Synthesis and Evaluation of Tetraazamacrocycles as Antiparasitic Agents

Caroline Mary Reid MSci

Thesis submitted in part fulfilment of the requirements for the Degree of Doctor of Philosophy

Supervisor: Prof. D. J. Robins

Department of Chemistry

Physical Sciences Faculty

UNIVERSITY
of
GLASGOW

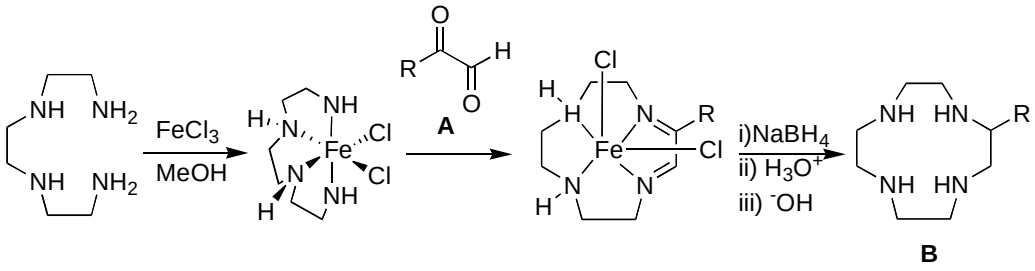
November 2006

Abstract

Human African Trypanosomiasis (HAT), commonly known as Sleeping Sickness, is endemic in over 36 countries in sub-Saharan Africa. It is caused by the parasitic subspecies *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, which is transmitted to humans by the tsetse fly. The World Health Organisation estimates that 0.5 million people are currently infected with the disease, with a further 60 million at risk. HAT is lethal if left untreated and there is no vaccine available. There are only four accessible drugs, which are all inadequate and highly toxic. Thus there is a vital need for novel anti-parasitic agents.

Compounds interfering with polyamine biosynthesis or function have shown potential use as anti-cancer, anti-HIV, anti-fungal and antiparasitic agents. The overall aim of this work was to synthesise novel macrocyclic polyamines containing different substituents to increase the toxicity against *T. brucei*.

Twenty racemic substituted tetraazamacrocycles **B** have been synthesised using an iron template method in good yields (54-100%) from triethylenetetraamine and aromatic glyoxal derivatives **A**. The R-groups were aromatic or heteroaromatic and were selected to give compounds with varying electronic demand and a broad range of log P values. Two derivatives contain parasite-specific recognition motifs were also prepared. The compounds were tested against *T. brucei* and several of the analogues displayed high activity.



Some of these azamacrocycles were also tested for activity against the malarial parasite *Plasmodium falciparum,* and for oligopeptidase B (OPB) inhibition, with a number of compounds exhibiting promising results.

Acknowledgements

This thesis is dedicated to my big sisters Sarah and Louise Reid.

"*Sisters, sisters, there were never such devoted sisters*"

Firstly I would like to thank my supervisor Prof. Robins for all his encouragement and guidance throughout this project, and also to Dr Andy Sutherland for his help and advice especially when the chemistry became trying!

The support received from other members of staff in the Chemistry Department has been second to none, and in particular I would like to mention Jim Gall, Dr David Rycroft and Dr David Adams (NMR spectroscopy), Jim Tweedy (mass spectroscopy), Arlene Douglas (IR spectroscopy) and Stuart Mackay (IT). Josie, you brighten up the C wing!

I thoroughly enjoyed being involved with and carrying out the biological evaluation of my compounds. A special mention goes to Dr Mike Barrett and members of his group Mhairi Stewart, Charles Ebikeme and Matt Gould for assistance throughout the trypanosome testing. Thanks also to Prof. Sylke Muller and Eva-Maria Patzewitz (malaria), Prof. Ian Gilbert (trypanosome) and Nick Bland (OBP).

I would also like to express my love and gratitude to my friends and work colleagues: Kathryn, Carolyn, Jenny, Rosie, Andy, Kate, Louise, Dave, Ally, Iain and Cameron, as well as the current and previous members of the Robins, Hartley and Sutherland research groups. It's been a ball!

To all the members of my church housegroup: your prayers and friendship are priceless. Mum, thanks for all your love and cards! I always knew your were worrying with me every step of the way.

To my Stephen – I hope there will always be chemistry between us…

"I can do everything through Him who gives me strength." Philippians 4:13

Preface

This thesis represents the original work of Caroline Mary Reid unless explicitly stated otherwise in the text. The research was carried out at the University of Glasgow in the Henderson and Loudon Laboratories under the supervision of Prof. David J. Robins and Dr Andrew Sutherland during the period of October 2003 to September 2006.

Abbreviations

Å	Ångstrom
AcOH	acetic acid
abs.	absolute
N'-AcSpd	N^1-acetyl spermidine
N'-AcSpm	N^1-acetyl spermine
ALDH	aldehyde dehydrogenase
Ar	aryl
ATP	adenosine triphosphate
aq	aqueous
B.C.	before Christ
Bn	benzyl
Boc	*tert*-butoxycarbonyl
br	broad
Bu	butyl
CBSS	Carter's Balanced Salt Solution
CI	chemical ionisation
CNS	central nervous system
cyclen	1,4,7,10-tetraazacyclododecane [2,2,2,2]
conc	concentrated
d	days (or doublet in NMR spectral data)
dd	doublet of doublets
DAPI	4'-6-diamidino-2-phenylindole
dba	bisdibenzylideneacetone
dcSAM	decarboxylated *S*-adenosylmethionine
DFMO	DL-α-difluoromethylornithine
DIBAL-H	diisobutylaluminium hydride
DIC	differential interference contrast
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-(dimethylamino)-pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribose nucleic acid
dt	doublet of triplets
EATRO	East African Trypanosomiasis Research Organisation
EI	electron impact ionisation
est.	estimate
Et	ethyl
EtOH	ethanol
eq	equivalents
FAB	fast atom bombardment
FDA	Food and Drug Administration
FITC	fluorescein-5-isothiocyanate
Fmoc	9-fluorenylmethoxycarbonyl
g	gram
h	hours
HAT	Human African Trypanosomiasis
HDM2	Human Double Minute gene 2
HEK	Human Embryonic Kidney
HIV	Human Immunodeficiency Virus
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry

Hz	Hertz
IC_{50}	concentration of a drug to inhibit 50% of enzyme activity
ICR	Institute for Cancer Research
IR	infra red
J	NMR spectra coupling constant
kDa	kilodalton
kg	kilogram
K_m	binding constant of P2 transporter
L	litre (or ligand in TETA complex with $FeCl_3$)
µL	microlitre
LEC	lowest effective concentration
LiHMDS	lithium hexamethyldisilazane
lit.	literature
LRMS	low resolution mass spectrometry
m	multiplet
M	molar (or metal in TETA complex with $FeCl_3$)
µm	micromole
µM	micromolar
λ_{max}	maximum ultraviolet-visible absorbance
ν_{max}	maximum velocity of adenosine uptake
MDM2	Mouse Double Minute gene 2
Me	methyl
Mel B	melarsoprol
MeOH	methanol
mg	milligram
MGBG	methyl glyoxal bis(guanylhydrazine)
MHz	mega Hertz
mmol	millimole
mM	millimolar
mL	millilitre
MOM	methoxymethyl
mp	melting point
Ms	methanesulfonyl
MSF	Médecins Sans Frontièrs
MTA	1,5'-methylthioadenosine
ng	nanogram
nm	nanomole
NMR	nuclear magnetic resonance
NMRI	Naval Medical Research Institute
OPB	oligopeptidase B
ODC	ornithine decarboxylase
P	partition coefficient
p53	p53 protein
p53	p53 gene
Ph	phenyl
PO	polyamine oxidase
ppm	parts per million
q	quartet
QSAR	Qualitative Structure-Activity Relationships
QY	catalyst
R_f	retention factor
RHOD	rhodamine
RNA	ribose nucleic acid
RPE	retinal pigment epithelial

ROS	reactive oxygen species
rt	room temperature
s	singlet
SAM	decarboxylated *S*-adenosylmethionine
SAMDC	*S*-adenosylmethionine decarboxylase
SDS-PAGE	sodium dodecyl sulfate poly-acrylamide gel electrophoresis
SpdSyn	spermidine synthase
SpmSyn	spermine synthase
STIB	Swiss Tropical Institute, Basel
t	temperature (or triplet in NMR spectral data)
TBDMSCl	*tert*-butyldimethylsilyl chloride
TEBACl	triethylbenzylammonium chloride
TETA	triethylene tetraamine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSI	trimethylsilyl iodide
tosyl	*para*-toluenesulfonyl
Tr	trityl
TR	trypanothione reductase
Ts	*para*-toluenesulfonyl
VSG	variant surface glycoprotein
UNC	University of North Carolina
UV	ultraviolet
WHO	World Health Organisation
XPhos	2-dicyclohyxylphosphino-2’-4’-6’-triisopropylbiphenyl

Table of Contents

1 Human African Trypanosomiasis 11

1.1 Human African Trypanosomiasis (HAT) 11
1.1.1 HAT Transmission 11
1.1.2 HAT History 12
1.1.3 Current Situation of HAT 12
1.2 The Trypanosome 13
1.3 Trypanosome Life Cycle 14
1.4 Disease and Diagnosis 15
1.5 Prevention and Control 16
1.6 Treatment 17
1.6.1 Suramin 17
1.6.2 Pentamidine 18
1.6.3 Melarsoprol 19
1.6.4 DL-α-Difluoromethylornithine 21
1.6.5 Additional Drugs 22
1.7 Drug Uptake by Trypanosomes 23
1.7.1 Trypanosome Transporters 23
1.7.2 Non-carrier-mediated uptake 27
1.8 Drug Resistance 27
1.8.1 Molecular Basis of Drug Resistance 28
1.8.2 Mechanisms of Drug Resistance 29
1.9 Future of HAT Treatment 30

2 Polyamine Metabolism 32

2.1 Functions of Polyamines 32
2.2 Polyamine History: Discovery and Elucidation 32
2.3 Polyamine Biosynthesis 33
2.3.1 Mammalian Polyamine Biosynthesis 33
2.3.2 Trypanosome Polyamine Biosynthesis 35
2.4 Polyamine Metabolism as a Therapeutic Target 36
2.4.1 Polyamine Metabolism as a Target for Cancer Chemotherapy 37
2.4.2 Polyamine Metabolism as a Target for Trypanocide Design 40
2.5 Previous Approaches Towards Antiparasitic Agents within the Robins Group 41

3 Synthesis and Evaluation of Tetraazamacrocycles as Antiparasitic Agents 43

3.1 Aims of this Work 43
3.2 Synthesis of 1,4,7,10-Tetraazacyclododecane (Cyclen) 43
3.2.1 Richman Atkins 44
3.2.2 Phase Transfer Conditions 45
3.2.3 Facile Route Utilising Bis-imidazoline 47
3.2.4 Tosylation of Cyclisation Precursors 49
3.2.5 Tosyl Deprotection Strategies 50
3.3 General Strategy Towards *C*-Functionalised Derivatives of Cyclen 52
3.3.1 Efficient Metal-Templated Synthesis 52
3.3.2 Synthesis of 1,2-Dicarbonyl Compounds 53
3.4 Synthesis and Biological Evaluation of *C*-Functionalised Derivatives of Cyclen 55

3.4.1 Synthesis of First Library *55*
3.4.2 Biological Evaluation of 66 - 72 ..... *56*
3.4.3 Synthesis of Further Analogues *58*
3.4.4 Biological Evaluation of 84 - 92 ..... *59*
3.4.5 Unsuccessful Syntheses *61*
3.5 Synthesis of Parasite-Specific Analogues 63
3.5.1 Melamine Derivative *63*
3.5.2 Benzamidine Derivative *67*
3.5.3 Guanidine Derivative *76*
3.5.4 Biological Evaluation of Parasite-Specific Analogues *77*
3.6 Summary of Synthetic Achievements 78
3.7 Biological Evaluation 79
3.7.1 Trypanosoma brucei *81*
3.7.2 Human Embryonic Kidney (HEK) Cells *81*
3.7.3 Plasmodium falciparum *(Malaria)* *82*
3.7.4 Oligopeptidase B (OPB) *82*
3.7.5 Fluorescence *83*
3.7.6 Polyamine Investigation *85*
3.8 Conclusion and Future Work 86

4 Synthesis of Enantiomerically Enhanced Tetraazamacrocycles 88

4.1 Introduction 88
4.2 Aims of this Work 88
4.3 Synthesis of Enantiomerically Enhanced Substituted Tetraazamacrocycles 88
4.3.1 Route I *88*
4.3.2 Route II *90*
4.3.3 Route III *92*
4.3.4 Route IV *93*
4.3.5 Removal of Tosyl Groups *95*
4.4 Conclusions and Future Work 95

5 Anti-Cancer Alkylating Agents 97

5.1 Cancer 97
5.2 Cancer Statistics 97
5.3 Cancer Therapy 97
5.3.1 Surgery *98*
5.3.2 Radiation Therapy *98*
5.3.3 Biotherapy *98*
5.3.4 Chemotherapy *98*
5.4 Alkylating Agents 98
5.4.1 Mustard Agents: Chemical Warfare to Chemotherapy *99*
5.4.2 Mechanism of Action *100*
5.4.3 Nitrogen Mustard Anti-Cancer Agents *102*
5.5 Development of *N*-Mustards within the Robins Group 103
5.6 Aim of this Work 106
5.7 Progress Towards Substituted Tetraazamacrocyclic *N*-Mustard Derivatives 107
5.7.1 N-*Hydroxyethylation of Cyclen* *107*
5.7.2 N-*Mustard Derivatives* *108*
5.7.3 N-*Hydroxyethylation of Racemic 2-Phenyl-1,4,7,10-Tetraazacyclododecane 66* *109*

5.8 Conclusions and Future Work 113

6 Synthesis of Pyrazolidine-3,5-diones for the Treatment of Cancer 115

6.1 Cancer-Causing Genes 115
6.1.1 Oncogenes 115
6.1.2 Suicide Genes 115
6.1.3 DNA-Repair Genes 115
6.1.4 Tumour Suppressor Genes 115
6.2 p53: “Guardian of the Genome” 116
6.3 p53-HDM2 as a Drug Target 118
6.3.1 Small Molecule HDM2 Antagonists 119
6.3.2 Small Molecule HDM2 Ubiquitin Ligase Inhibitors 121
6.4 Aim of this Work 122
6.5 Synthesis and Evaluation of Pyrazolidine-3,5-diones 122
6.5.1 Preparation of 1-phenylpyrazolidine-3,5-dione 241 123
6.5.2 Preparation of 4-(5-Bromo-2-furfurylidene)-1-phenyl-pyrazolidine-3,5-dione 240 125
6.5.3 Preparation of 4-(5-Bromo-2-furfurylidene)-1-phenyl-pyrazolidine-3,5-dione Analogues 126
6.5.4 Biological Evaluation of Library 128
6.6 Conclusion and Future Work 128

7 Experimental 129

7.1 General Experimental Details 129
7.2 Experimental to Chapter 3 129
7.2.1 General Procedures (3A – 3D) 129
7.2.2 Experimental Details 131
7.2.3 Biological Materials and Methods for Chapter 3 164
7.3 Experimental to Chapter 4 168
7.3.1 Experimental Details 168
7.4 Experimental to Chapter 5 181
7.4.1 Experimental Details 181
7.5 Experimental to Chapter 6 184
7.5.1 General Procedure (6A) 184
7.5.2 Experimental Details 184
7.5.3 Biological Materials and Methods for Chapter 6 190

8 References 191

1 Human African Trypanosomiasis

1.1 Human African Trypanosomiasis (HAT)

HAT, also known as sleeping sickness, is a vector-borne parasitic disease that is endemic in 36 countries in sub-Saharan Africa (see Figure 1). The World Health Organisation (WHO) estimate that over 60,000 people die annually from sleeping sickness, although many more infections and deaths go unreported.[1] Half a million people are currently infected and 60 million are at daily risk. HAT is one of the major factors constraining Africa's social and economic development. A subsistence farmer speaking at the 28th meeting of the International Scientific Council for Trypanosomiasis Research and Control, summed up the HAT problem: "*My child is dying of malaria, but it is African trypanosomiasis that is killing us*."[2]

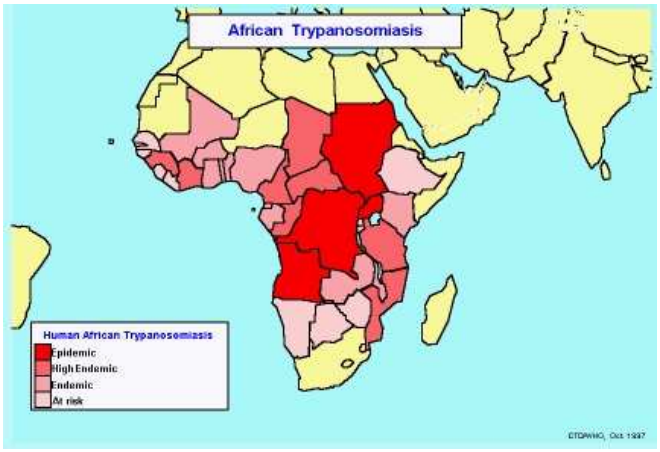


Figure 1

1.1.1 HAT Transmission

The parasites, named trypanosomes, are transmitted to humans and animals through the bite of the tsetse fly of the genus *Glossina* (Figure 2). Tsetse flies inhabit the vast savannah across sub-Saharan Africa, breeding in warm humid areas of vegetation along rivers, lakes, forests and scrubland.[1,3] Man, cattle and wild animals act as reservoirs of the parasite, and the tsetse fly feeds on their blood. The disease can be passed on in other ways; parasites can cross the placenta and infect the foetus causing abortion or perinatal death. Accidental infections in laboratories through handling infected blood are uncommon, and there is only one reported case of sexually transmitted sleeping sickness.[4]

Figure 2

1.1.2 HAT History

Trypanosomiasis was first recorded in the 14th century in the country of Mali. However the destructive force of this disease was not recognised until an epidemic in Uganda and the Congo Basin (1896-1906) killed half a million people.[1] The parasite was identified by two English doctors in 1902, Ford and Dutton, working in the Gambia, and the tsetse fly was recognised as the vector by Sir David Bruce (after whom *T. brucei* are named) in 1903.[5] There have been another two major epidemics in Africa since then, one affecting several countries in 1920, and one in 1970, which is still progressing today.[1]

1.1.3 Current Situation of HAT

Sleeping sickness is a daily threat, affecting rural and remote areas where the population is dependent on agriculture, fishing and hunting. These areas generally have little or no available health system, and the disease also follows social and economic problems such as poverty, displacement of population, political instability and war. The disease has a devastating impact on these areas by decreasing the labour force and hampering work capacity and production.[6] The disability adjusted life years lost due to sleeping sickness are two million.[7] In countries such as Sudan and Angola, the ability to deal with the epidemic situation of the disease has been surpassed, and the prevalence of the disease is greater than 20% of the population.[6]

Other subspecies of the parasite cause animal trypanosomiasis, for example domestic cattle suffer from Nagana (Figure 3). Nagana can cause a 25% decrease in milk production, a 20% decrease in calving as well as approximately three million livestock deaths per year, which also has a devastating effect on the community.[8]

Figure 3

1.2 The Trypanosome

Trypanosomes are protozoa of the family Trypanosomatidiae. The genus *Trypanosoma* may be divided into two groups, the stercoraria and the salivaria, which are both infective parasites (Figure 4). The stercoraria include the species *T. cruzi* that causes South American trypanosomiasis known as Chagas' disease. The salivarian group includes *T. brucei gambiense* and *T. brucei rhodesiense*, which are parasites of the subgenus *Trypanozoon*.[9]

Subkingdom	Protozoa						
Phylum	Sarcomastigophora						
Subphylum	Mastigophora						
Class	Zoomastigophora						
Order	Kinetoplastida						
Suborder	Trypanosomatina						
Family	Trypanosomatidiae						
Genus	*Trypanosoma*						
	Stercoraria			*Salivaria*			
Subgenus	*Herpetosoma*	*Megatrypanum*	*Schizotrypanum*	*Duttonella*	*Nannomonas*	*Trypanozoon*	*Pycnomonas*
Species	*T. (H.) lewisi* *T. (H.) musculi* *T. (H.) microti*	*T. (M.) theileri* *T. (M.) melophagium*	*T. (S.) cruzi* *T. (S.) dionisii*	*T. (D.) vivax* *T. (D.) uniforme*	*T. (N.) congolense* *T. (N.) simiae*	*T. (T.) equiperdum* *T. (T.) evansi* *T. (T.) b. brucei* *T. (T.) b. gambiense* *T. (T.) b. rhodesiense*	*T. (P.) suis*

Figure 4

There are two human forms of sleeping sickness, a chronic form and an acute form. The chronic form of the disease is caused by *T. brucei gambiense* and it affects Western and Central Africa with the disease lasting up to several years. Acute sleeping sickness, caused by *T. brucei rhodesiense*, is found in countries of Eastern and Southern Africa and lasts several weeks. Both forms if left untreated lead to death.[1,6] The two species are morphologically indistinguishable, and their body length range is 14-33 μm (Figure 5).[10]

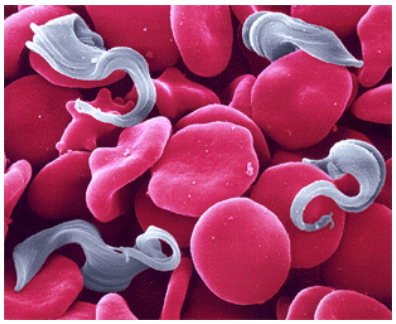


Figure 5

1.3 Trypanosome Life Cycle

During a blood meal, metacyclic trypomastigotes are injected into the host's skin by the fly. The parasites enter the lymphatic system and pass to the bloodstream. In the blood, the parasites are transformed into bloodstream trypomastigotes. These are replicated and are carried to other sites in the body, reaching other blood-fluids such as spinal and lymph fluid. A tsetse fly becomes infected with bloodstream trypomastigotes by taking a blood meal, and the parasites then transform into procyclic trypomastigotes in the fly's midgut. These proliferate *via* binary fission, leave the midgut and then convert into epimastigotes, which reach the fly's salivary glands. It is at this stage that the cycle begins again, with the cycle in the fly taking around three weeks (see Figure 6).[10]

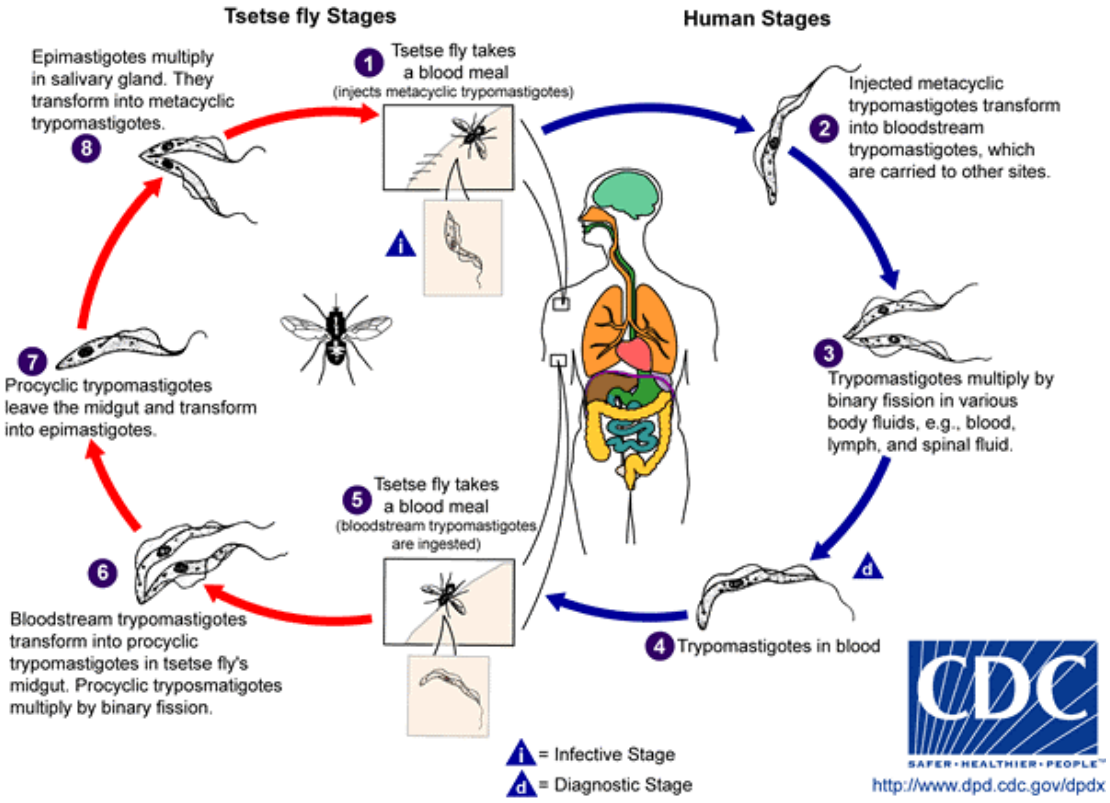


Figure 6

1.4 Disease and Diagnosis

The symptoms of first stage HAT are non-specific; fever, headaches, weakness, joint pain and itching. The parasite also invades the lymph system causing lymph nodes to swell up. Gradually the immune system is exhausted and the symptoms become more pronounced; anaemia, cardiovascular, endocrine and kidney disorders, abortion and oedema appear.[1] The parasite will then invade the central nervous system (CNS) and second stage symptoms begin. Depending on acute or chronic infection this can take as little as a few weeks, or several years after initial inoculation. At this time, the patients experience severe neurological and psychiatric problems. They lose the ability to concentrate and are prone to unpredictable and sudden mood swings, leading to confusion, violence and lethargy. Due to this torpor, eating, speaking and walking becomes a tremendous effort. One of the disease's most prominent symptoms is the inability to sleep at night, but exhaustion and times of sleep-like unconsciousness during the day. Eventually the disease leads to coma and death.[1]

Diagnosis is dependent on locating trypanosomes within the blood, lymphatic or cerebrospinal fluids of the patients. Early diagnosis of patients is rare due to the non-severe early symptoms plus sparse and inadequate health care. Therefore prognosis of the disease

is normally when the parasite has already invaded the CNS and the patient is displaying symptoms. The parasitaemia can be very low and difficult to detect and so techniques are used to concentrate (centrifugation or mini anion-exchange) and find parasites. Another way to detect infection is by measuring the presence of above normal levels of leukocytes (>5 cells/mL) or protein (>37 mg/100mL) within the CNS, or by the detection of low red blood cell count or by carrying out antibody and antigen tests.[9] These methods are invasive and difficult to use in resource-poor centres. Highly trained staff are also required for accurate results.

1.5 Prevention and Control

The WHO has set up a programme with three parts to give the most effective approach to sleeping sickness control. Of the 36 countries in which HAT is endemic, 22 are actively involved in this programme.[1]

The first and most crucial part is mobile medical monitoring with specialised staff using effective diagnostic equipment. Case-by-case screening is not practically possible in highly endemic regions and so systematic screening within identified foci is the best approach. Patients attend centres to determine what stage of the disease they are at, and what treatment is best. Patients then attend post-therapy follow-up care.[1]

Secondly, the availability of fixed-post medical services at hospitals, health centres and dispensaries is important, where blood samples are taken before analysis at reference centres.[1]

Finally, the most cheap and ecologically accepted method is vector control. In theory this is the most effective as the female flies only produce ten larvae in their short life span. Spraying the tsetse's habitat with insecticide from the ground and air, and destroying bushland are traditional schemes. Another way is to bait the flies using livestock that are sprayed with insecticide. These methods however require commitment and financial backing from the local government and community, which cannot be guaranteed in all areas. The most simple and effective form of control is using nets and traps for the flies.[1]

The epidemic in 1920 sparked the employment of control campaigns, mobile health teams and administration structures that screened millions of people at risk. The drug pentamidine was used as a preventative, with each person receiving one injection as it was wrongly thought that a low dose of this drug gave six months protection from the disease.

