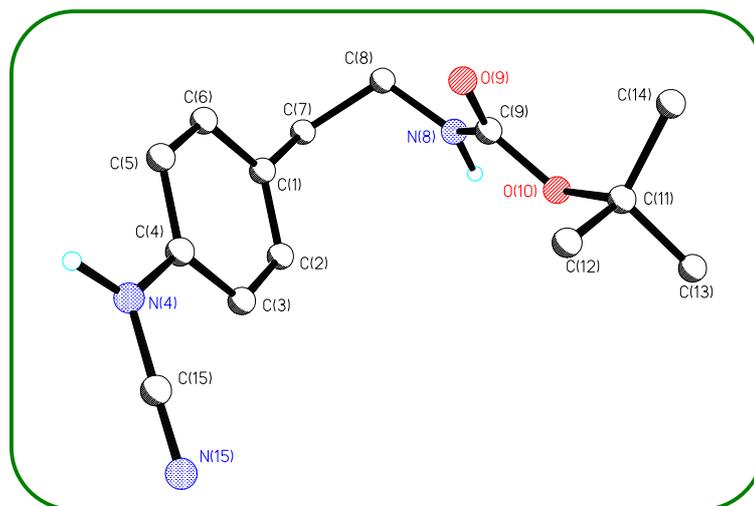


Figure 2.9 The X-ray crystal structure of *N*-(*tert*-butoxycarbonyl)-2-(4'-aminophenyl)ethylcyanamide (**113**)

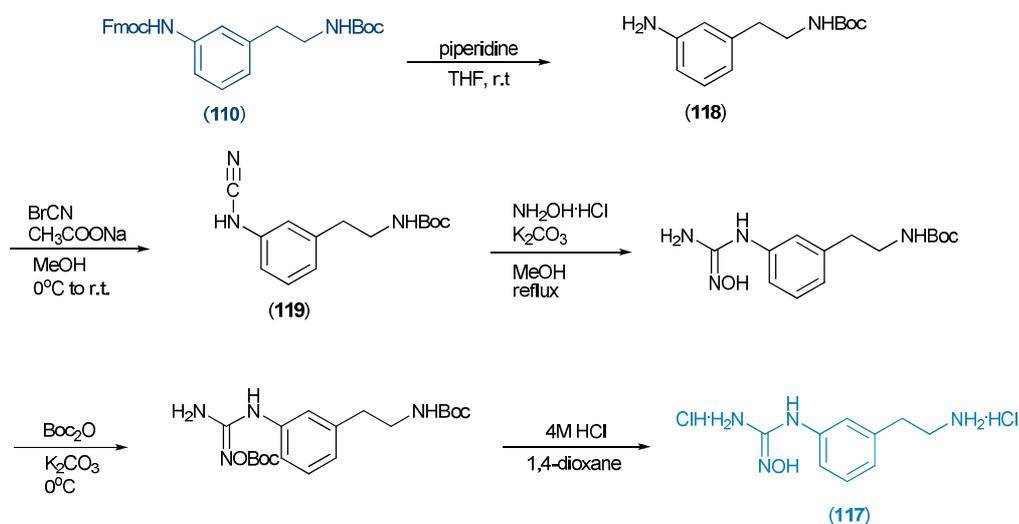
The bond length between C(15) and N(15) is equal to 1.153 Å, which is a correct value for the length of the triple bond for the nitrile. In addition, the bond angle of 179.4 degree for N(4)-C(15)-N(15) indicates that the bond has a linear geometry which is characteristic for the triple bond.

N-(*tert*-Butoxycarbonyl)-2-(4'-aminophenyl)ethylcyanamide (**113**) was treated with hydroxylamine hydrochloride in the presence of potassium carbonate, and the product (**114**) was taken without the characterization for the next step, which was Boc protection. It yielded the product (**115**) which was possible to purify by column chromatography and then subjected to the global Boc deprotection using 4M HCl in 1,4-dioxane to give *N*-phenyl-(4-ethylamine)-*N*²-hydroxyguanidine dihydrochloride (**116**) in 44% yield (over 3 steps). The final compound (**116**) was characterized by the ¹H and ¹³C NMR spectroscopy, IR spectroscopy, MS spectrometry and the purity confirmed by elemental analysis. The most indicative data were the characteristic peaks for the active protons (OH, NH, NH₂) in the lowfield region of the ¹H NMR spectrum. As usual an additional signal was seen at 10.86 ppm, confirming the protonated state of the hydroxyguanidine functionality. The elemental analysis (found: C 40.5, H 6.0, N 20.6%; calculated: C 40.5, H 6.0, N 20.9%) indicated that synthesized molecule is almost 100% pure. Surprisingly, just one isomer was present according to the ¹H and ¹³C NMR spectra, which may suggest as previously speculated that only direct attachment of the amino group to the phenyl

ring is responsible for the presence of two isomers in previous two analogues. It could be caused by various protonation states of aniline giving a mixture of di- and monohydrochlorides.

N-Boc-2-(3'-*N*-Fmocaminophenyl)ethylamine (**110**) was taken through same steps as in the case of the *para* analogue as shown in Scheme 2.15 to yield *N*-phenyl-(3-ethylamine)-*N*'-hydroxyguanidine dihydrochloride (**117**).

Scheme 2.15



Firstly the Fmoc group was successfully removed by piperidine to give Boc protected analogue (**118**) in 81% yield. The product was characterized by various analytical methods. The most indicative piece of data was appearance of broad singlet integrating for two protons at 4.94 ppm in ¹H NMR spectrum confirming the presence of NH₂ group. Additionally, the aromatic protons shifted up-field indicating presence of amino group in their chemical environment. *N*-Boc-2-(3'-aminophenyl)ethylamine (**118**) was reacted with cyanogen bromide to yield the corresponding cyanamide (**119**) in 85% yield. As in case of all other cyanamides, this one also had a very sharp peak at 2226 cm⁻¹ in the IR spectrum, as well as a weak signal in the ¹³C NMR spectrum for C≡N group at 112.4 ppm. *N*-(*tert*-Butoxycarbonyl)-2-(3'-aminophenyl)ethylcyanamide (**119**) was treated with hydroxylamine hydrochloride in the presence of potassium carbonate and the product was taken without characterization for the purification steps which included Boc protection and deprotection (Scheme 2.14) to give final product (**117**) in 40% yield over 3 steps. The desired *N*-hydroxyguanidine (**117**) was analyzed by

¹H and ¹³C NMR spectroscopy, IR spectroscopy and MS spectrometry. The most indicative data was appearance of characteristic peaks for the active protons (OH, NH, NH₂) in the ¹H NMR spectrum. The values of the chemical shifts were moved slightly down field, relative to the signals of the alkyl substituted *N*-hydroxyguanidines, due to the direct presence of the benzene ring next to the hydroxyguanidine functionality. The additional proton appeared at 10.91 and 10.18 ppm for NH/OH and at 10.07 ppm for NH. As in the case of all other derivatives with amino group, the NH₂ was also protonated and integrated for three protons at 8.27 ppm in the ¹H NMR spectrum. As previously, for the *para* analogue only one isomer was observed, confirming the theory that two isomers can form when the NH₂ group is directly attached to the benzene ring.

The synthesis of four novel *N*-arylalkyl-*N'*-hydroxyguanidines was tedious, but successful. The final compounds were synthesized in reasonable yields and purities.

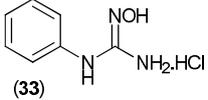
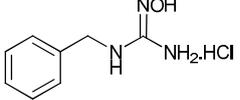
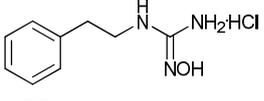
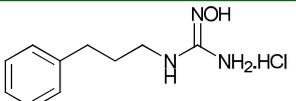
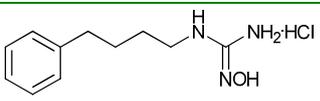
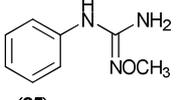
2.2 Biological results

2.2.1 Myography studies

Most of the *N*-hydroxyguanidines were tested in the rat aorta assay using myography to determine their vasodilatation activity. In general, the rat aorta was cut into small sections and mounted onto the myograph, and then stretched to 15 mN. Rat aorta rings were then washed and contracted to 80% of the maximum with phenylephrine. The concentration response curve was obtained within the range of 1-100 μM. Then, 50 μM ODQ was added to assess the reversibility of the relaxation. ODQ (1H-[1,2,4]Oxadiazole[4,3-a]quinoxalin-1-one) is a selective inhibitor of nitric oxide sensitive guanylate cyclase (GC), therefore significant reversibility is an indication that vasodilatation is mediated through NO. The criteria were set for a good vasodilator namely it must have an EC₅₀ of 30 μM or less and must show 80% reversibility with ODQ. The compounds that met above criteria were then examined in the isolated perfused kidney, to see the effect in the whole organ system.

The results presented are an average of six consecutive measurements. The log EC₅₀ values and reversibility percentages for the first class of *N*-arylalkyl-*N'*-hydroxyguanidines are summarized in Table 2.5.

Table 2.5 NO donor activity of *N*-arylalkyl-*N'*-hydroxyguanidines in rat aorta

Structure	Code No.	log EC ₅₀ [mol/dm ³]	ODQ induced reversibility[%]
 (33)	AK0	-4.8 ± 0.1	104 ± 13
 (34)	AK1	-4.9 ± 0.4	37 ± 10
 (120)	QZNO193	-4.6 ± 0.1	75 ± 16
 (35)	AK3	-4.8 ± 0.1	68 ± 12
 (36)	AK4	-3.2 ± 1.6	28 ± 14
 (95)	AK78	-2.4 ± 0.4	35 ± 17

The combined relaxation profiles for all the *N*-arylalkyl-*N'*-hydroxyguanidines tested at various concentrations are shown in Figure 2.10.

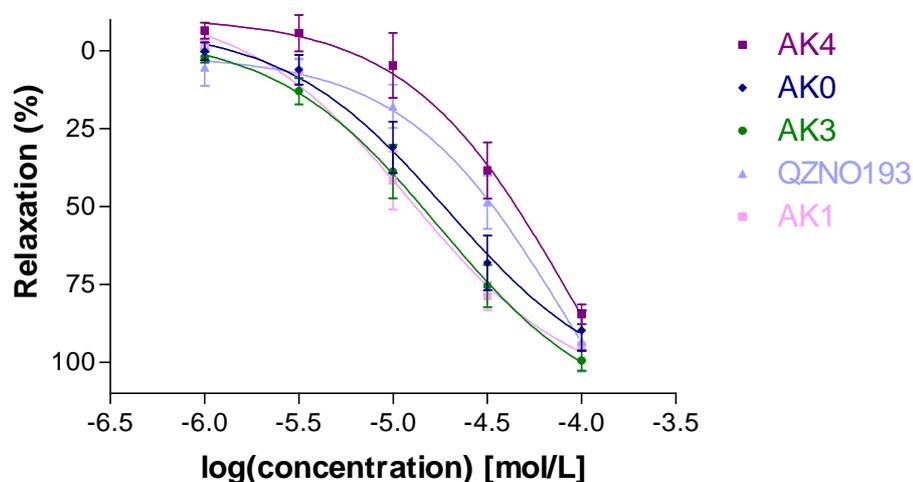


Figure 2.10 Vasorelaxation profiles of tested *N*-arylalkyl-*N'*-hydroxyguanidines

The biological results indicated that tested compounds act as potent nitric oxide donors. As seen in Table 2.4, AK0 has a better biological profile than the previous leading compound (QZNO193). Not only is a smaller concentration required to cause 50% relaxation, but also the ODQ experiment showed almost complete reversibility indicating that vasodilatation occurs mainly *via* generation of NO. However, based on the myography data the extension of the alkyl chain is beneficial only to three methylene groups. Further elongation of the alkyl chain caused significant deterioration of the biological activity. On the other hand, *N*-phenyl-*N'*-methoxyguanidine (AK78), which originally was designed as a control compound, caused 48% relaxation at the highest concentration. However, its mechanism of action is not clear and requires further investigation.

The vasodilatation by one compound, QZNO193, was examined in the presence of a range of additives as shown in Figure 2.11. The vasodilatation profile did not change in the presence of L-NME, a NOS inhibitor, implying the production of NO was not only caused by NO-synthase, but by other oxidizing enzymes present in the system. The clear inhibition by ODQ confirmed that the vasodilatation was soluble guanylate cyclase-dependent. In addition, the biological activity was not affected by haemoglobin, which is a known NO scavenger, indicating that vasodilatation occurs inside the cells.

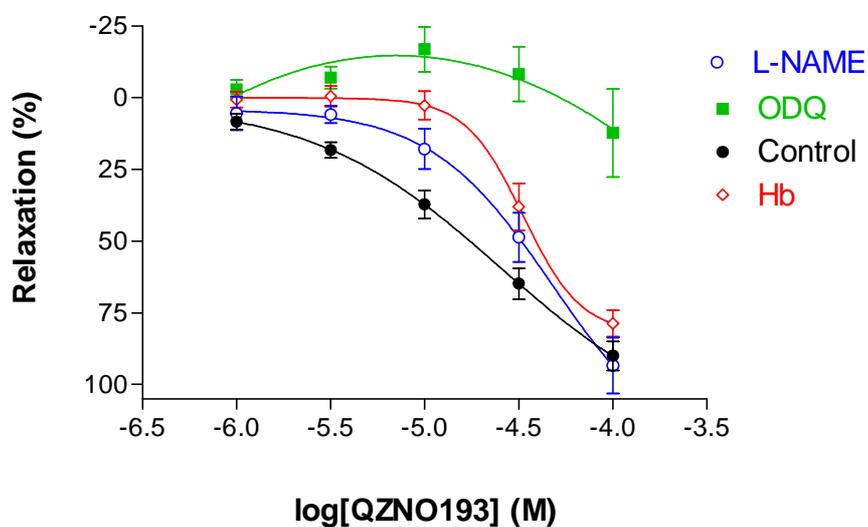
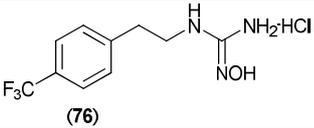
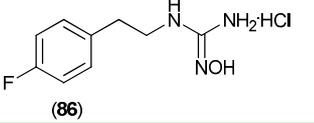
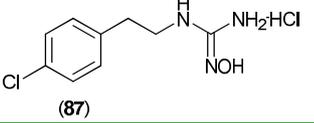
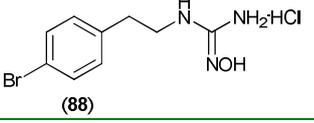
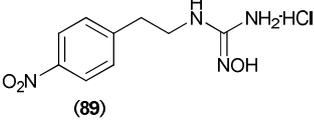
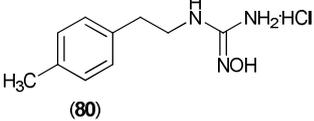
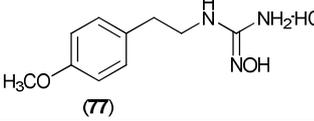
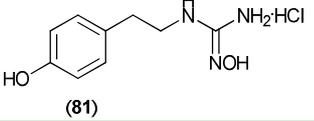
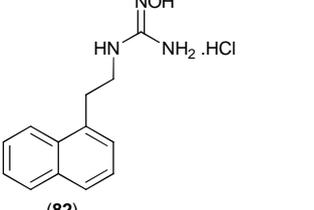
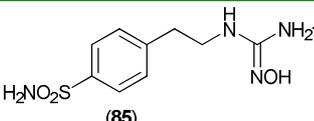


Figure 2.11 Vasorelaxation profile of QZNO193 in the presence of various additives

Some of the new derivatives of QZNO193 were also examined by myography and their biological activities are summarized in Table 2.6.

Table 2.6 NO donor activity of modified QZNO193 in a rat aorta

Structure	Code No.	$\log EC_{50}$ [mol/dm ³]	ODQ induced reversibility[%]
 (76)	AK2F	-5.1 ± 0.1	57 ± 9
 (86)	AW13	-4.2 ± 0.5	11 ± 15
 (87)	AW21	-4.0 ± 0.1	11 ± 14
 (88)	AK131	-4.8 ± 0.1	35 ± 14

 <p>(89)</p>	AK119	-4.6 ± 0.1	75 ± 10
 <p>(80)</p>	AK172	-4.9 ± 0.2	35 ± 14
 <p>(77)</p>	AK120	-4.3 ± 0.1	40.3 ± 3.7
 <p>(81)</p>	AK164	-4.2 ± 0.4	N/A
 <p>(82)</p>	AK52	-4.3 ± 0.6	0.5 ± 8
 <p>(85)</p>	AK169	no effect	N/A

The dose response curves are shown in Figure 2.12.

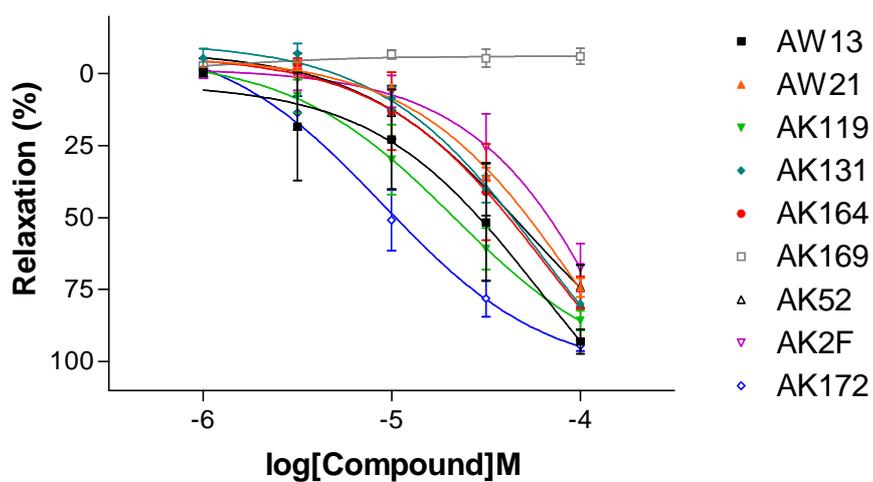
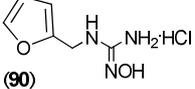
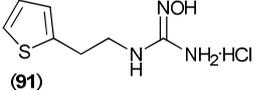
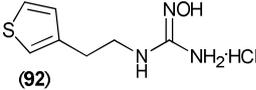
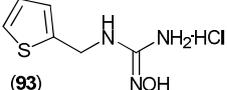


Figure 2.12 Vasorelaxation profiles of modified QZNO193 N-hydroxyguanidines

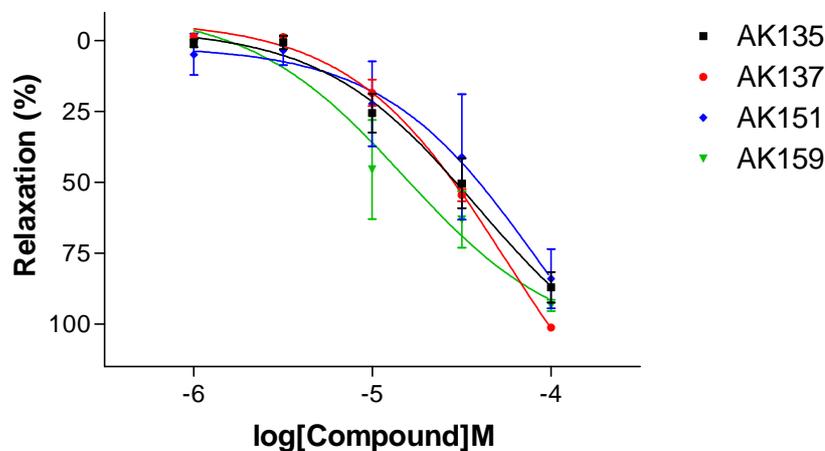
The biological data suggested that there is no correlation between the biological activity and electronic properties of the substituents. However, some data illustrate the relationship between the size of the substituents and vasodilatation properties. The EC₅₀ values indicated that the substitution of the phenyl ring with a trifluoromethyl group increased the activity. Almost the same activity was shown by the methyl substituted derivative, even though the CF₃ moiety is an electron-withdrawing group while CH₃ is an electron-donating. The lack of a correlation between the electronic properties is not a big surprise, because the hydroxyguanidine functionality is not directly attached to the phenyl ring. On the other hand, the CF₃ and CH₃ are almost the same size, which indicates that the size of the substituent is important. However, *N*-2-(4'-bromophenyl)ethyl-*N*'-hydroxyguanidine (AK131) has also a better biological profile (logEC₅₀ = -4.8) compared to the parent phenyl derivative (logEC₅₀ = -4.6). The rest of the tested compounds possessed higher EC₅₀ values, but still remained potent NO donors. Unfortunately, there is not really a pattern which can allow the most important structural features to be distinguished. As seen in Table 2.6, most of the new derivatives have poor ODQ reversibility which may imply that vasorelaxation is not only mediated through NO or that those compounds may be toxic. In some cases the ODQ errors are quite big. This can be a result of rat aorta deterioration with time. Interestingly, *N*-2-[4'-(sulfamoyl)phenyl]ethyl-*N*'-hydroxyguanidine (AK169) does not possess any biological activity. Since the detailed mechanism of vasodilatation is not known one can only speculate that complete loss of activity is due to the secondary pathway happening on the sulfonamide group such as oxidation, for example.

Additionally, a series of novel heterocyclic *N*-hydroxyguanidines has been synthesized and subjected to myography studies. The EC₅₀ values are summarized in Table 2.7.

Table 2.7 NO donor activity of heterocyclic analogues in rat aorta

Structure	Code No.	$\log EC_{50}$ [mol/dm ³]	ODQ induced reversibility[%]
 (90)	AK135	-4.4 ± 0.2	86.7 ± 13
 (91)	AK137	-4.2 ± 0.1	60.1 ± 41
 (92)	AK151	-4.1 ± 0.6	36.1 ± 22
 (93)	AK159	-4.8 ± 0.1	27.3 ± 12

Surprisingly, the entire class of heterocyclic compounds acts as nitric oxide donors. Two of them seemed to be very potent. AK135 and AK137 possessed almost the same NO donation profile as the lead compound QZNO193. Their vasodilatation profile is shown in Figure 2.13.

Figure 2.13 Vasorelaxation profiles of heterocyclic *N*-hydroxyguanidines

As in case of QZNO193, the vasodilatation profile of *N*-furfuryl-*N'*-hydroxyguanidine (AK135) was tested in the presence of various additives as illustrated in Figure 2.14. Essentially the same results were obtained for AK135 and QZNO193.

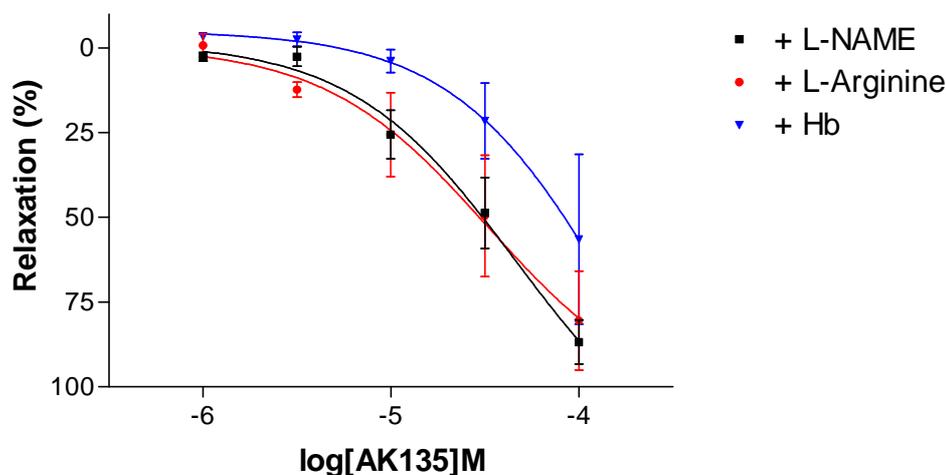


Figure 2.14 Vasorelaxation profile of AK135 in the presence of various additives

Since, the starting *meta*, *ortho* and *para* methoxyphenylacetonitriles were commercially available the corresponding hydroxyguanidines have been synthesized to test the effect of the substitution position on the vasodilatation. Their EC_{50} 's and ODQ data are gathered in Table 2.8.

Table 2.8 NO donor activity of methoxyphenylethyl-*N*'-hydroxyguanidines in rat aorta

Structure	Code No.	$\log EC_{50}$ [mol/dm ³]	ODQ induced reversibility[%]
 (77)	AK120	-4.3 ± 0.1	40.3 ± 3.7
 (78)	AK118	-5.4 ± 0.2	8.8 ± 1.7
 (79)	AK136	-4.7 ± 0.1	13.5 ± 18

All the methoxy derivatives cause vasodilatation under myography conditions. The EC_{50} values indicate that they are all potent NO donors with the best one being *meta* > *ortho* > *para* analogue. However, their ODQ reversibility is almost negligible, which may suggest that the

biological effect is not NO mediated and there is some secondary biological pathway or that these compounds are toxic. Their vasorelaxation curves are presented in Figure 2.15.

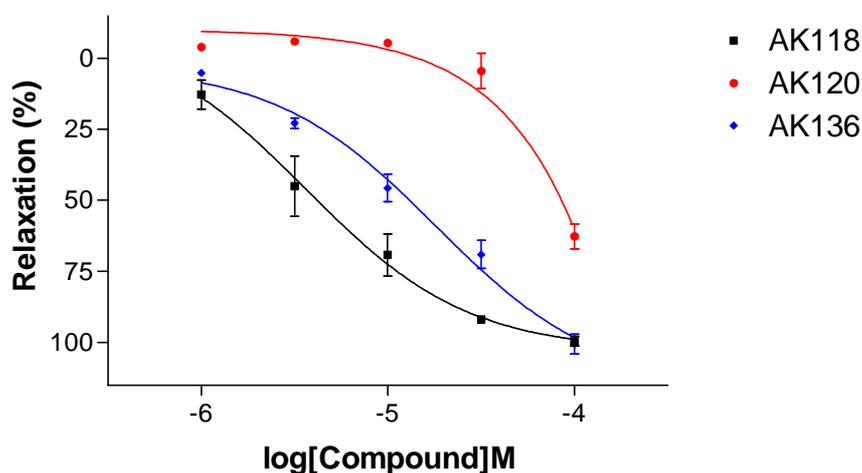
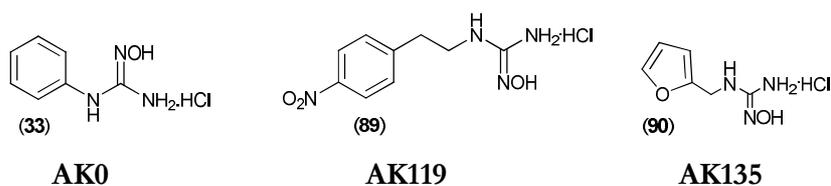


Figure 2.15 Vasorelaxation profiles of methoxyphenylethyl-*N'*-hydroxyguanidines

As mentioned at the beginning of this section, the compounds which met the preliminary criteria were tested in the isolated perfused kidney (IPK) model. The *N*-hydroxyguanidines selected for further investigation are presented in Scheme 2.14.

Scheme 2.14



The lead compound AK0 caused ~80% relaxation in the IPK, giving a log EC₅₀ of -4.2 (n=3) as illustrated by the dose response curve in Figure 2.16.

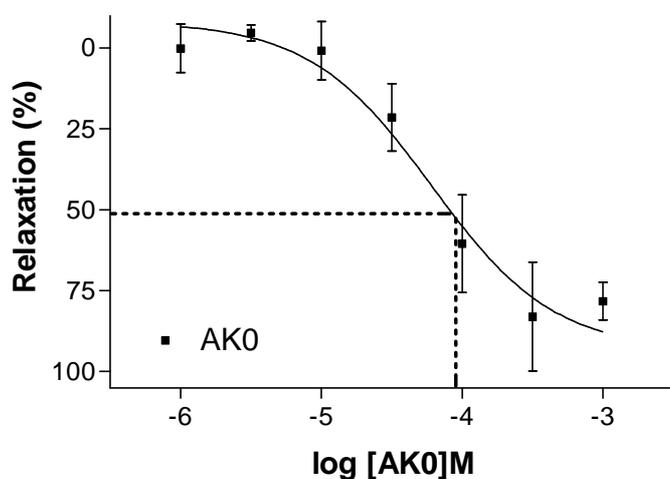


Figure 2.16 Vasorelaxation profile of AK0 (33) in isolated perfused kidney

The vasodilatation profile of AK0 is slightly worse than in the rat aorta, but still good relaxation is observed. However, when the AK119 was subjected to the same studies more complex results were observed. Vasorelaxation was seen initially, but then there was a subsequent vasoconstriction (Figure 2.17).

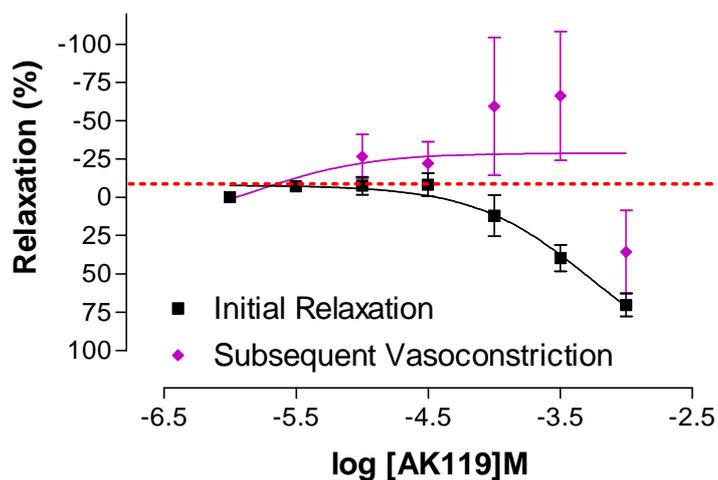


Figure 2.17 Vasorelaxation profile of AK119 in isolated perfused kidney

In this case, the IPK model was not a reflection of the rat aorta model, which can imply that the nitro analogue undergoes a different biological pathway in the rat aorta compared to the isolated organ. Therefore, this compound was not taken for further studies. On the other hand, *N*-furfuryl-*N'*-hydroxyguanidine hydrochloride (AK135) possessed a reasonably good biological profile in the isolated perfused kidney as presented in Figure 2.18.

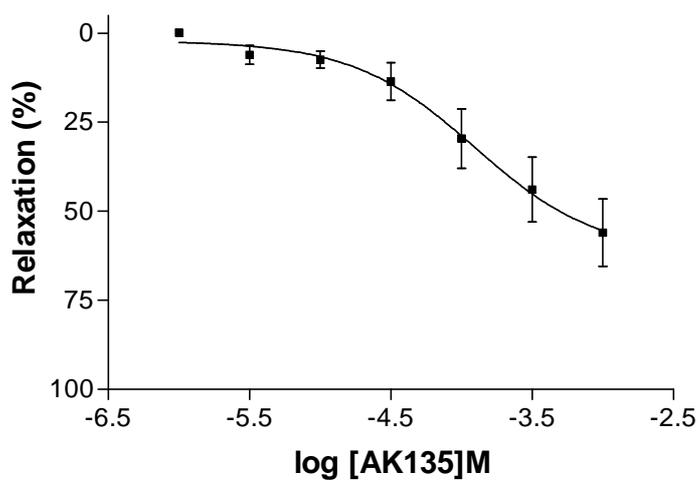


Figure 2.18 Vasorelaxation profile of AK135 (90) in isolated perfused kidney

The results from the myography as well as from the IPK studies, thus allowed the selection of two more *N*-hydroxyguanidines to be parent molecules for the corresponding pro-drugs.

2.3 Conclusions

Preliminary biological results indicated that almost all of the synthesized *N*-hydroxyguanidines (23 compounds) act as NO donors and cause smooth muscle relaxation in the rat aorta. The exception is *N*-2-[4'-(sulfamoyl)phenyl]ethyl-*N'*-hydroxyguanidine (AK169) which does not cause any vasorelaxant effect. Data for the series of *N*-arylalkyl-*N'*-hydroxyguanidines showed that the extension of the aliphatic chain is beneficial up to three methylene groups, while further elongation significantly reduced the biological activity. The compound with the best pharmacological profile turned, in the rat aorta assay, out to be *N*-phenyl-*N'*-hydroxyguanidine hydrochloride (AK0), ($EC_{50} = 19.9 \mu\text{M}$, ODQ = 104%). AK0 also caused significant vasodilatation in the IPK model. Changes to the benzene ring of *N*-phenylethyl-*N'*-hydroxyguanidine (QZNO193) demonstrated that substitution with trifluoromethyl group in the *para* position improved biological activity of the new derivative (QZNO193, $EC_{50} = 50.1 \mu\text{M}$; AK2F, $EC_{50} = 8.0 \mu\text{M}$). However, the ODQ test indicated worse reversibility, which may imply that the vasodilation does not occur mainly through NO mediated pathway. Additionally, *N*-2-(4'-nitrophenyl)ethyl-*N'*-hydroxyguanidine (AK119) had a very potent biological profile in the rat aorta assay ($EC_{50} = 25.1 \mu\text{M}$, ODQ = 75%). However, IPK model revealed that AK119 caused only initial vasorelaxation, but then a subsequent vasoconstriction was observed. *N*-Furfuryl-*N'*-hydroxyguanidine hydrochloride (AK135) also possessed a reasonably good biological profile in the rat aorta assay as well as in the isolated perfused kidney.

2.4 References

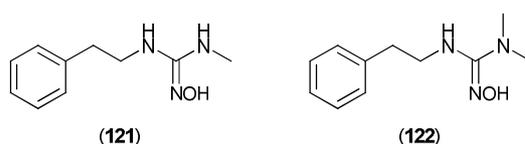
- 1 A. Renodon-Corniere, S. Dijols, C. Perollier, D. Lefevre-Groboillot, J. L. Boucher, R. Attias, M. A. Sari, D. Stuehr, and D. Mansuy, *J. Med. Chem.*, 2002, **45**, 944.
- 2 Q. Zhang and N. P. Botting, Unpublished Results, *University of St Andrews*, 2008.
- 3 A. J. H. Brian S. Furniss, Peter W.G. Smith, Autsin R. Tatchell, 'Vogel's Textbook of Practical Organic Chemistry', Longman Scientific & Technical, 1989.
- 4 S. Caddick, A. K. D. K. Haynes, D. B. Judd, and M. R. V. Williams, *Tetrahedron Lett.*, 2000, **41**, 3513.
- 5 S. Caddick, D. B. Judd, A. K. d. K. Lewis, M. T. Reich, and M. R. V. Williams, *Tetrahedron*, 2003, **59**, 5417.
- 6 D. J. Tantillo, J. M. Fukuto, B. M. Hoffman, R. B. Silverman, and K. N. Houk, *J. Am. Chem. Soc.*, 2000, **122**, 536.
- 7 T. B. Cai, D. Lu, and P. G. Wang, *Curr. Top. Med. Chem.*, 2005, **5**, 721.
- 8 I. Sucholeiki, V. Lynch, L. Phan, and C. S. Wilcox, *J. Org. Chem.*, 1988, **53**, 98.
- 9 S.-D. Cho, Y. D. Park, J. J. Kim, J. R. Falck, and Y. J. Yoon, *Bull. Korean Chem. Soc.*, 2004, **25**, 407.
- 10 N. Umino, T. Iwakuma, and N. Itoh, *Tetrahedron Lett.*, 1976, 2875.
- 11 J. H. Boal, A. Wilk, C. L. Scremin, G. N. Gray, L. R. Phillips, and S. L. Beaucage, *J. Org. Chem.*, 1996, **61**, 8617.
- 12 H. C. Brown, Y. M. Choi, and S. Narasimhan, *J. Org. Chem.*, 1982, **47**, 3153.
- 13 B. M. Adger and R. G. Young, *Tetrahedron Lett.*, 1984, **25**, 5219.
- 14 A. C. Spivey, J. McKendrick, R. Srikanan, and B. A. Helm, *J. Org. Chem.*, 2003, **68**, 1843.

***MONO- & DIMETHYL-
N-HYDROXYGUANIDINES***

3.1 Introduction

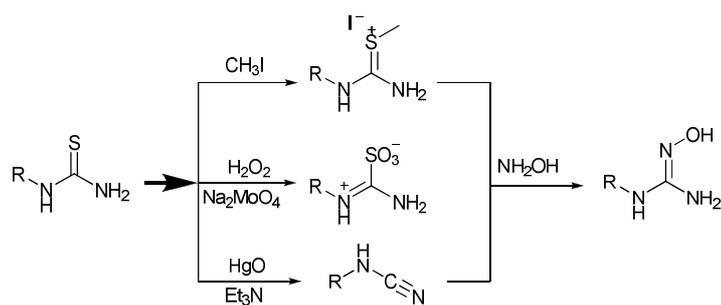
The synthesis of *N*-hydroxyguanidine pro-drugs in the Botting group has been hampered by problems occurring due to cyclisation reactions (see Section 6.1). The side reaction was caused by the presence of free amino group in the hydroxyguanidine functionality which easily reacted with a neighbouring carbonyl carbon to form an unwanted aromatic five-membered ring. Therefore, it was proposed that methylation of the free amino group might prevent cyclisation reactions, assuming that the corresponding *N*-hydroxyguanidines were still biologically active. The initial goal was therefore to synthesize the *N*-hydroxyguanidines shown in the Scheme 3.1 and investigate the role of the free NH₂ in the biological formation of NO.

Scheme 3.1

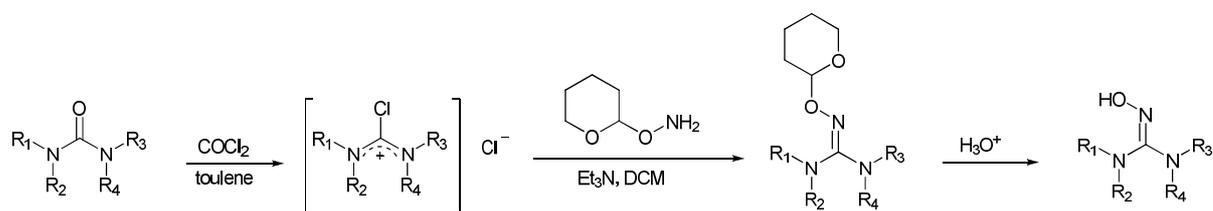


There are only a few publications which report suitable synthetic methodology for substituted hydroxyguanidines as presented previously in Section 1.5.1.^{1,2} Most synthetic routes involved thioureas as a key intermediate (Scheme 3.2.).

Scheme 3.2²

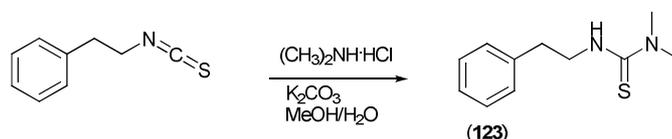


Ziman also reported the alternative employment of *C*-chloroformamidinium chlorides as reactive species in the synthesis of tri- and tetra-substituted hydroxyguanidines, which can be prepared by reaction of the appropriate urea (or thiourea) with phosgene as illustrated in Scheme 3.3.³

Scheme 3.3³

The synthesis of the dimethyl derivative (**122**) was investigated first, requiring access to the corresponding thiourea.⁴ Reaction of phenylethyl isothiocyanate with dimethylamine hydrochloride in the presence of base gave the *N,N*-dimethyl-*N'*-phenylethyl thiourea (**123**) as colourless crystals in 80% yield (Scheme 3.3). The product was analyzed by ¹H and ¹³C NMR spectroscopy, MS and elemental analysis. The presence of the methyl protons (6H, 3.12 ppm) in the ¹H NMR spectrum confirmed the chemical structure of the obtained compound. In addition, the characteristic peak (181.1 ppm) for the thiocarbonyl group was observed in the ¹³C NMR spectrum. The molecular weight was confirmed by the presence of the single peak in the negative ion mass spectrum at 207 (M-H)⁻.

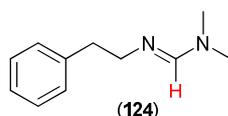
Scheme 3.3



Oxidation of the thiocarbonyl group to the sulfonic acid was chosen as a first route to investigate. According to the literature the reaction of the thiourea with hydrogen peroxide in the presence of sodium molybdate should lead to the corresponding aminoiminomethanesulfonic acid.⁵ Several attempts were made to synthesize *N,N*-dimethyl-*N'*-phenylethylaminoiminomethanesulfonic acid, however each time a yellow oil was isolated. The results of analysis by MS, ¹H and ¹³C NMR spectroscopy indicated the apparent loss of SO₃ due to the presence of a single peak in the mass spectrum at 177. The appearance of an additional peak in ¹H NMR spectrum at 10.22 ppm (1H, br s) was then identified as the proton between the two nitrogens in the unexpected product (**124**). There was also a weak signal in the ¹³C NMR

spectrum at 156 ppm which was identified as methine according to the PENDANT spectrum. This result implied that the transformation of the C=S group to the C-SO₃H was achievable, but than the corresponding aminoiminomethanesulfonic acid was unstable under the reaction conditions and underwent an elimination reaction with loss of sulfur trioxide to give either the anion or carbene which was then protonated to give compound (**124**).⁶⁻⁸

Scheme 3.4



In order to overcome this problem an alternative approach was investigated involving methylation of the *N,N*-dimethyl-*N'*-phenylethylthiourea (**123**) using methyl iodide, to give the methylsulfonium iodide (**125**), and producing a good leaving group for further reaction (Scheme 3.2).⁹ When this reaction was carried out the new methyl protons were observed at 2.26 ppm in the ¹H NMR spectrum. In addition, a shift of the quaternary carbon in the ¹³C NMR spectrum from 181.1 ppm (C=S) to 168.1 (C=S⁺-CH₃) as well as the presence of the single peak in the mass spectrum at 223 (M⁺) confirmed the structure of the product. The sulfonium iodide (**125**) was then reacted with hydroxylamine hydrochloride in the presence of silver nitrate and triethylamine. However, spectroscopic analyses indicated that instead of *N*-phenylethyl-*N'*-dimethyl-*N''*-hydroxyguanidine the corresponding urea (**126**) was obtained, which was confirmed by the presence of the single peak in the mass spectrum at 215 (M+Na⁺). Additionally, the X-ray crystal structure of the final compound, as shown in Figure 3.1, confirmed that the urea was the only product. It thus implied that hydrolysis to the urea had occurred rather than reaction with the hydroxylamine.

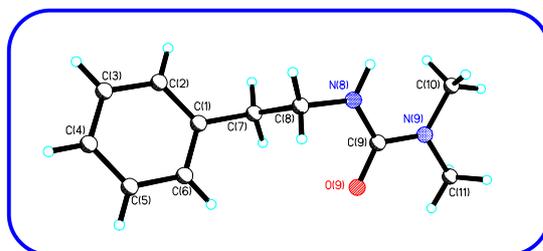
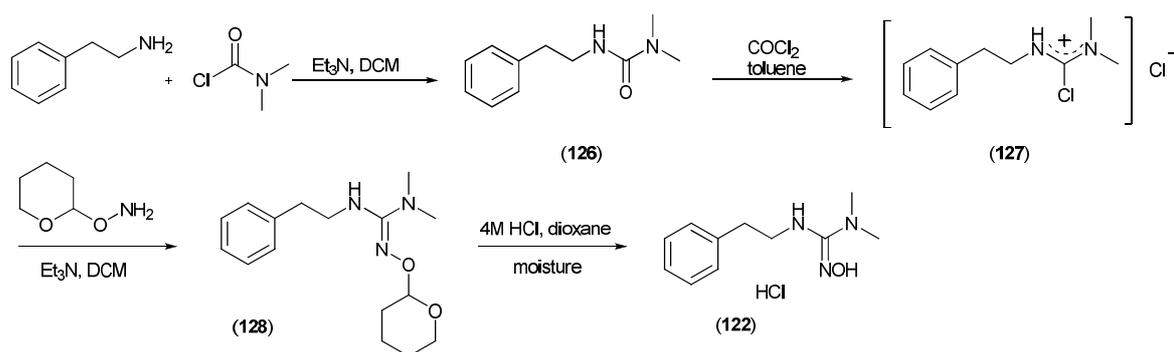


Figure 3.1 X-ray crystal structure of *N,N*-dimethyl-*N'*-phenethyl urea (**126**)

Instead of trying to improve this reaction it was decided to synthesize the *N,N*-dimethyl-*N'*-phenylethyl hydroxyguanidine (**122**) using the Ziman procedure (Scheme 3.5).³ The required *N,N*-dimethyl-*N'*-phenethyl urea (**126**) was synthesized from the phenylethylamine and dimethylcarbamoyl chloride in the presence of triethylamine. Since it is known compound, it was only analyzed by ¹H and ¹³C NMR spectroscopy and its purity was confirmed by the melting point (see experimental). In order to avoid the use of phosgene it was first attempted to obtain the *C*-chloroformamidinium chloride *via* the reaction of the urea with phosphorus oxychloride followed by the reaction with commercially available *O*-benzylhydroxylamine, as a model reaction.¹⁰ Unfortunately, this did not give satisfactory results. It was suspected that the key intermediate was not obtained since the *O*-benzylhydroxylamine was recovered after the work-up.

Scheme 3.5



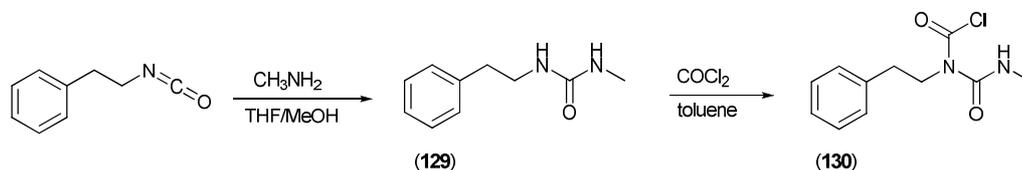
The urea (**126**) was then instead treated with a 1.93 M solution of phosgene (COCl₂) in toluene to give the *C*-chloroformamidinium chloride (**127**), which was reacted directly with *O*-tetrahydropyranyloxyamine in the presence of base to give the THP protected hydroxyguanidine (**128**).^{3, 11} The compound was obtained in 36% yield over the two steps and characterized by various analytical methods, such as ¹H and ¹³C NMR spectroscopy, IR spectroscopy and mass spectrometry. The most indicative piece of data was the appearance of a multiplet (4.97-5.00 ppm) corresponding to the CH proton from THP ring, as well as the aromatic protons (7.19-7.33 ppm) in the ¹H NMR spectrum. It confirmed the successful nucleophilic addition of the *O*-tetrahydropyranyloxyamine to the electrophilic centre of the

C-chloroformamidinium chloride. In addition, the correct mass was observed in HR-ESI-MS spectrum (292.2023 ((M+H)⁺. C₁₆H₂₆N₃O₂ requires 292.2025).

N,N-Dimethyl-*N'*-phenethyl-*N''*-*O*-tetrahydropyranyllhydroxyguanidine (**128**) was then easily deprotected using 4 M HCl in 1,4-dioxane/H₂O to give the desired product (**122**) in 73% yield. The structure of the compound was confirmed by the loss of peaks corresponding to the THP ring in the ¹H and ¹³C NMR spectra compared to the starting material. As usual, there were characteristic signals for the hydroxyguanidine group in the lowfield region of the spectrum. In addition, the X-ray crystal structure of the final product was obtained and a more detailed discussion of the structure will be given in the next section of this chapter.

The application of the *C*-chloroformamidinium chloride in the synthesis of *N,N*-dimethyl-*N'*-phenethyl-*N''*-hydroxyguanidine (**122**) resulted in a satisfactory yield and very good purity. Therefore, the same approach was employed for the synthesis of the monomethylated derivative. The corresponding urea (**129**) was a product of the reaction between phenylethyl isocyanate and methylamine as shown in Scheme 3.6.¹²

Scheme 3.6



N-Methyl-*N'*-phenethyl urea (**129**) was characterized by the ¹H and ¹³C NMR spectroscopy and its purity confirmed by elemental analysis (see experimental). The next step involved reaction of the urea with phosgene to form the reactive intermediate. However, several attempts to perform this reaction failed. Literature studies indicated that the products formed in the reaction of 1,3-disubstituted ureas with phosgene strongly depend on the substitution pattern of the starting urea.^{10, 13} As reported by Ulrich *et al.*, it was found that if the alkyl substituent was attached to the nitrogen by a primary carbon atom, then the reaction led predominately to attack on the nitrogen and the corresponding allophanoyl chlorides (**130**) were formed instead of *C*-chloroformamidinium chlorides (Scheme 3.6).^{13, 14} Since the above approach failed, it was decided to go back to the aminoiminomethanesulfonic acid as the key intermediate, especially as

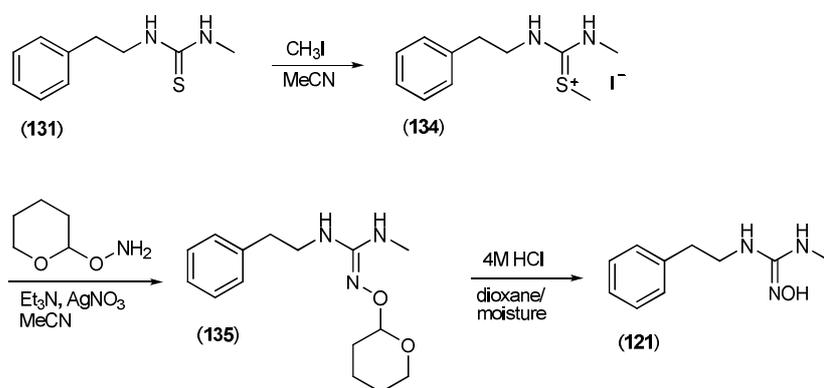
there are few reports describing the application of disubstituted aminoiminomethanesulfonic acids in the synthesis of disubstituted guanidines.^{5,8} However, this time the oxidation was carried out using freshly prepared peracetic acid as an oxidizing agent, instead of sodium molybdate and hydrogen peroxide. *N*-Methyl-*N'*-phenylethyl thiourea (**131**) was synthesized in the same way as in the case of the dimethyl derivative namely from phenylethyl isothiocyanate and methylamine instead of dimethylamine (Scheme 3.3). The desired product was obtained as an off-white solid in 90% yield. The presence of the methyl protons (3H, 2.89 ppm) in the ¹H NMR spectrum indicated the correct structure of the final compound. In addition, the characteristic peak (182.4 ppm) for the thicarbonyl group in the ¹³C NMR spectrum as well as the presence of the single peak in the mass spectrum at 217 (M+Na)⁺ confirmed the chemical structure. The results of elemental analysis confirmed the purity of *N*-methyl-*N'*-phenylethyl thiourea (**131**). *N*-Methyl-*N'*-phenylethyl thiourea (**131**) was then subjected to oxidation using freshly prepared peracetic acid to give the corresponding aminoiminomethanesulfonic acid (**132**) in only 25% yield, but in very good purity. The product was analyzed by various NMR techniques, MS and elemental analysis. The presence of the single peak in the mass spectrum at 265 (M+Na)⁺ indicated the correct molecular mass. The result of elemental analysis pointed out that synthesized product was nearly 100% pure. Interestingly, both the ¹H and ¹³C NMR spectra of aminoiminomethanesulfonic acid (**132**) in d₆-DMSO indicated that the synthesized molecule was actually a mixture of two isomers, which will be discussed in Section 3.2.2.

As the modified oxidation method was successful in the case of *N*-methyl-*N'*-phenylethyl thiourea (**131**), the same approach was used to synthesize the trisubstituted analogue, which had failed previously with the NaMoO₄/H₂O₂ oxidizing mixture. A syringe pump was also employed to control the rate of addition of the thiourea solution to the peracetic acid solution, as an additional precaution. The final product (**133**) was obtained as off-white crystals in 58% yield and was characterized by MS, ¹H and ¹³C NMR spectroscopy. However, the resulting data were misleading. Some of data indicated the presence of the final product while others the starting material. The correct molecular weight of *N,N*-dimethyl-*N'*-phenylethylaminoiminomethane sulfonic acid (**133**) was observed in the mass spectrum (M-H⁻ = 255), but the aminoiminomethanesulfonic acids generally give a strong signal in negative mode mass spectrometry. However, the ¹H and ¹³C NMR spectra looked identical to the starting material. Therefore, the crystal structure was obtained to confirm the identity of the final product, and indicated the presence of the desired product. Finally, the thin-layer chromatography showed

that the isolated crystals were actually a mixture of starting thiourea and the product. However, the sulfonic acid was only a very small percentage of the isolated solid. A few attempts were made in order to synthesize *N,N*-dimethyl-*N'*-phenylethylaminoiminomethane sulfonic acid (**133**) using various other oxidizing agents such as H_2O_2 , CF_3COOOH and using different solvents (methanol, acetonitrile, methanol/ H_2O_2), but none of them gave satisfactory results.^{8, 15-18} It has been already observed by others that trisubstituted thioureas are resistant to oxidation, but on the other hand forcing the reaction conditions caused SO_3 elimination as described previously.^{6, 15}

The presence of two isomers of *N*-phenylethyl-*N'*-methylaminomethanesulfonic acid (**132**) was expected to be a problem in the next step, namely the reaction with hydroxylamine hydrochloride. Therefore, the approach using methylated thiourea was employed as presented in Scheme 3.7.

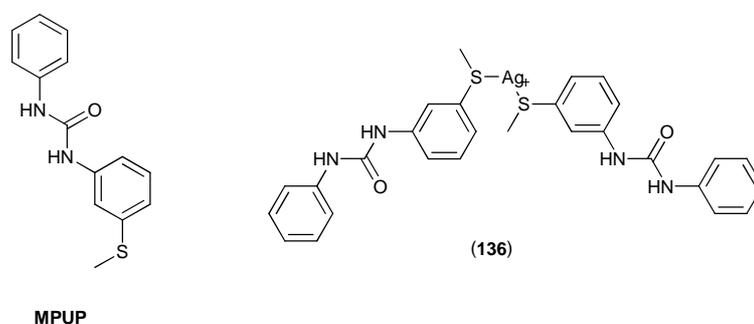
Scheme 3.7



Previously synthesized *N*-methyl-*N'*-phenylethyl thiourea (**131**) was reacted with methyl iodide to produce *N*-methyl-*N'*-phenylethyl-*S*-methylsulfonium iodide (**134**) in a good yield (70%). The structure was confirmed by ^1H and ^{13}C NMR spectroscopy and mass spectrometry. The shift of the quaternary carbon from 182.4 ppm ($\text{C}=\text{S}$) to 162.1 ppm ($\text{C}-\text{SCH}_3$) confirmed the successful reaction. The next step involved reaction of the methylated thiourea (**134**) with THP-protected hydroxylamine in the presence of triethylamine and silver nitrate. The thioether group is known to react with soft metal ions, particularly $\text{Ag}(\text{I})$ and $\text{Hg}(\text{II})$.¹⁹⁻²¹ Therefore, formation of the insoluble salt should facilitate the nucleophilic substitution. The final product

(**135**) gave the correct mass in the HR-ESI-MS spectrum (278.1866 ((M+H)⁺. C₁₅H₂₃N₃O₂ requires 278.1869). Interestingly, the ¹H and ¹³C NMR spectra indicated the presence of two isomers (see experimental), which were not studied in detail, mainly due to the very poor yield of 21% and the small amount of material obtained. Several attempts were made in order to improve the yield of this reaction, but none of them were very successful. It was suspected that some side reaction took place involving silver nitrate. Indeed, Steed *et al.* reported that 1-(3-methylsulfanyl-phenyl)-3-phenyl-1-urea (MPUP) reacted with AgNO₃ to form the complex (**136**) as shown in Scheme 3.8.²²

Scheme 3.8²²



Based on literature precedent it was expected that a similar reaction could occur with our substrate since a lot of inorganic solid was filtered off during the work-up. However, for the biological studies there was enough material to take it through the next step and obtain the final hydroxyguanidine (**121**). As shown in Scheme 3.7, the THP-protected hydroxyguanidine (**135**) was treated with a 4M HCl solution in 1,4-dioxane/H₂O to give *N*-methyl-*N'*-phenethyl-*N''*-hydroxyguanidine hydrochloride (**121**) in 71% yield. The product was characterized by the ¹H and ¹³C NMR spectroscopy, IR spectroscopy and MS. The characteristic signals for the hydroxyguanidine functional group were observed in the lowfield of the ¹H NMR spectrum indicating the successful deprotection. The novel, substituted hydroxyguanidines were therefore successfully synthesized and their structures were confirmed by various analytical techniques. In addition, along with the series of aminoiminomethanesulfonic acids, they were studied in more detail by X-ray crystallography and ¹⁵N NMR spectroscopy, which is described in the following section.

3.2 X-Ray crystallography and NMR spectroscopy studies

During the synthetic research it was discovered that aminoiminomethanesulfonic acids, as well as *N*-hydroxyguanidines, possessed very interesting structural properties. In addition, there is a very little known about this class of compounds from the spectroscopic point of view. Therefore, having these compounds in hand, some additional structural studies were performed using X-ray crystallography and NMR spectroscopy. A series of aminoiminomethanesulfonic acids and *N*-hydroxyguanidines were chosen for more detailed structural studies in order to determine their characteristics in the solid state as well as in solution and to compare the obtained data for two classes of compounds. *N*-phenethylaminoiminomethanesulfonic acid (**137**) was synthesized just for the purpose of these studies.

3.2.1 X-Ray Studies

The molecular structures of selected aminoiminomethanesulfonic acids, as determined by X-ray diffraction are presented in Figures 3.2, 3.3 and 3.4. The most informative X-ray data (selected bond lengths and torsion angles) are summarized in Table 3.1.

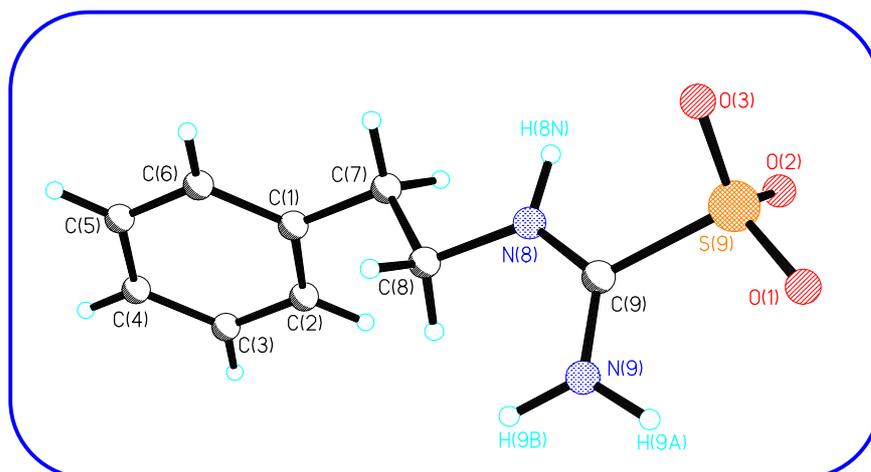


Figure 3.2 The X-ray crystal structure of *N*-phenethylaminoiminomethanesulfonic acid (**137**).

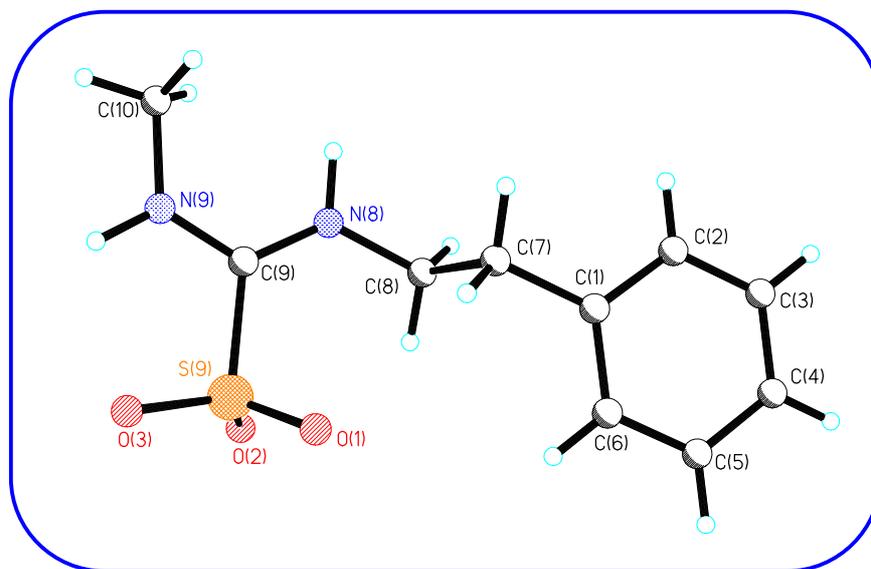


Figure 3.3 The X-ray crystal structure of *N*-methyl-*N'*-phenylethylaminoiminomethanesulfonic acid (132)

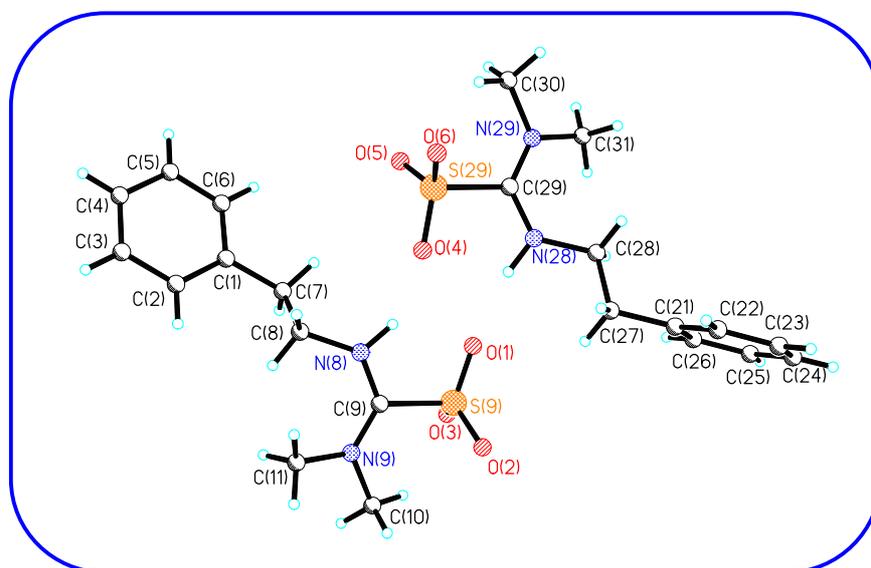
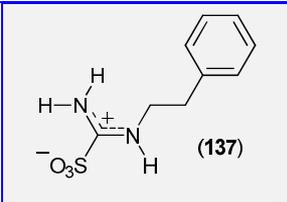
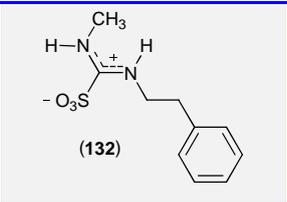
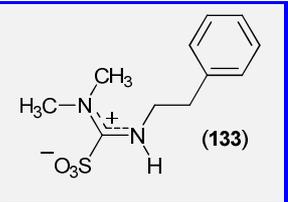


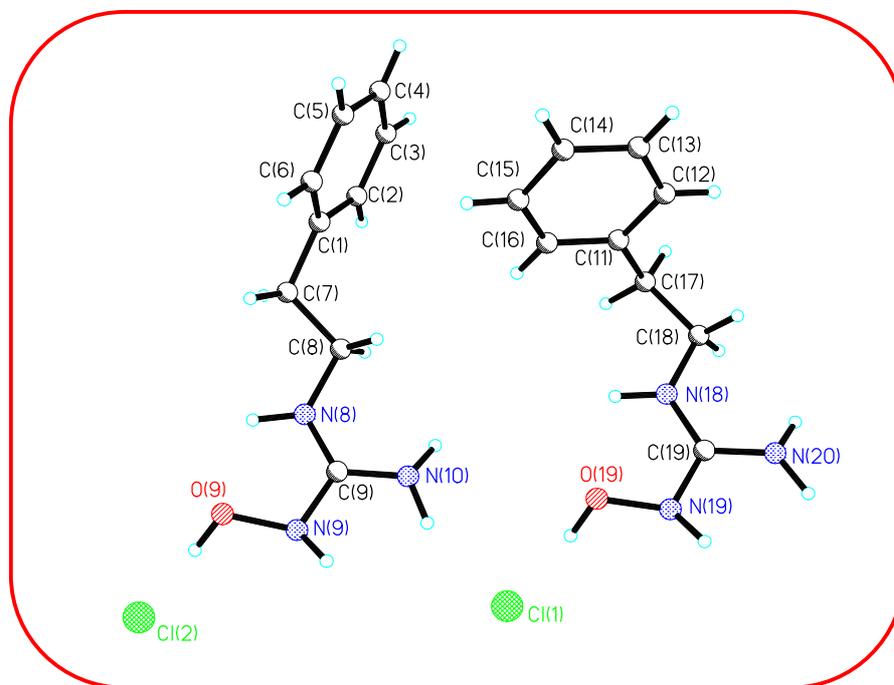
Figure 3.4 The X-ray crystal structure of *N,N*-dimethyl-*N'*-phenylethylaminoiminomethanesulfonic acid (133).

Table 3.1 Torsion angles [°] and bond lengths[Å] for aminoiminomethanesulfonic acids

			
<i>C(8)-N(8)-C(9)-N(9)</i>	-1.6(2)	177.79(17)	-14.1(7)[22.2(5)]
<i>C(8)-N(8)-C(9)-S(9)</i>	-179.73(12)	-3.7(2)	166.8(4)[-158.8(2)]
<i>N(9)-C(9)</i>	1.305(2)	1.303(2)	1.309(4)[1.311(4)]
<i>N(8)-C(9)</i>	1.302(2)	1.311(2)	1.320(5)[1.317(4)]
<i>C(9)-SO₃⁻</i>	1.824(15)	1.815(17)	1.839(3)[1.844(3)]

*two rotamers were found in the solid state

The X-ray structures were obtained for the corresponding *N*-hydroxyguanidines: (120) and (122), which were synthesized as HCl salts. Several attempts were made to crystallize the monomethylated derivative (121), but unfortunately none of them were successful. The X-ray crystal structures of selected *N*-hydroxyguanidines are shown in Figure 3.5 and 3.6. Selected bond lengths and torsion angles are given in Table 3.2.


Figure 3.5 The X-ray crystal structure of *N*-phenylethyl-*N'*-hydroxyguanidine hydrochloride (120).

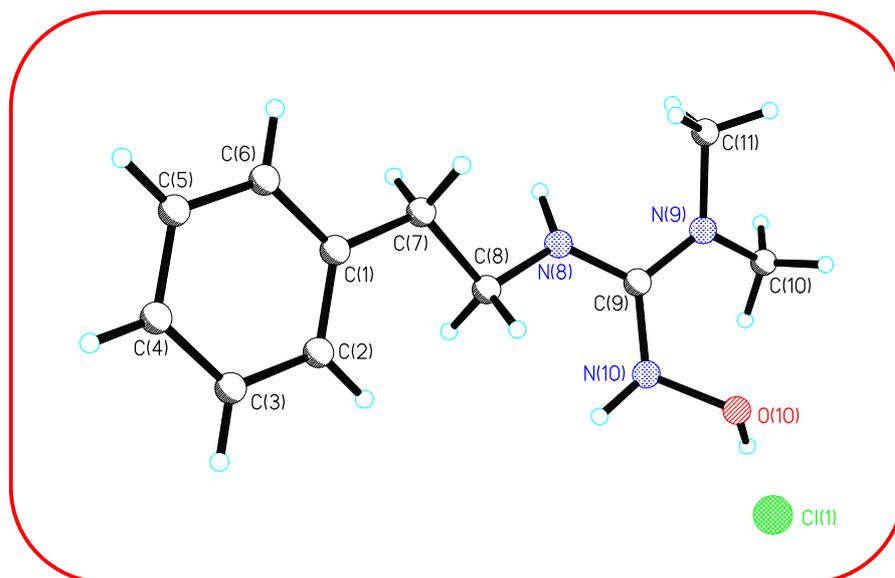
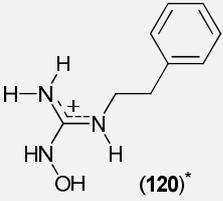
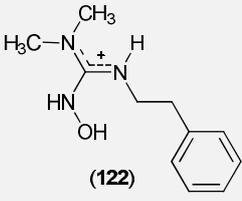


Figure 3.6 The X-ray crystal structure of *N,N*-dimethyl-*N'*-phenylethyl-*N''*-hydroxyguanidine hydrochloride (**122**).

Table 3.2 Torsion angles [°] and bond lengths[Å] for *N*-hydroxyguanidines

	 (120 *)	 (122)
<i>C(8)-N(8)-C(9)-N(9)</i>	172.0(3)[7.3(5)]	-163.22(17)
<i>C(8)-N(8)-C(9)-N(10)</i>	-8.1(6)[-171.9(3)]	19.0(3)
<i>C(9)-N(8)-N(10)-O(10)</i>	-19.6(5)[-15.4(5)]	-147.6(2)
<i>C(9)-N(9)-N(10)-O(10)</i>	160.5(3)[160.5(3)]	34.7(2)
<i>N(9)-C(9)</i>	1.329(5)[1.326(5)]	1.335(2)
<i>N(8)-C(9)</i>	1.323(5)[1.323(5)]	1.334(2)
<i>N(10)-C(9)</i>	1.466[1.470(5)]	1.466(2)

*two rotamers were found in the solid state

In the case of the aminoiminomethanesulfonic acids, all C(9)-N(8) and C(9)-N(9) bond lengths stay in a narrow range (1.302-1.320 Å) indicating that all three analogues of aminoiminomethanesulfonic acids possessed partial double bond character within the N(9)-C(9)-N(8) fragment and consequently the positive charge should be almost equally

distributed between two nitrogens. The same is observed in the case of *N*-hydroxyguanidines. However, the bond lengths of *N*-hydroxyguanidines (1.323-1.335 Å) are slightly longer indicating that both C-N bonds have more single bond character. On the other hand the N(10)-C(9) bond lengths (1.466-1.470 Å) imply essentially single bond character. Partial double bond character of C-N bonds is also in accordance with the protonation of N(8) and N(10) in the case of aminoiminomethanesulfonic acids and *N*-hydroxyguanidines, respectively. This was also confirmed by refinement of weakly bonded hydrogens.

The torsion angles imply that the geometry around the sulfonic acid as well as the hydroxyguanidine functionality is nearly planar. The deviation from planarity is the highest for **(133)** and **(122)** due to the presence of steric hindrance caused by two methyl groups. The planarity of *N*-hydroxyguanidines is more distorted which is in agreement with the more single bond character of the C(9)-N(10) bonds. While N(8), N(9), C(9) and N(10) adopt a nearly planar arrangement, O(10) diverts significantly from the plane as can be expected due to the single bond character of C(9)-N(10) bond.

In order to adopt the most energetically favorable configuration, compounds **(137)** and **(133)** exist in the solid state as *E*-isomers. On the other hand, analogue **(132)** in order to minimize steric repulsions adopts a *Z*-configuration at C(9)-N(8) bond and *E*-configuration at C(9)-N(9). According to the X-ray structure, *N*-phenylethyl-*N'*-hydroxyguanidine **(120)** exists in the solid state as a single *E*-isomer, similarly as its aminoiminomethanesulfonic acid counterpart **(137)**. However, **(122)** adopts opposite configuration and exists in the solid state as a single *Z*-isomer likely due to the larger steric hindrance of the *N,N*-dimethylamino group compared to the HN-OH group.

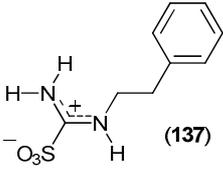
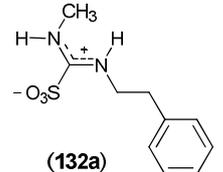
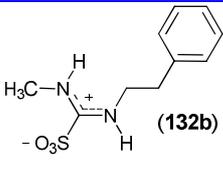
In addition, it was found that compounds **(133)** and **(120)** exist as two rotamers in the solid state, which differ only in the orientation of the phenyl ring.

3.2.2 NMR Spectroscopy Studies

Aminoiminomethanesulfonic Acids

A series of aminoiminomethanesulfonic acids (**132**) and (**137**) was analysed by various NMR techniques such as ^1H NMR, 1D gs-NOESY, ^1H - ^1H COSY, ^1H - ^{13}C HMBC and ^1H - ^{15}N HMBC. The values of the ^1H and ^{15}N chemical shifts are summarized in Table 3.3. Unfortunately, the aminoiminomethanesulfonic acid (**133**) could not be studied by NMR spectroscopy since it contained a large amount of starting thiourea, as described in Section 3.1.

Table 3.3 The values of ^1H and ^{15}N chemical shifts for aminoiminomethanesulfonic acids

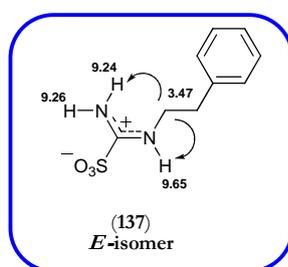
Structure	δ chemical shifts [ppm]*				
	^{15}N NMR data		^1H NMR data		
	N(8)	N(9)	N(8)H	N(9)H	N(9)CH ₃
 (137)	103.4	116.9	9.65	9.24 9.26	-
 (132a)	111.9	108.9	9.09	9.39	2.84
 (132b)	117.9	98.3	9.28	9.12	3.18

*all samples run in the d_6 -DMSO solutions at room temperature;

In the case of (**137**), the ^1H NMR spectrum showed three NH resonances at 9.24 (s), 9.26 (s) and 9.65 (t) ppm. The triplet corresponds to the NH which is adjacent to the CH_2 fragment and two singlets correspond to non-equivalent protons of the NH_2 . Since the singlet resonances at 9.24 and 9.26 coalesced at 300 K it can be concluded that in the d_6 -DMSO solution (**137**) has a

restricted rotation around C(9)-N(9) moiety, which is slow relative to the NMR time scale at room temperature. This is in agreement with delocalization of the double bond along the N(8)-C(9)-N(9) moiety found in the solid state. In order to determine the stereochemistry of the N-C-N fragment, (**137**) was also studied by 1D gs-NOESY (Scheme 3.9). Irradiation of the NCH₂ signal at 3.47 ppm resulted in NOE effects for NH resonances at 9.24 (s) and 9.65 (t) ppm. Observing NOE enhancement only for one of the two NH₂ resonances corresponded with restricted rotation around C(9)-N(9) fragment and also indicated that (**137**) existed in the d₆-DMSO solution as the *E*-isomer, as it also did in the solid state.

Scheme 3.9



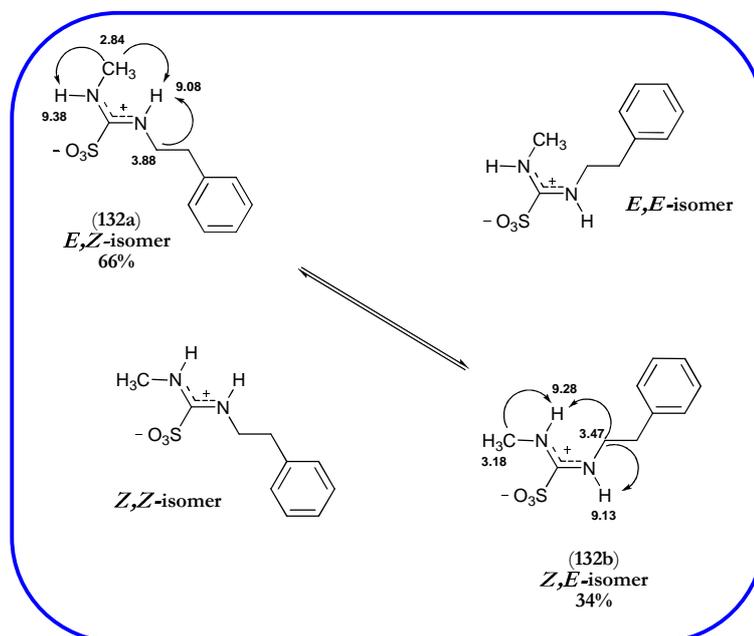
Although (**132**) gave a single peak with the correct mass (209.1112 M⁺. C₁₁H₁₇N₂S requires 209.1115) in the HR-ESI-MS spectrum, ¹H and ¹³C NMR spectra showed two sets of resonances in a 66:34 ratio. Moreover, ¹H-¹H COSY and ¹H-¹³C HMBC implied the same connectivity for both sets of resonances it is likely that there were two geometric isomers of (**132**) in d₆-DMSO solution. When the sample was heated to 350 K, the ¹H NMR spectra showed that the ratio changed from 66:34 to 61:39. Increasing the temperature also caused broadening of peaks, but coalescence was not achieved. The 1D gs-NOESY spectrum, recorded at 350 K, indicated significant saturation transfer (EXSY peak) upon selective irradiation of the NCH₂ resonance. The intensity of the EXSY peak decreased with temperature and became negligible at room temperature. Therefore it can be concluded that (**132a**) and (**132b**) are geometric isomers existing in the d₆-DMSO solution in a dynamic equilibrium with an energy barrier such that the interconversion is slow relative to the NMR timescale.

Due to the delocalization of double bond, four stereoisomers can be formed around the N-C-N system as illustrated in Scheme 3.10.

Therefore 1D gs-NOESY was employed to determine the stereochemistry of **(132a)** and **(132b)**. NOE was only observed at the neighbouring NH signal at 9.08 ppm for the NCH₂ signal of the major isomer **(132a)**, $\delta=3.88$ ppm). However, irradiation of the CH₃ resonance ($\delta=2.84$ ppm) resulted in NOE enhancement for both NH signals ($\delta=9.08$ and 9.38 ppm) which proves that **(132a)** exists as the *E,Z*-isomer. In the case of the minor isomer **(132b)**, the methyl resonance ($\delta=3.18$ ppm) showed NOE only to the adjacent NH. However, when selective irradiation was applied to the NCH₂ ($\delta=3.47$ ppm) signal, NOE enhancements were observed for both NH resonances ($\delta=9.13$ and 9.28 ppm), which identifies the *Z,E*-isomer.

The aminoiminomethanesulfonic acids were also investigated by ¹H-¹⁵N HMBC (Figure 3.7 and Figure 3.8). Since the spectra were recorded without using the low-pass filter, both long-range and single-bond correlations can be observed. Single-bond correlations yield strong doublets which easily allow ¹⁵N resonances to be assigned.

Scheme 3.10



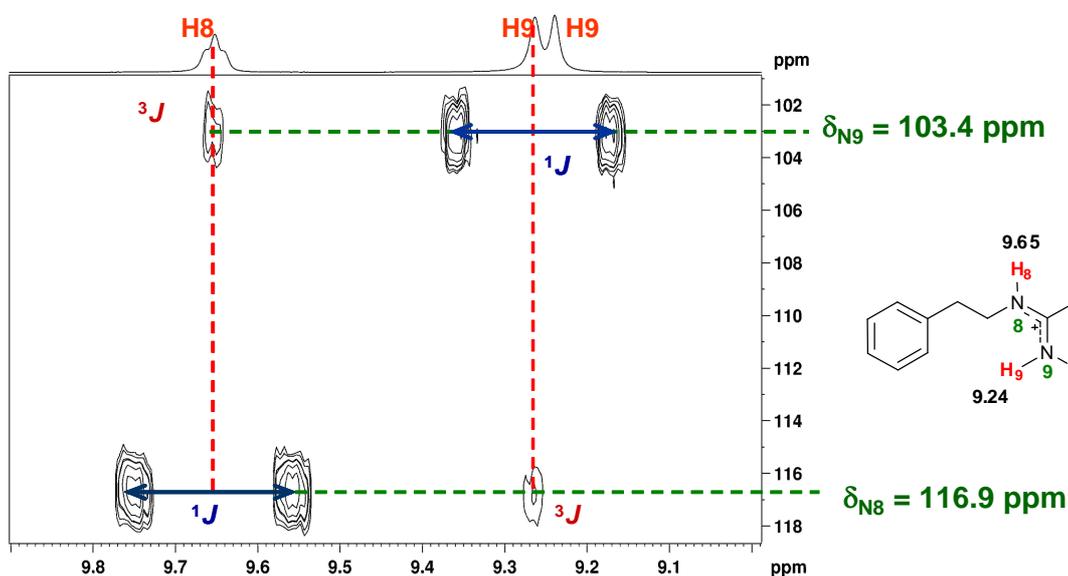


Figure 3.7 The expanded 2D ^1H - ^{15}N HMBC spectrum of (**137**) in d_6 -DMSO

The values the ^{15}N chemical shifts for (**137**) and (**132**) were found to be in a narrow range (100-120 ppm) which is consistent with the partial double bond character of the C-N bonds.²³ The weaker crosspeaks appear due to the long-range 3J correlation. While 1J crosspeaks enable easily derivation of 1J coupling constants, long-range coupling constants are significantly smaller and remain unresolved. However, the heteronuclear couplings < 10 Hz generally show a more or less linear response if the delay Δ for evolution of heteronuclear couplings is kept relatively short (40-50 ms). Therefore, the intensity of long-range crosspeaks can be used for rough estimation of small vs. large heteronuclear coupling constants.²⁴ As seen in Figure 3.7, proton H(9) ($\delta=9.24$ ppm) did not show a 3J crosspeak with nitrogen N(8) whilst protons H(9) ($\delta=9.26$ ppm) and H(8) have long-range crosspeaks with N(8) and N(9), respectively. Considering that (**137**) is the *E*-isomer, as was established by 1D-gs NOESY and X-ray it is obvious that the 3J correlation is observed only if proton and nitrogen are in the mutual trans-position and when the corresponding coupling constant is large.

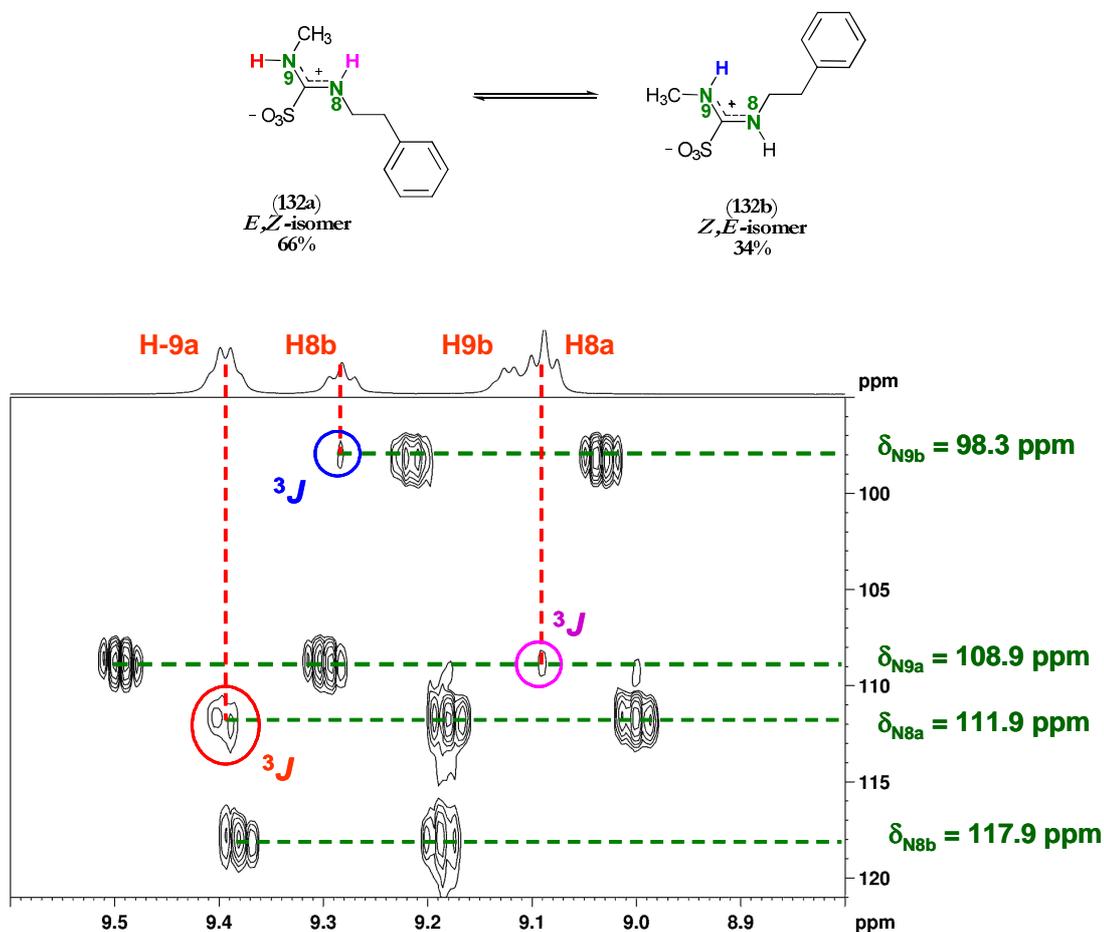


Figure 3.8 The expanded 2D ^1H - ^{15}N HMBC spectrum of (132) in d_6 -DMSO

The same procedure can be used to confirm the stereochemistry of the (132) isomers. As seen in Figure 3.8, in the case of major isomer (132a) two long-range crosspeaks are observed. The crosspeak of proton H(9a) with N(8a) is stronger than the crosspeak of proton H(8a) with N(9a). This implies that former pair of atoms is in the *trans*-arrangement while the latter one is in the *cis*-arrangement. Minor isomer (132b) shows only one long-range crosspeak for proton H(8b) and N(9b) showing that these atoms are in a mutual *trans*-orientation.

N-Hydroxyguanidines

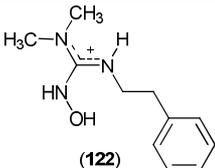
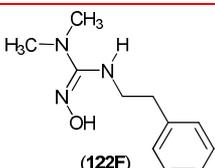
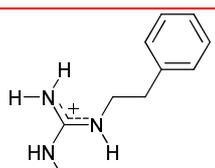
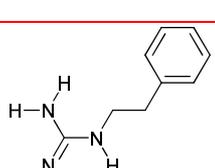
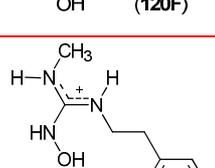
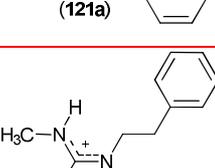
The *N*-hydroxyguanidines and their hydrochlorides were also studied by ^1H NMR, 1D gs-NOESY, ^1H - ^1H COSY, ^1H - ^{13}C HMBC and ^1H - ^{15}N HMBC. The values of the ^1H and ^{15}N chemical shifts are summarized in Table 3.4.

In the case of (**120**), there are characteristic broad signals in the low field region of the ^1H NMR spectrum, which indicate the presence of $-\text{OH}$, $-\text{NH}$ and $-\text{NH}_2$ groups. The singlet at 7.75 ppm with integral intensity of two protons can be assigned to NH_2 . The triplet at 8.00 ppm (1H) was identified as NH. In addition two further singlets were observed at 9.84 and 10.53 ppm. Similarly for (**122**), the triplet corresponding to N(8)H proton was seen at 7.84 ppm as well as two singlets were observed at 10.19 and 10.69 in the downfield region of the ^1H NMR spectrum. Since the singlets in the downfield region integrated for 1H each, they can be clearly assigned to OH and N(10)H protons. Neither the ^1H NMR nor 2D ^1H - ^{13}C nor ^1H - ^{15}N HBMC spectroscopy allows their discrimination. Unlike the analogous aminoiminomethanesulfonic acids, the NH_2 protons and the *N*-methyl groups of (**120**) and (**122**), respectively, are equivalent which implies free rotation around the C-N bond.

The values of the ^{15}N chemical shifts for N(8) and N(9) of (**120**) and (**122**), obtained indirectly by ^1H - ^{15}N HBMC spectroscopy (see Table 3.4), did not differ significantly which indicated the equal positive charge distribution along the N(8)-C(9)-N(9) fragment. For (**122**), N(9) is shifted upfield compared to (**120**) which can be ascribed to the presence of CH_3 groups.

Comparing the values of the ^{15}N chemical shifts with the analogous sulfonic acids reveals that chemical shifts of both N(8) and N(9) are slightly lower which is in agreement with more single bond character of the C(9)-N(8) and C(9)-N(9) bonds as already shown by X-ray crystallography.

Table 3.4 The values of ^1H and ^{15}N chemical shifts for *N*-hydroxyguanidines

Structure	δ chemical shifts [ppm]*								
	^{15}N NMR data			^1H NMR data					
	<i>N</i> (9)	<i>N</i> (8)	<i>N</i> (10)	<i>N</i> (8)H	<i>NCH</i> ₂	<i>NCH</i> ₃	<i>N</i> (9)H	<i>NH</i> / <i>OH</i>	
 (122)	64.1	86.6	139.8	7.84	3.47	2.82	-	10.19	10.69
 (122F)	39.0	62.5	264.3	4.98	3.14	2.25	-	8.67	-
 (120)	73.7	81.3	136.1	8.00	3.40	-	7.75	9.84	10.53
 (120F)	-	59.3	-	4.52	3.09	-	4.77	7.77	-
 (121a)	64.1	77.1	135						
 (121b)	71.9	83.0	135	7.84	3.40	2.71	7.98	9.91	10.59

*all samples run in the d_6 -DMSO solutions at room temperature; ^{15}N values of chemical shifts for (121) were recorded at 203K in d_3 -methanol.

Deprotonation of the nitrogen N(10) of (**122**) resulted in upfield shifts for N(8)H, NCH₂, NMe and OH resonances in the ¹H NMR spectrum. The significant ($\Delta\delta=124.5$ ppm) downfield shift of $\delta_{N(10)}$ was also seen in the ¹H-¹⁵N HMBC spectrum. In addition, considerable upfield shifts were observed for resonances of nitrogens N(8) and N(9). This implied that the double bond became essentially localized between the C(9) and N(10) fragment. Assuming free rotation about the N(8)-C(9) axis on the grounds of reduced double bond character, and given the enhanced C(9)-N(10) double bond character, (**122F**) might exist as two geometrical isomers, in principle, but only one set of signals was observed in the ¹H NMR spectrum at room temperature and at 203 K in d₄-methanol, which suggests that only one isomer exists in the solution. However, NOESY experiments did not allow determination of the stereochemistry of (**122F**), due to the exchange of weakly bonded hydrogens.

The 2D ¹H-¹⁵N HBMC experiment for free *N*-phenylethyl-*N'*-hydroxyguanidine (**120F**) did not provide the values of chemical shifts for all nitrogens. There was only one crosspeak present which corresponded to the nitrogen N(8). However, the upfield shift by 22 ppm with respect to the corresponding hydrochloride (**120**) implied that the N(10) nitrogen was deprotonated and the double bond was then localized between the C(9)-N(10) fragment, analogously to (**120F**). In addition, as seen in Table 3.4, there are significant upfield shifts for the values of δ_{N8H} , δ_{N8CH} and δ_{N9R} in the ¹H NMR spectrum.

Since the ¹H NMR spectrum of (**120F**) in d₆-DMSO at room temperature shows a broad signal for the CH₂ group ($\delta=3.09$ ppm), VT studies were performed to explain this phenomenon. As illustrated in Figure 3.9 the resonance corresponding to the CH₂ group gets narrower at higher temperatures. This can indicate the presence of a dynamic equilibrium between two possible stereoisomers which is likely the reason for missing crosspeaks in the ¹H-¹⁵N HMBC NMR spectrum.

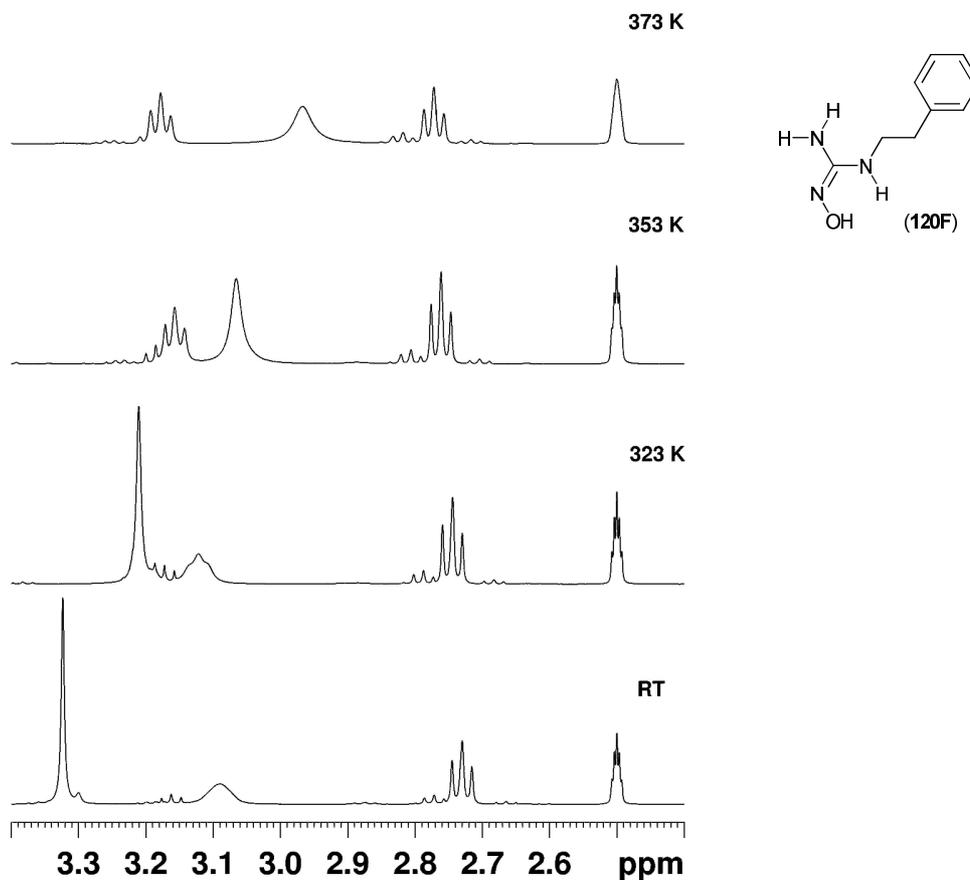


Figure 3.9 The expanded ^1H NMR VT spectrum of (120F) in d_6 -DMSO

Previous experiments indicated that (120) and (122) have a partial bond character along the N(8)-C(9)-N(9) fragment. However, this is in contradiction to the free rotation of the C-N bonds deduced from N(9)H and N(9)Me resonances (see above). In order to confirm delocalization of the double bond VT (variable temperature) NMR studies were performed in d_4 -methanol for *N,N*-dimethyl-*N'*-phenethyl-*N''*-hydroxyguanidine (122). As presented in Figure 3.10, the VT NMR spectra illustrate decoalescence of the N(9)Me resonance at about 209 K, which implies restricted rotation around either the N(8)-C(9) or N(9)-C(9) bond.

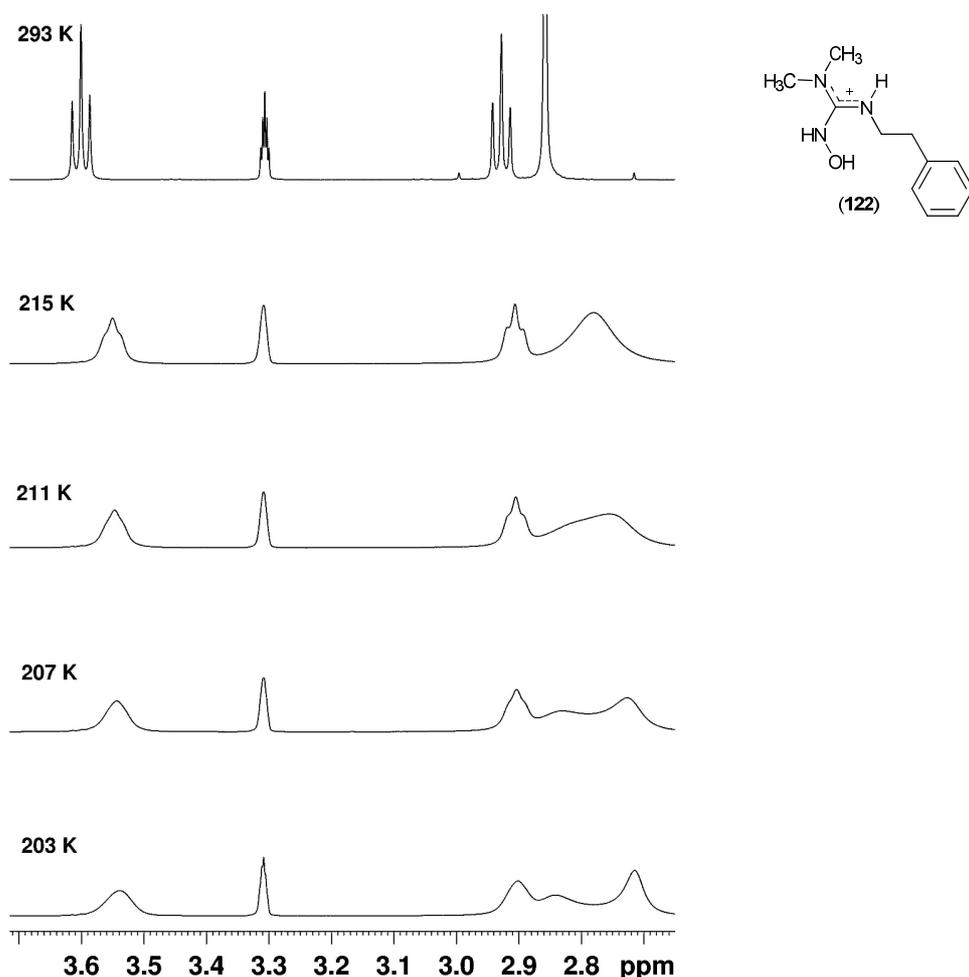


Figure 3.10 The expanded ^1H NMR VT spectrum of (122) in d_4 -methanol

VT studies were also performed for (120) in d_3 -methanol, which allowed observation of “weakly bonded” hydrogens. However, at 183 K the downfield region of the ^1H NMR spectrum becomes very complex which likely indicates both the presence of geometric isomers at lower temperature and the presence of non-equivalent NH_2 protons (Figure 3.11). So, these experiments stay in agreement with the previously stated assumption that the double bond is delocalized in the N(8)-C(9)-N(9) moiety, but the barrier for the rotation is much lower than that for the analogous aminoiminomethanesulfonic acids.

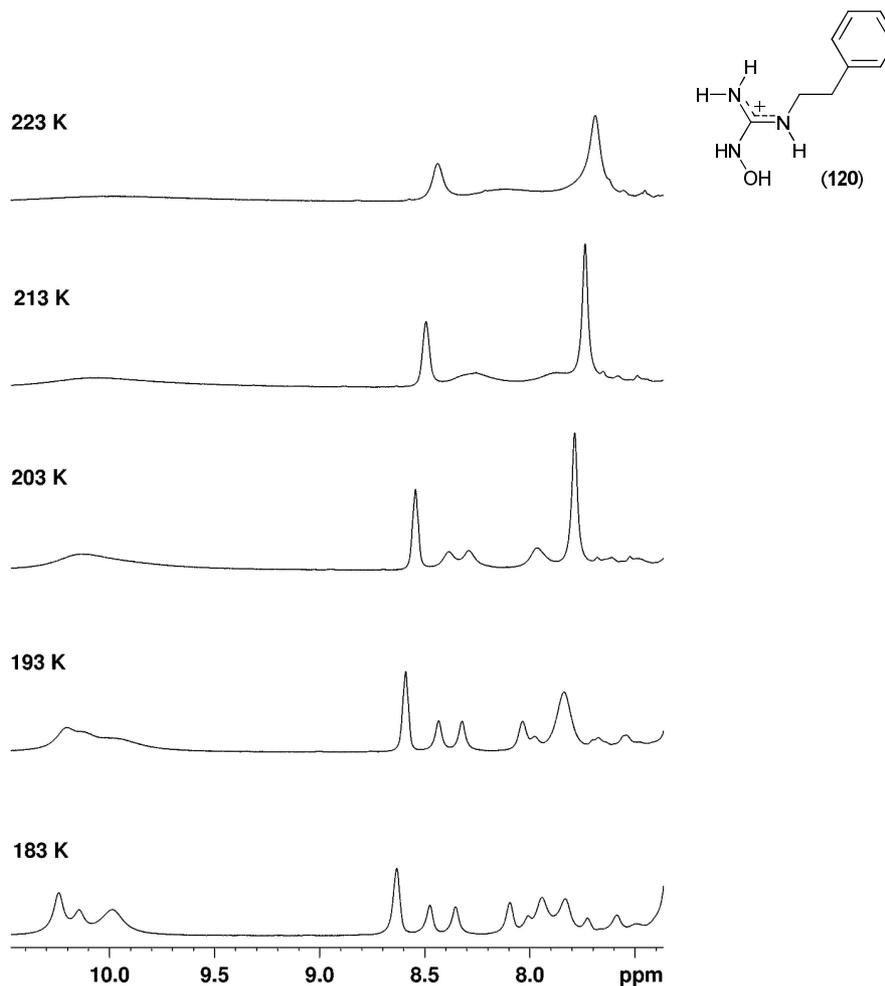


Figure 3.11 The expanded ^1H NMR VT spectrum of (120) in d_3 -methanol

The ^1H NMR spectrum of *N*-methyl-*N'*-phenethyl-*N''*-hydroxyguanidine (121) in d_6 -DMSO at room temperature showed broad resonances for the NCH_2 group ($\delta=3.40$ ppm) and NMe ($\delta=2.71$ ppm), which get narrower at higher temperatures as illustrated in Figure 3.12. This suggests a dynamic behavior of the molecule in solution.

The low temperature ^1H NMR study of (121) in d_3 -methanol confirmed this assumption as the two sets of resonances in a 1:1 ratio became apparent as shown in Figure 3.13.

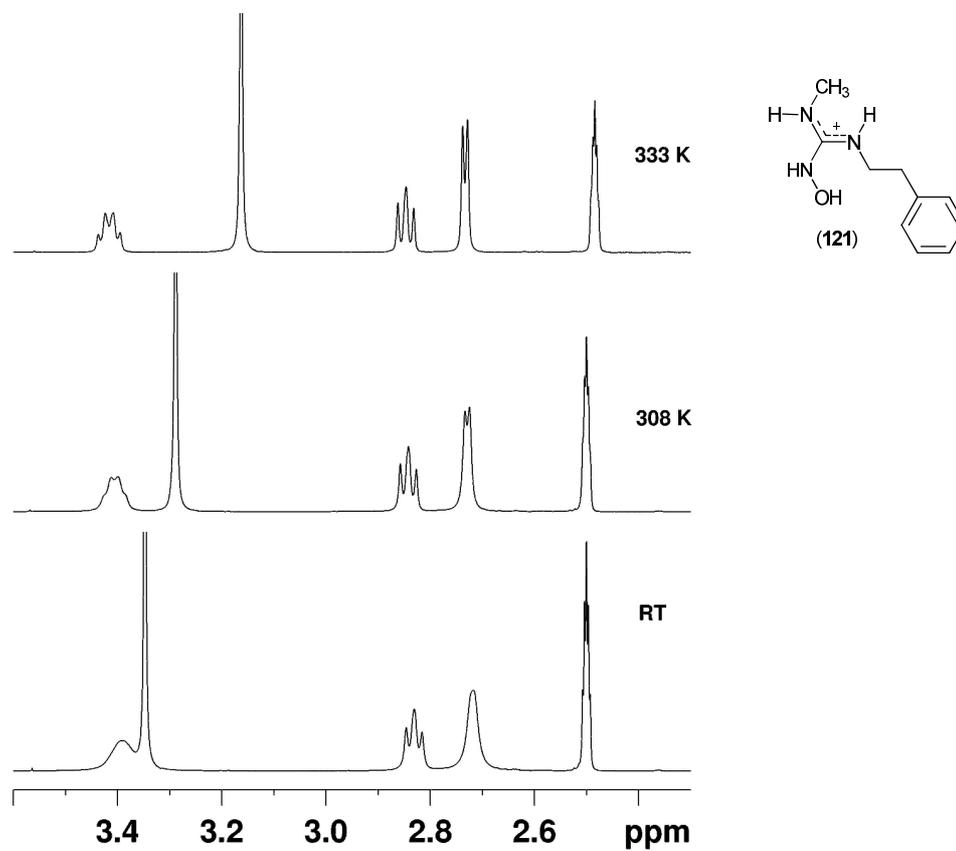


Figure 3.12 The expanded ¹H NMR VT spectrum of (121) in d₆-DMSO

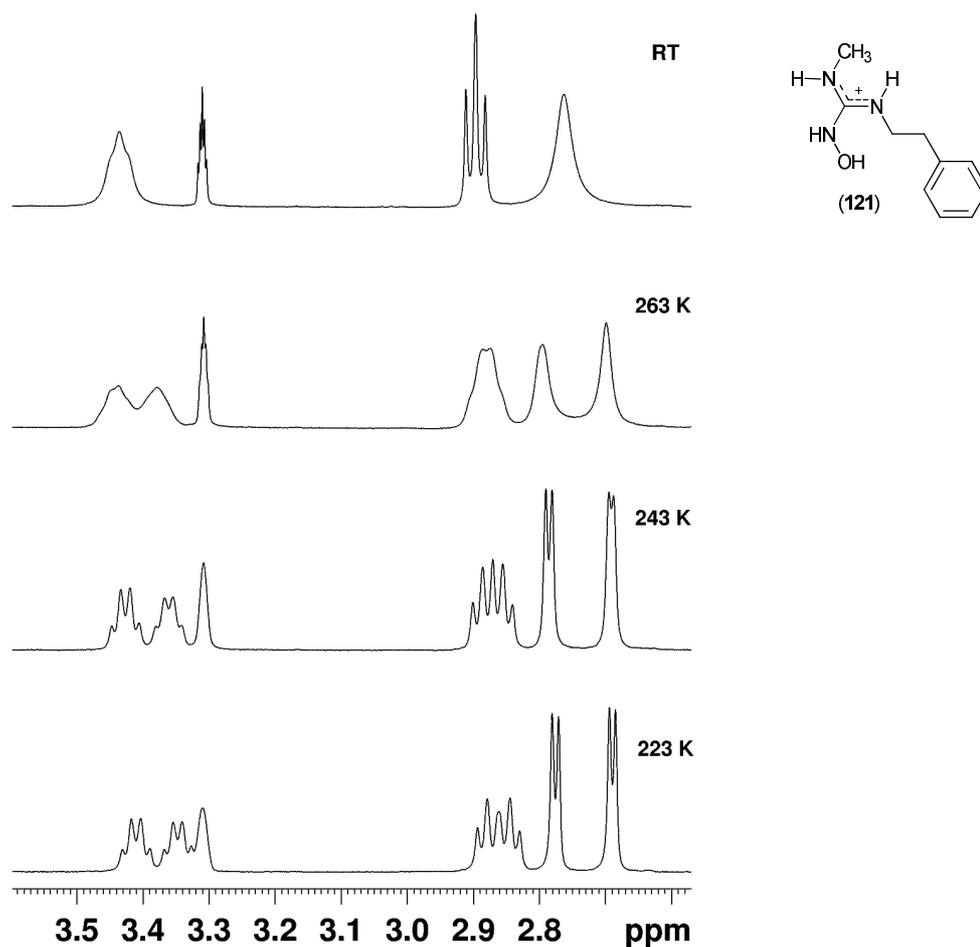
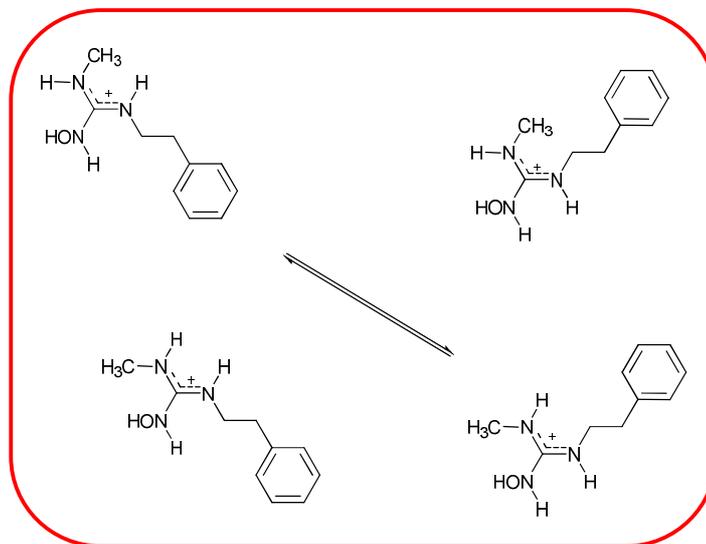


Figure 3.13 The expanded ¹H NMR VT spectrum of (121) in d₃-methanol

It was already proven that *N*-hydroxyguanidine hydrochlorides possessed the double bond delocalized along the N(8)-C(9)-N(9) fragment whilst the C(9)-N(10) bond had a single bond character. Therefore, it is likely that *N*'-methyl-*N*-phenethyl-*N*'-hydroxyguanidine (121) exists in the solution as two of four possible isomers as shown in Scheme 3.11.

Scheme 3.11



Due to the fast exchange, even at 233 K, it was impossible to identify the stereochemistry of these compounds by NOESY experiments. As d_3 -methanol was used for the low temperature ^1H NMR studies, the weakly bonded protons were observed at 233K. The resonances became sharp and the coupling was resolved revealing that multiplets at around 7.6 ppm and 8.1 ppm were overlapped triplet and quartet of N(8)H and N(9)H as illustrated in Figure 3.14.

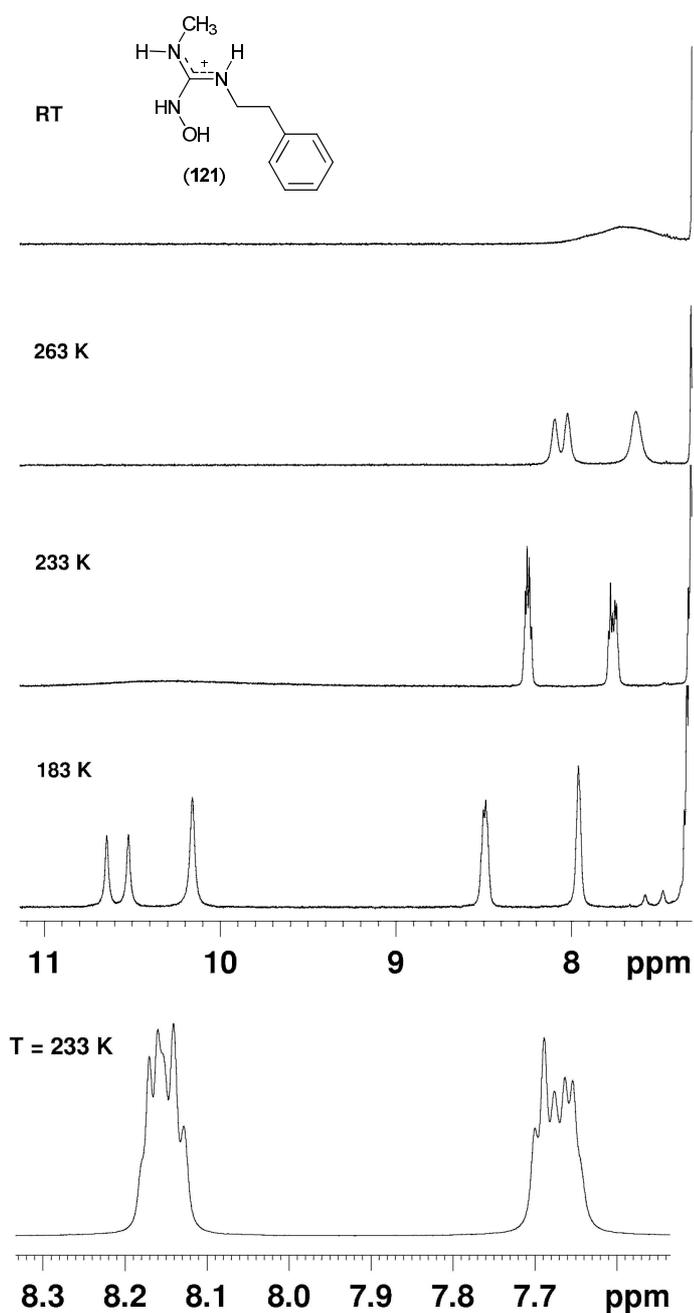


Figure 3.14 The expanded ^1H NMR VT spectrum of (121) in d_3 -methanol (weakly bonded hydrogens)

Both multiplets also showed crosspeaks with the NCH_3 and NCH_2 resonances in ^1H - ^1H COSY spectrum which confirms previous assignment. Furthermore, the resonances corresponding to $\text{N}(10)\text{H}$ and OH also became sharp and decoalescence was observed at 183K.

The 2D ^1H - ^{15}N HMBC spectrum of *N*'-methyl-*N*-phenethyl-*N*'-hydroxyguanidine (**121**) recorded at 240K (d_3 -methanol) showed crosspeaks for four ^{15}N resonances in the aliphatic region of the spectrum as presented in Figure 3.15.

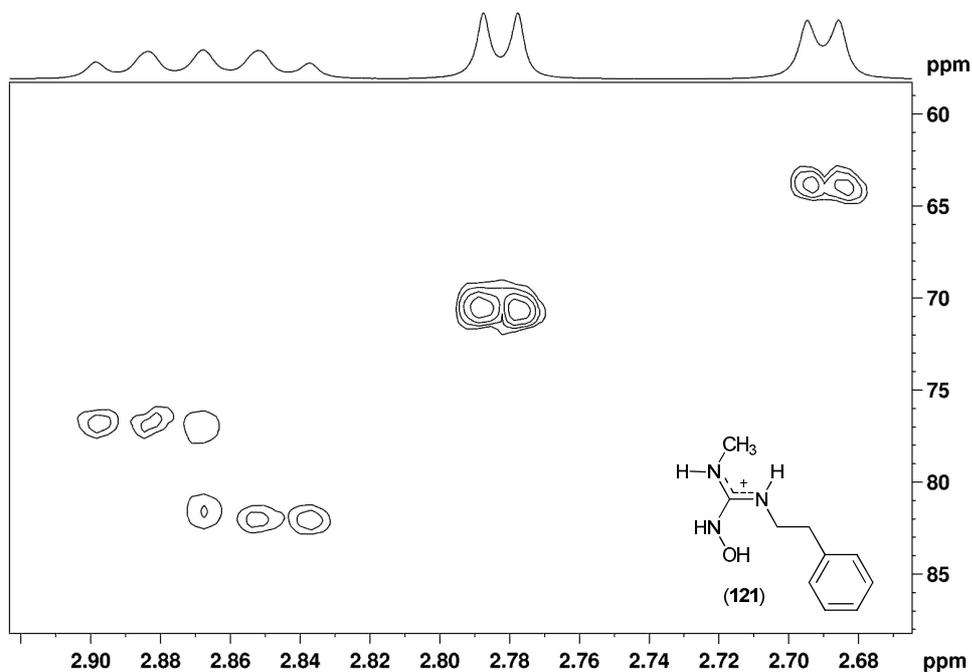


Figure 3.15 The expanded 2D ^1H - ^{15}N HMBC spectrum of (**121**) in d_3 -methanol at 240K

The two upfield shifted signals ($\delta=64$ and 72 ppm) possessed crosspeaks with methyl resonances which allowed to assign them as N(9). On the other hand, the downfield crosspeaks ($\delta=77$ and 82 ppm) can be determined as N(8) as they correlate to CH_2 groups.

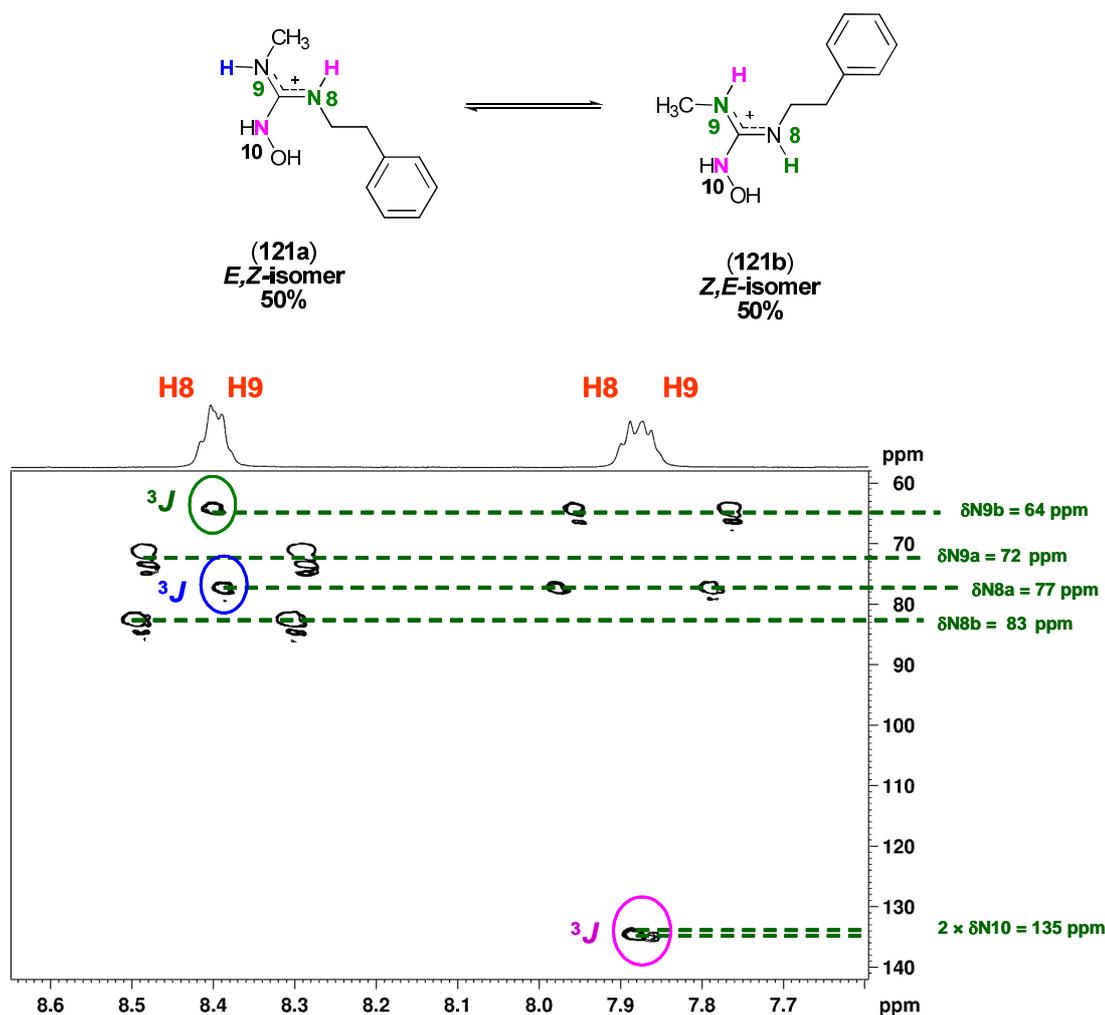


Figure 3.16 The expanded 2D ^1H - ^{15}N HMBC spectrum of (121) in d_3 -methanol at 203K

In order to see crosspeaks of weakly bonded hydrogens, the ^1H - ^{15}N HMBC spectrum was recorded one more time at 203K. This experiment revealed the two additional ^{15}N resonances at 135 ppm, which could be assigned to the nitrogen N(10) (Figure 3.16).

The ^{15}N resonances of N(9) at 64 ppm and N(8) at 77 ppm show 1J crosspeaks with ^1H signals overlapping at around 7.9 ppm and 3J crosspeaks with ^1H signals overlapping at around 8.4 ppm. Therefore these nitrogens must come from two different molecules. Consequently, the same applied to N(9) at 70 ppm and N(8) at 83 ppm, which show only 1J crosspeaks with ^1H signals overlapping at around 8.4 ppm. Both 1J and 3J crosspeaks of N(9) at 72 ppm and N(8) at 77 ppm are slightly shifted upfield in the F2 dimension, which indicated that they both belong to the same proton (H9). On the other hand, N(9) at 64 ppm and N(8) at 83 ppm correlate with

H8 shifted downfield in the F2 dimension. This enables assignment of the former ^1H and ^{15}N resonances to isomer (**121a**) and latter ones to isomer (**121b**).

In the case of isomer (**121a**), proton H(8) ($\delta=7.89$ ppm) has a 3J crosspeak with nitrogen N(10) ($\delta=135$ ppm) whilst proton H(9) ($\delta=8.39$ ppm) has long-range crosspeaks with N(8) ($\delta=77$ ppm). Considering that the 3J correlation is observed only if proton and nitrogen are in the mutual trans-position, as was established for aminoiminomethanesulfonic acids, it became evident that the isomer (**121a**) possesses *E,Z*-geometry Analogously, 3J crosspeak of H(8) ($\delta=8.40$ ppm) with N(9) ($\delta=64$ ppm) and H(9) ($\delta=7.86$ ppm) with N(10) ($\delta=135$ ppm) indicate that (**121b**) is *Z,E*-isomer.

3.3 Conclusions

The X-ray crystallography and NMR spectroscopy studies have shown that aminoiminomethanesulfonic acids and hydroxyguanidine hydrochlorides have analogous electronic structures. Both classes of compounds have the C-N double bond delocalised along the N(8)-C(9)-N(9) fragment with the positive charge equally distributed between the two nitrogens. In the case of aminoiminomethanesulfonic acids, the C-N bonds have considerable double bond character and therefore **137** exists as a single *E*-isomer in both the solid state and d_6 -DMSO solution. However, for the *N*-methyl analogue (**132**) only the *E,Z*-geometry for the N-C-N fragment was found in the solid state and there is a dynamic equilibrium between the *E,Z*-isomer (**132a**) and the *Z,E* isomer (**132b**) in d_6 -DMSO solution. Since the barrier for rotation of the N-C-N fragment is rather high, the exchange is slow compared to the NMR timescale at room temperature. In the solid state, hydroxyguanidine hydrochlorides (**120**) and (**122**) also exist as single *E*- and *Z*-isomers, respectively. As the C-N(8) and C-N(9) bonds have more single bond character, the N(8)-C-N(9) fragment freely rotates in solution. However, rotamers become apparent at low temperatures which implies that the rotation around C-N(8) and C-N(9) bonds is still slightly restricted due to double bond delocalisation. In the case of the *N*-methyl derivative (**121**), two rotamers, **121a** and **121b**, were found in d_3 -methanol at temperatures below 240 K, which were identified by ^1H - ^{15}N HMBC as the *E,Z*-isomer and *Z,E*-isomer, respectively. Observing only two of four possible isomers for both *N*-methyl derivatives (**121**) and (**132**) underlines the similarity of the electronic structures of aminoiminomethanesulfonic acids and *N*-hydroxyguanidine hydrochlorides and suggests that concerted flipping of C-N(8) and C-N(9) is a preferable pathway over stepwise flipping which would also yield *E,E*- and *Z,Z*-isomers. However, further experiments and computational studies are required to prove whether it is a kinetic or just thermodynamic effect.

3.4 References

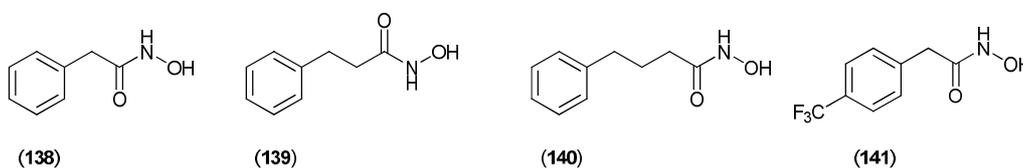
- 1 P. G. Wang, Cai, T.B., Taniguchi N., 'Nitric Oxide Donors - For Pharmaceutical and Biological Applications', Wiley-VCH Verlag GmbH & Co. KGaA, 2005.
- 2 T. B. Cai, D. Lu, and P. G. Wang, *Curr. Top. Med. Chem.*, 2005, **5**, 721.
- 3 S. D. Ziman, *J. Org. Chem.*, 1976, **41**, 3253.
- 4 R. Singh and S. K. Dikshit, *Polyhedron*, 1995, **14**, 1799.
- 5 A. E. Miller and J. J. Bischoff, *Synthesis*, 1986, 777.
- 6 A. E. Miller, J. J. Bischoff, and K. Pae, *Chem. Res. Toxicol.*, 1988, **1**, 169.
- 7 W. Kirmse, *Eur. J. Org. Chem.*, 2005, 237.
- 8 A. R. Katritzky and B. V. Rogovoy, *ARKIVOC* 2005, 49.
- 9 N. M. Olken and M. A. Marletta, *J. Med. Chem.*, 1992, **35**, 1137.
- 10 H. Ulrich and A. A. R. Sayigh, *J. Org. Chem.*, 1965, **30**, 2779.
- 11 S. Herres-Pawlis, A. Neuba, O. Seewald, T. Seshadri, H. Egold, U. Floerke, and G. Henkel, *Eur. J. Org. Chem.*, 2005, 4879.
- 12 T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, 1963, **6**, 669.
- 13 H. Ulrich and A. A. Sayigh, *Angew. Chem. Int. Ed. Engl.*, 1966, **5**, 704.
- 14 H. Ulrich, J. N. Tilley, and A. A. R. Sayigh, *J. Org. Chem.*, 1964, **29**, 2401.
- 15 J. J. Havel and R. Q. Kluttz, *Synth. Commun.*, 1974, **4**, 389.
- 16 W. Walter, H. Huehnerfuss, A. Neye, and K.-P. Ruess, *Liebigs Ann.*, 1973, 821.
- 17 W. Walter and K. P. Ruess, *Liebigs Ann.*, 1974, 253.
- 18 C. A. Maryanoff, R. C. Stanzione, J. N. Plampin, and J. E. Mills, *J. Org. Chem.*, 1986, **51**, 1882.
- 19 K. S. K. a. L. Qian, *Tetrahedron Lett.*, 1993, **34**, 7677.
- 20 V.-D. Le and C.-H. Wong, *J. Org. Chem.*, 2000, **65**, 2399.
- 21 S. C. Cherkofsky, in 'Mono- and disubstituted hydroxyguanidines in the treatment of depression', Application: US, 1976.
- 22 J. M. Russell, A. D. M. Parker, I. Radosavljevic-Evans, J. A. K. Howard, and J. W. Steed, *Cryst. Eng. Comm.*, 2006, **8**, 119.
- 23 R. Marek and A. Lycka, *Curr. Org. Chem.*, 2002, **6**, 35.
- 24 B. L. Marquez, W. H. Gerwick, and R. T. Williamson, *Magn. Reson. Chem.*, 2001, **39**, 499.

HYDROXAMIC ACIDS

4.1 Introduction

It has been reported that some of the physiological effects of hydroxamic acids such as their hypotensive activity may be due to their ability to release nitric oxide as described in Section 1.5.2.¹⁻⁷ It was therefore decided to examine a series of hydroxamic acids as NO donors in our assay system. Preliminary biological results indicated that the commercially available benzohydroxamic acid was capable of causing smooth muscle relaxation in rat aorta. The ODQ test implied that the relaxation may be mainly NO mediated since 70% reversibility was observed. In addition, the EC₅₀ value of 50.1 μ M was comparable to the activity of some *N*-hydroxyguanidines (see Section 2.2). Therefore, it was decided to prepare further homologues to explore their activity. Firstly, a series of homologues of benzohydroxamic acid (Scheme 4.1), with extended alkyl chains between the functional group and the benzene ring, were synthesized. In addition, a CF₃ substituted analogue was also prepared.

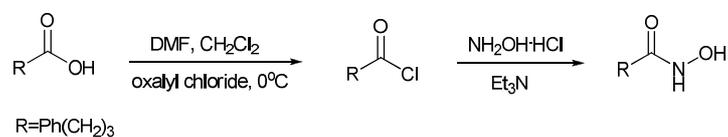
Scheme 4.1



4.1.1 Synthesis of hydroxamic acids

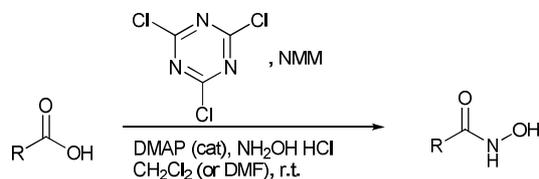
From acid chlorides

The synthesis of 4-phenylbutyrylhydroxamic acid (**140**) was carried out using the published procedure as in Scheme 4.2.⁸ Firstly, the acid chloride was prepared *via* reaction of 4-phenylbutyric acid with oxalyl chloride, in the presence of catalytic DMF. The crude acid chloride was then reacted with hydroxylamine hydrochloride in the presence of base.

Scheme 4.2⁸

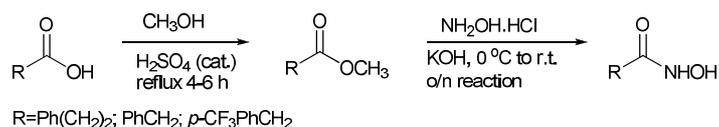
After purification by flash chromatography, the desired product (**140**) was obtained, although in a very poor 11% yield. However, literature data also indicated that there were difficulties with the isolation and purification steps.^{1,8,9} The final product was analyzed by ¹H and ¹³C NMR spectroscopy, MS and elemental analysis. The presence of single peaks corresponding to the NH proton (1H, 8.70 ppm) and the OH proton (1H, 10.37 ppm) confirmed the structural features of the hydroxamic acid. The presence of the single peak in the mass spectrum at 202.1 (M+Na)⁺, as well as results of elemental analysis, supported the correct structure and purity of the compound.

Recently, an improved one-step procedure has been published for the synthesis of various hydroxamic acids as shown in Scheme 4.3.¹⁰ This also employs formation of the acid chloride, but in this case *in situ* using cyanuric chloride, followed by its reaction with hydroxylamine hydrochloride in the presence of base. The initial goal was to use this method for the synthesis of the next three analogues. However, the published approach was employed several times with various modifications, but none of them gave the desired compounds as the major product.

Scheme 4.3¹⁰

From methyl esters

In order to overcome problems with the previous methods, a procedure which utilizes the corresponding methyl esters instead of the acid chlorides was employed as shown in Scheme 4.4.¹¹

Scheme 4.4¹¹

Methyl phenylacetate (**142**) was firstly prepared from the corresponding carboxylic acid by heating it under reflux in methanol with a catalytic amount of concentrated sulfuric acid. The methyl ester (**142**) was then reacted with a preformed slurry of hydroxylamine hydrochloride and potassium hydroxide. After work up, the crude product was obtained as a pale pink solid which was recrystallized from diethyl ether to give pure phenylacetohydroxamic acid (**138**) as pale pink crystals in a more acceptable 58% yield compared to 11% obtained from the acid chloride. The compound was characterized using ¹H and ¹³C NMR spectroscopy, MS and elemental analysis. The presence of the characteristic signals for the -NH and -OH groups in the lowfield region of the ¹H spectrum indicated the presence of the hydroxamic acid functionality. The disappearance of the peak corresponding to methyl group in the ¹H NMR spectrum confirmed the successful transformation. In addition, the single peak at 150 (M-H)⁻ in the mass spectrum as well as the results of elemental analysis confirmed the structure of the final product and its purity.

3-Phenylpropionohydroxamic acid (**139**) was obtained as described above, after recrystallization from petroleum ether. As in the case of other hydroxamic acids, the presence of two singlets at 8.72 ppm (NH) and 10.38 ppm (OH) showed the presence of the desired hydroxamic acid group. The results of elemental analysis (see experimental part) showed that the synthesized compound was nearly 100% pure and this was additionally confirmed by the presence of the single peak at 166 (M+H)⁺ in the mass spectrum. The X-ray crystal structure of the final product (**139**) is presented in Figure 4.1.

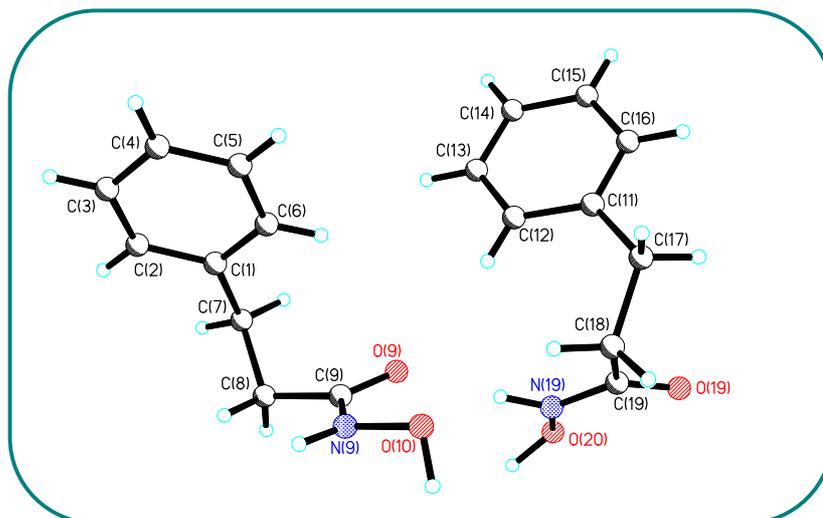


Figure 4.1 X-ray crystal structure of 3-phenylpropionohydroxamic acid (**139**)

4-(Trifluoromethyl)phenylacetohydroxamic acid (**141**) was also prepared as described above. Firstly, the methyl (4'-trifluoromethylphenyl)acetate (**143**) was synthesized by the acidic esterification of the corresponding carboxylic acid. As a known compound, only ^1H , ^{19}F and ^{13}C NMR spectroscopy were employed to confirm the structure of the product (see experimental). In the next step, the methyl ester (**143**) was treated with a suspension of hydroxylamine hydrochloride and potassium hydroxide. The final product (**141**) was obtained in 47% yield as pale pink crystals. The appearance of the characteristic signals (8.89 ppm – NH & 10.72 ppm – OH) in the low-field of the ^1H NMR spectrum confirmed the successful transformation. The purity was established by elemental analysis. The X-ray data proved the structure of the 4-(trifluoromethyl)phenylacetohydroxamic acid (**141**) as illustrated in Figure 4.2.

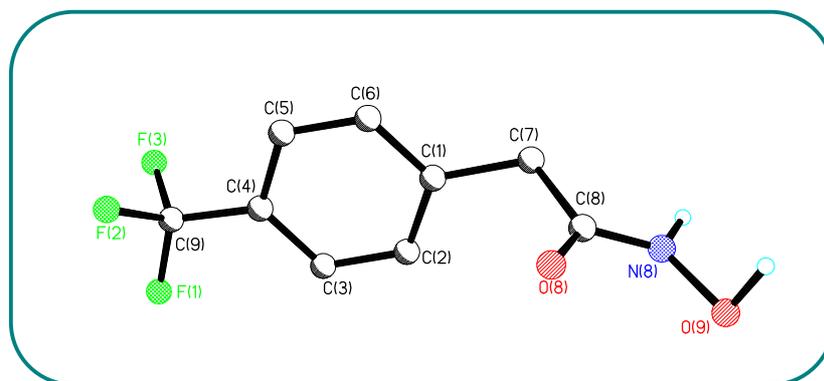


Figure 4.2 X-ray crystal structure of 4-(trifluoromethyl)phenylacetohydroxamic acid (**141**)

4.2 Biological Results

The synthesized series of hydroxamic acids was studied by the myography to investigate their NO donor activity. (Benzohydroxamic acid was commercially available). As in the case of the unsubstituted *N*-hydroxyguanidines, the hydroxamic acids were found to be good nitric oxide donors. The data are shown in Table 4.1.

Table 4.1 Biological activities of tested hydroxamic acids

Structure	Code No.	$\log EC_{50}$ [mol/dm ³]	ODQ induced reversibility[%]
 <chem>O=C(NO)c1ccccc1</chem>	BA	-4.3 ± 0.3	70.1 ± 9.8
 <chem>O=C(NO)CCOc1ccccc1</chem> (138)	AK42	-4.5 ± 0.2	96.6 ± 11.1
 <chem>O=C(NO)CCCOc1ccccc1</chem> (139)	AK46	-5.3 ± 0.1	95.8 ± 6.5
 <chem>O=C(NO)CCCCOc1ccccc1</chem> (140)	AKH4	-3.9 ± 0.1	50.0 ± 8.0

The results indicated that tested hydroxamic acids are very potent NO donors. Their EC_{50} values imply that extension of the alkyl chain caused an increase in their biological activity until the number of methylene groups reached 3. After that point, a higher dose of compound was required to achieve 50% relaxation. The ODQ test results suggested that the smooth muscle relaxation is mainly mediated *via* a NO dependent pathway. The hydroxamic acids seemed to be a promising class of novel NO donors. Their vasodilatation activity appeared to be as good as for *N*-hydroxyguanidines (Figure 4.3).

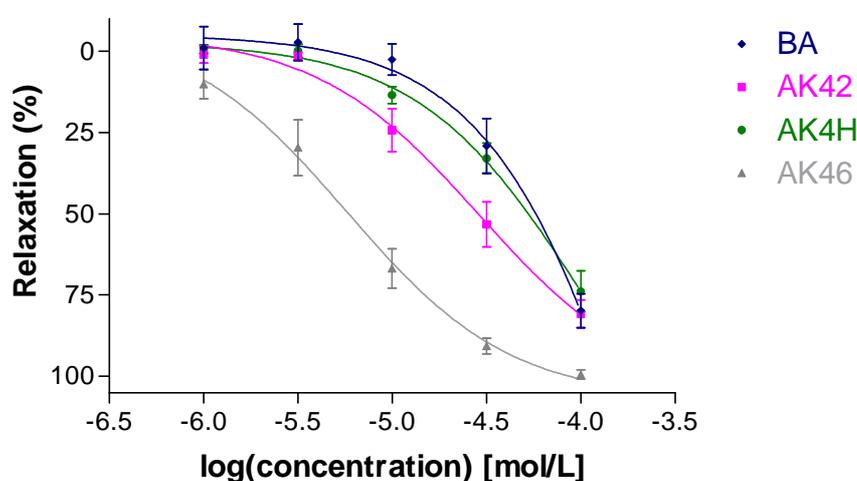


Figure 4.3 Vasorelaxation profiles of hydroxamic acids

3-Phenylpropionohydroxamic acid (**139**) possessed the best biological profile with $\log EC_{50} = -5.3$ [mol/dm³] and 96% ODQ induced reversibility. Therefore it was chosen for further investigation in the isolated perfused kidney (IPK) model and its vasodilatation activity is presented in Figure 4.4.

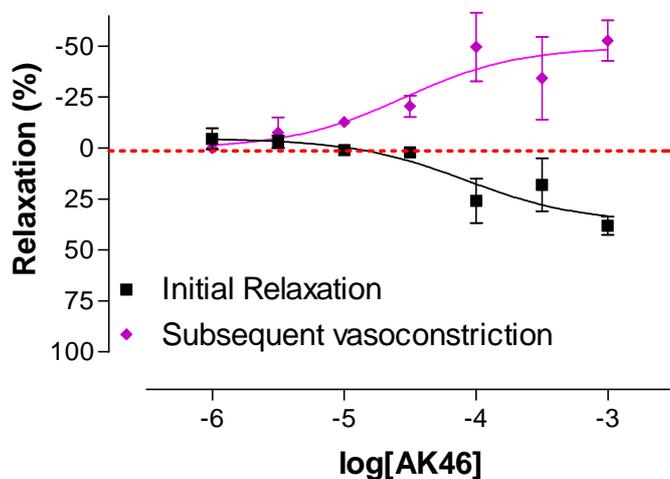


Figure 4.4 Vasorelaxation profile of AK46 (**139**) in isolated perfused kidney

The lead compound AK46 caused an initial vasodilatation of 38% at 1 mM, however, this was short lasting. The perfusion pressure through the kidney rapidly returned to the precontracted level. Additionally, a further vasoconstriction was observed at the higher concentrations of 3-phenylpropionhydroxamic acid. The reason for this unusual behaviour is unclear. However, this observation implies that AK46 must be producing further unexpected biological effects in the kidney assay.

4.3 Conclusions

Hydroxamic acids are a novel class of NO donors. Interestingly, the hydroxamic acids that were prepared possess promising pharmacological profiles. 3-Phenylpropionohydroxamic acid (AK46) revealed the most potent NO donation properties in the rat aorta assay. Data indicated that it met all of the required criteria, namely a low EC₅₀ value (6.4 μM) and almost 100% reversibility in the ODQ test.

The myography studies proved that hydroxamic acids will give smooth muscle relaxation in the rat aorta. However, their behavior in the IPK indicated that the whole kidney was more complex system and that hydroxamic acids did not undergo the same biological pathway as in the rat aorta. Further mechanistic studies should be performed in order to investigate this behavior in more detail.

4.4 References

- 1 J. B. Summers, H. Mazdiyasi, J. H. Holms, J. D. Ratajczyk, R. D. Dyer, and G. W. Carter, *J. Med. Chem.*, 1987, **30**, 574.
- 2 M. J. Miller, *Chem. Rev.*, 1989, **89**, 1563.
- 3 C. J. Marmion, T. Murphy, K. B. Nolan, and J. R. Docherty, *Chem. Comm.*, 2000, 1153.
- 4 L. N. Koikov, N. V. Alekseeva, E. A. Lisitza, E. S. Krichevsky, N. B. Grigoriev, A. V. Danilov, I. S. Severina, N. V. Pyatakova, and V. G. Granik, *Mendeleev Commun.*, 1998, 165.
- 5 C. J. Marmion, D. Griffith, and K. B. Nolan, *Eur. J. Inorg. Chem.*, 2004, 3003.
- 6 P. G. Wang, Cai, T.B., Taniguchi N., 'Nitric Oxide Donors - For Pharmaceutical and Biological Applications', Willey-VCH Verlag GmbH & Co. KGaA, 2005.
- 7 D. Griffith, K. Krot, J. Comiskey, K. B. Nolan, and C. J. Marmion, *J. Chem. Soc., Dalton Trans.*, 2008, 137.
- 8 M. A. Stolberg, W. A. Mosher, and T. Wagner-Jauregg, *J. Am. Chem. Soc.*, 1957, **79**, 2615.
- 9 J. B. Summers, B. P. Gunn, H. Mazdiyasi, A. M. Goetze, P. R. Young, J. B. Bouska, R. D. Dyer, D. W. Brooks, and G. W. Carter, *J. Med. Chem.*, 1987, **30**, 2121.
- 10 G. Giacomelli, A. Porcheddu, and M. Salaris, *Org. Lett.*, 2003, **5**, 2715.
- 11 I. T. Lim, S. O. Merouch, M. Lee, M. J. Heeg, and S. Mobashery, *J. Am. Chem. Soc.*, 2004, **126**, 10271.

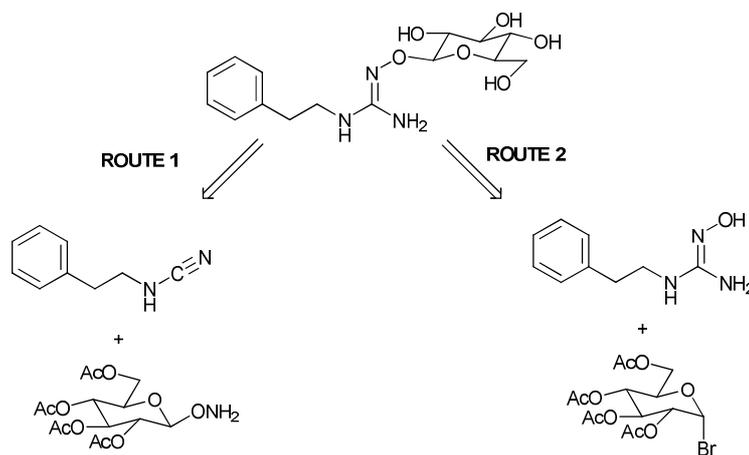
***NO-DONOR PRO-DRUGS
ACTIVATED BY GLYCOSIDASES***

5.1 Introduction

As described in Chapter 1, nitric oxide has beneficial physiological effects as well as harmful pathological effects. Due to its instability it is difficult to deliver NO directly to cells. In addition, global distribution of NO is not beneficial; therefore, it is essential to develop site selective drugs. There have been several approaches to achieve this goal, as discussed in the introduction. One of them employs a pro-drug strategy.^{1,2} In our case, the idea was based on coupling the active agent (*N*-hydroxyguanidine) with a carrier (enzyme recognized structure). The activation of the pro-drug would occur by hydrolysis (esters, amides, imines), oxidation or reduction.

NO has been found to be an anti-tumoral agent, but its mechanism of action in cancer cells is still not completely clear.³⁻⁵ In addition, glycosidases have been demonstrated to be elevated in the interstitial fluid of tumors, sera of animals and patients with tumors.⁶ Therefore, the aim was to couple the hydroxyguanidine moiety with a glucose molecule to produce the site selective pro-drug. Two possible retro synthetic approaches are shown in Scheme 5.1. The first route involves reaction of a cyanamide with a protected *O*-glycosyl hydroxylamine. In the second route a glycosyl donor (e.g. acetobromoglucose) is reacted with preformed *N*-hydroxyguanidine.