By the start of African independence in the 1960s, the disease had almost disappeared. However, after the initial success, effective monitoring ceased and so the disease reappeared in endemic form.[1]

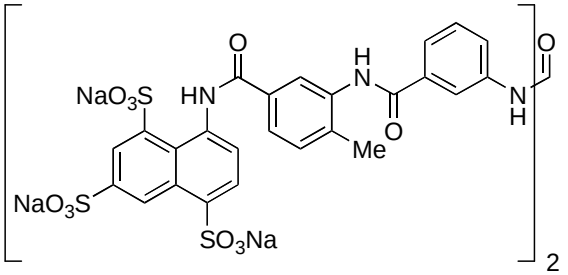
1.6 Treatment

An editorial in *Science* titled "*Trypanosomes at the Gates*" highlights that *T. brucei* is one of the species that causes one of the most major region-specific human diseases.[11] The disease has been studied for over a century but it still relies upon some of the most highly toxic and inadequate drugs. These drugs were invented almost 100 years ago! The reason for this is simple; the victims of the disease being among the world's poorest people, and there has been a lack of investment by the pharmaceutical industry.[12]

There is no vaccine against HAT due to an immuno-protective process using antigenic variation to protect the parasite. Trypanosomes have a variable surface glycoprotein (VSG) coat on their surface. The VSG has over 1000 different genes encoding for proteins, and it undergoes constant antigenic variation in order to evade the host's immune systems.[13] However, there are a handful of drugs available but they all come with serious side effects and drug resistance is on the increase. Four drugs are currently licensed to treat HAT which are all stage-specific.[5,14]

1.6.1 Suramin

Developed in 1916 by Oskar Dressel as a by-product in the German dye company Bayer, suramin **1** is a colourless polysulfonated symmetrical naphthalene derivative. It was first used an anti-sleeping sickness drug in 1922 after it had been observed that a number of naphthalene dye substances had trypanosomidal activity.[15] Suramin is a highly charged compound, containing six negative charges at physiological pH, and so it does not cross the blood-brain barrier. Suramin inhibits many enzymes by electrostatic interactions: For example, it inhibits reverse transcriptase, a key enzyme of the Human Immunodeficiency Virus (HIV).[16] The drug has also been tested against a variety of cancers, where it has been used in clinical trials against hormone-refractory prostate cancer.[17] However, suramin's mode of action has not been elucidated, although some suggestions are that it acts using an anti-angiogenesis effect along with interaction with growth factor receptors.[18,19]

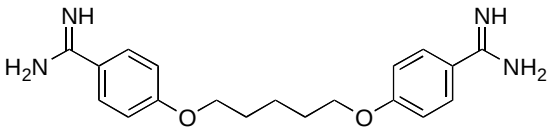


1

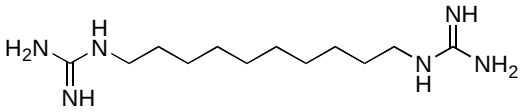
Suramin **1** is used in early stage HAT and it the preferred drug for use in East Africa. It is used in West Africa for the treatment of onchoceriasis (River blindness) and not for sleeping sickness. The drug is administered in 20 mg per kg body weight doses to patients by intravenous injection, and it circulates the blood associated with low-density lipoproteins and serum albumin. It is then taken up slowly by the parasite and host *via* receptor mediated endocytosis.[5]

1.6.2 Pentamidine

Pentamidine **2** is from a class of drugs called the diamidines, which were developed during the 1930s from the lead compound synthalin **3** which is a hypoglycaemic agent.[20] Pentamidine, also known as lomidine, is an aromatic diamidine which works directly against the parasites independent of the host. This drug, like suramin, is also used to treat early stage HAT because it doesn't cross the blood-brain barrier. It is active against *T. brucei gambiense* and it shouldn't be used against *T. brucei rhodiense* due to primary resistance in many areas of Africa.[5] Pentamidine is also used against antimony refractory leishmania and *Pneumocystis carinii* pneumonia.[12] The mode of action of diamidines has not been established, although their amphipathic character with charge and hydrophobicity makes them very like polyamines as they have the ability to interact with negatively charged intracellular components.[20] The interaction of pentamidine with intercatenated circular DNA molecules is extremely close, and these DNA units make up the mitochondrial genome of all kinetoplastid flagellates.[12] A number of alternative cellular targets have been proposed, however as the drug concentrates within the cells to mM levels, it is thought that its toxicity may arise simply by the inhibition of many cellular targets.[12] The recommended dose is seven intramuscular injections daily at 4 mg/kg body weight.[5]



2

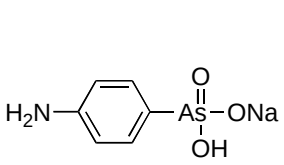


3

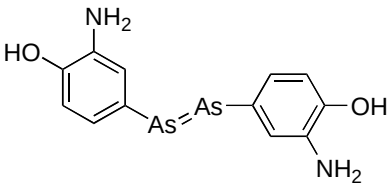
1.6.3 Melarsoprol

Despite being known as "the king of poisons", arsenic-containing compounds have been used as therapeutics since 2000 B.C. when arsenic trioxide (As_2O_3) was used as a drug and a poison in the Far East and India. In 400 B.C. Hippocrates and Aristotle administered orpiment (As_2S_3), and realger (AsS) as a treatment for ulcers. From the late 18th century until the 1950s, arsenic was used in Fowler's solution (1% $KAsO_2$) in Europe and America as a remedy for asthma, syphilis, rheumatism, skin disorders and was the first chemotherapeutic drug against leukaemia.[21,22]

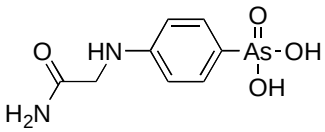
Thomas and Breinl observed in 1905 the trypanolytic activity of the aromatic arsenical atoxyl **4**. Paul Ehrlich developed the first clinically effective arsenical for the treatment of syphilis in 1910. This drug, named salvarsan **5**, or '606', is often considered to be *the* prototypic chemotherapeutic agent.[23] At this time, Ehrlich wrongly believed that trypanosomes and the venereally transmitted *T. equiperdum* (a spirochaete) were related. Tryparsomide **6**, developed in 1919 by Jacobs and Heidelberger, was the first clinically effective arsenical against HAT.[20]



4

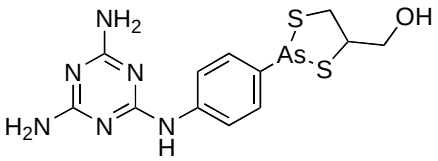


5



6

Freidheim's melaminophenyl arsenicals were developed in the 1940s, culminating with melarsoprol **7** (arsobal, Mel B) in 1947,[24] which is a drug that remains today as the most widely used trypanoside.[20] However, Mel B often has lethal side effects. A doctor working for Médecins Sans Frontièrs (MSF) in Uganda states, "*Melarsoprol is a terrible drug – you don't feel proud injecting it. It is caustic, it burns, and you don't know if you are going to save your patient or kill him*".[3]



7

Melarsoprol is very effective in the treatment of both early and late stages of HAT in both the Eastern and Western countries of Africa, against both the acute and chronic forms of the infection. The treatment consists of several series of injection using 3.6 mg/kg, with each series separated by at least a week.[5] However Mel B is only ever used in late stages of the disease due to its risks. Treatment using this drug is linked with acute, reactive encephalopathy (affects functions of the brain) in 5-10% of the patients, which results in paralysis, brain damage and in some cases (10-70%) death.[1,5]

Again, it is not fully understood how Mel B **7** or other arsenicals are toxic against trypanosomes, although it is understood that the drug is accumulated in the parasite using an unusual amino-purine transporter.[25] The loss of this transporter leads to drug resistance (see Chapter 1.8).[20,25] The trivalent oxidation state is more toxic than the pentavalent state, and trivalent arsenicals react aggressively and reversibly with vicinal dithiols in proteins and therefore, a number of proteins are inhibited by these compounds (Figure 7).[20]

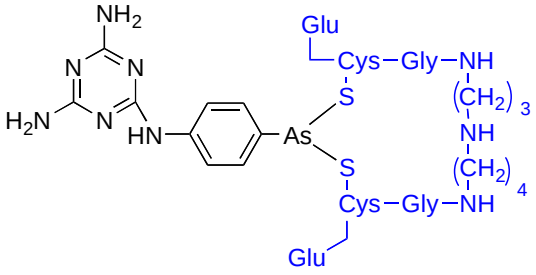
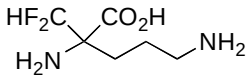


Figure 7

When treated with Mel B *in vitro*, the parasite quickly loses its motility, which is rapidly followed by lysis, resulting in cell death. It would therefore seem that Mel B has an effect on the cell's metabolism and membrane integrity.[20] Successful trials against leukaemia and myeloma using melarsoprol *in vitro* reached clinical trials although unfortunately the same reactive encephalopathy was observed.[26,27]

1.6.4 DL-α-Difluoromethylornithine

DL-α-Difluoromethylornithine **8** (DFMO), also known as eflornithine, is the only new drug to be developed in the last fifty years. Developed in the 1970s by Albert Sjoerdsmanot with clinical trials in the 1980s, DFMO is known as the 'resurrection drug' due to its effectiveness against late stage *T. brucei gambiense* infections that are resistant to Mel B.[28] Comatosed patients have been known to wake up rapidly and resume their usual activities on DFMO injection.



8

DFMO is not recommended as a treatment against the acute form of the disease as many *T. brucei rhodiense* are naturally resistant strains.[20] Therefore DFMO is efficiently used to treat early and late stages of West African sleeping sickness. Eflornithine was originally developed as an anticancer agent, however it remains at the trial stages as clinicians are still determining which malignancy it suits best.[22]

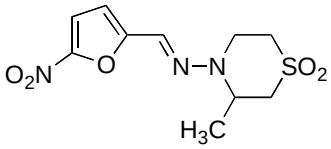
DFMO **8** is an analogue of ornithine, which is a specific suicide inhibitor of the enzyme ornithine decarboxylase (ODC). ODC is the first enzyme in the pathway of trypanosome polyamine synthesis (see Chapter 2.3.2). DFMO inhibits ODC with a similar affinity in both the trypanosome and mammalian host enzymes. However this drug is more specific to the parasite's enzyme because trypanosomes have an ODC that is degraded and reformed within the cells at a much lower rate than in mammals. DFMO therefore deprives the parasites of ODC resulting in delayed polyamine synthesis for a longer time than in the mammals. The growth-arrested parasites are then removed by a functional immune-system.[12]

In 1990, the drug was approved by the United States Food and Drug Administration (FDA), but unfortunately the company responsible for its production, Aventis, stopped its production in 1999. However, in 2001, Aventis signed a long-term agreement to manufacture and donate the drug, in association with MSF and WHO.[1,3]

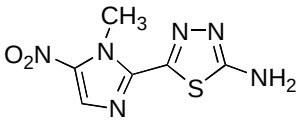
Eflornithine must be given in large quantities due to its rapid excretion. A full treatment contains 400 g taken over a period of two weeks, *via* an infusion followed by oral administration in fruit juice.[22]

1.6.5 Additional Drugs

There are a number of experimental nitroheterocyclic trypanoside compounds that are being investigated for the treatment of HAT. The first is nifurtimox **9**, which is a drug currently in use against Chagas' disease.[29] The nitro group of the compound is pivotal to its activity. Parasite death is thought to be *via* the one-electron reduction of the nitro group, which generates a free radical, which goes on to produce oxygen metabolites such as hydrogen peroxide, hydroxyl free radicals and superoxide.[30] Nifurimox has shown limited success against arsenical refractory *T. brucei* in trials within West Africa.[31] This drug is not very parasite-specific as it is thought to enter cells by passive diffusion.[12]



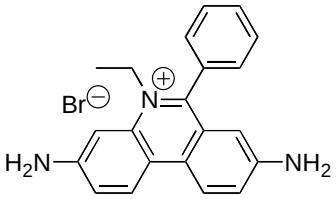
9



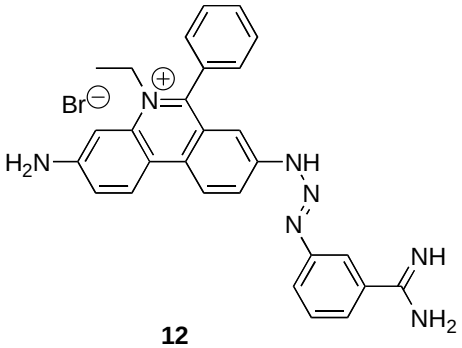
10

The second experimental nitro heterocyclic compound is called megazol **10**, which is a 5-nitroimidazole that shows good efficacy against *T. cruzi* and *T. brucei*.[32,33] Megazol enters the parasite by passive diffusion although the compound possesses a structural motif recognised by the amino-purine transporter responsible for the uptake of other trypanosomidal drugs.[25] *T. cruzi* is an intracellular parasite and therefore megazol activity indicates that the drug is specific to the parasite *via* a target of activation by the parasites.[12]

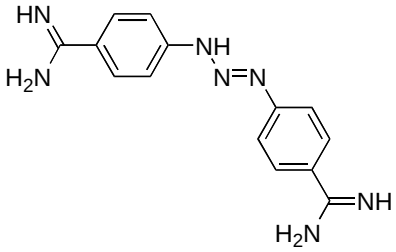
Drugs for the treatment of Nagana are ethidium **11**, isomethamidium **12** (samorin) and berenil **13**.[5] These treatments are effective but are suspected mutagens and teratogens because of their ability to intercalate DNA, and so cannot be used to treat HAT. A water-soluble analogue of melarsoprol called cymelarsen **14** has also recently been introduced.[20] Treated cattle may only be slaughtered for consumption several months after the end of the treatment, to ensure there are no drug residues in the meat.



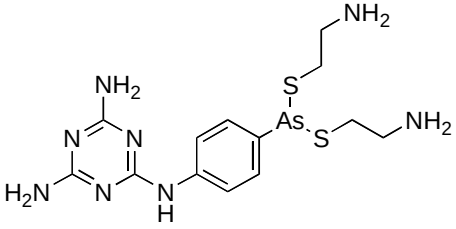
11



12



13



14

1.7 Drug Uptake by Trypanosomes

Trypanosomes live and move freely in the bloodstream and cerebral-spinal fluid of their host, and do not become intracellular. Therefore drugs to target these parasites only have to cross one membrane to reach the interior, and this gives us an opportunity to target drugs selectively to these cells.[14]

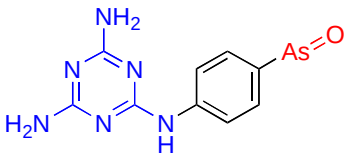
Hydrophobic drugs in general can directly enter the cells of trypanosomes and their mammalian host *via* passive diffusion across the lipid bilayer, but there is no selective accumulation within the parasite. Hydrophilic drugs on the other hand can only cross the cell's lipid membranes by means of active transport using specific channels or transporters. Drugs can therefore be chosen to exploit any differentiating uptake process between the host and parasite.[14]

1.7.1 Trypanosome Transporters

As early as 1938, studies by Hawking suggested that parasites actively accumulated some arsenical compounds.[34] He noticed that drug-resistant parasites did not accumulate drugs. In 1959, Williamson discovered that compounds containing the melamine motif could antagonise the anti-parasite action of known trypanosides, the diamidines and melaminophenyl arsenicals.[35] This was the birth of the idea that trypanosomes have a 'melamine receptor' on their surface. It was known that melaminophenyl arsenicals acted

intracellularly, and it was therefore proposed that the melamine ring facilitated the uptake of the drug from the extracellular environment into the interior. Melamine has similarities to a number of natural metabolites as it is a nitrogen rich heterocycle, and it was thought that the 'melamine receptor' would be a transporter for specific growth nutrients.[35]

However, back in the early 20th century when Ehrlich developed the first arsenicals, he also noticed that they were the first drugs to experience resistance.[23] He observed that cross-resistance between different classes of arsenical corresponded to the side chains on the aromatic ring and not on the arsenic moiety. Ehrlich proposed a theory of drug action based on the haptophore-toxophile biphasic mechanism of melarsen oxide **15** (Figure 8).[23] The 'anchorer' or haptophore (blue) part of the compound targets a specific cell receptor while independently the 'poisoner' or toxophile (red) moiety performs cytotoxic activity on the target. It is now known that the drug target receptors of the haptophore are cellular components such as membrane transport systems, cell wall components or intracellular targets such as enzymes.



15

Figure 8

The genome of *T. brucei* has been decoded and around 5-10% of the genes encode membrane transporter, which gives around 400 different genes.[36] Several classes of transporters of *T. brucei* have been characterised and exploited to target toxic chemicals specifically to this parasite.[36]

1.7.1.1 Purine Transporters

It wasn't until 1993 that it was discovered that the amino-purines and adenosine strongly inhibited lysis of trypanosomes. It was then proposed that the 'melamine receptor' introduced by Williams was actually an amino-purine carrier.[25]

T. brucei have a number of purine transporters in their plasma membrane (Figure 9). Two of these transporters identified by Carter and Fairlamb are adenosine transporters, the first is called P1 and it carries adenosine, guanosine and inosine. The second is called P2, and it carries adenosine and its nucleoside adenine.[25] P1 transporter functions as a general purine

nucleoside transporter whereas the P2 transporter also interacts with melaminophenyl arsenicals and diamidines due to the ability of these reagents to inhibit adenosine uptake.[37] Trypanosomes resistant to sodium melarsen were found to have lost their P2 transporter.[25] These data indicated that in wild-type *T. brucei* the P2 transporter is responsible for the uptake of melamine-based arsenicals and diamidines. Pentamidine however has been found to penetrate trypanosomes *via* alternative routes.[14] In 1999 both the genes for P1 and P2 transporters were cloned and the mutations linked to drug resistance were identified.[38,39]

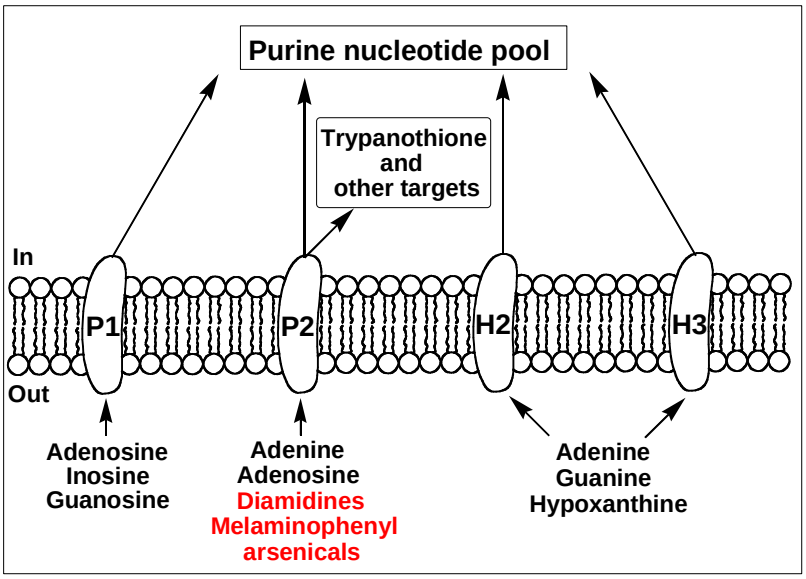


Figure 9

There is a recognition motif shared between diamidines **2**, melamine-based arsenicals **15** and amino-purines **16** (Figure 10).[20,37] Grafting this motif onto compounds that normally are not taken up into trypanosomes has facilitated the introduction of novel drugs into cells. The P2 recognition motif has been built into polyamine analogues with the result of inhibiting adenosine uptake by cells, plus incurring trypanocidal activity. This method has previously been used to introduce toxic tin and nitro-containing compounds into parasites.[40,41]

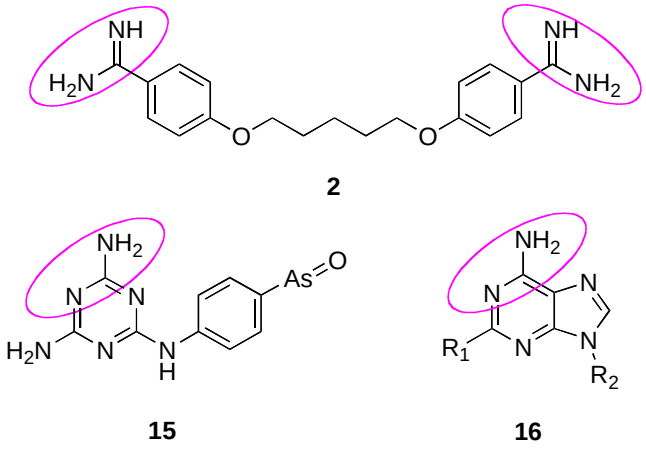


Figure 10

In addition to these amino-purine transporters, a number of purine nucleobase transporters have been identified.[42,43] Trypanosomes are heavily dependent on these systems due to their inability to synthesise their own purines. The investigation and characterisation of all the different purine transporters may lead to the elucidation of other unusual substrate recognition motifs.

1.7.1.2 Glucose Transporters

Glucose transporters from several trypanosomatids have been identified and characterised in order to exploit then as a means to target drugs.[44,45] Trypanosomes in their bloodstream form are highly dependent on substrate-level phosphorylation through glycolysis to produce adenosine triphosphate (ATP). Glycolysis occurs in trypanosomes within special organelles called glycosomes.

Trypanosomes are unique in that their different glucose transporters adapt depending on their environment, from the insect vector to the mammalian host. *T. brucei* has two transporters, one with low affinity and high capacity which functions in the bloodstream form where they are exposed to high glucose concentrations.[46] The second receptor is a high affinity transporter for use in the tsetse fly where glucose is rare. The genes of these receptors have been cloned and expressed in different systems. It was found that one could carry D-fructose as well as D-glucose,[47] plus a D-glucose analogue bearing substituents at the C2 and C6 positions.[48] The analogue carrying a C2 substituent exhibits competitive glucose-uptake inhibition. These analogues however are not taken up into the parasite interior despite binding to the receptor.[49] It is also understood that due to the blood glucose level being 5000 times higher than the adenosine concentration, using glucose transporters is not a key route for targeting anti-trypanosomiasis drugs.[49]

1.7.1.3 Amino Acid Transporters

There are a number of amino acids present in blood plasma in low millimolar concentrations.[50] Amino acids contain a vast range of ligands and therefore their transporters offer a potential portal of entry for drug molecules into cells. Proline is the main source of carbon and energy in procyclic trypanosomes, and its transporter has been studied extensively. Similarly the amino acid aspartic acid has been studied in depth, as it is the amino acid with the lowest plasma concentration. Aspartic acid does not have a high affinity transporter. Methionine was found to have a specific affinity transporter and its characterisation is currently underway. Targeting amino acid transporters may be a future route to tackling the HAT drug problem.[14]

1.7.2 Non-carrier-mediated uptake

As mentioned previously, passive diffusion (non-carrier-mediated uptake) is a major route to parasite drug entry due to the amphipathic nature of many drug compounds. The compound megazol has shown activity against *T. brucei* in rodent and primate infection models and shows potential as a novel HAT treatment.[51] Megazol **10** contains the recognition motif accepted by the P2 purine transporters, which inhibits adenosine P2 transportation and removes arsenical-induced lysis of the parasites *in vitro*. These data indicate that megazol could not be useful against HAT resistant to arsenicals (where P2 activity is altered). However, parasites selected for P2 deficiency that are resistant to melaminophenyl arsenicals and diamidine drugs showed no resistance to megazol.[52] Therefore megazol must have another major route of entry, and this is non-carrier mediated uptake.

It was found that even with a high concentration of adenosine and P2 transporters, megazol uptake was unaffected. The uptake was found to be biphasic, starting with rapid equilibration of the drug across the membrane simply due to passive diffusion. This was followed by slow accumulation over time due to metabolism of the drug intracellularly.[52]

1.8 Drug Resistance

There is an emerging threat of *T. brucei* becoming naturally resistant to the few available HAT treatments.[20] Drug resistance can be considered as the natural response of the parasite to the selective pressure of the drug. Resistance to other trypanocides is also on the increase and this threatens the current medical and vetinary treatment of these diseases. It

is vital to understand the current drugs' mode of action plus the mechanism of resistance, to give an insight into how chemotherapeutic strategies can be designed to overcome or reverse this problem. Doing this can also help to increase the therapeutic activity of current treatments by providing a basis for combination drug therapy, as it can reduce the toxicity of treatments and help decrease the rate at which resistance is appearing.[20]

In a Gambiensian disease report from Uganda in 1999, it was found that 30% of people treated for HAT using melarsoprol relapsed within two years,[53] and in an Angolan focus (2001) 25% of patients relapsed within 30 days.[54] Currently any resistance to the early stage treatments pentamidine and suramin are not considered a serious risk as any treatment failure may be due to misjudgement during stage-diagnosis. The innate resistance to DFMO by *T. brucei rhodesiense* has been demonstrated although this drug is effective against *T. brucei gambiense*. This is due to a difference in ODC turnover rates between the two species.[55]

Resistance to melarsoprol was initially introduced in laboratory strains of parasites, however it is now being observed in the field. This development of resistance to arsenicals and cross-resistance (tolerance to a usually toxic substance as a result of exposure to a similarly acting substance) to diamidines is thought to be due to the loss of the function of the P2 transporter as described earlier.[25] The melarsen arsenical resistant line created by Fairlamb and co-workers named *T. brucei* 427 cRU15 had reduced P1 activity and no P2 activity. This resistant strain also showed cross-resistance to some diamidines.

1.8.1 Molecular Basis of Drug Resistance

The gene that encodes the P2 transporter *TbAT1*, was discovered in 1999.[38] *TbAT1* encodes a 463 amino acid hydrophobic polypeptide with 11 transmembrane α-helices, a cytosolic loop, an extracellular carboxy terminus and a cytosolic amino terminus (Figure 11).

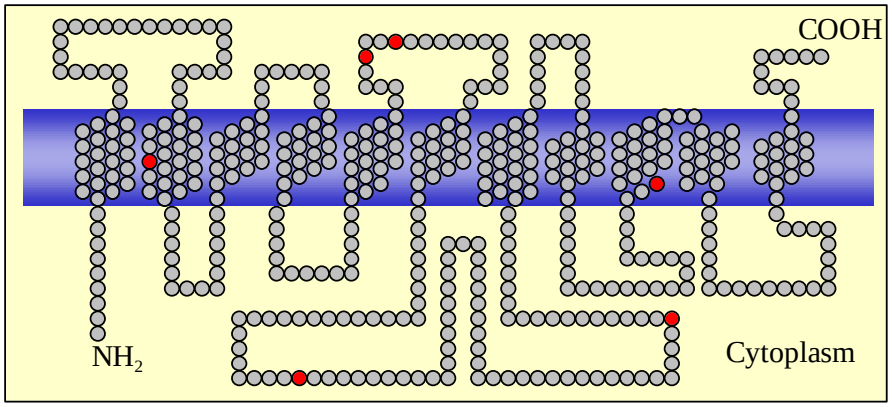


Figure 11

The P2 transporters from an arsenical resistant strain of *T. brucei* were cloned and expressed and it was found that the resistant strain had ten nucleotide alterations, which resulted in six changes at the amino acid level. The process in which the P2 transporter is altered is unclear, however it is likely to be due to either a point mutation within the gene, which alters K_m, ν_{max}, or both of the P2 transporter. Alternatively it may be due to the transporter being down-regulated.[56] A study of patients from north-western Uganda also found this mutation of *TbAT1* in trypanosomes of early stage infection and relapse patients. It was noted that many of the point mutations found in the wild species were the same as those generated in the laboratory.[57]

1.8.2 Mechanisms of Drug Resistance

Antimicrobial agents generally act through an interaction with a drug target and drug resistance can arise in a number of ways: i) as a consequence of a change in drug level; ii) by a change in the target; or iii) both of these ways (see Table 1).[25]

Drug Level	Target Level
By exclusion	Modified
Decreased drug import	Decreased target affinity
Increased drug export	Amplified
By sequestration	Increased drug sequestration
Drug-binding molecule	Increased enzyme activity above threshold
Drug compartmentalisation	Missing
By metabolism	Target bypass
Pro-drug not activated	Repaired/protected
Increased drug inactivation	Increased damage repair
	Protected by substrate

Table 1

Drug resistance can also be linked with the treatment of animal cases of trypanosomiasis. A publication in 2001 reported that the re-stocking of an area of Uganda with cattle from out-with the surrounding area resulted in an outbreak of *T. brucei rhodiense* HAT. This suggests that the cattle acted as a reservoir of the human infective parasites, and then the native tsetse flies spread the disease to the local population.[58]

The increase in drug resistance can be due to a number of factors; the drug's widespread use, the use of the drug as a prophylactic, self-administration, inappropriate dosing and exposing the parasite to sub-curative levels of the treatment.[58] The treatment of Nagana with berenil **13** is a classic case of all of these factors. Berenil was used as a prophylactic, bought on the black market and administered by farmers when it should be purchased and authorised by pharmacies. The black market products have been found to contain between 0 and 90% of the active ingredient, and therefore the doses varied widely. Farmers also underestimated the weight of their cattle and so did not give the correct dose, and were found to only treat animals that showed signs of illness. This also resulted in the probability of the trypanosome encountering sub-curative doses of the drug allowing resistance to grow.[59]

It is simple to select for cross-resistance *in vivo* and *in vitro* between berenil and melarsoprol. Cross-resistance has now been observed in the field with human infective trypanosomes infecting cattle, developing a resistance to berenil, and then being carried back to the human population with a cross-resistance to melarsoprol.[20]

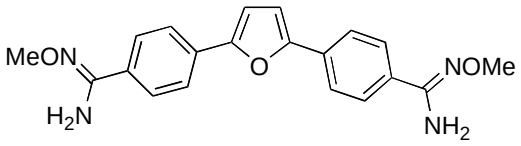
1.9 Future of HAT Treatment

When Aventis ceased the production of DFMO in 1995 due to its lack of profit, the future of HAT treatment looked bleak. However the agreement made in 2001 between WHO, MSF and Aventis after years of international pressure, found Aventis agreeing to donate enough DFMO, pentamidine and melarsoprol to cover global needs for five years.[3] They also agreed to financially support WHO's programmes for sleeping sickness research and treatment, plus providing technical assistance and technology to potential long-term manufacturers of the drugs. At this time the company Bayer announced that they would restart the production of two sleeping sickness drugs, and Bristol-Myers Squibb also became involved by paying for part of the first years supply of DFMO after they launched an eflornithine-based product for the removal of women's facial hair.[3]

Although this was an excellent turnaround for the drug companies, the value of Aventis' donation at around $5 million was still small in comparison to the $40 million per year cost for an effective pan-Africa HAT control programme.[3]

In early 2006, an announcement was made that the Bill and Melinda Gates Foundation was giving a $22.6 million grant to the University of North Carolina (UNC) at Chapel Hill which leads an international research consortium. The grant is to support the phase III clinical trial of a highly promising new drug for the treatment of sleeping sickness, which is the final step required before receiving approval from the US Food and Drug Administration.[60]

The new drug DB289 **17**, recently named pafuramidine, is far less toxic and more appropriate for use in the rural areas of Africa as it is an orally administered prodrug. In preliminary studies, DB289 had activity against *Pneumocystis carnii* in animal models, in HIV patients with Pneumocystis pneumonia and also against strains of *Plasmodium* (malaria) and *Cryptospiridium parvum* (another parasitic protozoan).[61] DB289 was tested for the first time in early stage sleeping sickness in a phase II trial in 2001 in Angola and the Democratic Republic of Congo which compared the activity of DB289 with pentamidine. Results indicated that DB289 was significantly safer than pentamidine although the treatment failure rate was higher than expected.[62]



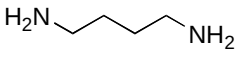
17

The phase III trials are currently underway and the UNC are conducting research into a paediatric formula for use in children under six years old and studying pafuramidine in children aged six to 12 years. UNC will also start an expanded access programme, along with studying the effectiveness of pafuramidine in treating the East African form of sleeping sickness.[60]

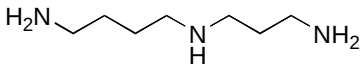
Therefore DB289 **17** is the first orally administered drug for the treatment of sleeping sickness and it is the first new HAT drug for over 50 years. Although test results look very promising for DB289, there is still a vital need for new sleeping sickness drugs to tackle the increasing problem of resistance and cross-resistance of current drugs, and their innate toxicity.