Scheme 5.1

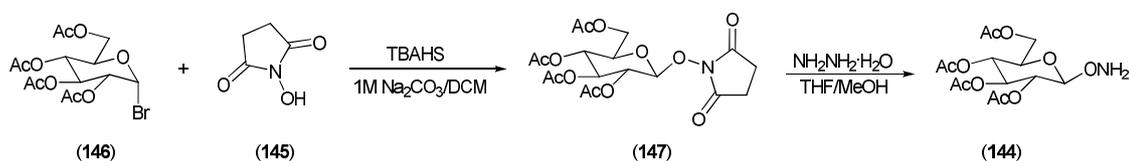


5.2 Synthesis

5.2.1 Route One

The first route required the synthesis of *O*-(tetra-*O*-acetyl-*D*-glucopyranosyl)hydroxylamine (**144**). There are few previously published procedures which describe the synthesis of this compound.⁷⁻¹³ The first approach employed the reaction of *N*-hydroxysuccinimide (**145**) with acetobromoglucose (**146**) and deprotection with hydrazine hydrate, as shown in Scheme 5.2.^{8,9}

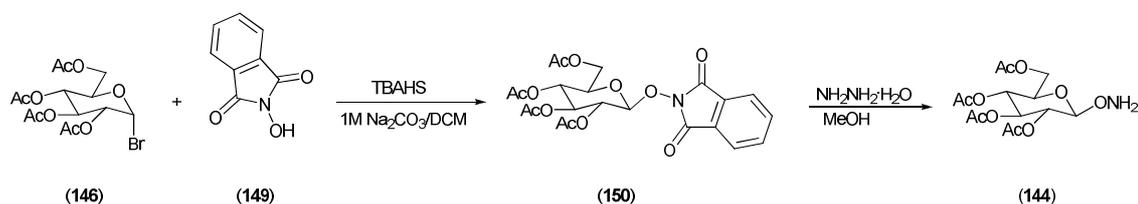
Scheme 5.2



However, this synthesis was difficult to repeat. The first step worked well and *O*-(tetra-*O*-acetyl- β -*D*-glucopyranose)-*N*-hydroxysuccinimide (**147**) was obtained in good yield (52%). The product (**147**) was analyzed using various analytical techniques such as ¹H and ¹³C NMR spectroscopy and mass spectrometry. The purity was confirmed by melting point which was exactly the same as literature value 182-184 °C. Unfortunately the deprotection with hydrazine hydrate proved very difficult. Since the reaction was performed under reflux conditions for about 10 minutes, it was very challenging to control the process. The problem was that hydrolysis of the acetate groups was observed to take place if the reaction was left too long. As a result a mixture of various glucose derivatives with different numbers of acetate groups was obtained which was impossible to separate. However, it was possible to completely deprotect *O*-(2, 3, 4, 6-tetra-*O*-acetyl- β -*D*-glucopyranosyl)-*N*-hydroxysuccinimide (**150**) using methodology which employed ammonium hydroxide at 75°C reported by Wiessler *et al.* to give *O*- β -*D*-glucopyranosylhydroxylamine (**148**).⁹ However, the final product was very difficult to work with due its high polarity.

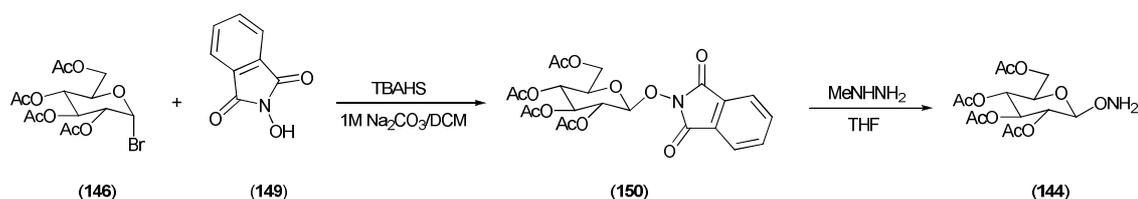
In order to synthesize *O*-(tetra-*O*-acetyl-*D*-glucopyranose)hydroxylamine (**144**), Thomas *et al* applied a similar route except that the *N*-hydroxyphthalimide (**149**) derivative was used instead of the *N*-hydroxysuccinimide (**146**), as illustrated in Scheme 5.3.^{14,15}

Scheme 5.3



Acetobromoglucose (146) was thus reacted with *N*-hydroxyphthalimide (149) using phase transfer conditions. *O*-(Tetra-*O*-acetyl-β-D-glucopyranose)-*N*-hydroxyphthalimide (150) was obtained in moderate 36-55% yield, in agreement with published yields.^{10,11} The compound (150) was characterized using ¹H and ¹³C NMR spectroscopy and mass spectrometry. The HR-ESI-MS gave the mass of 516.1115, C₂₂H₂₃NO₁₂Na requires 516.1118. Then, it was attempted to achieve selective deprotection as described in the publication.¹⁵ However, as in the case of the *N*-hydroxysuccinimide analogue, it was not possible to obtain the pure *O*-glycosylhydroxylamine. The very short reaction time and propensity of the acetates to undergo hydrolysis meant that either the product was a mixture of starting material and the required hydroxylamine, if the time was short, or a mixture of various partially deprotected *O*-hydroxylamine derivatives, when the reaction time was too long. The final successful reaction conditions (Scheme 5.4) employed methylhydrazine, which was a combination of the literature methodology and a previously applied procedure by the Botting group.¹⁰

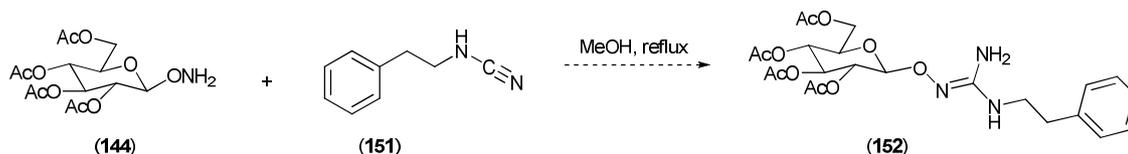
Scheme 5.4



O-(Tetra-*O*-acetyl-β-D-glucopyranose)hydroxylamine (144) was characterized using ¹H and ¹³C NMR spectroscopy. The most clear-cut indication of the product structure is the singlet at 5.82 ppm with an integration of two, signifying the presence of the NH₂ group. In addition, the absence of signals corresponding to the aromatic protons in the region of 7.75-7.83 ppm of the ¹H NMR spectrum as well as the aromatic carbons in the region of 105-135 ppm of the

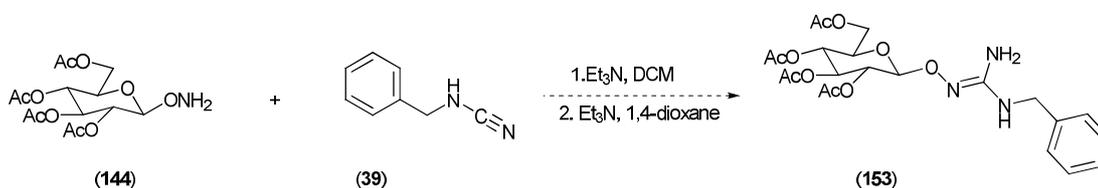
^{13}C NMR spectrum showed that no starting material remained. The synthesized hydroxylamine derivative (**144**) was then reacted with 2-phenylethylcyanamide (**151**) as shown in Scheme 5.5.

Scheme 5.5



The above transformation was never successful, either in the presence or absence of potassium carbonate, which was usually employed in the reaction of cyanamides and hydroxylamine hydrochloride. Thin-layer chromatography showed no reaction. However, prolonged heating of the reaction mixture caused hydrolysis of acetate groups. Since, methanol as a polar, protic solvent can accelerate the loss of acetates; the reaction was performed under slightly modified conditions, as illustrated in Scheme 5.6.

Scheme 5.6



Benzylcyanamide (**39**) and O-(tetra-O-acetyl-D-glucopyranose)hydroxylamine (**144**) were dissolved in dry dichloromethane and then triethylamine was added. The reaction mixture was heated under reflux for two days. The thin-layer chromatography indicated that major spots corresponded to the starting material, even though the reaction conditions were already quite harsh. In order to check the composition of the reaction mixture the crude product was analyzed by LC-MS. As shown in Figure 5.1, several peaks were present in the mass spectrum of the crude product. It illustrated that, except for the signals corresponding to starting materials (386 and 397), there were three other major peaks present which match a product with loss of one (538), two (496) and three (454) acetate groups, respectively. In addition, no intact product was observed in the spectrum.

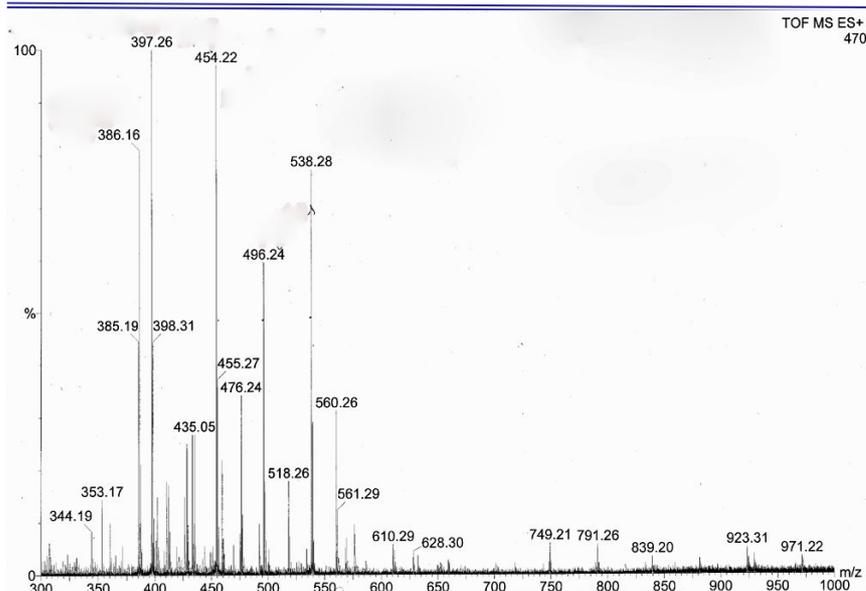


Figure 5.1 Mass spectrum of the crude product (**153**) (DCM used as a solvent).

Although, the new set of conditions did not improve the transformation, it helped to conclude that reaction needs to proceed at higher temperature and that the overall time of the heating should be shortened in order to avoid the hydrolysis of acetates. Unfortunately, changing the solvent from dichloromethane (bp 40 °C) to 1,4-dioxane (bp 101 °C) and heating under reflux for 7h instead of 2 days, did not force the reaction to go. As presented in Figure 5.2, the mass spectrum shows that mainly starting materials (**386**) are present in the crude mixture. This time there were no peaks which indicated the formation of desired product.

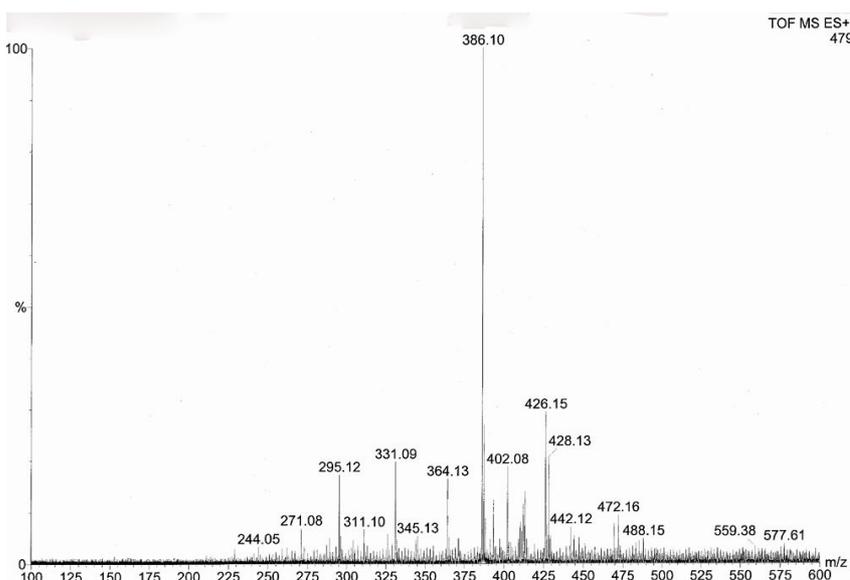
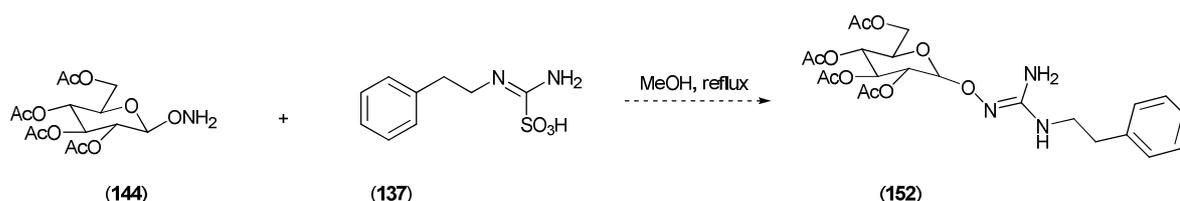


Figure 5.2 Mass spectrum of the crude product (**153**) (1,4-dioxane used as a solvent).

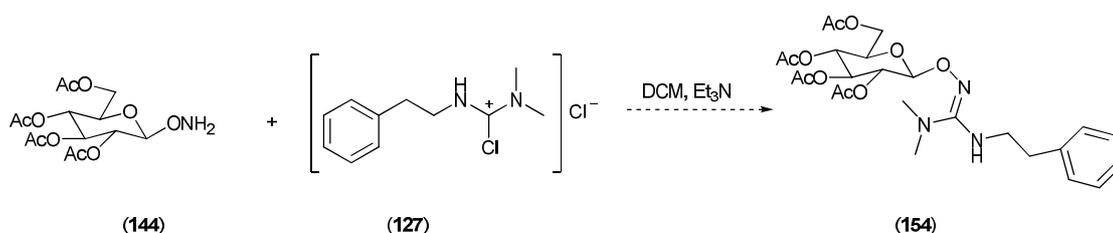
It was decided to employ alternative synthons for the guanidine moiety such as phenylethylaminomethanesulfonic acid (**137**) as already presented in Section 3.1 (Scheme 5.7) and chloroformamidinium chloride (**127**) (Scheme 5.8), which had been successfully reacted with the THP-protected hydroxylamine (see Section 3.1).

Scheme 5.7



The aminoiminomethanesulfonic acids are the products of oxidation of the corresponding thioureas. They had been reported as guanylation agents and successfully employed in the synthesis of various guanidines.^{16, 17} Therefore, they seemed to be suitable intermediates for our transformation. However, as in the case of previous reactions, thin-layer chromatography indicated the presence of only starting materials in the reaction mixture.

Scheme 5.8

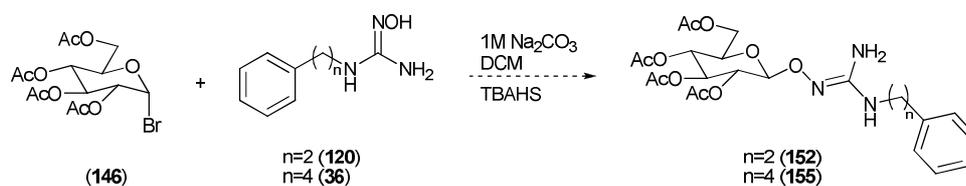


Reaction of ureas with phosgene leads to very reactive intermediates such as *C*-chloroformamidinium chlorides (**127**), which should rapidly undergo nucleophilic substitution and had been successfully employed in the synthesis of trisubstituted *N*-hydroxyguanidines (see Section 3.1).¹⁸ However, treatment of the corresponding *C*-chloroformamidinium chloride (**127**) with the sugar protected hydroxylamine (**144**), as shown in Scheme 10, did not give reasonable results. TLC analysis showed only the presence of starting materials. Unfortunately, neither of the newly employed reactive intermediates reacted with *O*-(tetra-*O*-acetyl-D-glucopyranose)hydroxylamine (**144**) to give the desired products.

5.1.2 Route Two

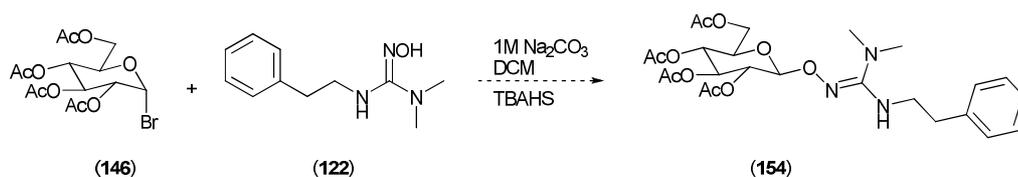
The complete lack of success with the first route led to an investigation of the second approach. Since both, *N*-hydroxysuccinimide (**145**) and *N*-hydroxyphthalimide (**149**) reacted with acetobromoglucose (**146**) under phase-transfer conditions; the same methodology was utilized to attempt to combine *N*-hydroxyguanidines and acetobromoglucose as shown in Scheme 5.9.

Scheme 5.9



However, the above reaction failed. In a number of attempts, a mixture of the starting materials was obtained according to thin layer chromatography. Since, in these hydroxyguanidines there are free amino- and hydroxyl- groups, we speculated that they may compete with each other for the electrophilic bromide. Therefore, we decided to treat *N,N'*-dimethyl-*N*-phenylethyl-*N'*-hydroxyguanidine (**122**) with acetobromoglucose (**146**) under the same set of conditions as illustrated in Scheme 5.10.

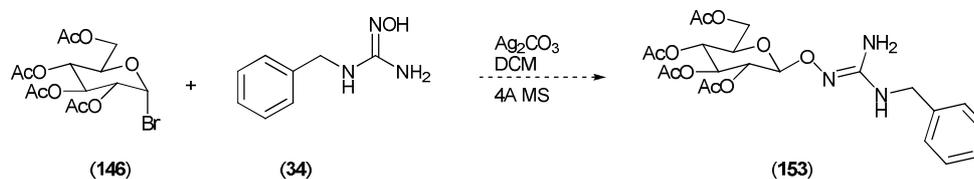
Scheme 5.10



Elimination of free amino group did not improve the overall transformation. Based on the TLC analysis, only starting materials were present in the reaction mixture. The reaction time did not affect the reaction, except that gradual loss of acetates from the acetobromoglucose was observed.

The Koenigs-Knorr reaction is the most popular set of conditions used for glycosylation of alcohols. Therefore an attempt was made to glycosylate *N*-hydroxyguanidines under Koenigs-Knorr conditions as shown in Scheme 5.11.¹⁹

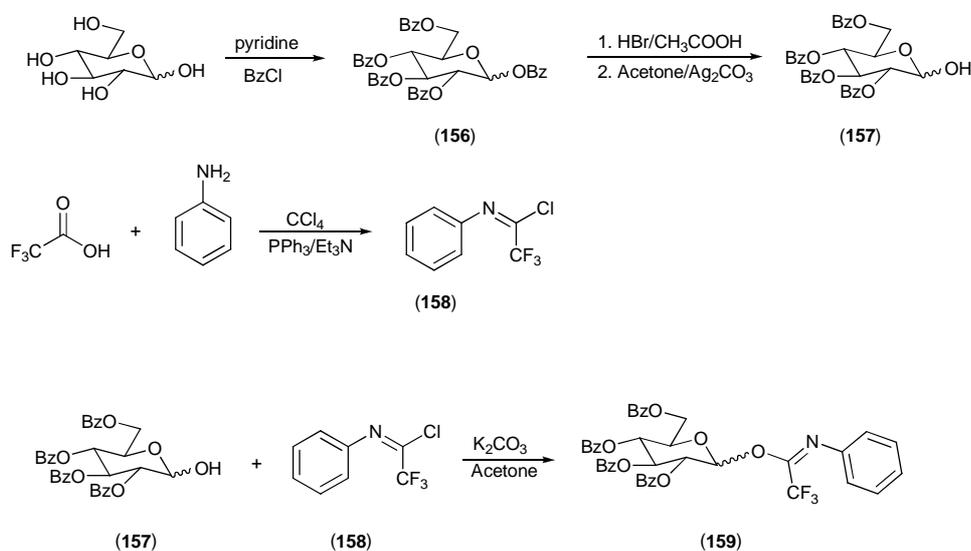
Scheme 5.11



Thin layer chromatography indicated mainly the presence of acetobromoglucose (146). Literature data showed that hydroxyguanidines could be easily oxidized using silver carbonate. In addition, M. Thomas *et al.* also could not glycosylate hydroxamic acids under Koenigs-Knorr conditions, probably due to the same reason. However, they successfully O-glycosylated hydroxamic acids, using glycosyl trichloroacetimidates as glycosyl donors.¹⁴

Therefore, the similar approach was employed to O-glycosylate N-hydroxyguanidines. Firstly, the glycosyl trifluoroacetimidate donor had to be synthesized according to Scheme 5.12.²⁰⁻²⁴

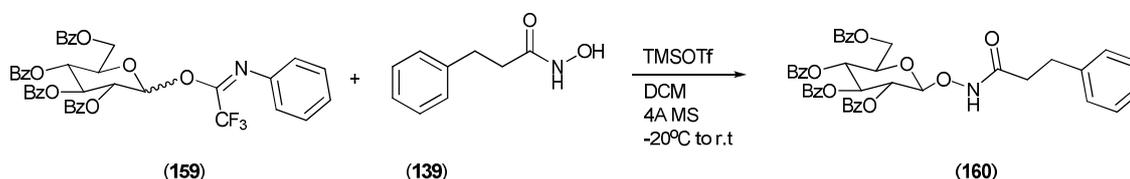
Scheme 5.12



Initially, glucose was treated with benzoyl chloride to give fully protected glucose (156) which was characterized by ¹H and ¹³C NMR spectroscopy. The data were compared with literature which confirmed the correct structure.²⁰ However, reported values of the melting point varied from 156 to 191 °C. Therefore, elemental analysis was performed to prove the purity of the final product (Found: C, 70.4; H, 4.6%. C₄₁H₃₂O₁₁ requires C, 70.3; H, 4.6%) and the melting

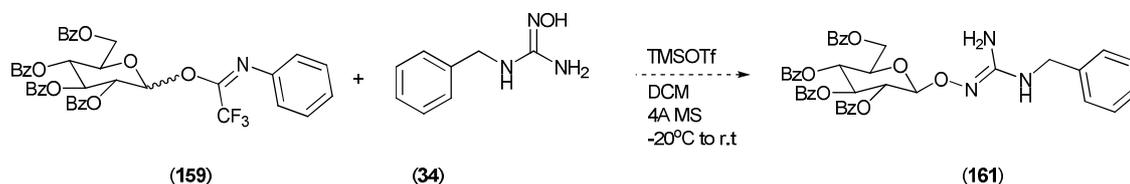
point was found to be 150-151 °C. In the next step, penta-*O*-benzoyl- β -D-glucopyranoside (**156**) was converted to the corresponding bromide using HBr in acetic acid, and it was taken without any characterization into the next reaction. The hydrolysis of the bromide in the presence of silver carbonate yielded 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranose (**157**) in an excellent yield (96%). The product was analyzed using ^1H and ^{13}C NMR spectroscopy. The spectra confirmed the structure of the product and were in agreement with literature data.^{20, 25} The trifluoroacetimidoyl chloride (**158**) was synthesized according to the published procedure by reacting aniline and TFA in the presence of triphenylphosphine and triethylamine.²¹ This gave the desired product (**158**) in 59% yield and the analytical data were in the agreement with literature.²¹ The glycosyl trifluoroacetimidate donor (**159**) was eventually obtained by coupling 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranose (**157**) and previously synthesized *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (**158**) in the presence of potassium carbonate.²⁰ The final product was synthesized as a mixture of α , β anomers, as reported in the literature.²⁰ The desired compound was characterized by various methods. The most significant indication of the structure was the presence of a signal at 6.43 ppm for the *ortho*- protons (-NPh group) in the ^1H NMR spectrum as well as appearance of a new signal at 171.2 ppm (OC(CF₃)NPh) for the quaternary carbon in the ^{13}C NMR spectrum. In addition, the correct mass was observed in HR-ESI-MS spectrum. The analytical results were compared with the published data to confirm the structure.²² 2,3,4,6-Tetra-*O*-benzoyl-D-glucopyranosyl-1-(*N*-phenyl)-2,2,2-trifluoroacetimidate (**159**) was then reacted with 3-phenylpropionohydroxamic acid (**139**) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst (Scheme 5.13).¹⁴

Scheme 5.13



Applying the above procedure it was possible to *O*-glycosylate phenethyl hydroxamic acid (**139**) according to the HR-ESI-MS (766.2264, C₄₃H₃₇NO₁₁Na requires 766.2266), even though a slightly different glycosyl donor was used to that reported by M. Thomas *et al.*¹⁴ The same methodology was then employed with the *N*-hydroxyguanidine as illustrated in Scheme 5.14.

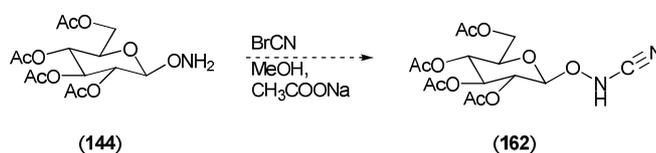
Scheme 5.14



Unfortunately, the above reaction did not give satisfactory results. After flash chromatography, mainly the starting glycosyl donor (159) was recovered. The *N*-benzyl-*N'*-hydroxyguanidine (34) is a very polar compound and probably stayed on the silica. The unsuccessful coupling indicated that even though, hydroxamic acids, as well as *N*-hydroxyguanidines, possess an N-OH moiety, their reactivity is significantly different. It indicates that the nucleophilicity of the hydroxyl groups must be different. Hydroxamic acids are classified as organic acids whilst *N*-hydroxyguanidines are basic.

A new strategy was then developed as shown in Scheme 5.15.

Scheme 5.15

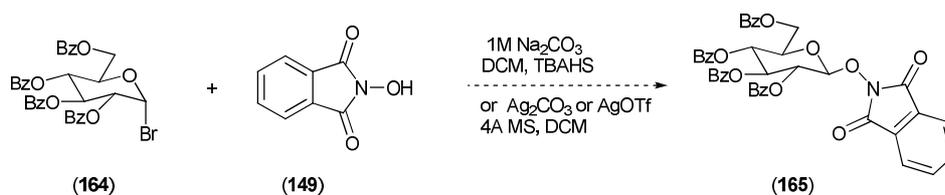


The idea was to treat *O*-(tetra-*O*-acetyl-*D*-glucopyranose)hydroxylamine (144) with cyanogen bromide in the presence of sodium acetate to obtain the corresponding cyanamide (162) which could be further reacted with a suitable amine. Unfortunately, the above reaction did not yield the desired cyanamide, but the starting material was recovered instead making the second step impossible to perform.

Based on the results obtained, a final attempt was designed. The most promising reaction thus far had been treatment of benzyl cyanamide (39) with *O*-(tetra-*O*-acetyl-*D*-glucopyranose)hydroxylamine (144) in the presence of triethylamine. However, the long reaction time caused the hydrolysis of acetate groups. Therefore, it was thought that replacement of the acetate groups with benzoyl groups would make the compound less susceptible towards

hydrolysis. Firstly, *O*-(tetra-*O*-benzoyl-D-glucopyranose)hydroxylamine (**163**) had to be synthesized. Two approaches were chosen as shown in Scheme 5.16.

Scheme 5.16



Unfortunately, the reactivity of bromosugar (**164**) and *N*-hydroxyphthalimide (**149**) was extremely low and therefore neither of the above reactions led to the desired product. Additionally, literature indicated that the *O*-(tetra-*O*-benzoyl-D-glucopyranose)hydroxylamine (**165**) had never been obtained before, which could suggest that the synthesis of this compound was difficult to perform.

5.3 Conclusions

In conclusion, it can be seen that a large number of attempts were made to synthesize a glycosylated *N*-hydroxyguanidine, using a variety of strategies. Unfortunately none of these were successful. Future work could involve designing a suitable linker between the sugar and hydroxyguanidine moiety to simplify the synthesis.

5.4 References

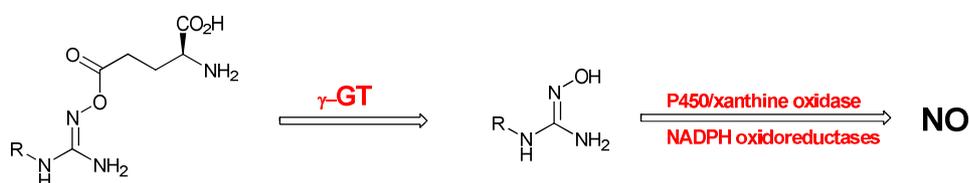
- 1 C. Napoli and L. J. Ignarro, *Annu. Rev. Pharmacol. Toxicol.*, 2003, **43**, 97.
- 2 G. R. J. Thatcher, *Curr. Top. Med. Chem.*, 2005, **5**, 597.
- 3 A. Jousserandot, J.-L. Boucher, Y. Henry, B. Niklaus, B. Clement, and D. Mansuy, *Biochemistry*, 1998, **37**, 17179.
- 4 B. Bonavida, S. Baritaki, S. Huerta-Yepez, M. I. Vega, D. Chatterjee, and K. Yeung, *Nitric Oxide*, 2008, **19**, 152.
- 5 S. Mocellin, V. Bronte, and D. Nitti, *Med. Res. Rev.*, 2007, **27**, 317.
- 6 R. J. Bernacki, M. J. Niedbala, and W. Korytnyk, *Cancer Metastasis Rev.*, 1985, **4**, 81.
- 7 S. Su, D. E. Acquilano, J. Arumugasamy, A. B. Beeler, E. L. Eastwood, J. R. Giguere, P. Lan, X. Lei, G. K. Min, A. R. Yeager, Y. Zhou, J. S. Panek, J. K. Snyder, S. E. Schaus, and J. A. Porco, Jr., *Org. Lett.*, 2005, **7**, 2751.
- 8 S. Cao, F. D. Tropper, and R. Roy, *Tetrahedron*, 1995, **51**, 6679.
- 9 B. L. Sorg, W. E. Hull, H.-C. Kliem, W. Mier, and M. Wiessler, *Carbohydr. Res.*, 2005, **340**, 181.
- 10 O. Renaudet and P. Dumy, *Tetrahedron Lett.*, 2001, **42**, 7575.
- 11 M. B. Mitchell and I. W. A. Whitcombe, *Tetrahedron Lett.*, 2000, **41**, 8829.
- 12 H. Brunner, M. Schonherr, and M. Zabel, *Tetrahedron: Asymmetry*, 2001, **12**, 2671.
- 13 P. R. Andreana, W. Xie, H. N. Cheng, L. Qiao, D. J. Murphy, Q.-M. Gu, and P. G. Wang, *Org. Lett.*, 2002, **4**, 1863.
- 14 M. Thomas, J.-P. Gesson, and S. Papot, *J. Org. Chem.*, 2007, **72**, 4262.
- 15 M. Thomas, F. Rivault, I. Tranoy-Opalinski, J. Roche, J.-P. Gesson, and S. Papot, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 983.
- 16 A. R. Katritzky and B. V. Rogovoy, *ARKIVOC* 2005, 49.
- 17 A. E. Miller and J. J. Bischoff, *Synthesis*, 1986, 777.
- 18 S. D. Ziman, *J. Org. Chem.*, 1976, **41**, 3253.
- 19 G. N. Bollenback, J. W. Long, D. G. Benjamin, and J. A. Lindquist, *J. Am. Chem. Soc.*, 1955, **77**, 3310.
- 20 B. N. A. Mbadugha and F. M. Menger, *Org. Lett.*, 2003, **5**, 4041.
- 21 K. Tamura, H. Mizukami, K. Maeda, H. Watanabe, and K. Uneyama, *J. Org. Chem.*, 1993, **58**, 32.
- 22 B. Yu and H. Tao, *J. Org. Chem.*, 2002, **67**, 9099.
- 23 C. Gauthier, J. Legault, M. Lebrun, P. Dufour, and A. Pichette, *Bioorg. Med. Chem.*, 2006, **14**, 6713.
- 24 N. Al-Maharik and N. P. Botting, *Tetrahedron Lett.*, 2006, **47**, 8703.
- 25 N. B. D'Accorso, I. M. E. Thiel, and M. Schueller, *Carbohydr Res.*, 1983, **124**, 177.

RENAL SELECTIVE NO-DONOR PRO-DRUG

6.1 Introduction

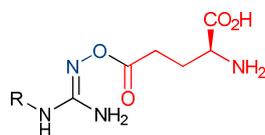
The first aim of previous studies was to design a renal selective pro-drug to treat acute renal failure (ARF), which affects up to 5% of all hospitalized patients and 10-30% of patients in critical care units.¹ This pathological condition is a result of a blood deficiency in the kidney caused by a constriction, or obstruction, of its blood vessels. So far, several therapies have been proposed to treat ARF condition, such as a renal-dose dopamine or mannitol, but their potential benefits still have to be confirmed. Ischemia is the most common cause of ARF. However, the pathology of ischemic conditions is very complex. It is known that reduced nitric oxide bioavailability is a major reason for this illness.² Recently, It has been suggested that ischemic ARF is the condition in which NO supplementation is probably the most beneficial approach.³⁻⁶ Therefore it would be highly useful to deliver NO which can cause vasorelaxation and maintain the proper blood flow in the kidney. However, due to its instability it is very difficult to introduce NO into biological systems. Therefore, one strategy is to combine a NO releasing molecule with an enzyme recognizable fragment to generate a pro-drug which can selectively deliver NO to the kidney. *N*-Hydroxyguanidines (NHG) have been shown to cause NO-mediated vasodilatation; therefore they were chosen to be a NO releasing part of the pro-drug. On the other hand, γ -glutamyl moiety was selected to protect the free hydroxyl group of NHG, since hopefully this can be reversibly cleaved off *in vivo* by the γ -glutamyl transpeptidase (γ -GT); an enzyme expressed almost exclusively in the kidney, liver and pancreas of most mammals, including man. In addition, γ -GT activity is considerably higher (5-10-fold) in the kidney than in liver and pancreas.^{1,2} The graphic representation of the pro-drug idea is presented in Scheme 6.1.

Scheme 6.1



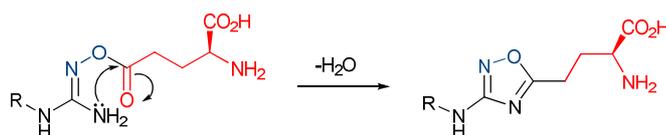
Zhang began work on the γ -GT derivatives for selective release in the kidney aiming to obtain simple glutamyl adducts as shown in Scheme 6.2.⁷

Scheme 6.2



Unfortunately, these targets were not synthetically achievable due to problems with intramolecular cyclization reactions. The side reaction was caused by the presence of a free amino group in the hydroxyguanidine functionality which easily reacted with a neighbouring carbonyl carbon to form an unwanted aromatic five-membered ring as shown in Scheme 6.3.

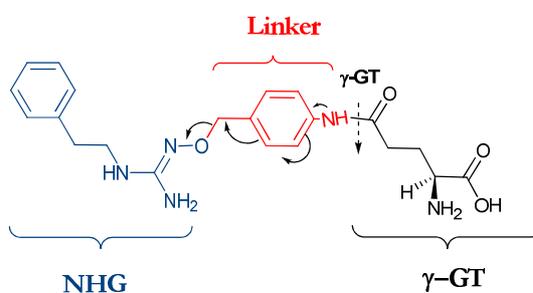
Scheme 6.3



Therefore, the next design involved the incorporation of an aliphatic linker which should prevent unwanted cyclizations. The new generation of pro-drugs was successfully synthesized; however the molecules were not active as substrates of γ -GT.

The final design was based on the incorporation of an aromatic linker as illustrated in Scheme 6.4. The corresponding molecule was synthetically achievable and γ -GT active, and also caused smooth muscle relaxation in the IPK model.

Scheme 6.4

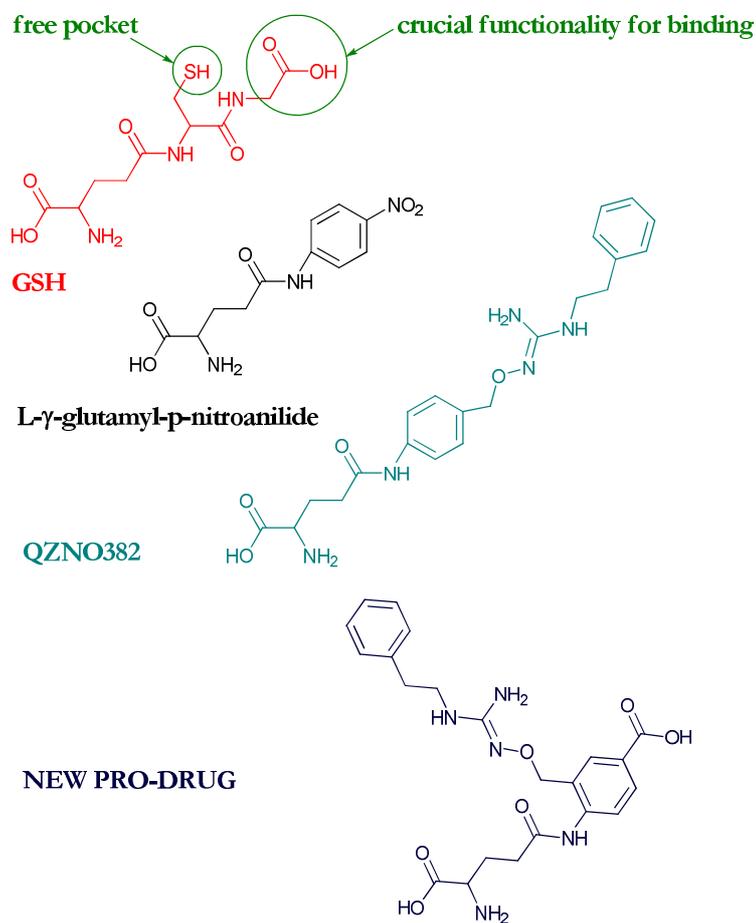


6.2 Improved Linker

6.2.1 Improved linker design

Smith *et al.* investigated the metabolism of glutathione (GSH) as well as the activity of γ -glutamyl transpeptidase (γ -GT) by inhibition studies using various inhibitors.^{8,9} γ -GT is an enzyme consisting of two nonidentical subunits, light and heavy, and is responsible for the cleavage of the γ -glutamyl bond of GSH and related compounds.⁹ In addition, Smith *et al.* mapped the active site of γ -GT with L- γ -glutamyl-*p*-nitroanilide and compared its binding with that of glutathione. They found out that there was a quite big pocket in the active site of γ -GT in the position of –SH group as shown in Scheme 6.5. In addition, the carboxylic acid functionality seemed to be crucial for binding to the active site of the enzyme. In the L- γ -glutamyl-*p*-nitroanilide, the nitro group was shown to bind in the place of the carboxyl group of glutathione.⁹ Based on these findings, the pro-drug QZNO382 can be modified to fit better into the active site of γ -GT. The new pro-drug has been proposed and a synthetic route designed. As presented in the Scheme 6.5, the current pro-drug, even though it is the substrate for γ -GT, does not take a full advantage of structural features within the active site of an enzyme. Therefore, the novel pro-drug will incorporate the hydroxyguanidine functionality in the position of –SH group of glutathione to fit better into the active site of γ -GT. In addition, the carboxylic acid moiety will be added to the structure to improve binding properties of the pro-drug.

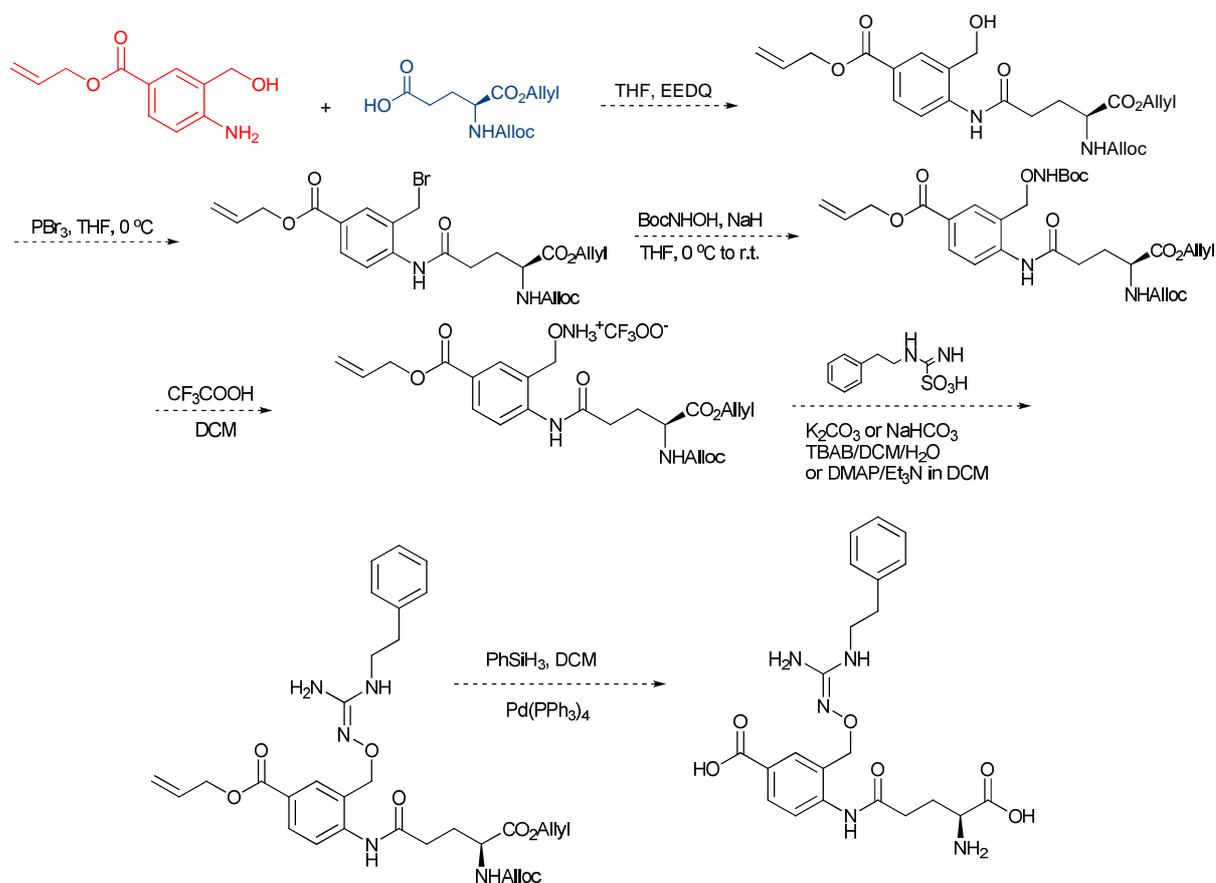
Scheme 6.5



6.2.2 Improved linker synthesis

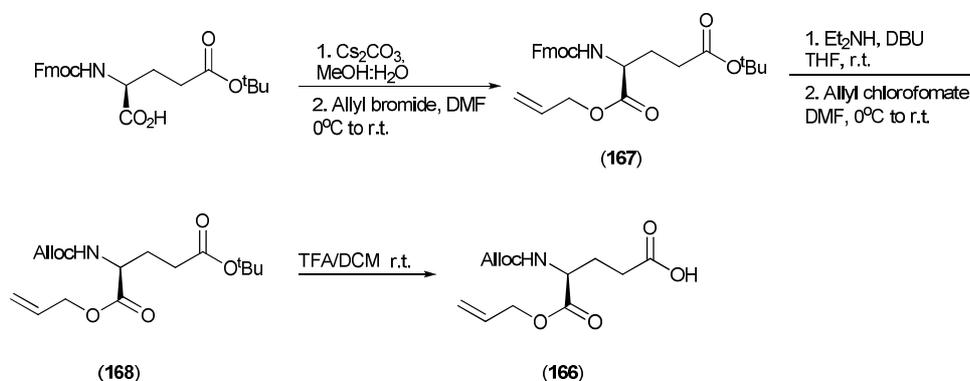
Since Zhang previously established an efficient synthetic methodology for the original pro-drug (QZNO382), a modified approach was firstly envisaged for the new target molecule as shown in Scheme 6.6.

Scheme 6.6



The key amino acid fragment, *N*-alloc *L*-glutamic acid α -allyl ester (**166**) was previously synthesized by Zhang and the same reaction pathway, shown in Scheme 6.7, was employed.

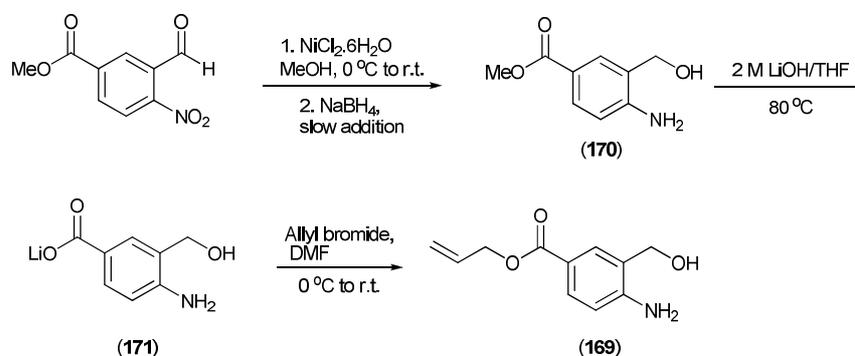
Scheme 6.7



The first reaction involved a conversion of the carboxylic acid into the cesium salt, which was then treated with allyl bromide to give *L*- α -allyl- γ -*tert*-butyl-*N*-9-fluorenylmethoxycarbonylglutamate (**167**) in 75% yield. The compound (**167**) was characterized by ¹H and ¹³C NMR spectroscopy and the purity was confirmed by melting point. Treatment of the product (**167**) with diethylamine in the presence of a catalytic amount of DBU allowed for removal of the Fmoc protecting group. The resulting crude product was then treated with allyl chloroformate to give *L*- α -allyl- γ -*tert*-butyl-*N*-allyloxycarbonylglutamate (**168**) in 56% yield. Its structure was confirmed by the ¹H and ¹³C NMR spectroscopy. The selective deprotection of the *tert*-butyl ester group was then achieved using trifluoroacetic acid to yield the final product (**166**), which was analyzed by the ¹H and ¹³C NMR spectroscopy (see experimental).

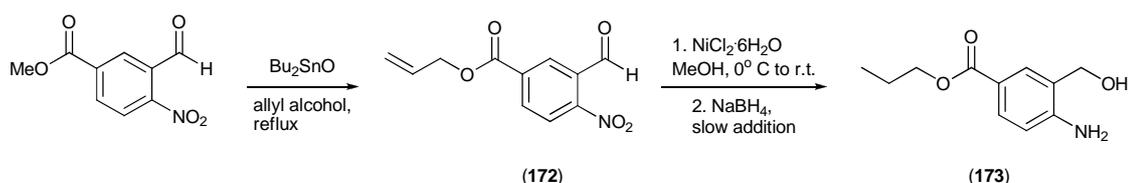
Once the glutamyl part of the new pro-drug had been successfully obtained, the next part of this project was focused on the synthesis of the new linker fragment derived from allyl 4-amino-3-(hydroxymethyl)benzoate (**169**). A commercially available starting material with suitable substituents in the required arrangement was methyl 3-formyl-4-nitrobenzoate. The initial approach is shown in Scheme 6.8. Methyl 3-formyl-4-nitrobenzoate was firstly reduced using *in situ* formed nickel boride to give methyl 4-amino-3-(hydroxymethyl)benzoate (**170**) in 55% yield.¹⁰

Scheme 6.8



The structure of the product **(170)** was confirmed by the ^1H and ^{13}C NMR spectroscopy and mass spectrometry. The presence of the CH_2 protons (2H, 4.37 ppm) and NH_2 protons (2H, 5.74 ppm) in the ^1H NMR spectrum confirmed the successful reduction of the aldehyde and nitro group to the alcohol and amine, respectively. In the next step, the ethyl ester was hydrolysed using lithium hydroxide and the resulting carboxylate **(171)** was reacted with allyl bromide. However, the desired allyl ester **(169)** was obtained in a poor 11% yield, which was not acceptable as it was just a starting material for more complex transformations as shown in Scheme 6.6. Therefore, a new synthetic route was derived as illustrated in Scheme 6.9.

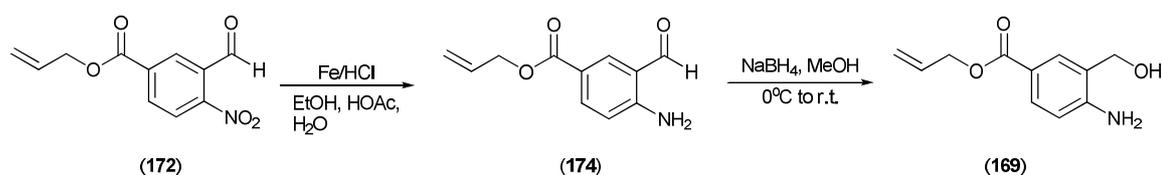
Scheme 6.9



Baumhof *et al.* reported the application of dibutyltin oxide (Bu_2SnO) as a catalyst for a transesterification of methyl esters with various alcohols including the allyl alcohol.¹¹ Therefore, the published procedure was used with methyl 3-formyl-4-nitrobenzoate which was heated for five hours under reflux in allyl alcohol with a catalytic amount of Bu_2SnO . The allyl 3-formyl-4-nitrobenzoate **(172)** was obtained in an acceptable 61% yield. The disappearance of the singlet corresponding to the methyl group and appearance of the new characteristic peaks for the allyl

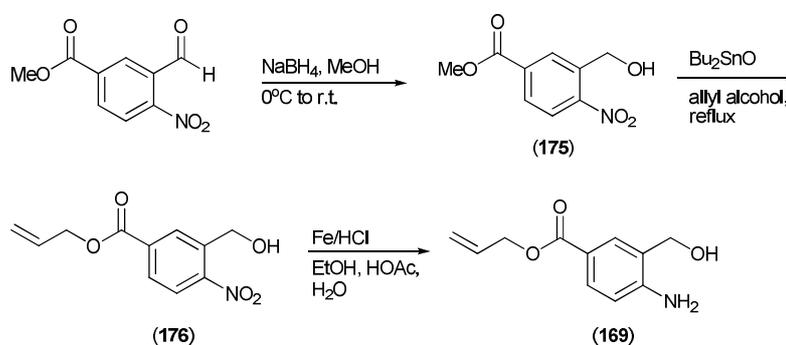
group in the ^1H NMR spectrum confirmed successful transesterification (see experimental). The purity was established by the elemental analysis which indicated that the final product was obtained as nearly 100% pure (Found: C, 56.1; H, 3.6; N, 5.8%. $\text{C}_{11}\text{H}_9\text{NO}_5$ requires C, 56.2; H, 3.9; N, 6.0%). The allyl derivative (**172**) was then subjected to the reduction using the nickel chloride and sodium borohydride procedure.¹⁰ However, the isolated compound was not the expected product. According to the results of ^1H NMR spectroscopy, the double bond of the allyl functionality had also been reduced giving the propyl ester (**173**). In order to overcome this problem, alternative reduction conditions were derived, which reduced the nitro and aldehyde groups in a stepwise fashion as shown in Scheme 6.10.

Scheme 6.10



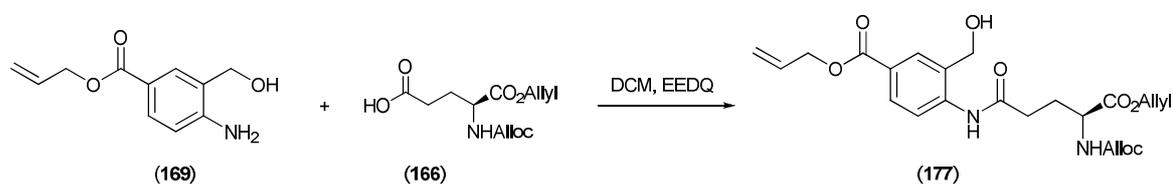
Zhang *et al.* recently reported a procedure for the selective reduction of nitro groups in the presence of aldehyde and ester groups using iron, acetic acid and a catalytic amount of concentrated hydrochloric acid.¹² Allyl 4-amino-3-formyl benzoate (**174**) was synthesized using this procedure in an excellent 94% yield. The appearance of the new singlet corresponding to the NH_2 protons (2H, 6.59 ppm) in the ^1H NMR spectrum confirmed the successful transformation. In addition, the characteristic peaks for the allyl group remained unchanged as well as the aldehyde singlet (see experimental). The product of reduction (**174**) was then treated with sodium borohydride in order to convert the aldehyde into the corresponding alcohol. The desired compound (**169**) was isolated in 53% yield. The most clear-cut indication of the product structure was the presence of new CH_2 protons (2H, 4.57 ppm) and the disappearance of the singlet (CHO) in the low-field of the ^1H NMR spectrum. However, this method failed to work on a larger scale. Therefore, the reaction pathway was further modified as presented in Scheme 6.11.

Scheme 6.11

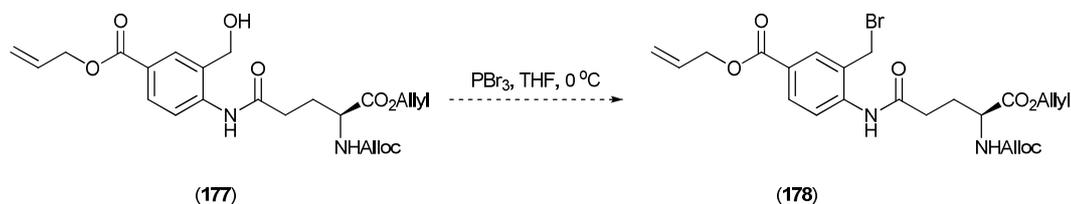


This time the methyl 3-formyl-4-nitrobenzoate was firstly treated with sodium borohydride to reduce the aldehyde and the desired product (**175**) was obtained in 71% yield, which was better than the reduction of allyl 4-amino-3-formyl benzoate (53%). In addition, the purification step was easier because it involved just simple recrystallization instead of column chromatography. The methyl 3-(hydroxymethyl)-4-nitrobenzoate (**175**) was analyzed by various methods. The structure was confirmed by the presence of CH_2 protons (2H, 5.02 ppm) and OH proton (1H, 2.34) in the ^1H NMR spectrum. Additionally, the HR-ESI-MS gave the mass of 234.0376 ($\text{M}+\text{Na}^+$) and $\text{C}_9\text{H}_9\text{NO}_5\text{Na}$ required 234.0378. The product was then transesterified to the allyl ester using the previously described Bu_2SnO catalyzed procedure.¹¹ Allyl 3-(hydroxymethyl)-4-nitrobenzoate (**176**) was synthesized in a very good yield (84%) and characterized by the ^1H and ^{13}C NMR spectroscopy, IR spectroscopy and mass spectrometry. The successful transesterification was confirmed by the disappearance of the singlet corresponding to the methyl group and appearance of the new characteristic peaks for the allyl group in the ^1H NMR spectrum. In addition, this time also the yield was significantly better and the purification was simplified to recrystallization and the column chromatography was excluded. The last step involved the reduction of the nitro group using the iron and acetic acid. Allyl 3-(hydroxymethyl)-4-aminobenzoate (**169**) was obtained in an excellent 99% yield. The most indicative piece of data was an upfield shift of aromatic protons in the ^1H NMR spectrum, which was characteristic for the presence of the amino substituent. Based on the obtained results, the route illustrated in Scheme 6.11 was chosen for the synthesis of allyl 3-(hydroxymethyl)-4-aminobenzoate (**169**). Having both starting materials in hand, the coupling reaction was then examined as shown in Scheme 6.12.

Scheme 6.12

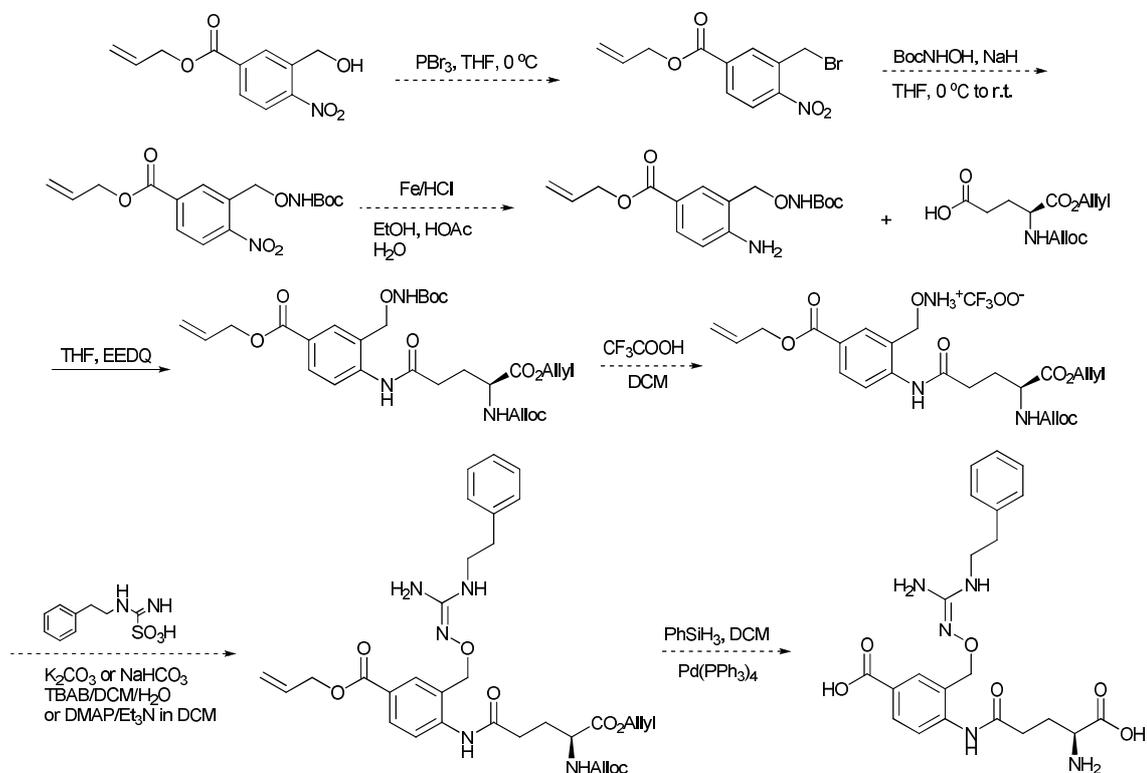


For the coupling EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) was employed rather than a carbodiimide to avoid racemisation of the amino acid. Thus, *N*-allyl *L*, α -allylglutamate (166) and allyl 3-(hydroxymethyl)-4-aminobenzoate (169) were dissolved in anhydrous DCM and EEDQ added. Unfortunately the reaction was quite complex, mainly from the purification point of view. Four different chromatography columns with various eluting systems from non-polar to pure ethyl acetate had to be performed in order to obtain the pure product. This significantly affected the yield of the reaction giving only 6% of isolated product. The next step involved bromination of the alcohol using phosphorus tribromide (Scheme 6.13).¹³

Scheme 6.13¹³

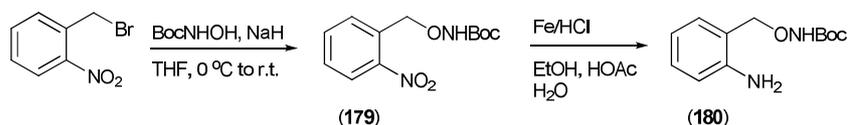
A number of attempts were made in order to successfully convert the alcohol (177) into the bromide (178). Firstly using PBr₃, the reaction was monitored by thin-layer chromatography and run at 0 ° and then at room temperature for about 48 hours. The TLC analysis indicated that only starting material was present in the reaction mixture. The next approach employed carbon tetrabromide (CBr₄) and triphenylphosphine (PPh₃)^{14, 15}, but as previously only the starting material was recovered. Therefore, we once more decided to change the strategy as shown in Scheme 6.14.

Scheme 6.14



However, there was one difficult to predict step in the reaction pathway, namely the reduction of the nitro group under acidic conditions in the presence of the Boc protected hydroxylamine moiety. Therefore, in order to determine the feasibility of this reaction a model compound was used and taken through the same set of transformations as presented in Scheme 6.15.

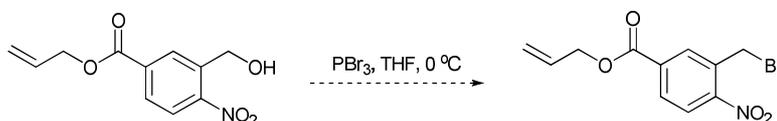
Scheme 6.15



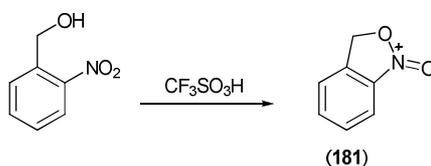
Commercially available 2-nitrobenzyl bromide was treated with Boc protected hydroxylamine, deprotonated using sodium hydride, to yield *tert*-butyl

2-nitrobenzyloxycarbamate (**179**). The structure was confirmed by ^1H NMR spectroscopy, by the appearance of a 9H singlet for the *tert*-butyl functionality. The product was then heated under reflux in the presence of iron, a catalytic amount of HCl and acetic acid to give *tert*-butyl 2-aminobenzyloxycarbamate (**180**) in a good yield.¹² The model reaction indicated that the hydroxylamine moiety remained untouched under the reduction conditions. Therefore, the next step involved the bromination of the allyl 3-(hydroxymethyl)-4-nitrobenzoate as shown in Scheme 6.16.

Scheme 6.16



Unfortunately, the bromination of the corresponding alcohol using either PBr₃ or CBr₄/PPh₃ did not give satisfactory results. The problem may be caused by the high reactivity of the bromide product and close proximity of the nitro group, which can react with each other to give a five membered ring. In addition, Austin *et al.* studied oxidation-reduction reactions which involved nitro groups.¹⁶ They discovered that when 2-nitrobenzyl alcohol was placed in trifluoromethanesulfonic acid at room temperature it was rapidly converted into the C-protonated conjugate acid of anthranil *N*-oxide (**181**) as shown in Scheme 6.17.

Scheme 6.17¹⁶

The same product was observed from 2-nitrobenzyl chloride at elevated temperature. It was suspected that the same transformation could occur during bromination reaction. In addition, the literature procedures for the synthesis of 2-nitrobenzyl bromide only involve bromination of 2-nitrotoluene.

6.3 Conclusions

The synthesis of the improved linker was not successful even though several approaches were employed to achieve the target molecule. The limiting step was coupling the hydroxyguanidine moiety with glutamyl fragment using EEDQ, which yielded the required product in a poor yield. Therefore, future work can focus on the optimization of this reaction. However, the preliminary results when EDC was employed as a coupling agent did not improve the overall transformation.

Additionally, the new synthetic route may be designed in which the bromination could be preformed using toluene derivative. This should help to avoid the difficulties encounter during bromination of 2-nitrobenzyl alcohol.

Even though the target compound has not been obtained, the synthetic studies allowed the exploration of a whole variety of novel chemical reactions.

6.4 References

- 1 P. A. Grace, *Br. J. Surg.*, 1994, **81**, 637.
- 2 A. B. Groeneveld, D. D. Tran, J. van der Meulen, J. J. Nauta, and L. G. Thijs, *Nephron*, 1991, **59**, 602.
- 3 H. Kurata, M. Takaoka, Y. Kubo, T. Katayama, H. Tsutsui, J. Takayama, M. Ohkita, and Y. Matsumura, *Eur. J. Pharmacol.*, 2005, **517**, 232.
- 4 G. Cirino, *Dig. Liver Dis.*, 2003, **35**, S2.
- 5 B. Persson Pontus, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2002, **283**, R1005.
- 6 L. A. Tome, L. Yu, I. De Castro, S. B. Campos, and A. C. Seguro, *Nephrology, Dialysis, Transplantation*, 1999, **14**, 1139.
- 7 Q. Zhang and N. P. Botting, Unpublished Results, *University of St Andrews*, 2008.
- 8 E. S. Stole, T. K. Smith; J.M. Mannings, and A. Meister, *J. Biol. Chem.*, 1994, **269**, 21435.
- 9 A. Meister and T.K. Smith, *J. Biol. Chem.*, 1995, **270**, 12476.
- 10 S. Caddick, D. B. Judd, A. K. d. K. Lewis, M. T. Reich, and M. R. V. Williams, *Tetrahedron*, 2003, **59**, 5417.
- 11 P. Baumhof, R. Mazitschek, and A. Giannis, *Angew. Chem., Int. Ed.*, 2001, **40**, 3672.
- 12 C. Zhang, C. K. De, R. Mal, and D. Seidel, *J. Am. Chem. Soc.*, 2008, **130**, 416.
- 13 J. H. Boal, A. Wilk, C. L. Scremin, G. N. Gray, L. R. Phillips, and S. L. Beaucage, *J. Org. Chem.*, 1996, **61**, 8617.
- 14 L. D. Fader, M. Boyd, and Y. S. Tsantrizos, *J. Org. Chem.*, 2001, **66**, 3372.
- 15 C. Mukai, M. Kobayashi, S. Kubota, Y. Takahashi, and S. Kitagaki, *J. Org. Chem.*, 2004, **69**, 2128.
- 16 R. P. Austin and J. H. Ridd, *J. Chem. Soc., Perkin Trans. 2, (1972-1999)*, 1994, 1411.

Summary and future work

In summary, thirty new *N*-hydroxyguanidines have been synthesized, but only twenty two were tested as nitric oxide donors. Preliminary biological results indicated that almost all of the synthesized *N*-hydroxyguanidines act as NO donors and cause smooth muscle relaxation in the rat aorta. Data for the series of *N*-phenylalkyl-*N'*-hydroxyguanidines showed that extension of the aliphatic chain was beneficial up to three methylene groups, while further elongation significantly reduced the biological activity. The compound with the best pharmacological profile turned out to be *N*-phenyl-*N'*-hydroxyguanidine hydrochloride (AK0), ($EC_{50} = 19.9 \mu\text{M}$, ODQ reversibility = 100%). Changes to the benzene ring of *N*-phenylethyl-*N'*-hydroxyguanidine (QZNO193) demonstrated that substitution with a trifluoromethyl group in the *para* position improved the biological activity of the new derivative (QZNO193, $EC_{50} = 50.1 \mu\text{M}$ whereas AK2F, $EC_{50} = 8.0 \mu\text{M}$). However, the ODQ test indicated worse reversibility, which may imply that the vasodilation does not occur mainly through NO mediated pathway. *N*-Furfuryl-*N'*-hydroxyguanidine (AK135) and *N*-2-(4'-nitrophenyl)ethyl-*N'*-hydroxyguanidine (AK119) appeared to be as good as the unsubstituted analogue based on the EC_{50} values as well as ODQ reversibility. Interestingly, *N*-2-[4'-(sulfamoyl)phenyl]ethyl-*N'*-hydroxyguanidine (AK169) did not possess any biological activity, which is probably due to the secondary biological pathway occurring on the sulfonamide group such as oxidation, for example. Unfortunately, there does not seem to be a logical structure activity relationship amongst this series of compounds which would allow the most important structural features for NO donor activity to be distinguished. Therefore, it would be beneficial to investigate the rat aorta assay in more detail and try to determine which enzyme is responsible for oxidation of *N*-hydroxyguanidines and eventually perform QSAR studies to be able to optimize the structure to produce the optimum NO donor molecule.

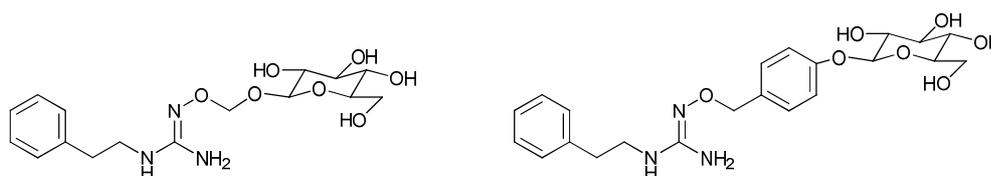
Methylated analogues of *N*-phenylethyl-*N'*-hydroxyguanidine were also synthesized, to investigate the effect of *N*-methylation on NO donor activity. However, these analogues have not yet been tested in the myography assay and so further biological studies need to be carried out in order to determine the vasorelaxation properties of compounds (121) and (122). During these synthetic studies it was discovered that these *N*-hydroxyguanidines along with their counterpart aminoiminomethanesulfonic acids revealed very interesting behaviour in the solid state as well as in solution. Therefore, detailed structural studies were performed using X-ray

crystallography and NMR spectroscopy, which showed that *N*-hydroxyguanidines and aminoiminomethanesulfonic acids possess analogous electronic structures. It would be interesting to carry out computational analysis on this set of compounds in order to calculate the rotational energy barriers for these molecules and compare the theory with the experimental data.

Interestingly, the hydroxamic acids that were prepared also possessed promising pharmacological profiles. 3-Phenylpropionohydroxamic acid (AK46) revealed the most potent biological profile with a low EC_{50} value (6.4 μ M) and almost 100% reversibility in the ODQ test. Unfortunately, preliminary biological studies in the isolated perfused kidney (IPK) model only showed initial vasorelaxation and then there was a subsequent unexpected vasoconstriction. This obviously excluded hydroxamic acids from further research towards renal selective NO donor pro-drugs. However, since there are very few publications related to the mechanism of NO donation by hydroxamic acids, more studies should be carried out to understand their behaviour. These studies should include chemical and enzymatic oxidation, using isolated NOS enzymes, to confirm that NO is indeed formed and elucidate the chemical mechanism of the process.

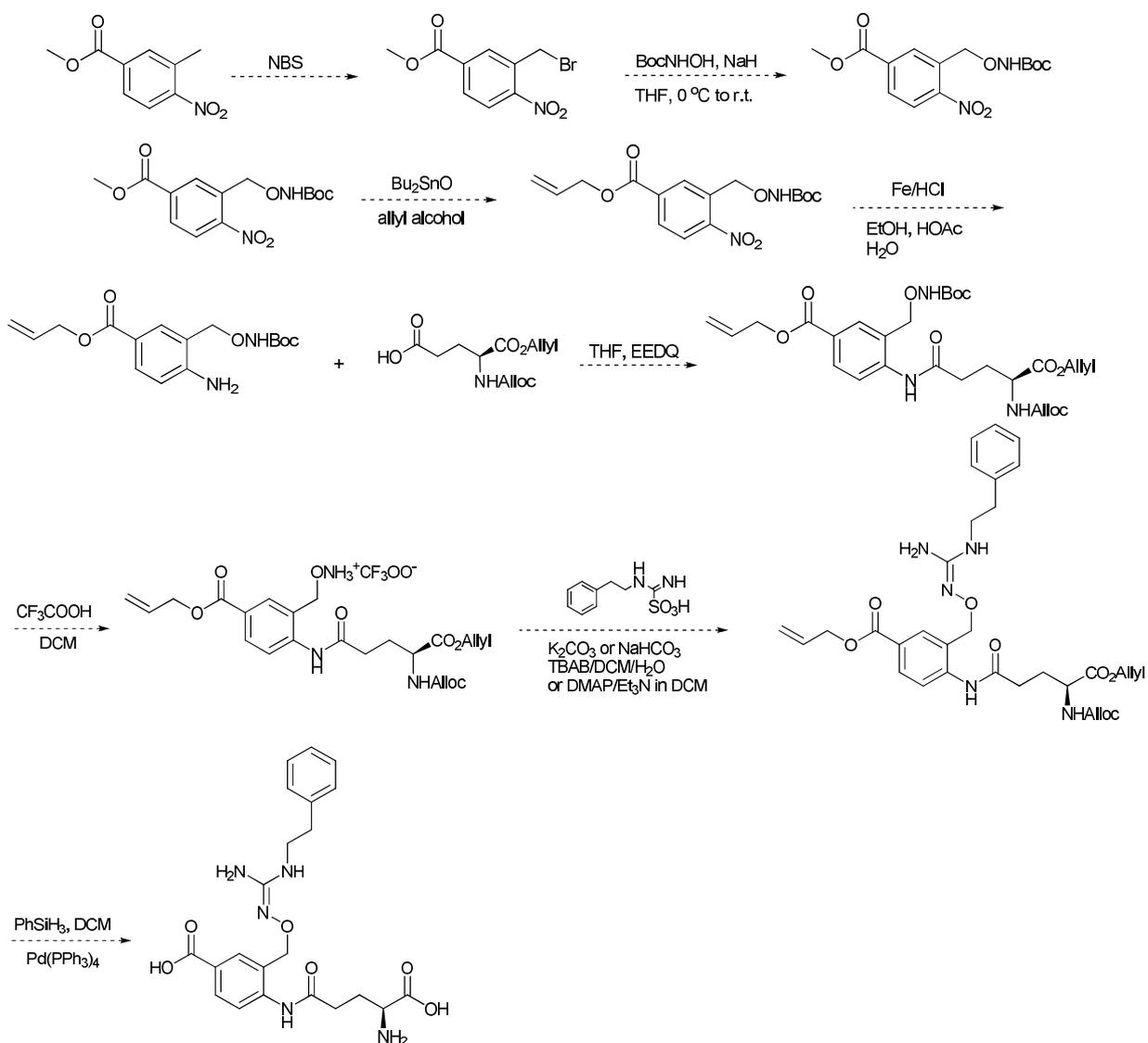
Two different types of enzyme-activated pro-drugs were designed using *N*-hydroxyguanidines as the NO donating molecule. The first was a glycoside derivative, where a glucose moiety was attached to the *N*-hydroxyguanidine and its release by a suitable glycosidase would be required before NO could be formed. Unfortunately, the synthesis of this compound proved to be very problematic and although a number of approaches were investigated, the target molecule was not successfully synthesised. Future work should focus on the design and synthesis of a suitable linker between the hydroxyguanidine and the sugar moiety, which based on the results of current studies, should be significantly easier to synthesise (Scheme 7.1). Once the target compound is synthetically achievable, biological studies should be performed to determine its affinity for glycosidases.