2 Polyamine Metabolism

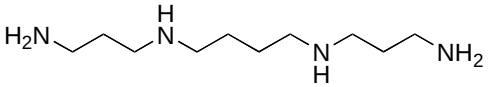
Polyamines are ubiquitous organic cations of low molecular weight found in all living cells examined to date. Most cells contain significant amounts of the polyamines putrescine **18**, spermidine **19** and spermine **20**, and their concentrations within cells are highly regulated.[63]



18



19



20

2.1 Functions of Polyamines

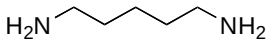
Although their full physiological function has been much debated and has not yet been fully elucidated, polyamines are crucial for cell growth and differentiation. They are positively charged at physiological conditions and can interact covalently with many cellular molecules, and during *in vitro* experiments, they have altered reaction rates and the dissociation of sub-cellular components.[64] Their polybasic character allows them to bind strongly to nucleic acids, and they can stabilise DNA by decreasing the repulsion between the strands by neutralising the negative charges on the phosphate groups. Polyamines can also influence protein synthesis by interaction with RNA, and assist the association of ribosomal subunits by binding to ribosomes. Polyamines also interact with receptor proteins such as the K^+ channel pore.[65]

Studies have indicated that rapidly growing and differentiating cells have higher concentrations of polyamines than those of quiescent cells, and the inhibition of polyamine biosynthetic enzymes can lead to cell growth cessation and even cell death.[65] Abnormal polyamine metabolism has also been found to influence the development of tumours.[65]

2.2 Polyamine History: Discovery and Elucidation

Putrescine and its homologue cadaverine were first discovered in the late 19th century by German chemists and medics studying the products of bacterial fermentation. Even as late as 1945 it was still thought that the presence of these amines in human tissues and fluids

was due to bacterial action.[66] Between 1920 and the 1940s, bacteria were studied and found to metabolise some amino acids, and these extracts were able to decarboxylate L-ornithine and L-lysine to putrescine **18** and cadaverine **21**, respectively.[66] Similarly, the presence of spermine **20** was first reported by Leeuwenhoek as a crystalline phosphate in human seminal fluid in the 17th century, but it wasn't until 1917 that Mary Rosenheim proved the presence of spermine in eukaryotic cells. Otto Rosenheim, Mary's husband, then carried out the proof of structure and chemical synthesis of spermine. He also discovered and synthesised the triamine spermidine **19**.[66] After this, little further research was carried out on the diamines, triamines or tetraamines until the end of World War II. At this time, Herbst and Snell found that a strain of influenza required putrescine for growth, and this led Herbst to carry out separations and investigations of various bacterial polyamines. This began 40 years of prolific research into the function and cellular metabolism of polyamines.[66]



21

Microbiology was established in the 1950s and 1960s that allowed the clarification of the activity and specificity of the amine oxidases. By 1980 the entire polyamine cycle, including the synthesis and degradation of compounds, and the numerous side branches yielding metabolic products, had been established.[66]

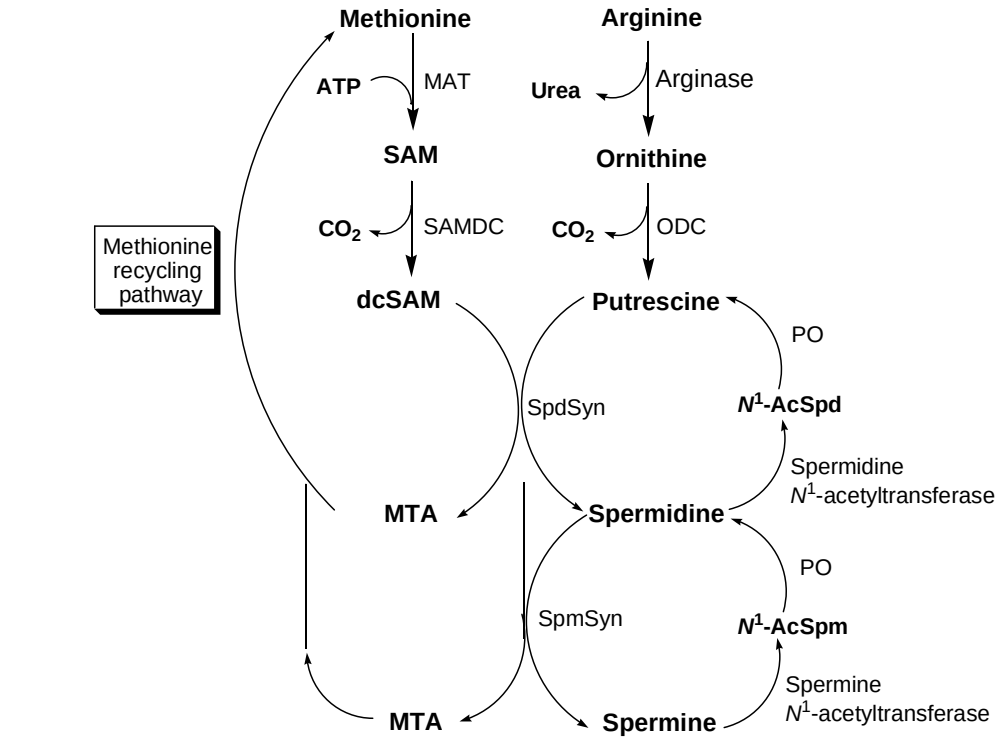
2.3 Polyamine Biosynthesis

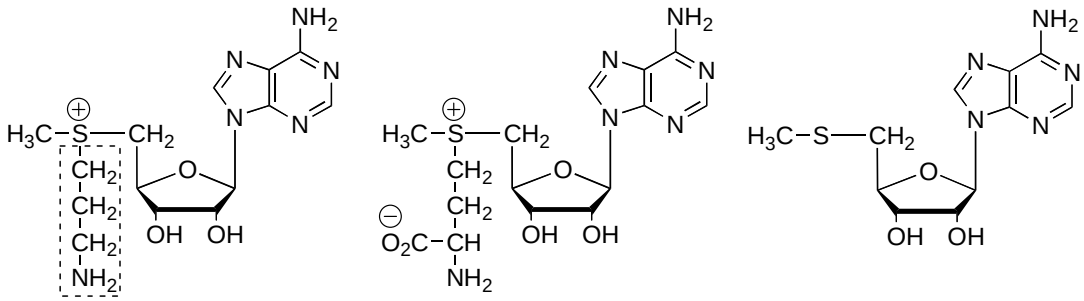
Polyamine synthesis is unique and not dependent on an external supply, but is entirely controlled by the cell.[64] Mammalian cells synthesise putrescine **18**, spermidine **19** and spermine **20**, whereas many bacteria and protozoa synthesise only putrescine and spermidine. Some microorganisms and higher plants can produce putrescine from agmatine (produced by the decarboxylation of arginine). However mammalian cells, fungi and protozoa do not have arginine decarboxylase and so they synthesise putrescine using the enzyme ornithine decarboxylase (ODC).[63]

2.3.1 Mammalian Polyamine Biosynthesis

Figure 12 shows the general pathway for polyamine biosynthesis in mammals, although they can also occasionally be obtained from the diet. Polyamines are derived from arginine

and methionine. Putrescine is formed from L-ornithine using ODC. Ornithine in mammalian cells is found in the plasma, or formed within the cells from arginine and the arginase enzyme.[63] ODC and arginine decarboxylase are pyridoxal phosphate-dependent enzymes. ODC is a homodimer with two active sites, and is found in very small concentration in inactive cells and is regulated by an ODC inhibitor. The activity of ODC can be increased by stimuli such as hormones, drugs and growth factors.[63]





22 **23** **24**

Figure 12

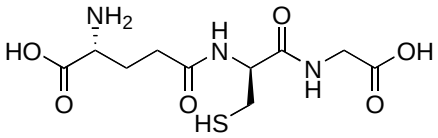
Putrescine is converted into spermidine and spermine *via* the action of two aminopropyl transferases followed by spermidine synthase (SpdSyn) and spermine synthase (SpmSyn), respectively. These enzymes are specific to their acceptor but both use decarboxylated *S*-adenosylmethionine **22** (dcSAM) as the aminopropyl donor.[65] The enzyme *S*-adenosylmethionine decarboxylase (SAMDC) then decarboxylates *S*-adenosylmethionine

23 (SAM). The cell concentration of dcSAM in mammalian cells is normally low, and its availability regulates the activity of aminopropyltransferases. The second product in the system of transferring an aminopropyl group from dcSAM to putrescine or spermidine is 1,5'-methylthioadenosine **24** (MTA). MTA is then degraded *via* the methionine recycling pathway using MTA phosphorylase to 5'-methylthioribose-1-phosphate and adenine, and the adenine is then converted into 5'-adeninemonophosphate using adenine phosphoribosyl transferase. 5'-Methylthioribose-1-phosphate is also converted back into methionine, and thus, the aminopropyl groups for the synthesis or spermidine and spermine are derived from methionine.[65]

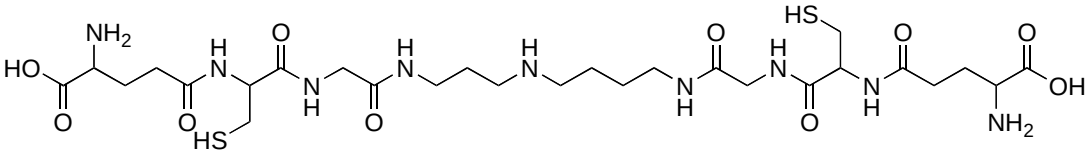
The enzymes polyamine oxidase (PO), spermidine N^1-acetyltransferase and spermine N^1-acetyltransferase degrade spermidine and spermine back to putrescine. This interconversion is a regulatory response when the levels of spermidine and spermine reach certain limits, and is induced by exposure to spermidine, toxic agents, and by fasting.[65]

2.3.2 Trypanosome Polyamine Biosynthesis

Trypanosome polyamine metabolism differs in several ways from that of their mammalian hosts with respect to their half-life, turnover, substrate specificity and regulation of enzymes, both within the main polyamine biosynthetic pathway and the related pathway of transmethylation.[64] African trypanosomes contain putrescine and spermidine, but not spermine. The spermidine content of trypanosomes varies from 10-30 nmol per 10^8 cells, and putrescine makes up 20-30% of the total polyamine content. The putrescine analogue cadaverine is not found in these parasites; however they do contain an unusual glutathione **25** derivative of spermidine which is N^1,N^8-bis(glutathionyl)spermidine, now known as trypanothione **26**. Trypanothione was characterised by Cerami and Fairlamb in 1992, and is a complex compound that replaces glutathione in the biosynthetic pathway and maintains the intracellular reducing environment, while performing the usual glutathione function as the trypanosomes' antioxidant.[67] Trypanothione is found at relatively constant levels in bloodstream trypanosomes at 1-2 nmol per 10^8 cells.[68] It has been found that trypanosome enzymes are specific to trypanothione and do not catalyse glutathione reactions.[66] Spermidine is essential in trypanosomes and its function is well defined. These protozoa have a specific trypanothione reductase (TR) enzyme that has been crystallised and its active sites analysed in the search for inhibitors as targets for chemotherapy.[66]



25



26

The starting point of polyamine biosynthesis in trypanosomes is ornithine and methionine (Figure 13). Ornithine is decarboxylated to putrescine using ODC, and methionine is transformed into SAM using ATP and SAM synthetase, before it is decarboxylated to dcSAM using SAMDC. The putrescine then accepts an aminopropyl group from dcSAM to form spermidine. The by-product MTA is phosphorylated *via* MTA phosphorylase to give adenine and methylthioribose-1-phosphate, and eventually to methionine and SAM using the methionine recycling pathway.[64]

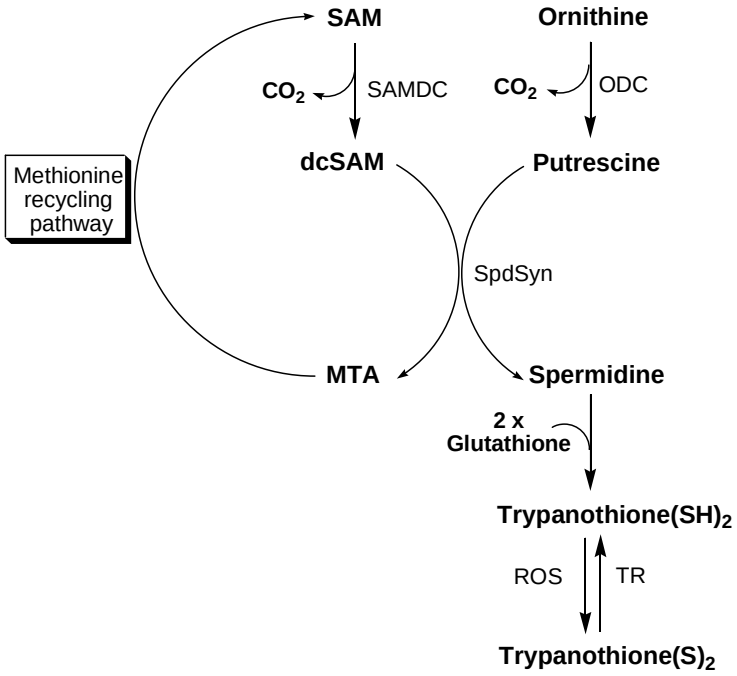


Figure 13

2.4 Polyamine Metabolism as a Therapeutic Target

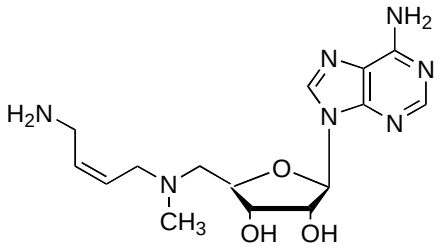
Pharmacological intervention that inhibits polyamine biosynthetic enzymes prevents cell growth, and causes cell death in almost every polyamine pathway studied.[69] Polyamine

pathways have been successfully exploited to design chemotherapeutics against a number of parasitic diseases and in cancer chemotherapy against a range of tumour cell lines.[69] The inhibition of key biosynthetic enzymes and polyamine transport as well as replacing naturally occurring polyamines with synthetic analogues are ways of directing drug design.[69]

2.4.1 Polyamine Metabolism as a Target for Cancer Chemotherapy

Polyamines that perturb polyamine biosynthesis and functionality have been shown to inhibit the growth of tumours efficiently.[69,70] Cancerous cells have an enhanced requirement for polyamines and so targeting their transport systems is an attractive vector for drug design.[65,66,69]

It was realised early on in the research into the activity and structures of polyamines that the enzyme ODC is the fundamental rate limiting and controlling step in the biosynthetic pathway.[70,71,72] In the 1970s DFMO **8**, a drug created by the Merrell-Dow research institute, was found to be the most active suicide inhibitor of ODC.[63,64] Over the last 40 years, DFMO has been used extensively as a tool in the elucidation of the synthesis and catabolism mechanisms of polyamines; in the polyamine mechanism of cells' proliferation and differentiation; and in the inhibition of cell growth as well as a number of other approaches to cancer chemotherapy.[66,70,73] Another key enzyme in the pathway is SAMDC and a drug discovered in 1963 called methylglyoxal bis(guanylhydrazone) **27** (MGBG) (Table 2) was found to be a potent inhibitor of eukaryotic SAMDC, resulting in the death of cancer cells.[66,74] This antileukaemic agent unfortunately showed some non-specific cytotoxic effects such as blocking the metabolism of fatty acids which resulted in damage to the mitochondrial membranes.[74] More recently however, an enzyme-activated SAMDC inhibitor which is an analogue of SAM called 5'-{[(*Z*)-4-amino-2-butenyl]-methylamino}-5'deoxyadenosine **28** (MDL73811) was synthesised, which showed low mammalian toxicity while being highly specific with significant antitumour activity.[75]



28

From the early work, it was established that mammalian cells contain four key enzymes in the polyamine synthetic pathway, ODC, SAMDC, SpdSyn and SpmSyn, and a multitude of powerful specific inhibitors have been found for all of these enzymes.[69] Several hundred polyamine synthesis inhibitors, catabolism stimulators and other polyamine derivatives have been tested against cancer cells.[66,69,70] These drug compounds focus mainly on these crucial enzymes but they also target the polyamine uptake/transport systems and other enzyme pathways in an attempt to stop cancer cell growth by decreasing the intracellular levels of putrescine, spermidine and spermine. Table 2 contains a selection of some inhibitors/activators of polyamine related enzymes.

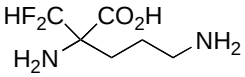
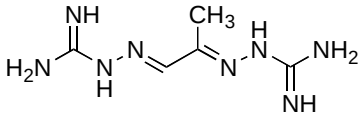
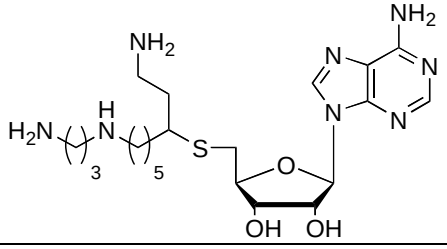
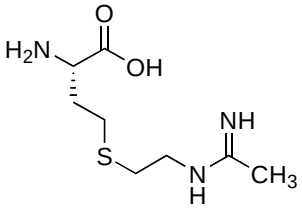
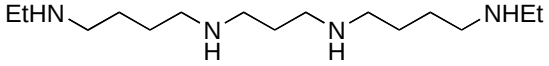
Enzyme Target	Inhibitor/Activator	Structure
ODC	**8** DFMO	
SAMDC	**27** MGBG	
SpdSyn/ SpmSyn	**29** AdoDATAD	
PO	**30** Aminoguanidine	
Unestablished molecular mechanism	**31** BE-4-4-4	

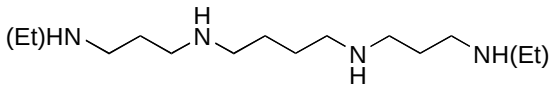
Table 2

Unfortunately these drugs do not completely inhibit cell growth as most mammalian cells (including cancer cells) have polyamine uptake and transport systems, which import

polyamines from neighbouring cells when polyamine concentrations are low. Mammalian cells have a rapid ODC turnover and have the ability to unblock the polyamine pathway by converting any spermine into spermidine *via* an acetylation pathway. To obtain maximum inhibition of cancer cells, a combination of inhibitors must be used.[70] For example DFMO **8** is relatively non-toxic and so can be used in conjunction with other drugs. DFMO and MGBG **27** together have shown success in tissue cultures, animal models and human trials. DFMO is first used to inhibit ODC and deplete the cells of polyamines before MGBG (a polyamine derivative) is administered. This is rapidly imported into cells to inhibit SAMDC. This drug combination has shown 99% proliferation inhibition in cell cultures.[69,76,77]

Polyamine transport systems are not strictly specific and a variety of synthetic polyamine derivatives and analogues can use them. In tumour cells, the activity of these systems is higher than in normal cells, and studies have shown that tumours take up higher amounts of radio-labelled polyamines than normal cells.[66,69] These systems can therefore be used to direct cancer chemotherapy in two ways: drugs can be designed to inhibit the system by preventing polyamine uptake; and uptake of potent polyamine analogues can interfere with or block key proliferation events.[70,78]

Structural analogues of polyamines are considered to be the compounds with the highest potential for interfering with polyamine action, and this has been investigated thoroughly in cancer cells.[66,69,70] One of the main advantages is that analogues disrupt the biosynthetic pathway in a number of ways, acting as polyamine antagonists that prevent growth related functions. Analogues including N^1,N^{12}-diethyl homospermidine **31** (BE-4-4-4) and N^1,N^{12}-bis(ethyl)spermine **32** (BE-3-4-3) have been shown to induce *N*'-AcSpm which leads to a loss in the polyamine content of treated cells.[79] These analogues also repress ODC and SAMDC activity in the same way as spermine. Therefore the uptake of BE-3-4-3 and BE-4-4-4 can result in a complete loss of normal polyamine activity and lead to cytotoxicity.[69]



32

2.4.2 Polyamine Metabolism as a Target for Trypanocide Design

In the design of drugs to be used against African trypanosomiasis, the progress in the search for anti-cancer drugs has been harnessed. The three main enzyme targets of trypanosomes are ODC, SAMDC and MTA phosphorylase.[80]

2.4.2.1 ODC Inhibition

After the success of DFMO **8** anti-cancer activity on a range of tumour cell lines, in the 1980s Bacchi and co-workers demonstrated that DFMO had an effect on the growth of the trypanosome. DFMO was used to cure acute infections of *T. brucei* in mice.[81] Infected animals were treated with 4% DFMO for 12 to 36 hours, and it was found that DFMO interrupted a number of areas of parasite metabolism. ODC was inactivated, while ornithine, spermine, putrescine and trypanothione levels were decreased by up to 95% and RNA and DNA production was reduced.[82] DFMO also blocked the synthesis of the VSG and other proteins. It was also found that SAM and dcSAM levels increase up to 400-fold which was an observation not seen in mammalian experiments. This difference has critical implications, as there are several biochemical distinctions between mammals and trypanosomes. The parasite has a long ODC half-life (four hours) and slow turnover, whereas mammals have a rapid ODC turnover with a half-life of 10-60 minutes. The trypanosomes' inability to synthesise spermine due to their lack of putrescine and spermidine transport is also a key difference.[64]

2.4.2.2 SAMDC Inhibition

The success of targeting cancer cells with the SAMDC inhibitor by blocking spermidine and spermine synthesis led to the investigation of SAMDC as a target in the search for antiparasitic drugs.[64] The trypanosome enzyme is unlike the mammalian version in a number of ways: its solubility and ionic properties are unique; and it does not cross react with human SAMDC. SAMDC is inhibited using the trypanosides berenil and pentamidine.[83]

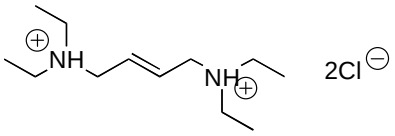
2.4.2.3 MTA Phosphorylase Inhibition

The third potential enzyme target is MTA phosphorylase, which is present in mammalian cells and some protozoa. This enzyme catalyses the conversion of MTA into adenine and

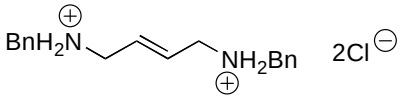
methylthioribose-1-phosphate. MTA analogues were synthesised to contain ribose moieties which on interaction with the enzyme, would block methionine recycling.[84]

2.5 Previous Approaches Towards Antiparasitic Agents within the Robins Group

L. Slater reported in her 1998 Ph.D thesis the synthesis of a number of putrescine analogues.[85] **33** was the lead compound as it displayed promising anti-malarial activity with a lowest effective concentration (LEC) against a culture of *P. falciparum* of 18-36 μM. Analogues had varying carbon chains and substitution patterns on the nitrogens, however the only compound to show any improved activity was the *N*,*N*'-bisbenzyl compound **34** (LEC 3-15 μM) although its activity *in vivo* was poor.

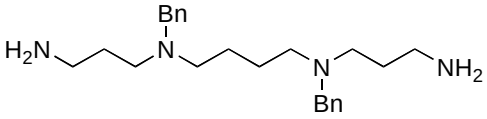


33



34

Slater then went on to prepare a number of spermine analogues with **35** showing the best result *in vitro* with a LEC value of 1-2 μM.



35

Later in 2003, L. Parker synthesised a number of polyazamacrocycles on the route to nitrogen mustard analogues. A selection of these compounds was tested against *L. mexicana* and *T. brucei* by Dr M. Barrett (University of Glasgow).[86] *N*-Substituted polyazamacrocycles had previously shown biological activity, displaying anti-tumour, anti-HIV, anti-malaria, anti-microbial, and anti-fungal properties.[86,87,88] The antiparasitic activity of polyazamacrocycles however, had not been published. Table 3 contains a number of the compounds tested with **39** displaying the best *in vitro* activity. There are no trends in the results with the activity profile of the macrocycles being different between the two parasite species.

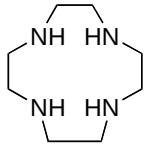
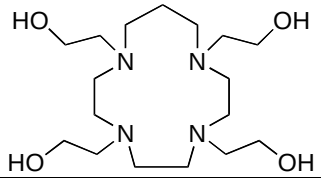
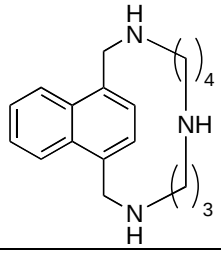
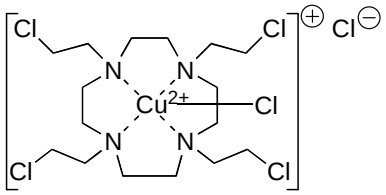
	Structure	*L. mexicana* IC_{50} (μM)	*T. brucei* IC_{50} (μM)
36		>3000	75
37		90	no effect
38		65	45
39		52	5

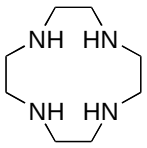
Table 3

This early work was the foundation for the synthesis of further analogues of 1,4,7,10-tetraazacyclododecane **36** (cyclen) with aromatic substituents in an attempt to increase the antiparasitic activity of polyazamacrocycles.

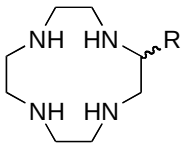
3 Synthesis and Evaluation of Tetraazamacrocycles as Antiparasitic Agents

3.1 Aims of this Work

Some investigation has been carried out within the Robins group to determine if the size, shape and heteroatom content of aza and oxoaza macrocycles affects their activity as antiparasitic agents.[85,86,89] The next endeavour was to vary the electronic demand on 1,4,7,10-tetraazacyclododecane **36** (commonly known as cyclen) by attaching various aryl groups at the C-2 position **40**. A number of analogues containing parasite-specific recognition motifs at the C-2 position were selected for preparation, and all compounds were to be tested *in vitro* for antiparasitic activity against *Trypanosoma brucei*. Cyclen **36** was chosen as our basic ring structure due to its promising activity in other biological test systems.[86] It was used as a reference in all the biological *in vitro* assays, as well as the starting material for the synthesis of anti-cancer alkylating agents (Chapter 5).



36



40

3.2 Synthesis of 1,4,7,10-Tetraazacyclododecane (Cyclen)

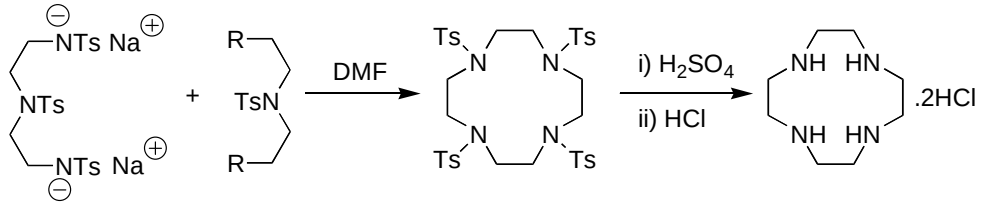
This first section describes an investigation to find the optimum strategy for the synthesis of cyclen **36**. As with other cyclisation reactions, the synthesis of polyazamacrocycles is notoriously tricky. There are numerous ways to prepare macrocycles ranging from high dilution reactions to metal or carbon templated cyclisations. In each case, the reaction conditions are designed to encourage intramolecular cyclisation over intermolecular oligomerisation.[90,91,92,93]

Cyclen is a 12-membered tetraazamacrocycle, which has become an important intermediate for the synthesis of diagnostic and therapeutic pharmaceutical agents.[94,95] In particular, one of the fastest growing medicinal uses of cyclen is in the development of magnetic resonance imaging contrast agents,[96] and more recently cyclen-based bifunctional chelating agents are being investigated for use as therapy against targeted cancer agents.[97]

Therefore there is an increasing demand for **36** on both a research and commercial scale, and considerable effort is being made towards efficient synthetic strategies. This first section focuses on the search for the best method to produce large quantities of high purity **36** in good yields.

3.2.1 Richman Atkins

Up until the early 1970s the most efficient method for the synthesis of polyazamacrocycles was by Stetter and Roos. This used high dilution conditions to condense terminal halides with bis-sulfonamide sodium salts.[92,98] This method gave moderate yields of macrocyclic sulfonamides. Richman and Atkins then developed their synthesis of polyazamacrocycles which didn't employ high dilution or template effects.[90] Their simple and adaptable preparation was used for the synthesis of nine to 21 membered rings containing three to seven heteroatoms, all with moderate to good yields. In this early publication **36** was synthesised by treating a stirred solution of **41** in DMF at 100 ^{o}C with one equivalent of **42** (where R is a different leaving group, OTs, OMs, Cl, Br, I) in DMF over one to two hours (Scheme 1). Addition of water to the cooled solution gave 1,4,7,10-tetrakis-(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane **43** in 80% yield. Hydrolysis to remove the tosyl protecting groups was carried out by heating **43** at 100 ^{o}C with concentrated sulfuric acid for 48 hours, which gave a 90% yield of cyclen after work-up. It was found that the sulfonate ester leaving groups on **42** along with the dipolar aprotic solvent removes the requirement for high dilution conditions.



41 **42** **43** **36**

Scheme 1

More recently, this reaction has often been carried out by the reaction of the dianion of a bis-toluenesulfonamide with a suitable bis-tosylated analogue in anhydrous DMF. The tosylamide salt can be formed by the reaction of sodium hydride in DMF or sodium ethoxide in ethanol, but more commonly it is formed *in situ* by the presence of caesium carbonate in the reaction mixture.[99] The caesium salt exhibits reactivity characteristics that have been dubbed the "caesium effect" due to two synthetic phenomena.[100] Firstly it

applies to the readiness with which caesium salts dissolved in DMF can be alkylated with alkyl halides, but the term also refers to the way that many macrocycles including very large rings and relatively apolar chain components can be synthesised *via* intramolecular anionic nucleophilic substitution of the precursor (Figure 14). In this case the intermolecular substitution is suppressed relative to the intramolecular process.

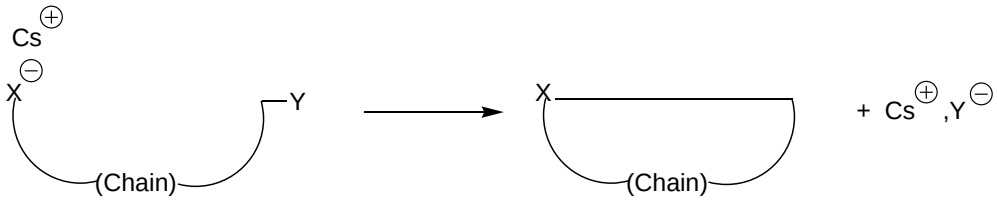


Figure 14

The tosyl groups serve two functions: firstly by rendering the secondary amine sufficiently acidic for salt formation under mild conditions; and secondly they protect the nitrogen allowing monoalkylation only.[101] In addition it has also been shown that the steric bulk of the *p*-toluenesulfonyl groups confer a pseudo-Thorpe-Ingold (gem-dialkyl) effect that encourages intramolecular reaction (Figure 15).[102,103] It is thought that the tosyl functionalities decrease the angle between the reacting termini, positioning them closer together and increasing the chance of reaction.

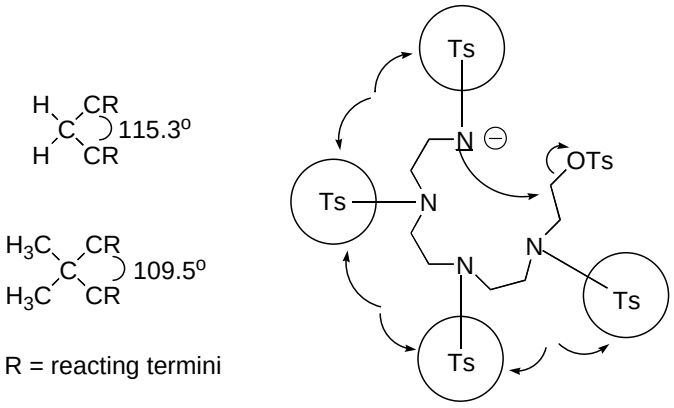
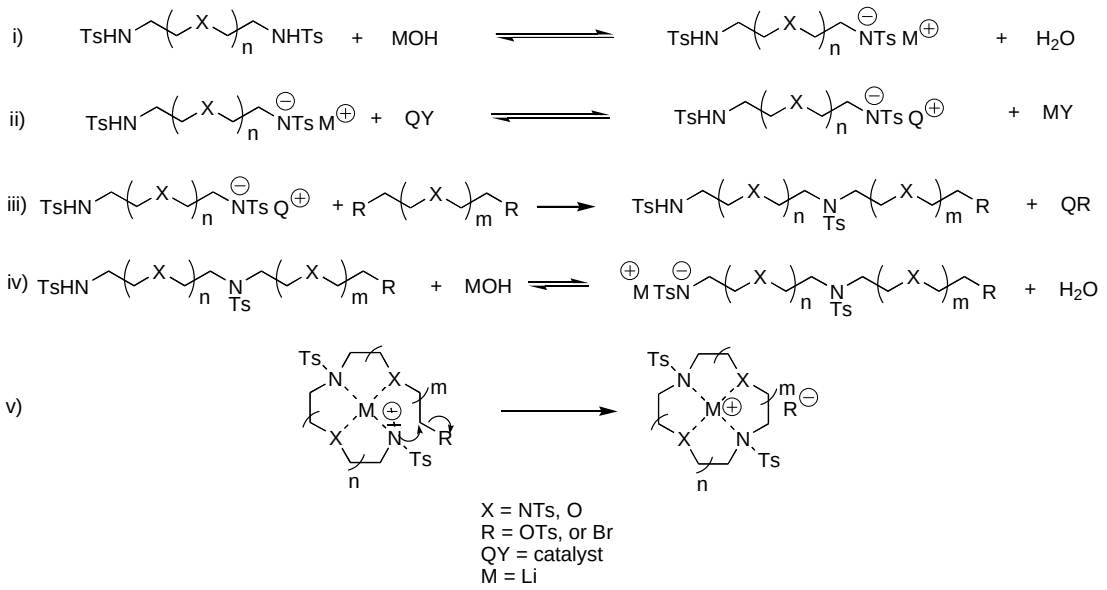


Figure 15

3.2.2 Phase Transfer Conditions

In 1988, Lukyanenko and co-workers described the synthesis of aza- and oxoaza-macrocycles using a biphasic system.[104] This procedure involves reacting bis-sulfonamide analogues with dibromides or ethylene glycol bis-(toluene-*p*-sulfonates) in aqueous alkali-toluene in the presence of quaternary ammonium salts as phase transfer catalysts.

This interphase reaction is presented in Scheme 2. Deprotonation of the sulfonamide groups with the alkali probably occurs successively (reactions i and iv). In the absence of catalyst (QY) no alkylation of sulfonamide occurs after the deprotonation of the first sulfonamide group (reaction i), however if catalyst is present, exchange takes place (reaction ii). The ionic pair that has been formed then undergoes alkylation in the organic phase (reaction iii). Deprotonation of the alkylated product results in the formation of a highly lipophilic anion (reaction iv) and this is able to solvate alkaline metal cations to form a cation-associated precursor. Intramolecular alkylation completes the process (reaction v).[104]

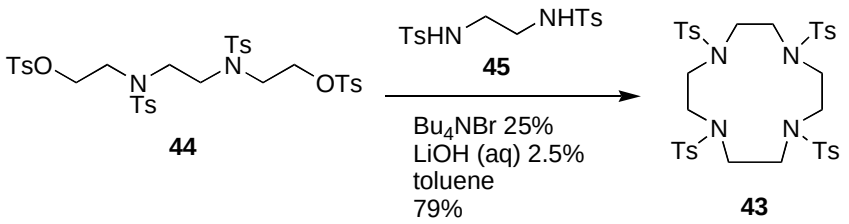


Scheme 2

This strategy was used to synthesise cyclen **36** in two ways.

3.2.2.1 Route I

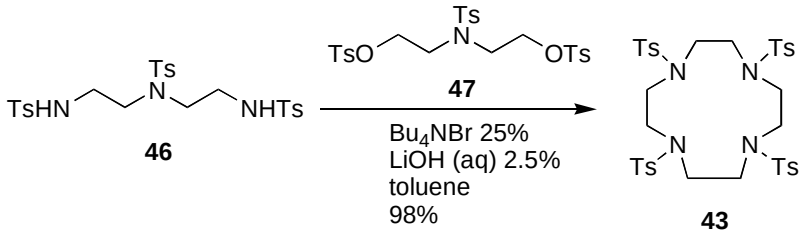
N,*N'*,*O*,*O'*-Tetra(*p*-toluenesulfonyl)-*N*,*N'*-bis(2-hydroxyethyl)-ethylenediamine **44** and ditosylated ethylenediamine **45** dissolved in toluene were added to a stirred mixture of tetrabutylammonium bromide and aqueous lithium hydroxide in toluene heated under reflux (Scheme 3) for 20 hours. The organic layer was then separated and dried to leave **43** as a pale yellow solid in 79% yield.



Scheme 3

3.2.2.2 Route II

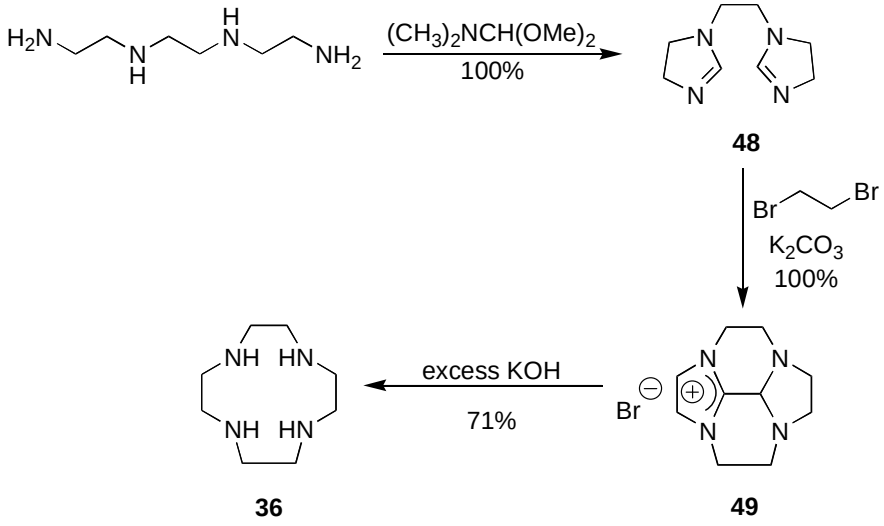
Using the same method, **43** was prepared from *N*,*N*',*N*''-tri(*p*-toluenesulfonyl)diethylene triamine **46** and *N*,*O*,*O*'-tri(*p*-toluenesulfonyl)diethanolamine **47**. In this case the reaction mixture was stirred and heated under reflux for seven days (Scheme 4). After work-up, **43** was obtained as a white solid in 98% yield.



Scheme 4

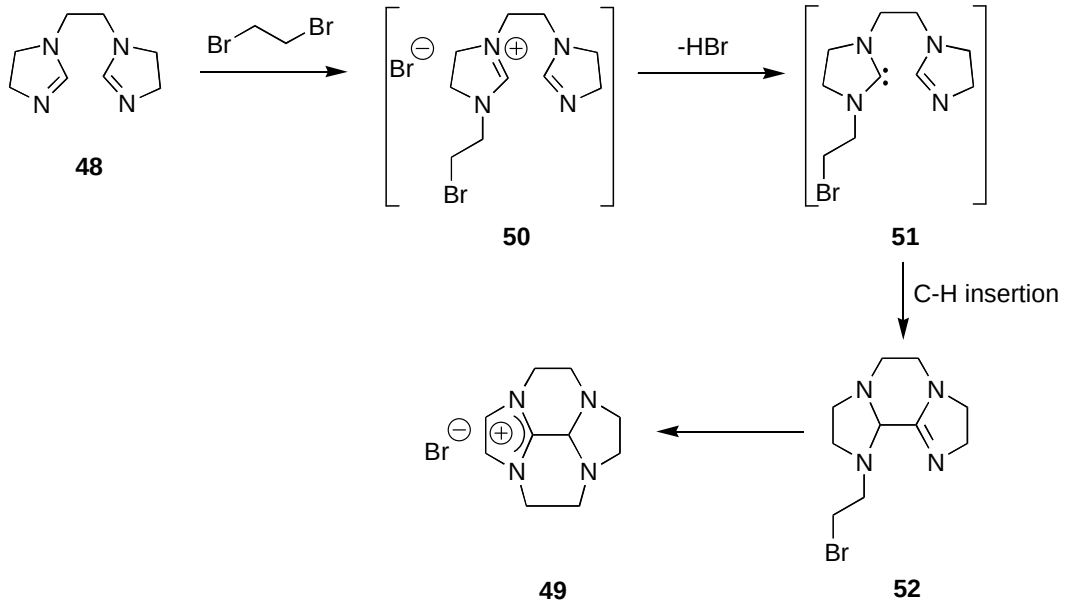
3.2.3 Facile Route Utilising Bis-imidazoline

By far the best method we found for the preparation of **36** on a large scale involved the use of a modern simple synthesis by Athey and Kiefer.[105] Around the time of this publication in the early 2000s, several new synthetic routes towards cyclen had been reported.[106-110] This method is a three-step route *via* bis-imidazoline **48** that can be carried out on a multigram scale (Scheme 5). The first step uses the reactivity of formamide acetals, previously known for converting primary amines and amides into amidines and acylated amidine respectively.[111] Bis-imidazoline **48** was prepared from triethylenetetraamine (TETA) and *N*,*N*-dimethylformamide dimethyl acetal under neat reaction conditions. Bis-imidazoline **48** can be recrystallised from THF although it can be taken onto the next step without further purification. Macrocyclisation of **48** with the dielectrophilic substrate 1,2-dibromoethane in acetonitrile with potassium carbonate gives the monoimidazolinium compound **49** in quantitative yield. Hot alkali hydrolysed **49** to afford 47 g of **36**, which is a 70% yield in three steps over two days.



Scheme 5

A possible stepwise mechanism for the generation of **49** from **48** is seen in Scheme 6. Bis-imidazoline **48** is alkylated by 1,2-dibromoethane producing imidazolinium intermediate **50**. **50** then loses hydrobromic acid to form carbene **51**, which undergoes an intramolecular carbene carbon-hydrogen insertion. This process will eventually lead to the electronically neutral intermediate **52**. This amidine may then readily displace the remaining bromine to form **49**. In comparison to the Richman-Atkins cyclisation, which goes through a 12-membered transition state for final macrocyclic ring closure, the mechanism from **52** to **49** passes through a conformationally favourable six-membered transition state.



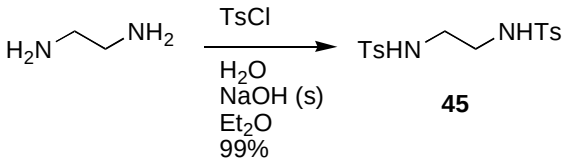
Scheme 6

3.2.4 Tosylation of Cyclisation Precursors

The starting materials for both the Richman-Atkins style cyclisation and the phase transfer conditions require tosyl protection of the corresponding amine or diol. Three different methods were investigated for the tosylation of the commercially available starting materials. Three trial reactions were carried out with *N*,*N*'-bis(2-hydroxyethyl)-ethylenediamine and the best method was then used to prepare all other tosylated reagents.

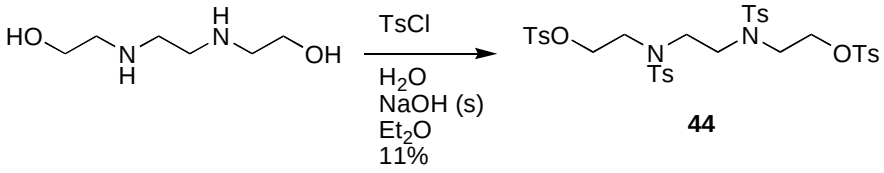
3.2.4.1 Route I

A previous member of the Robins group, F. Anderson, prepared *N*,*N*'-di(*p*-toluenesulfonyl)ethylenediamine **45**. Ethylenediamine was tosylated with *p*-toluenesulfonyl chloride in water, sodium hydroxide and diethyl ether (Scheme 7).[112]



Scheme 7

This method previously gave excellent yields but it was not so high yielding in our tosylation reactions. In the protection of *N*,*N*'-bis(2-hydroxyethyl)-ethylenediamine, this method achieved only 11% yield of product **44** (Scheme 8).

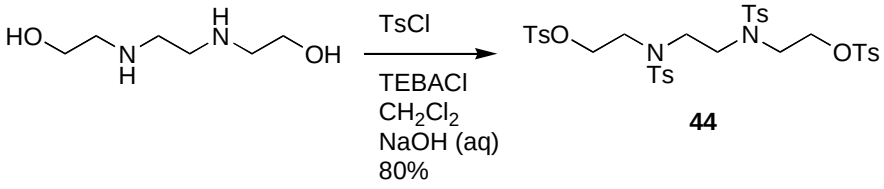


Scheme 8

3.2.4.2 Route II

Another method reported by F. Anderson was used to synthesise **44**.[112] A solution of *p*-toluenesulfonyl chloride in dichloromethane was added slowly to a stirred solution of *N*,*N*'-bis(2-hydroxyethyl)-ethylenediamine in dichloromethane (Scheme 9). A phase transfer catalyst benzyl triethylammonium chloride (TEBACl) was then added, followed by aqueous sodium hydroxide with vigorous stirring. After four hours at room temperature, the solution was poured onto water and the organic layer separated. Work-up gave the

product in good yields, however the product contained some non-tosylated starting material.

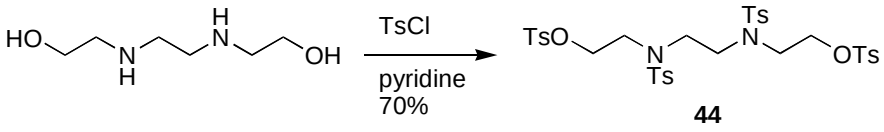


44

Scheme 9

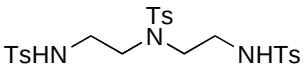
3.2.4.3 Route III

The most successful method for the synthesis of tosylated precursors followed a straightforward preparation by Dale *et al.*[113] The *N*,*N*'-bis(2-hydroxyethyl)-ethylenediamine was dissolved in dry pyridine at 0 ^{o}C, and a solution of *p*-toluenesulfonyl chloride in a small volume of dry pyridine was then added dropwise with stirring (Scheme 10). The reaction mixture was then stirred at room temperature overnight then poured into ice-water. The solid **44** was collected and recrystallised from ethanol in very high yields with excellent purity. This method was used to prepare **46** and **47** in 70% and 59% yields respectively.

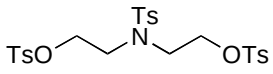


44

Scheme 10



46



47

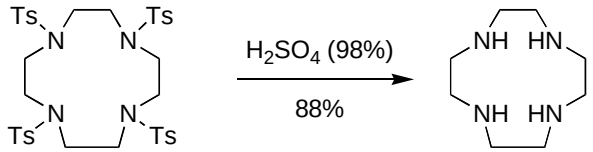
3.2.5 Tosyl Deprotection Strategies

In the case of cyclisation under phase transfer or Richman-Atkins conditions, the final step towards cyclen is the deprotection of the tosyl groups. There are a variety of methods described in the literature which include using concentrated sulfuric acid,[114] sodium/mercury amalgam,[115,116] potassium fluoride on alumina,[117] lithium aluminium hydride,[118] and hydrobromic acid of different concentrations in acetic acid with phenol.[101,104] However these harsh conditions result in very low yields (maximum 45%)[101]

and this makes the overall cyclisation method towards cyclen uneconomical. Two routes were employed to liberate the secondary amines of cyclen **36**.

3.2.5.1 Route I

In this method, **43** was heated at 110 ^{o}C in concentrated sulfuric acid for 40 hours before the flask was cooled in an ice bath and water was slowly added (Scheme 11).[101] Potassium hydroxide pellets were added to reach pH 13, then ethanol was added and the resulting precipitate was dissolved in hydrochloric acid and extracted with dichloromethane. The aqueous phase was basified with potassium hydroxide and extracted with chloroform. The combined organics were dried to yield a colourless oil. Recrystallisation from acetone gave cyclen **36** in 88% yield.

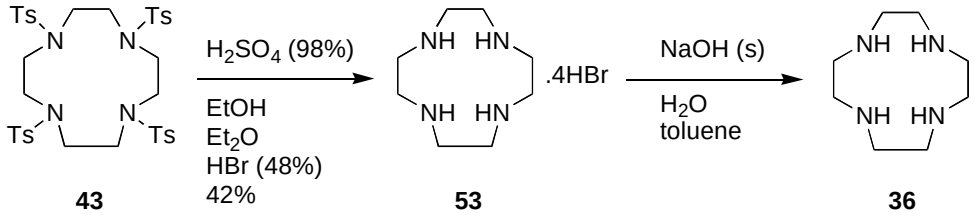


43 **36**

Scheme 11

3.2.5.2 Route II

A second method using hot sulfuric acid was more rapid (Scheme 12).[101] The sulfuric acid was heated to 165 ^{o}C before **43** was added. The solution was stirred vigorously at this temperature for six minutes before the flask was quickly cooled in an ice bath. The black liquid was added to a stirred flask of ethanol then diethyl ether was added and the solution was cooled to 0 ^{o}C. After filtration, a small volume of hot water and hydrobromic acid was added to the filtrate. Filtration removed the precipitated hydrobromide salt and the filtrate was concentrated *in vacuo* to give the tetrahydrobromide salt **53** in a modest 42% yield. To form the free base, **53** was stirred in an aqueous sodium hydroxide solution with toluene under reflux. A Dean-Stark apparatus was used to remove the water, and the toluene solution containing cyclen **36** can be decanted from the precipitated sodium bromide.[89]



43 **53** **36**

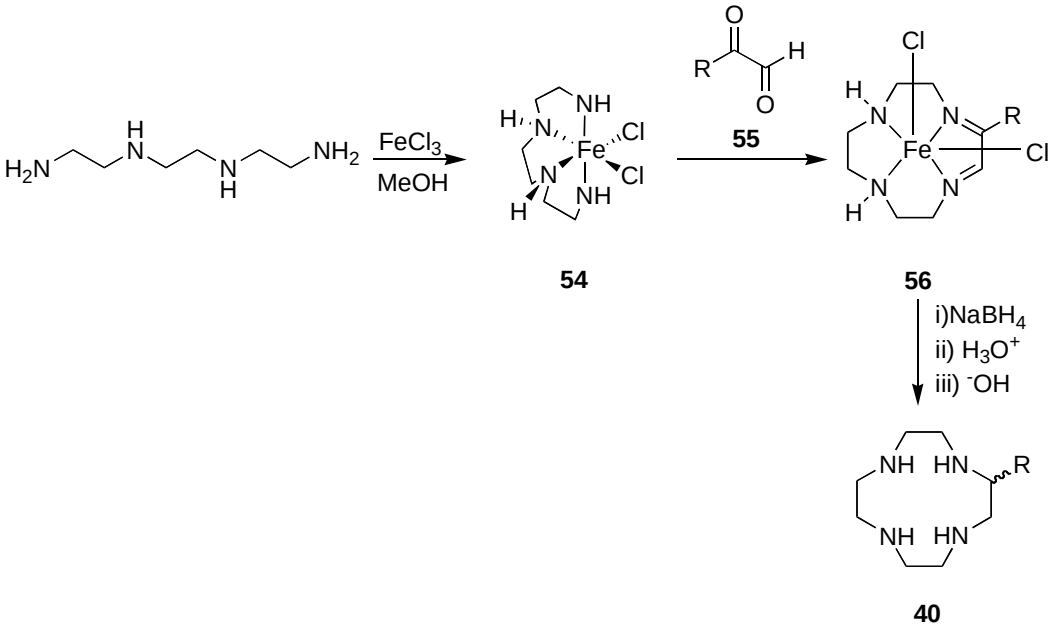
Scheme 12

3.3 General Strategy Towards *C*-Functionalised Derivatives of Cyclen

3.3.1 Efficient Metal-Templated Synthesis

Our goal was to find a route to *C*-substituted cyclen derivatives, and as in the preparation of other polyazamacrocycles, this normally requires high dilution syntheses using tosylamide cyclo-alkylation methodologies.[119-125] These reactions involve at least six steps with overall yields of 5-18%. However, a publication by Edlin and co-workers in 1996 reported that 12-membered tetraaza ring derivatives could be prepared using an efficient metal-templated strategy.[126]

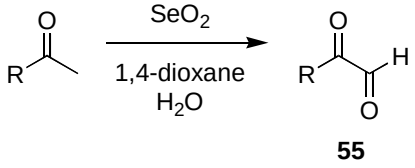
Triethylene tetraamine (TETA) forms well-defined *cis*-[$MLCl_2$] complexes with a range of first-row transition metals, and cyclo-co-condensation reactions with 1,2-dicarbonyl compounds are feasible.[127] The first step of the synthesis was to prepare the *cis*-[$FeLCl_2$]Cl complex by slowly adding a solution of TETA in methanol to a stirred solution of iron (III) chloride in methanol at room temperature (Scheme 13).[128] The diimine formed is likely to exist in the *cis*-conformation shown **54**, with the two terminal nitrogen atoms positioned in close proximity that would allow macrocyclisation. An aromatic glyoxal **55** was then added drop-wise in methanol, and the mixture was stirred for two hours. The intermediate **56** formed was generally a brown to green colour. After this time, an excess of sodium borohydride was added carefully to reduce the metal centre from iron (III) to iron (II). After heating and stirring the solution under reflux for four to 24 hours, acidic work-up removed the coordinated iron (II) ion. The racemic free base **40** was extracted using a chlorinated solvent in a high yield as an orange to dark brown viscous oil.



Scheme 13

3.3.2 Synthesis of 1,2-Dicarbonyl Compounds

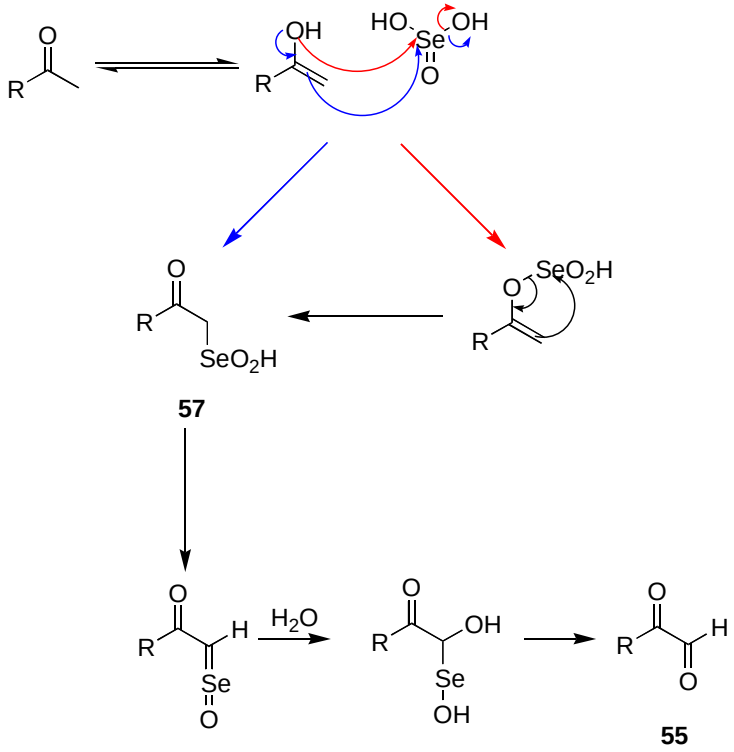
Although phenylglyoxal is commercially available, the other glyoxal derivatives required for the cyclisation process had to be prepared from the corresponding acetophenone. This was carried out by a simple preparation involving selenium dioxide oxidation in 1,4-dioxane and water using the commercially available acetophenones (Scheme 14).[129] These reactions could be followed to completion by thin layer chromatography and took up to 24 hours. Table 4 contains the glyoxal analogues prepared and the yields obtained.



Scheme 14

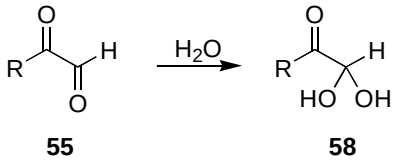
A publication in 2002 by Paul and co-workers showed that the same reactions could be completed using solvent-free microwave technology in seven to 10 minutes.[130] However in a number of test reactions it was found that microwave irradiation only gave partial conversion into the glyoxal and the microwave apparatus available could only be used to carry out reactions on a maximum 1.0 g scale. It was therefore more practical to use the traditional method.

The mechanism of α-oxidation of aldehydes and ketones by selenium dioxide has not been completely elucidated, and attack at both the carbon and oxygen of the enol has been proposed. The ketoselenic acid **57** formed in each case undergoes a Pummerer rearrangement, which gives the α-dicarbonyl compound **55** (Scheme 15).[131]



Scheme 15

Once the dicarbonyl was formed, it was converted to the di-hydrate compound **58** by heating and stirring in water (Scheme 16).[132] This often gave a crystalline products which were easier to handle than the glyoxal oils.

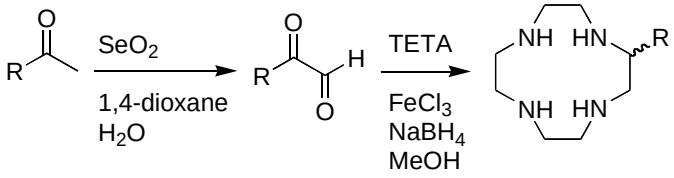


Scheme 16

3.4 Synthesis and Biological Evaluation of *C*-Functionalised Derivatives of Cyclen

3.4.1 Synthesis of First Library

Six aromatic glyoxal derivatives were prepared (**59** – **65**), and using the metal-templated strategy in Chapter 3.3, six substituted macrocycles (**66** – **72**) were synthesised (Table 4). Three of these compounds (**66**, **71**, **72**) have been made previously for lanthanide complexation.[126] No tetraazamacrocycles of this kind have been tested for antiparasitic activity.