Scheme 7.1



The second pro-drug design consisted of an *N*-hydroxyguanidine attached *via* a linker molecule to a γ -glutamyl functionality and was based on earlier studies in the group. This pro-drug should require the enzyme γ -GT for release of the active moiety, which would target its activity to the kidney. The new design described in the thesis was a modification of the first generation pro-drug, which was aimed at improving the binding of the pro-drug to the active site of γ -GT and, therefore, its substrate activity. Synthetic studies towards this target were carried out using various approaches. However, the target molecule was not achieved in this work as a number of problems were experienced, in particular in the final stages where cyclisation reactions appeared to be the major problem. In the light of these studies new approaches have been devised that could be attempted. For example, using a different starting material, methyl 3-methyl-4-nitrobenzoate as shown in Scheme 7.2, would allow an alternative radical bromination to be employed and avoid the intermediacy of the alcohol, the bromination of which appeared to be the main problem. In this new synthetic approach the coupling of the γ -glutamyl fragment could also be improved as removal of the hydroxyl group means that the amine cannot hydrogen bond to the adjacent hydroxyl, and so may be more reactive.

Scheme 7.2



EXPERIMENTAL

7.1 General

¹H NMR spectra were recorded on a Bruker Avance 300 (300.1 MHz) instrument, Bruker Avance 400 (400.1 Hz) instrument, Bruker Avance 500 (499.9 MHz) instrument or a Varian Gemini 2000 (300.0 MHz) instrument, using deuterated solvents such as CDCl₃, d₆-DMSO, d₄-methanol and D₂O as reference and internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS). The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); td (triplet of doublets); dd (doublet of doublets); ddd (doublet of doublet of doublets); q (quartet); or m (multiplet). The number of protons (n) for a given resonance is indicated by nH. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz.

¹³C NMR spectra were recorded on a Bruker Avance 300 (75.46 MHz) instrument and Bruker Avance 400 (100.10 Hz) instrument using the PENDANT and DEPTH sequence and internal deuterium lock or on a Varian Gemini 2000 (75.46 MHz) instrument using proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ in units of ppm relative to TMS. Where appropriate, coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz.

¹⁹F NMR spectra were recorded on a Bruker Avance 400 (376.5 MHz) instrument using proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ in units of ppm.

IR spectra were recorded on a Perkin-Elmer Paragon series 1000 FTIR spectrometer as thin films or nujol mulls between sodium chloride discs or as potassium bromide disks as indicated. Absorption maxima are reported in wavenumbers (cm⁻¹).

Melting points were determined in open capillary tubes using an Electrothermal Melting Point Apparatus and are uncorrected.

Analytical thin layer chromatography (TLC) was carried out on pre-coated plastic Flex 60A Macherey-Nagel silica gel plates. Visualisation was achieved by absorption of UV light, or thermal development after dipping in either a methanolic solution of 10% sulfuric acid or an aqueous solution of potassium permanganate, potassium carbonate and sodium hydroxide.

Flash Column chromatography was carried out on silica gel (Apollo Scientific Ltd 40-63 micron) under a positive pressure of compressed air.

Kugelrohr bulb-to-bulb distillations were carried out using a Büchi B580 instrument.

Solvents and Chemicals

Dichloromethane and methanol were distilled from calcium hydride in a recycling still. Diethyl ether was distilled from sodium in a recycling still using benzophenone ketyl as an indicator.¹ THF and toluene were purified using MBRAUN solvent purification systems (MB SPS-800). Chemicals were purchased from Acros UK, Aldrich UK, Avocado UK, Fisher UK or Fluka UK. All solvents and reagents were purified and dried, where necessary, by standard techniques.¹ Where appropriate and if not stated otherwise, all non aqueous reactions were performed under an inert atmosphere of nitrogen or argon. Brine refers to a saturated aqueous solution of sodium chloride. Hexane refers to *n*-hexane and petroleum ether to the fraction boiling between 40-60 °C. Room temperature (RT) refers to the temperature of 25 °C.

Hydroxamic Acid Test²

The drop, or a few small crystals of the test compound were dissolved in 1 ml of ethanol and 1 ml of 1M HCl was added. Then the prepared mixture was treated with one drop of 5% iron (III) chloride solution. The appearance of the deep wine color indicated the presence of the hydroxamic acid.

Biological Assays (performed by Dr Philip Milliken University of Edinburgh)

The biological testing was a staged screening process. Initially the compounds were examined in the rat aorta using myography, to determine their vasodilation activity. The compounds that meet the criteria below in these experiments were then examined in the isolated perfused kidney,

to see the effect in the whole organ system. Presented results are an average of six consecutive experiments.

The criteria set for the compounds in the myography study were:

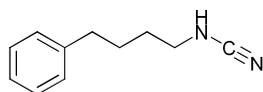
- compounds must have an EC_{50} of 30 [μ M] or less (this is the concentration that causes 50% of the maximum relaxation);
- 80% reversibility with ODQ (selective inhibitor of NO sensitive guanylate cyclase).

This allows selecting compounds with a good vasodilator profile, mediated through NO. If the relaxation is not reversed by ODQ, then the effect of the compound is not *via* NO, and the compound may be acting through other pathways, or could be toxic.

Myography

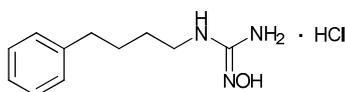
The rat aorta was cut into 2 mm sections and mounted onto the myograph, and stretched to 15 mN. Vessels were contracted with KCl to determine the maximal constriction. Vessels were washed and contracted to 80% of the maximum with phenylephrine. A concentration response curve was obtained for the compound (1-100 μ M). Then, 50 μ M ODQ was added to assess the reversibility of the relaxation.

Synthesis of 4-phenylbutylcyanamide (**37**)



To a suspension of 4-phenylbutylamine (1.83 g, 12.3 mmol) and sodium acetate (1.60 g, 19.5 mmol) in dry methanol (30 ml) was added dropwise a methanolic solution of BrCN (1.44 ml, 13.0 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the crude product was extracted from water with CH₂Cl₂ (3 x 15 ml). The organic layer was washed with water (30 ml), 1M HCl (30 ml) and brine (30 ml), dried over MgSO₄ and evaporated at reduced pressure. The pure 4-phenylbutylcyanamide (**37**) was obtained by column chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluting solvent. This gave the desired product as a pale yellow oil (1.10 g, 50%). ν_{\max} (PTFE card)/cm⁻¹ 3400 (NH), 2987 (CH aromatic), 2215 (C≡N), 1599 (C=C); δ_{H} (300 MHz, CDCl₃) 1.55-1.72 (4H, m, CH₂-2,3), 2.62 (2H, t, *J* 7 Hz, CH₂-4) 3.04 (2H, dt, *J* 7 Hz, *J* 6 Hz, CH₂-1) 3.57 (1H, br s, NH) 7.12-7.19 (3H, m, H-2', 4', 6') 7.23-7.29 (2H, m, H-3',5'); δ_{C} (75.46 MHz, CDCl₃) 28.4 (C-3), 29.7 (C-2), 35.7 (C-4), 46.6 (C-1), 116 (C≡N), 126.4 (C-4'), 128.8 (Ar-CH), 142.0 (C-1'); *m/z* (ES⁺) 197.1056 ((M+Na)⁺). C₁₁H₁₄N₂Na requires 197.1055; *m/z* (ES⁻) 173 (M-H⁻, 100%).

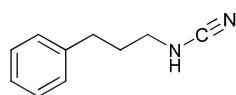
Synthesis of *N*-(4-phenylbutyl)-*N'*-hydroxyguanidine hydrochloride (**36**)



4-Phenylbutylcyanamide (**37**) (1.64 g, 9.4 mmol) was mixed with hydroxylamine hydrochloride (0.65 g, 9.4 mmol) in methanol (30 ml) and potassium carbonate (0.1 equiv) as a catalyst and heated under reflux for 4 h under an argon atmosphere. After cooling, the solvent was removed at reduced pressure and the desired product was recrystallized from CH₂Cl₂. Then the product was redissolved in minimum amount of methanol and precipitated out using CH₂Cl₂. A final crystallization from CH₂Cl₂ gave the pure product (**36**) as colourless crystals (1.27 g, 55%). mp 122-123 °C; (Found: C, 53.8; H, 7.8; N, 17.2%. C₁₁H₁₈ClNO₃ requires C, 54.2; H, 7.5; N, 17.3%).

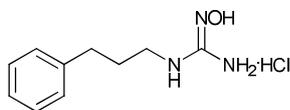
17.2%); $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$ 3428 (NH), 1625 (C=C); δ_{H} (300 MHz, d_6 -DMSO) 1.42-1.64 (4H, m, CH₂-2,3), 2.58 (2H, t, J 7 Hz, CH₂-4) 3.16 (2H, dt J 7 Hz, J 6 Hz, CH₂-1), 7.14-7.21 (3H, m, H-2', 4', 6'), 7.24-7.29 (2H, m, 3', 5'), 7.66 (2H, br s, NH₂), 7.92 (1H, t, J 7 Hz, NH), 9.76 (1H, br. s, NH/OH), 10.34 (1H, br. s, NH/OH); δ_{C} (75.46 MHz, d_6 -DMSO) 27.9 (C-3), 28.1 (C-2), 34.6 (C-4), 38.6 (C-1), 125.7 (C-4'), 128.3 (Ar-CH), 141.9 (C-1'), 158.2 (C=NOH); m/z (ES⁺) 208 ((M+H)⁺, 100%).

Synthesis of 3-phenylpropylcyanamide (40)



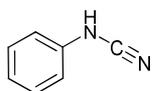
To a suspension of 3-phenyl-1-propylamine hydrochloride (5.00 g, 29.1 mmol) and sodium acetate (4.77 g, 58.2 mmol) in dry methanol (50 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (9.70 ml, 29.12 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the crude product was extracted with CH₂Cl₂ (3 x 30 ml). The organic layer was washed with brine (40 ml), dried over MgSO₄ and evaporated at reduced pressure. The pure cyanamide (**40**) was obtained by column chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluting solvents. This gave the desired product (**40**) as a pale yellow oil (3.05 g, 65%). $\nu_{\max}(\text{PTFE card})/\text{cm}^{-1}$ 3163, 2923 (NH), 2215 (C≡N), 1653 (C=C); δ_{H} (300 MHz, CDCl₃), 1.95 (2H, q, J 7 Hz, CH₂-2), 2.71 (2H, t, J 7 Hz, CH₂-3), 3.09 (2H, dt, J 7 Hz, J 6 Hz, CH₂-1), 3.63 (1H, br s, NH), 7.17-7.24 (3H, m, H-2', 4', 6'), 7.27-7.33 (2H, m, H-3',5'); δ_{C} (75.46 MHz, CDCl₃) 31.1 (C-3), 32.4 (C-2), 45.5 (C-1), 116.2 (C≡N), 126.3 (C-4'), 128.4 (C-3', 5'), 128.6 (C-2', 6'), 140.6 (C-1'); m/z (ES⁺) 183.0904 ((M+Na)⁺. C₁₀H₁₂N₂Na requires 183.0898); m/z (ES⁻) 159 (M-H⁺, 100%).

Synthesis of *N*-(3-phenylpropyl)-*N*'-hydroxyguanidine hydrochloride (**35**)



3-Phenylpropylcyanamide (**40**) (3.05 g, 19.1 mmol) was mixed with hydroxylamine hydrochloride (1.19 g, 17.2 mmol) in methanol (40 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 5 h under an argon atmosphere. After cooling, the solvent was removed at reduced pressure and the desired product was crystallized from CH_2Cl_2 and diethyl ether. Further purification was carried out by recrystallization from CH_2Cl_2 to give the pure product (**35**) as colourless crystals (2.28 g, 58%). mp 120-122 °C; (Found: C, 52.0; H, 6.9; N, 18.4%. $\text{C}_{10}\text{H}_{16}\text{ClN}_3\text{O}$ requires C, 52.3; H, 7.0; N, 18.3%); $\nu_{\text{max}}(\text{nujol})/\text{cm}^{-1}$ 3267, 3133 (NH, OH), 1578 (C=C); δ_{H} (300 MHz, d_6 -DMSO) 1.77 (2H, q, J 7 Hz, CH_2 -2), 2.59 (2H, t, J 7 Hz, CH_2 -3), 3.15 (2H, q, J 7 Hz, CH_2 -1), 7.14-7.23 (3H, m, H-2', 4', 6'), 7.25-7.31 (2H, m, H-3', 5'), 7.71 (2H, br s, NH_2), 8.05 (1H, t, J 7 Hz, NH), 9.82 (1H, br s, NH/OH), 10.43 (1H, br s, NH/OH); δ_{C} (75.46 MHz, D_2O) 29.4 (C-3), 31.9 (C-2), 40.4 (C-1), 126.2 (C-4'), 128.5 (C-2', 6'), 128.7 (C-3', 5'), 141.5 (C-1'), 158.6 (C=NOH); m/z (ES^+), 194 ($\text{M}+\text{H}^+$, 100%).

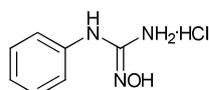
Synthesis of phenylcyanamide (**38**)³



To a suspension of aniline hydrochloride (10 g, 77.2 mmol) and dry sodium acetate (18.9 g, 231.5 mmol) in dry methanol (100 ml) was added dropwise 3 M solution of BrCN in CH_2Cl_2 (25.7 ml, 77.2 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the crude product was extracted with CH_2Cl_2 (3 x 40 ml). The organic layer was washed with brine (50 ml), dried over MgSO_4 and evaporated at reduced pressure. The pure phenylcyanamide (**38**) was obtained by column chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluting solvent. This gave the desired

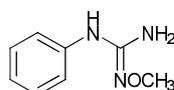
product (**38**) as a colourless oil (6.65 g, 73%). $\nu_{\max}(\text{neat})/\text{cm}^{-1}$ 3174, 3100 (NH), 2227 (C≡N), 1600 (C=C); δ_{H} (300 MHz, CDCl₃) 6.67 (1H, br s, NH), 6.92-7.17 (3H, m, H-2', 4', 6'), 7.27-7.41 (2H, m, H-3',5'); δ_{C} (75.46 MHz, CDCl₃) 111.3 (C≡N), 115.4 (C-2', 6'), 123.7 (C-4'), 129.8 (C-3', 5'), 137.2 (C-1'); m/z (ES⁻) 117 ((M-H)⁻, 100%).

Synthesis of *N*-phenyl-*N*'-hydroxyguanidine hydrochloride (**33**)³



Phenylcyanamide (**38**) (4.30 g, 36.4 mmol) was combined with hydroxylamine hydrochloride (2.28 g, 32.8 mmol) in absolute methanol (50 ml) with dry potassium carbonate (0.1 equiv) as a catalyst and heated under reflux for 6 h under an argon atmosphere. After cooling, the inorganic salt was filtered off and solvent was removed at reduced pressure. The desired product was crystallized from CH₂Cl₂ and diethyl ether. The pure product (**33**) was obtained by further recrystallization from CH₂Cl₂ to give the final product as an off-white solid (2.95 g, 47%). mp 126-128 °C (lit.,³ 128-129 °C); $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$ 3289 (NH), 1618, 1599 (C=C); δ_{H} (300 MHz, d₆-DMSO) 7.15-7.26 (3H, m, H-2', 4', 6'), 7.39 (2H, m, H-3', 5'), 7.94 (2H, br s, NH₂), 10.14 (1H, br s, NH), 10.24 (1H, br. s, NH/OH), 10.95 (1H, br. s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) 123.6 (C-2', 6'), 126.0 (C-4'), 129.5 (C-3', 5'), 135.3 (C-1'), 156.7 (C=NOH); m/z (ES⁺) 152 ((M+H)⁺, 100%), 153 (10).

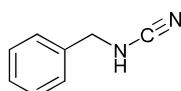
Synthesis of *N*-phenyl-*N*'-methoxyguanidine (**95**)



Phenylcyanamide (**38**) (3.8 g, 32.3 mmol) was combined with methoxylamine hydrochloride (1.8 g, 21.5 mmol) and dry potassium carbonate (4.45 g, 32.3 mmol) in dry 1,4-dioxane (50 ml) and heated under reflux for two days under an argon atmosphere. After cooling, the inorganic salt was filtered off and solvent was removed at reduced pressure. The desired product was purified by column chromatography on silica using ethyl acetate/diethyl ether (3:1) as the eluting solvent. The pure product (**95**) was obtained by crystallization from diethyl ether to give the final product

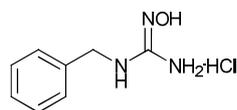
as a colourless solid (198 mg, 7%). mp 126-128 °C; (Found: C, 58.5; H, 6.7; N, 25.4%. $C_8H_{11}N_3O$ requires C, 58.2; H, 6.7; N, 25.4%); ν_{\max} (nujol)/ cm^{-1} 3469 3255, 3154, (NH), 1641, 1588 (C=C); δ_H (300 MHz, d_6 -DMSO) 3.57 (3H, s, CH_3), 5.21 (2H, br s, NH_2), 6.73-6.80 (1H, m, H-4'), 7.12-7.20 (2H, m, H-2', 6'), 7.29 (2H, d, J 8 Hz, H-3', 5'), 7.65 (1H, br s, NH); δ_C (75.46 MHz, d_6 -DMSO) 60.8 (CH_3), 117.1 (C-2', 6'), 119.8 (C-4'), 128.8 (C-3', 5'), 141.9 (C-1'), 151.6 (C=NOCH₃); m/z (ES⁺) 188 ((M+Na)⁺, 100%), 166 (20).

Synthesis of benzylcyanamide (**39**)³



To a suspension of benzylamine (10.00 g, 93.4 mmol) and sodium acetate (7.60 g, 93.4 mmol) in dry methanol (100 ml) was added dropwise a 3 M solution of BrCN in CH_2Cl_2 (31.0 ml, 93.4 mmol). The mixture was stirred in an ice bath for 5 h, and then the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the crude product was extracted with CH_2Cl_2 (3 x 40 ml). The organic layer was washed with brine (50 ml), dried over $MgSO_4$ and evaporated at reduced pressure. The pure benzylcyanamide was obtained by column chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluent. This gave the desired product (**39**) as an off-white solid (9.70 g, 79%). mp 39-40 °C; (Found: C, 72.2; H, 6.1; N, 21.2%. $C_8H_8N_2$ requires C, 72.7; H, 6.1; N, 20.9%); ν_{\max} (nujol)/ cm^{-1} 3252 (NH), 2214 (C≡N); δ_H (300 MHz, $CDCl_3$) 3.92 (1H, br s, NH), 4.13 (2H, d, J 6 Hz, CH_2 -1), 7.21-7.33 (5H, m, Ar-H); δ_C (75.46 MHz, $CDCl_3$) 50.3 (C-1), 116.0 (C≡N), 127.8 (C-4'), 128.6 (C-2', 6'), 129.0 (C-3', 5'), 136.2 (C-1') ; m/z (ES⁻) 131 ((M-H)⁻, 100%).

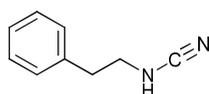
Synthesis of *N*-benzyl-*N'*-hydroxyguanidine hydrochloride (**34**)³



Benzylcyanamide (**39**) (9.55 g, 72.3 mmol) was heated under reflux with hydroxylamine hydrochloride (4.52 g, 65.1 mmol) and dry potassium carbonate (0.1 equiv) as a catalyst in absolute methanol (100 ml) for 6 h under an argon atmosphere. After cooling, the inorganic salt

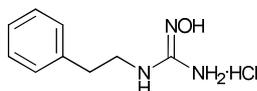
was filtered off and the solvent was removed at reduced pressure. The desired product was crystallized from CH_2Cl_2 and diethyl ether. The pure product (**34**) was obtained by further recrystallization from CH_2Cl_2 to give the final product as a colourless solid (5.92 g, 45%). mp 159-160 °C (lit.³, 158-159 °C); (Found: C, 47.5; H, 5.9; N, 21.0%. $\text{C}_8\text{H}_{12}\text{ClN}_3\text{O}$ requires C, 47.6; H, 6.0; N, 20.9%); ν_{max} (nujol)/ cm^{-1} 3244, 3089 (NH, OH), 1635, 1579 (C=C); δ_{H} (300 MHz, d_6 -DMSO) 4.42 (2H, d, J 6 Hz, CH_2 -1), 7.25-7.41 (5H, m, Ar-H), 7.81 (2H, br s, NH_2), 8.40 (1H, t, J 7Hz, NH), 9.88 (1H, br. s, NH/OH), 10.57 (1H, br. s, NH/OH); δ_{C} (75.46 MHz, d_6 -DMSO) 43.5 (C-1), 127.1 (C-2', 6'), 127.4 (C-4'), 128.4 (C-3', 5'), 137.3 (C-1'), 158.3 (C=NOH); m/z (ES^+) 166 ($\text{M}+\text{H}^+$, 100%).

Synthesis of phenylethylcyanamide (**151**)³



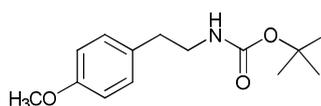
To a suspension of phenylethylamine (2.01 g, 16.5 mmol) and sodium acetate (2.71 g, 33.0 mmol) in dry methanol (100 ml) was added dropwise a 3 M solution of BrCN in CH_2Cl_2 (5.5 ml, 16.5 mmol). The mixture was stirred in an ice bath for 5 h, and then the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the crude product was extracted with CH_2Cl_2 (3 x 40 ml). The organic layer was washed with brine (50 ml), dried over MgSO_4 and evaporated at reduced pressure. The pure phenylethylcyanamide was obtained by column chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluant. This gave the desired product (**151**) as a pale yellow oil (1.46 g, 41%). δ_{H} (300 MHz, CDCl_3) 2.85 (2H, t, J 7 Hz, CH_2 -2), 3.24-3.35 (3H, m, CH_2 -1, NH), 7.11-7.31 (5H, m, Ar-H); δ_{C} (75.46 MHz, CDCl_3) 36.3 (C-2), 47.9 (C-1), 116.2 ($\text{C}\equiv\text{N}$), 127.5 (C-4'), 129.3 (C-2', 6'), 129.5 (C-3', 5'), 136.7 (C-1').

Synthesis of *N*-phenylethyl-*N'*-hydroxyguanidine hydrochloride (**120**)³



Phenylethylcyanamide (**151**) (4.31 g, 0.02 mol) was heated under reflux with hydroxylamine hydrochloride (1.39 g, 0.02 mol) and dry potassium carbonate (0.1 equiv) as a catalyst in absolute methanol (150 ml) for 6 h under an argon atmosphere. After cooling, the inorganic salt was filtered off and the solvent was removed at reduced pressure. The desired product was crystallized from CH₂Cl₂ and diethyl ether. The pure product (**120**) was obtained by further recrystallization from CH₂Cl₂ to give the final product as a colourless solid (3.10 g, 70%). mp 109-111 °C; (Found: C, 50.1; H, 6.4; N, 19.7%. C₉H₁₄ClN₃O requires C, 50.1; H, 6.5; N, 19.5 %); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3327, 3176, 3075 (NH, OH), 1635, 1579 (C=C); δ_{H} (300 MHz, d₆-DMSO) 2.79 (2H, t, *J* 7 Hz, CH₂-2), 3.43-3.67 (2H, m, CH₂-1) 7.18-7.24 (3H, m, H-2', 4', 6'), 7.27-7.32 (2H, m, H-3', 5'), 7.75 (2H, br s, NH₂), 8.00 (1H, t, *J* 6Hz, NH), 9.88 (1H, br. s, NH/OH), 10.53 (1H, br. s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) 34.5 (C-2), 42.5 (C-1), 127.2 (C-2', 6'), 129.1 (C-4'), 129.3 (C-3', 5'), 138.6 (C-1'), 159.0 (C=NOH); *m/z* (ES⁺) 180 (M+H⁺, 100%).

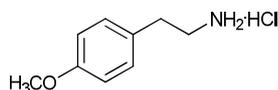
Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(4'-methoxyphenyl)ethylamine (**49**)



To a stirred solution of (4-methoxyphenyl)acetonitrile (2.00 g, 13.6 mmol) in dry methanol (80 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc₂O) (5.93 g, 27.2 mmol) and NiCl₂·6H₂O (0.32 g, 1.36 mmol). NaBH₄ (3.59 g, 95.1 mmol) was then added in small portions over 3 h. The resulting reaction mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and stirred for further 16 h, at which point diethylenetriamine (1.47 ml, 13.6 mmol) was added. The mixture was allowed to stir for 1.5 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (250 ml) and extracted with saturated aq. NaHCO₃ (3 x 80 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to yield a yellow oil. The crude product was

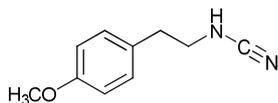
purified by column chromatography on silica with petroleum ether/diethyl ether (2:3) as eluting solvent. This gave the desired product (**49**) as a colourless solid (2.04 g, 60%). mp 56 °C; (Found: C, 66.9; H, 8.9; N, 5.5%. $C_{14}H_{21}NO_3$ requires C, 66.9; H, 8.4; N, 5.6%); ν_{\max} (nujol)/ cm^{-1} 3360 (NH), 1725 (C=O); δ_H (300 MHz, $CDCl_3$) 1.43 (9H, s, *t*-butyl), 2.73 (2H, t, *J* 7 Hz, CH_2 -2), 3.33 (2H, dt, *J* 7, *J* 6 Hz, CH_2 -1), 3.78 (3H, s, OCH_3), 4.55 (1H, br s, NH), 6.82-6.87 (2H, m, H-3', 5'), 7.07-7.13 (2H, m, H-2', 6'); δ_C (75.46 MHz, $CDCl_3$) 28.4 (CH_3), 35.3 (C-2), 42.0 (C-1), 55.3 (OCH_3), 79.2 (C-*t*-butyl), 114.0 (C-3', 5'), 129.7 (C-2', 6'), 131.0 (C-1'), 155.9 (C-4'), 158.2 (C=O); m/z (ES^+) 290 ((M+K)⁺, 100%), 274 ((M+Na)⁺, 65%).

Synthesis of 2-(4'-methoxyphenyl)ethylamine hydrochloride (**50**)⁴



A solution of *N*-(*tert*-butoxycarbonyl)-2-(4'-methoxyphenyl)ethylamine (**49**) (1.94 g, 7.73 mmol) in dry diethyl ether (3 ml) was treated with anhydrous HCl in 1,4-dioxane (4 M, 10 ml) at room temperature for 12 h. The excess solvent was decanted off and a colourless solid was filtered off and washed with petroleum ether. This gave the pure product (**50**) (1.45 g, 98%). mp 210-212 °C, (lit.,⁴ 211-213 °C); (Found: C, 57.6; H, 7.9; N, 7.8%. $C_9H_{14}ClNO$ requires: C, 57.6 ; H, 7.5; N, 7.5%; ν_{\max} (nujol)/ cm^{-1} 3651 (NH), 1601 (C=C aromatic); δ_H (300 MHz, d_6 -DMSO) 2.82 (2H, t, *J* 7 Hz, H-2), 2.88-3.03 (2H, m, H-1), 3.73 (3H, s, OCH_3), 6.86-6.92 (2H, m, H-3', 5'), 7.15-7.20 (2H, m, H-2',6'), 8.10 (3H, br s, NH_3^+); δ_C (75.46 MHz, d_6 -DMSO) 32.1 (C-2), 40.1 (C-1), 55.0 (OCH_3), 114.0 (C-3', 5'), 129.1 (C-1'), 129.6 (C-2', 6'), 158.0 (C-4'); m/z (ES^+) 152 ((M+H)⁺, 80%), 135 (100), 122 (100).

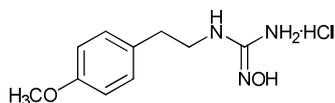
Synthesis of 2-(4'-methoxyphenyl)ethylcyanamide (**58**)



To a suspension of 2-(4'-methoxyphenyl)ethylamine hydrochloride (**50**) (1.32 g, 7.03 mmol) and sodium acetate (1.16 g, 14.2 mmol) in dry methanol (30 ml) was added dropwise a 3M solution

of BrCN in CH₂Cl₂ (2.34 ml, 7.03 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (30 ml). The crude product was extracted with CH₂Cl₂ (3 x 20 ml). The organic layer was washed with brine (20 ml), dried over MgSO₄ and evaporated at reduced pressure. The pure product was obtained by chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluting solvent. This gave the desired product (**58**) as a pale yellow oil (0.99 g, 80%). $\nu_{\max}(\text{neat})/\text{cm}^{-1}$ 3219, 3004 (NH), 2936 (CH aromatic), 2222 (C≡N), 1612 (C=C aromatic); δ_{H} (300 MHz, d₆-DMSO) 2.70 (2H, t, *J* 7 Hz, CH₂-2), 3.07-3.15 (2H, m, CH₂-1), 3.72 (3H, s, OCH₃), 6.77 (1H, t, *J* 6 Hz, NH), 6.84-6.89 (2H, m, H-3', 5'), 7.12-7.19 (2H, m, H-2', 6'); δ_{C} (75.46 MHz, d₆-DMSO) 34.5 (C-2), 46.1 (C-1), 54.9 (OCH₃), 113.8 (C-3', 5'), 117.2 (C≡N), 129.7 (C-2', 6'), 130.1 (C-1'), 157.8 (C-4'); m/z (ES⁺) 199.0848 ((M+Na)⁺. C₁₀H₁₂N₂ONa requires 199.0847); m/z (ES⁻) 175 ((M-H)⁻, 100%).

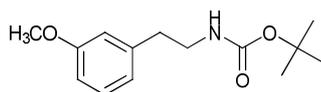
Synthesis of *N*-2-(4'-methoxyphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (**77**)



2-(4'-Methoxyphenyl)ethylcyanamide (**58**) (0.76 g, 4.28 mmol) was mixed with hydroxylamine hydrochloride (0.30 g, 4.28 mmol) in dry methanol (25 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 6 h under an argon atmosphere. After cooling, the solvent was removed at reduced pressure and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Further purification was achieved by redissolving the crude product in minimum amount of methanol and the insoluble solid was filtered off. The addition of CH₂Cl₂ and diethyl ether gave the pure product (**77**) as colourless crystals (0.61 g, 58%). mp 90-92 °C; (Found: C, 49.2; H, 6.3; N, 16.9%. C₁₀H₁₆ClN₃O₂ requires C, 48.9; H, 6.6; N, 17.1%); $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$ 3414, 3308, 3140 (NH, OH), 1581 (C=C aromatic); δ_{H} (300 MHz, d₆-DMSO) 2.72 (2H, t, *J* 7 Hz, CH₂-2), 3.30-3.38 (2H, m, CH₂-1), 3.72 (3H, s, OCH₃), 6.83-6.89 (2H, m, H-3', 5'), 7.16-7.22 (2H, m, H-2', 6'), 7.69 (2H, br s, NH₂), 7.89 (1H, t, *J* 6 Hz, NH), 9.83 (1H, br s, NH/OH), 10.45 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) 34.1 (C-2), 42.6

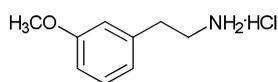
(C-1), 55.4 (OCH₃), 114.1 (C-3', 5'), 130.2 (C-2', 6'), 130.7 (C-1'), 157.8 (C-4'), 158.1 (C=NOH); m/z (ES⁺), 210 ((M+H)⁺, 100%).

Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(3'-methoxyphenyl)ethylamine (**45**)



To a stirred solution of 3-methoxyphenylacetonitrile (2.00 g, 13.6 mmol) in dry methanol (80 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc₂O) (5.93 g, 27.2 mmol) and NiCl₂ · 6H₂O (0.32 g, 1.36 mmol). NaBH₄ (3.59 g, 95.1 mmol) was then added in small portions over 3 h. The resulting reaction mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and stirred for further 4 h, at which point diethylenetriamine (1.47 ml, 13.6 mmol) was added. The mixture was allowed to stir for 1.5 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (250 ml) and extracted with saturated aq. NaHCO₃ (3 x 80 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to yield yellow oil. The crude product was purified by column chromatography on silica with petroleum ether/diethyl ether (1:2) as eluting solvent. This gave the desired product (**45**) as a colourless solid (2.07g, 61%). mp 36 °C; (Found: C, 67.1; H, 8.8; N, 5.7%. C₁₄H₂₁NO₃ requires C, 66.9; H, 8.4; N, 5.6%); ν_{\max} (nujol)/cm⁻¹ 3343 (NH), 1725 (C=O), 1603 (C=C aromatic); δ_{H} (300 MHz, CDCl₃) 1.45 (9H, s, *t*-butyl), 2.79 (2H, t, *J* 7 Hz, CH₂-2), 3.39 (2H, t, *J* 7Hz, CH₂-1), 3.82 (3H, s, OCH₃), 4.56 (1H, br s, NH), 6.73-6.83 (3H, m, H-2', 4', 6'), 7.21-7.28 (1H, m, H-5'); δ_{C} (75.46 MHz, CDCl₃) 28.3 (CH₃), 36.3 (C-2), 41.7 (C-1), 55.2 (OCH₃), 79.3 (C-*t*-butyl), 111.8 (C-4'), 114.5 (C-2'), 121.2 (C-6'), 129.6 (C-5'), 140.6 (C-1'), 155.9 (C=O), 159.8 (C-3'); m/z (ES⁺) 290 ((M+K)⁺, 100%), 261 (70).

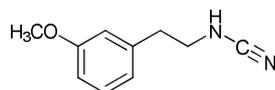
Synthesis of 2-(3'-methoxyphenyl)ethylamine hydrochloride (**46**)⁵



A solution of *N*-(*tert*-butoxycarbonyl)-2-(3'-methoxyphenyl)ethylamine (**45**) (2.03 g, 8.07 mmol) in dry diethyl ether (3 ml) was treated with anhydrous HCl in 1,4-dioxane (4 M, 10 ml) at room

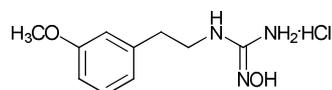
temperature for 12 h. The excess of solvent was decanted off and the colourless solid was filtered off and washed with petroleum ether. This gave the pure product (**46**) (1.51 g, 86%). mp 132 °C, (lit.,⁵ 132 °C); (Found: C, 57.4; H, 7.9; N, 7.5%. C₉H₁₄ClNO requires: C, 57.6 ; H, 7.5; N, 7.5%); ν_{\max} (nujol)/cm⁻¹ 3651 (NH), 1602 (C=C aromatic); δ_{H} (300 MHz, d₆-DMSO) 2.84-2.91 (2H, m, CH₂-2), 2.95-3.05 (2H, m, CH₂-1), 3.74 (3H, s, OCH₃), 6.79-6.85 (3H, m, H-2', 4', 6'), 7.20-7.27 (1H, m, H-5'), 8.19 (3H, br s, NH₃⁺); δ_{C} (75.46 MHz, d₆-DMSO) 32.9 (C-2), 39.9 (C-1), 54.9 (OCH₃), 112.2 (C-4'), 114.2 (C-2'), 120.8 (C-6'), 129.6 (C-5'), 138.9 (C-1'), 159.4 (C-3'); m/z (ES⁺) 152 ((M+H)⁺, 100%).

Synthesis of 2-(3'-methoxyphenyl)ethylcyanamide (**59**)



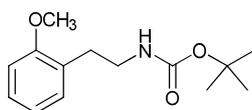
To a suspension of 2-(3'-methoxyphenyl)ethylamine hydrochloride (**46**) (1.20 g, 6.39 mmol) and sodium acetate (1.05 g, 12.8 mmol) in dry methanol (30 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (2.13 ml, 6.39 mmol). The mixture was stirred in an ice bath for 2 h, then the ice bath was removed and reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (30 ml). The crude product was extracted with CH₂Cl₂ (3 x 20 ml). The organic layer was washed with brine (20 ml), dried over MgSO₄ and evaporated. The pure product was obtained by column chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluting solvents. This gave the desired product (**59**) as a colourless oil (0.81 g, 72%). ν_{\max} (neat)/cm⁻¹ 3221, 3003 (NH), 2940 (CH aromatic), 2221 (C≡N), 1602 (C=C aromatic); δ_{H} (300 MHz, d₆-DMSO) 2.74 (2H, t, J 7 Hz, CH₂-2), 3.11-3.20 (2H, m, CH₂-1), 3.74 (3H, s, OCH₃), 6.76-6.84 (4H, m, C-2', 4', 6', NH), 7.18-7.24 (1H, m, H-5'); δ_{C} (75.46 MHz, d₆-DMSO) 35.4 (C-2), 45.8 (C-1), 54.9 (OCH₃), 111.8 (C-4'), 114.4 (C-2'), 117.2 (C≡N), 121.0 (C-6'), 129.3 (C-5'), 139.8 (C-1'), 159.3 (C-3'); m/z (ES⁺) 199.0850 ((M+Na)⁺. C₁₀H₁₂N₂ONa requires 199.0847); m/z (ES⁻) 175 ((M-H)⁻, 100%).

Synthesis of *N*-2-(3'-methoxyphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (**78**)



2-(3'-Methoxyphenyl)ethylcyanamide (**59**) (0.81 g, 4.57 mmol) was mixed with hydroxylamine hydrochloride (0.32 g, 4.57 mmol) in dry methanol (30 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 6 h under an argon atmosphere. After cooling, the solvent was removed and the desired product was crystallized from CH_2Cl_2 and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtered off. Addition of CH_2Cl_2 and diethyl ether gave the pure product (**78**) as colourless solid (0.60 g, 53%). mp 88-90 °C; (Found: C, 48.2; H, 6.7; N, 17.6%. $\text{C}_{10}\text{H}_{16}\text{ClN}_3\text{O}_2$ requires C, 48.9; H, 6.6; N, 17.1%); ν_{max} (nujol)/ cm^{-1} 3318 (NH), 1639, 1586 (C=C aromatic); δ_{H} (300 MHz, d_6 -DMSO) 2.76 (2H, t, J 7 Hz, H-2), 3.38-3.43 (2H, m, H-1), 3.74 (3H, s, OCH_3), 6.75-6.89 (3H, m, H-2', 4', 6'), 7.21 (1H, t, J 8 Hz, H-5'), 7.72 (2H, br s, NH_2), 7.92 (1H, t, J 6 Hz, NH), 9.84 (1H, br s, NH/OH), 10.29 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d_6 -DMSO) 34.9 (C-2), 42.2 (C-1), 55.3 (OCH_3), 112.3 (C-4'), 114.8 (C-2'), 121.4 (C-6'), 129.7 (C-5'), 140.21 (C-1'), 158.5 (C=NOH), 159.6 (C-3'); m/z (ES^+) 210 ((M+H)⁺, 100%).

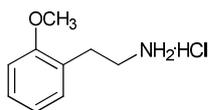
Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(2'-methoxyphenyl)ethylamine (**47**)



To a stirred solution of 2-methoxyphenylacetonitrile (2.50 g, 17.0 mmol) in dry methanol (100 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc_2O) (7.42 g, 34.0 mmol) and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.40 g, 1.70 mmol). NaBH_4 (4.50 g, 119 mmol) was then added in small portions over 4 h. The resulting reaction mixture, containing a finely divided black precipitate was allowed to warm to room temperature and stirred for a further 16 h, at which point diethylenetriamine (1.75 ml, 17.0 mmol) was added. The mixture was allowed to stir for 1.5 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (250 ml) and

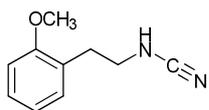
extracted with saturated aq. NaHCO_3 (3 x 100 ml). The organic layer was dried over MgSO_4 and the solvent was removed at reduced pressure to yield a yellow oil. The crude product was purified by column chromatography on silica with dichloromethane/ethyl acetate (1:1) as eluting solvent. This gave the desired product (**47**) as a colourless solid (3.18 g, 75%). mp 52-54 °C; (Found: C, 67.2; H, 8.6; N, 5.3%. $\text{C}_{14}\text{H}_{21}\text{NO}_3$ requires C, 66.9; H, 8.4; N, 5.6%); ν_{max} (nujol)/ cm^{-1} 3324 (NH), 1687 (C=O), 1602, 1588 (C=C aromatic); δ_{H} (300 MHz, CDCl_3) 1.35 (9H, s, *t*-butyl), 2.73 (2H, t, *J* 7 Hz, CH_2 -2), 3.27 (2H, t, *J* 7 Hz, CH_2 -1), 3.75 (3H, s, OCH_3), 4.55 (1H, br s, NH), 6.73-6.85 (2H, m, H-3', 5'), 7.01-7.19 (2H, m, H-4', 6'); δ_{C} (75.46 MHz, CDCl_3) 28.4 (CH_3), 30.8 (C-2), 40.8 (C-1), 55.2 (OCH_3), 79.1 (C-*t*-butyl), 110.3 (C-3'), 120.5 (C-5'), 127.4 (C-1'), 127.7 (C-4'), 130.6 (C-6'), 157.6 (C-2'), 156.1 (C=O); m/z (ES^+) 274 ((M+Na) $^+$, 100%).

Synthesis of 2-(2'-methoxyphenyl)ethylamine hydrochloride (**48**)⁶



A solution of *N*-(*tert*-butoxycarbonyl)-2-(2'-methoxyphenyl)ethylamine (**47**) (2.74 g, 10.9 mmol) in dry dioxane (3 ml) was treated with anhydrous HCl in 1,4-dioxane (4 M, 14 ml) at room temperature for 12 h. The excess of solvent was decanted off and the colourless solid was filtered off and washed with petroleum ether. This gave the pure product (**48**) (1.36 g, 68%). mp 139-140 °C, (lit.,⁶ 141-142°C); δ_{H} (300 MHz, D_2O) 2.89 (2H, t, *J* 7 Hz, CH_2 -2), 3.13 (2H, t, *J* 7 Hz, CH_2 -1), 3.76 (3H, s, OCH_3), 6.87-7.00 (2H, m, H-3', 5'), 7.14-7.19 (1H, m, H-4'), 7.22-7.30 (1H, m, H-6'); δ_{C} (75.46 MHz, D_2O) 27.8 (C-2), 39.6 (C-1), 55.3 (OCH_3), 111.4 (C-3'), 121.1 (C-5'), 124.7 (C-1'), 129.0 (C-4'), 130.8 (C-6'), 157.4 (C-2').

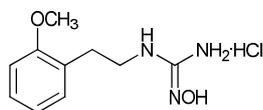
Synthesis of 2-(2'-methoxyphenyl)ethylcyanamide (**60**)



To a suspension of 2-(2'-methoxyphenyl)ethylamine hydrochloride (**48**) (1.30 g, 6.93 mmol) and sodium acetate (1.13 g, 13.8 mmol) in dry methanol (25 ml) was added dropwise a 3M solution

of BrCN in CH₂Cl₂ (2.31 ml, 6.93 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (30 ml). The crude product was extracted with CH₂Cl₂ (3 x 20 ml). The organic layer was washed with brine (20 ml), dried over MgSO₄ and evaporated at reduced pressure. The pure product was obtained by chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluting solvent. This gave the desired product (**60**) as a pale yellow oil (0.56 g, 46%). $\nu_{\max}(\text{neat})/\text{cm}^{-1}$ 3214 (NH), 2939, 2838 (CH aromatic), 2221 (C≡N), 1602, 1589 (C=C aromatic); δ_{H} (300 MHz, CDCl₃) 2.84 (2H, t, *J* 7 Hz, CH₂-2), 3.22-3.29 (2H, m, CH₂-1), 3.55 (1H, br s, NH), 3.76 (3H, s, OCH₃), 6.78-6.89 (2H, m, H-3', 5'), 7.06-7.11 (1H, m, H-4'), 7.14-7.22 (1H, m, H-6'); δ_{C} (75.46 MHz, CDCl₃) 31.0 (C-2), 46.4 (C-1), 55.3 (OCH₃), 110.5 (C-3'), 116.1 (C≡N), 120.8 (C-5'), 125.6 (C-1'), 128.5 (C-4'), 130.9 (C-6'), 157.5 (C-2'); m/z (ES⁺) 199.0854 ((M+Na)⁺. C₁₀H₁₂N₂ONa requires 199.0847).

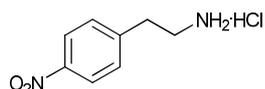
Synthesis of *N*-2-(2'-methoxyphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (**79**)



2-(2'-Methoxyphenyl)ethylcyanamide (**60**) (0.52 g, 2.95 mmol) was mixed with hydroxylamine hydrochloride (0.18 g, 2.65 mmol) in dry methanol (20 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 5 h under an argon atmosphere. After cooling, the solvent was removed and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtered off. Addition of CH₂Cl₂ and diethyl ether gave the pure product (**79**) as colourless crystals (0.65 g, 46%). mp 90-92 °C; (Found: C, 49.0; H, 6.6; N, 17.1%. C₁₀H₁₆ClN₃O₂ requires C, 48.9; H, 6.6; N, 17.1%); $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$ 3746, 3353 (NH), 1586 (C=C aromatic); δ_{H} (300 MHz, d₆-DMSO) 2.78 (2H, t, *J* 7 Hz, CH₂-2), 3.28-3.36 (2H, m, CH₂-1), 3.78 (3H, s, OCH₃), 6.84-6.99 (2H, m, H-3', 5'), 7.17-7.25 (2H, m, H-4',6'), 7.65 (2H, br s, NH₂), 7.85 (1H, t, *J* 6 Hz, NH), 9.79 (1H, br s, NH/OH), 10.38 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) 28.9 (C-2), 40.7 (C-1), 55.3

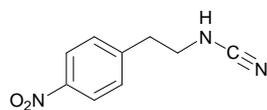
(OCH₃), 110.6 (C-3'), 120.2 (C-5'), 126.0 (C-1'), 127.9 (C-4'), 130.1 (C-6'), 157.1 (C-2'), 158.2 (C=NOH); m/z (ES⁺), 210 ((M+H)⁺, 100%).

Synthesis of 2-(4'-nitrophenyl)ethylamine hydrochloride (**97**)⁷



4-Nitrophenylacetonitrile (5.00 g, 30.8 ml) was dissolved in dry THF (60 ml) and BF₃·Et₂O complex (11.6 ml, 92.4 mmol) was added. The solution was cooled to 0 °C in an ice bath and treated with small portions of NaBH₄ (1.16 g, 30.8 mmol). Then, the ice bath was removed and the mixture left to stir at room temperature for 16 h. The solvent was evaporated at reduced pressure and the residue extracted between water (50 ml) and CH₂Cl₂ (3 × 60 ml). The organic layer was washed with a saturated solution of sodium bicarbonate (70 ml) and dried over MgSO₄. A red oil was obtained after solvent evaporation and dissolved in few drops of MeOH. Addition of CH₂Cl₂ and diethyl ether caused precipitation. The crude product was filtered off and recrystallized from CH₂Cl₂ to give the final product (**97**) as a yellow solid (0.54g, 11%). mp 199-200 °C, (lit.,⁷ 200 °C); δ_H (300 MHz, D₂O) 3.08 (2H, t, *J* 7 Hz, CH₂-2), 3.29 (2H, t, *J* 7 Hz, CH₂-1), 7.47 (2H, d, *J* 8 Hz, H-2', 6'), 8.16 (2H, d, *J* 8 Hz, H-3',5'); δ_C (75.46 MHz, d₆-DMSO) 33.0 (C-2), 40.3 (C-1), 124.4 (C-3', 5'), 130.3 (C-2', 6'), 145.1 (C-4'), 147.2 (C-1'); m/z (ES⁺) 237 (100%), 167 ((M+H)⁺, 20%).

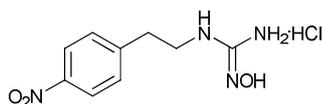
Synthesis of 2-(4'-nitrophenyl)ethylcyanamide (**70**)



To a suspension of 2-(4'-nitrophenyl)ethylamine hydrochloride (**101**) (0.49 g, 2.40 mmol) and sodium acetate (0.40 g, 4.80 mmol) in dry methanol (25 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (0.80 ml, 2.40 mmol). The mixture was stirred in an ice bath for 2 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water

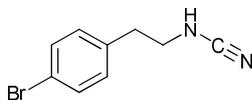
(30 ml). The crude product was extracted with ethyl acetate (3 x 20 ml). The organic layer was washed with brine (20 ml), dried over MgSO₄ and evaporated. The pure product was obtained by column chromatography on silica using ethyl acetate/diethyl ether (2:1) as the eluting solvent. This gave the desired product (**70**) as a yellow solid (0.21 g, 47%). mp 88 °C; ν_{\max} (neat)/cm⁻¹ 3200 (NH), 2220 (C≡N), 1597 (C=C aromatic), 1517, 1346; δ_{H} (300 MHz, d₆-DMSO) 2.92 (2H, t, *J* 7 Hz, CH₂-2), 3.21-3.28 (2H, m, CH₂-1), 6.85 (1H, t, *J* 6 Hz, NH), 7.53-7.58 (2H, m, H-2', 6'), 8.16-8.21 (2H, m, H-3', 5'); δ_{C} (75.46 MHz, CDCl₃) 35.1 (C-2), 45.2 (C-1), 116.9 (C≡N), 123.4 (C-3', 5'), 130.2 (C-2', 6'), 146.2 (C-4'), 146.8 (C-1'); m/z (ES⁺) 214.0594 ((M+Na)⁺). C₉H₉N₃O₂Na requires 214.0592; m/z (ES⁻) 190 ((M-H)⁻, 100%).

Synthesis of *N*-2-(4'-nitrophenyl)ethyl-*N*'-hydroxyguanidine hydrochloride (**89**)



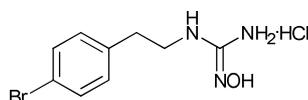
2-(4'-Nitrophenyl)ethylcyanamide (**70**) (0.21 g, 1.08 mmol) was mixed with hydroxylamine hydrochloride (0.08 g, 1.08 mmol) in dry methanol (15 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 8 h under an argon atmosphere. After cooling, the solvent was removed at reduced pressure and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Further purification was achieved by redissolving the crude product in minimum amount of methanol and the insoluble solid was filtered off. Addition of CH₂Cl₂ gave the pure product (**89**) as a pale yellow solid (0.28 g, 53%). mp 92-94 °C; ν_{\max} (nujol)/cm⁻¹ 3428, 3316 (NH), 1653, 1597 (C=C aromatic), 1511; δ_{H} (300 MHz, d₆-DMSO) 2.95 (2H, t, *J* 7 Hz, CH₂-2), 3.42-3.50 (2H, m, CH₂-1), 7.44 (2H, br s, NH₂), 7.55-7.60 (2H, m, H-2', 6'), 7.99 (1H, t, *J* 6 Hz, NH), 8.15-8.20 (2H, m, H-3',5'), 9.85 (1H, br s, NH/OH), 10.48 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) 34.7 (C-2), 41.6 (C-1), 123.8 (C-3', 5'), 130.6 (C-2', 6'), 146.6 (C-4'), 147.1 (C-1'), 158.5 (C=NOH); m/z (ES⁺) 225.0986 ((M+H)⁺). C₉H₁₃N₄O₃ requires 225.0988 (ES⁺), 225 ((M+H)⁺, 100%).

Synthesis of 2-(4'-bromophenyl)ethylcyanamide (**69**)



To a suspension of 2-(4'-bromophenyl)ethylamine hydrochloride (1.34 g, 5.69 mmol) and sodium acetate (0.93 g, 11.4 mmol) in dry methanol (30 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (1.90 ml, 5.69 mmol). The mixture was stirred in an ice bath for 2 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (30 ml). The crude product was extracted with CH₂Cl₂ (3 x 20 ml). The organic layer was washed with brine (20 ml), dried over MgSO₄ and evaporated. The pure product was obtained by chromatography on silica using petroleum ether/ethyl acetate (1:1) as the eluting solvent. This gave the desired product (**69**) as a colourless oil (0.71 g, 56%). $\nu_{\max}(\text{neat})/\text{cm}^{-1}$ 3215 (NH), 2928 (CH aromatic), 2221 (C≡N), 1637 (C=C aromatic); δ_{H} (300 MHz, CDCl₃) 2.83 (2H, t, *J* 7 Hz, CH₂-2), 3.25 (2H, dt, *J* 6 Hz, *J* 7 Hz, CH₂-1), 4.26 (1H, t, *J* 6 Hz, NH), 7.05-7.11 (2H, m, H-2', 6'), 7.40-7.46 (2H, m, H-3', 5'); δ_{C} (75.46 MHz, CDCl₃) 35.3 (C-2), 46.9 (C-1), 116.2 (C≡N), 120.8 (C-4'), 127.0 (C-2', 6'), 128.8 (C-2', 6'), 130.6 (C-3', 5'), 131.8 (C-3', 5'), 136.4 (C-1'); m/z (ES⁺) 246.9846 ((M+Na)⁺. C₉H₉N₂Na⁷⁹Br requires 246.9847), 248.9829 ((M+Na)⁺. C₉H₉N₂Na⁸¹Br requires 248.9826); m/z (ES⁻) 223 ((M-H)⁻, 100%), 225 ((M-H)⁻, 92%).

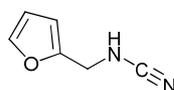
Synthesis of *N*-2-(4'-bromophenyl)ethyl-*N*'-hydroxyguanidine hydrochloride (**88**)



2-(4'-Bromophenyl)ethylcyanamide (**69**) (0.47 g, 2.09 mmol) was mixed with hydroxylamine hydrochloride (0.13 g, 1.88 mmol) in dry methanol (20 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 6 h under an argon atmosphere. After cooling, the solvent was removed at reduced pressure and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtered off.

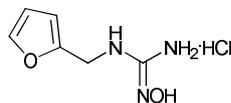
Addition of CH_2Cl_2 gave the pure product (**88**) as a colourless solid (0.37 g, 67%). mp 132-134 °C; (Found: C, 36.8; H, 4.2; N, 14.3%. $\text{C}_9\text{H}_{13}\text{BrClN}_3\text{O}$ requires C, 36.7; H, 4.4; N, 14.3%); $\nu_{\text{max}}(\text{nujol})/\text{cm}^{-1}$ 3125 (NH), 1590 (C=C aromatic); δ_{H} (300 MHz, d_6 -DMSO) 2.77 (2H, t, J 7 Hz, CH_2 -2), 3.35-3.43 (2H, m, CH_2 -1), 7.22-7.27 (2H, m, H-2', 6'), 7.47-7.52 (2H, m, H-3',5'), 7.70 (2H, br s, NH_2), 7.91 (1H, t, J 6 Hz, NH), 9.81 (1H, br s, NH/OH), 10.42 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d_6 -DMSO) 33.8 (C-2), 41.6 (C-1), 119.5 (C-4'), 131.1 (C-2', 3', 5', 6'), 137.7 (C-1'), 158.1 (C=NOH); m/z (ES^+) 260 ($(\text{M}+\text{H})^+$, 100%).

Synthesis of furfurylcyanamide (**71**)



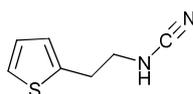
To a suspension of furfurylamine (3.00 g, 30.9 mmol) and sodium acetate (2.53 g, 30.9 mmol) in dry methanol (70 ml) was added dropwise a 3M solution of BrCN in CH_2Cl_2 (10.30 ml, 30.9 mmol). The mixture was stirred in an ice bath for 2 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (80 ml). The crude product was extracted with ethyl acetate (3 x 50 ml). The organic layer was dried over MgSO_4 . Evaporation of the solvent at reduced pressure gave the desired product (**71**) as a yellow oil (2.95 g, 78%). $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$ 3205 (NH), 2925 (CH aromatic), 2227 ($\text{C}\equiv\text{N}$), 1601 (C=C aromatic); δ_{H} (300 MHz, CDCl_3) 1.70 (1H, br s, NH), 4.20 (2H, d, J 6 Hz, CH_2 -1), 6.32-6.37 (2H, m, H-3', 4'), 7.41 (1H, t, J 1.4 Hz, H-5'); δ_{C} (75.46 MHz, CDCl_3) 42.9 (C-1), 115.2 ($\text{C}\equiv\text{N}$), 109.2 (C-3'), 110.6 (C-4'), 143.3 (C-5'), 149.4 (C-2'); m/z (ES^+) 145.0375 ($(\text{M}+\text{Na})^+$). $\text{C}_6\text{H}_6\text{N}_2\text{ONa}$ requires 145.0378); m/z (ES^-) 121 ($(\text{M}-\text{H})^-$, 100%).

Synthesis of *N*-furfuryl-*N'*-hydroxyguanidine hydrochloride (**90**)



Furfurylcyanamide (**71**) (1.63 g, 13.3 mmol) was mixed with hydroxylamine hydrochloride (0.83 g, 12.0 mmol) in dry methanol (40 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 6 h under an argon atmosphere. After cooling, the solvent was removed and the desired product was crystallized from ethyl acetate and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtered off. Addition of ethyl acetate gave the pure product (**90**) as a pale brown solid (0.43 g, 20%). mp 108-110 °C; (Found: C, 37.7; H, 4.9; N, 21.6%. $C_6H_{10}ClN_3O_2$ requires C, 37.6; H, 5.2; N, 21.9%); ν_{\max} (nujol)/ cm^{-1} 3333 (NH), 1579 (C=C); δ_H (300 MHz, d_6 -DMSO) 4.43 (2H, d, J 6 Hz, CH_2 -1), 6.38-6.44 (2H, m, H-3', 4'), 7.62-7.64 (1H, m, H-5') 7.86 (2H, br s, NH_2), 8.29 (1H, t, J 6 Hz, NH), 9.91 (1H, br s, NH/OH), 10.65 (1H, br s, NH/OH); δ_C (75.46 MHz, d_6 -DMSO) 37.3 (C-1), 108.0 (C-3'), 110.5 (C-4'), 142.8 (C-5'), 150.1 (C-2'), 158.1 (C=NOH); m/z (ES^+) 156 (($M+H$)⁺, 100%).

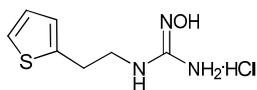
Synthesis of thiophene-2-ethylcyanamide (**72**)



To a suspension of thiophene-2-ethylamine (2.50 g, 19.6 mmol) and sodium acetate (2.42 g, 29.5 mmol) in dry methanol (50 ml) was added dropwise a 3M solution of BrCN in CH_2Cl_2 (6.55 ml, 19.6 mmol). The mixture was stirred in an ice bath for 2 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (80 ml). The crude product was extracted with CH_2Cl_2 (3 x 40 ml). The organic layer was dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The pure product was obtained by chromatography on silica using petroleum ether/ethyl acetate (1:1) as the eluting solvent. This gave the desired product (**72**) as a pale yellow oil (2.32 g, 78%). ν_{\max} (neat)/ cm^{-1} 3212 (NH), 2934

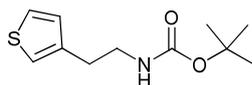
(CH aromatic), 2222 (C≡N), 1610 (C=C aromatic); δ_{H} (300 MHz, CDCl₃) 3.13 (2H, t, J 7 Hz, CH₂-2), 3.33 (2H, quartet, J 7 Hz, CH₂-1), 3.91 (1H, br s, NH), 6.86-7.00 (2H, m, H-3', 5'), 7.16-7.22 (1H, m, H-4'); δ_{C} (75.46 MHz, CDCl₃) 30.2 (C-2), 42.9 (C-1), 115.8 (C≡N), 124.6 (C-5'), 126.1 (C-3'), 127.3 (C-4'), 139.3 (C-2'); m/z (ES⁺) 175.0307 ((M+Na)⁺. C₇H₈N₂NaS requires 175.0306); m/z (ES⁻) 151 ((M-H)⁻, 100%).

Synthesis of *N*-(thiophene-2-ethyl)-*N'*-hydroxyguanidine hydrochloride (**91**)



Thiophene-2-ethylcyanamide (**72**) (2.09 g, 13.7 mmol) was mixed with hydroxylamine hydrochloride (0.86 g, 12.3 mmol) in dry methanol (40 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 7 h under an argon atmosphere. After cooling, the solvent was removed and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtered off. Addition of CH₂Cl₂ gave the pure product (**91**) as a pale yellow solid (1.25 g, 46%). mp 100-102 °C; ν_{max} (nujol)/cm⁻¹ 3393 (NH), 1578 (C=C); (Found: C, 37.5; H, 5.1; N, 18.8%. C₇H₁₂ClN₃OS requires C, 37.9; H, 5.5; N, 18.9%; δ_{H} (300 MHz, d₆-DMSO) 3.01 (2H, t, J 7 Hz, CH₂-2), 3.38-3.47 (2H, m, CH₂-1), 6.94-6.98 (2H, m, H-3', 4'), 7.36 (1H, br t, J 3 Hz, H-5'), 7.75 (2H, br s, NH₂), 8.00 (1H, t, J 6 Hz, NH), 9.89 (1H, br s, NH/OH), 10.55 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) 28.7 (C-2), 42.0 (C-1), 124.3 (C-5'), 125.7 (C-3'), 127.0 (C-4'), 140.2 (C-2'), 158.1 (C=NOH); m/z (ES⁺) 186 ((M+H)⁺, 100%).

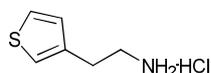
Synthesis of *N*-(*tert*-butoxycarbonyl)-thiophene-3-ethylamine (**53**)



To a stirred solution of thiophene-3-acetonitrile (2.00 g, 16.2 mmol) in dry methanol (100 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc₂O) (7.09 g, 32.5 mmol) and

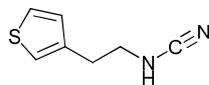
NiCl₂ · 6H₂O (0.39 g, 1.62 mmol). NaBH₄ (4.30 g, 114 mmol) was then added in small portions over 4 h. The resulting reaction mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and stirred for a further 16 h, at which point diethylenetriamine (1.68 ml, 16.2 mmol) was added. The mixture was allowed to stir for 1.5 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (250 ml) and extracted with saturated aq. NaHCO₃ (3 x 80 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to yield a yellow oil. The crude product was purified by column chromatography on silica with petroleum ether/diethyl ether (1:2) as eluting solvent. This gave the desired product (**53**) as a colourless oil (1.99 g, 54%). $\nu_{\max}(\text{neat})/\text{cm}^{-1}$ 3382 (NH), 1726 (C=O); δ_{H} (300 MHz, CDCl₃) 1.45 (9H, s, *t*-butyl), 2.84 (2H, t, *J* 7 Hz, CH₂-2), 3.40 (2H, quartet, *J* 7 Hz, CH₂-1), 4.59 (1H, br s, NH), 6.95-7.04 (2H, m, H-2', 4'), 7.27-7.32 (1H, m, H-5'); δ_{C} (75.46 MHz, CDCl₃) 28.4 (CH₃), 30.7 (C-2), 41.1 (C-1), 79.3 (C-*t*-butyl), 121.3 (C-2'), 125.8 (C-5'), 128.1 (C-4'), 139.3 (C-3'), 156.0 (C=O); m/z (ES⁺) 250.0873 ((M+Na)⁺. C₁₁H₁₇NO₂NaS requires 250.0878).

Synthesis of thiophene-3-ethylamine hydrochloride (**54**)⁸



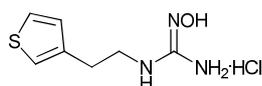
A solution of *N*-(*tert*-butoxycarbonyl)-thiophene-3-ethylamine (**53**) (1.90 g, 8.34 mmol) in dry diethyl ether (3 ml) was treated with anhydrous HCl in 1,4-dioxane (4 M, 10 ml) at room temperature for 10 h. The excess solvent was decanted off and the colourless solid was filtered off and washed with diethyl ether. This gave the pure product (**54**) (1.18 g, 87%). mp 198-200 °C; δ_{H} (300 MHz, D₂O) 2.96 (2H, t, *J* 7 Hz, CH₂-2), 3.20 (2H, t, *J* 7 Hz, CH₂-1), 7.02 (1H, dd, *J* 2, *J* 3 Hz, H-4'), 7.18-7.21 (1H, m, H-2'), 7.40 (1H, quartet, *J* 2.5 Hz, H-5'); δ_{C} (75.46 MHz, D₂O) 27.3 (C-2), 40.0 (C-1), 123.0 (C-2'), 127.0 (C-5'), 127.9 (C-4'), 136.7 (C-3'); m/z (ES⁺) 128.0531 ((M+H)⁺. C₆H₁₀NS requires 128.0534).

Synthesis of thiophene-3-ethylcyanamide (**73**)



To a suspension of thiophene-3-ethylamine hydrochloride (**54**) (1.13 g, 6.90 mmol) and sodium acetate (1.13 g, 13.8 mmol) in dry methanol (30 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (2.30 ml, 6.90 mmol). The mixture was stirred in an ice bath for 2 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (50 ml). The crude product was extracted with CH₂Cl₂ (3 x 25 ml). The organic layer was dried over MgSO₄ and the solvent removed under reduced pressure. The pure product was obtained by chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluting solvent. This gave the desired product (**73**) as a yellow oil (1.05 g, 40%). $\nu_{\max}(\text{neat})/\text{cm}^{-1}$ 3375 (NH), 3099 (CH aromatic), 2221vs (C≡N), 1606 (C=C aromatic); δ_{H} (300 MHz, CDCl₃) 2.96 (2H, t, *J* 7 Hz, CH₂-2), 3.33 (2H, dt, *J* 7, *J* 6 Hz, CH₂-1), 3.48 (1H, br s, NH), 6.93-6.98 (1H, m, H-4'), 7.05-7.10 (1H, m, H-2'), 7.30-7.35 (1H, m, H-5'); δ_{C} (75.46 MHz, CDCl₃) 30.4 (C-2), 46.8 (C-1), 122.3 (C-2'), 126.6 (C-5'), 127.8 (C-4'), 137.3 (C-3'); m/z (ES⁺) 153.0489 ((M+H)⁺). C₇H₉N₂S requires 153.0486; m/z (ES) 151 ((M-H), 100%).

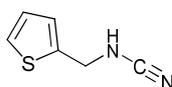
Synthesis of *N*-thiophene-3-ethyl-*N*'-hydroxyguanidine hydrochloride (**92**)



Thiophene-3-ethylcyanamide (**73**) (0.38 g, 2.50 mmol) was mixed with hydroxylamine hydrochloride (0.16 g, 2.25 mmol) in dry methanol (20 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 6 h under an argon atmosphere. After cooling, the solvent was removed and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtered off. Addition of CH₂Cl₂ gave the pure product (**92**) as an off-white solid (0.55 g, 51%). mp 114-116 °C; (Found: C, 37.9; H, 5.3; N, 18.8%. C₇H₁₂ClN₃OS requires C, 37.9; H, 5.5; N, 18.9%);

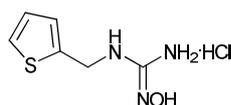
ν_{\max} (nujol)/ cm^{-1} 3395 (NH), 1582 (C=C); δ_{H} (300 MHz, d_6 -DMSO) 2.81 (2H, t, J 7 Hz, CH_2 -2), 3.36-3.45 (2H, m, CH_2 -1), 7.08 (1H, dd, J 2, J 3 Hz, H-4'), 7.27-7.30 (1H, m, H-2'), 7.47 (1H, dd, J 2, J 3 Hz, H-5') 7.74 (2H, br s, NH_2), 7.97 (1H, t, J 6 Hz, NH), 9.86 (1H, br s, NH/OH), 10.50 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d_6 -DMSO) 29.1 (C-2), 41.3 (C-1), 121.8 (C-2'), 125.9 (C-5'), 128.5 (C-4'), 138.5 (C-3'), 158.1 (C=NOH); m/z (ES^+) 186 ((M+H)⁺, 100%).

Synthesis of thiophene-2-methylcyanamide (75)



To a suspension of thiophene-2-methylamine (1.50 g, 13.2 mmol) and sodium acetate (2.17 g, 26.4 mmol) in dry methanol (40 ml) was added dropwise a 3M solution of BrCN in CH_2Cl_2 (4.42 ml, 13.2 mmol). The mixture was stirred in an ice bath for 2 h, then the ice bath was removed and reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (50 ml). The crude product was extracted with CH_2Cl_2 (3 x 25 ml). The organic layer was dried over MgSO_4 . The pure product was obtained by chromatography on silica using diethyl ether/ethyl acetate (2:1) as the eluting solvents. This gave the desired product (75) as a pale yellow, low melting solid (1.37 g, 75%). (Found: C, 51.8; H, 4.4; N, 19.9%. $\text{C}_6\text{H}_6\text{N}_2\text{S}$ requires C, 52.2; H, 4.4; N, 20.3%); ν_{\max} (neat)/ cm^{-1} 3931, 3815 (NH), 2224 ($\text{C}\equiv\text{N}$); δ_{H} (300 MHz, CDCl_3) 4.30 (1H, br s, NH), 4.38 (2H, d, J 7 Hz, CH_2 -1), 6.96-7.00 (1H, m, H-3'), 7.03-7.07 (1H, m, H-4'), 7.29-7.32 (1H, m, H-5'); δ_{C} (75.46 MHz, CDCl_3) 45.2 (C-1), 115.9 ($\text{C}\equiv\text{N}$), 126.9 (C-5'), 126.7 (C-3'), 127.6 (C-4'), 138.9 (C-2'); m/z (ES^+) 139.0325 ((M+H)⁺. $\text{C}_7\text{H}_6\text{N}_2\text{S}$ requires 139.0330); m/z (ES^+) 97 (100%).

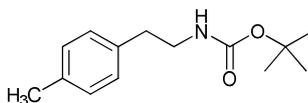
Synthesis of *N*-thiophene-2-methyl-*N'*-hydroxyguanidine hydrochloride (93)



Thiophene-2-methylcyanamide (75) (1.28 g, 9.25 mmol) was mixed with hydroxylamine hydrochloride (0.58 g, 8.32 mmol) in dry methanol (40 ml) and potassium carbonate (0.1 equiv)

was added as a catalyst. The reaction mixture was heated under reflux for 6 h under an argon atmosphere. After cooling, the solvent was removed and the desired product was crystallized from CH_2Cl_2 and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtrated off. Addition of CH_2Cl_2 gave the pure product (**93**) as a dark yellow solid (0.65 g, 34%). mp 108-110 °C; (Found: C, 35.0; H, 4.8; N, 20.0%. $\text{C}_6\text{H}_{10}\text{ClN}_3\text{OS}$ requires C, 34.7; H, 4.8; N, 20.2%); $\nu_{\text{max}}(\text{nujol})/\text{cm}^{-1}$ 3367 (NH), 1576 (C=C); δ_{H} (300 MHz, d_6 -DMSO) 4.62 (2H, d, J 7 Hz, CH_2 -1), 6.97-7.01 (1H, m, H-3'), 7.08-7.12 (1H, m, H-4'), 7.47 (1H, dd, J 2, J 3 Hz H-5'), 7.88 (2H, br s, NH_2), 8.41 (1H, t, J 6 Hz, NH), 9.92 (1H, br s, NH/OH), 10.68 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d_6 -DMSO) 125.8 (C-5'), 126.4 (C-3'), 126.8 (C-4'), 139.8 (C-2'), 157.9 (C=NOH); m/z (ES^+) 172 ((M+H)⁺, 100%).

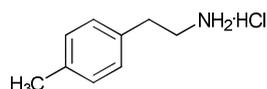
Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(4'-methylphenyl)ethylamine (**49**)



To a stirred solution of 4-methylphenylacetonitrile (1.00 g, 7.62 mmol) in dry methanol (50 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc_2O) (3.33 g, 15.2 mmol) and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.18 g, 0.76 mmol). NaBH_4 (2.02 g, 53.4 mmol) was then added in small portions over 1.5 h. The resulting reaction mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and stirred for further 16 h, at which point diethylenetriamine (0.82 ml, 7.62 mmol) was added. The mixture was allowed to stir for 1.5 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (150 ml) and extracted with saturated aq. NaHCO_3 (3 x 50 ml). The organic layer was dried over MgSO_4 and the solvent was removed at reduced pressure to yield yellow oil. The crude product was purified by column chromatography on silica with petroleum ether/diethyl ether (1:1) as eluting solvent. This gave the desired product (**49**) as colourless crystals (1.79 g, 72%). mp 38-40 °C; (Found: C, 71.5; H, 9.3; N, 5.9%. $\text{C}_{14}\text{H}_{21}\text{NO}_2$ requires C, 71.5; H, 9.0; N, 5.9%); $\nu_{\text{max}}(\text{nujol})/\text{cm}^{-1}$ 3372 (NH), 1899 (C=O), 1604 (C=C aromatic); δ_{H} (300 MHz, CDCl_3) 1.43 (9H, s, *t*-butyl), 2.33 (3H, s, CH_3), 2.75 (2H, t, J 7 Hz, CH_2 -2), 3.29-3.41 (2H, m, CH_2 -1), 4.54 (1H, br s, NH), 7.05-7.14 (4H, m, Ar-H); δ_{C} (75.46 MHz, CDCl_3) 21.0 (CH_3), 28.4 (CH_3), 35.8 (C-2), 41.9 (C-1), 79.1

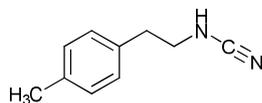
(*t*-butyl), 128.7 (C-2', 6'), 129.3 (C-3', 5'), 135.8 (C-4'), 135.9 (C-1'), 155.9 (C=O); m/z (ES⁺) 258 ((M+Na)⁺, 100%).