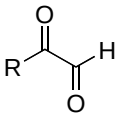
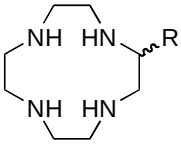
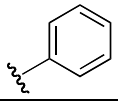
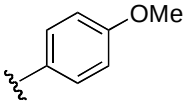
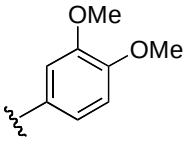
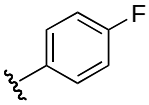
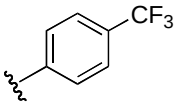
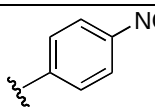
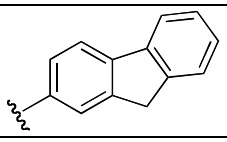
R	Glyoxal 	Yield (%)	Macrocycle 	Yield (%)	Novel?	Est. log P
	**59**	100	**66**	84	no[126]	-0.19
	**60**	100	**67**	83	yes	-0.11
	**61**	100	**68**	81	yes	-0.55
	**62**	100	**69**	80	yes	0.01
	**63**	100	**70**	64	yes	0.77
	**64**	80	**71**	67	no[126]	-0.37
	**65**	42	**72**	100	no[126]	1.83

Table 4

3.4.1.1 Log P

The estimated log P value for each compound is also included in Table 4. These were calculated using an online program.[133] Log P measurements show the degree to which the compound is partitioned between water and a non-miscible solvent such as octanol. P is the partition coefficient and it is defined as the ratio of concentration of the compound (as the neutral molecule) in aqueous phase to the concentration in the immiscible solvent. In practice the log P will vary according to the conditions under which it is measured and the choice of partitioning solvent. However, using this programme, we can get a general understanding about the variation in these values between the different analogues. Relationships between log P and biological activity are often found in series of compounds. Hansch in 1964 showed that these relationships are often parabolic, leading to an optimum value for the log P for a desired activity.[134] For both CNS and gastric absorption the relationship appears to be parabolic with an optimum log P value of around 2 ± 1 (Figure 16).[134,135]

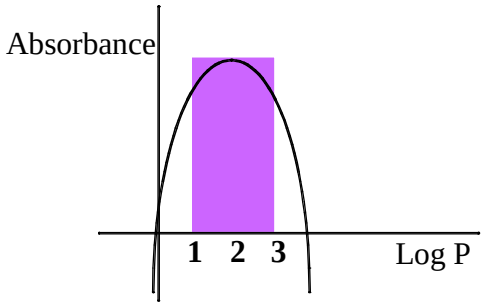
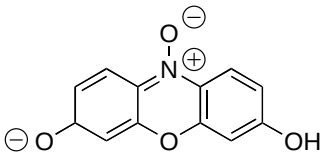


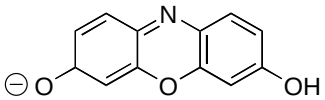
Figure 16

3.4.2 Biological Evaluation of 66 - 72

Compounds **66** - **72** were tested for antiparasitic activity against *Trypanosoma brucei* along with non-substituted cyclen **36** as reference using an Alamar Blue assay. Cultures of the parasites are treated with serial concentrations of the compounds and left for 48 hours at 26 ^{o}C before staining with Alamar Blue for 24 hours. Alamar Blue is a redox indicator that monitors the reducing environment of the cells.[136] When added to the cells, Alamar Blue is in the resazurin form **73** that is a deep purple colour. If the parasites are alive, the compound is converted to resorufin **74**, which is colourless.

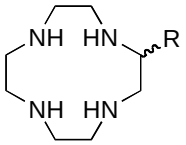


73



74

The IC_{50} values were calculated from the spectrophotometric data taken using a fluorimeter, and two compounds (**70** and **72**) were found to have activity at a concentration < 7 μM (Table 5). Interestingly the compounds with the highest activity correspond to those with the log P value in the range for optimum *in vivo* absorption.



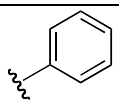
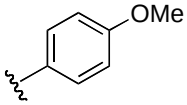
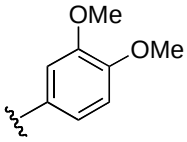
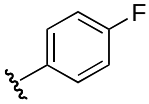
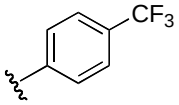
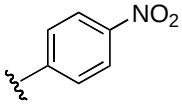
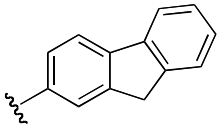
	R	Est. log P	*T. brucei* IC_{50} (μM)	HEK IC_{50} (μM)
36	H	-1.83	>200	5.0
66		-0.19	>200	>200
67		-0.11	>200	>200
68		-0.55	>200	>200
69		0.01	>200	>200
70		0.77	6.8	73
71		-0.37	>200	>200
72		1.83	2.6	26.0

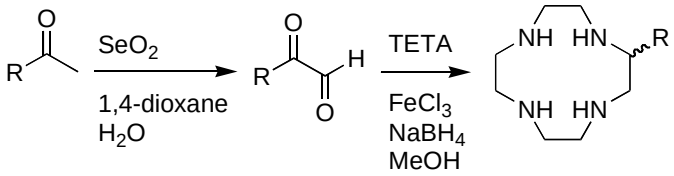
Table 5

To discover if these compounds have any parasite-selectivity, another Alamar Blue assay was carried out using Human Embryonic Kidney (HEK) cells. A selective drug would be active against the parasite but not against human cells. There is no dramatic parasite specificity observed between the results in Table 5, and therefore these compounds would

not make ideal antiparasite drugs. However, the outstanding activity of **72** prompted the synthesis of further analogues in an attempt to increase the activity and selectivity of the macrocycles against *T. brucei*.

3.4.3 Synthesis of Further Analogues

A further nine analogues were prepared, containing different heterocycles, larger aromatic moieties and a non-aromatic substituent, using the same methodology (Table 6).



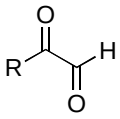
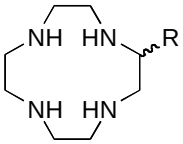
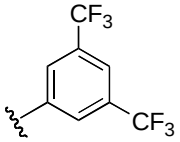
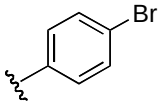
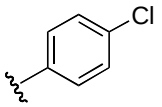
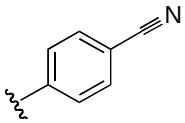
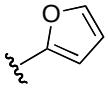
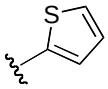
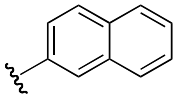
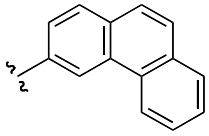
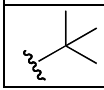
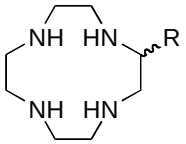
R	Glyoxal 	Yield (%)	Macrocycle 	Yield (%)	Novel?	Est. log P
	**75**	100	**84**	54	yes	1.73
	**76**	100	**85**	71	yes	0.70
	**77**	100	**86**	78	yes	0.77
	**78**	100	**87**	72	no[126]	-0.65
	**79**	75	**88**	54	yes	-0.82
	**80**	95	**89**	79	yes	-0.37
	**81**	83	**90**	77	yes	0.98
	**82**	43	**91**	79	yes	2.16
	**83**	100	**92**	63	yes	-0.05

Table 6

3.4.4 Biological Evaluation of 84 - 92

Compounds **84** – **92** were tested *in vitro* in the same way against *T. brucei* and HEK cells using Alamar Blue assays, and their IC_{50} values were calculated (Table 7).



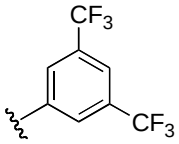
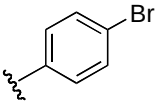
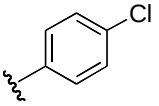
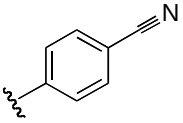
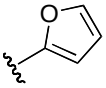
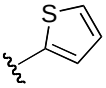
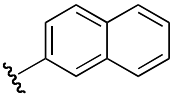
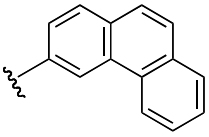
	R	Est. log P	*T. brucei* IC_{50} (μM)	HEK IC_{50} (μM)
84		1.73	6.8	61.0
85		0.70	21.8	>200
86		0.77	2.8	181.2
87		-0.65	>200	>200
88		-0.82	127.8	>200
89		-0.37	72.3	>200
90		0.98	6.9	>200
91		2.16	1.26	24.9
92		-0.05	133.0	>200

Table 7

Again, compounds with a log P value within the optimum range have shown the highest activity against *T. brucei* and HEK cells. **91** has the best antiparasite activity so far with an IC_{50} value of 1.26 μM. This permitted some *in vivo* testing that was carried out using mice infected with *T. brucei.* **91** was administered interperitoneally in 20mg/kg doses and an experiment using melarsoprol in 5mg/kg doses served as a control. Melarsoprol **7** cured all mice at 5 mg/kg, however **91** given at 20 mg/kg did not.

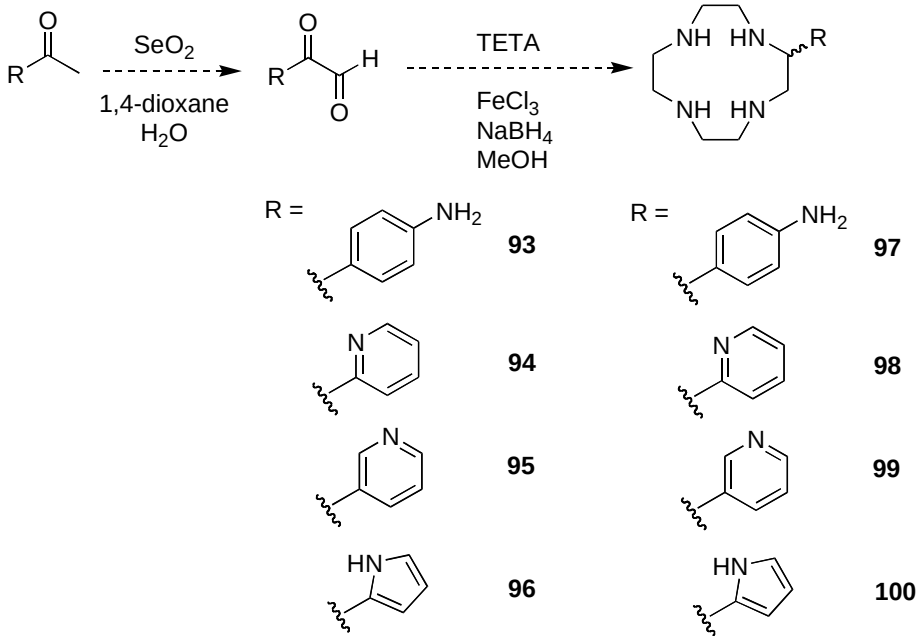
A number of compounds demonstrated some parasite-specificity: **85**, **86** and **90** are more active against *T. brucei* than the human cell line, however their activity is not potent enough to warrant further testing in animal models.

3.4.5 Unsuccessful Syntheses

In addition to the compounds produced in Chapters 3.4.1 and 3.4.3, the synthesis of other substituted tetraazamacrocycles was unsuccessfully attempted.

3.4.5.1 Amine Analogues

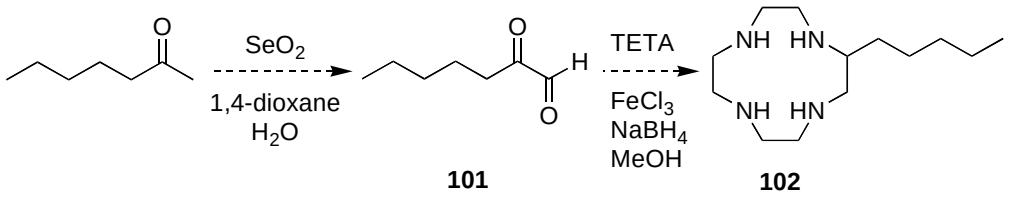
To complete the library of compounds, the synthesis of some nitrogen-containing compounds would be advantageous (Scheme 17). Unfortunately the amine function of the acetophenone starting materials was also oxidised by the selenium dioxide, resulting in degraded material. No product or starting material could be isolated in any case.



Scheme 17

3.4.5.2 Non-Aromatic Analogue

The synthesis of another non-aromatic analogue **102** was attempted, however the methyl ketone does not form a stable glyoxal **101** (Scheme 18). In the case of **83** however, we were able to form the glyoxal due to stabilisation by the tertiary carbon. Interestingly in Edlin's report of the metal-templated synthesis of tetraazamacrocycles, it was suggested that an aryl group was necessary to stabilise the diimine cyclisation intermediate.[128]

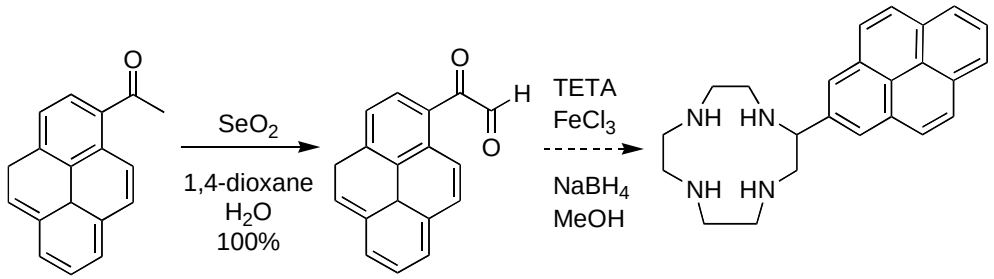


101 **102**

Scheme 18

3.4.5.3 Pyrene Derivative

The biological results indicate that the compounds with a log P value within the optimum range demonstrate the greatest toxicity. It was therefore desirable to synthesis the pyrene derivative **104** that has an estimated log P value of 2.82. It was postulated that it would also have high antiparasitic activity if it could cross the lipid bilayer of the parasite cell membrane by passive diffusion. 1-Acetylpyrene was successfully transformed into glyoxal **103** using selenium dioxide in quantitative yield, however the insolubility of **103** in methanol prevented the cyclisation (Scheme 19).

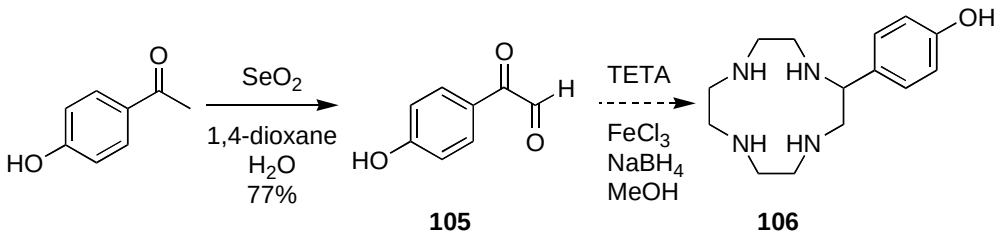


103 **104**

Scheme 19

3.4.5.4 Hydroxyl Analogue

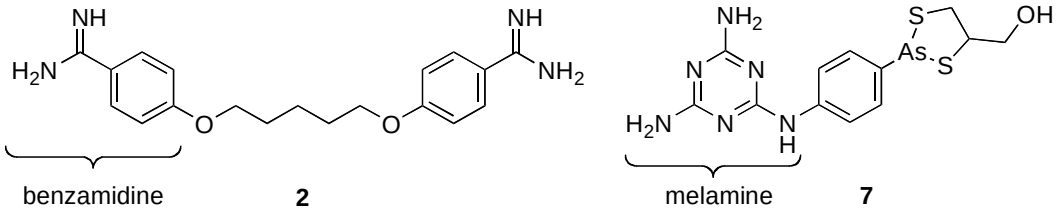
The synthesis of the substituted aromatic derivative bearing a hydroxyl group was attempted (Scheme 20). 4'-Hydroxyacetophenone was successfully converted into (4-hydroxy-phenyl)-oxo-acetaldehyde **105** using selenium dioxide in a good yield. The cyclisation step to form the macrocycle **106** however was unsuccessful for unknown reasons.



Scheme 20

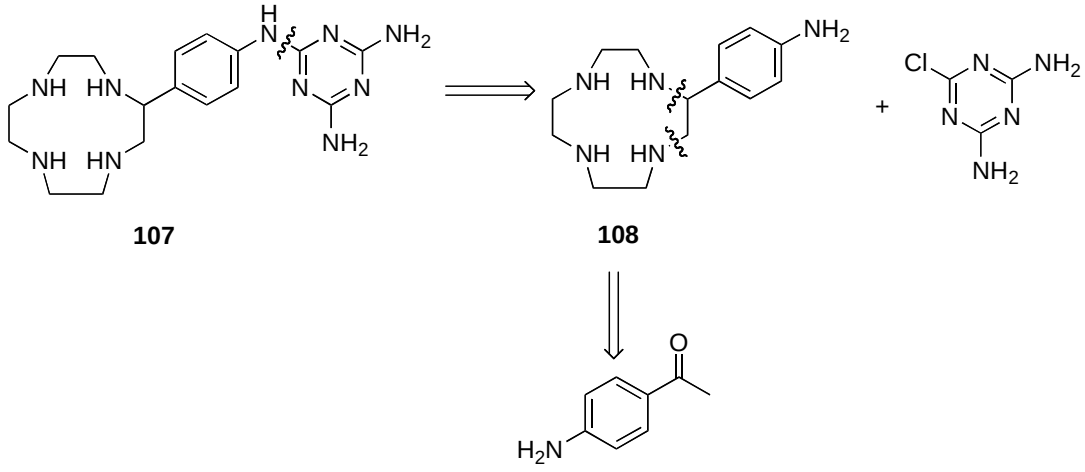
3.5 Synthesis of Parasite-Specific Analogues

The next synthetic aim was to prepare tetraazamacrocycles derivatives that contain parasite P2 recognition motifs, which are found in the HAT drugs pentamidine **2** and melarsoprol **7** (see Chapter 1).[20,35,37] Synthesising compounds with melamine or benzamidine motifs may increase the specificity and toxicity of these cyclen analogues.



3.5.1 Melamine Derivative

Target compound **107** was retrosynthesised to commercially available 4'-aminoacetophenone and 2-chloro-4,6-diamino-1,3,5-triazine (Scheme 21). The first step in the synthesis would require oxidation of the acetyl to a 1,2-dicarbonyl, however it has been previously shown that this is not possible as the selenium dioxide destroys the amine group (Chapter 3.4.5). Therefore a protecting group strategy was designed to protect the amine during the oxidation and cyclisation steps. Selective removal of the protecting group should allow the coupling of the melamine group to form **107**.

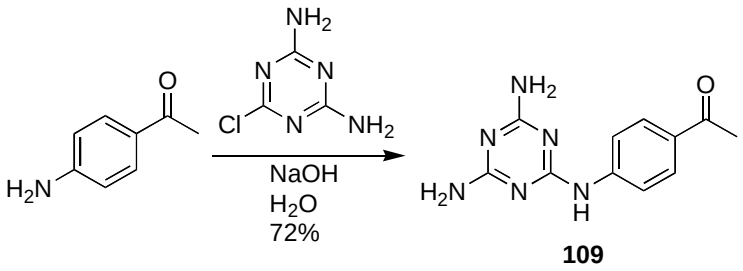


107 **108**

Scheme 21

3.5.1.1 Test Coupling

Firstly a trial coupling reaction was carried out using 2-chloro-4,6-diamino-1,3,5-triazine and 4'-aminoacetophenone under basic aqueous conditions to give **109** as a white solid (Scheme 22).[41,137,138]

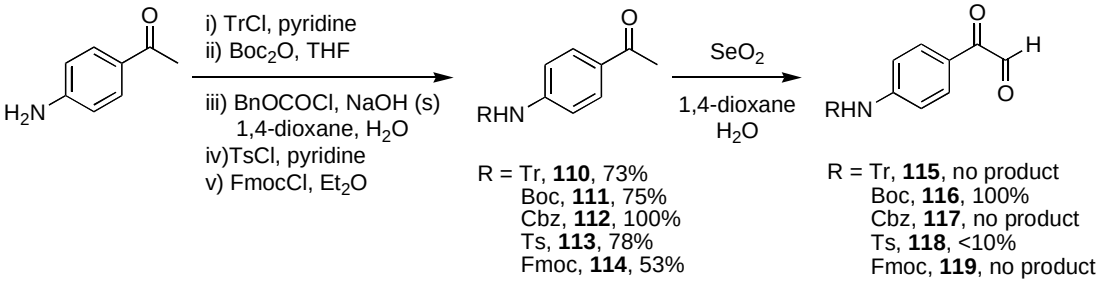


109

Scheme 22

3.5.1.2 Amine Protection

A number of different protecting groups were used to protect the amine functionality with varying success (Scheme 23).

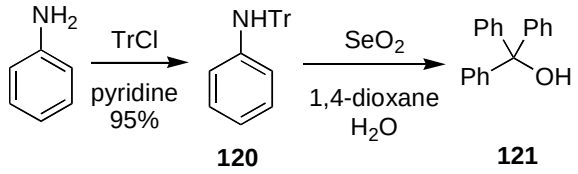


Scheme 23

Trityl

The first protecting group used was the trityl group. Amine **110** was synthesised from 4'-acetylbenzylamine and triphenylmethyl chloride in a good yield (Scheme 23).[139] The oxidation using selenium dioxide however was unsuccessful as the trityl group was removed under these conditions resulting in degradation of the compound. No starting material or product was isolated from this reaction.

Aniline was also trityl-protected to confirm that the selenium dioxide deprotects the trityl group and oxidises the amino group. **120** was formed in excellent yield under the same conditions, and then reaction with selenium dioxide gave triphenylmethyl hydroxide **121**, and signs of degraded aniline (Scheme 24).



Scheme 24

tert-*Butoxycarbonyl*

Using one equivalent of di-*tert*-butyl dicarbonate to protect 4'-aminoacetophenone gave Boc-protected amine **111** in a good yield after purification by flash chromatography (Scheme 23).[140] **111** was then successfully oxidised to give **116** in a quantitative yield.

Benzyloxycarbonyl

112 was synthesised in an excellent yield using benzylchloroformate however the Cbz group did not survive the oxidation step (Scheme 23).[141]

Tosyl

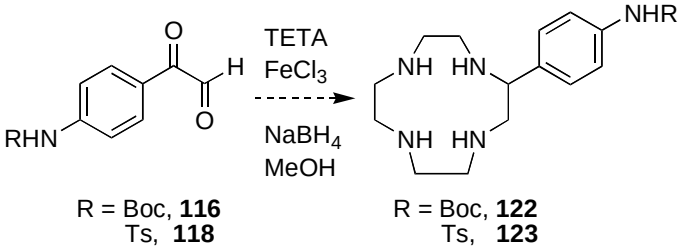
p-Toluenesulfonyl chloride was used to protect 4'-aminoacetophenone to give **113** (Scheme 23),[113] and then oxidation gave **118** in a very low yield.

9-Fluorenylmethoxycarbonyl

9-Fluorenylmethylchloroformate was used to protect the amine in moderate yields **114** (Scheme 23).[142] Due to time constrains towards the end of this project, the oxidation was attempted but the product was not isolated. Thin layer chromatography of the reaction mixture showed only starting material after stirring and heating for a number of days, and there were also signs of degraded amine.

3.5.1.3 Cyclisation

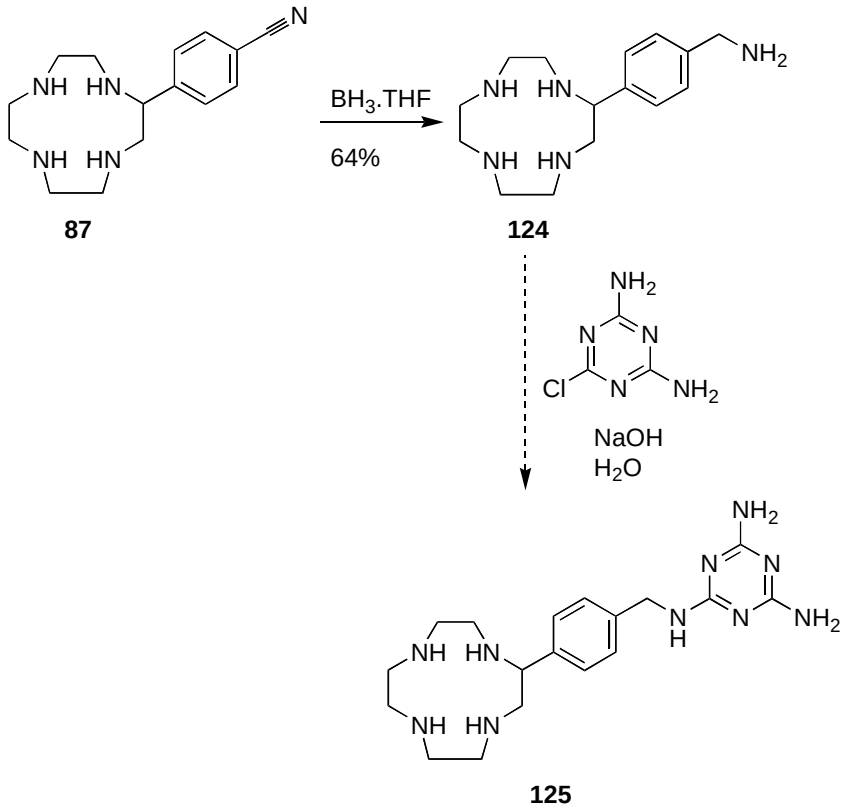
The only groups that survived the oxidation step from the five different protecting strategies were Boc and tosyl. The next cyclisation step using the metal-template strategy however was not successful and no product was isolated in either case (Scheme 25). It is thought that the harsh reduction conditions followed by work-up using concentrated hydrochloric acid may have removed the protecting group resulting in the product being too polar to extract into the chlorinated solvent.



Scheme 25

3.5.1.4 Another Route

Although the protecting group strategy was not successful, another way to synthesise an amino-derivative is by reducing the nitrile derivative **87** prepared earlier. This was done using borane, to give **124** in a good yield as a cream, viscous oil (Scheme 26).[143] When the coupling reaction was carried out under the same conditions as before using 2-chloro-4,6-diamino-1,3,5-triazine,[41,137,138] the target material **125** could not be isolated. At this stage we moved away from the melamine-based compounds as their solubility in test reactions was a problem. It is unlikely that **125** would dissolve in water or DMSO for *in vitro* testing.



Scheme 26

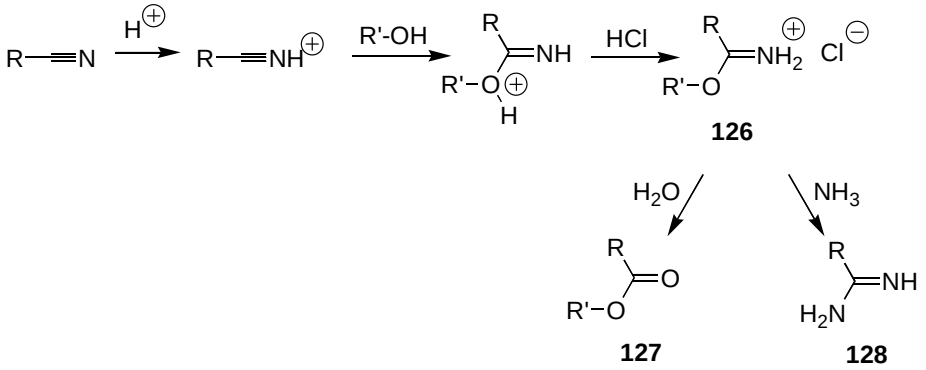
3.5.2 Benzamidine Derivative

The amidine functionality has been found in many natural products, and amidine-containing compounds are often a critical part of many biological processes. Substituted amidines are useful intermediates in the synthesis of many heterocyclic compounds, and consequently a plethora of methods has been developed for their preparation.[111,144] The most common route for synthesis are from amides, nitriles or thioamides.[145-149] This section describes the strategies attempted for the preparation of benzamidine-substituted macrocycles.

3.5.2.1 Pinner

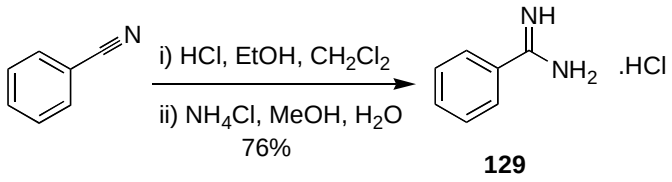
The Pinner reaction is an organic reaction of a nitrile with an alcohol under acid catalysis.[150] If hydrochloric acid is used for example, the product formed is the hydrochloric acid salt of an imino ester or an alkyl imidate **126**. These "Pinner salts" can react with an excess of alcohol to form the ortho-ester, with water to form an ester **127**, or with ammonia or an amine to form an amidine **128** (Scheme 27). A. Pinner developed this reaction and studied the nature of imidates in the late 19th century.[151,152] The reaction is

normally carried out at 0 °C in an anhydrous solvent such as chloroform or diethyl ether, although an excess of alcohol can also be used as the diluent in some cases.[111,144,150]



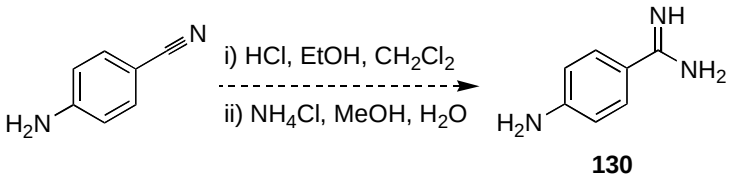
Scheme 27

A trial reaction under Pinner conditions was carried out using benzonitrile (Scheme 28).[153] Benzonitrile was dissolved in dry dichloromethane and two equivalents of absolute ethanol were added. The solution was cooled to -8 °C using a sodium chloride-ice bath, and then the solution was saturated with anhydrous hydrochloric acid. Addition of diethyl ether led to a precipitate that was extracted from the basic solution to give an oil. Ammonium chloride in aqueous methanol was added and the mixture was heated under reflux for six hours. After work-up, benzamidine **129** was achieved as a white solid in good yield.



Scheme 28

Before this reaction was carried out with macrocycle **87**, another test reaction was carried out using an amine. 4'-Aminobenzonitrile was under the same conditions as before, however 100% starting material was recovered (Scheme 29). It is understood that the first step using hydrochloric acid formed the salt of the amine, which precipitated out of solution and the imidate did not form. Thus work-up just produced the free base of the starting material. This reaction was repeated a number of times using alcohol as the solvent in an attempt to dissolve the amine salt formed, and the volume of hydrochloric acid bubbled through the solution was increased, however we did not isolate any 4'-amino benzamidine **130**.