Synthesis of 2-(4'-methylphenyl)ethylamine hydrochloride (**50**)⁵



A solution of *N*-(*tert*-butoxycarbonyl)-2-(4'-methylphenyl)ethylamine (**49**) (1.08, 3.11 ml) in dry diethyl ether (2 ml) was treated with anhydrous HCl in 1,4-dioxane (4 M, 4.0 ml) at room temperature for 12 h. The excess of solvent was decanted off and the colourless solid was filtered off and washed with petroleum ether. This gave the pure product (**50**) (0.53 g, 100%). mp 216-217 °C, (lit.,⁵ 217-218 °C); δ_{H} (300 MHz, *d*₄-methanol) 2.31 (3H, s, CH₃), 2.92 (2H, t, *J* 7 Hz, CH₂-2), 3.12-3.18 (2H, m, CH₂-1), 7.14-7.16 (4H, br s, Ar-H); δ_{C} (75.46 MHz, *d*₄-methanol) 21.1 (CH₃), 34.2 (C-2), 42.1 (C-1), 129.7 (C-2', 6'), 130.6 (C-3', 5'), 134.8 (C-4'), 138.0 (C-1').

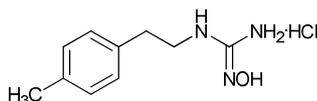
Synthesis of 2-(4'-methylphenyl)ethylcyanamide (**61**)



To a suspension of 2-(4'-methoxyphenyl)ethylamine hydrochloride (**50**) (0.48 g, 2.78 mmol) and sodium acetate (0.46 g, 5.56 mmol) in dry methanol (20 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (0.93 ml, 2.78 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (30 ml). The crude product was extracted with CH₂Cl₂ (3 x 20 ml). The organic layer was washed with brine (20 ml), dried over MgSO₄ and evaporated. The pure product was obtained by chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluting solvent. This gave the desired product (**61**) as a colourless oil (0.18 g, 39%). ν_{max} (neat)/cm⁻¹ 3228, 3021 (NH), 2220 (C≡N), 1616, 1576 (C=C); δ_{H} (400 MHz, CDCl₃) 2.33 (3H, s, CH₃), 2.87 (2H, t, *J* 7 Hz, CH₂-2), 3.32 (2H, t, *J* 7 Hz, CH₂-1), 3.44 (1H, br s, NH), 7.08-7.16 (4H, m, Ar-H); δ_{C} (100 MHz,

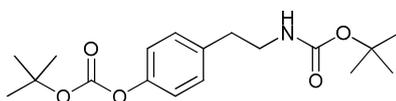
CDCl₃) 21.1 (CH₃), 35.4 (C-2), 47.6 (C-1), 115.3 (C≡N), 128.7 (C-2', 6'), 129.6 (C-3', 5'), 134.0 (C-4'), 136.7 (C-1'); *m/z* (ES⁺) 183.0901 ((M+Na)⁺. C₁₀H₁₂N₂Na requires 183.0898); *m/z* (ES⁻) 159 ((M-H)⁻, 100%).

Synthesis of *N*-2-(4'-methylphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (**80**)



2-(4'-Methylphenyl)ethylcyanamide (**61**) (0.15 g, 0.92 mmol) was mixed with hydroxylamine hydrochloride (0.06 g, 0.83 mmol) in dry methanol (15 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 7 h under an argon atmosphere. After cooling, the solvent was removed at reduced pressure and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtered off. Addition of CH₂Cl₂ gave the pure product (**80**) as a colourless solid (63 mg, 30%). mp 118-120 °C; (Found: C, 52.5; H, 6.9; N, 18.4%. C₁₀H₁₆ClN₃O requires C, 52.3; H, 7.0; N, 18.3%); ν_{\max} (nujol)/cm⁻¹ 3306, 3110 (NH), 1590 (C=C), 1517; δ_{H} (400 MHz, d₆-DMSO) 2.26 (3H, s, CH₃), 2.74 (2H, t, *J* 7 Hz, CH₂-2), 7.13 (4H, 2 x d, *J* 8, Ar-H), 7.69 (2H, br s, NH₂), 7.89 (1H, t, *J* 6 Hz, NH), 9.82 (1H, br s, NH/OH), 10.36 (1H, br s, NH/OH); δ_{C} (100 MHz, d₆-DMSO) 21.1 (CH₃), 34.5 (C-2), 42.5 (C-1), 129.2 (C-2', 6'), 129.4 (C-3', 5'), 135.6 (C-4'), 135.8 (C-1'), 158.6 (C=NOH); *m/z* (ES⁺) 194 ((M+H)⁺, 100%).

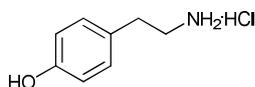
Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(4'-hydroxyphenyl)ethylamine (**51**)



To a stirred solution of 4-hydroxyphenylacetonitrile (1.52 g, 11.4 mmol) in dry methanol (50 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc₂O) (4.99 g, 22.8 mmol) and NiCl₂ · 6H₂O (0.27 g, 1.14 mmol). NaBH₄ (3.03 g, 80.0 mmol) was then added in small portions over 2 h. The resulting reaction mixture, containing a finely divided black precipitate, was

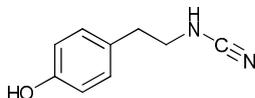
allowed to warm to room temperature and stirred for further 16 h, at which point diethylenetriamine (1.18 ml, 11.4 mmol) was added. The mixture was allowed to stir for 1.5 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (250 ml) and extracted with saturated aq. NaHCO₃ (3 x 80 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to yield a yellow oil. The crude product was purified by column chromatography on silica with petroleum ether/diethyl ether (2:3) as eluting solvent. This gave the desired product (**51**) as a colourless solid (2.11 g, 55%). mp 68 °C; (Found: C, 64.2; H, 8.2; N, 4.0%. C₁₈H₂₇NO₅ requires C, 64.1; H, 8.1; N, 4.1%); ν_{\max} (nujol)/cm⁻¹ 3408 (NH), 1752 (C=O), 1595 (C=C, aromatic); δ_{H} (300 MHz, CDCl₃) 1.43 (9H, s, carbonate, *t*-butyl), 1.55 (9H, s, carbamate, *t*-butyl), 2.78 (2H, t, *J* 7 Hz, CH₂-2), 3.30-3.40 (2H, m, CH₂-1), 4.54 (1H, br s, NH), 7.06-7.13 (2H, m, H-3', 5'), 7.15-7.22 (2H, m, H-2', 6'); δ_{C} (75.46 MHz, CDCl₃) 27.7 (CH₃), 28.4 (CH₃), 35.6 (C-2), 41.7 (C-1), 65.9 (C-(CH₃)₃), 83.5 (C-(CH₃)₃), 121.4 (C-3', 5'), 129.7 (C-2', 6'), 136.5 (C-1'), 149.6 (O-C=O), 152.1 (C-4'), 155.8 (N-C=O); m/z (ES⁺) 360 ((M+H)⁺, 100%).

Synthesis of 2-(4'-hydroxyphenyl)ethylamine hydrochloride (**52**)⁹



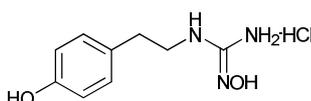
A solution of *N*-(*tert*-butoxycarbonyl)-2-(4'-hydroxyphenyl)ethylamine (**51**) (2.11 g, 6.24 mmol) in dry diethyl ether (3 ml) was treated with anhydrous HCl in dioxane (4 M, 8.0 ml) at room temperature for 12 h. The excess solvent was decanted off and the colourless solid was filtered off and washed with petroleum ether. The solid was then dissolved in dry 1,4-dioxane (3 ml) and treated again with anhydrous HCl in 1,4-dioxane (4 M, 4.0 ml) at room temperature for 12 h. This gave the pure product (**52**) as a colourless solid (0.77 g, 71%). mp 270-272 °C (dec.), (lit.⁹, 270-272 °C); δ_{H} (300 MHz, d₆-DMSO) 2.72-2.80 (2H, m, CH₂-2), 2.89-2.96 (2H, m, CH₂-1), 6.68-6.75 (2H, m, H-3', 5'), 7.00-7.06 (2H, m, H-2',6'), 8.05 (3H, br s, NH₃⁺), 9.37 (1H, s, OH); δ_{C} (75.46 MHz, d₆-DMSO) 32.1 (C-2), 38.6 (C-1), 115.3 (C-3', 5'), 127.2 (C-1'), 129.5 (C-2', 6'), 156.1 (C-4').

Synthesis of 2-(4'-hydroxyphenyl)ethylcyanamide (**62**)



To a suspension of 2-(4'-hydroxyphenyl)ethylamine hydrochloride (**52**) (0.77 g, 4.42 mmol) and sodium acetate (0.72 g, 8.84 mmol) in dry methanol (20 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (1.47 ml, 4.42 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (30 ml). The crude product was extracted with CH₂Cl₂ (3 x 20 ml). The organic layer was washed with brine (20 ml), dried over MgSO₄ and the solvent was removed at reduced pressure. This gave the desired product (**62**) as an off-white solid (0.45 g, 63%). mp 102-103 °C; $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$ 3173 (NH), 2235 (C≡N), 1889, 1615, 1598 (C=C); δ_{H} (300 MHz, d₆-DMSO) 2.64 (2H, t, *J* 7 Hz, CH₂-2), 3.04-3.11 (2H, m, CH₂-1), 6.64-6.71 (2H, m, H-3', 5'), 6.75 (1H, t, *J* 6 Hz, NH), 6.98-7.05 (2H, m, H-2', 6'), 9.22 (1H, s, OH); δ_{C} (75.46 MHz, d₆-DMSO) 35.1 (C-2), 46.8 (C-1), 115.6 (C-3', 5'), 117.7 (C≡N), 128.7 (C-1'), 130.1 (C-2', 6'), 156.3 (C-4'); *m/z* (ES⁺) 185.0695 ((M+Na)⁺). C₉H₁₀N₂ONa requires 185.0691; *m/z* (ES⁻) 161 ((M-H)⁻, 100%).

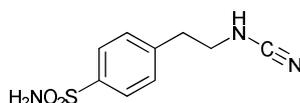
Synthesis of *N*-2-(4'-hydroxyphenyl)ethyl-*N*'-hydroxyguanidine hydrochloride (**81**)



2-(4'-Hydroxyphenyl)ethylcyanamide (**62**) (0.14 g, 0.88 mmol) was mixed with hydroxylamine hydrochloride (0.06 g, 0.78 mmol) in dry methanol (15 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 6 h under an argon atmosphere. After cooling, the solvent was removed at reduced pressure and the desired product was crystallized from ethyl acetate and CH₂Cl₂. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtered off. Addition of ethyl acetate gave the pure product (**81**) as a colourless solid (81 mg, 40%). mp 120-122 °C; δ_{H} (300 MHz, d₆-DMSO) 2.67 (2H, t, *J* 7 Hz, CH₂-2), 3.26-3.73 (2H, m, CH₂-1),

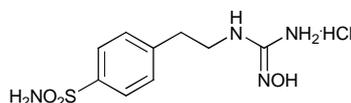
6.66-6.73 (2H, m, H-3', 5'), 7.01-7.08 (2H, m, H-2',6'), 7.65 (2H, br s, NH₂), 7.81 (1H, t, *J* 6 Hz, NH), 9.29 (1H, s, phenyl-OH), 9.77 (1H, br s, NH/ OH), 10.38 (1H, br s, NH/OH); δ_C (75.46 MHz, d₆-DMSO) 33.6 (C-2), 42.3 (C-1), 115.1 (C-3', 5'), 128.2 (C-1'), 129.6 (C-2', 6'), 155.9 (C-4'), 158.1 (C=NOH); m/z (ES⁺) 196.1083 ((M+H)⁺). C₉H₁₄N₃O₂ requires 196.1086; m/z (ES⁺) 196 ((M+H)⁺, 100%).

Synthesis of 2-(4'-(sulfamoyl)phenyl)ethylcyanamide (**66**)



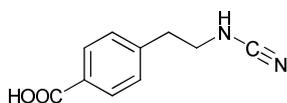
To a suspension of 2-[4'-(sulfamoyl)phenyl]ethylamine (2.00 g, 9.99 mmol) and sodium acetate (1.64 g, 20.0 mmol) in dry methanol (50 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (3.33 ml, 9.99 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (50 ml). The crude product was extracted with CH₂Cl₂ (3 x 30 ml). The organic layer was washed with brine (30 ml), dried over MgSO₄ and the solvent was removed at reduced pressure. This gave the desired product (**66**) as a colourless oil (1.63 g, 73%). ν_{\max} (neat)/cm⁻¹ 3340 (NH), 2220 (C≡N), 1598 (C=C), 1332, 1157 (SO₂NH₂); δ_H (400 MHz, d₆-DMSO) 2.85 (2H, t, *J* 7 Hz, CH₂-2), 3.17-3.25 (2H, m, CH₂-1), 6.83 (1H, t, *J* 6 Hz, NH), 7.31 (2H, br s, NH₂), 7.41-7.49 (2H, m, H-2', 6'), 7.73-7.78 (2H, m, H-3', 5'); δ_C (100 MHz, d₆-DMSO) 35.1 (C-2), 45.5 (C-1), 117.0 (C≡N), 129.1 (C-3', 5'), 129.3 (C-2', 6'), 142.3 (C-4'), 142.5 (C-1'); m/z (ES⁺) 248.0473 ((M+Na)⁺). C₉H₁₁N₃O₂NaS requires 248.0470).

**Synthesis of *N*-2-[4'-(sulfamoyl)phenyl]ethyl-
N'-hydroxyguanidine hydrochloride (**85**)**



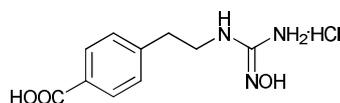
2-[4'-(Sulfamoyl)phenyl]ethylcyanamide (**66**) (1.50 g, 6.66 mmol) was mixed with hydroxylamine hydrochloride (0.42 g, 5.99 mmol) in dry methanol (40 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 7 h under an argon atmosphere. After the completion, the mixture was cooled in an ice bath and di-*tert*-butyl dicarbonate (1.45 g, 6.66 mmol) and potassium carbonate (0.92 g, 6.66 mmol) were added. The solution was left to stir for an hour. The solvent was then removed at reduced pressure and the residue was partitioned between water (20 ml) and ethyl acetate (40 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to give a yellow oil. Further purification was achieved by column chromatography of the protected hydroxyguanidine (petroleum: diethyl ether: ethyl acetate-gradient). The product was then dissolved in dry THF (10 ml) and treated with anhydrous HCl in 1,4-dioxane (4 M, 10.0 ml) at room temperature for 12 h. The solid was filtered off and washed with diethyl ether to give the pure product (**85**) as a colourless solid (0.361 g, 21%); mp 136-138 °C; (Found: C, 37.0; H, 5.3; N, 18.7%. C₉H₁₅ClN₄O₃S requires C, 36.7; H, 5.1; N, 19.0%); ν_{\max} (nujol)/cm⁻¹ 3478, 3311, 3275, 3156 (NH, OH), 1646, 1597 (C=C); δ_{H} (300 MHz, d₆-DMSO) 2.87 (2H, t, *J* 7 Hz, CH₂-2), 3.36-3.47 (2H, m, CH₂-1), 7.32 (2H, br s, NH₂), 7.42-7.48 (2H, m, H-2', 6'), 7.69 (2H, br s, NH₂), 7.73-7.79 (2H, m, H-3', 5'), 7.87 (1H, br s, NH), 9.75 (1H, br s, NH/OH), 10.34 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) 34.6 (C-2), 42.0 (C-1), 126.1 (C-3', 5'), 129.7 (C-2', 6'), 142.8 (C-4'), 142.9 (C-1'), 158.6 (C=NOH); *m/z* (ES⁺) 259 ((M+H)⁺, 100%).

Synthesis of 2-(4'-carboxyphenyl)ethylcyanamide (**65**)



To a suspension of 4-(2-aminoethyl)benzoic acid (2.00 g, 9.92 mmol) and sodium acetate (1.63 g, 19.94 mmol) in dry methanol (50 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (3.31 ml, 9.92 mmol). The mixture was stirred in an ice bath for 2 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in water (50 ml). The crude product was extracted with CH₂Cl₂ (3 x 30 ml). The organic layer was washed with brine (30 ml), dried over MgSO₄ and the solvent was removed at reduced pressure. The crude product was purified by column chromatography on silica with petroleum ether/diethyl ether (1:2) as eluting solvent. This gave the desired product (**65**) as a pale yellow solid (0.77 g, 41%). mp 114-116 °C; (Found: C, 62.7; H, 5.2; N, 14.6%. C₁₀H₁₀N₂O₂ requires C, 63.1; H, 5.3; N, 14.7%); ν_{\max} (nujol)/cm⁻¹ 3325 (NH), 2676 (CH aromatic), 2209 (C≡N), 1700, 1684 (C=O), 1611, 1576 (C=); δ_{H} (400 MHz, d₆-DMSO) 2.84 (2H, t, *J* 7 Hz, CH₂-2), 3.20 (2H, t, *J* 7 Hz, CH₂-1), 6.85 (1H, br s, NH), 7.34-7.38 (2H, m, H-2', 6'), 7.84-7.90 (2H, m, H-3', 5'); δ_{C} (100 MHz, d₆-DMSO) 35.8 (C-2), 46.0 (C-1), 117.5 (C≡N), 129.3 (C-2', 6'), 129.8 (C-3', 5'), 129.3 (C-4'), 143.8 (C-1'), 167.8 (C=O); *m/z* (ES⁺) 191.0824 ((M+H)⁺. C₁₀H₁₁N₂O₂ requires 191.0821).

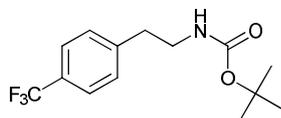
Synthesis of N-2-(4'-carboxyphenyl)ethyl-N'-hydroxyguanidine hydrochloride (**84**)



2-(4'-Carboxyphenyl)ethylcyanamide (**65**) (0.21 g, 1.11 mmol) was mixed with hydroxylamine hydrochloride (0.07 g, 1.00 mmol) in dry methanol (20 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 7 h under an argon atmosphere. After cooling, the solvent was removed at reduced pressure and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtered off.

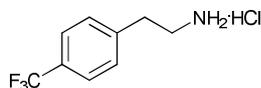
Addition of CH_2Cl_2 gave the pure product (**84**) as a colourless solid (0.21 mg, 83%). mp 168-170 °C; ν_{max} (nujol)/ cm^{-1} 3379 (NH), 1693 (C=O), 1660, 1613 (C=C); δ_{H} (300 MHz, d_6 -DMSO) 2.87 (2H, t, J 7 Hz, CH_2 -2), 3.42 (2H, quartet, J 7 Hz, CH_2 -1), 7.37-7.42 (2H, m, H-2', 6'), 7.71 (2H, br s, NH_2), 7.85-7.91 (2H, m, H-3', 5'), 7.94 (1H, br t, J 6 Hz, NH), 9.81 (1H, br s, NH/OH), 10.42 (1H, br s, NH/OH); δ_{C} (75.46 MHz, d_6 -DMSO) 34.4 (C-2), 41.5 (C-1), 129.0 (C-3', 5'), 129.1 (C-4'), 129.3 (C-2', 6'), 143.5 (C-1'), 158.1 (C=NOH), 167.2 (C=O); m/z (ES^+) 224.1032 ((M+H)⁺. $\text{C}_{10}\text{H}_{14}\text{N}_3\text{O}_3$ requires 224.1035); m/z (ES^+) 224 ((M+H)⁺, 100%).

Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(4'-trifluoromethylphenyl)ethylamine (**41**)



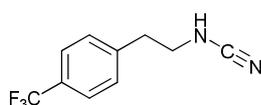
To a stirred solution of 4-(trifluoromethyl)phenylacetonitrile (1.05 g, 5.7 mmol) in dry methanol (40.5 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc_2O) (2.40 g, 11.0 mmol) and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.13 g, 0.6 mmol). NaBH_4 (1.46 g, 38.6 mmol) was then added in small portions over 1.5 h. The resulting reaction mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and stirred for further 2 h, at which point diethylenetriamine (0.58 ml, 5.4 mmol) was added. The mixture was allowed to stir for 1.5 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (150 ml) and washed with saturated aq. NaHCO_3 (2 x 40 ml). The organic layer was dried over MgSO_4 and the solvent was removed at reduced pressure to yield a colourless precipitate. The crude product was purified by column chromatography on silica with petroleum ether/diethyl ether (1:1) as eluting solvent. This gave the desired product (**41**) as a colourless solid (1.00g, 61%). mp 78-79 °C; (Found: C, 58.2; H, 6.1; N, 4.6%. $\text{C}_{14}\text{H}_{18}\text{F}_3\text{NO}_2$ requires C, 58.1; H, 6.2; N, 4.8%); ν_{max} (nujol)/ cm^{-1} 3361 (NH), 1672 (C=O), 1525; δ_{H} (300 MHz, CDCl_3) 1.58 (9H, s, *t*-butyl), 2.86 (2H, t, J 7 Hz, CH_2 -2), 3.39 (2H, q, J 7 Hz, CH_2 -1) 4.54 (1H, br s, NH), 7.31 (2H, d, J 8 Hz, H-2', 6'), 7.56 (2H, d, J 8 Hz, H-3',5'); δ_{C} (75.46 MHz, CDCl_3) 28.4 (CH_3), 36.1 (C-2), 41.5 (C-1), 79.4 (C-*t*-butyl), 125.3 (q, J 271 Hz [^{13}C - ^{19}F], CF_3), 125.4 (q, J 4 Hz [^{13}C - ^{19}F], C-3', 5'), 128.6 (q, J 32 Hz [^{13}C - ^{19}F], C-4'), 129.2 (C-2', 6'), 143.2 (C-1'), 155.8 (C=O); δ_{F} (376.5 MHz, CDCl_3), -62.8 (CF_3); m/z (ES^+) 312 ((M+Na)⁺, 100%).

Synthesis of 2-(4'-trifluoromethylphenyl)ethylamine hydrochloride (42)



A solution of *N*-(*tert*-butoxycarbonyl)-2-(4'-trifluoromethylphenyl)ethylamine (**41**) (0.70 g, 2.4 mmol) in dry 1,4-dioxane (2 ml) was treated with anhydrous HCl in 1,4-dioxane (4 M, 10 ml) at room temperature for 6 h. At that point a few drops of petroleum ether were added to aid precipitation. The solution was left overnight. The excess of solvent was decanted off and yellowish solid was filtered off and washed with petroleum ether. This gave the pure product (**42**) as a colourless solid (0.5 g, 90%). mp 178-179 °C; (Found: C, 48.0; H, 4.7; N, 6.0%. $C_9H_{11}ClF_3N$ requires: C, 47.9; H, 4.9; N, 6.2%); ν_{\max} (nujol)/ cm^{-1} 3424 (NH), 1638, 1602 (C=C); δ_H (300 MHz, D_2O) 2.95 (2H, t, J 7 Hz, CH_2-2), 3.18 (2H, t, J 7 Hz, CH_2-1) 7.35 (2H, d, J 8 Hz, H-2', 6') 7.58 (2H, d, J 8 Hz, H-3',5'); δ_C (75.46 MHz, D_2O) 32.9 (C-2), 40.5 (C-1), 125.7 (C-3', 5'), 128.9 (C-4'), 129.3 (C-2', 6'), 140.9 (C-1') δ_C (125.7 MHz, $CDCl_3$) 32.5 (C-2), 40.3 (C-1), 124.1 (q, J 271 Hz [$^{13}C-^{19}F$], CF_3), 125.7 (q, J 4 Hz [$^{13}C-^{19}F$], C-3', 5'), 128.7 (q, J 32 Hz [$^{13}C-^{19}F$], C-4'), 129.3 (C-2', 6'), 140.9 (C-1'); δ_F (376.5 MHz, D_2O), -62.8 (CF_3); m/z (ES^+) 190 ((M+H) $^+$, 100%), 173 (15), 155 (4).

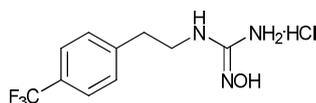
Synthesis of 2-(4'-trifluoromethylphenyl)ethylcyanamide (57)



To a suspension of 2-(4'-trifluoromethylphenyl)ethylamine hydrochloride (**42**) (1.07 g, 4.7 mmol) and sodium acetate (1.16 g, 14.2 mmol) in dry methanol (25 ml) was added dropwise a 3M solution of BrCN in CH_2Cl_2 (1.60 ml, 4.7 mmol). The mixture was stirred in an ice bath for 3 h, than the ice bath was removed and reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the crude product was extracted with CH_2Cl_2 (3 x 15 ml). The organic layer was washed with brine (20 ml), dried over $MgSO_4$ and evaporated. The pure product was obtained by column chromatography on silica using

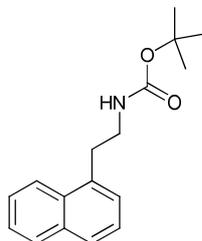
petroleum ether/diethyl ether (1:2) as the eluent. This gave the desired product (**57**) as a yellow oil (0.68 g, 76%). ν_{\max} (neat)/ cm^{-1} 3211 (NH), 2937 (CH aromatics), 2225 (C \equiv N), 1725, 1619 (C=C); δ_{H} (300 MHz, CDCl₃) 2.93 (2H, t, *J* 7 Hz, CH₂-2), 3.30 (2H, q, *J* 7 Hz, CH₂-1), 3.63 (1H, br s, NH), 7.27 (2H, d, *J* 8 Hz, H-2', 6'), 7.53 (2H, d, *J* 8 Hz, H-3', 5'); δ_{C} (75.46 MHz, CDCl₃) 35.6 (C-2), 47.1 (C-1), 125.8 (C-3', 5'), 129.2 (C-2', 6'); δ_{C} (125.7 MHz, CDCl₃) 35.5 (C-2), 46.5 (C-1), 116.2 (C \equiv N), 124.0 (q, *J* 272 Hz [¹³C-¹⁹F], CF₃), 125.4 (q, *J* 4 Hz [¹³C-¹⁹F], C-3', 5'), 128.9 (q, *J* 32 Hz [¹³C-¹⁹F], C-4'), 129.1 (C-2', 6'), 141.5 (C-1'); δ_{F} (376.5 MHz, CDCl₃), -63.0 (CF₃); m/z (ES⁺) 215.0791 ((M+H)⁺). C₁₀H₁₀N₂F₃ requires 215.0796).

Synthesis of *N*-2-(4'-trifluoromethylphenyl)ethyl-*N'*-hydroxyguanidine hydrochloride (76**)**

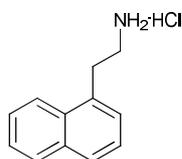


2-(4'-Trifluoromethylphenyl)ethylcyanamide (**57**) (0.62 g, 3.3 mmol) was mixed with hydroxylamine hydrochloride (0.21 g, 3.0 mmol) in dry methanol (25 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 4 h under an argon atmosphere. After cooling down, the solvent was removed at reduced pressure and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Further purification was done by redissolving the crude product in minimum amount of methanol and precipitated out from CH₂Cl₂ and diethyl ether. A final crystallization from CH₂Cl₂ gave the pure product (**76**) as colourless crystals (0.52 g, 62%); mp 118-120 °C; (Found: C, 41.8; H, 4.4; N, 14.8%. C₁₀H₁₃ClF₃N₃O requires C, 42.3; H, 4.6; N, 14.8%); ν_{\max} (nujol)/ cm^{-1} 3353, 3309, 3108 (NH), 1649, 1589 (C=C); δ_{H} (300 MHz, d₆-DMSO) 2.90 (2H, t, *J* 7 Hz, CH₂-2), 3.43 (2H, q, *J* 7 Hz, CH₂-1), 7.50 (2H, d, *J* 8 Hz, H-3', 5'), 7.68 (2H, d, *J* 8 Hz, H-2', 6'), 7.73 (2H, br s, NH₂), 7.94 (1H, t, *J* 7 Hz, NH), 9.82 (1H, br s, NH/OH), 10.43 (1H, br s, NH/OH); δ_{C} (125.7 MHz, d₆-DMSO) 34.1 (C-2), 41.7 (C-1), 124.3 (q, *J* 269 Hz [¹³C-¹⁹F], CF₃), 125.4 (q, *J* 4 Hz [¹³C-¹⁹F], C-3', 5'), 128.4 (q, *J* 32 Hz [¹³C-¹⁹F], C-4'), 129.7 (C-2', 6'), 143.3 (C-1'), 158.1 (C=NOH); δ_{F} (376.5 MHz, CDCl₃), -61.2 (CF₃); m/z (ES⁺) 248 ((M+H)⁺, 100%).

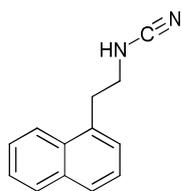
Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(1'-naphthyl)ethylamine (**55**)



To a stirred solution of 1-naphthylacetonitrile (3.00 g, 17.9 mmol) in dry methanol (60 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc₂O) (7.83 g, 35.9 mmol) and NiCl₂·6H₂O (0.43 g, 1.8 mmol). NaBH₄ (4.75 g, 125.6 mmol) was then added in small portions over 3 h. The resulting reaction mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and stirred for further 2 h, at which point diethylenetriamine (1.94 ml, 17.9 mmol) was added. The mixture was allowed to stir for 3 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (250 ml) and extracted with saturated aq. NaHCO₃ (2 x 60 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to yield a colourless precipitate. The crude product was purified by column chromatography on silica with petroleum ether/diethyl ether (1:2) as eluting solvent. This gave the desired product, which was recrystallized from diethyl ether. The final product (**55**) was obtained as a colourless solid (3.05 g, 63%); mp 84–86 °C; (Found: C, 75.3; H, 7.8; N, 5.1%. C₁₇H₂₁NO₂ requires C, 75.3; H, 7.8; N, 5.2%); ν_{\max} (nujol)/cm⁻¹ 3337 (NH), 1681 (C=O); δ_{H} (500 MHz, CDCl₃) 1.47 (9H, s, *t*-butyl), 3.29 (2H, t, *J* 7 Hz, CH₂-2), 3.51 (2H, br s, CH₂-1), 4.69 (1H, br s, NH), 7.34 (1H, d, *J* 8 Hz, H-2'), 7.42 (1H, t, *J* 8 Hz, H-3'), 7.50 (1H, t, *J* 8 Hz, H-6'), 7.55 (1H, t, *J* 8 Hz, H-7'), 7.76 (1H, d, *J* 8 Hz, H-4'), 7.87 (1H, d, *J* 8 Hz, H-5'), 8.10 (1H, d, *J* 8 Hz, H-8'); δ_{C} (125.70 MHz, CDCl₃) 28.4 (CH₃), 33.4 (C-2), 41.4 (C-1), 79.2 (C-*t*-butyl), 123.8 (C-9'), 125.7 (C-7', 8'), 126.8 (C-2', 3'), 127.2 (C-4'), 128.8 (C-6'), 132.0 (C-10'), 133.9 (C-5'), 135.2 (C-1'), 156.0 (C=O); m/z (ES⁺) 294 ((M+Na)⁺, 100%), 310 ((M+K)⁺, 100%).

Synthesis of 2-(1'-naphthyl)ethylamine hydrochloride (56)

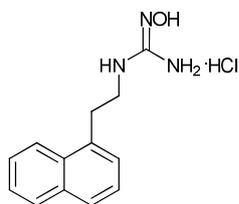
A solution of *N*-(*tert*-butoxycarbonyl)-2-(1'-naphthyl)ethylamine (**55**) (2.83 g, 10.4 mmol) in dry 1,4-dioxane (10 ml) was treated with anhydrous HCl in 1,4-dioxane (4 M, 15 ml) at room temperature and left to stir overnight. The excess of solvent was decanted off and the off-white solid was filtered off and washed with diethyl ether. This gave the pure product (**56**) as a colourless solid (2.04 g, 94%). mp 250 °C (dec.); (Found: C, 68.9; H, 6.5; N, 6.8%. C₁₂H₁₄ClN requires: C, 69.3; H, 6.7; N, 6.8%); ν_{\max} (nujol)/cm⁻¹ 3424 (NH), 1638, 1594 (C=C); δ_{H} (500 MHz, D₂O) 3.28 (2H, t, *J* 7 Hz, CH₂-1), 3.38 (2H, t, *J* 7 Hz, CH₂-2), 7.36-7.44 (2H, m, H-6', 7'), 7.48-7.58 (2H, m, H-2', 3'), 7.81 (1H, d, *J* 8 Hz, H-4'), 7.90 (1H, d, *J* 8 Hz, H-5'), 7.99 (1H, d, *J* 8 Hz, H-8'); δ_{C} (125.7 MHz, D₂O) 29.9 (C-2), 39.7 (C-1), 123.0 (C-9'), 125.7 (C-7'), 126.3 (C-8'), 127.4 (C-2', 3'), 128.0 (C-4'), 128.9 (C-6'), 131.0 (C-10'), 132.4 (C-5'), 133.6 (C-1'); m/z (ES⁺) 172 ((M+H)⁺, 100%).

Synthesis of 2-(1'-naphthyl)ethylcyanamide (63)

To a suspension of 2-(1'-naphthyl)ethylamine hydrochloride (**56**) (1.90 g, 9.2 mmol) and sodium acetate (2.25 g, 27.4 mmol) in dry methanol (25 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (3.05 ml, 9.2 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the crude product was extracted with CH₂Cl₂ (3 x 20 ml). The organic layer was washed with brine (30 ml), dried over MgSO₄ and evaporated at the solvent was removed at reduced pressure. The pure cyanamide was obtained by column

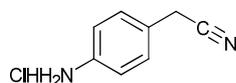
chromatography on silica using petroleum ether/diethyl ether (1:1) as the eluting solvent. This gave the desired product (**63**) as a yellow oil (1.05 g, 58%). $\nu_{\max}(\text{neat})/\text{cm}^{-1}$ 3211, 3062, (NH), 2937 (CH), 2223 (C \equiv N), 1719, 1597 (C=C); δ_{H} (300 MHz, CDCl₃) 3.36-3.52 (5H, m, CH₂-1, 2, NH), 7.36-7.46 (2H, m, H-2', 3'), 7.49-7.59 (2H, m, H-7', 8'), 7.79 (1H, d, *J* 8 Hz, H-4'), 7.88 (1H, d, *J* 8 Hz, H-5'), 7.97 (1H, d, *J* 8 Hz, H-8'); δ_{C} (75.46 MHz, CDCl₃) 33.5 (C-2), 47.1 (C-1), 116.1 (C \equiv N), 123.4 (C-9'), 125.9 (C-7'), 126.3 (C-8'), 126.9 (C-2'), 127.7 (C-3'), 128.4 (C-4'), 129.5 (C-6'), 131.9 (C-10'), 133.4 (C-5'), 134.4 (C-1'); m/z (ES⁺) 219.0906 ((M+Na)⁺. C₁₃H₁₂N₂Na requires 219.0898); m/z (ES⁻) 195 ((M+H)⁻, 100%)

Synthesis of *N*-2-(1'-naphthyl)ethyl-*N*'-hydroxyguanidine hydrochloride (**82**)



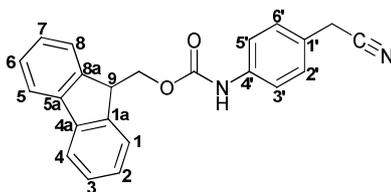
2-(1'-Naphthyl)ethylcyanamide (**63**) (0.92 g, 4.7 mmol) was mixed with hydroxylamine hydrochloride (0.29 g, 4.2 mmol) in dry methanol (25 ml) and potassium carbonate was added (0.1 equiv) as a catalyst. The reaction mixture was heated under reflux for 5 h under an argon atmosphere. After cooling down, the solvent was removed at reduced pressure and the desired product was crystallized from CH₂Cl₂ and diethyl ether. Crystallization from CH₂Cl₂ gave the pure product (**82**) as an off-white solid (0.88 g, 79%); mp 94-96 °C; (Found: C, 58.5; H, 6.1; N, 15.9%. C₁₃H₁₆ClN₃O requires C, 58.8; H, 6.1; N, 15.8%); $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$ 3436, 3323 (NH), 1617, 1577 (C=C); δ_{H} (300 MHz, d₆-DMSO) 3.24-3.32 (2H, m, CH₂-1), 3.45-3.55 (2H, m, CH₂-2), 7.36-7.46 (2H, m, H-6', 7'), 7.43-7.56 (2H, m, H-2', 3'), 7.73 (2H, br s, NH₂), 7.82 (1H, dt, *J* 5 Hz, *J* 3 Hz, H-5'), 7.94 (1H, d, *J* 8 Hz, H-8'), 8.05 (1H, br t, *J* 7 Hz, NH), 9.85 (1H, br s, NH/OH), 10.46 (1H, br. s, NH/OH); δ_{H} (300 MHz, D₂O) 3.24 (2H, t, *J* 7 Hz, H-1), 3.47 (2H, t, *J* 7 Hz, H-2), 7.27-7.42 (2H, m, H-6', 7'), 7.43-7.55 (2H, m, H-2', 3'), 7.76 (1H, d, *J* 8 Hz, H-4'), 7.86 (1H, d, *J* 8 Hz, H-5'), 7.98 (1H, d, *J* 8 Hz, H-8'); δ_{C} (75.46 MHz, d₆-DMSO) 31.5 (C-2), 41.3 (C-1), 123.6 (C-9'), 125.5 (C-7'), 125.7 (C-8'), 126.2 (C-2'), 126.9 (C-3'), 127.1 (C-4'), 128.6 (C-6'), 131.4 (C-10'), 133.4 (C-5'), 134.2 (C-1'); 158.1 (C=NOH); m/z (ES⁺) 230 ((M+H)⁺, 100%).

Synthesis of 4-aminophenylacetonitrile hydrochloride (**98**)¹⁰



To a magnetically stirred solution of 4-nitrophenylacetonitrile (3.00 g, 18.5 mmol) in ethanol (30 ml) was added 10% Pd-Charcoal catalyst (480 mg). Hydrazine hydrate (2.25 ml, 46.3 mmol) was added dropwise maintaining the temperature in the range 20-25 °C. When effervescence had ceased the catalyst was removed by filtration through celite. Evaporation of the filtrate at reduced pressure afforded 4-aminophenylacetonitrile as a yellow oil, which was converted to the hydrochloride salt by the addition of 4M HCl (5 ml) in 1,4-dioxane. The final product (**98**) was obtained as a pale yellow solid (2.31 g, 74%). mp 218 °C (dec.), (lit.¹⁰, 220 °C); ν_{\max} (nujol)/cm⁻¹ 2565, 2594 (NH), 2248 (C≡N); (Found: C, 56.8; H, 5.3; N, 16.3%. C₈H₉ClN₂ requires C, 57.0; H, 5.4; N, 16.6%); δ_{H} (400 MHz, d₆-DMSO, free amine) 3.76 (2H, s, H-1), 5.11 (2H, s, NH₂), 6.54 (2H, d, *J* 8Hz, H-3',5'), 6.96 (2H, d, *J* 8 Hz, H-2', 6'); δ_{C} (75.46 MHz, d₆-DMSO, HCl salt) 21.9 (C-1), 119.0 (C≡N), 123.5 (C-3', 5'), 129.4 (C- 2', 5'), 130.8 (C-4'), 131.8 (C-1').

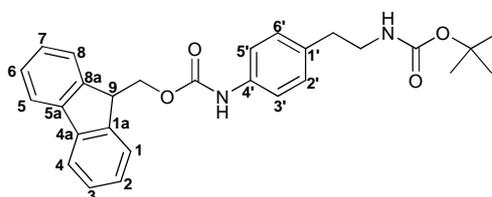
Synthesis of 4-(*N*-9-fluorenylmethoxycarbonylamino)phenylacetonitrile (**100**)



To a solution of 4-aminophenylacetonitrile hydrochloride (**98**) (1.26 g, 7.5 mmol) and pyridine (1.44 ml, 18.0 mmol) in CH₂Cl₂ (20 ml) at 0 °C was added 9-fluorenylmethoxycarbonyl chloride (2.13 g, 8.2 mmol). The solution was stirred at 0 °C for further 15 minutes, before the ice bath was removed and stirring continued at room temperature for 1 h. CH₂Cl₂ (30 ml) was added and the solution washed with H₂O (2×20 ml). The organic layer was dried (MgSO₄) and concentrated under reduced pressure to give a yellow solid which was recrystallized from CH₂Cl₂/petroleum ether to give the final compound (**100**) as an off-white solid (2.03g, 77%). mp 156 °C; ν_{\max} (nujol)/cm⁻¹ 3278 (NH), 2248 (C≡N), 1700 (C=O), 1597 (C=C); (Found: C,

77.5; H, 5.0; N, 7.6%. $C_{23}H_{18}N_2O_2$ requires C, 77.9; H, 5.1; N, 7.9%); δ_H (300 MHz, $CDCl_3$) 3.71 (2H, s, CH_2CN), 4.28 (1H, t, J 7 Hz, H-9), 4.57 (2H, d, J 7 Hz, CH_2O), 6.67 (1H, br s, NH), 7.22-7.46 (8H, m, H-1, 2, 3, 6, 7, 8, H-2', 6'), 7.62 (2H, d, J 8 Hz, H-3', 5'), 7.79 (2H, d, J 8 Hz, H-4, 5); δ_C (75.46 MHz, $CDCl_3$) 23.0 (CH_2), 44.1 (C-9), 66.9 (CH_2O), 120.1 (C-3', 5'), 124.9 (C-3, 6), 127.2 (C-2, 3, 6, 7), 127.9 (C-1, 8), 128.7 (C-2', 6'), 137.6 (C-1'), 141.4 (C-4a, 5a), 143.3 (C-1a, 8a), 143.6 (C-4'); m/z (ES^+) 377 ($(M+Na)^+$, 100%).

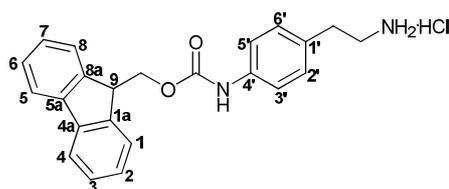
**Synthesis of *N*-(*tert*-butoxycarbonyl)-
-2-(4'-*N*-9-fluorenylmethoxycarbonylaminophenyl)ethylamine (101)**



To a stirred solution of 4-(*N*-9-fluorenylmethoxycarbonyl)phenylacetone (100) (4.27 g, 12.1 mmol) in a mixture of dry THF (125 ml) and dry methanol (125 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc_2O) (5.28 g, 24.2 mmol) and $NiCl_2 \cdot 6H_2O$ (0.29 g, 1.21 mmol). $NaBH_4$ (3.19 g, 84.4 mmol) was then added in small portions over 2 h. The resulting reaction mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and stirred for further 24 h, at which point diethylenetriamine (1.30 ml, 12.1 mmol) was added. The mixture was allowed to stir for 1.5 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (250 ml) and extracted with saturated aq. $NaHCO_3$ (3 x 80 ml). The organic layer was dried over $MgSO_4$ and the solvent was removed at reduced pressure to yield a pale yellow solid. The crude product was washed with diethyl ether to remove unreacted starting material. This gave the desired product (101) as an off-white solid (3.23 g, 58%). mp 166-168 °C; ν_{max} (*nujol*)/ cm^{-1} 3332 (NH), 1700, 1697 (C=O), 1653 (C=C); (Found: C, 73.6; H, 6.3; N, 6.1%. $C_{28}H_{30}N_2O_4$ requires C, 73.3; H, 6.6; N, 6.1%); δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, *t*-butyl), 2.75 (2H, t, J 7 Hz, CH_2-2), 3.31-3.39 (2H, m, CH_2-1), 4.28 (1H, t, J 7 Hz, H-9), 4.54-4.59 (3H, m, CH_2O , NH), 6.55 (1H, br s, NH), 7.12 (2H, d, J 8 Hz, H-2', 6'), 7.28-7.33 (4H, m, H-2, 3, 6, 7), 7.42 (2H, t, J 8 Hz, H-1, 8), 7.62 (2H, d, J 8 Hz, H-3', 5'), 7.79 (2H, d, J 8 Hz, H-4, 5); δ_C (75.46 MHz, $CDCl_3$) 28.4 (CH_3), 35.5 (C-2), 41.8 (C-1), 47.2 (C-9),

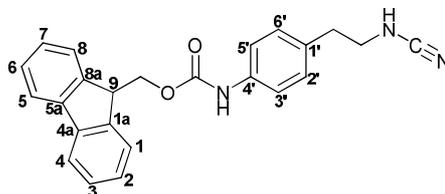
66.8 (CH₂O), 79.3 (C-*t*-butyl), 119.3 (C-3', 6'), 120.1 (C-3, 6), 125.0 (C-2, 4, 5, 7), 127.2 (C-2', 6'), 127.8 (C-2, 6'), 129.4 (C-1, 8), 134.8 (C-4'), 136.1 (C-1'), 141.4 (C-4a, 5a), 143.8 (C-1a, 8a), 155.9 (C=O); *m/z* (ES⁺) 480 ((M+Na)⁺, 100%), 481 (48).

Synthesis of 2-(4'-*N*-9-fluorenylmethoxycarbonylaminophenyl)ethylamine hydrochloride (102**)**



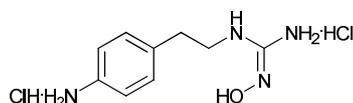
A solution of *N*-(*tert*-butoxycarbonyl)-2-(4'-*N*-9-fluorenylmethoxycarbonylphenyl)ethylamine (**101**) (1.63 g, 3.5 mmol) in dry THF (5 ml) was treated with anhydrous HCl in 1,4-dioxane (4 M, 9.0 ml) at room temperature for 16 h. The solid was filtered off and washed with diethyl ether to give the pure product (**102**) as a colourless solid (1.02 g, 73%). mp 220 °C (dec); ν_{\max} (nujol)/cm⁻¹ 3336, 2067 (NH), 1697 (C=O), 1606 (C=C); (Found: C, 69.6; H, 5.8; N, 6.9%. C₂₃H₂₃ClN₂O₂ requires: C, 69.9; H, 5.9; N, 7.1%); δ_{H} (300 MHz, d₄-methanol) 2.82 (2H, t, *J* 7 Hz, CH₂-2), 3.06 (2H, t, *J* 7 Hz, CH₂-1), 4.19 (1H, t, *J* 7 Hz, H-9), 4.40 (2H, t, *J* 7 Hz, CH₂O), 7.11 (2H, d, *J* 8 Hz, H-2', 6'), 7.21-7.27 (2H, m, H-2, 7), 7.29-7.38 (4H, m, H-1, 3, 6, 8), 7.62 (2H, d, *J* 8 Hz, H-3', 5), 7.73 (2H, d, *J* 8 Hz, H-4, 5); δ_{C} (75.46 MHz, d₄-methanol) 32.4 (C-2), 40.4 (C-1), 46.9 (C-9), 66.2 (CH₂O), 119.4 (C-3', 5'), 124.6 (C-3, 6), 126.7 (C-2, 4), 127.3 (C-5, 7), 128.7 (C-2', 6'), 130.8 (C-1, 8), 137.8 (C-1', 4'), 141.1 (C-4a, 5a), 143.8 (C-1a, 8a), 154.4 (C=O); *m/z* (ES⁺) 380 ((M+Na)⁺ 50%), 358 ((M+H)⁺, 100%).

Synthesis of 2-(4'-N-9-fluorenylmethoxycarbonylaminophenyl)ethylcyanamide (**103**)



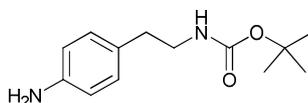
To a suspension of 2-[4'-(N-9-fluorenylmethoxycarbonyl)phenyl]ethylamine hydrochloride (**102**) (1.02 g, 2.6 mmol) and sodium acetate (0.42 g, 5.16 mmol) in dry methanol (25 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (0.86 ml, 2.6 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature for 48 h. The solvent was removed at reduced pressure and the crude product was extracted with CH₂Cl₂ (6 x 100 ml). The organic layer was washed with 2M HCl (100 ml) and then with H₂O (2 x 100 ml) and finally with brine (100 ml), dried over MgSO₄ and the solvent was removed at reduced pressure. The pure product was obtained by recrystallization from CH₂Cl₂/petroleum ether. This gave the desired product (**103**) as an off-white solid (0.56 g, 56%). mp 170 °C; ν_{\max} (nujol)/cm⁻¹ 3318 (NH), 2218 (C≡N), 1696 (C=O); δ_{H} (400 MHz, d₆-DMSO) 2.70 (2H, t, *J* 7 Hz, CH₂-2), 3.08-3.14 (2H, m, CH₂-1), 4.30 (1H, t, *J* 7 Hz, H-9), 4.47 (2H, t, *J* 7 Hz, CH₂O), 6.77 (1H, br t, *J* 6 Hz, NH), 7.14 (2H, d, *J* 8 Hz, H-2', 6'), 7.33-7.43 (6H, m, H-1, 2, 3, 6, 7, 8), 7.75 (2H, d, *J* 8 Hz, H-3', 5'), 7.91 (2H, d, *J* 8 Hz, H-4, 5), 9.61 (1H, br s, NH); δ_{C} (100.6 MHz, d₆-DMSO) 34.5 (C-2), 45.9 (C-1), 46.5 (C-9), 65.5 (CH₂O), 117.4 (C≡N), 118.5 (C-3', 5'), 120.1 (C-3, 6), 125.0 (C-2, 4, 5, 7), 127.2 (C-2', 6'), 127.7 (C-2', 6'), 129.1 (C-1, 8), 132.5 (C-1', 4'), 140.7 (C-4a, 5a), 143.6 (C-1a, 8a), 153.6 (C=O); *m/z* (ES⁺) 406.1524 ((M+Na⁺)). C₂₄H₂₁N₃O₂Na requires 406.1532; *m/z* (ES⁺) 406 ((M+Na⁺), 100%).

Synthesis of *N*-2-(4'-aminophenyl)ethyl-*N*'-hydroxyguanidine dihydrochloride (**107**)



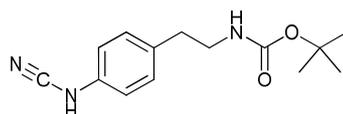
2-(4'-*N*-9-Fluorenylmethoxycarbonylamino phenyl)ethylcyanamide (**103**) (1.07 g, 2.8 mmol) was mixed with hydroxylamine hydrochloride (0.58 g, 8.3 mmol) in dry methanol (100 ml) and acetonitrile (30 ml) and sodium acetate (0.2 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 8 h under an argon atmosphere. Then, the reaction mixture was cooled to 0 °C in an ice bath and di-*tert*-butyl dicarbonate (0.61 g, 2.8 mmol) and potassium carbonate (0.40 g, 2.8 mmol) were added. The solution was left to stir for an hour. The solvent was then removed at reduced pressure and the residue was partitioned between water (20 ml) and ethyl acetate (40 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to give a yellow oil. The residue was dissolved in dry THF (20 ml) and piperidine (20% w/w solution) was added. The reaction was left to stir for another hour. The solvent was removed at reduced pressure and the crude product was purified by column chromatography (petroleum: diethyl ether: ethyl acetate-gradient). The product was then dissolved in dry THF (5 ml) and treated with anhydrous HCl in 1,4-dioxane (4 M, 10.0 ml) at room temperature for 16 h. The solid was filtered off and washed with diethyl ether to give the pure product (**107**) as an off white solid. (175 mg, 23%). mp 210-212 °C (dec.); ν_{\max} (nujol)/cm⁻¹ 3160, 2590 (NH), 1643 (C=C); δ_{H} (300 MHz, d₆-DMSO) **major isomer**: 2.91 (2H, t, *J* 7 Hz, CH₂-2), 3.75 (2H, t, *J* 7 Hz, CH₂-1), 6.74 (1H, br s), 7.31-7.74 (4H, m, Ar-H), 7.74 (2H, s, NH₂), 7.97 (1H, br t, *J* 5 Hz, NH), 9.84 (1H, br s, NH/OH), 10.47 (3H, br s, NH₃⁺), 10.91 (1H, s, NH/OH); **minor isomer**: 2.82 (2H, t, *J* 7 Hz, CH₂-2), 3.40 (2H, t, *J* 7 Hz, CH₂-1), 6.74 (1H, br s), 7.31-7.74 (4H, m, Ar-H), 7.74 (2H, s, NH₂), 7.97 (1H, br t, *J* 5 Hz, NH), 9.84 (1H, br s, NH/OH), 10.47 (3H, br s, NH₃⁺), 10.91 (1H, s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) **major isomer**: 34.2 (C-1), 42.0 (C-2), 123.5 (C-3', 5'), 124.5 (C-1'), 130.4 (C-2', 6'), 130.7 (C-4'), 158.6 (C=NOH); **minor isomer**: 32.9 (C-1), 39.9 (C-2), 122.4 (C-3', 5'), 124.5 (C-1'), 129.2 (C-2', 6'), 130.7 (C-4'), 157.9 (C=NOH); m/z (ES⁺) 195.1240 ((M+H)⁺. C₉H₁₅N₄O requires 196.1246); m/z (ES⁺) 195 ((M+H)⁺, 100%).

Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(4'-aminophenyl)ethylamine (**112**)



N-(*tert*-Butoxycarbonyl)-2-(4'-*N*-9-fluorenylmethoxycarbonylphenyl)ethylamine (**101**) (1.54 g, 3.36 mmol) was dissolved in THF (20 ml) and piperidine (5 ml) was added. The reaction mixture was left to stir over night. After removal of solvent under reduced pressure and the residue was purified by column chromatography using gradient elution with diethyl ether and ethyl acetate. The pure product (**112**) was obtained as a pale yellow solid (0.33 g, 79%). mp 68 °C; (Found: C, 66.5; H, 8.9; N, 11.5%. $C_{13}H_{20}N_2O_2$ requires C, 66.1; H, 8.5; N, 11.8%); ν_{\max} (nujol)/ cm^{-1} 3378, 3182 (NH), 1684 (C=O), 1528; δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, CH_3), 2.68 (2H, t, J 7 Hz, H-2), 3.31 (2H, dt, J 7 Hz, H-1), 3.60 (2H, br s, NH_2), 4.52 (1H, br s, NH), 6.64 (2H, d, J 8 Hz, H-3', 5'), 6.97 (2H, t, J 8 Hz, H-2', 6'); δ_C (75.46 MHz, $CDCl_3$) 28.4 (CH_3), 35.5 (C-2), 41.8 (C-1), 115.4 (C-3', 5'), 129.6 (C-2', 6'), 137.8 (C-1'), 144.8 (C-4'), 155.9 (C=O); m/z (ES^+) 259 ($(M+Na)^+$, 100%).

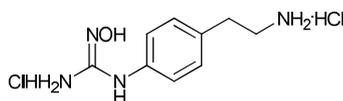
Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(4'-aminophenylethyl)cyanamide (**113**)



To a suspension of *N*-(*tert*-butoxycarbonyl)-2-(4'-aminophenyl)ethylamine (**112**) (0.99 g, 4.2 mmol) and sodium acetate (0.69 g, 8.4 mmol) in dry methanol (20 ml) was added dropwise a 3M solution of BrCN in CH_2Cl_2 (1.40 ml, 4.2 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the crude product was extracted with CH_2Cl_2 (3 x 20 ml). The organic layer was washed with brine (20 ml), dried over $MgSO_4$ and the solvent was removed at reduced pressure. The pure cyanamide was obtained by column chromatography on silica using gradient of petroleum ether/diethyl ether (1:1) to ethyl acetate as the eluting solvents. This gave the desired product (**113**) as colourless crystals (0.46 g, 42%).

mp 104-106 °C; (Found: C, 64.3; H, 7.7; N, 16.2%. $C_{14}H_{19}N_3O_2$ requires C, 64.3; H, 7.3; N, 16.1%); ν_{\max} (nujol)/ cm^{-1} 3344, 3147, 3079 (NH), 2222 (C≡N), 1680 (C=O); δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, CH_3), 2.75 (2H, t, J 7 Hz, CH_2 -2), 3.33 (2H, dt, J 7 Hz, CH_2 -1), 4.60 (1H, br s, NH), 6.50 (1H, br s, NH), 6.94 (2H, d, J 8 Hz, H-3', 5'), 7.13 (2H, d, J 8 Hz, H-2', 6'); δ_C (75.46 MHz, $CDCl_3$) 28.4 (CH_3), 35.5 (C-2), 41.9 (C-1), 79.8 (C-*t*-butyl), 111.2 (C≡N), 115.6 (C-3', 5'), 130.1 (C-2', 6'), 134.2 (C-1'), 136.0 (C-4'), 156.2 (C=O); m/z (ES^+) 284.1382 ((M+Na⁺). $C_{14}H_{19}N_3O_2Na$ requires 284.1375); m/z (ES^+) 284 ((M+Na⁺), 100%), m/z (ES^-) 260 ((M-H)⁻, 100%).

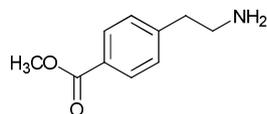
Synthesis of *N*-phenyl-(4-ethylamine)-*N'*-hydroxyguanidine hydrochloride (**116**)



N-(*tert*-Butoxycarbonyl)-2-(4'-aminophenyl)cyanamide (**115**) (0.72 g, 2.7 mmol) was mixed with hydroxylamine hydrochloride (0.20 g, 2.7 mmol) in dry methanol (50 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 7 h under an argon atmosphere. After the completion, the mixture was cooled in an ice bath and di-*tert*-butyl dicarbonate (0.60 g, 2.7 mmol) and potassium carbonate (0.38 g, 2.7 mmol) were added. The solution was left to stir for an hour. The solvent was then removed at reduced pressure and the residue was partitioned between water (10 ml) and ethyl acetate (20 ml). The organic layer was dried over $MgSO_4$ and the solvent was removed at reduced pressure to give a yellow oil. Further purification was achieved by column chromatography of the protected hydroxyguanidine (petroleum: diethyl ether: ethyl acetate-gradient). The product was then dissolved in dry THF (5 ml) and treated with anhydrous HCl in 1,4-dioxane (4 M, 10.0 ml) at room temperature for 16 h. The solid was filtered off and washed with diethyl ether to give the pure product (**116**) as a pale yellow solid (478 mg, 44%). mp 142-144 °C (dec.); (Found: C, 40.5; H, 6.0; N, 20.6%. $C_9H_{16}Cl_2N_4O$ requires C, 40.5; H, 6.0; N, 20.9%); ν_{\max} (nujol)/ cm^{-1} 3382, 2448 (NH), 1609 (C=C), 1511; δ_H (300 MHz, d_6 -DMSO) 2.87-3.05 (4H, m, CH_2 -1, 2), 7.17 (2H, d, J 8 Hz, H-2', 6'), 7.31 (2H, d, J 8 Hz, H-3', 5'), 7.88 (2H, br s, NH_2), 8.26 (3H, br s, NH_3^+), 10.00 (1H, br s, NH), 10.13 (1H, br s, NH/OH), 10.86 (1H, s, NH/OH); δ_C (75.46 MHz, d_6 -DMSO) 32.6 (C-1), 66.7 (C-2), 125.0 (C-2', 6'), 130.2 (C-3',5'), 134.2 (C-4'), 135.9 (C-1'), 157.3

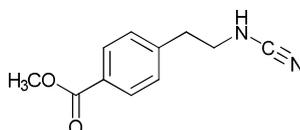
(C=NOH); m/z (ES^+) 195.1235 ($(M+H)^+$). $C_9H_{15}N_4O$ requires 195.1246; m/z (ES^+) 195 ($(M+H)^+$, 100%).

Synthesis of methyl 4-(2-aminoethyl)benzoate (**178**)¹¹



4-(2-Aminoethyl)benzoic acid hydrochloride (2.05 g, 10.1 mmol) was dissolved in distilled water (40 ml) and basified with 2 M NaOH to pH 8-9. The aqueous layer was extracted with ethyl acetate (3 x 30 ml), dried ($MgSO_4$) and the solvent was removed at reduced pressure to give the free amine, which was then dissolved in MeOH (10 ml) and one drop of concentrated H_2SO_4 was added. The reaction mixture was heated under reflux for 5 h. After cooling, the solvent was removed under reduced pressure and the residue was partitioned between CH_2Cl_2 (30 ml) and H_2O (30 ml). The organic layer was washed with saturated sodium bicarbonate solution (2 x 20 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give the pure product (**178**) as a colourless solid (1.04 g, 57%). mp 54-56 °C; δ_H (400 MHz, d_4 -methanol) 2.90 (2H, t, J 7 Hz, CH_2 -1), 3.05 (2H, t, J 7 Hz, CH_2 -2), 3.81 (3H, s, CH_3), 7.31 (2H, d, J 8 Hz, H-3', 5'), 7.91 (2H, d, J 8 Hz, H-2', 6'); δ_C (75.46 MHz, d_4 -methanol) 35.1 (C-1), 41.7 (C-2), 52.7 (CH_3), 130.1 (C-2', 6'), 130.7 (C-2', 6'), 131.1 (C-3', 5'), 144.3 (C-1'), 168.4 (C-4'), 180.4 (C=O); m/z (ES^+) 180 ($(M+H)^+$, 70%), 163 (100).

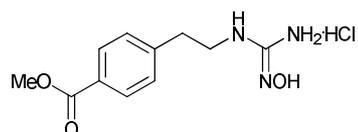
Synthesis of methyl 4-(2-cyanamidoethyl)benzoate (**64**)



To a suspension of methyl 4-(2-aminoethyl)benzoate (**178**) (1.00 g, 5.59 mmol) and sodium acetate (0.92 g, 11.2 mmol) in dry methanol (30 ml) was added dropwise a 3M solution of BrCN in CH_2Cl_2 (1.86 ml, 5.59 mmol). The mixture was stirred in an ice bath for 2 h, then the ice bath was removed and reaction mixture was allowed to stir at room temperature overnight. The

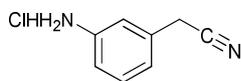
solvent was removed at reduced pressure and the residue was dissolved in water (30 ml). The crude product was extracted with CH_2Cl_2 (3 x 30 ml). The organic layer was washed with brine (30 ml), dried over MgSO_4 and evaporated. The pure product was obtained by chromatography on silica using petroleum ether/diethyl ether (1:2) as the eluting solvent. This gave the desired product (**64**) as a colourless oil (0.32 g, 30%). $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$ 3336 (NH), 2223 ($\text{C}\equiv\text{N}$), 1700 ($\text{C}=\text{O}$), 1576 ($\text{C}=\text{C}$); δ_{H} (400 MHz, CDCl_3) 2.94 (2H, t, J 7 Hz, CH_2 -2), 3.32 (2H, dt, J 6 Hz, J 7 Hz, CH_2 -1), 3.86 (3H, s, CH_3), 4.64 (1H, br s, NH), 7.22-7.28 (2H, m, C-2', 6'), 7.95 (2H, d, J 8 Hz, C-3', 5'); δ_{C} (75.46 MHz, CDCl_3) 36.3 (C-2), 47.6 (C-1), 60.8 (CH_3), 129.3 (C-2', 6'), 130.5 (C-3', 5'), 143.0 (C-4'), 151.1 (C-1'), 167.4 ($\text{C}=\text{O}$); m/z (ES⁻) 203.0819 ((M-H)⁻. $\text{C}_{11}\text{H}_{11}\text{N}_2\text{O}_2$ requires 203.0821); m/z (ES⁺) 227 ((M+Na)⁺, 100%).

Synthesis of methyl 4-(2-(2-hydroxyguanidino)ethyl)benzoate hydrochloride (**87**)



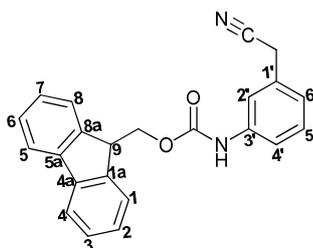
Methyl 4-(2-cyanamidoethyl)benzoate (**66**) (0.30 g, 1.45 mmol) was mixed with hydroxylamine hydrochloride (0.10 g, 1.45 mmol) in dry methanol (20 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for 3 h under an argon atmosphere and then stirred for further 12 h at room temperature. The solvent was removed at reduced pressure, and the product was crystallized from CH_2Cl_2 and diethyl ether. Further purification was achieved by redissolving the crude product in the minimum amount of methanol and the insoluble solid was filtrated off. Addition of CH_2Cl_2 gave the pure product (**87**) as a colourless solid (128 mg, 32%). mp 148-150 °C; (Found: C, 46.8; H, 5.9; N, 14.9%. $\text{C}_{10}\text{H}_{16}\text{ClN}_3\text{O}$; 0.4 H_2O requires C, 47.0; H, 6.0; N, 14.9%); $\nu_{\text{max}}(\text{nujol})/\text{cm}^{-1}$ 3345, 3139 (NH), 1714 ($\text{C}=\text{O}$), 1653 ($\text{C}=\text{C}$), 1284 ($\text{C}-\text{O}$); δ_{H} (400 MHz, d_6 -DMSO) 2.88 (2H, t, J 7 Hz, CH_2 -1), 3.36-3.40 (2H, m, CH_2 -2), 3.84 (3H, s, CH_3), 7.43 (2H, d, J 8 Hz, H-3', 5'), 7.68 (2H, br s, NH_2), 7.88-7.95 (3H, m, H-2', 6' and NH), 9.80 (1H, br s, OH); δ_{C} (100 MHz, d_6 -DMSO) 34.4 (C-1), 41.2 (C-2), 52.0 (CH_3), 127.8 (C-1'), 129.2 (C-3', 5'), 129.3 (C-2', 6'), 144.1 (C-4'), 158.1 ($\text{C}=\text{NOH}$), 166.1 ($\text{C}=\text{O}$); m/z (ES⁺) 238 ((M+H)⁺, 100%).

Synthesis of 3-aminophenylacetonitrile hydrochloride (**108**)¹²



To a stirred solution of 3-nitrophenylacetonitrile (2.50 g, 15.4 mmol) in ethanol (25 ml) was added 10% Pd-charcoal catalyst (400 mg). Hydrazine hydrate (1.88 ml, 38.5 mmol) was then added dropwise maintaining the temperature in the range 20-25 °C. When effervescence had ceased the catalyst was removed by filtration through celite. Removal of the solvent at reduced pressure afforded 3-aminophenylacetonitrile as a yellow oil, which was converted to the hydrochloride salt by the addition of 4M HCl in 1,4-dioxane (5 ml). The final product (**108**) was obtained as a pale yellow solid (2.25 g, 87%). mp 210 °C (dec.), (lit.¹², 209-210 °C); ν_{\max} (nujol)/cm⁻¹ 2628, 2591 (NH), 2251 (C≡N), 1603 (C=C); δ_{H} (300 MHz, d₄-methanol) 3.92 (2H, s, CH₂), 7.26-7.31 (1H, m, H-2'), 7.34-7.50 (3H, m, H-4', 5', 6'); δ_{C} (75.46 MHz, d₄-methanol) 23.7 (CH₂), 119.3 (C≡N), 124.1 (C-4'), 124.3 (C-6'), 130.5 (C-2'), 132.4 (C-3'), 133.1 (C-1'), 136.0 (C-5').

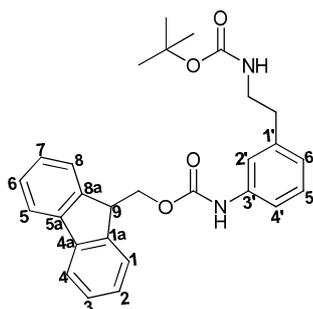
Synthesis of 3-(*N*-9-fluorenylmethoxycarbonylamino)phenylacetonitrile (**109**)



To a solution of 3-aminophenylacetonitrile hydrochloride (**108**) (4.27 g, 25.3 mmol) and pyridine (4.89 ml, 60.8 mmol) in CH₂Cl₂ (80 ml) at 0 °C was added 9-fluorenylmethoxycarbonyl chloride (7.21 g, 27.9 mmol). The solution was stirred at 0 °C for 15 minutes, before the ice bath was removed and stirring continued at room temperature for 1 h. CH₂Cl₂ (60 ml) was added and the solution washed with H₂O (2 × 50 ml). The organic layer was dried (MgSO₄) and the solvent was removed at reduced pressure to give a yellow solid which was purified by column chromatography using petroleum ether/diethyl ether (1:2) as an eluting solvent giving the final

compound (**109**) as an off-white solid (5.87g, 65%). mp 108-109 °C; ν_{\max} (nujol)/cm⁻¹ 3312 (NH), 2361 (C≡N), 1737, 1699 (C=O), 1612 (C=C); (Found: C, 78.1; H, 5.1; N, 7.2%. C₂₃H₁₈N₂O₂ requires C, 77.9; H, 5.1; N, 7.9%); δ_{H} (400 MHz, CDCl₃) 3.70 (2H, s, CH₂CN), 4.27 (1H, t, *J* 7 Hz, H-9), 4.56 (2H, d, *J* 7 Hz, CH₂O), 6.77 (1H, br s, NH), 7.01-7.06 (1H, m, H-6'), 7.27-7.47 (8H, m, H-1, 2, 3, 6, 7, 8, H-4', 5'), 7.62-7.64 (1H, m, H-2'), 7.79 (2H, d, *J* 8 Hz, H-4, 5); δ_{C} (75.46 MHz, CDCl₃) 23.6 (CH₂), 47.1 (C-9), 66.9 (CH₂O), 117.7 (C≡N), 118.1 (C-1, 8), 120.1 (C-4'), 122.9 (C-2'), 125.1 (C-6'), 127.2 (C-2, 7), 127.9 (C-3, 4, 5, 6), 129.9 (C-5'), 131.0 (C-1'), 138.5 (C-3'), 141.4 (C-4a, 5a), 143.7 (C-1a, 8a), 152.8 (C=O); *m/z* (ES⁺) 377.1257 ((M+Na)⁺. C₂₃H₁₈N₂O₂Na requires 377.1266); *m/z* (ES⁺) 377 ((M+Na)⁺, 100%).

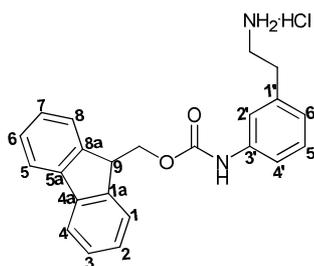
**Synthesis of *N*-(*tert*-butoxycarbonyl)-
-2-(3'-*N*-9-fluorenylmethoxycarbonylaminophenyl)ethylamine (**110**)**



To a stirred solution of 3-(*N*-9-fluorenylmethoxycarbonyl)phenylacetone (**109**) (5.58 g, 15.7 mmol) in a mixture of dry THF (150 ml) and dry methanol (150 ml), cooled to 0 °C, were added di-*tert*-butyl dicarbonate (Boc₂O) (6.87 g, 31.5 mmol) and NiCl₂·6H₂O (0.37 g, 1.57 mmol). NaBH₄ (4.17 g, 110 mmol) was then added in small portions over 3 h. The resulting reaction mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and stirred for further 24 h, at which point diethylenetriamine (1.70 ml, 15.7 mmol) was added. The mixture was allowed to stir for 1.5 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (250 ml) and extracted with saturated aq. NaHCO₃ (3 x 80 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to yield a pale yellow solid. The crude product was purified by the column chromatography using diethyl ether/petroleum ether (2:1) as an eluting solvent. This gave the starting material (0.80 g), Fmoc-deprotected amine (0.64 g) and the desired product (**110**) as an off-white solid (1.93 g,

28%). mp 60-61 °C; ν_{\max} (nujol)/ cm^{-1} 3303 (NH), 1699, 1686 (C=O), 1612, 1596 (C=C); (Found: C, 73.6; H, 6.9; N, 6.2%. $\text{C}_{28}\text{H}_{30}\text{N}_2\text{O}_4$ requires C, 73.3; H, 6.6; N, 6.1%); δ_{H} (300 MHz, CDCl_3) 1.48 (9H, s, *t*-butyl), 2.79 (2H, t, *J* 7 Hz, CH_2 -2), 3.42 (2H, t, *J* 7 Hz, CH_2 -1), 4.28 (1H, t, *J* 7 Hz, H-9), 4.54 (2H, d, *J* 7 Hz, CH_2O), 6.65 (1H, br s, NH), 6.92-6.97 (1H, m, 6'), 7.12 (2H, d, *J* 8 Hz, H-2, 7), 7.28-7.33 (4H, m, H-3, 6, 4', 5'), 7.42 (2H, t, *J* 8 Hz, H-1, 8), 7.62-7.65 (1H, m, H-2'), 7.79 (2H, d, *J* 8 Hz, H-4, 5); δ_{C} (75.46 MHz, CDCl_3) 28.4 (CH_3), 36.2 (C-2), 41.9 (C-1), 47.2 (C-9), 68.9 (CH_2O), 120.1 (C-3', 6'), 124.1 (C-3, 6), 125.0 (C-2, 4, 5, 7), 127.2 (C-2', 6'), 127.8 (C-2, 6'), 129.3 (C-1, 8), 138.0 (C-4'), 140.1 (C-1'), 141.4 (C-4a, 5a), 143.8 (C-1a, 8a), 152.6 (C=O); m/z (ES^+) 481 ($(\text{M}+\text{Na})^+$, 100).