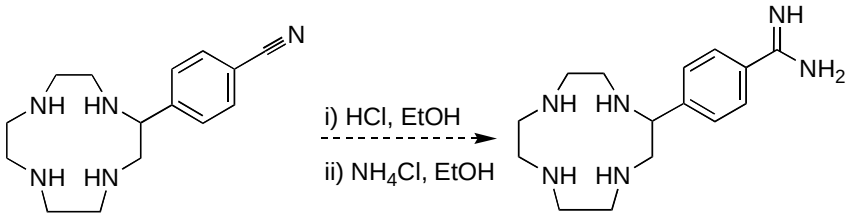


130

Scheme 29

In the paper "The Chemistry of Imidates", Roger and Neilson mention that if the nitriles have an amino group it should be protected (using *p*-toluenesulfonyl chloride) to prevent precipitation of the aminonitrile hydrohalide.[150] Protecting the amines in our macrocyclic starting material is possible, however it adds an extra two steps into the synthesis, and as mentioned previously, tosyl protecting groups are notoriously difficult to remove.

Baksheev and Gavrilov published the successful preparation of imido esters of amino acids by using methanol saturated with hydrochloric acid to react with aminonitrile hydrochlorides.[154] Using an adapted method we attempted the synthesis of our target compound **131** (Scheme 30).[155] **87** was cooled to -8 ^{o}C in absolute ethanol, and hydrochloric acid was bubbled through the solution for 25 mins. The concentrated product in ethanol was heated at 60 ^{o}C with ammonium chloride and the yellow solid obtained after work-up consisted mainly of ammonium chloride and starting material but no target material. The Pinner strategy towards our target material **131** was therefore unsuccessful.



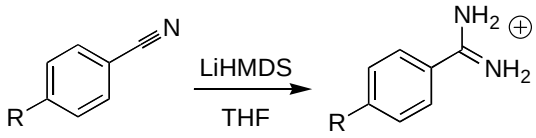
87 **131**

Scheme 30

3.5.2.2 LiHMDS

A number of pentamidine and other amidine-containing analogues needed for biological activity have been prepared by the conversion of a nitrile functional groups into amidines using lithium hexamethyldisilazide (LiHMDS).[156,157] LiHMDS is a strong base which is employed as a metallating agent. LiHMDS can be prepared *in situ* from *n*-BuLi and 1,1,1,3,3,3-hexamethyldisilazane in THF,[158] however we used the commercially available reagent. A number of successful test reactions were carried out with benzonitrile and 4'-

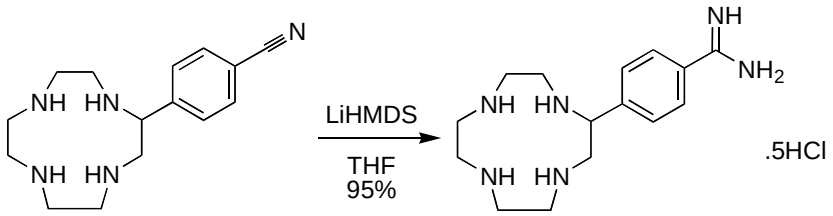
acetobenzonitrile using two equivalents of LiHMDS at -78 ^{o}C (Scheme 31). Following work-up, both benzamidine **129** and 4'-acetyl benzamidine **132** were isolated in good yields with high purity.



R = H, **129**, 64%
$COCH_3$, **132**, 65%

Scheme 31

This method was then attempted using macrocycle **87**, however a number of modifications were required to complete the reaction. Using two equivalents of LiHMDS gave only starting material, and increasing the number of equivalents to 5.5 only produced a small amount of product. After a number of unsuccessful attempts involving changing the amount of reagents, temperature and reaction time, amidine **131** was synthesised in excellent yield (Scheme 32). Five equivalents of LiHMDS were used at -78 ^{o}C and the worked up product was treated with hydrochloric acid in diethyl ether. The product was recrystallised from ethanol and diethyl ether to give **131** as a pentahydrochloride salt. This extremely hygroscopic product had to be handled carefully under anhydrous conditions. Characterisation of the product was problematic as the product could only be identified by ^{13}C NMR spectroscopic data. The nitrile quaternary carbon of **87** comes at δ 111.2 (Figure 17) and the amidine quaternary carbon of **131** comes at δ 163.3 (Figure 18).



87 **131**

Scheme 32

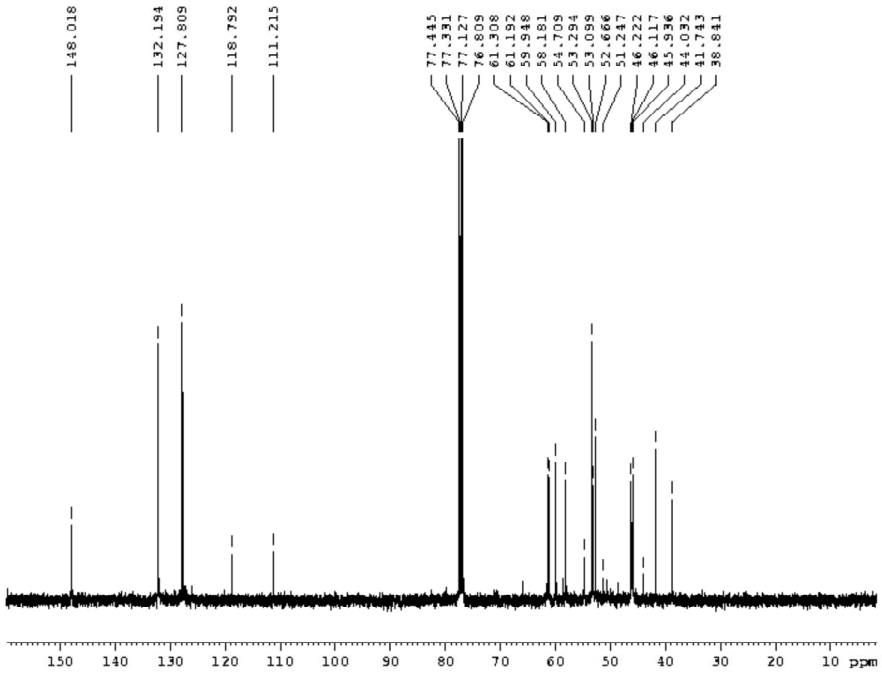


Figure 17

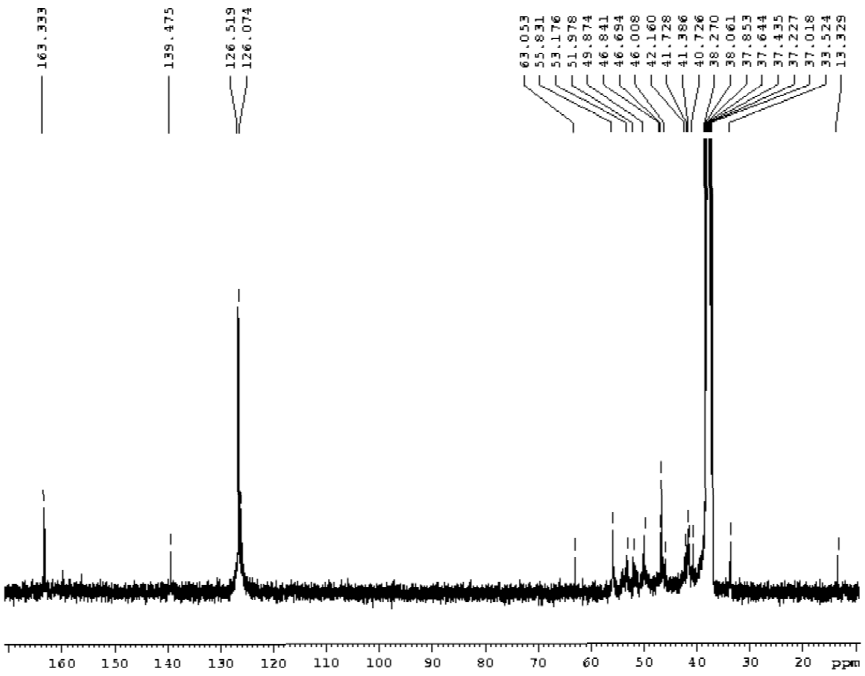
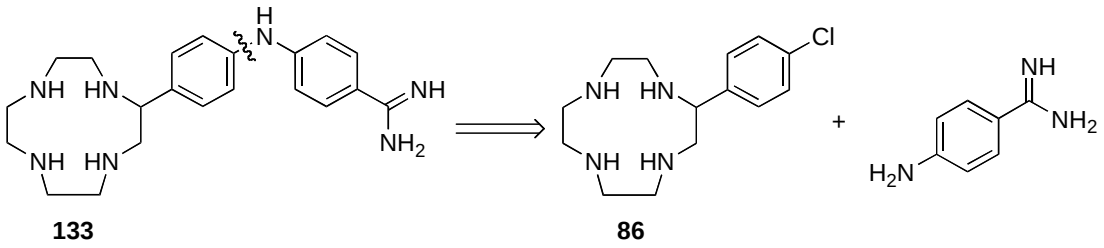


Figure 18

3.5.2.3 Buchwald-Hartwig Amination

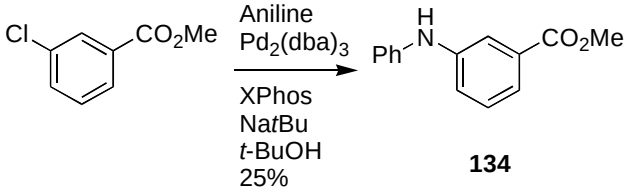
Another target benzamidine compound is **133**, which can be retrosynthesised to macrocycle **86** and commercially available 4'-aminobenzamidine (Scheme 33).



133 **86**

Scheme 33

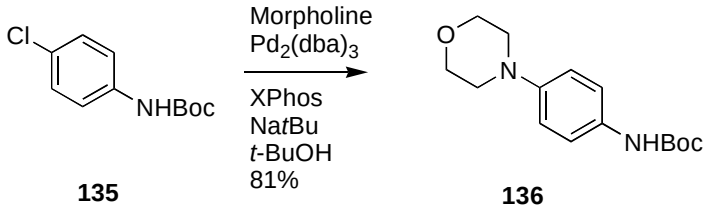
A publication by Huang *et al.* in 2003 described how a range of different compounds can undergo palladium-catalysed amination reactions.[159] A test reaction was carried out to synthesise methyl 3-phenylaminobenzoate **134** (Scheme 34). This reaction was done in a Schlenk tube using $Pd_2(dba)_3$ and the XPhos ligand under argon in a modest yield.[159,160]



134

Scheme 34

In Huang's publication, this strategy had been successfully used to couple Boc-protected amines without removing the protecting group (Scheme 35).[159]

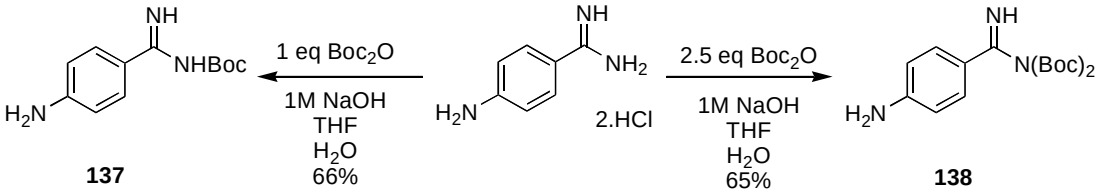


135 **136**

Scheme 35

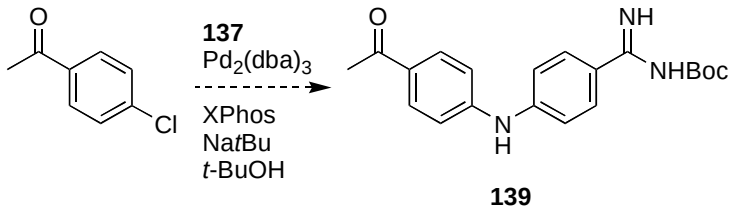
The aim therefore was to couple macrocycle **86** with Boc-protected 4'-aminobenzamidine under these conditions before removing the Boc group to give the benzamidine compound. The hydrochloride salt of 4'-aminobenzamidine was Boc-protected using di-*tert*-butyl dicarbonate under basic conditions (Scheme 36). The primary amine of the benzamidine is

the most reactive and so the Boc protecting group was chosen to allow C-N bond formation at the aromatic amine.[161] Both the mono- **137** and di-Boc **138** protected amines were prepared,[161,162] however mono-protected **137** was used in all coupling reactions.

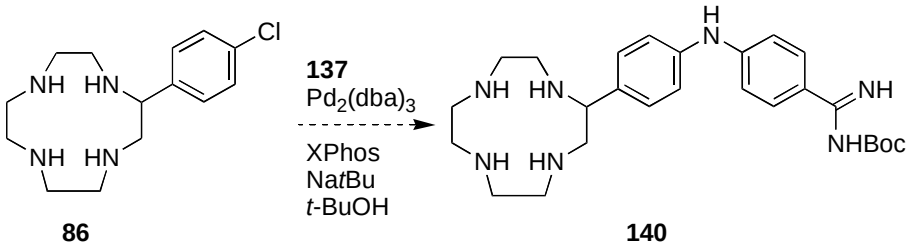


Scheme 36

Unfortunately amination reactions carried out with 4'-chloroacetophenone and **137** (Scheme 37), and **86** with **137** (Scheme 38), were both unsuccessful and therefore this amination route was abandoned.



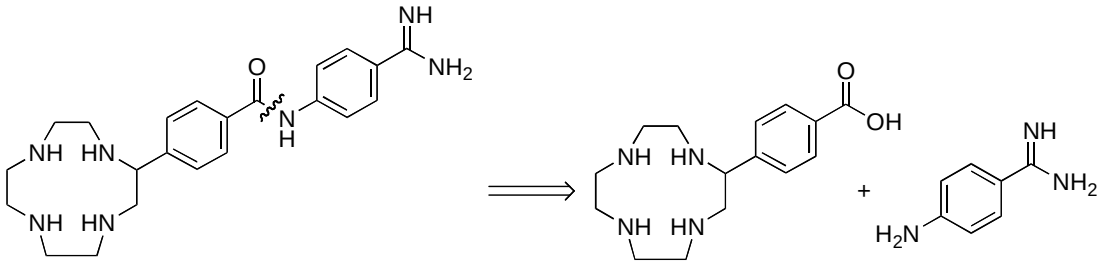
Scheme 37



Scheme 38

3.5.2.4 Amide Coupling

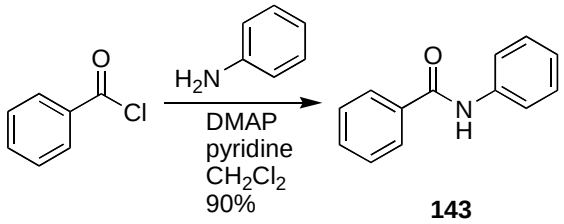
The benzamidine moiety may also be coupled to the macrocycle *via* an amide bond. Target material **141** can be retrosynthesised to carboxylic acid **142** and 4'-aminobenzamidine (Scheme 39).



141 **142**

Scheme 39

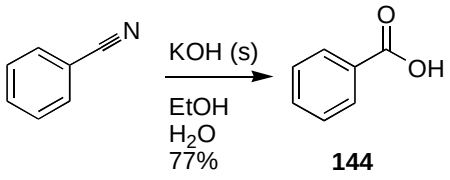
A test reaction to find the optimum conditions for amide coupling was carried out using aniline and benzoyl chloride to give benzanilide **143** (Scheme 40).[163]



143

Scheme 40

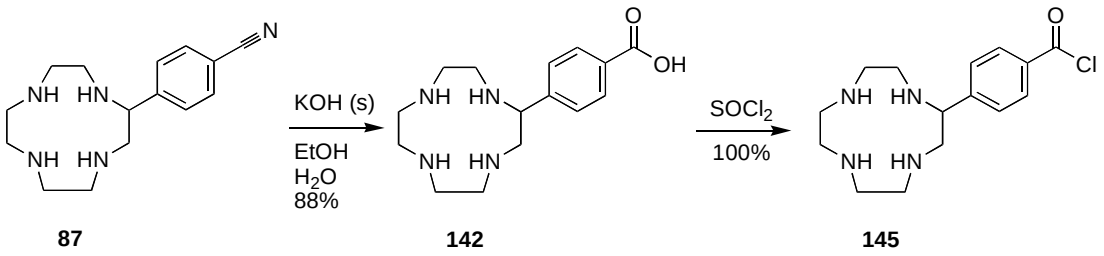
A trial reaction was also carried out to find the best conditions for the hydrolysis of a nitrile to a carboxylic acid functional group. This was done with benzonitrile using potassium hydroxide (Scheme 41).[164] Work-up gave beautiful white crystals of benzoic acid **144** in a good yield.



144

Scheme 41

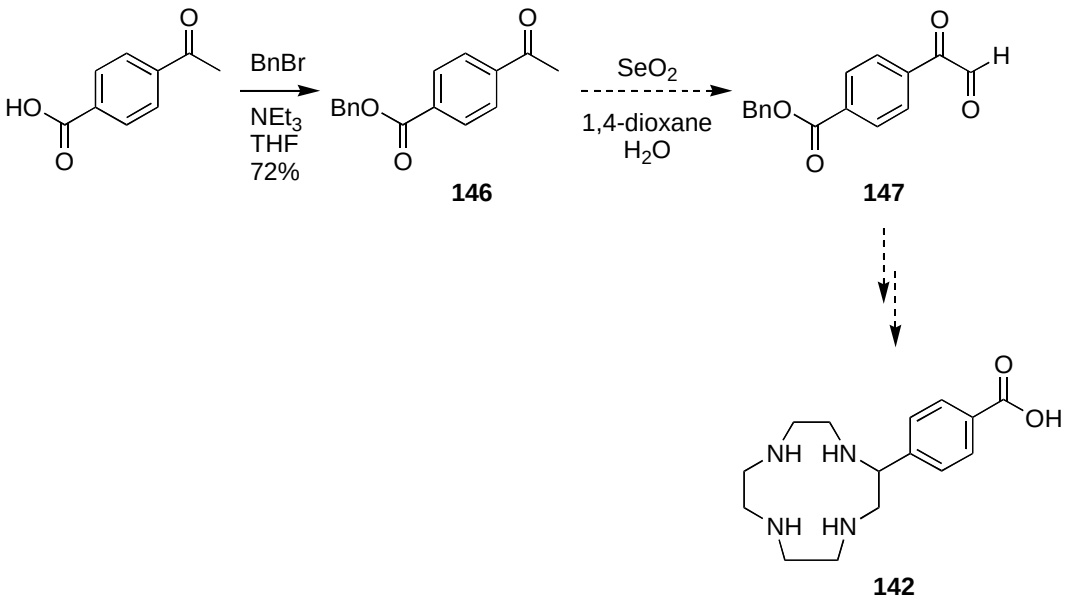
142 was prepared directly from **87** under the same conditions in an excellent yield, before **142** was converted into the acid chloride **145** using thionyl chloride (Scheme 42).[165]



87 **142** **145**

Scheme 42

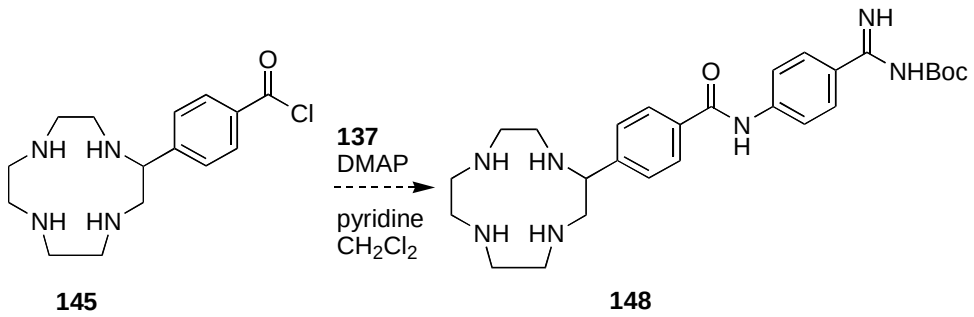
Another route toward carboxylic acid was studied (Scheme 43). 4'-Acetyl benzoic acid was protected using benzyl bromide to give **146** in a good yield before carrying out an oxidation using selenium dioxide.[166] It was thought that glyoxal **147** could be cyclised and then deprotected to give **142**. This route was undesirable for a number of reasons: the starting material was reasonably expensive; glyoxal **147** required further purification by flash chromatography that resulted in low yields; and the overall scheme required the extra protection and deprotection steps which were time consuming. This route was therefore incomplete and not used for the production of **142**.



146 **147** **142**

Scheme 43

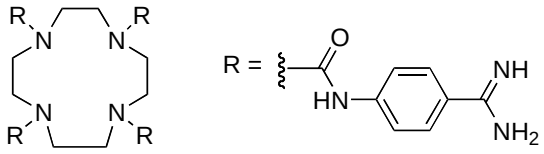
Acid chloride **145** was carried straight onto the next step without purification or characterisation and reacted with **137** using DMAP (Scheme 44). Unfortunately after work-up the ^{1}H and ^{13}C NMR spectroscopic analysis revealed only the Boc-protected starting material **137**, and the aqueous phase contained the macrocycle **145**. This route towards a benzamidine derivative was therefore discarded.



Scheme 44

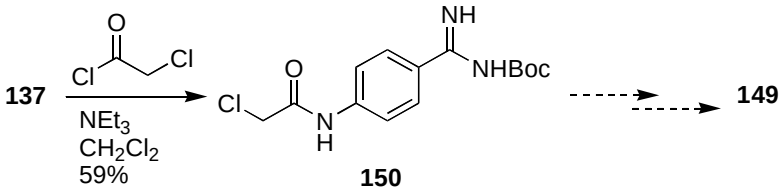
3.5.2.5 Four Benzamidine Moieties

Another benzamidine-containing target compound **149** is a non-substituted azamacrocycle with four benzamidine moieties connected through the nitrogen atoms.



149

Using a method used to prepare a number of trypanocides, Boc-protected **137** was coupled with chloroacetyl chloride using triethylamine in dichloromethane in a good yield (Scheme 45).[161] The coupling of **150** with cyclen **36** was unsuccessful. We were unable to obtain any analysis from the product despite numerous recrystallisations from various solvent systems. Due to time constraints this route was also discarded.

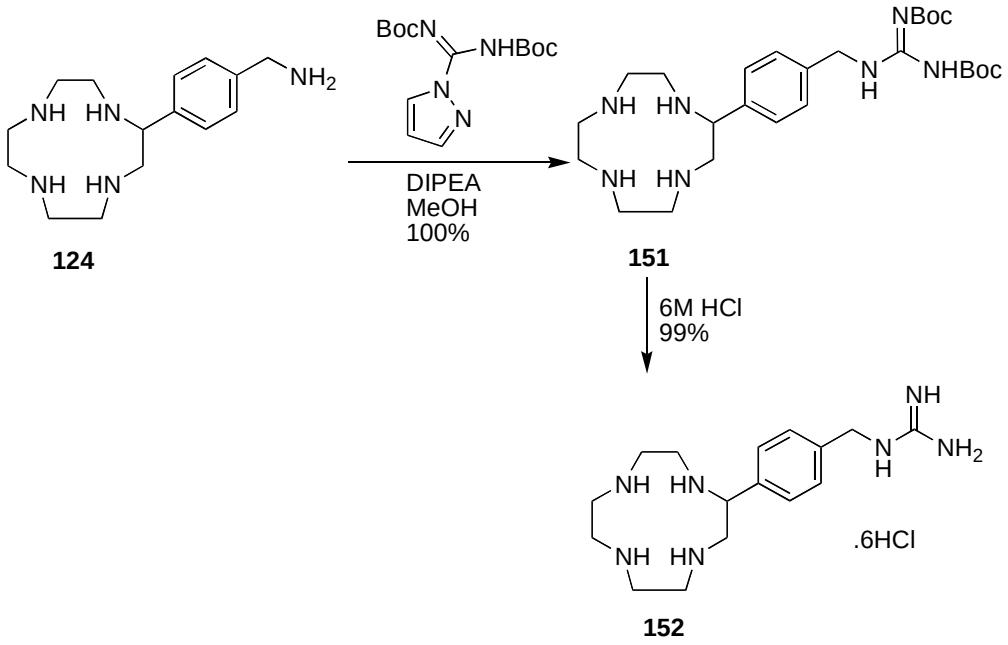


Scheme 45

3.5.3 Guanidine Derivative

A guanidine derivative **152** (which also contains the P2 recognition motif) was prepared using amine **124**, *N*,*N*'-di-Boc-1*H*-pyrazole-1-carboxamidine and *N*,*N*-diisopropylethylamine (Scheme 46).[167] The Boc protecting groups were removed from **151**

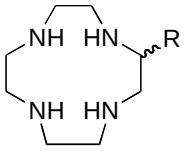
using hydrochloric acid. The yellow residue was recrystallised from methanol and diethyl ether in excellent yield to give the hexahydrochloride salt **152** as a white solid.



Scheme 46

3.5.4 Biological Evaluation of Parasite-Specific Analogues

Two tetraazamacrocycles containing a trypanosome P2 purine transporter recognition motif were prepared (**131** and **152**), along with two other novel macrocycles (**124** and **142**). These four compounds were tested for antiparasite activity, and toxicity against HEK cells (Table 8).



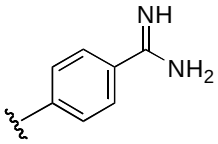
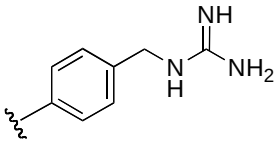
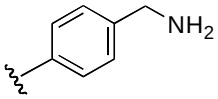
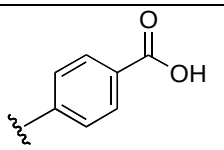
	R	Est. log P	*T. brucei* IC_{50} (μM)	HEK IC_{50} (μM)
131		-1.44	22.0	>200
152		-1.64	41.2	>200
124		-1.12	>200	>200
142		-0.31	>200	>200

Table 8

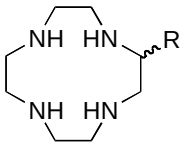
Compounds **124** and **142** unsurprisingly show no activity against trypanosomes or the human cell line, as their log P values suggest that they would not be taken up *in vitro*. **131** and **152** however show trypanocidal activity but are ineffective against the HEK cells. Both compounds have log P values outwith the optimum range for passive diffusion, therefore the P2 recognition motif has caused the parasites to transport the compounds actively across the cell wall. The absence of a P2 transporter in the mammalian cells means that they are unaffected by the drug dose of **131** and **152**.

3.6 Summary of Synthetic Achievements

Cyclen **36** was prepared in large quantities for synthesis of anti-cancer alkylating agents, and as a standard compound for all biological testing. Twenty racemic *C*-substituted analogues were prepared using an iron-template method. Fourteen of these are novel, including two analogues containing a P2 recognition motif required for parasite-specific activity. The synthesis of a number of other parasite-specific analogues was investigated without success.

3.7 Biological Evaluation

All 20 compounds were tested as their hydrochloride salts against *T. brucei* and HEK cells along with non-substituted cyclen **36**. They were also tested against the malarial parasite *Plasmodium falciparum,* and a handful were tested for oligopeptidase B (OPB) inhibition. Table 9 contains the full list of compounds and their biological test results. Fluorescence experiments and a study of the effects of polyamines were also carried out later in an attempt to elucidate the mode of antiparasitic action.



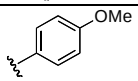
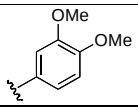
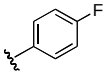
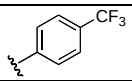
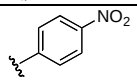
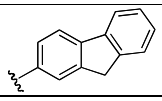
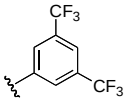
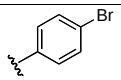
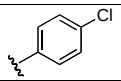
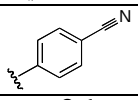
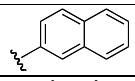
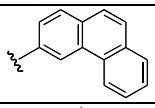
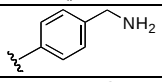
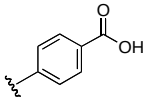
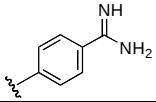
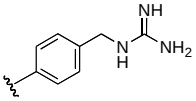
	R	Est. log P	*T. brucei* IC_{50} (μM)	HEK IC_{50} (μM)	*P. falciparum* IC_{50} (μM)	OPB IC_{50} (μM)
36	H	-1.83	>200	5.0	>100	0.19
66		-0.19	>200	>200	26.0	-
67		-0.11	>200	>200	26.0	-
68		-0.55	>200	>200	>100	-
69		0.01	>200	>200	24.0	-
70		0.77	6.8	73	5.0	-
71		-0.37	>200	>200	13.0	-
72		1.83	2.6	26.0	1.3	0.24
84		1.73	6.8	61.0	4.8	-
85		0.70	21.8	>200	5.8	-
86		0.77	2.8	181.2	2.3	-
87		-0.65	>200	>200	>100	-
88		-0.82	127.8	>200	>100	5.08
89		-0.37	72.3	>200	46.7	-
90		0.98	6.9	>200	1.7	0.27
91		2.16	1.26	24.9	<1.5	0.27
92		-0.05	133.0	>200	>100	-
124		-1.12	>200	>200	>100	-
142		-0.31	>200	>200	>100	-
131		-1.44	22.0	>200	>100	0.36
152		-1.64	41.2	>200	>100	-

Table 9

3.7.1 Trypanosoma brucei

In general from the test results it can be hypothesised that the log P value plays a large part in the activity of the compound. In general, those compounds with a log P value within the optimum range for gastric and CNS absorption have the most striking activity. **72**, **86** and **91** showed the greatest toxicity. Figure 19 contains an example IC_{50} curve, which represents the activity of **91**.

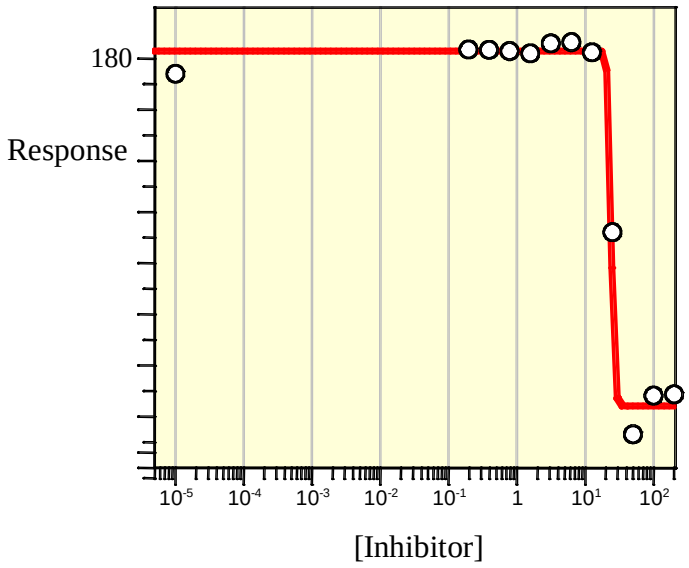


Figure 19

Incorporating the trypanosome P2 purine recognition motif onto **131** and **152** has allowed the parasites to uptake compounds which otherwise could not have passively diffused into the cells. Adding the recognition motif did not however improve on the toxicity of **91**. **91** was tested *in vivo* against *T. brucei* by Dr Vanessa Yardley (London School of Hygiene and Tropical Medicine), however a dose of 20 mg/kg of **91** did not cure trypanosomiasis.

3.7.2 Human Embryonic Kidney (HEK) Cells

Alamar Blue assays determining the activity of the compounds against HEK cells highlights any selectivity of the compounds towards the parasites. A selective drug should have toxicity against *T. brucei* at less than 1-2 μM, but would be inactive against human cell lines. The most active drugs (**72**, **86** and **91**) against *T. brucei* also show high activity against HEK cells, and the compounds with the greatest parasite-selectivity are **84**, **85**, **90**, **131** and **152**. The compounds with the P2 recognition motif (**131** and **152**) display parasite

selectivity, but their activity against the trypanosomes is not high enough to permit *in vivo* testing.

3.7.3 Plasmodium falciparum *(Malaria)*

Plasmodium flaciparum is one of four species of *Plasmodium* protozoa that cause malaria. Malaria is a parasitic disease transmitted by the *Anopheles* mosquito, which affects approximately 300 million people worldwide, with at least 90% of cases occurring in tropical Africa. Every year between 1 and 1.5 million people die from malaria.[168]

E.-M. Patzewitz from the group of Prof. S. Muller (University of Glasgow) tested all 21 tetraazamacrocycles against *P. falciparum*. The results in Table 9 show that analogues with large aromatic moieties, and electronegative heteroatoms, have the greatest activity. **91** displayed the best activity with an IC_{50} value lower than 1.5 μM, and this assay should be repeated to obtain a more accurate result. Interestingly the compounds containing the trypanosome P2 recognition motif (**131** and **152**) show no activity against the malaria parasite.

3.7.4 Oligopeptidase B (OPB)

OPB is a serine peptidase with a substrate specificity similar to trypsin, which is a major digestive enzyme. OPB hydrolyses peptide bonds in low molecular mass peptides on the carboxy side of basic residues (for example arginine and lysine).[169] No crystal structure for OPB is available, however the structure of the closely related prolyl oligopeptidase has been determined. This revealed the presence of two domains; an N-terminal propeller domain, and a C-terminal hydrolase domain, which contains the substrate-binding and catalytic residues.

N. Bland working for Prof. J. Mottram and Prof. G. Coombs (Universities of Glasgow and Strathclyde) used some of these macrocyclic polyamines to study the inhibition of OPB cloned from *Leishmania major*. Although the compounds are not exceedingly active (Table 9), they do inhibit the enzyme no matter what substituent is present, which is surprising as polyamines have previously been shown to be mild stimulants of these types of enzymes.[169] Therefore it is hypothesised that there is a non-substrate-like interaction occurring, for example at an allosteric site such as the pore of the propeller domain. The compounds may block access to the active site of the enzyme.

3.7.5 Fluorescence

Drug analogues **72**, **90** and **91**, which displayed the greatest *in vitro* activity, also fluoresce at certain wavelengths. A number of experiments were carried out to determine where the compound accumulates within the parasite as it causes toxicity. Using a fluorescence microscope, tests were carried out using **72** and **91** on wild type *T. brucei* 427, mutant clone knockout *T. brucei ΔTbat1* (P2 purine transporter null), and *Leishmania mexicana*.

The trypanosomes were viewed under normal light (DIC) and then excited at different wavelengths to view fluorescence. Figure 20 contains the absorption and fluorescence emission spectra of fluorescein-5-isothiocyanate-labelled goat anti-mouse IgG antibody in pH 8.0 buffer (FITC) (green), rhodamine phalloidin in pH 7.0 buffer (RHOD) (red) and DAPI bound to DNA (DAPI) (blue) which were the three regions used to observe fluorescence.[170]

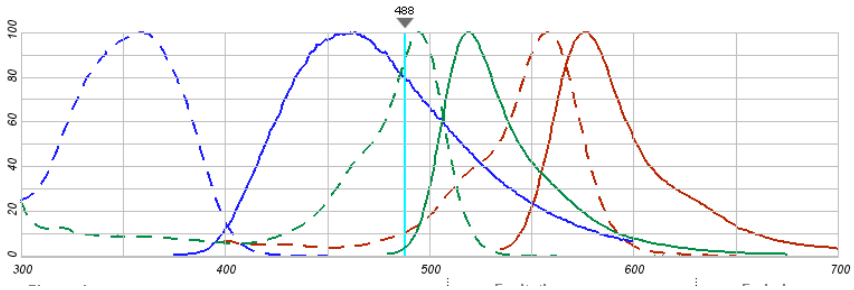


Figure 20

3.7.5.1 *Trypanosoma brucei*

Using both the wet slide technique and blood smears, the fluorescence of compounds **72** and **91** was observed at various time points under normal light and then using different wavelengths. They were dispersed within the parasite cells within 30 minutes (Figure 21 shows trypanosome after 30 minutes with compound **91** at x100 magnification).

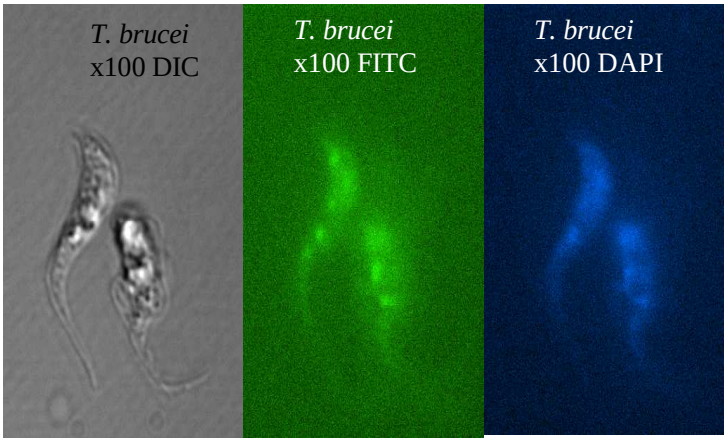


Figure 21

91 had also become accumulated in some of the parasite's intracellular organelles after 60 minutes (Figure 22 shows trypanosome after one hour with compound **91**). It is impossible at this stage to determine into which organelle the drug is accumulating, and further experiments need to be carried out using known organelle stains. Conclusions about the mode of toxicity of these macrocycles may then be made.

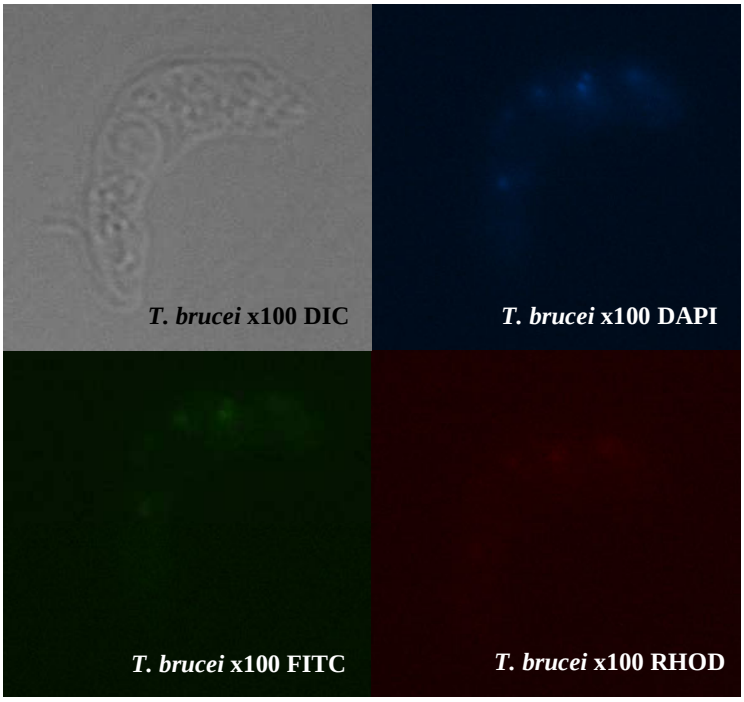


Figure 22

Drug **91** was also given to mutant clone knockout *T. brucei* and the same procedure carried out. As before, the drug became cytosolic after around 30 minutes, and then after an hour had become more punctate in some areas. This suggests that these compounds do not enter

the trypanosomes *via* the P2 purine transporter, but probably enter by passive diffusion across the cell membrane.

3.7.5.2 *Leishmania mexicana*

Leishmaniasis is another parasitic disease spread by the bite of infected sand flies in tropical and subtropical countries. There are a number of different forms of this disease, but the most common are cutaneous leishmaniasis, which causes skin sores, and visceral leishmaniasis, which affects some of the internal organs of the body.[171] Although these polyamines have not yet been tested for toxicity against *leishmania*, compounds **72** and **91** were used to view drug uptake into *L. mexicana*. Using the wet slide technique, **72** and **91** were found to become cytosolic after 30 minutes, and were concentrated in specific organelles after one hour, similar to the activity within trypanosomes. Alamar Blue assays are required to define any general toxicity.

3.7.6 Polyamine Investigation

An investigation was carried to establish how these novel polyamines kill the parasites. A number of Alamar Blue assays were carried out to determine if adding a concentration of some of the parasite's natural polyamines affects the drug activity. This may indicate which biochemical pathway the compounds are interrupting.

Six compounds (**36**, **66**, **72**, **90**, **91**, **84**) were tested against *T. brucei* as before, by treating the parasite with serial concentrations of the drug, this time dissolved in a 0.1 M solution of the polyamine (putrescine **18**, spermidine **19** or spermine **20**) in HMI-9 medium. IC_{50} values were calculated from the spectrophotometric data, and it was found that adding putrescine has no effect on the drug activity (Figure 23). However adding spermine or spermidine killed all the trypanosomes on the plate. Unfortunately 0.1 M of spermine and spermidine is toxic to the trypanosomes and so this experiment must be repeated at a lower polyamine concentration.

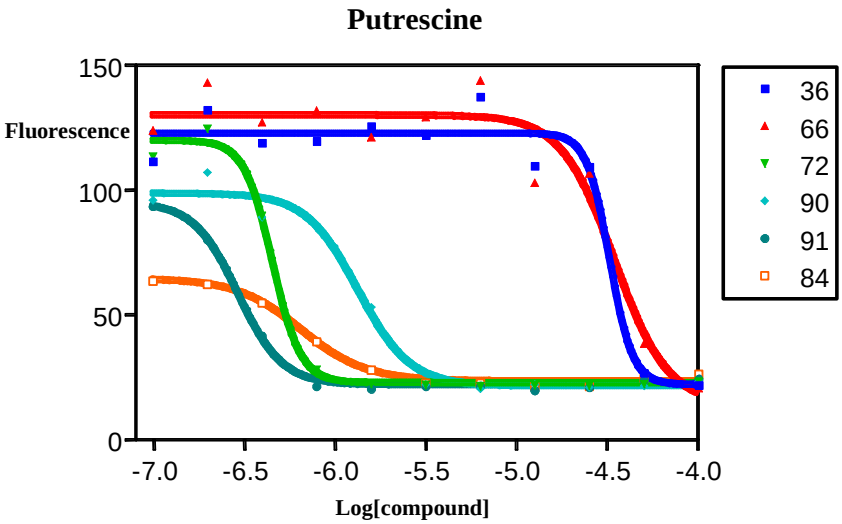


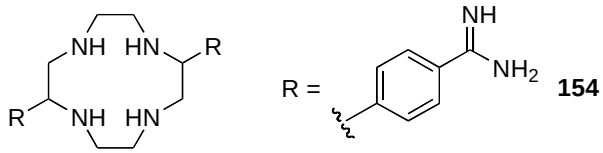
Figure 23

3.8 Conclusion and Future Work

A library of 21 tetraazamacrocyles was prepared and tested against *T. brucei*, *P. falciparum*, HEK cells and for activity as OPB inhibitors. A number of compounds showed potent toxicity against the parasites, in particular **91** which underwent *in vivo* testing. The library also contained two compounds bearing a P2 purine transporter recognition motif, which led to parasite specificity of these compounds. Fluorescence investigation and further Alamar Blue assays gave an insight into the mode of entry and action of these tetraazamacrocycles.

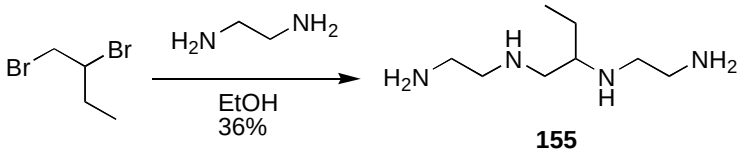
Further fluorescence work should be carried out to determine the exact organelles in which the compounds are accumulating, and this may tell us more about which biological pathway the drugs are affecting. The ability of the macrocycle to coordinate with metals such as iron and copper should also be investigated as the cause of cell death in biological systems.

Further analogues should be prepared bearing more than one substituent (for example **153**), which may increase the log P value of the macrocycle and assist the uptake into the parasites. There is also the opportunity to have more than one benzamidine moiety on the macrocycle **154**, creating an analogue of pentamidine **2**, which may be more toxic *in vitro*.



153 **154**

A route towards substituted TETA analogues was attempted. N^1,N^2-Bis-(2-aminoethyl)-butane-1,2-diamine **155** was prepared from 1,2-dibromobutane and 1,2-diaminoethane in a modest yield as a racemate (Scheme 47).[172] A variety of dibromo compounds are commercially available and so a range of substituted compounds may be prepared in this way. Cyclisation using the iron-template method has not yet been carried out.



155

Scheme 47

4 Synthesis of Enantiomerically Enhanced Tetraazamacrocycles

4.1 Introduction

Over the past fifteen to twenty years drug chirality, and in particular the use of single enantiomers instead of racemic mixtures, has become an area of great interest. This has resulted in considerable advances in the synthesis, analysis and separation of chiral compounds, as well as an increased appreciation of the potential significance of the differential biological properties of the enantiomers of chiral drugs that are administered as racemates.[173-176]

After scientific meetings in the late 1980s and early 1990s, the American FDA and European guidelines recognised the significance of chirality in pharmacology and therapeutics. This led with increasing frequency to the synthesis of new chiral chemical compounds as single enantiomers as opposed to racemic mixtures. In addition, many established drugs marketed as racemates were re-evaluated and re-marketed as single enantiomers. This development is known as “The Chiral Switch”.[177]

4.2 Aims of this Work

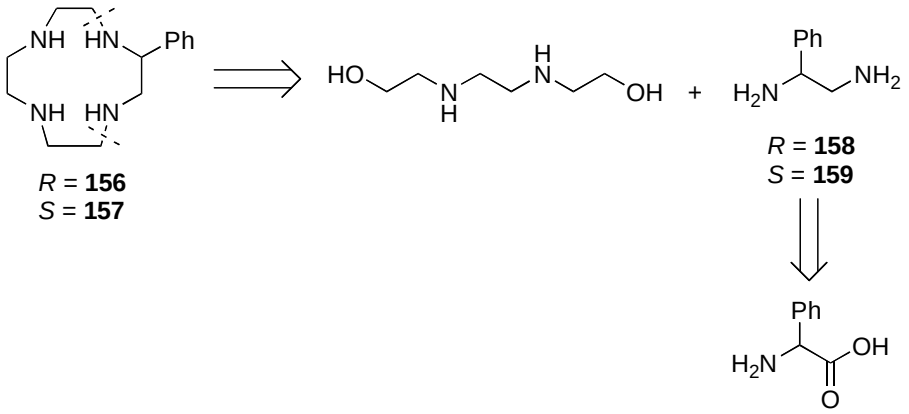
It has been demonstrated that a number of substituted tetraazamacrocycles have moderate to high *in vitro* activity against trypanosomes (Chapter 3). These compounds are currently synthesised as racemic mixtures, and so the aim was to develop a general strategy for the synthesis of single stereoisomers of each compound. This may result in the halving of the required dose of the active drugs.

4.3 Synthesis of Enantiomerically Enhanced Substituted Tetraazamacrocycles

4.3.1 Route I

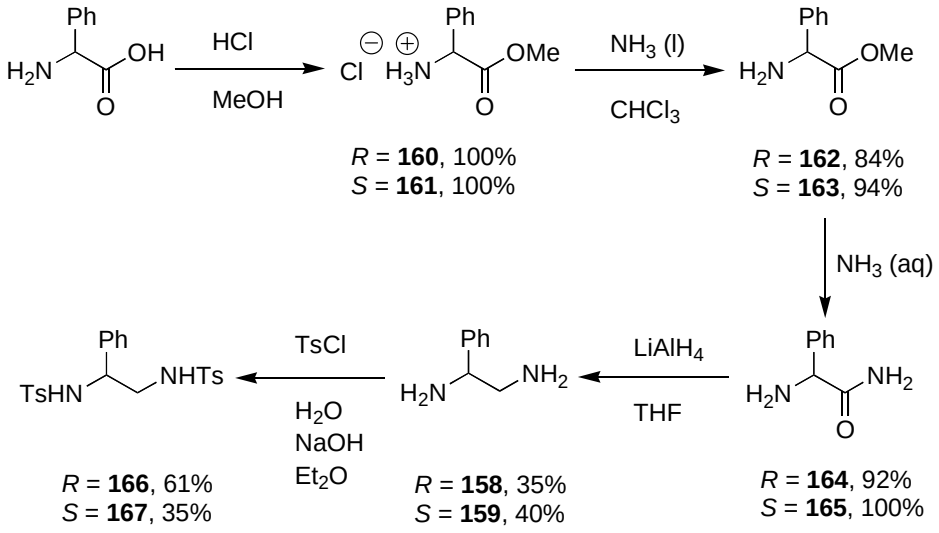
In Chapter 3.2.2 tosylated cyclen **43** was successfully synthesised in 79% yield from ditosylated ethylenediamine **45** and **44**. Retrosynthesis of our target chiral compounds **156**

and **157** therefore suggests that the synthesis could start from an amino acid, for example phenylglycine, which is commercially available in both the *R* and *S* forms (Scheme 48).



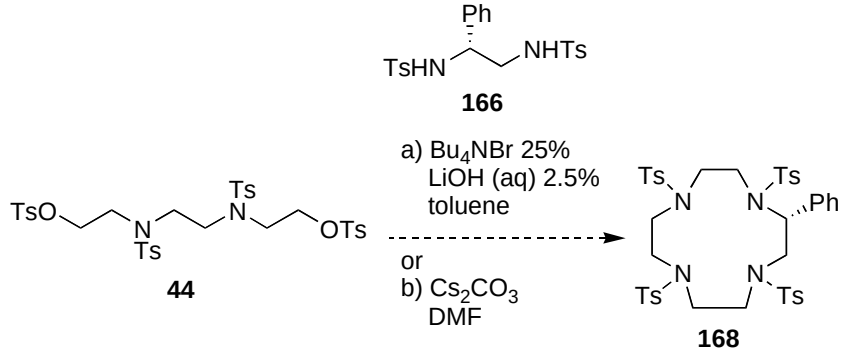
Scheme 48

Ditosylated diamines **166** and **167** were synthesised over five steps from phenylglycine (Scheme 49).[178,179,180]



Scheme 49

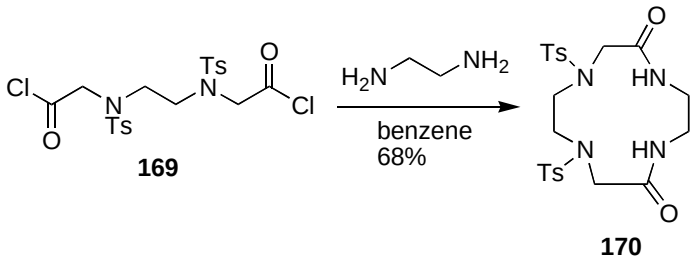
Using the (*R*)-isomer **166**, cyclisation with *N,N',O,O'*-tetra(*p*-toluenesulfonyl)-*N,N'*-bis(2-hydroxyethyl)-ethylenediamine **44** was carried out under phase-transfer conditions using tetrabutylammonium bromide (Scheme 50).[104] However, only starting material was present at the end of the reaction time. This step was also attempted using traditional Richman-Atkins conditions with caesium carbonate and DMF,[90] however no product was observed in the NMR spectra. Increasing the reaction time did not affect this result and it was concluded that the aryl group provides a steric clash, which interrupts the pseudo-Thorpe-Ingold effect created by the tosyl groups.



Scheme 50

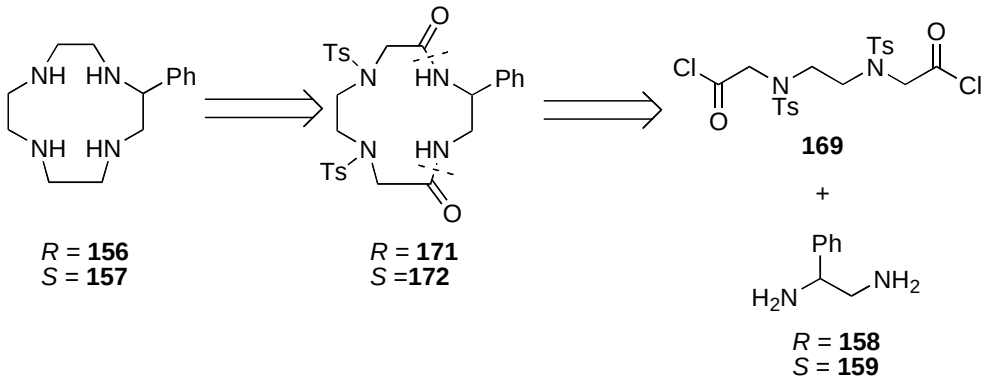
4.3.2 Route II

Stetter and Mayer in 1961 had successfully cyclised a di-acid chloride **169** with 1,2-diamino ethane to form 1,4-ditosyl-6,11-dioxo-1,4,7,10-tetraazacyclododecane **170** (Scheme 51).[165]



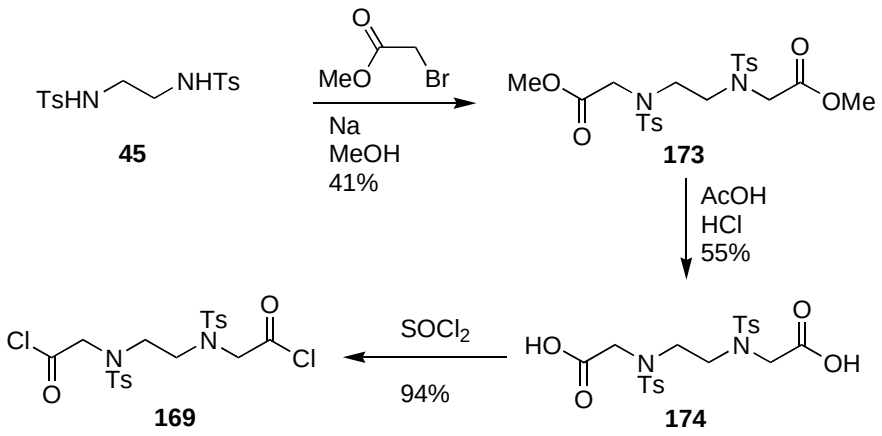
Scheme 51

The target material was retrosynthesised to **169** and diamines **158** and **159**, which had been prepared previously (Scheme 52).



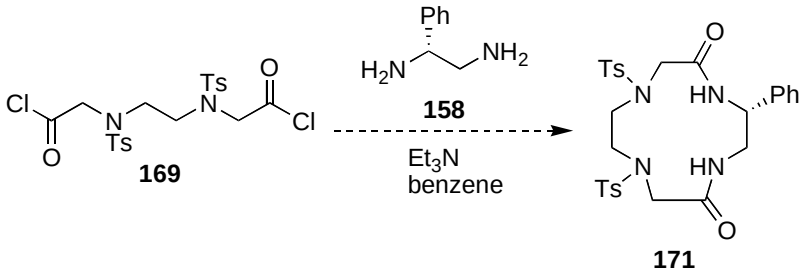
Scheme 52

Starting material **169** was prepared over four steps from tosylated ethylenediamine **45** (Scheme 53).[119,165]



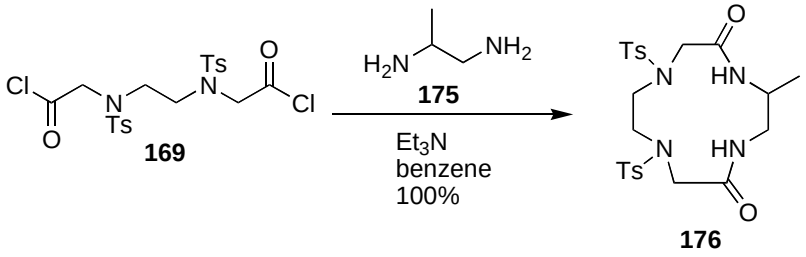
Scheme 53

The cyclisation step was then carried out under high dilution conditions in benzene, using triethylamine, **169** and **158**; however the expected product **171** was not observed (Scheme 54).



Scheme 54

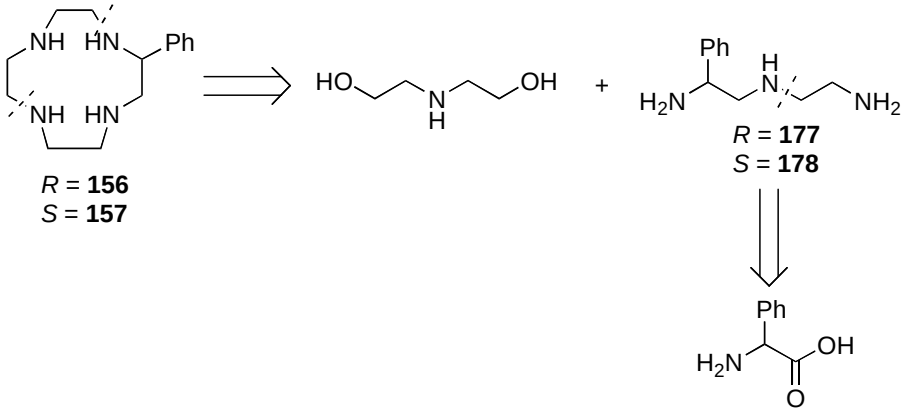
The reaction was repeated using racemic 1,2-diaminopropane **175** and **169**, which gave compound **176** quantitatively (Scheme 55). Therefore in this method, the aromatic ring present in **158** is also affecting the cyclisation reaction. This route towards enantiomerically enhanced macrocycles was abandoned for two reasons: firstly the high dilution conditions in benzene are not advisable for safety reasons; and secondly, after cyclisation the macrocycle would require a further two steps to reduce the amide groups and remove the tosyl protecting groups.



Scheme 55

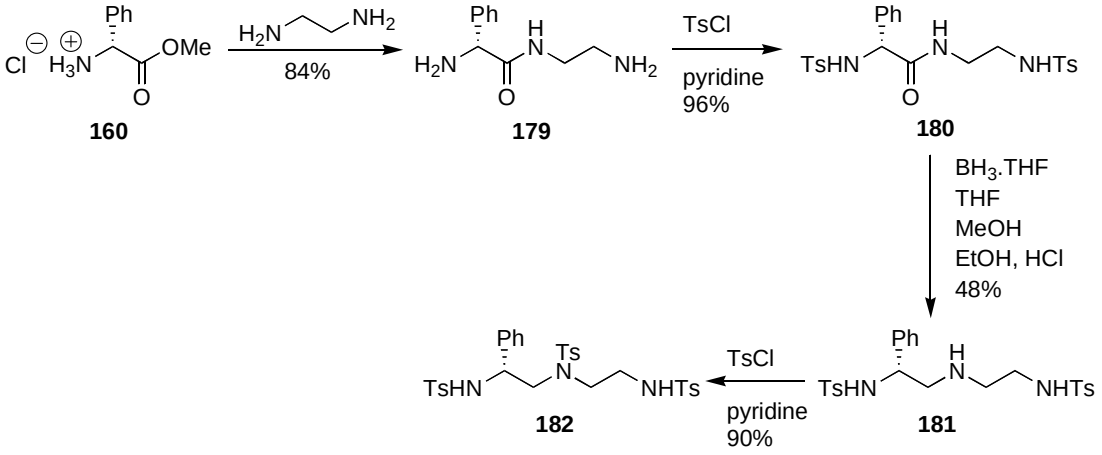
4.3.3 Route III

Retrosynthesis of our target material in a slightly different way from route I led to an alternative strategy, which resembles the route by Garrity.[120] We can begin our synthesis with phenylglycine, or any other amino acid, and diethanolamine (Scheme 56).