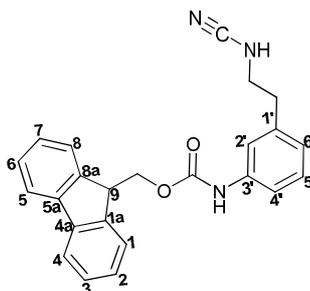
Synthesis of 2-(3'-*N*-9-fluorenylmethoxycarbonylaminophenyl)ethylamine hydrochloride (**179**)



A solution of *N*-(*tert*-butoxycarbonyl)-2-(3'-*N*-9-fluorenylmethoxycarbonylphenyl)ethylamine (**114**) (1.92 g, 4.2 mmol) in dry THF (5 ml) was treated with anhydrous HCl in 1,4-dioxane (4 M, 10.5 ml) at room temperature for 16 h. The precipitate was filtered off and washed with diethyl ether to give the pure product (**179**) as a colourless solid (1.13 g, 69%). mp 198-200 °C (dec); (Found: C, 69.5; H, 5.8; N, 6.8%. $\text{C}_{23}\text{H}_{23}\text{ClN}_2\text{O}_2$ requires: C, 69.9; H, 5.9; N, 7.1%); ν_{\max} (nujol)/ cm^{-1} 3163, 3079, 2692, 2596 (NH), 1709 (C=O), 1597 (C=C); δ_{H} (300 MHz, d_4 -methanol) 2.92 (2H, t, *J* 7 Hz, CH_2 -2), 3.16 (2H, t, *J* 7 Hz, CH_2 -1), 4.28 (1H, t, *J* 7 Hz, H-9), 4.49 (2H, d, *J* 7 Hz, CH_2O), 6.62-6.98 (1H, m, H-6'), 7.23-7.44 (6H, m, H-2, 3, 6, 7, 4', 5'), 7.47 (1H, br s, NH), 7.70 (2H, d, *J* 8 Hz, H-1, 8), 7.81-7.90 (3H, m, H-4, 5, 2'); δ_{C} (75.46 MHz, d_4 -methanol) 35.0 (C-2), 42.3 (C-1), 48.8 (C-9), 68.1 (CH_2O), 119.4 (C-5'), 120.6 (C-3, 6), 121.4 (C-2, 4), 124.8 (C-5, 7), 126.5, 128.6 (C-2', 6'), 129.3, 130.9 (C-1, 8), 139.0 (C-1', 4'), 141.1 (C-4a,

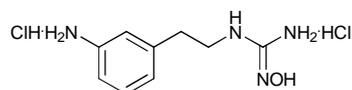
5a), 143.1 (C-1a, 8a), 145.6 (C-3'), 155.0 (C=O); m/z (ES^+) 359.1750 ($(M+H^+)$). $C_{23}H_{23}N_2O_2$ requires 359.1760); m/z (ES^+) 359 ($(M+H^+)$, 100%).

Synthesis of 2-(3'-*N*-9-fluorenylmethoxycarbonylamino)phenylethylcyanamide (**180**)



To a suspension of 2-[3'-(*N*-9-fluorenylmethoxycarbonyl)phenyl]ethylamine hydrochloride (**179**) (2.30 g, 5.8 mmol) and sodium acetate (1.43 g, 17.5 mmol) in dry methanol (75 ml) was added dropwise a 3M solution of BrCN in CH_2Cl_2 (1.94 ml, 5.8 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and the reaction mixture was allowed to stir at room temperature for 10 h. The solvent was removed at reduced pressure and the crude product was extracted with ethyl acetate (3 x 100 ml). The organic layer was washed with 2M HCl (100 ml) and then with H_2O (2 x 100 ml) and finally with brine (100 ml), dried over $MgSO_4$ and the solvent was removed at reduced pressure. The pure product was obtained by recrystallization from CH_2Cl_2 /petroleum ether. This gave the desired product (**180**) as an off-white solid (1.58 g, 71%). mp 84-85 °C; ν_{max} (nujol)/ cm^{-1} 3308, 2727 (NH), 2218 ($C\equiv N$), 1695 (C=O), 1611 (C=C); δ_H (400 MHz, $CDCl_3$) 2.88 (2H, t, J 7 Hz, CH_2 -2), 3.29-3.36 (2H, m, CH_2 -1), 3.56 (1H, br s, NH), 4.27 (1H, t, J 7 Hz, H-9), 4.54 (2H, d, J 7 Hz, CH_2 O), 6.76 (1H, br t, J 6 Hz, NH), 6.91 (1H, d, J 8 Hz, H-6'), 7.18-7.45 (6H, m, H-4', 5', 2, 3, 6, 7), 7.62 (2H, d, J 8 Hz, H-1, 8), 7.78-7.85 (3H, m, H-4, 5, 2'); δ_C (100.6 MHz, $CDCl_3$) 36.2 (C-2), 47.5 (C-1), 47.7 (C-9), 67.3 (CH_2 O), 119.5 (C-2', 4'), 120.5 (C-1, 8), 124.5 (C-6'), 125.3, 125.5 (C-2, 7), 127.6 (C-4, 5), 128.3 (C-3, 6), 130.0 (C-5'), 138.8 (C-1'), 141.8 (C-4a, 5a), 144.1 (C-1a, 8a); m/z (ES^+) 406.1535($(M+Na^+)$). $C_{24}H_{21}N_3O_2Na$ requires 406.1532); m/z (ES^+) 406 ($(M+Na^+)$, 100%).

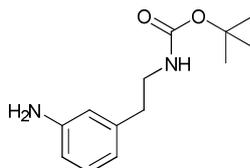
Synthesis of *N*-2-(3'-aminophenyl)ethyl-*N*'-hydroxyguanidine dihydrochloride (**111**)



2-(3'-*N*-9-Fluorenylmethoxycarbonylamino)phenylethylcyanamide (**180**) (637 mg, 1.7 mmol) was mixed with hydroxylamine hydrochloride (127 mg, 1.8 mmol) in dry methanol (20 ml) and sodium acetate (13.6 mg, 0.17 mmol) was added as a catalyst. The reaction mixture was heated under reflux for 8 h under an argon atmosphere. Then, the reaction mixture was cooled to 0 °C in an ice bath and di-*tert*-butyl dicarbonate (0.29 g, 1.34 mmol) and potassium carbonate (0.17 g, 1.21 mmol) were added. The solution was left to stir for an hour. Then, the solvent was removed at reduced pressure and the residue was partitioned between water (10 ml) and ethyl acetate (20 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to give a yellow oil. The residue was dissolved in dry THF (10 ml) and piperidine (20% w/w solution) was added. The reaction was left to stir for another hour. The solvent was removed at reduced pressure and the residue was dissolved in dry methanol (10 ml). Then, the reaction mixture was cooled to 0 °C in an ice bath and di-*tert*-butyl dicarbonate (0.29 g, 1.34 mmol) and potassium carbonate (0.17 g, 1.21 mmol) were added. The solution was left to stir for an hour. Then, the solvent was removed at reduced pressure and the residue was partitioned between water (10 ml) and ethyl acetate (20 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to give a yellow oil. The crude product was purified by column chromatography (petroleum: diethyl ether: ethyl acetate-gradient). The pure product was then dissolved in dry THF (1 ml) and treated with anhydrous HCl in 1,4-dioxane (4 M, 5 ml) at room temperature for 16 h. The solid was filtered off and washed with diethyl ether to give the pure product (**111**) as an off-white solid. (121 mg, 37%). mp 169-170 °C (dec.); ν_{\max} (nujol)/cm⁻¹ 3118, 2613 (NH), 1639 (C=C); δ_{H} (300 MHz, d₆-DMSO) **major isomer:** 2.90 (2H, t, *J* 7 Hz, CH₂-2), 3.73 (2H, t, *J* 7 Hz, CH₂-1), 6.69 (2H, br s), 7.11-7.24 (3H, m, H-2', 4', 6'), 7.37 (1H, t, *J* 8 Hz, H-5'), 7.72 (2H, s, NH₂), 7.92 (1H, br t, NH), 9.79 (3H, br s, NH₃⁺), 10.41 (1H, s, NH/OH); **minor isomer:** 2.82 (2H, t, *J* 7 Hz, CH₂-2), 3.34-3.42 (2H, m, CH₂-1), 6.69 (2H, br s), 7.11-7.24 (3H, m, H-2', 4', 6'), 7.37 (1H, t, *J* 8 Hz, H-5'), 7.72 (2H, s, NH₂), 7.92 (1H, br t, NH), 9.79 (3H, br s, NH₃⁺), 10.41 (1H, s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) **major isomer:** 33.2 (C-1), 41.9 (C-2), 120.5 (C-4'), 122.5 (C-2'),

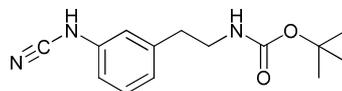
127.0 (C-6'), 130.1 (C-5'), 157.2 (C=NOH); **minor isomer:** 34.2 (C-1), 42.1 (C-2), 120.5 (C-4'), 122.5 (C-2'), 127.0 (C-6'), 130.1 (C-5'), 157.2 (C=NOH); m/z (ES⁺) 195.1252((M+H)⁺. C₉H₁₅N₄O requires 195.1246); m/z (ES⁺) 195 ((M+H)⁺, 100%).

Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(3'-aminophenyl)ethylamine (**118**)



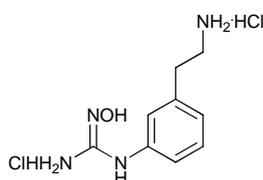
N-(*tert*-Butoxycarbonyl)-2-(3'-*N*-9-fluorenylmethoxycarbonylphenyl)ethylamine (**110**) (0.92 g, 2.01 mmol) was dissolved in THF (15 ml) and piperidine (2.5 ml) was added. The reaction mixture was left to stir over night. After removal of the solvent under reduced pressure, the residue was purified by column chromatography using gradient elution with diethyl ether and ethyl acetate. The pure product (**118**) was obtained as a pale yellow solid (0.38 g, 81%). mp 100-101 °C; (Found: C, 66.4; H, 8.8; N, 11.7%. C₁₃H₂₀N₂O₂ requires C, 66.1; H, 8.5; N, 11.8%); ν_{\max} (nujol)/cm⁻¹ 3430, 3376, 3350 (NH), 1674 (C=O), 1604, 1589 (C=C); δ_{H} (400 MHz, d₆-DMSO) 1.37 (9H, s, CH₃), 2.48-2.52 (2H, m, H-2), 3.01-3.09 (2H, m, H-1), 4.94 (2H, br s, NH₂), 6.31 (1H, d, *J* 8 Hz, H-4'), 6.35-6.40 (2H, m, H-2', 6'), 6.83 (1H, br t, *J* 6 Hz, NH), 6.87-6.92 (1H, m, H-5'); δ_{C} (75.46 MHz, d₆-DMSO) 28.2 (CH₃), 35.9 (C-2), 41.7 (C-1), 77.4 (C-*t*-butyl), 111.8 (C-4'), 114.1 (C-2'), 116.0 (C-6'), 128.8 (C-5'), 139.8 (C-1'), 148.6 (C-3'), 155.4 (C=O); m/z (ES⁺) 259.1416 ((M+Na)⁺). C₁₃H₂₀N₂O₂Na requires 259.1422); m/z (ES⁺) 259 ((M+Na)⁺, 100%).

Synthesis of *N*-(*tert*-butoxycarbonyl)-2-(3'-aminophenyl)cyanamide (**119**)



To a suspension of *N*-(*tert*-butoxycarbonyl)-2-(3'-aminophenyl)ethylamine (**118**) (0.95 g, 4.0 mmol) and sodium acetate (0.66 g, 8.0 mmol) in dry methanol (40 ml) was added dropwise a 3M solution of BrCN in CH₂Cl₂ (1.4 ml, 4.0 mmol). The mixture was stirred in an ice bath for 3 h, then the ice bath was removed and reaction mixture was allowed to stir at room temperature overnight. The solvent was removed at reduced pressure and the crude product was extracted with ethyl acetate (3 x 50 ml). The organic layer was washed with brine (50 ml), dried over MgSO₄ and the solvent was removed at reduced pressure. The pure cyanamide was obtained by column chromatography on silica using dichloromethane/ethyl acetate (1:1) as the eluting solvents. This gave the desired product (**119**) as a yellow oil (0.88 g, 85%). ν_{\max} (nujol)/cm⁻¹ 3321, 3065, 2977 (NH), 2226 (C≡N), 1710 (C=O), 1609 (C=C); δ_{H} (400 MHz, CDCl₃) 1.42 (9H, s, CH₃), 2.74 (2H, t, *J* 7 Hz, CH₂-2), 3.26-3.39 (2H, m, CH₂-1), 4.75 (1H, br s, NH), 6.82-6.91 (3H, m, H-2', 4', 6'), 7.22 (1H, t, *J* 8 Hz, H-5'), 8.06 (1H, br s, NH); δ_{C} (75.46 MHz, CDCl₃) 28.8 (CH₃), 36.5 (C-2), 42.0 (C-1), 66.3 (C-*t*-butyl), 112.4 (C≡N), 113.9 (C-6'), 116.1 (C-2'), 124.1 (C-4'), 130.3 (C-5'), 138.5 (C-1'), 141.1 (C-3'), 156.7 (C=O); m/z (ES⁺) 284.1375 ((M+Na⁺). C₁₄H₁₉N₃O₂Na requires 284.1373); m/z (ES⁺) 284 ((M+Na⁺), 100%), m/z (ES⁻) 260 ((M-H)⁻, 100%).

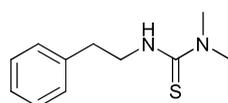
Synthesis of *N*-phenyl-(3-ethylamine)-*N*'-hydroxyguanidine hydrochloride (**117**)



N-(*tert*-Butoxycarbonyl)-2-(3'-aminophenyl)cyanamide (**119**) (0.79 g, 3.0 mmol) was mixed with hydroxylamine hydrochloride (0.21 g, 3.0 mmol) in dry methanol (30 ml) and potassium carbonate (0.1 equiv) was added as a catalyst. The reaction mixture was heated under reflux for

7 h under an argon atmosphere. After the completion, the mixture was cooled in an ice bath and di-*tert*-butyl dicarbonate (1.62 g, 3.73 mmol) and potassium carbonate (0.54 g, 3.73 mmol) were added. The solution was left to stir for an hour. The solvent was then removed at reduced pressure and the residue was partitioned between water (10 ml) and ethyl acetate (20 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to give a yellow oil. Further purification was achieved by column chromatography of the protected hydroxyguanidine (petroleum: diethyl ether: ethyl acetate-gradient). The pure product was then dissolved in dry THF (2 ml) and treated with anhydrous HCl in 1,4-dioxane (4 M, 5 ml) at room temperature for 14 h. The solid was filtered off and washed with diethyl ether to give the pure product (**117**) as a yellow solid (150 mg, 22%). mp 159-160 °C (dec.); ν_{\max} (nujol)/cm⁻¹ 3322, 2458 (NH), 1600, 1598 (C=C); δ_{H} (300 MHz, d₆-DMSO) 2.92 (2H, t, *J* 7 Hz, CH₂-1), 2.98-3.06 (2H, m, CH₂-2), 7.07 (1H, d, *J* 8 Hz, H-6'), 7.13-7.19 (2H, m, H-2', 4'), 7.35 (1H, t, *J* 8 Hz, H-5'), 7.93 (2H, br s, NH₂), 8.27 (3H, br s, NH₃⁺), 10.07 (1H, br s, NH), 10.18 (1H, br s, NH/OH), 10.91 (1H, s, NH/OH); δ_{C} (75.46 MHz, d₆-DMSO) 33.0 (C-1), 40.1 (C-2), 120.7 (C-6'), 123.2 (C-2'), 125.0 (C-4'), 127.2 (C-5'), 130.2 (C-1'), 138.9 (C-3'), 156.7 (C=NHOH); m/z (ES⁺) 195.1246 ((M+H)⁺). C₉H₁₅N₄O requires 195.1246; m/z (ES⁺) 195 ((M+H)⁺, 100%).

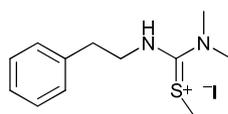
Synthesis of *N,N*-dimethyl-*N'*-phenethyl thiourea (**123**)^{13,14}



Phenylethyl isothiocyanate (5.0 g, 30.6 mmol) was dissolved in methanol (45 ml) and water (5 ml), and then potassium carbonate (4.23 g, 30.6 mmol) was added. Dimethylamine hydrochloride (2.5 g, 30.6 mmol) was dissolved in methanol (10 ml) and added dropwise to the reaction mixture. The solution was allowed to stir overnight at room temperature. The inorganic salt was filtered off and product (**123**) was crystallized from methanol as colourless crystals (5.08 g, 80%). mp 110-112 °C (lit.¹⁴, 112 °C); (Found: C, 63.4; H, 7.9; N, 13.5%. C₁₁H₁₆N₂S requires C, 63.4; H, 7.7; N, 13.4%); ν_{\max} (nujol)/cm⁻¹ 3424 (NH), 1638 (C=C), 1535; δ_{H} (300 MHz, d₆-DMSO) 2.83 (2H, t, *J* 7, CH₂-2), 3.12 (6H, s, 2×CH₃), 3.60-3.67 (2H, m, CH₂-1), 7.15-7.23 (3H, m, H-2', 4', 6'), 7.25-7.32 (2H, m, H-3', 5'), 7.46 (1H, t, *J* 5 Hz, NH); δ_{C} (75.46 MHz, d₆-DMSO) 35.0 (C-2), 40.1 (CH₃), 46.8 (C-1), 126.0 (C-4'), 128.3 (C-2', 6'), 128.6 (C-3', 5'), 139.6

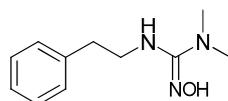
(C-1'), 181.1 (C=S); m/z (ES⁺) 209 ((M+H)⁺, 100%), 231 (7), 247 (40); m/z (ES⁻) 207 ((M-H)⁻, 100%);

Synthesis of *N,N*-dimethyl-*N'*-phenethyl-*S*-methylsulfonium iodide (**125**)



N,N-Dimethyl-*N'*-phenethylthiourea (**123**) (1.53 g, 7.4 mmol) was dissolved in acetonitrile (30 ml) and then iodomethane (2.09 g, 14.7 mmol) was added dropwise. The reaction mixture was stirred under an argon atmosphere overnight. The solvent was removed at reduced pressure and trapped solvent was treated with bleach. The residue was partitioned between water (20 ml) and CH₂Cl₂ (2 × 20 ml), the organic layer was washed with aq. NaHCO₃ (30 ml) and dried over MgSO₄. Removal of solvent at reduced pressure gave the final product (**125**) as pale yellow crystals (1.56 g, 61%). mp 66-67 °C; δ_H (500 MHz, CDCl₃) 2.26 (3H, s, CH₃), 3.17 (2H, t, *J* 7 Hz, CH₂-2), 3.41 (6H, s, 2×CH₃), 3.99 (2H, dt, *J* 6 Hz, *J* 7 Hz, CH₂-1), 7.18-7.31 (5H, m, Ar-H), 10.00 (1H, br t, *J* 6 Hz, NH); δ_C (125.7 MHz, CDCl₃) 17.0 (S-CH₃), 36.3 (C-2), 43.8 (CH₃), 49.1 (C-1), 53.5 (CH₃), 126.9 (C-4'), 128.7 (C-2', 6'), 129.3 (C-3', 5'), 137.5 (C-1'), 168.1 (C=S-CH₃); m/z (ES⁺) 223.1268 ((M)⁺. C₁₂H₁₉N₂S requires 223.1269).

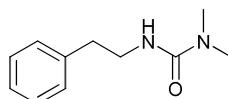
Attempted synthesis of *N,N*-dimethyl-*N'*-phenethyl-*N''*-hydroxyguanidine (**122**)



N,N-Dimethyl-*N'*-phenethyl-*S*-methylsulfonium iodide (**125**) (2.13 g, 6.1 mmol), hydroxylamine hydrochloride (0.42 g, 6.1 mmol) and triethylamine (2.53 g, 18.24 mmol) were dissolved in CH₃CN (40 ml) and cooled to 0 °C. Then AgNO₃ (1.14 g, 6.7 mmol) was dissolved in CH₃CN (5ml) and added dropwise to the reaction mixture which was stirred at 0 °C for 3 hours. Then the suspension was filtered through celite to remove the grey-black silver salts. The filtrate was concentrated and residue dissolved in ethyl acetate (30 ml). The organic layer was washed with 1M H₃PO₄ (2 × 30 ml) and brine (30 ml), then dried over MgSO₄ and concentrated at reduced

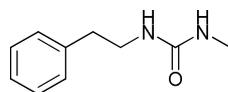
pressure to give a brown oil. The addition of diethyl ether caused precipitation and the colourless solid was filtered off and recrystallized from diethyl ether. However, the synthesized product according to results of ^1H , ^{13}C NMR, mass spectrometry, elemental analysis and X-ray was *N,N*-dimethyl-*N'*-phenethyl urea (**126**) (453 mg, 21%). mp 95-96 °C, (lit.¹⁵, 98 °C); The rest of data are the same as below.

Synthesis of *N,N*-dimethyl-*N'*-phenethyl urea (**126**)¹⁵



To a solution of phenylethylamine (11.17 g, 70.9 mmol) and triethylamine (19.75 ml, 141.7 mmol) in CH_2Cl_2 (100 ml) was added dropwise dimethylcarbamoyl chloride (6.51 ml, 70.9 mmol). The reaction mixture was stirred for about 6 h and then it was washed with water (3×40 ml). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give a desired product (**126**) as pale yellow crystals (11.45 g, 84%). mp 98-99 °C, (lit.¹⁵, 98 °C); δ_{H} (300 MHz, d_6 -DMSO) 2.70 (2H, t, J 7 Hz, CH_2 -2), 2.76 (6H, s, $2 \times \text{CH}_3$), 3.16-3.25 (2H, m, CH_2 -1), 6.36 (1H, br t, J 6 Hz, NH), 7.15-7.21 (3H, m, H-2', 4', 5'), 7.24-7.32 (2H, m, 3', 6'); δ_{C} (75.46 MHz, d_6 -DMSO) 35.8 (CH_3), 36.2 (C-2), 42.0 (C-1), 125.9 (C-4'), 128.2 (C-2', 6'), 128.6 (C-3', 5'), 139.9 (C-1'), 158.1 (C=O).

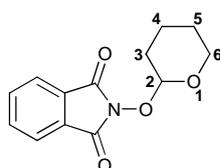
Synthesis of *N*-methyl-*N'*-phenethyl urea (**129**)¹⁶



To a solution of phenethyl isocyanate (4.7 g, 32 mmol) in dry THF (60 ml) was added dropwise a 2M solution of methylamine in methanol (16 ml, 32 mmol). The reaction mixture was stirred for about 12 h and the solvents were removed under reduced pressure. The residue was recrystallized from petroleum ether and CH_2Cl_2 to give a final product (**129**) as pale yellow solid (4.45 g, 78%). mp 70-72 °C; (Found: C, 67.6; H, 7.9; N, 15.8%. $\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}$ requires C, 67.4;

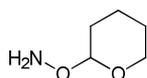
H, 7.9; N, 15.7%); δ_{H} (300 MHz, CDCl_3) 2.61 (3H, br s, CH_3), 2.70 (2H, t, J 7 Hz, CH_2 -2), 3.32 (2H, t, J 7Hz, CH_2 -1), 4.78 (2H, br s, $2\times\text{NH}$), 7.06-7.16 (3H, m, H-2', 4', 5'), 7.17-7.25 (2H, m, 3', 6'); δ_{C} (75.46 MHz, CDCl_3) 27.0 (CH_3), 36.5 (C-2), 41.7 (C-1), 126.4 (C-4'), 128.6 (C-2', 6'), 128.8 (C-3', 5'), 139.2 (C-1'), 159.4 (C=O).

Synthesis of *O*-Tetrahydropyranyl-*N*-hydroxyphthalimide (**181**)¹⁷



N-Hydroxyphthalimide (6.24 g, 38.2 mmol) was dissolved in dry CH_2Cl_2 (100 ml). Then, 3,4-dihydropyran (5.23 ml, 57.4 mmol) and pyridinium-*p*-toluenesulfonate (0.46 g, 3.82 mmol) were added. The reaction was left to stir for 17 h under an argon atmosphere. The reaction mixture was then diluted with diethyl ether (500 ml) and washed with brine (3×200 ml). The organic layer was dried over MgSO_4 . Removal of the solvent at reduced pressure yielded a colourless solid which was recrystallized from ethyl acetate to give a final product (**181**) as a colourless solid (5.37 g, 60%). mp 122-124 °C (lit.¹⁷, 123 °C); δ_{H} (300 MHz, CDCl_3) 1.58-2.18 (6H, m, CH_2 -3,4,5), 3.61-3.70 (1H, m, H-6a), 4.45-4.57 (1H, m, H-6b), 5.39-5.43 (1H, m, CH_2 -2), 7.69-7.76 (2H, m, H-3', 4'), 7.78-7.86 (2H, m, H-2', 5'); δ_{C} (75.46 MHz, CDCl_3) 17.6 (C-4), 24.8 (C-5), 27.7 (C-3), 62.3 (C-6), 103.1 (C-2), 123.4 (C-2', 5'), 129.1 (C-3', 4'), 134.3 (C-1', 6'), 163.8 (C=O); m/z (ES^+) 270 ($(\text{M}+\text{Na})^+$, 100%).

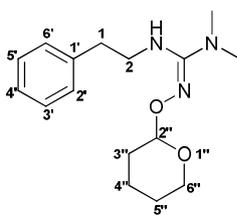
Synthesis of *O*-Tetrahydropyranylhydroxylamine (**182**)



O-Tetrahydropyranyl-*N*-hydroxyphthalimide (**181**) (5.37 g, 21.7 mmol) was dissolved in ethanol (300 ml) and hydrazine hydrate (1.06 ml, 21.7 mmol) was added. The reaction mixture was heated under reflux for 5 h and then cooled to room temperature. The by-product was removed

by filtration and the filtrate concentrated under reduced pressure to give off-white solid to which another portion of ethanol (50 ml) was added. The by-product was filtrated off and the removal of solvent gave a yellow residue, which was then distilled under reduced pressure using Kugelrohr apparatus to give the final product (**182**) as colourless oil (1.71 g, 50 %). δ_{H} (300 MHz, d_6 -DMSO) 1.53-1.71 (2H, m, CH_2 -3), 1.36-1.53 (4H, m, CH_2 -4,5), 3.38-3.45 (1H, m, H-6a), 3.71-3.81 (1H, m, H-6b), 4.54-4.58 (1H, m, H-2), 5.98 (2H, br s, NH_2); δ_{C} (75.46 MHz, d_6 -DMSO) 19.4 (C-4), 24.8 (C-5), 28.5 (C-3), 61.6 (C-6), 101.5 (C-2); m/z (ES^+), 140.0687 ($(\text{M}+\text{Na})^+$. $\text{C}_5\text{H}_{11}\text{NO}_2\text{Na}$ requires 140.0686); m/z (ES^+) 140 (100%).

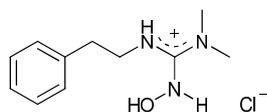
**Synthesis of *N,N*-dimethyl-*N'*-phenethyl-
N''-*O*-tetrahydropyranylhydroxyguanidine (**128**)**



N,N-Dimethyl-*N'*-phenethyl urea (**126**) (5.67 g, 29.5 mmol) was dissolved in dry toluene (150 ml) and cooled to $-22\text{ }^{\circ}\text{C}$. Then, a 1.93 M solution of COCl_2 in toluene (15.29 ml, 29.5 mmol) was added at such rate to maintain the temperature at $-22\text{ }^{\circ}\text{C}$. The reaction mixture was stirred at $-22\text{ }^{\circ}\text{C}$ for 6 h and then left so as to stir at room temperature for another 14 h. The intermediate *C*-chloroformamidinium chloride (**127**) was formed as a white, moisture-sensitive solid and was used for next step without characterization. The toluene was decanted off and the solid was redissolved in dry CH_2Cl_2 (35 ml). The solution was then added dropwise to a cool ($-22\text{ }^{\circ}\text{C}$) solution of *O*-tetrahydropyranylhydroxylamine (3.51 g, 29.5 mmol) and triethylamine (8.23 ml, 59.0 mmol) in dry CH_2Cl_2 (10 ml). The reaction mixture was warmed to the room temperature and stirred for another 4 h. Then, more CH_2Cl_2 (40 ml) was added and the organic layer was washed with water (6×40 ml) and dried over MgSO_4 . After removal of the solvent at reduced pressure, the residue was purified by column chromatography on silica using ethyl acetate/diethyl ether (3:1) as the eluting solvent. This gave the desired product (**128**) as a colourless oil (3.09 g, 36%). δ_{H} (400 MHz, CDCl_3) 1.42-1.61 (4H, m, CH_2 -4'', 5''), 1.65-1.80 (2H,

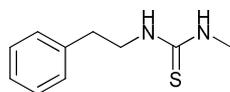
m, CH₂-3''), 2.68 (6H, s, 2 × CH₃), 2.81 (2H, t, *J* 7 Hz, CH₂-1), 3.25-3.99 (2H, m, CH₂-2), 3.51-3.58 (1H, m, H-6''a), 3.77-3.84 (1H, m, H-6''b), 4.76 (1H, br s, NH), 4.97-5.00 (1H, m, H-2''), 7.19-7.33 (5H, m, Ar-H); δ_C (100 MHz, CDCl₃) 20.4 (C-4''), 25.3 (C-5''), 29.4 (C-3''), 37.0 (C-1), 39.7 (CH₃), 45.2 (C-2), 63.3 (C-6''), 100.8 (C-2''), 126.5 (C-4'), 128.6 (C-2', 6'), 128.9 (C-3', 5'), 138.9 (C-1'), 160.8 (C=N); *m/z* (ES⁺) 292.2023 ((M+H)⁺. C₁₆H₂₆N₃O₂ requires 292.2025); *m/z* (ES⁻) 207 (100%).

Synthesis of *N,N*-dimethyl-*N'*-phenethyl-*N''*-hydroxyguanidine hydrochloride (**122**)



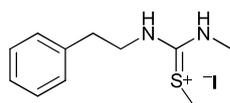
N,N-Dimethyl-*N'*-phenethyl-*N''*-*O*-tetrahydropyranylhdroxyguanidine (**128**) (1.00 g, 3.4 mmol) was dissolved in 1,4-dioxane (5 ml) and water (1 ml) and then a 4M HCl solution in 1,4-dioxane (8.6 ml, 34.3 mmol) was added. The reaction mixture was heated under reflux for 4 h. After cooling, the solvent was removed at reduced pressure and the residue was extracted between CH₂Cl₂ (25 ml) and H₂O (25 ml). Removal of water at reduced pressure yielded a brown oil, which was dissolved in the minimum amount of CH₃CN and crystallized from EtOAc/Et₂O to give the final product (**122**) as pale pink crystals (71 mg, 73%). mp 122-123 °C; ν_{max}(KBr)/cm⁻¹ 3198, 3089, 2853 (NH), 1869, 1641, 1571 (C=C); (Found: C, 54.3; H, 7.6; N, 17.0%. C₁₁H₁₈ClN₃O requires C, 54.2; H, 7.4; N, 17.2%); δ_H (300 MHz, d₆-DMSO) 2.82 (6H, s, 2 × CH₃), 2.85 (2H, t, *J* 7 Hz, CH₂-2), 3.47 (2H, dt, *J* 7 Hz, CH₂-1), 7.17-7.33 (5H, m, Ar-H), 7.76 (1H, t, *J* 6 Hz, NH), 10.19 (1H, s, NH/OH), 10.69 (1H, br s, NH/OH); δ_C (75.46 MHz, d₆-DMSO) 35.6 (C-2), 39.2 (CH₃), 45.3 (C-1), 126.8 (C-4'), 128.8 (C-3', 5'), 129.3 (C-2', 6'), 138.9 (C-1'), 158.9 (C=NOH); *m/z* (ES⁺) 207 (100%).

Synthesis of *N*-methyl-*N'*-phenethylthiourea (**131**)¹⁸



Phenylethyl isothiocyanate (2.5 g, 15.3 mmol) was dissolved in methanol (25 ml), and then an aqueous solution of methylamine (11.95 ml, 15.3 mmol, 40%) was added. The solution was allowed to stir overnight at room temperature. The solvent was removed at reduced pressure. Addition of cold water caused precipitation and the crude product was filtered off. Pure *N*-methyl-*N'*-phenethyl thiourea (**131**) was obtained by crystallization from cold water/methanol (9:1) as a colourless solid (2.67 g, 90%). mp 62-64 °C, (lit.¹⁸, 66-67 °C); (Found: C, 61.7; H, 7.8; N, 14.5%. C₁₀H₁₄N₂S requires C, 61.8; H, 7.3; N, 14.4%); ν_{\max} (nujol)/cm⁻¹ 3400, 3214 (NH), 1638, 1560; δ_{H} (300 MHz, CDCl₃) 2.89 (3H, s, CH₃), 2.95 (2H, t, *J* 7 Hz, CH₂-2), 3.78 (2H, t, *J* 7 Hz, CH₂-1), 7.20-7.30 (3H, m, H-2', 4', 6'), 7.30-7.38 (2H, m, H-3', 5'); δ_{C} (75.46 MHz, CDCl₃) 30.4 (CH₃), 35.2 (C-2), 45.7 (C-1), 126.8 (C-4'), 128.8 (C-2', 6'), 128.9 (C-3', 5'), 138.3 (C-1'), 182.4 (C=S); m/z (ES⁺) 217 ((M+Na)⁺, 100%).

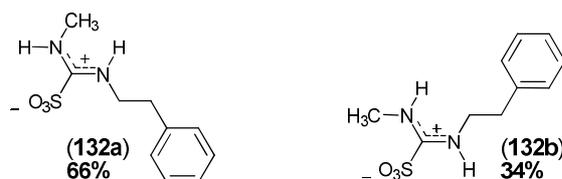
Synthesis of *N*-methyl-*N'*-phenethyl-*S*-methylsulfonium iodide (**134**)



N-Methyl-*N'*-phenethylthiourea (**131**) (5.04 g, 26.0 mmol) was dissolved in dry acetonitrile (150 ml) and then iodomethane (7.37 g, 51.9 mmol) was added drop wise. The reaction mixture was stirred under an argon atmosphere overnight. The solvent was removed at reduced pressure and trapped solvent was treated with bleach. The residue was partitioned between water (80 ml) and CH₂Cl₂ (2 × 60 ml), the organic layer was washed with aq. NaHCO₃ (60 ml) and dried over MgSO₄. Removal of solvent at reduced pressure led to the final product (**134**) as pale yellow low melting crystals (6.05 g, 70%). δ_{H} (300 MHz, CDCl₃) 2.52 (3H, s, CH₃), 2.97 (2H, t, *J* 7 Hz, CH₂-2), 3.04 (3H, br s, CH₃), 3.67 (2H, t, *J* 7 Hz, CH₂-1), 6.28 (1H, br s, NH), 7.16-7.31 (5H, m, Ar-H); δ_{C} (75.46 MHz, CDCl₃) 15.6 (SCH₃), 33.1 (CH₃), 36.3 (C-2), 47.4 (C-1), 127.1 (C-4'),

129.0 (C-2', 6'), 129.4 (C-3', 5'), 138.8 (C-1'), 162.1 (C=S); m/z (ES⁺) 209.1112 ((M)⁺. C₁₁H₁₇N₂S requires 209.1115).

Synthesis of *N*-methyl-*N*'-phenylethylaminomethane sulfonic acid (**132**)



Peracetic acid was prepared by slowly adding acetic anhydride (25 ml, 266 mmol) to 30% H₂O₂ (22.5 ml, 625 mmol) cooled in an ice bath. The addition of two drops of concentrated H₂SO₄ caused a violent reaction. After the reaction was completed, acetic anhydride (55 ml, 582 mmol) was slowly added to the cooled mixture. The mixture was left to stand for one day. A cooled solution of *N*-methyl-*N*'-phenethyl thiourea (**131**) (1.10 g, 5.7 mmol) in CH₂Cl₂ (50 ml) was added to the freshly prepared peracetic acid (3 equiv.) at such a rate so as to maintain the temperature of the reaction at 2-5 °C. The mixture was then warmed to room temperature and left overnight. The yellow solid was filtered off and recrystallized from EtOH to give the final product (**132**) as colourless crystals (342 mg, 25%). mp 203-204 °C; (Found: C, 49.3; H, 5.6; N, 11.5%. C₁₀H₁₄N₂O₃S requires C, 49.6; H, 5.8; N, 11.6%); ν_{\max} (PTFE card)/cm⁻¹ 1273s (SO₃H), m/z (ES⁺) 265 ((M+Na)⁺, 100%).

The NMR data showed the presence of two geometric isomers:

δ_{H} (300 MHz, d₆-DMSO)*

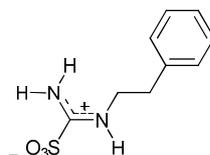
Protons	δ_{H} 132a (66%)	δ_{H} 132b (34%)
-CH ₃	2.82 (3H, d, <i>J</i> 6 Hz)	3.17 (3H, d, <i>J</i> 6 Hz)
H-1	3.84-3.90 (2H, m)	3.43-3.50 (2H, m)
H-2	2.86-2.95 (2H, m)	2.86-2.95 (2H, m)
-NH ⁺	9.07 (1H, t, <i>J</i> 6 Hz)	9.26 (1H, t, <i>J</i> 6 Hz)
-NHCH ₃	9.36-9.41 (1H, m)	9.10-9.14 (1H, m)
Aromatic	7.19-7.35 (5H, m)	7.19-7.35 (5H, m)

δ_C (75.46 MHz, d_6 -DMSO)*

Carbons	δ_C 132a (66%)	δ_C 132b (34%)
-CH ₃	31.4	29.3
C-1	46.1	43.2
C-2	36.1	33.2
C-1'	138.5	138.3
C-2', 6'	128.9	128.7
C-3', 5'	129.1	129.0
C-4'	126.8	126.8
C-SO ₃ ⁻	164.5	163.8

* The data are supported by ¹H-¹H COSY, ¹H-¹³C HMBC and 1D gs-NOESY experiments, which also established the configuration and ratio of two isomers.

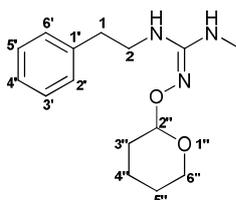
Synthesis of *N*-phenylethylaminomethane sulfonic acid (**137**)



Peracetic acid was prepared by slowly adding acetic anhydride (25 ml, 266 mmol) to 30% H₂O₂ (22.5 ml, 625 mmol) cooled in an ice bath. The addition of two drops of concentrated H₂SO₄ caused a violent reaction. After the reaction was completed, acetic anhydride (55 ml, 582 mmol) was slowly added to the cooled mixture. The mixture was left to stand for one day. A cooled solution of *N*-phenethyl thiourea (1.02 g, 5.7 mmol) in MeOH (51 ml) was added to the freshly prepared peracetic acid (3 equiv.) at such a rate so as to maintain the temperature of the reaction at 2-5 °C. The mixture was then warmed to room temperature and left overnight. The yellow solid was filtered off and recrystallized from EtOH to give the final product (**137**) as colourless crystals (0.98, 76%). mp 135-137 °C ; (Found: C, 47.7; H, 5.40; N, 12.1%. C₉H₁₂N₂O₃S requires C, 47.4; H, 5.30; N, 12.3 %); ν_{\max} (nujol)/cm⁻¹ 3100 (NH), 1250 (R-SO₃⁻); δ_H (300 MHz, d_6 -DMSO) 2.82 (2H, d, *J* 7 Hz, CH₂-2), 3.41-3.50 (2H, m, CH₂-1), 7.18-7.33 (5H, m, Ar-H), 9.24 (1H, s, NH), 9.26 (1H, s, NH), 9.64 (1H, br t, *J* 6 Hz, NH); δ_C (75.46 MHz, d_6 -DMSO) 33.0

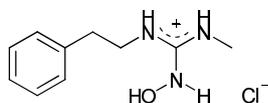
(C-2), 43.3 (C-1), 126.4(C-4'), 128.3 (C-2', 6'), 128.8 (C-3', 5'), 137.9 (C-1'), 165.3 (C-SO₃); m/z (ES) 227 (M-H⁺, 100%).

Synthesis of *N*-methyl-*N'*-phenethyl-*N''*-*O*-tetrahydropyranlylhydroxyguanidine (**135**)



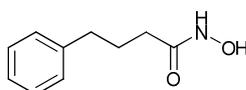
A solution of *N*-methyl-*N'*-phenethyl-*S*-methylsulfonium iodide (**134**) (2.00 g, 5.9 mmol), triethylamine (0.99 ml, 7.1 mmol) and *O*-tetrahydropyranlylhydroxylamine (1.04 g, 8.9 mmol) in dry acetonitrile (40 ml) was added to a solution of AgNO₃ (2.53 g, 14.9 mmol) in dry acetonitrile (5 ml) at such a rate so as to keep the temperature at 0 °C. A yellow precipitate was formed. The reaction mixture was left to stir for 2h in the ice bath. Then, the inorganic solid was filtered through celite and the filtrate was concentrated under reduced pressure to give a yellow oil. The residue was purified by flash chromatography on silica gel using a gradient of diethyl ether and ethyl acetate to afford the final product (**135**) as a pale yellow oil (343 mg, 21%). δ_{H} (400 MHz, CDCl₃) 1.40-1.56 (4H, m, H-4'', 5'', CH₂), 1.62-1.75 (2H, m, H-3'', CH₂), 2.57 (3H, br d, *J* 6 Hz, CH₃), 2.77 (2H, t, *J* 7 Hz, H-1, CH₂), 3.20-3.28 (2H, m, H-2, CH₂), 3.43-3.51 (1H, m, H-6'', CH), 3.75-3.86 (1H, m, NH), 4.61-4.65 (1H, m, H-2'', CH-**minor isomer**), 4.67-4.86 (1H, m, H-2'', CH-**major isomer**), 7.17-7.30 (5H, m, Ar-H); δ_{C} (100 MHz, CDCl₃) 19.6, 20.8 (two isomers, C-4''), 25.3, 25.4 (two isomers, C-5''), 28.5 (CH₃), 28.8, 29.7 (C-3'', two isomers), 37.0 (C-1), 42.3 (C-2), 62.5, 63.7 (two isomers, C-6''), 101.0, 102.5 (two isomers, C-2''), 126.5 (C-4'), 128.6 (C-3', 5'), 128.9 (C-4', 6'), 138.0 (C-1'), 156.7 (C=N); m/z (ES⁺), 278.1866 ((M+H)⁺. C₁₅H₂₃N₃O₂ requires 278.1869); m/z (ES⁺) 300 (100%), 278 (53%).

Synthesis of *N*-methyl-*N*'-phenethyl-*N*''-hydroxyguanidine hydrochloride (**121**)



N-Methyl-*N*'-phenethyl-*N*''-*O*-tetrahydropyranylhydroxyguanidine (**135**) (139 mg, 0.5 mmol) was dissolved in 1,4-dioxane (1 ml) and water (0.1 ml) and then a 4M HCl solution in 1,4-dioxane (1.3 ml, 5.0 mmol) were added. The reaction mixture was heated under reflux for 4 h. After cooling, the solvent was removed at reduced pressure and residue extracted between CH₂Cl₂ (10 ml) and H₂O (10 ml). The removal of water yielded a brown oil, which was dissolved in the minimum amount of CH₃CN and crystallized from EtOAc/Et₂O to give the final product (**121**) as an off-white solid (81 mg, 71%). mp 170-171 °C; δ_H (400 MHz, d₆-DMSO) 2.71 (3H, br, CH₃), 2.83 (2H, t, *J* 7 Hz, CH₂-2), 7.15-7.42 (5H, m, Ar-H), 7.88 (1H, br s, NH), 7.98 (1H, br d, *J* 6 Hz, NH), 9.91 (1H, s, NH/OH), 10.59 (1H, br s, NH/OH), [CH₂-1 overlapped with solvent]; δ_H (400 MHz, d₄-methanol) 2.76 (3H, br, CH₃), 2.90 (2H, t, *J* 7 Hz, CH₂-2), 3.43 (2H, br t, *J* 7 Hz, CH₂-1), 7.20-7.27 (3H, m, H-2', 4', 6'), 7.29-7.34 (2H, m, H-3', 5'); δ_C (75.46 MHz, d₆-DMSO) 27.6 (CH₃), 34.4 (C-2), 41.9 (C-1), 126.3 (C-4'), 128.3 (C-3', 5'), 128.8 (C-2', 6'), 138.4 (C-1'), 156.9 (C=NOH); *m/z* (ES⁺), 194.1300 ((M+H)⁺. C₁₀H₁₆N₃O requires 194.1293); *m/z* (ES⁺) 194 (100%).

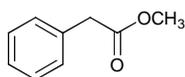
Synthesis of 4-phenylbutyrohydroxamic acid (**140**)¹⁹



4-Phenylbutyric acid (2.38 g, 14.5 mmol) and DMF (few drops) were dissolved in CH₂Cl₂ (100 ml) and cooled to 0 °C. Oxalyl chloride (4.4 g, 33.6 mmol) was added slowly to the reaction mixture. After being stirred for 40 minutes, the mixture was added to a solution of hydroxylamine hydrochloride (3.75 g, 58 mmol) and triethylamine (8.8 g, 87 mmol) in THF (50 ml)/H₂O (10 ml). After being stirred for an additional 30 minutes, the mixture was poured into 2 M HCl (20 ml) and extracted with CH₂Cl₂ (2 x 20 ml). The organic phase was dried over MgSO₄ and the solvent was removed at reduced pressure. The residue was washed with

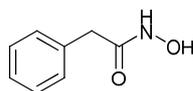
aq. saturated NaHCO_3 (30 ml) and extracted with ethyl acetate (2 x 30 ml). The solvent was removed at reduced pressure and crude product was purified by flash chromatography on silica, using diethyl ether/petroleum ether (2:1) as the eluting solvent. The final product (**140**) was recrystallized from diethyl ether to give tan yellow crystals (0.28 g, 11%). mp 72-74 °C (lit.¹⁹, 77.5-79 °C); (Found: C, 66.6; H, 7.3; N, 7.7%. $\text{C}_{10}\text{H}_{13}\text{NO}_2$ requires C, 67.0; H, 7.1; N, 7.8%); δ_{H} (300 MHz, d_6 -DMSO) 1.73-1.83 (2H, m, CH_2 -3), 1.97 (2H, t, J 7 Hz, CH_2 -4) 2.55 (2H, t, J 7 Hz, CH_2 -2) 7.15-7.20 (3H, m, H-2', 4', 6') 7.25-7.31 (2H, m, H-3', 5') 8.70 (1H, s, NH), 10.37 (1H, s, OH); δ_{C} (75.46 MHz, d_6 -DMSO) 26.9 (C-2), 31.7 (C-1), 34.5 (C-3), 125.7 (C-4'), 128.3 (C-3', 5'), 129.4 (C-2', 6'), 141.6 (C-1'), 168.8 (C=O); m/z (ES^+) 202 ($(\text{M}+\text{Na})^+$, 100%).

Synthesis of methyl phenylacetate (**142**)²⁰



Phenylacetic acid (2.67 g, 19.9 mmol) was heated under reflux for 8 h in methanol (10 ml) with a catalytic amount of concentrated sulfuric acid. After cooling, the solvent was removed at reduced pressure and the residue was partitioned between CH_2Cl_2 (30 ml) and H_2O (30 ml). The organic layer was washed with aq. saturated NaHCO_3 (2 x 30 ml) and dried over MgSO_4 . Then the solvent was removed at reduced pressure to give the pure product (**142**) as a colourless oil (2.73 g, 91%). δ_{H} (300 MHz, CDCl_3) 3.63 (2H, s, CH_2 -2), 3.70 (3H, s, OCH_3), 7.26-7.37 (5H, m, Ar-H); δ_{C} (75.46 MHz, CDCl_3) 41.2 (C-2), 52.0 (OCH_3), 127.1 (C-4'), 128.6 (C-3', 5'), 129.3 (C-2', 6'), 134.0 (C-1'), 172.0 (C=O).

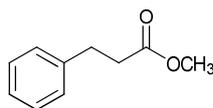
Synthesis of phenylacetohydroxamic acid (**138**)¹⁹



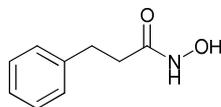
Methyl phenylacetate (**142**) (1.68 g, 11.2 mmol) in dry methanol (25 ml) was cooled in an ice bath and a preformed slurry of hydroxylamine hydrochloride (1.56 g, 22.4 mmol) and potassium hydroxide (2.51 g, 44.8 mmol) in dry methanol (15 ml) was added. The reaction mixture was stirred under an argon atmosphere overnight and was then acidified to pH 4 with HCl. The

solvent was removed at reduced pressure to give a colourless solid, which was repeatedly boiled in ethyl acetate and filtered until the ferrous chloride test was negative confirming that the entire product had been extracted. The combined filtrate was concentrated under reduced pressure to give phenylacetohydroxamic acid as a pale pink solid. The crude product was recrystallized from diethyl ether to give the pure hydroxamic acid (**138**) as pale pink crystals (0.97 g, 58%). mp 120-121 °C (lit.¹⁹, 122-123 °C); (Found: C, 63.7; H, 6.1; N, 9.3%. C₈H₉NO₂ requires C, 63.6; H, 6.0; N, 9.3%); δ_{H} (300 MHz, d₆-DMSO) 3.35 (2H, br s, CH₂-1), 7.18-7.35 (5H, m, Ar-H), 8.83 (1H, s, NH), 10.65 (1H, s, OH); δ_{C} (75.46 MHz, d₆-DMSO) 40.3 (C-2), 126.4 (C-4'), 128.1 (C-3', 5'), 128.9 (C-2', 6'), 136.0 (C-1'), 167.0 (C=O), m/z (ES⁺) 174 ((M+Na)⁺, 100%); m/z (ES⁻) 150 ((M-H)⁻, 100%).

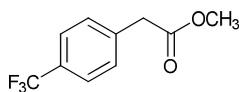
Synthesis of methyl 3-phenylpropionate (**183**)²¹



Hydrocinnamic acid (5.0 g, 33.3 mmol) was heated under reflux for 8 h in methanol (15 ml) with a catalytic amount of concentrated sulfuric acid. After cooling, the solvent was removed at reduced pressure and the residue was partitioned between CH₂Cl₂ (40 ml) and H₂O (40 ml). The organic layer was washed with saturated sodium bicarbonate solution (2 × 40 ml) and dried over MgSO₄. Then the solvent was removed at reduced pressure to give the pure product (**183**) as a colourless oil (4.08 g, 74%). δ_{H} (300 MHz, CDCl₃) 2.67 (2H, t, *J* 7 Hz, CH₂-1), 2.99 (2H, t, *J* 7 Hz, CH₂-2), 3.70 (3H, s, CH₃), 7.20-7.36 (5H, m, Ar-H); δ_{C} (75.46 MHz, CDCl₃) 31.0 (C-2), 35.7 (C-3), 51.6 (CH₃), 126.3 (C-4'), 128.3 (C-3', 5'), 128.5 (C-2', 6'), 140.5 (C-1'), 173.3 (C=O).

Synthesis of 3-phenylpropionohydroxamic acid (139)¹⁹

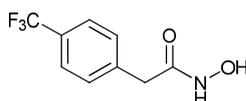
A solution of methyl 3-phenylpropionate (**183**) (3.96 g, 24.1 mmol) in dry methanol (40 ml) was cooled in an ice bath and a preformed slurry of hydroxylamine hydrochloride (3.35 g, 48.3 mmol) and potassium hydroxide (5.41 g, 96.6 mmol) in dry methanol (30 ml) was added. The reaction mixture was stirred under an argon atmosphere overnight and was then acidified to pH 4 with HCl. The solvent was removed at reduced pressure to give a colourless solid, which was repeatedly boiled in ethyl acetate and filtered until the ferrous chloride test was negative. The combined filtrates were concentrated under reduced pressure to give a pale pink solid. The crude product was recrystallized from petroleum ether to give the pure hydroxamic acid (**139**) as colourless crystals (2.67 g, 67%). mp 78-79 °C (lit.¹⁹, 79-80 °C); (Found: C, 65.0; H, 6.7; N, 8.6%. C₉H₁₁NO₂ requires C, 65.4; H, 6.7; N, 8.5%); δ_{H} (300 MHz, d₆-DMSO) 2.25 (2H, t, *J* 7 Hz, CH₂-1), 2.80 (2H, t, *J* 7 Hz, CH₂-2), 7.12-7.21 (3H, m, H-2', 4', 6'), 7.22-7.30 (2H, m, H-3', 5'), 8.72 (1H, br s, NH), 10.38 (1H, s, OH); δ_{C} (75.46 MHz, d₆-DMSO) 30.8 (C-2), 33.8 (C-3), 125.9 (C-4'), 128.2 (C-3', 5'), 128.3 (C-2', 6'), 141.0 (C-1'), 168.2 (C=O); *m/z* (ES⁺), 166 ((M+H)⁺, 100%), 188 (20).

Synthesis of methyl (4'-trifluoromethylphenyl)acetate (143)

4-(Trifluoromethyl)phenylacetic acid (2.50 g, 12.2 mmol) was heated under reflux for 8 h in methanol (20 ml) with a catalytic amount of concentrated sulfuric acid. After cooling, the solvent was removed at reduced pressure and the residue was partitioned between CH₂Cl₂ (30 ml) and H₂O (30 ml). The organic layer was washed with saturated sodium bicarbonate solution (2 × 30 ml) and dried over MgSO₄. Then the solvent was removed under reduced pressure to give the pure product (**143**) as a colourless oil (2.08 g, 78%). δ_{H} (300 MHz, CDCl₃) 3.69 (2H, s, CH₂), 3.71 (3H, s, CH₃), 7.40 (2H, d, *J* 8 Hz, H-2', 6'), 7.59 (2H, d, *J* 8 Hz, H-3', 5'); δ_{C} (75.46

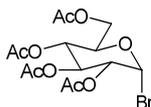
MHz, CDCl₃) 40.9 (C-2), 52.3 (CH₃), 125 (C-4'), 128.6 (C-3', 5'), 129.3 (C-2', 6'), 137.9 (C-1'), 171.2 (C=O); δ_F (376.5 MHz, CDCl₃), -63.0 (CF₃).

Synthesis of 4-(trifluoromethyl)phenylacetohydroxamic acid (**141**)



Methyl (4'-trifluoromethyl)phenylacetate (**143**) (2.00 g, 9.20 mmol) in dry methanol (25 ml) was cooled in an ice bath, and charged with preformed slurry of hydroxylamine hydrochloride (1.27 g, 18.3 mmol) and potassium hydroxide (2.06 g, 36.8 mmol) in dry methanol (15 ml). The reaction mixture was stirred under an argon atmosphere overnight and then was acidified to pH 4 with HCl. The solvent was removed at reduced pressure to give a colourless solid, which was repeatedly boiled in ethyl acetate and filtered until the ferrous chloride test was negative confirming that the entire product had been extracted. The combined filtrate was concentrated under reduced pressure to give 4-(trifluoromethyl)phenylacetohydroxamic acid as a pale pink solid. The crude product was recrystallized from diethyl ether to give the pure hydroxamic acid (**141**) as pale pink crystals (0.95 g, 47%). mp 138-139 °C; (Found: C, 49.4; H, 3.4; N, 6.2%. C₉H₈F₃NO₂ requires C, 49.3; H, 3.7; N, 6.4%); ν_{max}(nujol)/cm⁻¹ 3201 (NH, OH), 1636 (C=O), 1585 (C=C); δ_H (400 MHz, d₆-DMSO) 3.40 (2H, br s, CH₂), 7.48 (2H, d, *J* Hz, H-2', 6'), 7.67 (2H, d, *J* Hz, H-3', 5'), 8.89 (1H, s, NH), 10.72 (1H, s, OH); δ_C (75.46 MHz, d₆-DMSO) 40.3 (C-2), 125.2 (q, *J* 4 Hz [¹³C-¹⁹F], C-3', 5'), 126.5 (q, *J* 271 Hz [¹³C-¹⁹F], CF₃), 127.3 (q, *J* 32 Hz [¹³C-¹⁹F], C-4'), 129.7 (C-2', 6'), 130.5 (C-1'), 166.2 (C=O); δ_F (376.5 MHz, CDCl₃), -61.3 (CF₃); *m/z* (ES⁺) 174 ((M+Na)⁺, 100%); *m/z* (ES) 150 ((M-H)⁻, 100%).

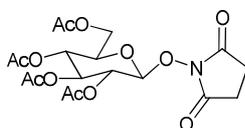
Synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**146**)²²



45% w/v Hydrogen bromide in acetic acid (60 ml) was added dropwise to D-glucose (39.0 g, 216 mmol) in acetic anhydride (150 ml) at 0 °C under an argon atmosphere. After 4 hours

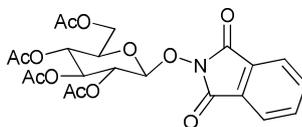
further 45% w/v hydrogen bromide in acetic acid (180 ml) was added and the solution stirred at room temperature overnight. The reaction mixture was taken up in DCM (300 ml) and poured into ice/water (500 ml). The organic layer was then added carefully to an ice/saturated NaHCO₃ solution (600 ml) with stirring. Once the gas evolution became less vigorous the organic phase was separated and dried over MgSO₄. The solvent was removed at reduced pressure to give a golden oil which solidified upon cooling to 0 °C. The product was recrystallized from diethyl ether to give 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide (**146**) as a colourless solid (84.9 g, 96%). mp 87-88 °C (lit.²², 88-89 °C); δ_{H} (400 MHz, CDCl₃) 2.02 (3H, s, CH₃), 2.04 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.09 (3H, s, CH₃), 4.09-4.15 (1H, m, H-5), 4.25-4.35 (2H, m, H_{6a, 6b}), 4.82 (1H, dd, J 5 Hz, J 10 Hz, H-2), 5.15 (1H, t, J 10 Hz, H-4), 5.54 (1H, t, J 10 Hz, H-3), 6.59 (1H, t, J 5 Hz, H-1); δ_{C} (100.6 MHz, CDCl₃) 20.54 (CH₃), 20.61 (CH₃), 20.64 (CH₃), 20.65 (CH₃), 60.9 (C-6), 67.2 (C-4), 70.2 (C-2), 70.6 (C-3), 72.3 (C-5), 86.6 (C-1), 169.5 (C=O), 169.7 (C=O), 169.8 (C=O), 170.5 (C=O).

Synthesis of *O*-(tetra-*O*-acetyl- β -D-glucopyranose)-*N*-hydroxysuccinimide (**147**)²³



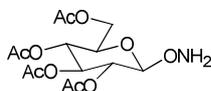
Acetobromoglucose (**146**) (5.00 g, 12.2 mmol) was dissolved in CH₂Cl₂ (100 ml) while *N*-hydroxysuccinimide (4.90 g, 42.6 mmol) and tetrabutylammonium hydrogen sulfate (4.54 g, 13.4 mmol) were dissolved in 1M Na₂CO₃ (100 ml). The two solutions were added together and vigorously stirred at room temperature for 12 h. To the solution more CH₂Cl₂ (50 ml) was added and organic phase was washed with distilled water (2 × 70 ml) and brine (50 ml). The organic extracts were dried over Na₂SO₄ and evaporated under reduced pressure to give an off-white solid (**147**) (2.84 g, 52%). mp 182-184 °C (lit.²³, 182-184 °C); δ_{H} (400 MHz, CDCl₃) 2.01 (6H, s, 2×CH₃), 2.06 (3H, s, CH₃), 2.11 (3H, s, CH₃), 2.72 (4H, s, succinimide-CH₂), 3.71-3.78 (1H, m, H-5), 4.14 (1H, dd, $J_{5,6b}$ 3 Hz, $J_{6a,6b}$ 12 Hz, H-6b), 4.28 (1H, dd, $J_{5,6a}$ 3 Hz, $J_{6a,6b}$ 12 Hz, H-6a), 5.05 (1H, d, J 7 Hz, H-1), 5.17-5.53 (3H, m, H-2, 3, 4); δ_{C} (100.6 MHz, CDCl₃) 20.57 (CH₃), 20.60 (CH₃), 20.65 (CH₃), 20.73 (CH₃), 25.4 (CH₂), 61.7 (C-4), 68.1 (C-2), 69.6 (C-3), 72.2 (C-6), 72.4 (C-5), 103.8 (C-1), 169.3 (NC=O), 169.4 (C=O), 170.0 (C=O), 170.1 (C=O), 170.6 (C=O); m/z (ES⁺) 468.1127 (M+Na⁺). C₁₈H₂₃NO₁₂Na requires 468.1118).

Synthesis of *O*-(tetra-*O*-acetyl- β -D-glucopyranose)-*N*-hydroxyphthalimide (**150**)²⁴



Acetobromoglucose (**146**) (10.00 g, 24.3 mmol) was dissolved in CH_2Cl_2 (200 ml) while *N*-hydroxyphthalimide (11.90 g, 73.0 mmol) and tetrabutylammonium hydrogen sulfate (9.08 g, 26.8 mmol) were dissolved in 1M Na_2CO_3 (200 ml). The two solutions were added together and vigorously stirred at room temperature for 12 h. To the solution more CH_2Cl_2 (100 ml) was added and organic phase was washed with distilled water (2×100 ml) and brine (50 ml). The organic extracts were dried over Mg_2SO_4 and concentrated under reduced pressure to give a colorless oil, which was further purified by the column chromatography (ethyl acetate: petroleum ether – 2:1) to yield the pure product (**150**) as a colorless solid (2.72 g, 23%). mp 160-161 °C; δ_{H} (400 MHz, CDCl_3) 2.02 (3H, s, CH_3), 2.04 (3H, s, CH_3), 2.05 (3H, s, CH_3), 2.19 (3H, s, CH_3), 3.74-3.80 (1H, m, H-5), 4.13 (1H, dd, $J_{5,6b}$ 3 Hz, $J_{6a,6b}$ 12 Hz, H-6b), 4.34 (1H, dd, $J_{5,6a}$ 3 Hz, $J_{6a,6b}$ 12 Hz, H-6a), 5.09-5.12 (1H, m, H-1), 5.20-5.28 (1H, m, H-2), 5.28-5.32 (2H, m, H-3, 4), 7.75-7.80 (2H, m, H-2', 5'), 7.83-7.88 (2H, m, H-3', 4'); δ_{C} (100.6 MHz, CDCl_3) 20.58 (CH_3), 20.62 (CH_3), 20.70 (CH_3), 61.8 (C-4), 68.1 (C-2), 69.0 (C-3), 72.4 (C-6), 72.5 (C-5), 105.2 (C-1), 123.8 (C-2', 5'), 128.7 (C-3', 4'), 134.8 (C-1', 6'), 162.6 (NC=O), 169.3 (C=O), 169.5 (C=O), 170.2 (C=O), 170.6 (C=O); m/z (ES^+) 516.1115 ($\text{M}+\text{Na}^+$). $\text{C}_{22}\text{H}_{23}\text{NO}_{12}\text{Na}$ requires 516.1118).

Synthesis of *O*-(tetra-*O*-acetyl- β -D-glucopyranose)hydroxylamine (**144**)²²



Method 1:

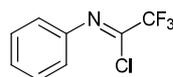
O-(Tetra-*O*-acetyl- β -D-glucopyranose)-*N*-hydroxysuccinimide (0.76 g, 1.72 mmol) (**147**) was stirred in dry THF (10 ml) and dry methanol (5 ml) and hydrazine hydrate (0.17 ml, 3.42 mmol) was added. The reaction mixture was heated under reflux for 10 minutes and then cooled to room temperature. The suspension was diluted with aqueous sodium bicarbonate solution

(5%, 10 ml) and washed with diethyl ether (2×15 ml), dried over MgSO_4 and concentrated under reduced pressure to yield a colourless solid (**144**) (170 mg, 48%). mp 120-121 °C; δ_{H} (400 MHz, CDCl_3) 2.00 (3H, s, CH_3), 2.03 (3H, s, CH_3), 2.07 (3H, s, CH_3), 2.10 (3H, s, CH_3), 3.72-3.77 (1H, m, H-5), 4.17 (1H, dd, H-6b, $J_{5,6b}$ 3 Hz, $J_{6a,6b}$ 12 Hz), 4.30 (1H, dd, H-6a, $J_{5,6a}$ 3 Hz, $J_{6a,6b}$ 12 Hz), 4.72 (1H, d, J 8 Hz, H-1), 5.10-5.22 (3H, m, H-2, 3, 4), 5.82 (2H, s, NH_2); δ_{C} (100.6 MHz, CDCl_3) 20.6 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 61.8 (C-4), 68.2 (C-2), 69.6 (C-3), 71.8 (C-6), 72.9 (C-5), 103.4 (C-1), 169.5 (C=O), 170.2 (C=O), 170.7 (C=O); m/z (ES^+) 386.1062 ((M+Na⁺). $\text{C}_{14}\text{H}_{21}\text{NO}_{10}\text{Na}$ requires 386.1063).

Method 2:

O-(Tetra-O-acetyl- β -D-glucopyranose)-N-hydroxyphthalimide (**150**) (1.03 g, 2.03 mmol) was stirred in dry THF (10 ml) and methylhydrazine was added (0.22 ml, 4.05 mmol). The reaction mixture was heated under reflux for 15 minutes (the progress of the reaction was controlled by TLC) and then cooled to room temperature. The suspension was diluted with aqueous sodium bicarbonate solution (5%, 10 ml) and washed with diethyl ether (2×15 ml), dried over MgSO_4 and concentrated under reduced pressure to yield a colourless solid (**144**) (0.44 g, 59%). Analytical data are the same as above.

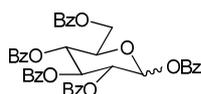
Synthesis of N-phenyl-2, 2, 2-trifluoroacetimidoyl chloride (158**)²⁵**



Triphenylphosphine (18.6 g, 71 mmol), triethylamine (4.0 ml, 28 mmol) and trifluoroacetic acid (1.8 ml, 24 mmol) were dissolved in carbon tetrachloride (12 ml). The solution was stirred for about 10 minutes in an ice bath. Aniline (2.65 g, 28 mmol) was dissolved in carbon tetrachloride (12 ml) and added to the reaction mixture. The solution was then heated under reflux for 3 h. The solvents were removed at reduced pressure and the residue was diluted with hexane (20 ml) and filtered. The residual solid comprising of Ph_3PO , Ph_3P and $\text{Et}_3\text{N}\cdot\text{HCl}$, was washed with hexane several times. The filtrate was concentrated under reduced pressure and the residue was distilled under reduced pressure using the Kugelrohr apparatus to give the final product (**158**) as a pale yellow oil (2.90 g, 59%). δ_{H} (400 MHz, CD_3Cl) 7.09-7.13 (2H, m, H-2', 6'), 7.29-7.34 (1H,

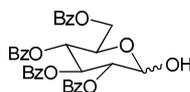
m, H-4'), 7.42-7.47 (2H, m, H-3', 5'); δ_C (75.46 MHz, CD₃Cl) 115.4 (CF₃), 121.0 (C-2',6'), 127.8 (C-4'), 129.6 (C-3', 5'), 143.3 (C-1'); δ_F (400 MHz, CD₃Cl) -71.62 (CF₃).

Synthesis of 1, 2, 3, 4, 6-penta-*O*-benzoyl-D-glucopyranoside (**156**)²⁶



To glucose (20.0 g, 110 mmol) stirring in pyridine (240 ml) for 20 minutes was added benzoyl chloride (0.67 mol, 80 ml). The dark yellow solution was stirred in an oil bath at 60-65 °C for an hour. The resulting suspension was quenched with water (10 ml) and allowed to stir at room temperature for 10 minutes after which more water (100 ml) was added. The mixture was then poured into 2 litres of iced water and allowed to stir for an hour. The white precipitate was filtered and recrystallized repeatedly from acetone/methanol to give the final product (**156**) as a colourless solid (48.0 g, 62%). mp 150-151 °C (lit.²⁶, 172-174 °C); (Found: C, 70.4; H, 4.6%. C₄₁H₃₂O₁₁ requires C, 70.3; H, 4.6%); δ_H (400 MHz, CDCl₃) 4.39-4.45 (1H, m, H-5), 4.52 (1H, dd, $J_{5,6b}$ 4.7 Hz, $J_{6a,6b}$ 12 Hz, H-6b), 4.67 (1H, dd, $J_{5,6a}$ 3 Hz, $J_{6a,6b}$ 12 Hz, H-6a), 5.80-5.88 (2H, m, H-2, 4), 6.05 (1H, t, J 9.5 Hz, H-3), 6.31 (1H, d, J 8.0 Hz, H-1), 7.27-7.58 (15H, m, Ar-H), 7.80-8.10 (10H, m, Ar-ortho-H); δ_C (75.46 MHz, CDCl₃) 62.7 (C-6), 69.1 (C-4), 70.9 (C-2), 72.8 (C-3), 73.2 (C-5), 92.7 (C-1), 128.4, 128.5, 128.6 (Ar-ortho), 128.8, 128.7, 129.6 (Ar-quaternary), 129.9, 130.3 (Ar-meta), 133.1, 133.4, 133.5, 133.6, 133.9 (Ar-para), 164.6 (C=O), 165.2 (C=O), 165.7 (C=O), 166.1 (C=O); m/z (ES⁺) 723 ((M+Na)⁺, 100%).

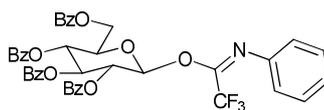
Synthesis of 2, 3, 4, 6-tetra-*O*-benzoyl-D-glucopyranose (**157**)²⁷



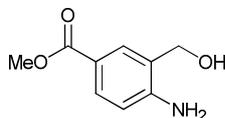
1, 2, 3, 4, 6-Penta-*O*-benzoyl-D-glucopyranoside (**156**) (46.0 g, 65.6 mmol), was stirred in DCM (150 ml) and HBr/HOAc (30%, 30 ml) at room temperature, forming a clear orange solution. After 3h, the solution was washed with saturated NaHCO₃ (2 × 40 ml) and brine (40 ml) and finally with water (40 ml). The organic layer was dried over MgSO₄ and then concentrated under

reduced pressure to give a yellow oil. The residue solidified to an off-white solid under high vacuum and was dissolved in acetone (40 ml) and water (2 ml). The solution was treated with AgCO_3 (9.05 g, 32.8 mmol) for 2 h and filtered over celite to give the final product (**157**) as a pale-pink solid (37.7 g, 96%). δ_{H} (400 MHz, CDCl_3) 4.41-4.54 (2H, m, H-3, 4), 4.60-4.74 (2H, m, H-5, 6b), 5.33 (1H, dd, $J_{5,6a}$ 3 Hz, $J_{6a,6b}$ 12 Hz, H-6a), 5.74-5.80 (1H, m, H-2), 6.24-6.30 (1H, m, H-1), 7.26-8.20 (20H, m, Ar-H); δ_{C} (75.46 MHz, CDCl_3) 63.4 (C-6), 68.1 (C-4), 69.9 (C-2), 70.9 (C-3), 72.9 (C-5), 90.9 (C-1), 128.9, 129.3, 129.6 (Ar-ortho, meta), 130.2, 130.3, 130.5 (Ar-quaternary), 133.6, 134.0, 134.0, 134.4 (Ar-para), 165.6 (C=O), 165.8 (C=O), 166.3 (C=O), 166.8 (C=O); m/z (ES^+) 619.1569 ($\text{M}+\text{Na}^+$). $\text{C}_{34}\text{H}_{28}\text{O}_{10}\text{Na}$ requires 619.1580).

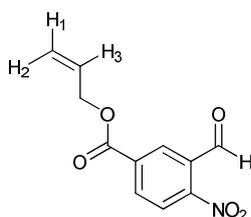
**Synthesis of 2, 3, 4, 6-tetra-O-benzoyl- β -D-glucopyranosyl
1-(*N*-phenyl)-2, 2, 2-trifluoroacetimidate (**159**)²⁸**



Tetra-O-benzoyl-D-glucopyranose (**157**) (4.21 g, 7.0 mmol), *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (**158**) (2.90 g, 14.0 mmol) and potassium carbonate (1.93 g, 14.0 mmol) were suspended in acetone (100 ml) and stirred for 3 hours. The inorganic salt was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography using ethyl acetate and petroleum ether (3:1) as an eluting solvent to give final product (**159**) as an off white solid (2.14 g, 40%). mp 78-80 °C; δ_{H} (400 MHz, CDCl_3) 4.51 (1H, dd, $J_{5,6b}$ 4.7 Hz, $J_{6a,6b}$ 12.2 Hz H-6b), 4.60 (1H, br m, H-5), 4.67 (1H, d, $J_{6a,6b}$ 12.2 Hz, H-6a), 5.61 (1H, br d, J 9.7 Hz, H-2), 5.82 (1H, t, J 9.7 Hz, H-4), 6.25 (1H, t, J 9.7 Hz, H-3), 6.43 (2H, br m, Ar-H), 6.83 (1H, br m, H-1), 6.99-8.12 (23H, m, Ar-H); δ_{C} (75.46 MHz, CDCl_3) 62.4 (C-6), 68.6 (C-4), 70.0 (C-2), 70.5 (C-3), 70.8 (C-5), 119.1 (C-1), 120.2 (phenyl-ortho), 124.4 (phenyl-para), 128.4, 128.5, 128.6 (Bz-meta), 129.4 (quaternary), 129.5, 129.8, 129.9 (Bz-ortho), 131.9 (phenyl-meta), 133.2, 133.4, 133.6, 133.7 (Bz-para), 142.8 (quaternary), 165.2 (C=O), 165.3 (C=O), 165.7 (C=O), 166.0 (C=O), 171.2 (C=NHPh); m/z (ES^+) 790.1876 ($\text{M}+\text{Na}^+$). $\text{C}_{42}\text{H}_{32}\text{F}_3\text{NO}_{10}\text{Na}$ requires 790.1876).

Synthesis of methyl 4-amino-3-(hydroxymethyl)benzoate (170)

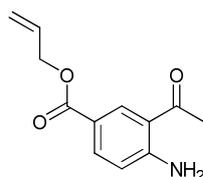
To a stirred solution of methyl 3-formyl-4-nitrobenzoate (1.05 g, 5.0 mmol) in dry methanol (40.5 ml), cooled to 0 °C, was added NiCl₂·6H₂O (0.12 g, 0.5 mmol). NaBH₄ (1.34 g, 35.4 mmol) was then added in small portions over 1.5 h. The resulting reaction mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and stirred for further 10 h, at which point diethylenetriamine (0.6 ml, 5.0 mmol) was added. The mixture was allowed to stir for 1 h before solvent evaporation. The purple residue was then dissolved in ethyl acetate (150 ml) and washed with saturated aq. NaHCO₃ (2 x 40 ml). The organic layer was dried over MgSO₄ and the solvent was removed at reduced pressure to yield a pale yellow solid (**170**) (0.51g, 55%). δ_{H} (400 MHz, d₆-DMSO) 3.73 (3H, s, OCH₃), 4.37 (2H, d, *J* 6 Hz, CH₂), 5.11 (1H, t, *J* 6 Hz, OH), 5.74 (2H, s, NH₂), 6.61 (1H, d, *J* 8 Hz, H-5), 7.57 (1H, dd, *J* 2 Hz, *J* 8 Hz, H-6), 7.73 (1H, d, *J* 2 Hz, H-2); δ_{C} (75.46 MHz, d₆-DMSO) 56.4 (CH₂), 65.5 (CH₃), 118.6 (C-5), 121.0 (C-1), 129.5 (C-3), 134.2 (C-2), 134.7 (C-6), 156.0 (C-4), 171.8 (C=O); *m/z* (CI) 182 ((M+H)⁺, 100%); 182.0823 (M+H)⁺ C₉H₁₁NO₃, requires 182.0817).

Synthesis of allyl 3-formyl-4-nitrobenzoate (172)

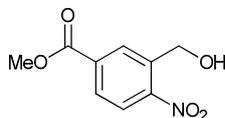
Methyl 3-formyl-4-nitrobenzoate (2.0 g, 9.6 mmol) was dissolved in allyl alcohol (80 ml). After the addition of dibutyltin oxide (0.24 g, 0.96 mmol) the mixture was heated under reflux for 5 h. After completion of the reaction, the solution was poured into a saturated sodium bicarbonate solution (100 ml) and extracted with ethyl acetate (3 × 100 ml). The combined organic layers, which contained Bu₂SnO as a fine white precipitate, were filtered through celite and dried over

Na_2SO_4 . After removal of solvent at reduced pressure, the product was purified by column chromatography using diethyl ether and petroleum ether (1:2) as an eluting solvent to give final product (**172**) as a pale green solid (1.37 g, 61%). mp 46-47 °C; (Found: C, 56.1; H, 3.6; N, 5.8%. $\text{C}_{11}\text{H}_9\text{NO}_5$ requires C, 56.2; H, 3.9; N, 6.0%); ν_{max} (nujol)/ cm^{-1} 3101, 1722 (C=O), 1690 (C=O), 1650 (NO_2), 1586 (C=C); δ_{H} (400 MHz, CDCl_3) 4.89 (2H, dt, J 1.3 Hz, J 5.8 Hz, allyl CH_2), 5.36 (1H, dd, J 1.3 Hz, J 10 Hz, H_1), 5.44 (1H, dd, J 1.3 Hz, J 17 Hz, H_2), 5.98-6.11 (1H, m, H_3), 8.16 (1H, d, J 8 Hz, H-5), 8.41 (1H, dd, J 2 Hz, J 8 Hz, H-6), 8.60 (1H, d, J 2 Hz, H-2), 10.41 (1H, s, CHO); δ_{C} (CDCl_3) 66.9 (CH_2), 119.7 ($\text{CH}_2=$), 124.8 (C-5), 131.1 ($\text{CH}=$), 131.3 (C-6), 134.7 (C-2), 155.7 (C-4), 164.2 (C=O), 187.0 ($\text{HC}=\text{O}$); m/z (CI) 236 (($\text{M}+\text{H}$) $^+$, 100%); 236.0557 ($\text{M}+\text{H}$) $^+$ $\text{C}_{11}\text{H}_9\text{NO}_5$ requires 236.0559).

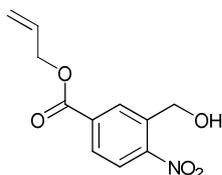
Synthesis of allyl 4-amino-3-formyl benzoate (**174**)



Allyl 3-formyl-4-nitrobenzoate (**172**) (0.56 g, 2.4 mmol), iron powder (1.02 g, 18.4 mmol) and conc. HCl (2 drops), were added to a mixture of ethanol, acetic acid and water (2:2:1, 25 ml). The resulting suspension was heated under reflux for 15 minutes and then stirred at 25 °C for 30 minutes. Subsequently, it was filtered, diluted with water (100 ml) and extracted with ethyl acetate (3 × 100 ml). The organic layer was washed with saturated NaHCO_3 (2 × 100 ml) and water (2 × 100 ml), dried over MgSO_4 , and concentrated under reduced pressure to give the final compound (**174**) as a yellow solid (0.46 g, 94%). mp 78-80 °C; ν_{max} (nujol)/ cm^{-1} 3443, 3336 (NH), 1686, 1655 (C=O), 1618 (C=C); δ_{H} (300 MHz, CDCl_3) 4.80 (2H, dd, J 1.3 Hz, J 5.8 Hz, allyl CH_2), 5.28 (1H, dd, J 1.3 Hz, J 10 Hz, H_1), 5.40 (1H, dd, J 1.3 Hz, J 17 Hz, H_2), 5.96-6.11 (1H, m, H_3), 6.59 (2H, s, NH_2), 6.65 (1H, d, J 8 Hz, H-5), 7.92-8.00 (1H, m, H-6'), 8.22-8.28 (1H, m, H-2'), 9.90 (1H, s, CHO); δ_{C} (75.46 MHz, d_4 -methanol) 66.3 (CH_2), 116.9 (C-5), 118.3 (C-3), 118.7 ($\text{CH}_2=$), 134.0 (C-2), 136.5 ($\text{CH}=$), 140.0 (C-6), 155.6 (C-4), 167.1 (C=O), 195.2 ($\text{HC}=\text{O}$); m/z (ES^+) 228 (($\text{M}+\text{H}$) $^+$, 100%), 441 (78); 228.0632 ($\text{M}+\text{Na}$) $^+$ $\text{C}_{11}\text{H}_{11}\text{NO}_3\text{Na}$ requires 228.0637).

Synthesis of methyl 3-(hydroxymethyl)-4-nitrobenzoate (175)

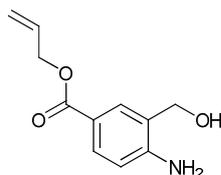
Methyl 3-formyl-4-nitrobenzoate (3.0 g, 14.3 mmol) was dissolved in dry methanol (100 ml). The reaction mixture was cooled to 0 °C and NaBH₄ (0.81 g, 21.5 mmol) was slowly added over a period of 20 minutes. The resulting reaction mixture was allowed to warm to room temperature and then diluted with water (100 ml). The suspension was extracted with DCM (3 x 100 ml). The combined organic layers were washed with water (2 x 100 ml) and brine (50 ml) and finally dried over MgSO₄. Removal of the solvent at reduced pressure gave a red oil, which solidified under high vacuum. The crude product was recrystallized from diethyl ether to give the desired compound (**175**) as ivory needle crystals (2.15 g, 71%). mp 58-59 °C; (Found: C, 51.6; H, 4.0; N, 6.7%. C₉H₉NO₅ requires C, 51.2; H, 4.3; N, 6.6%); ν_{\max} (nujol)/cm⁻¹ 3259 (OH), 1725 (C=O), 1612, 1589 (C=C); δ_{H} (300 MHz, CDCl₃) 2.34 (1H, br s, OH), 3.97 (3H, s, CH₃), 5.02 (2H, br s, CH₂), 8.10-8.11 (2H, m, H-2,6), 8.42-8.43(1H, m, H-5); δ_{C} (75.46 MHz, CDCl₃) 53.0 (CH₃), 62.2 (CH₂), 125.2 (C-5), 129.7 (C-2), 131.2 (C-6), 135.0 (C-3), 137.3 (C-1), 165.4 (C=O); m/z (ES⁺) 234.0376 (M+Na⁺). C₉H₉NO₅Na requires 234.0378).

Synthesis of allyl 3-(hydroxymethyl)-4-nitrobenzoate (176)

Methyl 3-(hydroxymethyl)-4-nitrobenzoate (**175**) (1.65 g, 7.9 mmol) was dissolved in allyl alcohol (65 ml). After the addition of dibutyltin oxide (0.20 g, 0.79 mmol) the mixture was heated under reflux for 5 h. After the completion of the reaction the solution was poured into a saturated sodium bicarbonate solution and extracted with ethyl acetate (3 × 100 ml). The combined organic layers, which contained Bu₂SnO as a fine white precipitate, were filtered through celite

and dried over Na_2SO_4 . The removal of solvent gave a brown oil, which solidified under high vacuum. The crude product was recrystallized from diethyl ether to yield the final compound (**176**) as pale yellow crystals (1.56 g, 84%). mp 68-70 °C; (Found: C, 55.5; H, 4.6; N, 6.0%. $\text{C}_{11}\text{H}_{11}\text{NO}_5$ requires C, 55.7; H, 4.7; N, 5.9%); $\nu_{\text{max}}(\text{nujol})/\text{cm}^{-1}$ 3320 (OH), 1716 (C=O), 1650, 1611, 1588 (C=C); δ_{H} (300 MHz, CDCl_3) 2.62 (1H, br s, OH), 4.86 (2H, dt, J 1.3 Hz, J 5.8 Hz, allyl CH_2), 5.02 (2H, br s, CH_2), 5.33 (1H, dd, J 1.3 Hz, J 10 Hz, H_1), 5.43 (1H, dd, J 1.3 Hz, J 17 Hz, H_2), 5.95-6.11 (1H, m, H_3), 8.09-8.13 (2H, m, H-2, 6), 8.43 (1H, d, J 8 Hz, H-5); δ_{C} (75.46 Hz, CDCl_3) 62.0 (CH_2), 66.5 (CH_2), 119.2 ($\text{CH}_2=$), 125.0 (C-5), 129.0 (C-2), 129.5 (C-6), 131.0 ($\text{CH}=\text{C}$), 134.8 (C-3), 137.1 (C-1), 150.0 (C-4), 164.4 (C=O); m/z (ES^+) 260.0535 ($\text{M}+\text{Na}^+$). $\text{C}_{11}\text{H}_{11}\text{NO}_5\text{Na}$ requires 260.0535).

Synthesis of allyl 4-amino-3-(hydroxymethyl)benzoate (**169**)



Method 1:

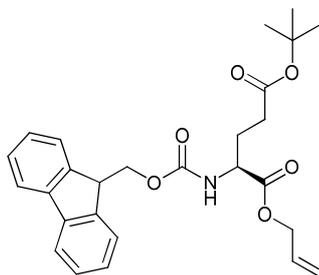
Allyl 4-amino-3-formylbenzoate (**174**) (0.20 g, 0.96 mmol) was dissolved in dry methanol (20 ml). The reaction mixture was cooled to 0 °C and NaBH_4 (0.25 g, 6.7 mmol) was slowly added. The resulting reaction mixture was allowed to warm to room temperature and stirred for further 2 h. After removal of the solvent, the residue was dissolved in ethyl acetate (50 ml) and extracted with water (2 x 20 ml). The organic layer was dried over MgSO_4 and the solvent was removed at reduced pressure to yield a yellow oil, which was purified by column chromatography using ethyl acetate and diethyl ether as an eluting solvent (1:2). The pure product (**169**) was obtained as a pale yellow oil (0.10g, 53%). $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$ 3371, 3085, 2923 (NH, OH), 2680, 1911, 1694 (C=O), 1505; δ_{H} (400 MHz, d_4 -methanol) 4.57 (2H, s, CH_2), 4.73 (2H, dt, J 6 Hz, J 1.6 Hz, H_1), 5.24 (1H, dd, J 10 Hz, J 1.6 Hz, H_2), 5.37 (1H, dd, J 17 Hz, J 1.6 Hz, H_3), 5.97-6.09 (1H, m, allyl CH), 6.70 (1H, d, J 8 Hz, H-5), 7.73 (1H, dd, J 2 Hz, J 8 Hz, H-6), 7.80 (1H, d, J 2 Hz, H-2); δ_{C} (75.46 MHz, d_4 -methanol) 52.0 (CH), 63.2 (CH_2), 65.9, 66.1 (O- CH_2), 115.3 (C-5), 117.9 (allyl

CH₂), 118.6 (C-1), 125.1 (C-2), 131.7 (C-6), 131.8 (allyl CH), 134.2 (C-3), 153.1 (C-4), 168.4 (C=O); m/z (CI) 190 ((M-H₂O+H)⁺, 100%); 208.0976 (M+H)⁺ C₁₁H₁₄NO₃ requires 208.0974).

Method 2:

Allyl 3-(hydroxymethyl)-4-nitrobenzoate (**176**) (1.04 g, 4.4 mmol), iron powder (1.94 g, 34.0 mmol) and conc. HCl (3 drops), were added to a mixture of ethanol, acetic acid and water (2:2:1, 50 ml). The resulting suspension was heated under reflux for 15 minutes and then stirred at 25 °C for 30 minutes. Subsequently, it was filtered, diluted with water (100 ml) and extracted with ethyl acetate (3 × 100 ml). The organic layer was washed with aq. saturated NaHCO₃ (3 × 100 ml) and water (3 × 100 ml), dried over MgSO₄, and concentrated under reduced pressure to give the final compound (**169**) as a yellow oil (0.91 g, 99%). Analytical data are the same as above.

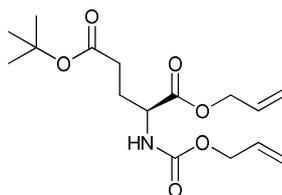
Synthesis of *L*-α-allyl-γ-*tert*-butyl-*N*-9-fluorenylmethoxycarbonylglutamate (167**)**



Fmoc-Glu(O^tBu)-OH (12.75 g, 30 mmol) in aqueous methanol (90%, 100 ml) was titrated with a solution of CsCO₃ (5.17 g, 15.7 mmol, 25% in water) to pH 7. The solvents were evaporated at reduced pressure and coevaporated with toluene (2 × 100 ml). The residue was dried under vacuum overnight. The cesium salt was suspended in anhydrous DMF (100 ml), cooled to 0 °C, and treated with allyl bromide (5.2 ml, 60 mmol) by dropwise addition over 20 minutes. After 1 h stirring the reaction mixture was allowed to warm to room temperature and stirring was continued for a further 10 h. The solvent was removed at reduced pressure and the residue was partitioned between water (100 ml) and ethyl acetate (3 × 100 ml). The combined organic layers were dried over MgSO₄ and the solvent removed at reduced pressure. The residue was purified by column chromatography using petroleum ether: diethyl ether (2:1) as a eluting solvent giving

a pure product as a colourless solid which was recrystallized from diethyl ether to give a final compound (**167**) as needle crystals (9.76 g, 75%). mp 79-80 °C; δ_{H} (400 MHz, CDCl_3) 1.45 (9H, s, CH_3), 1.93-2.03 (1H, m, Glu- β , CH), 2.14-2.25 (1H, m, Glu- β , CH), 2.29-2.37 (2H, m, Glu- γ , 2 \times CH), 4.22 (1H, t, J 7 Hz, Fmoc, CH), 4.33-4.55 (3H, m, Glu- α , CH and Fmoc, CH_2), 4.65 (2H, d, J 6 Hz, allyl, CH_2), 5.26 (1H, dd, J 1.1 Hz, J 10 Hz, allyl, CH), 5.34 (1H, dd, J 1.1 Hz, J 17 Hz, allyl, CH), 5.48 (1H, d, J 8 Hz, NH), 5.84-5.98 (1H, m, allyl CH), 7.32 (2H, t, J 8 Hz), 7.40 (2H, t, J 8 Hz), 7.57-7.63 (2H, m), 7.76 (2H, d, J 8 Hz); δ_{C} (75.46 MHz, CDCl_3) 27.6 (CH_2), 28.1 (CH_3), 31.5 (CH_2), 47.2 (Fmoc CH), 53.6 (CH), 66.2 (O- CH_2), 67.1 (O- CH_2), 80.9 (*t*-butyl), 119.0 (allyl CH_2), 120.0 (C-3), 125.2 (C-4), 127.1 (C-6), 127.7 (C-5), 131.5 (allyl CH), 141.3 (C-7), 143.7 (C-2), 143.9 (C-2), 156.0 (C=O), 171.8 (C=O), 172.1 (C=O).

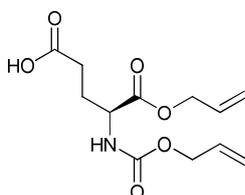
Synthesis of *L*- α -allyl- γ -*tert*-butyl-*N*-allyloxycarbonylglutamate (**168**)



To a solution of *L*- α -allyl- γ -*tert*-butyl-*N*-9-fluorenylmethoxycarbonylglutamate (**167**) (6.22 g, 13.4 mmol) in dry THF (100 ml) was added freshly distilled diethylamine (28 ml, 267 mmol) and DBU (0.41 ml, 2.7 mmol). The mixture was stirred under an argon atmosphere in darkness for 12 h at room temperature. The solvents were removed under reduced pressure and residue was taken up in 1,4-dioxane (100 ml) and aqueous Na_2CO_3 (20%). The reaction mixture was cooled to 0 °C and allyl chloroformate (2.12 ml, 20.0 mmol) was added dropwise. Then, the resulting solution was allowed to stir for 2 h at 0 °C before it was diluted with water (100 ml) and extracted with ethyl acetate (3 \times 100 ml). The combined organic layers were washed with water (2 \times 50 ml), brine (50 ml) and dried over MgSO_4 . The solvent was removed under reduced pressure to give the crude product as a yellow oil, which was purified using column chromatography (petroleum ether : diethyl ether-2:1) to yield the final compound (**168**) as a colourless oil (2.55 g, 58%). ν_{max} (neat)/ cm^{-1} 3346, 3086, 2979, 2936 (NH), 1729 (C=O), 1529 (C=C), 1257, 1154 (COO); δ_{H} (400 MHz, CDCl_3) 1.43 (9H, s, CH_3), 1.89-2.01 (1H, m, Glu- β , CH), 2.10-2.21 (1H, m, Glu- β , CH), 2.24-2.41 (2H, m, Glu- γ , 2 \times CH), 4.35-4.43 (1H, m, Glu- α),

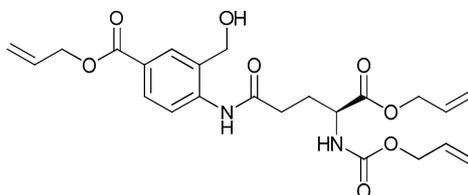
4.56 (2H, d, J 6 Hz, allyl, CH₂), 4.64 (2H, dt, J 6 Hz, J 1.1 Hz, allyl, CH), 5.19-5.39 (5H, m, allyl, 2×CH and NH), 5.85-5.96 (2H, m, allyl CH); δ_{C} (75.46 MHz, CDCl₃) 27.6 (CH₂), 28.1 (CH₃), 31.5 (CH₂), 53.6 (CH), 65.9 (O-CH₂), 66.1(O-CH₂), 80.9(*t*-butyl), 117.8 (allyl CH₂), 119.0 (allyl CH₂), 131.5 (allyl CH), 132.6 (allyl CH), 167.3 (C=O), 171.7 (C=O), 172.0 (C=O); m/z (ES⁺) 350.1580 (M+Na⁺). C₁₆H₂₅NO₆Na requires 350.1577).

Synthesis of allyl *N*-alloc *L*-glutamate (**166**)



L- α -Allyl- γ -*tert*-butyl-*N*-allyloxycarbonylglutamate (**168**) (2.50 g, 7.6 mmol) was treated with TFA in dry DCM (50:50, 20 ml) at room temperature for 12 h. The solvents were removed at reduced pressure and the residue was co-evaporated, several times, with toluene to give the product (**166**) as a pale yellow oil (2.00 g, 97%). δ_{H} (300 MHz, CDCl₃) 1.91-2.07 (1H, m, Glu- β , CH), 2.16-2.31 (1H, m, Glu- β , CH), 2.38-2.57 (2H, m, Glu- γ , 2×CH), 4.37-4.49 (1H, m, Glu- α), 4.57 (2H, d, J 6 Hz, allyl, CH₂), 4.64 (2H, dt, J 1.3 Hz, J 6 Hz, allyl, CH), 5.18-5.38 (4H, m, allyl, 2×CH), 5.44 (1H, d, J 8 Hz, NH), 5.83-5.99 (2H, m, allyl CH); δ_{C} (75.46 MHz, CDCl₃) 27.5 (CH₂), 29.9 (CH₂), 53.6 (CH), 66.0 (O-CH₂), 66.3 (O-CH₂), 118.0 (allyl CH₂), 119.2 (allyl CH₂), 131.3 (allyl CH), 132.5 (allyl CH), 155.9 (C=O), 171.9 (C=O), 177.7 (C=O); m/z (ES⁺) 294.0954 (M+Na⁺). C₁₂H₁₇NO₆Na requires 294.0950).

Synthesis of (S)-vinyl 4-(5-(allyloxy)-4-(allyloxycarbonylamino)-5-oxopentanamido)-3-(hydroxymethyl)benzoate (177)



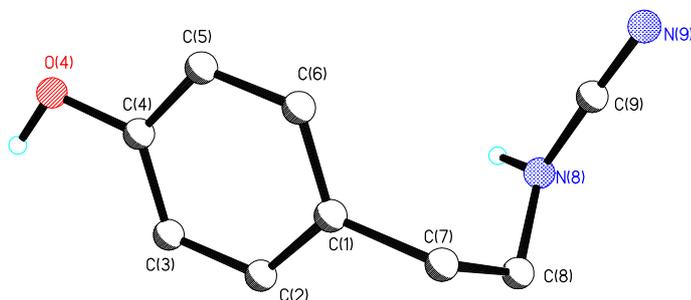
To a solution of allyl *N*-alloc *L*-glutamate (**166**) (1.28 g, 4.73 mmol) and allyl 4-amino-3-(hydroxymethyl)benzoate (**169**) (1.11 g, 5.36 mmol) in dry DCM (100 ml) was added EEDQ (1.32 g, 5.36 g). The mixture was stirred under an argon atmosphere at room temperature for 16 h and poured into 1N HCl solution (100 ml). The layers were separated and the aqueous layer was extracted with DCM (2 × 100 ml). The combined organic layers were washed with water (2 × 100 ml), brine (50 ml) and dried over MgSO₄. After removal of solvent, the crude product was purified four times by column chromatography with various eluting systems. The first column was performed using petroleum ether:diethyl ether (1:2) in order to separate the major product. This was analyzed by ¹H NMR spectroscopy and it was still a mixture of more than two compounds. Therefore, another eluting system (DCM:ethyl acetate, 2:1) was chosen and the purification was performed again, but still the product was not pure. In order to obtain reasonably pure material two more chromatography columns were done, finally using pure ethyl acetate as an eluting solvent. This gave a desired product (**177**) as a yellow oil (131 mg, 6%). δ_{H} (300 MHz, CDCl₃) 1.88-2.05 (1H, m, Glu- β , CH), 2.18-2.29 (1H, m, Glu- β , CH), 2.43-2.53 (2H, m, Glu- γ , 2×CH), 4.36-4.46 (1H, m, Glu- α), 4.51-4.63 (4H, m, allyl, CH₂), 4.79 (1H, dt, *J* 1.3 Hz, *J* 6 Hz, allyl, CH), 5.17-5.39 (7H, m, allyl, 2×CH), 5.46 (1H, br d, *J* 8 Hz, NH), 5.82-5.95 (2H, m, allyl CH), 5.96-6.08 (1H, m, allyl CH), 7.04 (1H, br d, *J* 8 Hz, H-5), 7.90-8.00 (2H, m, H-2,6); δ_{C} (75.46 MHz, CDCl₃) 27.5 (CH₂), 29.9 (CH₂), 58.7 (CH), 60.4 (aromatic O-CH₂), 64.1 (O-CH₂), 65.4 (O-CH₂), 66.9 (O-CH₂), 115.2 (C-5), 117.9 (allyl CH₂), 118.3 (allyl CH₂), 119.3 (allyl CH₂), 131.1 (C-2), 131.2 (C-6), 131.3 (allyl CH), 132.5 (allyl CH), 133.7 (allyl CH), 150.3 (C-1), 150.4 (C-4), 155.9 (C=O), 166.4 (C-3), 171.9 (C=O), 173.0 (C=O), 175.8 (C=O); *m/z* (ES⁺) 483 (M+Na⁺).

7.2 References

- 1 D. D. A. Perrin and W.L.F. Armarego, 'Purification of Laboratory Chemicals', 3rd ed. P. Press, Oxford, 1989.
- 2 Brian S. Furniss, Antony J. Hannaford, Peter W.G. Smith, Autsin R. Tatchell, 'Vogel's Textbook of Practical Organic Chemistry', Longman Scientific & Technical, London, 1989.
- 3 A. Renodon-Corniere, S. Dijols, C. Perollier, D. Lefevre-Groboillot, J.-L. Boucher, R. Attias, M.-A. Sari, D. Stuehr, and D. Mansuy, *J. Med. Chem.*, 2002, **45**, 944.
- 4 R. A. Glennon, S. M. Liebowitz, and G. M. Anderson, III, *J. Med. Chem.*, 1980, **23**, 294.
- 5 E. Goessnitzer and A. Punkenhofer, *Monatsh. Chem.*, 2003, **134**, 909.
- 6 Clark L. C, Jr., F. Benington, and R. D. Morin, *Alabama J. Med. Sci.*, 1964, **1**, 417.
- 7 U. M. Joshi, B. S. Kopal, and H. V. Joshi, *Synth. Commun.*, 2003, **33**, 1829.
- 8 M. B. Arnold, Y. Dao, K. M. Gardinier, D. J. Garmene, S. J. Green, E. J. Hembre, and J. Lu, in 'Thienopyridines as melanin-concentrating hormone receptor antagonists, their preparation, pharmaceutical compositions, and use in therapy', Application: WO, 2007.
- 9 K. S. Masahiko, S. Murahashi, and S. Shigehiro, *Bull. Chem. Soc. Jpn.*, 1990, **63**, 1252.
- 10 M. J. Kornet, A. M. Crider, and E. O. Magarian, *J. Med. Chem.*, 1977, **20**, 1210.
- 11 W. Grell, R. Hurnaus, G. Griss, R. Sauter, E. Rupprecht, M. Mark, P. Luger, H. Nar, H. Wittneben, and P. Mueller, *J. Med. Chem.*, 1998, **41**, 5219.
- 12 R. C. Elderfield and K. L. Burgess, *J. Am. Chem. Soc.*, 1960, **82**, 1975.
- 13 R. Singh and S. K. Dikshit, *Polyhedron*, 1995, **14**, 1799.
- 14 J. v. Braun and H. Deutsch, *Ber.*, 1912, **45**, 2188.
- 15 P. Borgna, L. Vicarini, and G. Calderara, *Farmaco, Edizione Scientifica*, 1979, **34**, 338.
- 16 T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, 1963, **6**, 669.
- 17 R. N. C. Warrener, E.N., *Angew. Chem. Int. Ed. Engl.*, 1996, 511.
- 18 Y. Xu, J. Zhang, W. Hua, and D. Zhu, *Yaoxue Xuebao*, 2003, **38**, 586.
- 19 J. B. Summers, B. P. Gunn, H. Mazdiyasi, A. M. Goetze, P. R. Young, J. B. Bouska, R. D. Dyer, D. W. Brooks, and G. W. Carter, *J. Med. Chem.*, 1987, **30**, 2121.
- 20 J.-S. Fruchart, G. Lippens, C. Kuhn, H. Gras-Masse, and O. Melnyk, *J. Org. Chem.*, 2002, **67**, 526.
- 21 I. V. P. Raj and A. Sudalai, *Tetrahedron Lett.*, 2005, **46**, 8303.
- 22 S. Su, D. E. Acquilano, J. Arumugasamy, A. B. Beeler, E. L. Eastwood, J. R. Giguere, P. Lan, X. Lei, G. K. Min, A. R. Yeager, Y. Zhou, J. S. Panek, J. K. Snyder, S. E. Schaus, and J. A. Porco, Jr., *Org. Lett.*, 2005, **7**, 2751.
- 23 B. L. Sorg, W. E. Hull, H.-C. Kliem, W. Mier, and M. Wiessler, *Carbohydr. Res.*, 2005, **340**, 181.
- 24 O. Renaudet and P. Dumy, *Tetrahedron Lett.*, 2001, **42**, 7575.

- 25 K. Tamura, H. Mizukami, K. Maeda, H. Watanabe, and K. Uneyama, *J. Org. Chem.*, 1993, **58**, 32.
- 26 N. B. D'Accorso, I. M. E. Thiel, and M. Schueller, *Carbohydr. Res.*, 1983, **124**, 177.
- 27 B. N. A. Mbadugha and F. M. Menger, *Org. Lett.*, 2003, **5**, 4041.
- 28 B. Yu and H. Tao, *J. Org. Chem.*, 2002, **67**, 9099.

2-(4'-hydroxyphenyl)ethylcyanamide (62)



Crystal data and structure refinement for (62)			
Empirical formula	C ₉ H ₁₀ N ₂ O	Index ranges	-7 ≤ h ≤ 5, -9 ≤ k ≤ 7, -7 ≤ l ≤ 11
Formula weight	162.19	Reflections collected	2380
Temperature	93(2) K	Independent reflections	796 [R(int) = 0.0437]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	94.9 %
Crystal system	Monoclinic	Absorption correction	Multiscan
Space group	P2(1)	Max. and min. transmission	1.0000 and 0.9445
Unit cell dimensions	a = 5.835(2) Å, α = 90° b = 7.606(3) Å, β = 101.066(15)° c = 9.857(4) Å, γ = 90°	Refinement method	Full-matrix least-squares on F ²
Volume	429.3(3) Å ³	Data / restraints / parameters	796 / 3 / 119
Z	2	Goodness-of-fit on F ²	1.128
Density (calculated)	1.255 Mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0441, wR2 = 0.1001
Absorption coefficient	0.085 mm ⁻¹	R indices (all data)	R1 = 0.0571, wR2 = 0.1067
F(000)	172	Absolute structure parameter	0(10)
Crystal size	0.100 x 0.100 x 0.010 mm ³	Extinction coefficient	0.024(16)
Theta range for data collection	3.41 to 25.33°	Largest diff. peak and hole	0.183 and -0.146 e.Å ⁻³

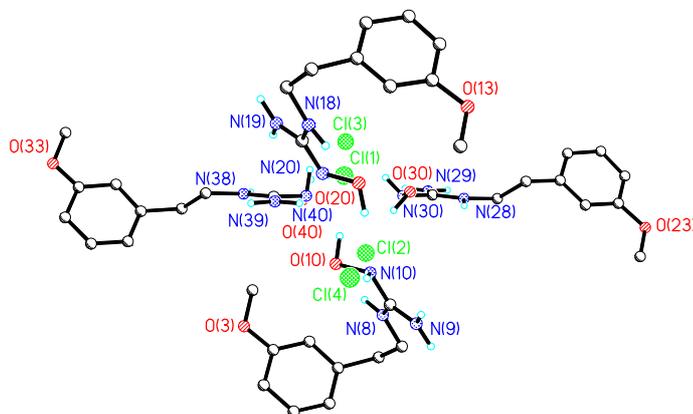
Bond lengths [Å] and angles [°] for (62)

C(1)-C(6)	1.393(5)
C(1)-C(2)	1.395(5)
C(1)-C(7)	1.507(5)
C(2)-C(3)	1.381(5)
C(3)-C(4)	1.386(5)
C(4)-O(4)	1.376(4)
C(4)-C(5)	1.385(5)
C(5)-C(6)	1.382(5)
C(7)-C(8)	1.513(6)
C(8)-N(8)	1.468(5)
N(8)-C(9)	1.315(5)
C(9)-N(9)	1.148(5)

C(6)-C(1)-C(2)	117.7(3)
C(6)-C(1)-C(7)	121.9(3)
C(2)-C(1)-C(7)	120.4(3)
C(3)-C(2)-C(1)	121.3(3)
C(2)-C(3)-C(4)	119.9(3)
O(4)-C(4)-C(5)	117.8(3)
O(4)-C(4)-C(3)	122.3(3)
C(5)-C(4)-C(3)	119.9(3)
C(6)-C(5)-C(4)	119.7(3)
C(5)-C(6)-C(1)	121.5(3)
C(1)-C(7)-C(8)	114.3(3)
N(8)-C(8)-C(7)	113.1(3)
C(9)-N(8)-C(8)	120.3(3)
N(9)-C(9)-N(8)	178.0(4)

Torsion angles [°] for (62)

C(6)-C(1)-C(2)-C(3)	-0.4(5)
C(7)-C(1)-C(2)-C(3)	179.8(3)
C(1)-C(2)-C(3)-C(4)	0.7(5)
C(2)-C(3)-C(4)-O(4)	179.5(3)
C(2)-C(3)-C(4)-C(5)	-0.7(5)
O(4)-C(4)-C(5)-C(6)	-179.9(3)
C(3)-C(4)-C(5)-C(6)	0.3(6)
C(4)-C(5)-C(6)-C(1)	0.0(5)
C(2)-C(1)-C(6)-C(5)	0.0(5)
C(7)-C(1)-C(6)-C(5)	179.7(3)
C(6)-C(1)-C(7)-C(8)	-106.7(4)
C(2)-C(1)-C(7)-C(8)	73.1(4)
C(1)-C(7)-C(8)-N(8)	60.5(4)
C(7)-C(8)-N(8)-C(9)	62.7(4)
C(8)-N(8)-C(9)-N(9)	90(11)

***N*-2-(3'-methoxyphenyl)ethyl-*N*'-hydroxyguanidine hydrochloride (78)**

Crystal data and structure refinement for (78)			
Empirical formula	C ₁₀ H ₁₆ ClN ₃ O ₂	Index ranges	-8 ≤ h ≤ 8, -14 ≤ k ≤ 9, -32 ≤ l ≤ 35
Formula weight	245.71	Reflections collected	15862
Temperature	93(2) K	Independent reflections	7649 [R(int) = 0.0572]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	99.8 %
Crystal system	Monoclinic	Absorption correction	Multiscan
Space group	Pc	Max. and min. transmission	1.0000 and 0.8204
Unit cell dimensions	a = 6.8502(16) Å, α = 90° b = 12.383(3) Å, β = 95.292(7)° c = 29.607(7) Å, γ = 90°	Refinement method	Full-matrix least-squares on F ²
Volume	2500.7(10) Å ³	Data / restraints / parameters	7649 / 22 / 618
Z	8	Goodness-of-fit on F ²	1.054
Density (calculated)	1.305 Mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0722, wR2 = 0.1574
Absorption coefficient	0.296 mm ⁻¹	R indices (all data)	R1 = 0.1039, wR2 = 0.1890
F(000)	1040	Absolute structure parameter	0.01(12)
Crystal size	0.1000 x 0.0300 x 0.0100 mm ³	Extinction coefficient	N/A
Theta range for data collection	1.64 to 25.35°	Largest diff. peak and hole	0.943 and -0.445 e.Å ⁻³

Bond lengths [Å] and angles [°] for (78)

C(1)-C(6)	1.405(4)
C(1)-C(2)	1.407(4)
C(1)-C(7)	1.548(4)
C(2)-C(3)	1.370(4)
C(2)-H(2A)	0.9500
C(3)-O(3)	1.348(3)
C(3)-C(4)	1.424(4)
O(3)-C(10)	1.432(4)
C(4)-C(5)	1.408(5)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.323(4)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.547(4)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(8)	1.458(3)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
N(8)-C(9)	1.333(3)
N(8)-H(8N)	0.9800(8)
C(9)-N(9)	1.343(3)
C(9)-N(10)	1.375(3)
N(9)-H(9A)	0.9800
N(9)-H(9B)	0.9800
N(10)-O(10)	1.4031(12)
N(10)-H(10N)	0.9800(8)
O(10)-H(10O)	0.9800(8)
C(10)-H(10A)	0.9800
C(10)-H(10B)	0.9800
C(10)-H(10C)	0.9800
C(11)-C(12)	1.358(4)
C(11)-C(16)	1.392(4)
C(11)-C(17)	1.474(4)
C(12)-C(13)	1.393(4)
C(12)-H(12A)	0.9500
C(13)-C(14)	1.379(4)
C(13)-O(13)	1.383(4)
O(13)-C(20)	1.418(4)
C(14)-C(15)	1.338(5)
C(14)-H(14A)	0.9500
C(15)-C(16)	1.444(4)
C(15)-H(15A)	0.9500
C(16)-H(16A)	0.9500
C(17)-C(18)	1.518(4)
C(17)-H(17A)	0.9900
C(17)-H(17B)	0.9900
C(18)-N(18)	1.463(3)
C(18)-H(18A)	0.9900
C(18)-H(18B)	0.9900
N(18)-C(19)	1.345(3)
N(18)-H(18N)	0.9800(8)
C(19)-N(19)	1.319(3)
C(19)-N(20)	1.321(3)

N(19)-H(19A)	0.9800
N(19)-H(19B)	0.9800
N(20)-O(20)	1.4345(11)
N(20)-H(20N)	0.9799(8)
O(20)-H(20O)	0.9800(8)
C(20)-H(20A)	0.9800
C(20)-H(20B)	0.9800
C(20)-H(20C)	0.9800
C(21)-C(22)	1.357(4)
C(21)-C(26)	1.364(4)
C(21)-C(27)	1.491(4)
C(22)-C(23)	1.408(4)
C(22)-H(22A)	0.9500
C(23)-C(24)	1.356(4)
C(23)-O(23)	1.388(3)
O(23)-C(30)	1.432(4)
C(24)-C(25)	1.358(4)
C(24)-H(24A)	0.9500
C(25)-C(26)	1.450(4)
C(25)-H(25A)	0.9500
C(26)-H(26A)	0.9500
C(27)-C(28)	1.534(4)
C(27)-H(27A)	0.9900
C(27)-H(27B)	0.9900
C(28)-N(28)	1.477(3)
C(28)-H(28A)	0.9900
C(28)-H(28B)	0.9900
N(28)-C(29)	1.325(3)
N(28)-H(28N)	0.9800(8)
C(29)-N(29)	1.343(3)
C(29)-N(30)	1.349(3)
N(29)-H(29A)	0.9800
N(29)-H(29B)	0.9800
N(30)-O(30)	1.4467(13)
N(30)-H(30N)	0.9800(8)
O(30)-H(30O)	0.9800(8)
C(30)-H(30A)	0.9800
C(30)-H(30B)	0.9800
C(30)-H(30C)	0.9800
C(31)-C(36)	1.412(4)
C(31)-C(32)	1.413(4)
C(31)-C(37)	1.540(4)
C(32)-C(33)	1.383(3)
C(32)-H(32A)	0.9500
C(33)-O(33)	1.354(3)
C(33)-C(34)	1.426(3)
O(33)-C(40)	1.427(3)
C(34)-C(35)	1.387(4)
C(34)-H(34A)	0.9500
C(35)-C(36)	1.344(4)
C(35)-H(35A)	0.9500
C(36)-H(36A)	0.9500
C(37)-C(38)	1.498(4)
C(37)-H(37A)	0.9900
C(37)-H(37B)	0.9900
C(38)-N(38)	1.478(3)
C(38)-H(38A)	0.9900

C(38)-H(38B)	0.9900
N(38)-C(39)	1.312(3)
N(38)-H(38N)	0.9800(8)
C(39)-N(39)	1.327(3)
C(39)-N(40)	1.371(2)
N(39)-H(39A)	0.9800
N(39)-H(39B)	0.9800
N(40)-O(40)	1.3961(14)
N(40)-H(40N)	0.9799(8)
O(40)-H(40O)	0.9800(8)
C(40)-H(40A)	0.9800
C(40)-H(40B)	0.9800
C(40)-H(40C)	0.9800
C(6)-C(1)-C(2)	119.5(3)
C(6)-C(1)-C(7)	121.5(3)
C(2)-C(1)-C(7)	118.9(2)
C(3)-C(2)-C(1)	119.6(2)
C(3)-C(2)-H(2A)	120.2
C(1)-C(2)-H(2A)	120.2
O(3)-C(3)-C(2)	124.5(2)
O(3)-C(3)-C(4)	114.3(2)
C(2)-C(3)-C(4)	121.0(3)
C(3)-O(3)-C(10)	116.9(2)
C(5)-C(4)-C(3)	116.5(3)
C(5)-C(4)-H(4A)	121.8
C(3)-C(4)-H(4A)	121.8
C(6)-C(5)-C(4)	123.2(3)
C(6)-C(5)-H(5A)	118.4
C(4)-C(5)-H(5A)	118.4
C(5)-C(6)-C(1)	120.0(3)
C(5)-C(6)-H(6A)	120.0
C(1)-C(6)-H(6A)	120.0
C(8)-C(7)-C(1)	109.7(2)
C(8)-C(7)-H(7A)	109.7
C(1)-C(7)-H(7A)	109.7
C(8)-C(7)-H(7B)	109.7
C(1)-C(7)-H(7B)	109.7
H(7A)-C(7)-H(7B)	108.2
N(8)-C(8)-C(7)	109.1(2)
N(8)-C(8)-H(8A)	109.9
C(7)-C(8)-H(8A)	109.9
N(8)-C(8)-H(8B)	109.9
C(7)-C(8)-H(8B)	109.9
H(8A)-C(8)-H(8B)	108.3
C(9)-N(8)-C(8)	123.12(17)
C(9)-N(8)-H(8N)	118.1(8)
C(8)-N(8)-H(8N)	118.7(7)
N(8)-C(9)-N(9)	123.4(2)
N(8)-C(9)-N(10)	117.90(19)
N(9)-C(9)-N(10)	118.7(2)
C(9)-N(9)-H(9A)	120.0
C(9)-N(9)-H(9B)	120.0
H(9A)-N(9)-H(9B)	120.0
C(9)-N(10)-O(10)	114.29(15)
C(9)-N(10)-H(10N)	104.0(7)
O(10)-N(10)-H(10N)	116.7(6)

N(10)-O(10)-H(10O)	100.55(11)
O(3)-C(10)-H(10A)	109.5
O(3)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
O(3)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
C(12)-C(11)-C(16)	118.1(2)
C(12)-C(11)-C(17)	119.7(2)
C(16)-C(11)-C(17)	121.8(3)
C(11)-C(12)-C(13)	122.6(3)
C(11)-C(12)-H(12A)	118.7
C(13)-C(12)-H(12A)	118.7
C(14)-C(13)-O(13)	116.0(3)
C(14)-C(13)-C(12)	118.0(3)
O(13)-C(13)-C(12)	126.0(3)
C(13)-O(13)-C(20)	116.4(2)
C(15)-C(14)-C(13)	123.0(3)
C(15)-C(14)-H(14A)	118.5
C(13)-C(14)-H(14A)	118.5
C(14)-C(15)-C(16)	117.7(3)
C(14)-C(15)-H(15A)	121.1
C(16)-C(15)-H(15A)	121.1
C(11)-C(16)-C(15)	120.5(3)
C(11)-C(16)-H(16A)	119.7
C(15)-C(16)-H(16A)	119.7
C(11)-C(17)-C(18)	112.1(2)
C(11)-C(17)-H(17A)	109.2
C(18)-C(17)-H(17A)	109.2
C(11)-C(17)-H(17B)	109.2
C(18)-C(17)-H(17B)	109.2
H(17A)-C(17)-H(17B)	107.9
N(18)-C(18)-C(17)	109.4(2)
N(18)-C(18)-H(18A)	109.8
C(17)-C(18)-H(18A)	109.8
N(18)-C(18)-H(18B)	109.8
C(17)-C(18)-H(18B)	109.8
H(18A)-C(18)-H(18B)	108.2
C(19)-N(18)-C(18)	124.59(16)
C(19)-N(18)-H(18N)	107.65(18)
C(18)-N(18)-H(18N)	126.56(16)
N(19)-C(19)-N(20)	118.6(2)
N(19)-C(19)-N(18)	121.4(2)
N(20)-C(19)-N(18)	120.0(2)
C(19)-N(19)-H(19A)	120.0
C(19)-N(19)-H(19B)	120.0
H(19A)-N(19)-H(19B)	120.0
C(19)-N(20)-O(20)	113.70(14)
C(19)-N(20)-H(20N)	117.67(18)
O(20)-N(20)-H(20N)	112.18(11)
N(20)-O(20)-H(20O)	114.76(11)
O(13)-C(20)-H(20A)	109.5
O(13)-C(20)-H(20B)	109.5
H(20A)-C(20)-H(20B)	109.5
O(13)-C(20)-H(20C)	109.5
H(20A)-C(20)-H(20C)	109.5
H(20B)-C(20)-H(20C)	109.5

C(22)-C(21)-C(26)	118.4(2)
C(22)-C(21)-C(27)	120.6(2)
C(26)-C(21)-C(27)	120.8(3)
C(21)-C(22)-C(23)	122.4(3)
C(21)-C(22)-H(22A)	118.8
C(23)-C(22)-H(22A)	118.8
C(24)-C(23)-O(23)	116.6(2)
C(24)-C(23)-C(22)	118.4(3)
O(23)-C(23)-C(22)	125.0(2)
C(23)-O(23)-C(30)	117.2(2)
C(23)-C(24)-C(25)	122.3(3)
C(23)-C(24)-H(24A)	118.8
C(25)-C(24)-H(24A)	118.8
C(24)-C(25)-C(26)	117.6(3)
C(24)-C(25)-H(25A)	121.2
C(26)-C(25)-H(25A)	121.2
C(21)-C(26)-C(25)	120.9(3)
C(21)-C(26)-H(26A)	119.6
C(25)-C(26)-H(26A)	119.6
C(21)-C(27)-C(28)	110.9(2)
C(21)-C(27)-H(27A)	109.5
C(28)-C(27)-H(27A)	109.5
C(21)-C(27)-H(27B)	109.5
C(28)-C(27)-H(27B)	109.5
H(27A)-C(27)-H(27B)	108.0
N(28)-C(28)-C(27)	107.9(2)
N(28)-C(28)-H(28A)	110.1
C(27)-C(28)-H(28A)	110.1
N(28)-C(28)-H(28B)	110.1
C(27)-C(28)-H(28B)	110.1
H(28A)-C(28)-H(28B)	108.4
C(29)-N(28)-C(28)	125.79(16)
C(29)-N(28)-H(28N)	115.6(3)
C(28)-N(28)-H(28N)	117.7(2)
N(28)-C(29)-N(29)	123.1(2)
N(28)-C(29)-N(30)	120.9(2)
N(29)-C(29)-N(30)	115.9(2)
C(29)-N(29)-H(29A)	120.0
C(29)-N(29)-H(29B)	120.0
H(29A)-N(29)-H(29B)	120.0
C(29)-N(30)-O(30)	111.56(14)
C(29)-N(30)-H(30N)	115.43(17)
O(30)-N(30)-H(30N)	105.61(11)
N(30)-O(30)-H(30O)	99.99(12)
O(23)-C(30)-H(30A)	109.5
O(23)-C(30)-H(30B)	109.5
H(30A)-C(30)-H(30B)	109.5
O(23)-C(30)-H(30C)	109.5
H(30A)-C(30)-H(30C)	109.5
H(30B)-C(30)-H(30C)	109.5
C(36)-C(31)-C(32)	119.8(2)
C(36)-C(31)-C(37)	119.4(3)
C(32)-C(31)-C(37)	120.8(2)
C(33)-C(32)-C(31)	119.7(2)
C(33)-C(32)-H(32A)	120.2
C(31)-C(32)-H(32A)	120.2
O(33)-C(33)-C(32)	123.2(2)

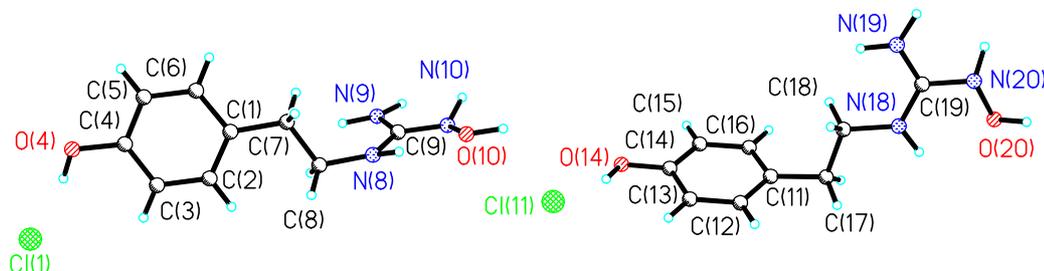
O(33)-C(33)-C(34)	116.7(2)
C(32)-C(33)-C(34)	120.1(2)
C(33)-O(33)-C(40)	118.6(2)
C(35)-C(34)-C(33)	117.7(2)
C(35)-C(34)-H(34A)	121.2
C(33)-C(34)-H(34A)	121.2
C(36)-C(35)-C(34)	123.7(3)
C(36)-C(35)-H(35A)	118.1
C(34)-C(35)-H(35A)	118.1
C(35)-C(36)-C(31)	118.9(3)
C(35)-C(36)-H(36A)	120.5
C(31)-C(36)-H(36A)	120.5
C(38)-C(37)-C(31)	113.3(2)
C(38)-C(37)-H(37A)	108.9
C(31)-C(37)-H(37A)	108.9
C(38)-C(37)-H(37B)	108.9
C(31)-C(37)-H(37B)	108.9
H(37A)-C(37)-H(37B)	107.7
N(38)-C(38)-C(37)	107.9(2)
N(38)-C(38)-H(38A)	110.1
C(37)-C(38)-H(38A)	110.1
N(38)-C(38)-H(38B)	110.1
C(37)-C(38)-H(38B)	110.1
H(38A)-C(38)-H(38B)	108.4
C(39)-N(38)-C(38)	125.30(16)
C(39)-N(38)-H(38N)	108.0(6)
C(38)-N(38)-H(38N)	126.7(6)
N(38)-C(39)-N(39)	124.5(2)
N(38)-C(39)-N(40)	117.9(2)
N(39)-C(39)-N(40)	117.6(2)
C(39)-N(39)-H(39A)	120.0
C(39)-N(39)-H(39B)	120.0
H(39A)-N(39)-H(39B)	120.0
C(39)-N(40)-O(40)	114.64(13)
C(39)-N(40)-H(40N)	95.54(14)
O(40)-N(40)-H(40N)	102.41(15)
N(40)-O(40)-H(40O)	112.59(13)
O(33)-C(40)-H(40A)	109.5
O(33)-C(40)-H(40B)	109.5
H(40A)-C(40)-H(40B)	109.5
O(33)-C(40)-H(40C)	109.5
H(40A)-C(40)-H(40C)	109.5
H(40B)-C(40)-H(40C)	109.5

Torsion angles [°] for (78)

C(6)-C(1)-C(2)-C(3)	0.9(4)
C(7)-C(1)-C(2)-C(3)	-176.8(2)
C(1)-C(2)-C(3)-O(3)	176.8(3)
C(1)-C(2)-C(3)-C(4)	2.4(4)
C(2)-C(3)-O(3)-C(10)	-0.5(4)
C(4)-C(3)-O(3)-C(10)	174.3(3)
O(3)-C(3)-C(4)-C(5)	179.9(3)
C(2)-C(3)-C(4)-C(5)	-5.2(4)

C(3)-C(4)-C(5)-C(6)	4.9(5)
C(4)-C(5)-C(6)-C(1)	-1.8(5)
C(2)-C(1)-C(6)-C(5)	-1.3(4)
C(7)-C(1)-C(6)-C(5)	176.4(3)
C(6)-C(1)-C(7)-C(8)	-77.6(3)
C(2)-C(1)-C(7)-C(8)	100.0(3)
C(1)-C(7)-C(8)-N(8)	-57.7(3)
C(7)-C(8)-N(8)-C(9)	161.6(2)
C(8)-N(8)-C(9)-N(9)	-0.4(3)
C(8)-N(8)-C(9)-N(10)	178.90(19)
N(8)-C(9)-N(10)-O(10)	20.6(2)
N(9)-C(9)-N(10)-O(10)	-160.03(16)
C(16)-C(11)-C(12)-C(13)	-0.2(4)
C(17)-C(11)-C(12)-C(13)	172.6(3)
C(11)-C(12)-C(13)-C(14)	2.0(4)
C(11)-C(12)-C(13)-O(13)	179.8(3)
C(14)-C(13)-O(13)-C(20)	-178.2(3)
C(12)-C(13)-O(13)-C(20)	4.0(4)
O(13)-C(13)-C(14)-C(15)	178.4(3)
C(12)-C(13)-C(14)-C(15)	-3.5(5)
C(13)-C(14)-C(15)-C(16)	3.0(5)
C(12)-C(11)-C(16)-C(15)	-0.4(4)
C(17)-C(11)-C(16)-C(15)	-173.0(3)
C(14)-C(15)-C(16)-C(11)	-1.0(5)
C(12)-C(11)-C(17)-C(18)	-99.8(3)
C(16)-C(11)-C(17)-C(18)	72.6(4)
C(11)-C(17)-C(18)-N(18)	58.7(3)
C(17)-C(18)-N(18)-C(19)	-163.2(2)
C(18)-N(18)-C(19)-N(19)	1.7(3)
C(18)-N(18)-C(19)-N(20)	-179.95(19)
N(19)-C(19)-N(20)-O(20)	162.21(16)
N(18)-C(19)-N(20)-O(20)	-16.2(2)
C(26)-C(21)-C(22)-C(23)	-0.4(4)
C(27)-C(21)-C(22)-C(23)	-175.1(3)
C(21)-C(22)-C(23)-C(24)	0.9(4)
C(21)-C(22)-C(23)-O(23)	179.4(3)
C(24)-C(23)-O(23)-C(30)	176.4(3)
C(22)-C(23)-O(23)-C(30)	-2.2(4)
O(23)-C(23)-C(24)-C(25)	179.7(3)
C(22)-C(23)-C(24)-C(25)	-1.7(4)
C(23)-C(24)-C(25)-C(26)	1.9(4)
C(22)-C(21)-C(26)-C(25)	0.7(4)
C(27)-C(21)-C(26)-C(25)	175.3(3)
C(24)-C(25)-C(26)-C(21)	-1.4(4)
C(22)-C(21)-C(27)-C(28)	-97.3(3)
C(26)-C(21)-C(27)-C(28)	88.2(3)
C(21)-C(27)-C(28)-N(28)	-179.0(2)
C(27)-C(28)-N(28)-C(29)	146.2(2)
C(28)-N(28)-C(29)-N(29)	-0.6(4)
C(28)-N(28)-C(29)-N(30)	179.0(2)
N(28)-C(29)-N(30)-O(30)	14.2(3)
N(29)-C(29)-N(30)-O(30)	-166.22(17)
C(36)-C(31)-C(32)-C(33)	-0.4(4)
C(37)-C(31)-C(32)-C(33)	179.4(2)
C(31)-C(32)-C(33)-O(33)	-176.9(2)
C(31)-C(32)-C(33)-C(34)	1.2(4)
C(32)-C(33)-O(33)-C(40)	-6.5(4)

C(34)-C(33)-O(33)-C(40)	175.4(2)
O(33)-C(33)-C(34)-C(35)	176.8(2)
C(32)-C(33)-C(34)-C(35)	-1.4(4)
C(33)-C(34)-C(35)-C(36)	0.8(5)
C(34)-C(35)-C(36)-C(31)	-0.1(5)
C(32)-C(31)-C(36)-C(35)	-0.2(4)
C(37)-C(31)-C(36)-C(35)	-179.9(3)
C(36)-C(31)-C(37)-C(38)	-82.4(3)
C(32)-C(31)-C(37)-C(38)	97.8(3)
C(31)-C(37)-C(38)-N(38)	177.4(2)
C(37)-C(38)-N(38)-C(39)	-151.2(2)
C(38)-N(38)-C(39)-N(39)	4.4(4)
C(38)-N(38)-C(39)-N(40)	-178.12(19)
N(38)-C(39)-N(40)-O(40)	-13.0(2)
N(39)-C(39)-N(40)-O(40)	164.61(18)

***N*-2-(4'-hydroxyphenyl)ethyl-*N*'-hydroxyguanidine hydrochloride (81)**

Crystal data and structure refinement for (81)			
Empirical formula	C ₉ H ₁₄ ClN ₃ O ₂	Index ranges	-6 ≤ h ≤ 6, -8 ≤ k ≤ 8, -32 ≤ l ≤ 30
Formula weight	231.68	Reflections collected	9403
Temperature	93(2) K	Independent reflections	3820 [R(int) = 0.1191]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	95.6 %
Crystal system	Triclinic	Absorption correction	Multiscan
Space group	P-1	Max. and min. transmission	1.0000 and 0.9169
Unit cell dimensions	a = 5.640(6) Å, α = 90.39(3)° b = 7.270(8) Å, β = 95.16(3)° c = 26.698(14) Å, γ = 91.24(2)°	Refinement method	Full-matrix least-squares on F ²
Volume	1090.0(18) Å ³	Data / restraints / parameters	3820 / 12 / 306
Z	4	Goodness-of-fit on F ²	1.153
Density (calculated)	1.412 Mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.1164, wR2 = 0.3084
Absorption coefficient	0.335 mm ⁻¹	R indices (all data)	R1 = 0.1339, wR2 = 0.3317
F(000)	488	Absolute structure parameter	N/A
Crystal size	0.2000 x 0.1000 x 0.0100 mm ³	Extinction coefficient	0.002(8)
Theta range for data collection	2.30 to 25.35°	Largest diff. peak and hole	0.665 and -0.594 e.Å ⁻³

Bond lengths [Å] and angles [°] for (81)

C(1)-C(2)	1.374(8)
C(1)-C(6)	1.408(8)
C(1)-C(7)	1.502(7)
C(2)-C(3)	1.392(7)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.376(8)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.361(8)
C(4)-O(4)	1.384(6)
O(4)-H(4O)	0.980(3)
C(5)-C(6)	1.376(7)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.505(8)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(8)	1.450(6)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
N(8)-C(9)	1.321(7)
N(8)-H(8N)	0.980(3)
C(9)-N(9)	1.338(7)
C(9)-N(10)	1.356(7)
N(9)-H(9A)	0.980(3)
N(9)-H(9B)	0.980(3)
N(10)-O(10)	1.424(6)
N(10)-H(10N)	0.980(3)
O(10)-H(10O)	0.9800
C(11)-C(12)	1.393(8)
C(11)-C(16)	1.395(8)
C(11)-C(17)	1.512(7)
C(12)-C(13)	1.376(7)
C(12)-H(12A)	0.9500
C(13)-C(14)	1.405(8)
C(13)-H(13A)	0.9500
C(14)-O(14)	1.363(6)
C(14)-C(15)	1.375(8)
O(14)-H(14O)	0.980(3)
C(15)-C(16)	1.388(8)
C(15)-H(15A)	0.9500
C(16)-H(16A)	0.9500
C(17)-C(18)	1.523(8)
C(17)-H(17A)	0.9900
C(17)-H(17B)	0.9900
C(18)-N(18)	1.476(6)
C(18)-H(18A)	0.9900
C(18)-H(18B)	0.9900
N(18)-C(19)	1.319(7)
N(18)-H(18N)	0.980(3)
C(19)-N(19)	1.316(7)
C(19)-N(20)	1.360(7)
N(19)-H(19A)	0.980(3)
N(19)-H(19B)	0.980(3)
N(20)-O(20)	1.408(6)
N(20)-H(20N)	0.980(3)

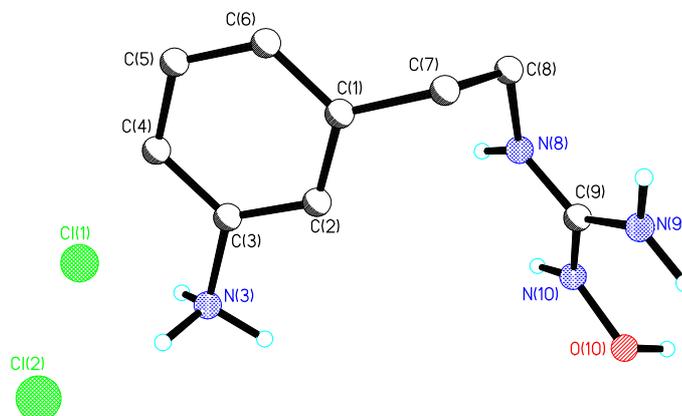
O(20)-H(20O)	0.980(3)
C(2)-C(1)-C(6)	117.1(5)
C(2)-C(1)-C(7)	124.2(5)
C(6)-C(1)-C(7)	118.7(5)
C(1)-C(2)-C(3)	121.7(5)
C(1)-C(2)-H(2A)	119.2
C(3)-C(2)-H(2A)	119.2
C(4)-C(3)-C(2)	119.3(5)
C(4)-C(3)-H(3A)	120.3
C(2)-C(3)-H(3A)	120.3
C(5)-C(4)-C(3)	120.6(5)
C(5)-C(4)-O(4)	118.5(5)
C(3)-C(4)-O(4)	120.9(5)
C(4)-O(4)-H(4O)	115(4)
C(4)-C(5)-C(6)	119.8(5)
C(4)-C(5)-H(5A)	120.1
C(6)-C(5)-H(5A)	120.1
C(5)-C(6)-C(1)	121.4(5)
C(5)-C(6)-H(6A)	119.3
C(1)-C(6)-H(6A)	119.3
C(1)-C(7)-C(8)	113.5(4)
C(1)-C(7)-H(7A)	108.9
C(8)-C(7)-H(7A)	108.9
C(1)-C(7)-H(7B)	108.9
C(8)-C(7)-H(7B)	108.9
H(7A)-C(7)-H(7B)	107.7
N(8)-C(8)-C(7)	113.2(4)
N(8)-C(8)-H(8A)	108.9
C(7)-C(8)-H(8A)	108.9
N(8)-C(8)-H(8B)	108.9
C(7)-C(8)-H(8B)	108.9
H(8A)-C(8)-H(8B)	107.8
C(9)-N(8)-C(8)	124.9(5)
C(9)-N(8)-H(8N)	97(4)
C(8)-N(8)-H(8N)	136(4)
N(8)-C(9)-N(9)	123.2(5)
N(8)-C(9)-N(10)	120.6(5)
N(9)-C(9)-N(10)	116.2(5)
C(9)-N(9)-H(9A)	109(4)
C(9)-N(9)-H(9B)	116(4)
H(9A)-N(9)-H(9B)	134(6)
C(9)-N(10)-O(10)	111.7(4)
C(9)-N(10)-H(10N)	120(4)
O(10)-N(10)-H(10N)	114(4)
N(10)-O(10)-H(10O)	109.5
C(12)-C(11)-C(16)	118.8(5)
C(12)-C(11)-C(17)	121.1(5)
C(16)-C(11)-C(17)	120.0(5)
C(13)-C(12)-C(11)	120.6(5)
C(13)-C(12)-H(12A)	119.7
C(11)-C(12)-H(12A)	119.7
C(12)-C(13)-C(14)	120.1(5)
C(12)-C(13)-H(13A)	120.0
C(14)-C(13)-H(13A)	120.0
O(14)-C(14)-C(15)	118.6(5)
O(14)-C(14)-C(13)	121.6(5)

C(15)-C(14)-C(13)	119.7(5)
C(14)-O(14)-H(14O)	105(4)
C(14)-C(15)-C(16)	119.9(5)
C(14)-C(15)-H(15A)	120.0
C(16)-C(15)-H(15A)	120.0
C(15)-C(16)-C(11)	120.8(5)
C(15)-C(16)-H(16A)	119.6
C(11)-C(16)-H(16A)	119.6
C(11)-C(17)-C(18)	109.0(5)
C(11)-C(17)-H(17A)	109.9
C(18)-C(17)-H(17A)	109.9
C(11)-C(17)-H(17B)	109.9
C(18)-C(17)-H(17B)	109.9
H(17A)-C(17)-H(17B)	108.3
N(18)-C(18)-C(17)	110.9(5)
N(18)-C(18)-H(18A)	109.5
C(17)-C(18)-H(18A)	109.5
N(18)-C(18)-H(18B)	109.5
C(17)-C(18)-H(18B)	109.5
H(18A)-C(18)-H(18B)	108.1
C(19)-N(18)-C(18)	120.4(5)
C(19)-N(18)-H(18N)	117(4)
C(18)-N(18)-H(18N)	121(4)
N(19)-C(19)-N(18)	121.9(5)
N(19)-C(19)-N(20)	118.3(5)
N(18)-C(19)-N(20)	119.7(5)
C(19)-N(19)-H(19A)	119(4)
C(19)-N(19)-H(19B)	118(4)
H(19A)-N(19)-H(19B)	122(6)
C(19)-N(20)-O(20)	113.2(4)
C(19)-N(20)-H(20N)	111(4)
O(20)-N(20)-H(20N)	119(4)
N(20)-O(20)-H(20O)	94(5)

Torsion angles [°] for (81)

C(6)-C(1)-C(2)-C(3)	0.8(8)
C(7)-C(1)-C(2)-C(3)	-179.1(5)
C(1)-C(2)-C(3)-C(4)	0.4(8)
C(2)-C(3)-C(4)-C(5)	-2.0(8)
C(2)-C(3)-C(4)-O(4)	179.8(5)
C(3)-C(4)-C(5)-C(6)	2.3(8)
O(4)-C(4)-C(5)-C(6)	-179.4(5)
C(4)-C(5)-C(6)-C(1)	-1.1(8)
C(2)-C(1)-C(6)-C(5)	-0.5(7)
C(7)-C(1)-C(6)-C(5)	179.4(5)
C(2)-C(1)-C(7)-C(8)	14.9(7)
C(6)-C(1)-C(7)-C(8)	-165.0(5)
C(1)-C(7)-C(8)-N(8)	178.1(4)
C(7)-C(8)-N(8)-C(9)	-84.4(7)
C(8)-N(8)-C(9)-N(9)	-0.2(9)
C(8)-N(8)-C(9)-N(10)	-178.8(5)
N(8)-C(9)-N(10)-O(10)	-13.7(7)
N(9)-C(9)-N(10)-O(10)	167.7(5)
C(16)-C(11)-C(12)-C(13)	-0.4(8)
C(17)-C(11)-C(12)-C(13)	175.2(5)

C(11)-C(12)-C(13)-C(14)	-0.7(8)
C(12)-C(13)-C(14)-O(14)	179.3(5)
C(12)-C(13)-C(14)-C(15)	2.2(8)
O(14)-C(14)-C(15)-C(16)	-179.7(5)
C(13)-C(14)-C(15)-C(16)	-2.5(8)
C(14)-C(15)-C(16)-C(11)	1.4(8)
C(12)-C(11)-C(16)-C(15)	0.1(8)
C(17)-C(11)-C(16)-C(15)	-175.6(5)
C(12)-C(11)-C(17)-C(18)	-102.8(6)
C(16)-C(11)-C(17)-C(18)	72.7(7)
C(11)-C(17)-C(18)-N(18)	-174.6(5)
C(17)-C(18)-N(18)-C(19)	171.2(5)
C(18)-N(18)-C(19)-N(19)	4.8(8)
C(18)-N(18)-C(19)-N(20)	-177.3(5)
N(19)-C(19)-N(20)-O(20)	-165.1(5)
N(18)-C(19)-N(20)-O(20)	16.9(7)

***N*-2-(3'-aminophenyl)ethyl-*N*'-hydroxyguanidine dihydrochloride (111)**

Crystal data and structure refinement for (111)			
Empirical formula	C ₉ H ₁₆ Cl ₂ N ₄ O	Index ranges	-16 ≤ h ≤ 14, -13 ≤ k ≤ 12, -12 ≤ l ≤ 18
Formula weight	267.16	Reflections collected	13265
Temperature	93(2) K	Independent reflections	2266 [R(int) = 0.0625]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	99.3 %
Crystal system	Orthorhombic	Absorption correction	Multiscan
Space group	Pbca	Max. and min. transmission	1.0000 and 0.9541
Unit cell dimensions	a = 14.276(3) Å, α = 90° b = 11.091(3) Å, β = 90° c = 15.796(4) Å, γ = 90°	Refinement method	Full-matrix least-squares on F ²
Volume	2501.2(11) Å ³	Data / restraints / parameters	2266 / 0 / 149
Z	8	Goodness-of-fit on F ²	1.171
Density (calculated)	1.419 Mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0873, wR2 = 0.2595
Absorption coefficient	0.505 mm ⁻¹	R indices (all data)	R1 = 0.1020, wR2 = 0.2772
F(000)	1120	Absolute structure parameter	N/A
Crystal size	0.200 x 0.200 x 0.020 mm ³	Extinction coefficient	0.002(2)
Theta range for data collection	3.39 to 25.35°	Largest diff. peak and hole	1.804 and -0.663 e.Å ⁻³

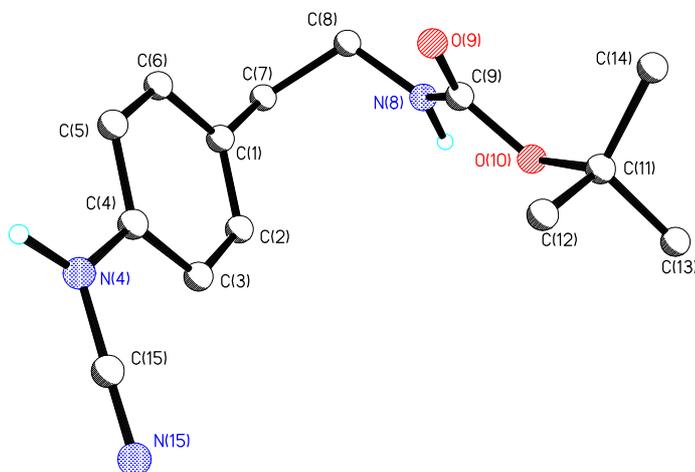
Bond lengths [Å] and angles [°] for (111)

C(1)-C(6)	1.385(7)
C(1)-C(2)	1.388(7)
C(1)-C(7)	1.505(7)
C(2)-C(3)	1.378(6)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.388(7)
C(3)-N(3)	1.467(6)
N(3)-H(3A)	0.9801
N(3)-H(3B)	0.9801
N(3)-H(3C)	0.9801
C(4)-C(5)	1.380(7)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.376(7)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.522(7)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(8)	1.454(6)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
N(8)-C(9)	1.310(6)
N(8)-H(8N)	0.9800
C(9)-N(9)	1.311(6)
C(9)-N(10)	1.373(7)
N(9)-H(9A)	0.9800
N(9)-H(9B)	0.9800
N(10)-O(10)	1.325(7)
N(10)-H(10N)	0.9800
O(10)-H(10O)	0.9800
C(6)-C(1)-C(2)	118.1(4)
C(6)-C(1)-C(7)	120.8(4)
C(2)-C(1)-C(7)	121.1(4)
C(3)-C(2)-C(1)	119.7(4)
C(3)-C(2)-H(2A)	120.2
C(1)-C(2)-H(2A)	120.2
C(2)-C(3)-C(4)	122.1(4)
C(2)-C(3)-N(3)	119.1(4)
C(4)-C(3)-N(3)	118.8(4)
C(3)-N(3)-H(3A)	109.5
C(3)-N(3)-H(3B)	109.5
H(3A)-N(3)-H(3B)	109.5
C(3)-N(3)-H(3C)	109.5
H(3A)-N(3)-H(3C)	109.5
H(3B)-N(3)-H(3C)	109.5
C(5)-C(4)-C(3)	118.0(4)
C(5)-C(4)-H(4A)	121.0
C(3)-C(4)-H(4A)	121.0
C(6)-C(5)-C(4)	120.1(4)
C(6)-C(5)-H(5A)	119.9
C(4)-C(5)-H(5A)	119.9
C(5)-C(6)-C(1)	122.0(4)
C(5)-C(6)-H(6A)	119.0
C(1)-C(6)-H(6A)	119.0

C(1)-C(7)-C(8)	113.9(4)
C(1)-C(7)-H(7A)	108.8
C(8)-C(7)-H(7A)	108.8
C(1)-C(7)-H(7B)	108.8
C(8)-C(7)-H(7B)	108.8
H(7A)-C(7)-H(7B)	107.7
N(8)-C(8)-C(7)	113.3(4)
N(8)-C(8)-H(8A)	108.9
C(7)-C(8)-H(8A)	108.9
N(8)-C(8)-H(8B)	108.9
C(7)-C(8)-H(8B)	108.9
H(8A)-C(8)-H(8B)	107.7
C(9)-N(8)-C(8)	124.4(4)
C(9)-N(8)-H(8N)	117.8
C(8)-N(8)-H(8N)	117.8
N(8)-C(9)-N(9)	123.7(5)
N(8)-C(9)-N(10)	113.7(5)
N(9)-C(9)-N(10)	122.6(5)
C(9)-N(9)-H(9A)	120.0
C(9)-N(9)-H(9B)	120.0
H(9A)-N(9)-H(9B)	120.0
O(10)-N(10)-C(9)	112.4(5)
O(10)-N(10)-H(10N)	123.8
C(9)-N(10)-H(10N)	123.8
N(10)-O(10)-H(10O)	109.5

Torsion angles [°] for (111)

C(6)-C(1)-C(2)-C(3)	0.8(6)
C(7)-C(1)-C(2)-C(3)	-177.4(4)
C(1)-C(2)-C(3)-C(4)	-0.9(6)
C(1)-C(2)-C(3)-N(3)	-178.3(4)
C(2)-C(3)-C(4)-C(5)	0.5(7)
N(3)-C(3)-C(4)-C(5)	177.9(4)
C(3)-C(4)-C(5)-C(6)	0.1(7)
C(4)-C(5)-C(6)-C(1)	-0.2(7)
C(2)-C(1)-C(6)-C(5)	-0.2(7)
C(7)-C(1)-C(6)-C(5)	178.0(4)
C(6)-C(1)-C(7)-C(8)	-100.4(5)
C(2)-C(1)-C(7)-C(8)	77.8(5)
C(1)-C(7)-C(8)-N(8)	-65.9(5)
C(7)-C(8)-N(8)-C(9)	-78.9(5)
C(8)-N(8)-C(9)-N(9)	-0.6(7)
C(8)-N(8)-C(9)-N(10)	-179.4(4)
N(8)-C(9)-N(10)-O(10)	-173.8(4)
N(9)-C(9)-N(10)-O(10)	7.4(7)

N-(*tert*-butoxycarbonyl)-2-(4'-aminophenyl)ethylcyanamide (113)

Crystal data and structure refinement for (113)			
Empirical formula	C ₁₄ H ₁₉ N ₃ O ₂	Index ranges	-9 ≤ h ≤ 6, -10 ≤ k ≤ 10, -12 ≤ l ≤ 13
Formula weight	261.32	Reflections collected	4755
Temperature	93(2) K	Independent reflections	2600 [R(int) = 0.0444]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	97.7 %
Crystal system	Triclinic	Absorption correction	Multiscan
Space group	P-1	Max. and min. transmission	1.0000 and 0.8793
Unit cell dimensions	a = 8.304(8) Å, α = 98.592(11)° b = 8.733(7) Å, β = 102.328(13)° c = 11.538(9) Å, γ = 112.065(15)°	Refinement method	Full-matrix least-squares on F ²
Volume	732.8(11) Å ³	Data / restraints / parameters	2600 / 2 / 182
Z	2	Goodness-of-fit on F ²	1.075
Density (calculated)	1.184 Mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0670, wR2 = 0.1642
Absorption coefficient	0.081 mm ⁻¹	R indices (all data)	R1 = 0.0937, wR2 = 0.1842
F(000)	280	Absolute structure parameter	N/A
Crystal size	0.2000 x 0.0500 x 0.300 mm ³	Extinction coefficient	0.044(9)
Theta range for data collection	1.87 to 25.35°	Largest diff. peak and hole	0.259 and -0.321 e.Å ⁻³

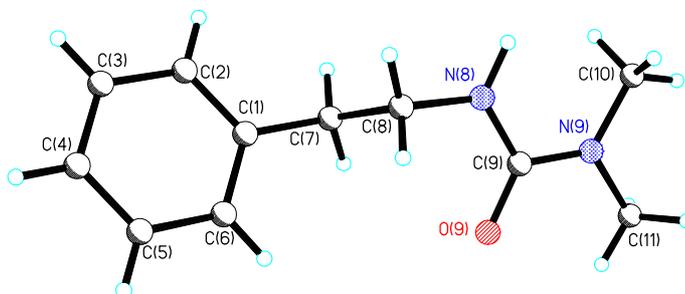
Bond lengths [Å] and angles [°] for (113)

C(1)-C(2)	1.378(4)
C(1)-C(6)	1.401(4)
C(1)-C(7)	1.512(3)
C(2)-C(3)	1.396(3)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.389(4)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.384(4)
C(4)-N(4)	1.425(3)
C(5)-C(6)	1.387(3)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.534(4)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(8)	1.457(3)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
N(8)-C(9)	1.347(3)
N(8)-H(8N)	0.979(3)
O(9)-C(9)	1.226(3)
C(9)-O(10)	1.346(3)
O(10)-C(11)	1.491(3)
C(11)-C(12)	1.514(3)
C(11)-C(14)	1.517(4)
C(11)-C(13)	1.523(4)
C(12)-H(12A)	0.9800
C(12)-H(12B)	0.9800
C(12)-H(12C)	0.9800
C(13)-H(13A)	0.9800
C(13)-H(13B)	0.9800
C(13)-H(13C)	0.9800
C(14)-H(14A)	0.9800
C(14)-H(14B)	0.9800
C(14)-H(14C)	0.9800
N(4)-C(15)	1.327(4)
N(4)-H(4N)	0.980(3)
C(15)-N(15)	1.153(3)
C(2)-C(1)-C(6)	118.1(2)
C(2)-C(1)-C(7)	121.2(2)
C(6)-C(1)-C(7)	120.7(2)
C(1)-C(2)-C(3)	122.0(2)
C(1)-C(2)-H(2A)	119.0
C(3)-C(2)-H(2A)	119.0
C(4)-C(3)-C(2)	118.8(2)
C(4)-C(3)-H(3A)	120.6
C(2)-C(3)-H(3A)	120.6
C(5)-C(4)-C(3)	120.4(2)
C(5)-C(4)-N(4)	118.6(2)
C(3)-C(4)-N(4)	121.0(2)
C(4)-C(5)-C(6)	119.9(2)
C(4)-C(5)-H(5A)	120.1
C(6)-C(5)-H(5A)	120.1
C(5)-C(6)-C(1)	120.9(2)

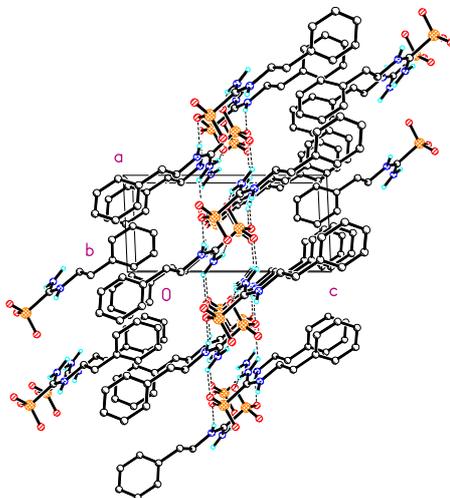
C(5)-C(6)-H(6A)	119.6
C(1)-C(6)-H(6A)	119.6
C(1)-C(7)-C(8)	112.9(2)
C(1)-C(7)-H(7A)	109.0
C(8)-C(7)-H(7A)	109.0
C(1)-C(7)-H(7B)	109.0
C(8)-C(7)-H(7B)	109.0
H(7A)-C(7)-H(7B)	107.8
N(8)-C(8)-C(7)	112.5(2)
N(8)-C(8)-H(8A)	109.1
C(7)-C(8)-H(8A)	109.1
N(8)-C(8)-H(8B)	109.1
C(7)-C(8)-H(8B)	109.1
H(8A)-C(8)-H(8B)	107.8
C(9)-N(8)-C(8)	123.1(2)
C(9)-N(8)-H(8N)	117.6(14)
C(8)-N(8)-H(8N)	118.7(14)
O(9)-C(9)-O(10)	124.6(2)
O(9)-C(9)-N(8)	125.2(2)
O(10)-C(9)-N(8)	110.2(2)
C(9)-O(10)-C(11)	121.13(19)
O(10)-C(11)-C(12)	109.74(18)
O(10)-C(11)-C(14)	110.10(19)
C(12)-C(11)-C(14)	113.2(2)
O(10)-C(11)-C(13)	101.69(19)
C(12)-C(11)-C(13)	110.6(2)
C(14)-C(11)-C(13)	110.9(2)
C(11)-C(12)-H(12A)	109.5
C(11)-C(12)-H(12B)	109.5
H(12A)-C(12)-H(12B)	109.5
C(11)-C(12)-H(12C)	109.5
H(12A)-C(12)-H(12C)	109.5
H(12B)-C(12)-H(12C)	109.5
C(11)-C(13)-H(13A)	109.5
C(11)-C(13)-H(13B)	109.5
H(13A)-C(13)-H(13B)	109.5
C(11)-C(13)-H(13C)	109.5
H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5
C(11)-C(14)-H(14A)	109.5
C(11)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	109.5
C(11)-C(14)-H(14C)	109.5
H(14A)-C(14)-H(14C)	109.5
H(14B)-C(14)-H(14C)	109.5
C(15)-N(4)-C(4)	122.0(2)
C(15)-N(4)-H(4N)	118.6(16)
C(4)-N(4)-H(4N)	119.2(17)
N(15)-C(15)-N(4)	179.4(3)

Torsion angles [°] for (113)

C(6)-C(1)-C(2)-C(3)	0.7(4)
C(7)-C(1)-C(2)-C(3)	178.7(2)
C(1)-C(2)-C(3)-C(4)	-1.2(4)
C(2)-C(3)-C(4)-C(5)	0.6(4)
C(2)-C(3)-C(4)-N(4)	179.9(2)
C(3)-C(4)-C(5)-C(6)	0.5(4)
N(4)-C(4)-C(5)-C(6)	-178.9(2)
C(4)-C(5)-C(6)-C(1)	-0.9(4)
C(2)-C(1)-C(6)-C(5)	0.3(4)
C(7)-C(1)-C(6)-C(5)	-177.7(2)
C(2)-C(1)-C(7)-C(8)	-96.6(3)
C(6)-C(1)-C(7)-C(8)	81.4(3)
C(1)-C(7)-C(8)-N(8)	65.5(3)
C(7)-C(8)-N(8)-C(9)	-106.5(3)
C(8)-N(8)-C(9)-O(9)	-2.7(4)
C(8)-N(8)-C(9)-O(10)	177.8(2)
O(9)-C(9)-O(10)-C(11)	-5.7(4)
N(8)-C(9)-O(10)-C(11)	173.77(18)
C(9)-O(10)-C(11)-C(12)	68.2(3)
C(9)-O(10)-C(11)-C(14)	-57.1(3)
C(9)-O(10)-C(11)-C(13)	-174.7(2)
C(5)-C(4)-N(4)-C(15)	-168.8(2)
C(3)-C(4)-N(4)-C(15)	11.8(4)
C(4)-N(4)-C(15)-N(15)	14(32)

N,N-dimethyl-*N*'-phenylethyl urea (126)

Crystal data and structure refinement for (126)			
Empirical formula	C ₁₁ H ₁₆ N ₂ O	Index ranges	-12 ≤ h ≤ 9, -11 ≤ k ≤ 10, -22 ≤ l ≤ 24
Formula weight	192.26	Reflections collected	11892
Temperature	93(2) K	Independent reflections	1887 [R(int) = 0.0220]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	96.3 %
Crystal system	Orthorhombic	Absorption correction	Multiscan
Space group	Pbca	Max. and min. transmission	1.0000 and 0.5917
Unit cell dimensions	a = 10.417(2) Å, α = 90° b = 9.860(2) Å, β = 90° c = 20.882(5) Å, γ = 90°	Refinement method	Full-matrix least-squares on F ²
Volume	2144.7(8) Å ³	Data / restraints / parameters	1887 / 1 / 134
Z	8	Goodness-of-fit on F ²	1.064
Density (calculated)	1.191 Mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0443, wR2 = 0.1200
Absorption coefficient	0.078 mm ⁻¹	R indices (all data)	R1 = 0.0462, wR2 = 0.1220
F(000)	832	Absolute structure parameter	N/A
Crystal size	0.1500 x 0.1500 x 0.0300 mm ³	Extinction coefficient	N/A
Theta range for data collection	2.76 to 25.34°	Largest diff. peak and hole	0.249 and -0.250 e.Å ⁻³

N-phenylethylaminoiminomethanesulfonic acid (137)

Crystal data and structure refinement for (137)			
Empirical formula	$C_9H_{12}N_2O_3S$	Index ranges	$-6 \leq h \leq 5, -8 \leq k \leq 10, -10 \leq l \leq 13$
Formula weight	228.27	Reflections collected	3126
Temperature	93(2) K	Independent reflections	1495 [R(int) = 0.0133]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	93.4 %
Crystal system	Monoclinic	Absorption correction	Multiscan
Space group	P2(1)	Max. and min. transmission	1.0000 and 0.8547
Unit cell dimensions	a = 5.3683(12) Å, $\alpha = 90^\circ$ b = 8.428(2) Å, $\beta = 92.506(7)^\circ$ c = 11.189(3) Å, $\gamma = 90^\circ$	Refinement method	Full-matrix least-squares on F^2
Volume	505.7(2) Å ³	Data / restraints / parameters	1495 / 4 / 149
Z	2	Goodness-of-fit on F^2	1.043
Density (calculated)	1.499 mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0191, wR2 = 0.0474
Absorption coefficient	0.308 mm ⁻¹	R indices (all data)	R1 = 0.0192, wR2 = 0.0475
F(000)	240	Absolute structure parameter	0.03(5)
Crystal size	0.1000 x 0.1000 x 0.1000 mm ³	Extinction coefficient	N/A
Theta range for data collection	3.03 to 25.37°	Largest diff. peak and hole	0.265 and -0.186 e.Å ⁻³

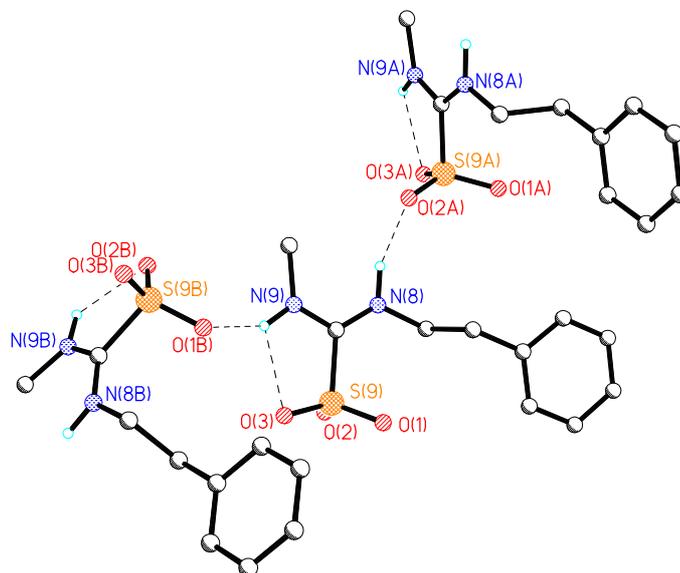
Bond lengths [Å] and angles [°] for (137)

C(1)-C(6)	1.384(2)
C(1)-C(2)	1.394(2)
C(1)-C(7)	1.516(2)
C(2)-C(3)	1.388(2)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.381(3)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.387(2)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.391(2)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.523(2)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(8)	1.4803(18)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
N(8)-C(9)	1.301(2)
N(8)-H(8N)	0.978(3)
C(9)-N(9)	1.305(2)
C(9)-S(9)	1.8242(15)
N(9)-H(9A)	0.979(3)
N(9)-H(9B)	0.978(3)
S(9)-O(2)	1.4403(12)
S(9)-O(3)	1.4432(12)
S(9)-O(1)	1.4466(12)
C(6)-C(1)-C(2)	119.12(15)
C(6)-C(1)-C(7)	121.22(14)
C(2)-C(1)-C(7)	119.65(16)
C(3)-C(2)-C(1)	120.21(16)
C(3)-C(2)-H(2A)	119.9
C(1)-C(2)-H(2A)	119.9
C(4)-C(3)-C(2)	120.49(15)
C(4)-C(3)-H(3A)	119.8
C(2)-C(3)-H(3A)	119.8
C(3)-C(4)-C(5)	119.50(15)
C(3)-C(4)-H(4A)	120.2
C(5)-C(4)-H(4A)	120.2
C(4)-C(5)-C(6)	120.14(16)
C(4)-C(5)-H(5A)	119.9
C(6)-C(5)-H(5A)	119.9
C(1)-C(6)-C(5)	120.52(15)
C(1)-C(6)-H(6A)	119.7
C(5)-C(6)-H(6A)	119.7
C(1)-C(7)-C(8)	112.29(13)
C(1)-C(7)-H(7A)	109.1
C(8)-C(7)-H(7A)	109.1
C(1)-C(7)-H(7B)	109.1
C(8)-C(7)-H(7B)	109.1
H(7A)-C(7)-H(7B)	107.9
N(8)-C(8)-C(7)	108.35(13)
N(8)-C(8)-H(8A)	110.0
C(7)-C(8)-H(8A)	110.0

N(8)-C(8)-H(8B)	110.0
C(7)-C(8)-H(8B)	110.0
H(8A)-C(8)-H(8B)	108.4
C(9)-N(8)-C(8)	124.75(13)
C(9)-N(8)-H(8N)	119.8(11)
C(8)-N(8)-H(8N)	115.4(11)
N(8)-C(9)-N(9)	126.48(13)
N(8)-C(9)-S(9)	116.07(11)
N(9)-C(9)-S(9)	117.42(11)
C(9)-N(9)-H(9A)	118.7(14)
C(9)-N(9)-H(9B)	123.5(13)
H(9A)-N(9)-H(9B)	118(2)
O(2)-S(9)-O(3)	114.96(7)
O(2)-S(9)-O(1)	113.95(7)
O(3)-S(9)-O(1)	114.81(7)
O(2)-S(9)-C(9)	104.76(7)
O(3)-S(9)-C(9)	102.29(7)
O(1)-S(9)-C(9)	104.05(7)

Torsion angles [°] for (137)

C(6)-C(1)-C(2)-C(3)	-1.0(2)
C(7)-C(1)-C(2)-C(3)	-179.99(15)
C(1)-C(2)-C(3)-C(4)	1.2(3)
C(2)-C(3)-C(4)-C(5)	-0.5(3)
C(3)-C(4)-C(5)-C(6)	-0.5(3)
C(2)-C(1)-C(6)-C(5)	0.0(2)
C(7)-C(1)-C(6)-C(5)	179.01(15)
C(4)-C(5)-C(6)-C(1)	0.7(2)
C(6)-C(1)-C(7)-C(8)	104.53(18)
C(2)-C(1)-C(7)-C(8)	-76.50(19)
C(1)-C(7)-C(8)-N(8)	175.02(13)
C(7)-C(8)-N(8)-C(9)	-171.64(14)
C(8)-N(8)-C(9)-N(9)	-1.6(2)
C(8)-N(8)-C(9)-S(9)	-179.73(12)
N(8)-C(9)-S(9)-O(2)	-61.96(13)
N(9)-C(9)-S(9)-O(2)	119.76(12)
N(8)-C(9)-S(9)-O(3)	58.31(13)
N(9)-C(9)-S(9)-O(3)	-119.96(12)
N(8)-C(9)-S(9)-O(1)	178.13(11)
N(9)-C(9)-S(9)-O(1)	-0.15(13)

N-methyl-*N*'-phenylethylaminoiminomethanesulfonic acid (132)

Crystal data and structure refinement for (132)			
Empirical formula	C ₁₀ H ₁₄ N ₂ O ₃ S	Index ranges	-13 ≤ h ≤ 13, -13 ≤ k ≤ 12, -9 ≤ l ≤ 8
Formula weight	242.29	Reflections collected	6525
Temperature	93(2) K	Independent reflections	1931 [R(int) = 0.0249]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	96.6 %
Crystal system	Orthorhombic	Absorption correction	Multiscan
Space group	Pna2(1)	Max. and min. transmission	1.0000 and 0.7812
Unit cell dimensions	a = 11.814(2) Å, α = 90° b = 11.656(2) Å, β = 90° c = 8.6208(16) Å, γ = 90°	Refinement method	Full-matrix least-squares on F ²
Volume	1187(4) Å ³	Data / restraints / parameters	1931 / 3 / 156
Z	4	Goodness-of-fit on F ²	1.051
Density (calculated)	1.356 mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0232, wR2 = 0.0576
Absorption coefficient	0.267 mm ⁻¹	R indices (all data)	R1 = 0.0245, wR2 = 0.0582
F(000)	512	Absolute structure parameter	-0.03(6)
Crystal size	0.080 x 0.080 x 0.080 mm ³	Extinction coefficient	0.0023(12)
Theta range for data collection	2.45 to 25.33°	Largest diff. peak and hole	0.207 and -0.174 e.Å ⁻³

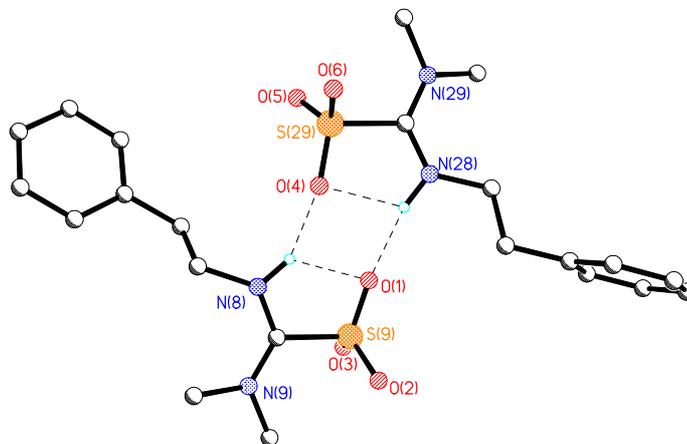
Bond lengths [Å] and angles [°] for (132)

C(1)-C(2)	1.386(3)
C(1)-C(6)	1.395(3)
C(1)-C(7)	1.505(3)
C(2)-C(3)	1.385(3)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.369(4)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.382(4)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.386(4)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.520(2)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(8)	1.468(2)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
N(8)-C(9)	1.311(2)
N(8)-H(8N)	0.979(3)
C(9)-N(9)	1.303(2)
C(9)-S(9)	1.8147(17)
N(9)-C(10)	1.455(2)
N(9)-H(9N)	0.978(3)
C(10)-H(10A)	0.9800
C(10)-H(10B)	0.9800
C(10)-H(10C)	0.9800
S(9)-O(3)	1.4341(13)
S(9)-O(1)	1.4369(14)
S(9)-O(2)	1.4442(13)
C(2)-C(1)-C(6)	118.36(19)
C(2)-C(1)-C(7)	121.03(16)
C(6)-C(1)-C(7)	120.60(16)
C(3)-C(2)-C(1)	121.24(19)
C(3)-C(2)-H(2A)	119.4
C(1)-C(2)-H(2A)	119.4
C(4)-C(3)-C(2)	120.0(2)
C(4)-C(3)-H(3A)	120.0
C(2)-C(3)-H(3A)	120.0
C(3)-C(4)-C(5)	119.8(2)
C(3)-C(4)-H(4A)	120.1
C(5)-C(4)-H(4A)	120.1
C(4)-C(5)-C(6)	120.6(2)
C(4)-C(5)-H(5A)	119.7
C(6)-C(5)-H(5A)	119.7
C(5)-C(6)-C(1)	120.0(2)
C(5)-C(6)-H(6A)	120.0
C(1)-C(6)-H(6A)	120.0
C(1)-C(7)-C(8)	111.92(14)
C(1)-C(7)-H(7A)	109.2
C(8)-C(7)-H(7A)	109.2
C(1)-C(7)-H(7B)	109.2
C(8)-C(7)-H(7B)	109.2
H(7A)-C(7)-H(7B)	107.9

N(8)-C(8)-C(7)	110.88(13)
N(8)-C(8)-H(8A)	109.5
C(7)-C(8)-H(8A)	109.5
N(8)-C(8)-H(8B)	109.5
C(7)-C(8)-H(8B)	109.5
H(8A)-C(8)-H(8B)	108.1
C(9)-N(8)-C(8)	127.62(15)
C(9)-N(8)-H(8N)	118.8(13)
C(8)-N(8)-H(8N)	113.1(13)
N(9)-C(9)-N(8)	122.89(16)
N(9)-C(9)-S(9)	115.54(13)
N(8)-C(9)-S(9)	121.56(14)
C(9)-N(9)-C(10)	123.09(15)
C(9)-N(9)-H(9N)	118.7(13)
C(10)-N(9)-H(9N)	118.0(13)
N(9)-C(10)-H(10A)	109.5
N(9)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
N(9)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
O(3)-S(9)-O(1)	114.42(8)
O(3)-S(9)-O(2)	115.43(8)
O(1)-S(9)-O(2)	113.87(8)
O(3)-S(9)-C(9)	103.63(8)
O(1)-S(9)-C(9)	105.31(8)
O(2)-S(9)-C(9)	102.15(7)

Torsion angles [°] for (132)

C(6)-C(1)-C(2)-C(3)	0.3(3)
C(7)-C(1)-C(2)-C(3)	179.33(17)
C(1)-C(2)-C(3)-C(4)	-0.2(3)
C(2)-C(3)-C(4)-C(5)	0.0(3)
C(3)-C(4)-C(5)-C(6)	0.2(4)
C(4)-C(5)-C(6)-C(1)	-0.1(4)
C(2)-C(1)-C(6)-C(5)	-0.2(3)
C(7)-C(1)-C(6)-C(5)	-179.2(2)
C(2)-C(1)-C(7)-C(8)	103.07(18)
C(6)-C(1)-C(7)-C(8)	-77.9(2)
C(1)-C(7)-C(8)-N(8)	-177.50(14)
C(7)-C(8)-N(8)-C(9)	-96.89(19)
C(8)-N(8)-C(9)-N(9)	177.79(17)
C(8)-N(8)-C(9)-S(9)	-3.7(2)
N(8)-C(9)-N(9)-C(10)	1.6(3)
S(9)-C(9)-N(9)-C(10)	-177.02(13)
N(9)-C(9)-S(9)-O(3)	-11.65(15)
N(8)-C(9)-S(9)-O(3)	169.74(14)
N(9)-C(9)-S(9)-O(1)	-132.15(14)
N(8)-C(9)-S(9)-O(1)	49.25(16)
N(9)-C(9)-S(9)-O(2)	108.63(14)
N(8)-C(9)-S(9)-O(2)	-69.97(15)

***N*-dimethyl-*N*'-phenylethylaminoiminomethanesulfonic acid (133)**

Crystal data and structure refinement for (133)			
Empirical formula	C ₁₁ H ₁₆ N ₂ O ₃ S	Index ranges	-25 ≤ h ≤ 21, -10 ≤ k ≤ 8, -12 ≤ l ≤ 14
Formula weight	256.32	Reflections collected	13988
Temperature	93(2) K	Independent reflections	3991 [R(int) = 0.0322]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	98.0 %
Crystal system	Orthorhombic	Absorption correction	Multiscan
Space group	Pca2(1)	Max. and min. transmission	1.0000 and 0.8622
Unit cell dimensions	a = 21.828(3) Å, α = 90° b = 9.0868(13) Å, β = 90° c = 12.3809(19) Å, γ = 90°	Refinement method	Full-matrix least-squares on F ²
Volume	2455.7(6) Å ³	Data / restraints / parameters	3991 / 3 / 321
Z	8	Goodness-of-fit on F ²	1.057
Density (calculated)	1.387 mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0403, wR2 = 0.0855
Absorption coefficient	0.262 mm ⁻¹	R indices (all data)	R1 = 0.0497, wR2 = 0.0898
F(000)	1088	Absolute structure parameter	0.33(8)
Crystal size	0.100 x 0.100 x 0.030 mm ³	Extinction coefficient	0.0015(3)
Theta range for data collection	2.92 to 25.35°	Largest diff. peak and hole	0.317 and -0.391 e.Å ⁻³

Bond lengths [Å] and angles [°] for (133)

C(1)-C(6)	1.374(5)
C(1)-C(2)	1.395(6)
C(1)-C(7)	1.521(6)
C(2)-C(3)	1.401(5)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.370(5)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.380(5)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.381(5)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.367(7)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(8)	1.475(6)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
N(8)-C(9)	1.320(5)
N(8)-H(8N)	0.979(3)
C(9)-N(9)	1.309(4)
C(9)-S(9)	1.839(3)
S(9)-O(2)	1.436(2)
S(9)-O(3)	1.440(2)
S(9)-O(1)	1.441(2)
N(9)-C(11)	1.458(4)
N(9)-C(10)	1.468(4)
C(10)-H(10A)	0.9800
C(10)-H(10B)	0.9800
C(10)-H(10C)	0.9800
C(11)-H(11A)	0.9800
C(11)-H(11B)	0.9800
C(11)-H(11C)	0.9800
C(21)-C(22)	1.386(4)
C(21)-C(26)	1.396(5)
C(21)-C(27)	1.512(5)
C(22)-C(23)	1.404(5)
C(22)-H(22A)	0.9500
C(23)-C(24)	1.369(5)
C(23)-H(23A)	0.9500
C(24)-C(25)	1.374(5)
C(24)-H(24A)	0.9500
C(25)-C(26)	1.386(5)
C(25)-H(25A)	0.9500
C(26)-H(26A)	0.9500
C(27)-C(28)	1.520(5)
C(27)-H(27A)	0.9900
C(27)-H(27B)	0.9900
C(28)-N(28)	1.477(5)
C(28)-H(28A)	0.9900
C(28)-H(28B)	0.9900
N(28)-C(29)	1.311(4)
N(28)-H(28N)	0.979(3)
C(29)-N(29)	1.317(4)
C(29)-S(29)	1.844(3)

S(29)-O(5)	1.435(2)
S(29)-O(6)	1.439(2)
S(29)-O(4)	1.448(2)
N(29)-C(30)	1.467(4)
N(29)-C(31)	1.475(4)
C(30)-H(30A)	0.9800
C(30)-H(30B)	0.9800
C(30)-H(30C)	0.9800
C(31)-H(31A)	0.9800
C(31)-H(31B)	0.9800
C(31)-H(31C)	0.9800
C(6)-C(1)-C(2)	118.3(4)
C(6)-C(1)-C(7)	119.6(4)
C(2)-C(1)-C(7)	122.1(4)
C(1)-C(2)-C(3)	120.4(4)
C(1)-C(2)-H(2A)	119.8
C(3)-C(2)-H(2A)	119.8
C(4)-C(3)-C(2)	119.9(4)
C(4)-C(3)-H(3A)	120.0
C(2)-C(3)-H(3A)	120.0
C(3)-C(4)-C(5)	119.8(3)
C(3)-C(4)-H(4A)	120.1
C(5)-C(4)-H(4A)	120.1
C(4)-C(5)-C(6)	120.1(4)
C(4)-C(5)-H(5A)	119.9
C(6)-C(5)-H(5A)	119.9
C(1)-C(6)-C(5)	121.4(4)
C(1)-C(6)-H(6A)	119.3
C(5)-C(6)-H(6A)	119.3
C(8)-C(7)-C(1)	118.2(4)
C(8)-C(7)-H(7A)	107.8
C(1)-C(7)-H(7A)	107.8
C(8)-C(7)-H(7B)	107.8
C(1)-C(7)-H(7B)	107.8
H(7A)-C(7)-H(7B)	107.1
C(7)-C(8)-N(8)	114.3(4)
C(7)-C(8)-H(8A)	108.7
N(8)-C(8)-H(8A)	108.7
C(7)-C(8)-H(8B)	108.7
N(8)-C(8)-H(8B)	108.7
H(8A)-C(8)-H(8B)	107.6
C(9)-N(8)-C(8)	130.6(4)
C(9)-N(8)-H(8N)	110(3)
C(8)-N(8)-H(8N)	119(3)
N(9)-C(9)-N(8)	127.0(3)
N(9)-C(9)-S(9)	119.4(3)
N(8)-C(9)-S(9)	113.6(2)
O(2)-S(9)-O(3)	114.97(16)
O(2)-S(9)-O(1)	114.58(15)
O(3)-S(9)-O(1)	114.24(15)
O(2)-S(9)-C(9)	103.19(15)
O(3)-S(9)-C(9)	104.62(14)
O(1)-S(9)-C(9)	103.21(14)
C(9)-N(9)-C(11)	123.0(3)
C(9)-N(9)-C(10)	124.1(3)
C(11)-N(9)-C(10)	112.6(3)

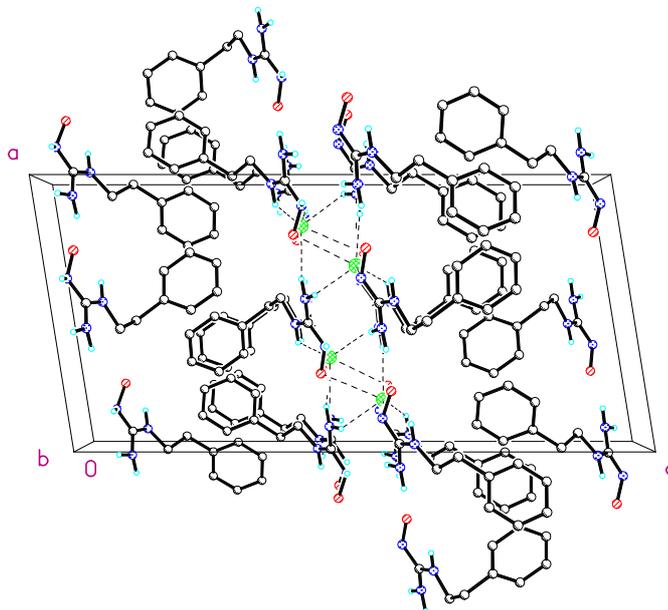
N(9)-C(10)-H(10A)	109.5
N(9)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
N(9)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
N(9)-C(11)-H(11A)	109.5
N(9)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
N(9)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
C(22)-C(21)-C(26)	118.5(3)
C(22)-C(21)-C(27)	121.6(3)
C(26)-C(21)-C(27)	119.8(3)
C(21)-C(22)-C(23)	120.5(3)
C(21)-C(22)-H(22A)	119.8
C(23)-C(22)-H(22A)	119.8
C(24)-C(23)-C(22)	119.9(3)
C(24)-C(23)-H(23A)	120.0
C(22)-C(23)-H(23A)	120.0
C(23)-C(24)-C(25)	120.1(3)
C(23)-C(24)-H(24A)	119.9
C(25)-C(24)-H(24A)	119.9
C(24)-C(25)-C(26)	120.6(3)
C(24)-C(25)-H(25A)	119.7
C(26)-C(25)-H(25A)	119.7
C(25)-C(26)-C(21)	120.4(3)
C(25)-C(26)-H(26A)	119.8
C(21)-C(26)-H(26A)	119.8
C(21)-C(27)-C(28)	110.8(3)
C(21)-C(27)-H(27A)	109.5
C(28)-C(27)-H(27A)	109.5
C(21)-C(27)-H(27B)	109.5
C(28)-C(27)-H(27B)	109.5
H(27A)-C(27)-H(27B)	108.1
N(28)-C(28)-C(27)	110.0(3)
N(28)-C(28)-H(28A)	109.7
C(27)-C(28)-H(28A)	109.7
N(28)-C(28)-H(28B)	109.7
C(27)-C(28)-H(28B)	109.7
H(28A)-C(28)-H(28B)	108.2
C(29)-N(28)-C(28)	128.9(3)
C(29)-N(28)-H(28N)	117(2)
C(28)-N(28)-H(28N)	114(2)
N(28)-C(29)-N(29)	126.5(3)
N(28)-C(29)-S(29)	113.9(2)
N(29)-C(29)-S(29)	119.7(2)
O(5)-S(29)-O(6)	115.20(15)
O(5)-S(29)-O(4)	114.33(14)
O(6)-S(29)-O(4)	113.91(14)
O(5)-S(29)-C(29)	104.75(15)
O(6)-S(29)-C(29)	104.39(14)
O(4)-S(29)-C(29)	102.29(15)
C(29)-N(29)-C(30)	123.4(3)
C(29)-N(29)-C(31)	123.7(3)
C(30)-N(29)-C(31)	112.9(3)

N(29)-C(30)-H(30A)	109.5
N(29)-C(30)-H(30B)	109.5
H(30A)-C(30)-H(30B)	109.5
N(29)-C(30)-H(30C)	109.5
H(30A)-C(30)-H(30C)	109.5
H(30B)-C(30)-H(30C)	109.5
N(29)-C(31)-H(31A)	109.5
N(29)-C(31)-H(31B)	109.5
H(31A)-C(31)-H(31B)	109.5
N(29)-C(31)-H(31C)	109.5
H(31A)-C(31)-H(31C)	109.5
H(31B)-C(31)-H(31C)	109.5

Torsion angles [°] for (133)

C(6)-C(1)-C(2)-C(3)	-0.1(6)
C(7)-C(1)-C(2)-C(3)	-178.7(4)
C(1)-C(2)-C(3)-C(4)	-0.1(6)
C(2)-C(3)-C(4)-C(5)	-0.3(5)
C(3)-C(4)-C(5)-C(6)	0.7(5)
C(2)-C(1)-C(6)-C(5)	0.5(6)
C(7)-C(1)-C(6)-C(5)	179.2(4)
C(4)-C(5)-C(6)-C(1)	-0.9(5)
C(6)-C(1)-C(7)-C(8)	147.3(6)
C(2)-C(1)-C(7)-C(8)	-34.1(8)
C(1)-C(7)-C(8)-N(8)	-168.5(5)
C(7)-C(8)-N(8)-C(9)	-117.3(6)
C(8)-N(8)-C(9)-N(9)	-14.1(7)
C(8)-N(8)-C(9)-S(9)	166.8(4)
N(9)-C(9)-S(9)-O(2)	-51.4(3)
N(8)-C(9)-S(9)-O(2)	127.8(3)
N(9)-C(9)-S(9)-O(3)	69.2(3)
N(8)-C(9)-S(9)-O(3)	-111.6(3)
N(9)-C(9)-S(9)-O(1)	-170.9(3)
N(8)-C(9)-S(9)-O(1)	8.2(3)
N(8)-C(9)-N(9)-C(11)	-15.9(5)
S(9)-C(9)-N(9)-C(11)	163.1(3)
N(8)-C(9)-N(9)-C(10)	171.3(3)
S(9)-C(9)-N(9)-C(10)	-9.7(4)
C(26)-C(21)-C(22)-C(23)	0.0(5)
C(27)-C(21)-C(22)-C(23)	179.0(3)
C(21)-C(22)-C(23)-C(24)	0.8(5)
C(22)-C(23)-C(24)-C(25)	-1.3(5)
C(23)-C(24)-C(25)-C(26)	1.0(5)
C(24)-C(25)-C(26)-C(21)	-0.1(5)
C(22)-C(21)-C(26)-C(25)	-0.4(5)
C(27)-C(21)-C(26)-C(25)	-179.3(3)
C(22)-C(21)-C(27)-C(28)	113.1(3)
C(26)-C(21)-C(27)-C(28)	-67.9(4)
C(21)-C(27)-C(28)-N(28)	179.9(3)
C(27)-C(28)-N(28)-C(29)	146.2(3)
C(28)-N(28)-C(29)-N(29)	22.2(5)
C(28)-N(28)-C(29)-S(29)	-158.8(2)
N(28)-C(29)-S(29)-O(5)	-124.8(2)
N(29)-C(29)-S(29)-O(5)	54.3(3)
N(28)-C(29)-S(29)-O(6)	113.8(2)

N(29)-C(29)-S(29)-O(6)	-67.2(3)
N(28)-C(29)-S(29)-O(4)	-5.2(3)
N(29)-C(29)-S(29)-O(4)	173.9(2)
N(28)-C(29)-N(29)-C(30)	-167.1(3)
S(29)-C(29)-N(29)-C(30)	14.0(4)
N(28)-C(29)-N(29)-C(31)	13.8(5)
S(29)-C(29)-N(29)-C(31)	-165.1(2)

***N*-phenylethyl-*N'*-hydroxyguanidine hydrochloride (120)**

Crystal data and structure refinement for (120)			
Empirical formula	C ₉ H ₁₄ ClN ₃ O	Index ranges	-14 ≤ h ≤ 10, -8 ≤ k ≤ 7, -28 ≤ l ≤ 29
Formula weight	215.68	Reflections collected	10496
Temperature	93(2) K	Independent reflections	3283 [R(int) = 0.0317]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	84.7 %
Crystal system	Monoclinic	Absorption correction	Multiscan
Space group	P2(1)/c	Max. and min. transmission	1.0000 and 0.8445
Unit cell dimensions	a = 11.980(3) Å, α = 90° b = 7.3875(15) Å, β = 99.080(6)° c = 24.609(7) Å γ = 90°	Refinement method	Full-matrix least-squares on F ²
Volume	2150.6(10) Å ³	Data / restraints / parameters	3283 / 10 / 295
Z	8	Goodness-of-fit on F ²	0.910
Density (calculated)	1.332 Mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0675, wR2 = 0.1366
Absorption coefficient	0.328 mm ⁻¹	R indices (all data)	R1 = 0.0750, wR2 = 0.1404
F(000)	912	Absolute structure parameter	N/A
Crystal size	0.1000 x 0.1000 x 0.0100 mm ³	Extinction coefficient	0.0018(4)
Theta range for data collection	2.21 to 25.32°	Largest diff. peak and hole	0.503 and -0.350 e.Å ⁻³

Bond lengths [Å] and angles [°] for (120)

C(1)-C(2)	1.381(6)
C(1)-C(6)	1.400(6)
C(1)-C(7)	1.514(6)
C(2)-C(3)	1.385(6)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.377(7)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.386(7)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.383(6)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.512(6)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(8)	1.467(5)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
N(8)-C(9)	1.323(5)
N(8)-H(8N)	0.969(19)
C(9)-N(9)	1.329(5)
C(9)-N(10)	1.360(5)
N(9)-H(9A)	0.983(19)
N(9)-H(9B)	0.968(19)
N(10)-O(10)	1.412(5)
N(10)-H(10N)	0.97(2)
O(10)-H(10O)	0.97(2)
C(11)-C(16)	1.384(6)
C(11)-C(12)	1.398(6)
C(11)-C(17)	1.516(5)
C(12)-C(13)	1.386(6)
C(12)-H(12A)	0.9500
C(13)-C(14)	1.385(7)
C(13)-H(13A)	0.9500
C(14)-C(15)	1.381(6)
C(14)-H(14A)	0.9500
C(15)-C(16)	1.393(6)
C(15)-H(15A)	0.9500
C(16)-H(16A)	0.9500
C(17)-C(18)	1.521(6)
C(17)-H(17A)	0.9900
C(17)-H(17B)	0.9900
C(18)-N(18)	1.470(5)
C(18)-H(18A)	0.9900
C(18)-H(18B)	0.9900
N(18)-C(19)	1.327(5)
N(18)-H(18N)	0.97(2)
C(19)-N(19)	1.321(5)
C(19)-N(20)	1.350(5)
N(19)-H(19A)	0.97(2)
N(19)-H(19B)	0.98(2)
N(20)-O(20)	1.412(5)
N(20)-H(20N)	0.962(19)
O(20)-H(20O)	0.97(2)

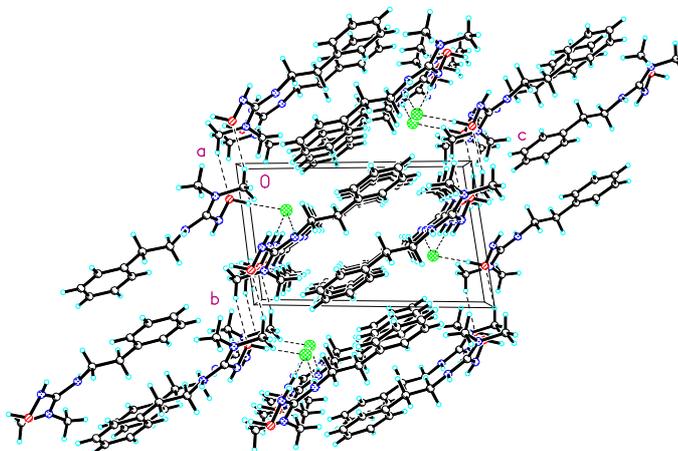
C(2)-C(1)-C(6)	119.2(4)
C(2)-C(1)-C(7)	121.6(4)
C(6)-C(1)-C(7)	119.1(4)
C(1)-C(2)-C(3)	120.9(4)
C(1)-C(2)-H(2A)	119.6
C(3)-C(2)-H(2A)	119.6
C(4)-C(3)-C(2)	119.9(5)
C(4)-C(3)-H(3A)	120.1
C(2)-C(3)-H(3A)	120.1
C(3)-C(4)-C(5)	119.8(4)
C(3)-C(4)-H(4A)	120.1
C(5)-C(4)-H(4A)	120.1
C(6)-C(5)-C(4)	120.7(4)
C(6)-C(5)-H(5A)	119.6
C(4)-C(5)-H(5A)	119.6
C(5)-C(6)-C(1)	119.5(4)
C(5)-C(6)-H(6A)	120.2
C(1)-C(6)-H(6A)	120.2
C(8)-C(7)-C(1)	112.8(4)
C(8)-C(7)-H(7A)	109.0
C(1)-C(7)-H(7A)	109.0
C(8)-C(7)-H(7B)	109.0
C(1)-C(7)-H(7B)	109.0
H(7A)-C(7)-H(7B)	107.8
N(8)-C(8)-C(7)	109.0(4)
N(8)-C(8)-H(8A)	109.9
C(7)-C(8)-H(8A)	109.9
N(8)-C(8)-H(8B)	109.9
C(7)-C(8)-H(8B)	109.9
H(8A)-C(8)-H(8B)	108.3
C(9)-N(8)-C(8)	123.9(4)
C(9)-N(8)-H(8N)	112(3)
C(8)-N(8)-H(8N)	123(3)
N(8)-C(9)-N(9)	122.6(4)
N(8)-C(9)-N(10)	119.4(4)
N(9)-C(9)-N(10)	118.0(4)
C(9)-N(9)-H(9A)	118(3)
C(9)-N(9)-H(9B)	115(3)
H(9A)-N(9)-H(9B)	120(4)
C(9)-N(10)-O(10)	112.7(3)
C(9)-N(10)-H(10N)	107(3)
O(10)-N(10)-H(10N)	123(3)
N(10)-O(10)-H(10O)	106(4)
C(16)-C(11)-C(12)	118.6(4)
C(16)-C(11)-C(17)	121.0(4)
C(12)-C(11)-C(17)	120.3(4)
C(13)-C(12)-C(11)	120.4(4)
C(13)-C(12)-H(12A)	119.8
C(11)-C(12)-H(12A)	119.8
C(14)-C(13)-C(12)	120.3(4)
C(14)-C(13)-H(13A)	119.8
C(12)-C(13)-H(13A)	119.8
C(15)-C(14)-C(13)	119.7(4)
C(15)-C(14)-H(14A)	120.1
C(13)-C(14)-H(14A)	120.1
C(14)-C(15)-C(16)	119.9(4)
C(14)-C(15)-H(15A)	120.0

C(16)-C(15)-H(15A)	120.0
C(11)-C(16)-C(15)	120.9(4)
C(11)-C(16)-H(16A)	119.5
C(15)-C(16)-H(16A)	119.5
C(11)-C(17)-C(18)	115.2(4)
C(11)-C(17)-H(17A)	108.5
C(18)-C(17)-H(17A)	108.5
C(11)-C(17)-H(17B)	108.5
C(18)-C(17)-H(17B)	108.5
H(17A)-C(17)-H(17B)	107.5
N(18)-C(18)-C(17)	110.7(3)
N(18)-C(18)-H(18A)	109.5
C(17)-C(18)-H(18A)	109.5
N(18)-C(18)-H(18B)	109.5
C(17)-C(18)-H(18B)	109.5
H(18A)-C(18)-H(18B)	108.1
C(19)-N(18)-C(18)	122.1(4)
C(19)-N(18)-H(18N)	117(4)
C(18)-N(18)-H(18N)	121(4)
N(19)-C(19)-N(18)	122.3(4)
N(19)-C(19)-N(20)	117.8(4)
N(18)-C(19)-N(20)	119.9(4)
C(19)-N(19)-H(19A)	128(4)
C(19)-N(19)-H(19B)	117(3)
H(19A)-N(19)-H(19B)	108(5)
C(19)-N(20)-O(20)	114.9(3)
C(19)-N(20)-H(20N)	110(3)
O(20)-N(20)-H(20N)	123(3)
N(20)-O(20)-H(20O)	105(4)

Torsion angles [°] for (120)

C(6)-C(1)-C(2)-C(3)	-1.2(7)
C(7)-C(1)-C(2)-C(3)	-179.4(4)
C(1)-C(2)-C(3)-C(4)	0.1(7)
C(2)-C(3)-C(4)-C(5)	1.2(7)
C(3)-C(4)-C(5)-C(6)	-1.4(7)
C(4)-C(5)-C(6)-C(1)	0.4(7)
C(2)-C(1)-C(6)-C(5)	0.9(7)
C(7)-C(1)-C(6)-C(5)	179.2(4)
C(2)-C(1)-C(7)-C(8)	73.9(6)
C(6)-C(1)-C(7)-C(8)	-104.3(5)
C(1)-C(7)-C(8)-N(8)	161.3(4)
C(7)-C(8)-N(8)-C(9)	-161.5(4)
C(8)-N(8)-C(9)-N(9)	-8.2(6)
C(8)-N(8)-C(9)-N(10)	171.9(4)
N(8)-C(9)-N(10)-O(10)	-19.6(5)
N(9)-C(9)-N(10)-O(10)	160.5(3)
C(16)-C(11)-C(12)-C(13)	-1.3(6)
C(17)-C(11)-C(12)-C(13)	176.5(4)
C(11)-C(12)-C(13)-C(14)	0.6(7)
C(12)-C(13)-C(14)-C(15)	1.4(7)
C(13)-C(14)-C(15)-C(16)	-2.8(7)
C(12)-C(11)-C(16)-C(15)	0.0(7)
C(17)-C(11)-C(16)-C(15)	-177.8(4)
C(14)-C(15)-C(16)-C(11)	2.1(7)

C(16)-C(11)-C(17)-C(18)	-116.0(5)
C(12)-C(11)-C(17)-C(18)	66.2(5)
C(11)-C(17)-C(18)-N(18)	71.9(4)
C(17)-C(18)-N(18)-C(19)	179.9(3)
C(18)-N(18)-C(19)-N(19)	7.3(6)
C(18)-N(18)-C(19)-N(20)	-172.0(4)
N(19)-C(19)-N(20)-O(20)	165.2(3)
N(18)-C(19)-N(20)-O(20)	-15.4(5)

***N,N*-dimethyl-*N'*-phenylethyl-*N*-hydroxyguanidine hydrochloride (122)**

Crystal data and structure refinement for (122)			
Empirical formula	C ₁₁ H ₁₈ ClN ₃ O	Index ranges	-5 ≤ h ≤ 8, -7 ≤ k ≤ 9, -14 ≤ l ≤ 15
Formula weight	243.73	Reflections collected	3917
Temperature	93(2) K	Independent reflections	2097 [R(int) = 0.0327]
Wavelength	0.71073 Å	Completeness to theta = 25.00°	92.9 %
Crystal system	Triclinic	Absorption correction	Multiscan
Space group	P-1	Max. and min. transmission	1.0000 and 0.6247
Unit cell dimensions	a = 6.9008(16) Å, α = 78.99(2)° b = 7.6974(18) Å, β = 75.15(2)° c = 12.576(3) Å γ = 76.77(3)°	Refinement method	Full-matrix least-squares on F ²
Volume	622.3(3) Å ³	Data / restraints / parameters	2097 / 3 / 161
Z	2	Goodness-of-fit on F ²	1.006
Density (calculated)	1.301 Mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0407, wR2 = 0.0724
Absorption coefficient	0.292 mm ⁻¹	R indices (all data)	R1 = 0.0538, wR2 = 0.0783
F(000)	260	Absolute structure parameter	N/A
Crystal size	0.1500 x 0.0500 x 0.0500 mm ³	Extinction coefficient	0.0012(16)
Theta range for data collection	2.75 to 25.32°	Largest diff. peak and hole	0.231 and -0.237 e.Å ⁻³

Bond lengths [Å] and angles [°] for (122)

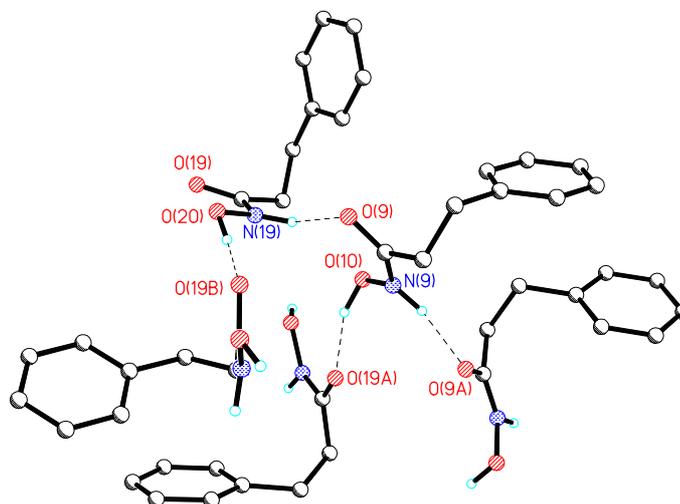
C(1)-C(2)	1.386(3)
C(1)-C(6)	1.391(2)
C(1)-C(7)	1.515(2)
C(2)-C(3)	1.396(3)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.372(3)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.378(3)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.383(3)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.518(3)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(8)	1.461(2)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
N(8)-C(9)	1.334(2)
N(8)-H(8N)	0.979(3)
C(9)-N(9)	1.335(2)
C(9)-N(10)	1.348(2)
N(9)-C(11)	1.457(2)
N(9)-C(10)	1.466(2)
C(10)-H(10A)	0.9800
C(10)-H(10B)	0.9800
C(10)-H(10C)	0.9800
C(11)-H(11A)	0.9800
C(11)-H(11B)	0.9800
C(11)-H(11C)	0.9800
N(10)-O(10)	1.4117(19)
N(10)-H(10N)	0.979(3)
O(10)-H(10O)	0.979(3)
C(2)-C(1)-C(6)	117.90(18)
C(2)-C(1)-C(7)	123.42(17)
C(6)-C(1)-C(7)	118.69(18)
C(1)-C(2)-C(3)	120.50(18)
C(1)-C(2)-H(2A)	119.8
C(3)-C(2)-H(2A)	119.8
C(4)-C(3)-C(2)	120.7(2)
C(4)-C(3)-H(3A)	119.6
C(2)-C(3)-H(3A)	119.6
C(3)-C(4)-C(5)	119.26(18)
C(3)-C(4)-H(4A)	120.4
C(5)-C(4)-H(4A)	120.4
C(4)-C(5)-C(6)	120.25(19)
C(4)-C(5)-H(5A)	119.9
C(6)-C(5)-H(5A)	119.9
C(5)-C(6)-C(1)	121.4(2)
C(5)-C(6)-H(6A)	119.3
C(1)-C(6)-H(6A)	119.3
C(1)-C(7)-C(8)	114.69(16)
C(1)-C(7)-H(7A)	108.6
C(8)-C(7)-H(7A)	108.6

C(1)-C(7)-H(7B)	108.6
C(8)-C(7)-H(7B)	108.6
H(7A)-C(7)-H(7B)	107.6
N(8)-C(8)-C(7)	108.77(16)
N(8)-C(8)-H(8A)	109.9
C(7)-C(8)-H(8A)	109.9
N(8)-C(8)-H(8B)	109.9
C(7)-C(8)-H(8B)	109.9
H(8A)-C(8)-H(8B)	108.3
C(9)-N(8)-C(8)	124.92(16)
C(9)-N(8)-H(8N)	114.5(13)
C(8)-N(8)-H(8N)	118.1(13)
N(8)-C(9)-N(9)	119.66(17)
N(8)-C(9)-N(10)	119.19(16)
N(9)-C(9)-N(10)	121.12(16)
C(9)-N(9)-C(11)	120.72(15)
C(9)-N(9)-C(10)	123.10(15)
C(11)-N(9)-C(10)	115.43(14)
N(9)-C(10)-H(10A)	109.5
N(9)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
N(9)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
N(9)-C(11)-H(11A)	109.5
N(9)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
N(9)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
C(9)-N(10)-O(10)	116.86(13)
C(9)-N(10)-H(10N)	123.3(12)
O(10)-N(10)-H(10N)	110.2(12)
N(10)-O(10)-H(10O)	105.7(13)

Torsion angles [°] for (122)

C(6)-C(1)-C(2)-C(3)	0.4(3)
C(7)-C(1)-C(2)-C(3)	-179.89(17)
C(1)-C(2)-C(3)-C(4)	0.2(3)
C(2)-C(3)-C(4)-C(5)	-0.4(3)
C(3)-C(4)-C(5)-C(6)	0.1(3)
C(4)-C(5)-C(6)-C(1)	0.4(3)
C(2)-C(1)-C(6)-C(5)	-0.7(3)
C(7)-C(1)-C(6)-C(5)	179.59(18)
C(2)-C(1)-C(7)-C(8)	-0.1(3)
C(6)-C(1)-C(7)-C(8)	179.63(17)
C(1)-C(7)-C(8)-N(8)	-178.88(15)
C(7)-C(8)-N(8)-C(9)	159.21(17)
C(8)-N(8)-C(9)-N(9)	-163.22(17)
C(8)-N(8)-C(9)-N(10)	19.0(3)
N(8)-C(9)-N(9)-C(11)	18.3(3)
N(10)-C(9)-N(9)-C(11)	-164.03(16)
N(8)-C(9)-N(9)-C(10)	-151.36(18)
N(10)-C(9)-N(9)-C(10)	26.3(3)
N(8)-C(9)-N(10)-O(10)	-147.57(16)
N(9)-C(9)-N(10)-O(10)	34.7(2)

3-phenylpropionohydroxamic acid (139)



Crystal data and structure refinement for (139)			
Empirical formula	C ₉ H ₁₁ NO ₂	Index ranges	-9 ≤ h ≤ 9, -8 ≤ k ≤ 8, -30 ≤ l ≤ 30
Formula weight	165.19	Reflections collected	14529
Temperature	93(2) K	Independent reflections	1286 [R(int) = 0.1240]
Wavelength	1.54178 Å	Completeness to theta = 25.00°	96.2 %
Crystal system	Orthorhombic	Absorption correction	Multiscan
Space group	Pbca	Max. and min. transmission	1.0000 and 0.7177
Unit cell dimensions	a = 10.5232(9) Å, α = 90° b = 9.5588(9) Å, β = 90° c = 33.738(3) Å γ = 90°	Refinement method	Full-matrix least-squares on F ²
Volume	3393.7(5) Å ³	Data / restraints / parameters	1286 / 4 / 235
Z	16	Goodness-of-fit on F ²	1.053
Density (calculated)	1.293 mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.0606, wR2 = 0.1562
Absorption coefficient	0.753 mm ⁻¹	R indices (all data)	R1 = 0.0646, wR2 = 0.1607
F(000)	1408	Absolute structure parameter	N/A
Crystal size	0.100 x 0.100 x 0.010 mm ³	Extinction coefficient	0.0011(3)
Theta range for data collection	4.95 to 44.67°	Largest diff. peak and hole	0.217 and -0.227 e.Å ⁻³

Bond lengths [Å] and angles [°] for (139)

C(1)-C(6)	1.380(5)
C(1)-C(2)	1.387(5)
C(1)-C(7)	1.499(5)
C(2)-C(3)	1.363(5)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.371(6)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.368(5)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.391(5)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.530(5)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-C(9)	1.492(5)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
C(9)-O(9)	1.251(4)
C(9)-N(9)	1.314(4)
N(9)-O(10)	1.394(3)
N(9)-H(9N)	0.979(3)
O(10)-H(10O)	0.980(3)
C(11)-C(12)	1.388(5)
C(11)-C(16)	1.392(5)
C(11)-C(17)	1.506(5)
C(12)-C(13)	1.379(5)
C(12)-H(12A)	0.9500
C(13)-C(14)	1.387(5)
C(13)-H(13A)	0.9500
C(14)-C(15)	1.379(5)
C(14)-H(14A)	0.9500
C(15)-C(16)	1.373(5)
C(15)-H(15A)	0.9500
C(16)-H(16A)	0.9500
C(17)-C(18)	1.544(5)
C(17)-H(17A)	0.9900
C(17)-H(17B)	0.9900
C(18)-C(19)	1.486(5)
C(18)-H(18A)	0.9900
C(18)-H(18B)	0.9900
C(19)-O(19)	1.257(4)
C(19)-N(19)	1.316(5)
N(19)-O(20)	1.405(4)
N(19)-H(19N)	0.980(3)
O(20)-H(20O)	0.980(3)
C(6)-C(1)-C(2)	117.1(3)
C(6)-C(1)-C(7)	121.9(3)
C(2)-C(1)-C(7)	121.0(3)
C(3)-C(2)-C(1)	122.1(4)
C(3)-C(2)-H(2A)	118.9
C(1)-C(2)-H(2A)	118.9
C(2)-C(3)-C(4)	120.4(4)
C(2)-C(3)-H(3A)	119.8

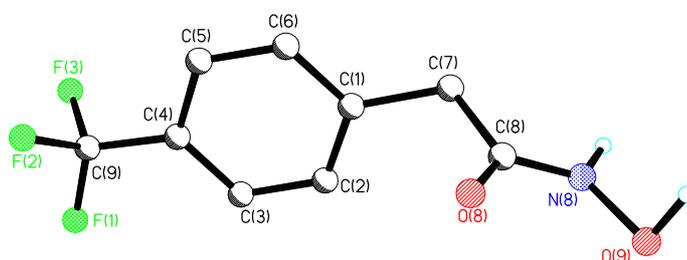
C(4)-C(3)-H(3A)	119.8
C(5)-C(4)-C(3)	119.1(4)
C(5)-C(4)-H(4A)	120.5
C(3)-C(4)-H(4A)	120.5
C(4)-C(5)-C(6)	120.5(4)
C(4)-C(5)-H(5A)	119.7
C(6)-C(5)-H(5A)	119.7
C(1)-C(6)-C(5)	120.8(4)
C(1)-C(6)-H(6A)	119.6
C(5)-C(6)-H(6A)	119.6
C(1)-C(7)-C(8)	114.8(3)
C(1)-C(7)-H(7A)	108.6
C(8)-C(7)-H(7A)	108.6
C(1)-C(7)-H(7B)	108.6
C(8)-C(7)-H(7B)	108.6
H(7A)-C(7)-H(7B)	107.5
C(9)-C(8)-C(7)	113.3(3)
C(9)-C(8)-H(8A)	108.9
C(7)-C(8)-H(8A)	108.9
C(9)-C(8)-H(8B)	108.9
C(7)-C(8)-H(8B)	108.9
H(8A)-C(8)-H(8B)	107.7
O(9)-C(9)-N(9)	122.7(3)
O(9)-C(9)-C(8)	121.4(4)
N(9)-C(9)-C(8)	115.9(4)
C(9)-N(9)-O(10)	118.9(3)
C(9)-N(9)-H(9N)	127.3(17)
O(10)-N(9)-H(9N)	113.2(17)
N(9)-O(10)-H(10O)	102(2)
C(12)-C(11)-C(16)	117.6(3)
C(12)-C(11)-C(17)	122.4(4)
C(16)-C(11)-C(17)	120.0(3)
C(13)-C(12)-C(11)	121.2(4)
C(13)-C(12)-H(12A)	119.4
C(11)-C(12)-H(12A)	119.4
C(12)-C(13)-C(14)	120.4(4)
C(12)-C(13)-H(13A)	119.8
C(14)-C(13)-H(13A)	119.8
C(15)-C(14)-C(13)	118.9(4)
C(15)-C(14)-H(14A)	120.5
C(13)-C(14)-H(14A)	120.5
C(16)-C(15)-C(14)	120.5(4)
C(16)-C(15)-H(15A)	119.7
C(14)-C(15)-H(15A)	119.7
C(15)-C(16)-C(11)	121.4(3)
C(15)-C(16)-H(16A)	119.3
C(11)-C(16)-H(16A)	119.3
C(11)-C(17)-C(18)	116.5(3)
C(11)-C(17)-H(17A)	108.2
C(18)-C(17)-H(17A)	108.2
C(11)-C(17)-H(17B)	108.2
C(18)-C(17)-H(17B)	108.2
H(17A)-C(17)-H(17B)	107.3
C(19)-C(18)-C(17)	112.5(3)
C(19)-C(18)-H(18A)	109.1
C(17)-C(18)-H(18A)	109.1
C(19)-C(18)-H(18B)	109.1

C(17)-C(18)-H(18B)	109.1
H(18A)-C(18)-H(18B)	107.8
O(19)-C(19)-N(19)	121.7(3)
O(19)-C(19)-C(18)	122.8(3)
N(19)-C(19)-C(18)	115.4(3)
C(19)-N(19)-O(20)	118.9(3)
C(19)-N(19)-H(19N)	123.1(19)
O(20)-N(19)-H(19N)	116.4(19)
N(19)-O(20)-H(20O)	106(3)

Torsion angles [°] for (139)

C(6)-C(1)-C(2)-C(3)	-0.1(5)
C(7)-C(1)-C(2)-C(3)	179.3(3)
C(1)-C(2)-C(3)-C(4)	-0.3(6)
C(2)-C(3)-C(4)-C(5)	0.3(6)
C(3)-C(4)-C(5)-C(6)	0.1(6)
C(2)-C(1)-C(6)-C(5)	0.5(5)
C(7)-C(1)-C(6)-C(5)	-178.9(3)
C(4)-C(5)-C(6)-C(1)	-0.5(6)
C(6)-C(1)-C(7)-C(8)	74.2(4)
C(2)-C(1)-C(7)-C(8)	-105.2(4)
C(1)-C(7)-C(8)-C(9)	-74.1(4)
C(7)-C(8)-C(9)-O(9)	-55.3(4)
C(7)-C(8)-C(9)-N(9)	125.6(3)
O(9)-C(9)-N(9)-O(10)	3.1(5)
C(8)-C(9)-N(9)-O(10)	-177.8(2)
C(16)-C(11)-C(12)-C(13)	-1.1(5)
C(17)-C(11)-C(12)-C(13)	177.1(3)
C(11)-C(12)-C(13)-C(14)	-0.2(5)
C(12)-C(13)-C(14)-C(15)	0.5(5)
C(13)-C(14)-C(15)-C(16)	0.4(5)
C(14)-C(15)-C(16)-C(11)	-1.8(5)
C(12)-C(11)-C(16)-C(15)	2.1(5)
C(17)-C(11)-C(16)-C(15)	-176.1(3)
C(12)-C(11)-C(17)-C(18)	28.3(5)
C(16)-C(11)-C(17)-C(18)	-153.5(3)
C(11)-C(17)-C(18)-C(19)	62.4(4)
C(17)-C(18)-C(19)-O(19)	59.8(4)
C(17)-C(18)-C(19)-N(19)	-120.9(3)
O(19)-C(19)-N(19)-O(20)	-5.4(4)
C(18)-C(19)-N(19)-O(20)	175.3(3)

4-(trifluoromethyl)phenylacetohydroxamic acid (141)



Crystal data and structure refinement for (141)			
Empirical formula	C ₉ H ₈ F ₃ NO ₂	Index ranges	-32 ≤ h ≤ 42, -6 ≤ k ≤ 4, -11 ≤ l ≤ 11
Formula weight	219.16	Reflections collected	5651
Temperature	93(2) K	Independent reflections	1697 [R(int) = 0.1554]
Wavelength	1.54178 Å	Completeness to theta = 25.00°	98.7 %
Crystal system	Monoclinic	Absorption correction	Multiscan
Space group	C2/c	Max. and min. transmission	1.0000 and 0.9676
Unit cell dimensions	a = 35.291(7) Å, α = 90° b = 5.6375(11) Å, β = 95.27(3)° c = 9.4844(19) Å γ = 90°	Refinement method	Full-matrix least-squares on F ²
Volume	1878.9(6) Å ³	Data / restraints / parameters	1697 / 2 / 145
Z	8	Goodness-of-fit on F ²	1.046
Density (calculated)	1.550 mg/m ³	Final R indices [I > 2σ(I)]	R1 = 0.1194, wR2 = 0.3016
Absorption coefficient	0.148 mm ⁻¹	R indices (all data)	R1 = 0.1947, wR2 = 0.3619
F(000)	896	Absolute structure parameter	N/A
Crystal size	0.3000 x 0.1000 x 0.0100 mm ³	Extinction coefficient	N/A
Theta range for data collection	2.32 to 25.40°	Largest diff. peak and hole	0.439 and -0.425 e.Å ⁻³

Bond lengths [Å] and angles [°] for (141)

C(1)-C(6)	1.384(10)
C(1)-C(2)	1.397(9)
C(1)-C(7)	1.523(9)
C(2)-C(3)	1.375(9)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.386(10)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.401(9)
C(4)-C(9)	1.482(10)
C(5)-C(6)	1.360(10)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.503(9)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-O(8)	1.248(8)
C(8)-N(8)	1.317(9)
N(8)-O(9)	1.398(7)
N(8)-H(8N)	0.980(3)
O(9)-H(9O)	0.980(3)
C(9)-F(1)	1.323(9)
C(9)-F(2)	1.326(8)
C(9)-F(3)	1.366(9)
C(6)-C(1)-C(2)	118.6(6)
C(6)-C(1)-C(7)	120.9(6)
C(2)-C(1)-C(7)	120.5(6)
C(3)-C(2)-C(1)	121.1(7)
C(3)-C(2)-H(2A)	119.4
C(1)-C(2)-H(2A)	119.4
C(2)-C(3)-C(4)	119.5(6)
C(2)-C(3)-H(3A)	120.2
C(4)-C(3)-H(3A)	120.2
C(3)-C(4)-C(5)	119.5(6)
C(3)-C(4)-C(9)	121.8(6)
C(5)-C(4)-C(9)	118.6(7)
C(6)-C(5)-C(4)	120.3(6)
C(6)-C(5)-H(5A)	119.9
C(4)-C(5)-H(5A)	119.9
C(5)-C(6)-C(1)	121.0(6)
C(5)-C(6)-H(6A)	119.5
C(1)-C(6)-H(6A)	119.5
C(8)-C(7)-C(1)	114.8(6)
C(8)-C(7)-H(7A)	108.6
C(1)-C(7)-H(7A)	108.6
C(8)-C(7)-H(7B)	108.6
C(1)-C(7)-H(7B)	108.6
H(7A)-C(7)-H(7B)	107.5
O(8)-C(8)-N(8)	123.5(6)
O(8)-C(8)-C(7)	122.8(6)
N(8)-C(8)-C(7)	113.7(6)
C(8)-N(8)-O(9)	122.2(6)
C(8)-N(8)-H(8N)	122(3)
O(9)-N(8)-H(8N)	115(3)
N(8)-O(9)-H(9O)	94(6)

F(1)-C(9)-F(2)	107.4(6)
F(1)-C(9)-F(3)	105.6(6)
F(2)-C(9)-F(3)	104.3(6)
F(1)-C(9)-C(4)	113.7(7)
F(2)-C(9)-C(4)	114.0(5)
F(3)-C(9)-C(4)	111.1(6)

Torsion angles [°] for (141)

C(6)-C(1)-C(2)-C(3)	1.5(10)
C(7)-C(1)-C(2)-C(3)	-177.7(6)
C(1)-C(2)-C(3)-C(4)	-0.1(11)
C(2)-C(3)-C(4)-C(5)	-0.2(10)
C(2)-C(3)-C(4)-C(9)	177.9(7)
C(3)-C(4)-C(5)-C(6)	-1.0(10)
C(9)-C(4)-C(5)-C(6)	-179.2(7)
C(4)-C(5)-C(6)-C(1)	2.6(10)
C(2)-C(1)-C(6)-C(5)	-2.8(10)
C(7)-C(1)-C(6)-C(5)	176.5(6)
C(6)-C(1)-C(7)-C(8)	118.0(7)
C(2)-C(1)-C(7)-C(8)	-62.8(9)
C(1)-C(7)-C(8)-O(8)	-40.8(9)
C(1)-C(7)-C(8)-N(8)	139.1(6)
O(8)-C(8)-N(8)-O(9)	-1.6(10)
C(7)-C(8)-N(8)-O(9)	178.5(5)
C(3)-C(4)-C(9)-F(1)	8.0(10)
C(5)-C(4)-C(9)-F(1)	-173.9(6)
C(3)-C(4)-C(9)-F(2)	131.5(7)
C(5)-C(4)-C(9)-F(2)	-50.4(9)
C(3)-C(4)-C(9)-F(3)	-111.0(8)
C(5)-C(4)-C(9)-F(3)	67.1(8)