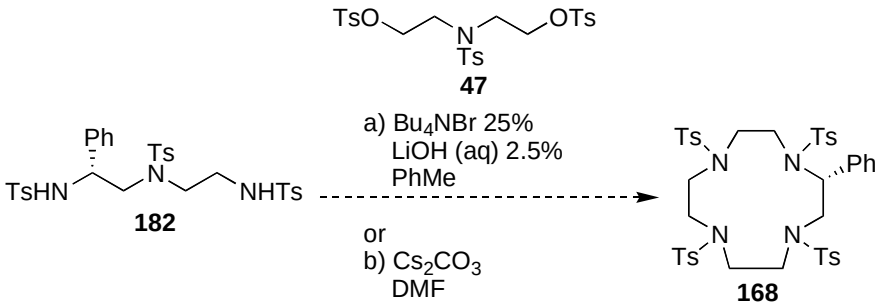
Scheme 56

The tritosylated (*R)*-amine **182** was prepared over four steps from phenylglycine (Scheme 57).[113,180,181,182]



Scheme 57

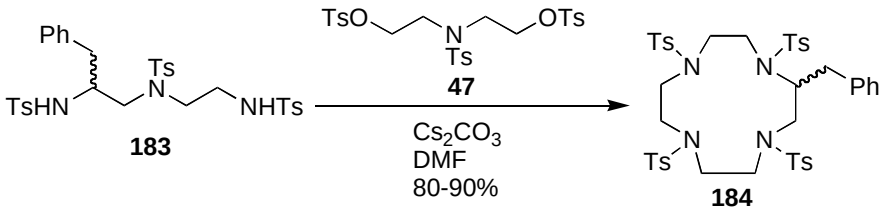
Cyclisation was investigated under Lukyanenko's phase transfer conditions, and then with the traditional caesium carbonate method; however cyclisation did not go to completion (Scheme 58). Varying the reaction times and conditions made no difference, and only starting material was recovered from the reaction mixture in each case. Again it seems that the proximity of the extra aryl group on starting material **182** is disturbing the pseudo-Thorpe-Ingold effect.



Scheme 58

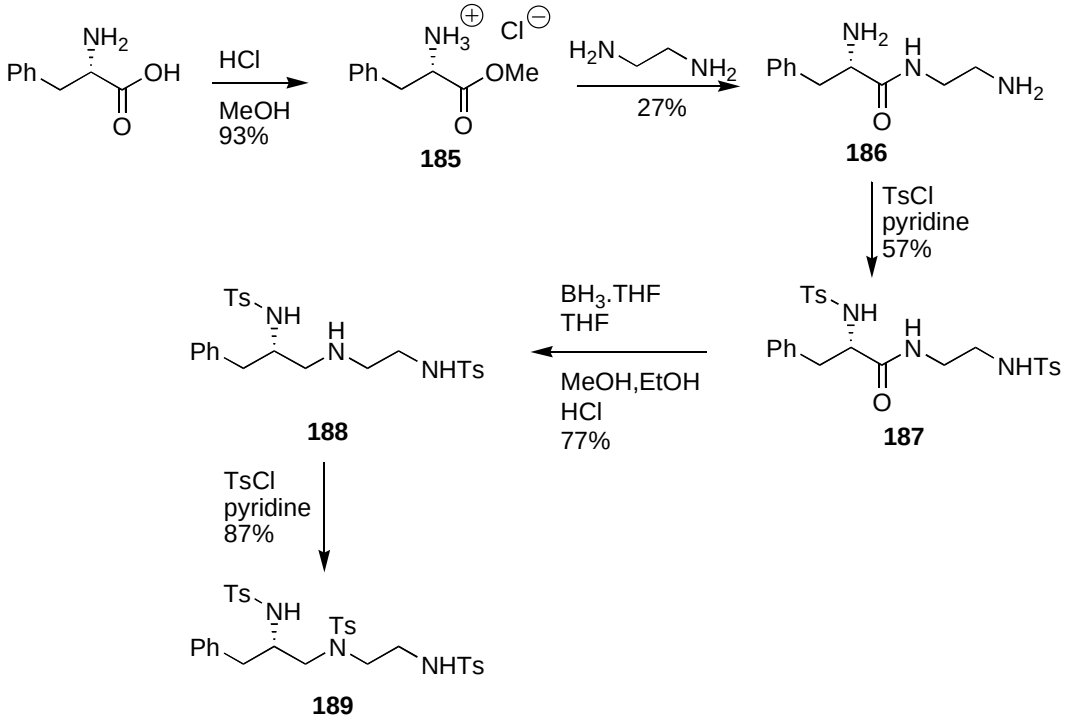
4.3.4 Route IV

A final attempt to synthesise an enantiomerically enhanced tetraazamacrocycles referred back to Garrity's publication, where racemic compound **184** was synthesised under Richman-Atkins conditions (Scheme 59).[120]



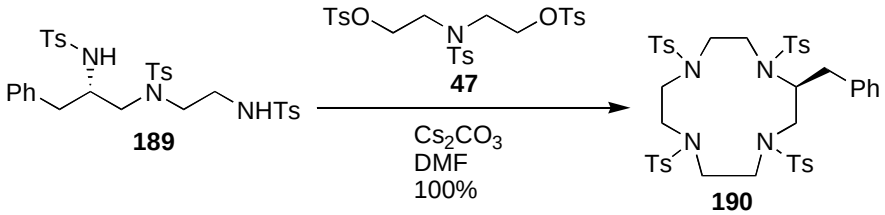
Scheme 59

It was proposed that the extra carbon between the polyamine and the phenyl group is necessary to prevent steric clashes. Therefore using a route described previously, (*S*)-phenylalanine was converted into **189** over five steps (Scheme 60).[113,180,181,182]



Scheme 60

189 was then successfully cyclised with **47** under Richman-Atkins conditions to give **190** in an excellent yield (Scheme 61).

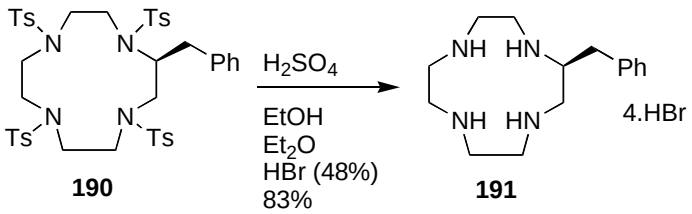


Scheme 61

This reaction was also attempted using phase-transfer conditions described earlier;[104] however the product was impure and obtained in a low yield.

4.3.5 Removal of Tosyl Groups

There are a number of reported methods for the removal of tosyl protecting groups, however many are highly inefficient (see Chapter 3.2.5). Garrity used lithium aluminium hydride to deprotect tosyl groups.[120] However previous attempts within the Robins group using this method have failed. In a preparation reported by Parker *et al*. and adapted by S. Jones from the Robins group, **190** was deprotected using sulfuric acid in ethanol, and the tetrahydrobromide salt was formed using hydrobromic acid (Scheme 62).[89,101]



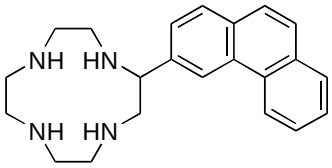
Scheme 62

191 requires conversion into the free base and purification before full characterisation. Unfortunately due to time constraints this work was not completed.

4.4 Conclusions and Future Work

A strategy has been found for the synthesis of single enantiomers of substituted tetraazamacrocycles. The starting materials are commercially available amino acids and so a wide range of compounds may be prepared. Unfortunately this route is lengthy and requires an undesirable tosyl deprotection.

The most biologically active substituted macrocycle is **91**, which has a large aromatic moiety and this compound cannot be retrosynthesised to a commercially available amino acid. Also, it is thought that the large ring structure may impede the cyclisation process of our preferred route. Another way to prepare single enantiomers of this compound, and other related aromatic macrocycles, may be by utilising chiral HPLC apparatus to separate the enantiomers of a racemate.



91

5 Anti-Cancer Alkylating Agents

5.1 Cancer

Cancer is the name given to illness caused by the uncontrolled growth and division of cells. Cancer is found everywhere in the plant and animal kingdoms. The earliest reference to human cancer is from an Egyptian papyrus written between 3000 and 1500 BC describing tumours of the breast.[183] Cancer is caused by numerous effects and can occur in any cell of the body.

Our body's cells undergo constant growth and multiplication under rigorous control within our tissues and organs. To divide, a cell first replicates its DNA to make an exact copy. The cell then splits to form two identical 'daughter' cells. If the strict control over this process is lost, it can lead to cells multiplying irregularly to form a lump or 'tumour' of abnormal cancerous tissue.[183]

A tumour alone can cause discomfort, for example by blocking the digesting system, or in the case of leukaemia (cancer of white blood cells), normal blood function is lost. Tumours can also press against nerves and alter normal body functions by releasing hormones. If a tumour becomes malignant it can spread *via* metastasis. Cancerous cells break away from the tumour and start multiplying in another part of the body.[183]

5.2 Cancer Statistics

There are over 200 different types of cancer although lung, breast, large bowel (colorectal) and prostate cancer are the most common accounting for over half of all new cases. 275,000 people are diagnosed with cancer every year and it causes 26% of all deaths annually in the United Kingdom.[184]

5.3 Cancer Therapy

Modern medicine offers us a number of ways to tackle cancer. The four main methods are as follows.

5.3.1 Surgery

Although only 13% of cancers are cured by surgery, it is carried out in 90% of cancer patients. Tissue biopsies are critical for diagnosis, and it is also used as a preventive in certain cancers.[183]

5.3.2 Radiation Therapy

Electromagnetic radiation is used to disrupt the atoms within tumour cells. This stops cell proliferation and growth and causes cell death. Radiation has the greatest effect on rapidly dividing cancer cells; however it does also affect normal cells.[183]

5.3.3 Biotherapy

This therapy employs the body's natural defence systems to control the cancer. Some examples are using interleukins to increase the activity of lymphocytes; interferons to increase the cell-killing activity of the immune system; and gene therapy, which involves inserting genes into tumour cells to change their proliferation activity.[183]

5.3.4 Chemotherapy

Chemotherapy uses cytotoxic drugs to control tumour growth, shrink tumours, remove metastatic cancer growths and relieve pain. There are a number of different types of anti-cancer agents including antibiotics, antimitotics, hormones, antimetabolites, inorganic compounds and alkylating agents.[183]

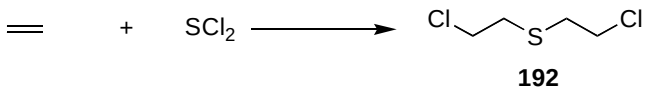
5.4 Alkylating Agents

Scientists have compared alkylating agents to the ancient Roman god Janus, who had two faces on one head facing in opposite directions (Figure 24). These chemical compounds, like Janus, have a dual nature with the power to cause great damage as well as the ability to cure disease. The history of the use of the alkylating agent mustard gas demonstrates this analogy well.[185]

Figure 24

5.4.1 Mustard Agents: Chemical Warfare to Chemotherapy

Mustard gas is the common name of 1,1-thiobis(2-chloroethane) **192**, which is also known as blister gas, sulfur mustard, Kampstoff Lost, and yperite. It was first synthesised in 1860 by Frederick Guthrie who reacted SCl_2 with ethylene (Scheme 63).[186] Guthrie also noted the toxic effects of the product on his skin.



192

Scheme 63

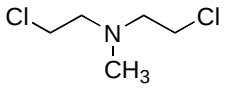
Despite its name, this agent is not gaseous but a clear to pale yellow viscous oil with a melting point of 14 ^{o}C. Mustard gas is odourless, however when it is mixed with other chemicals and dispersed as an aerosol it has a distinctive odour that has been compared to mustard, garlic or horseradish.[187]

Mustard gas was first used as a chemical warfare agent in July 1917 during World War I in Belgium. It is a deadly and debilitating poison, particularly dangerous due to its ability to penetrate all protective clothing and the masks that were available at the time.[188] Exposure to the chemical of more than 50% of body surface area is usually fatal. It is a vesicant (blister-inducing agent) and despite no immediate symptoms, 4-24 h after exposure, skin will turn into deep, burning and itching blisters. The eyes become sore and the eyelids swollen, possibly leading to conjunctivitis and blindness. If the aerosol is inhaled, the mucous membranes of the respiratory system will bleed and blister leading to pulmonary oedema.[188,189] Mustard gas was only fatal in 1% of cases and so its efficiency was mainly due to its incapacitation effect: a wounded soldier slows an army more than a dead soldier.[189]

Since 1917 mustard gas has been used in a number of other conflicts: for example by the UK against Iraqi rebels (1920); by the Soviet Union in China (1930); by Italy against Ethiopia (1935-1940); and Iraq against Iran (1983-1988).[189]

A number of epidemiology studies quickly linked the exposure of mustard gas (whether through military or occupational contact) to an increased risk of lung and other respiratory tract cancer, and later with cancers of other tissues.[187] Mustard gas was found to cause DNA damage and genetic mutations in all test systems including fungi, bacteria, *in vitro* rodent cells and *in vivo* mammalian experiments.[187]

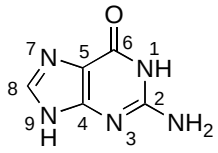
In 1943 during World War II an incident occurred in the Italian port of Bari where German bombers sank 16 ships, one of which contained 100 tonnes of mustard gas. Dr Cornelius Packard Rhoads made a startling discovery while treating survivors by noticing a large reduction in their white blood cell counts.[185] He then proposed that the ingredient of the chemical agent could be used to treat cancer, in particular leukaemia (Hodgkin's disease). Around the same time, two pharmacologists (Goodman and Gilman) at Yale University showed that nitrogen mustard analogues affected rapidly dividing cells.[190] This research led to the development of mechlorethamine **193**, a bifunctional nitrogen mustard analogue of mustard gas that is still used today in the treatment of leukaemia.[183]



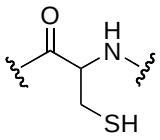
193

5.4.2 Mechanism of Action

The mechanism of action of these chemicals is *via* the formation of strong covalent bonds by alkylation of various nucleophilic moieties.[191] Both cytotoxic and chemotherapeutic effects are due to the alkylation of DNA primarily at the *N*-7 position in guanine **194**, although other DNA bases and protein residues (for example cysteine **195**) can also be alkylated in this way.[192,193]



194



195

Mutagenesis and teratogenesis occur when one bond is made with a nucleophile; however, compounds containing two reactive chloroethyl side chains can act as bifunctional alkylating agents (Figure 25). If the agent reacts with two guanine residues on the same strand the result is 'limpet attachment' which does not prevent the separation of the DNA

stands, but does prevent vital processing enzymes from accessing the DNA resulting in apoptosis.[192]

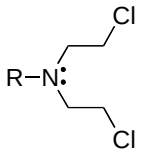
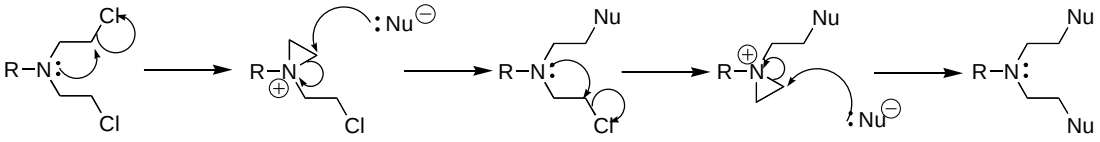


Figure 25

Bifunctional alkylating agents cross-link between two nucleic acid chains or between a protein and a nucleic acid. The cytotoxicity corresponds to the formation of inter-strand DNA cross-links *via* the formation of an aziridinium ion with subsequent alkylation within the DNA major groove (Scheme 64).[192]



Scheme 64

The compound then alkylates again to form a cross-link that prevents the DNA unwinding and this prevents any further cell replication processes, ultimately resulting in cell death (Figure 26).

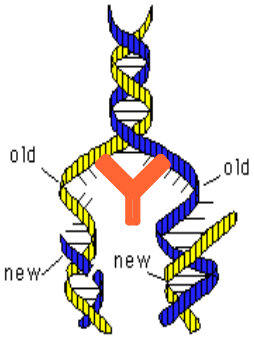


Figure 26

Alkylating agents are not cell-cycle-phase specific although they are found to have the greatest effect during DNA synthesis, as cells exposed to the drug in earlier stages, such as G1, have enough time to repair DNA damage (Figure 27).[194] These drugs are also proliferation dependent, displaying highest potency in rapidly growing and diving cells. Nascent cells have a longer time to correct any DNA damage before mutagenesis occurs.[194]

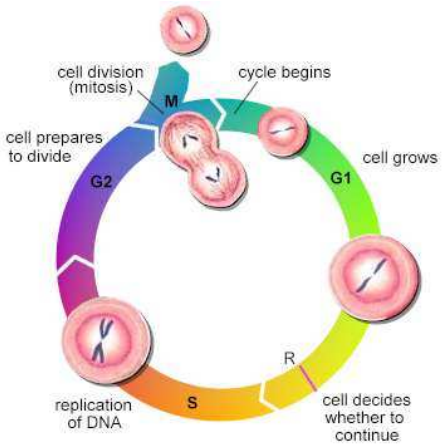
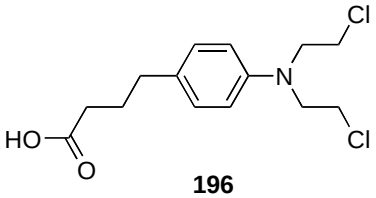


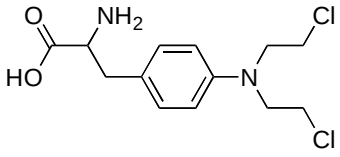
Figure 27

5.4.3 Nitrogen Mustard Anti-Cancer Agents

Since the discovery of the first prototypic anti-cancer agent mechlorethamine **193** in the 1940s, a number of analogues have been developed and used to treat a variety of tumours. Mechlorethamine (commonly known as Mustargen) was used to treat prostate cancer. Modifications of this nitrogen mustard were made to improve solubility, stability and membrane transport, and this led to the development of chlorambucil **196**, which underwent clinical trials in the 1950s.[195]



196

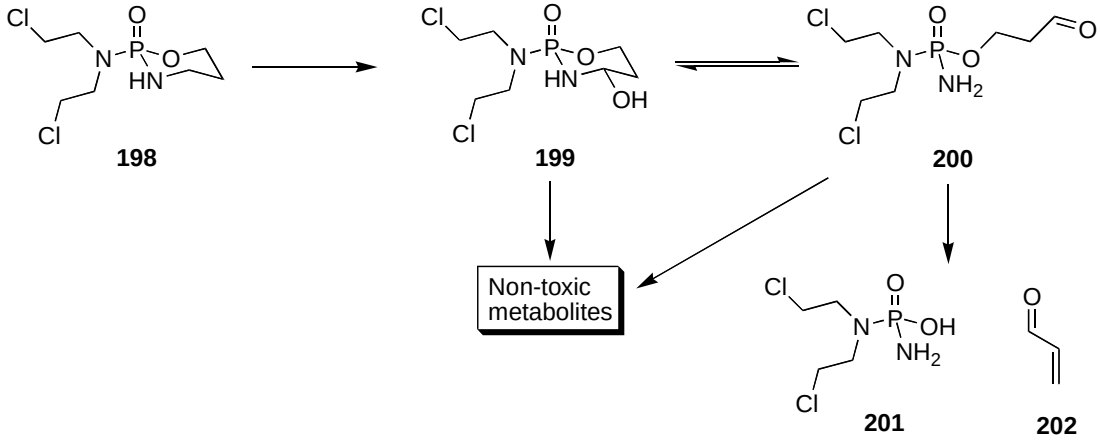


197

Chlorambucil is an orally administered drug for the treatment of chronic lymphocytic leukaemia, ovarian cancers, trophoblastic neoplasms and Waldenström macroglobulinemia, as well as being used as an immunosuppressive drug.[195] The main side effect of chlorambucil is bone marrow suppression. Another *N*-mustard analogue is melphalan **197**, a phenylalanine derivative of mechlorethamine, which is used to treat multiple myeloma, malignant melanoma and ovarian cancer.[195]

Despite the relative success of these *N*-mustard alkylating agents against cancer, they still had dangerous and severe side effects from the blistering of mucous membranes, reduced immunity and seizures, to the induction of further cancers.[196,197] These undesirable and life threatening side effects encouraged the development of prodrugs to minimise side effects and increase the selectivity of the drugs to cancerous tissue. One of the first *N*-mustard

prodrugs was cyclophosphamide **198**, which is used to treat a number of cancers including Hodgkin's disease and solid tumours, as well as treating some autoimmune disorders.[198] Developed in 1958, cyclophosphamide was designed to be cleaved by phosphoramidase enzymes *in vivo*, liberating the active mustard moiety. However, in practice the drug is oxidised by enzymes in the liver to give the active metabolite 4-hydroxycyclophosphamide **199**, which exists in equilibrium with its tautomer aldophosphamide **200** (Scheme 65). Most of the aldophosphamide is oxidised by aldehyde dehydrogenase (ALDH) to form carboxyphosphamide and a small amount is converted into phosphoramide mustard **201** that forms cross-links within DNA. Acrolein **202** is also produced which is toxic to the bladder epithelium leading to hemorrhagic cystitis.[198]



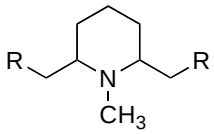
Scheme 65

Despite these side effects, cyclophosphamide **198** is still the most common anti-cancer alkylating agent used today.[183,198]

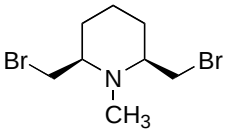
5.5 Development of *N*-Mustards within the Robins Group

In the mid 1990s, during her PhD research, N. Henderson synthesised a range of bifunctional mustard compounds based on piperidine **203**.[199] The conformational restriction imposed by the piperidine ring was investigated to determine if it would affect the aziridinium ion formation. Compound **204** showed cytotoxicity against human colon carcinoma cell lines with an IC_{50} of 8.5 μM.[200] Henderson then aimed to increase the selectivity of the alkylation at the guanine *N*-7 site. A number of bispiperidine derivatives **205** were synthesised which were cytotoxic in three cisplatin resistant cell lines, and they showed increased guanine selectivity when compared to melphalan.[201] However the IC_{50}

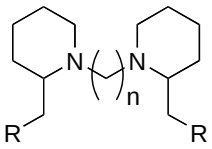
values were not as low as melphalan despite effective cross-linking of DNA at lower concentrations.



203

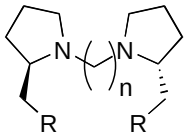


204

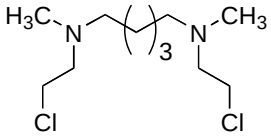


205

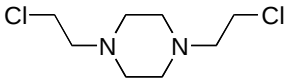
At this point it was observed that there was a relationship between the chain length of the carbon-linker and the biological activity, indicating an optimum distance between the nitrogens. In 1999 F. Anderson investigated this area further in her PhD research, and produced a series of homochiral bispyrrolidine mustard derivatives **206**, along with a number of alkyl linear and cyclic mustards (such as **207** and **208**).[112] It was found that compounds with 2-, 5-, and 6- carbon linkers gave the best alkylation data whereas 3- and 4-carbon linkers gave no DNA cross-linking activity.[202]



206

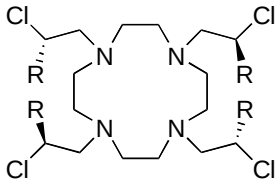


207



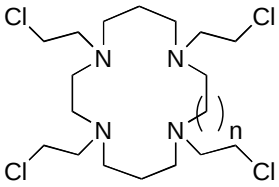
208

This result contrasts with that of conventional mustard drugs. Unfortunately none of these novel compounds gave high cytotoxicity and so Dr S. Lacy began working on the use of macrocyclic polyamines as a framework to synthesise alkylating agents. Macrocyclic polyamines have been used widely as ligands for metal chelation.[203-206] They also offer a route to compounds with a range of ring sizes, a vast variety of structures, and can have different substituents on the carbon and nitrogen atoms. Dr Lacy synthesised tetrakis-(2-chloroethyl)-1,4,7,10-tetraazacyclododecane **209**, an alkylating agent that is a derivative of commercially available cyclen **36**. **209** was found to have exceptional DNA cross-linking activity, with 100% cross-linking at 0.1 μM, and in tests against a human colon carcinoma cell line, **209** showed a higher cytotoxicity (IC_{50} = 22 μM) than chlorambucil **196** (IC_{50} = 45 μM).[207]

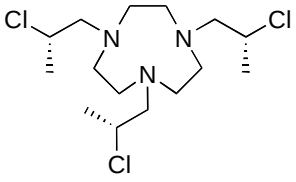


209, R = H
210, R = CH_3

Following this discovery, a number of poly-2-chloroethylated macrocyclic polyamines were developed with varying ring sizes, numbers of nitrogens, substituents on carbons, and carbon chain lengths between the nitrogens. These compounds (**209** – **213**) were tested for biological activity with a number of promising results (see Table 10).



211, n = 1
212, n= 2

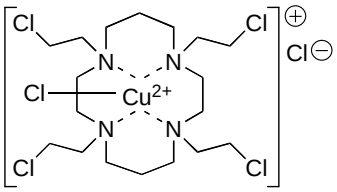


213

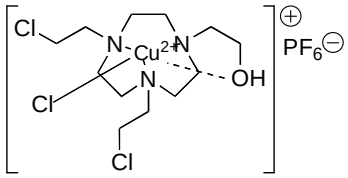
Compound	DNA cross-linking at 0.1 μM (%)	IC_{50} (μM)
209	100	22
210	64	10
211	94	8
212	100	9
213	30	13

Table 10

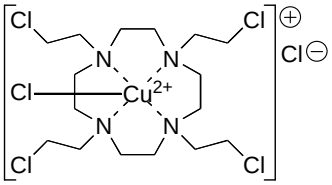
Because of difficulties experienced in the synthesis of some target macrocycles, L. Parker aimed to develop a flexible, reliable method for the synthesis of azamacrocycles of variable sizes with different carbon chain lengths between the nitrogens.[86,208] Parker also targeted the low selectivity of current chemotherapeutic agents against cancer by designing redox-active compounds as prodrugs.[209] These Cu (II) complexes are electrochemically reduced in hypoxic (low oxygen) tissue to release the *N*-mustard cytotoxin. Mustard complexes **214** and **215** showed irreversible redox behaviour and low thermodynamic stability and so behaved as typical mustard drugs but had no hypoxia selectivity. Complex **39** however displayed reversible redox behaviour and high thermodynamic stability under aqueous conditions exhibiting excellent hypoxia selectivity against a lung tumour cell line.[209]



214



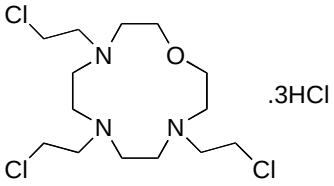
215



39

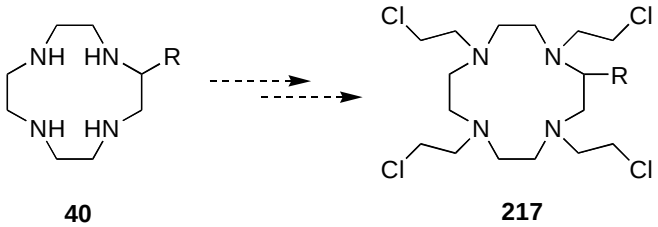
5.6 Aim of this Work

Further investigation has been carried out within the Robins group to discover if the size and shape of aza and oxoaza macrocycles affects their activity as alkylating agents. **216** prepared by S. Jones was found to have an IC_{50} value of 4.0 μM which is the most toxic of these types of compound prepared so far.[89]



216

Cyclen-based alkylating agents therefore remain the most active compounds *in vitro,* and so all novel macrocycles were based on the lead compound (**36**). The aim of this project is to convert the novel substituted tetraazamacrocycles synthesised in Chapter 3 into anti-cancer alkylating agents **217** (Scheme 66). These compounds bear aryl groups with different functional groups that vary the electronic demand on the macrocyclic ring system. This may affect the aziridinium ion formation and thus increase or decrease the rate of DNA alkylation.



40 **217**

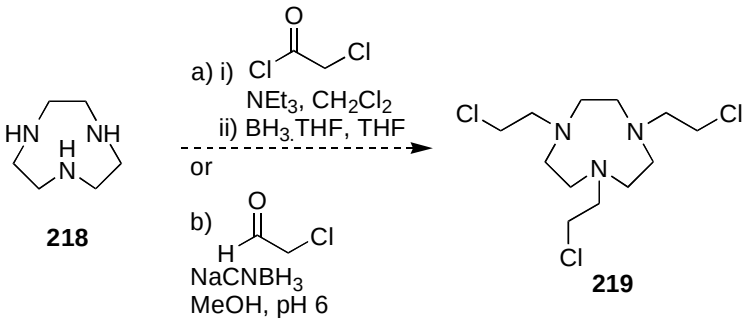
Scheme 66

5.7 Progress Towards Substituted Tetraazamacrocyclic *N*-Mustard Derivatives

In this section the progress towards the preparation of substituted tetrakis-(2-chloroethyl)-1,4,7,10-tetraazacyclododecane derivatives will be discussed. The test reactions were carried out on non-substituted, commercially available cyclen **36** before applying them to the substituted compounds.

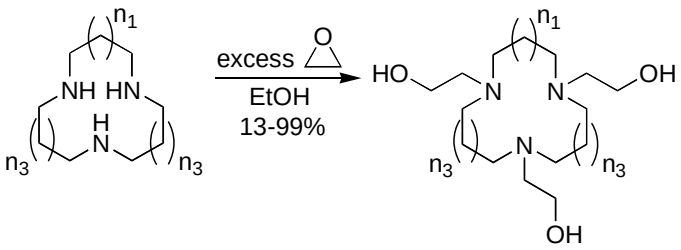
5.7.1 N-*Hydroxyethylation of Cyclen*

In her quest to optimise the synthesis of polyazamacrocyclic *N*-mustard derivatives, Parker tried a number of routes to reach the poly-*N*-(2-chloroethyl) compounds but was unsuccessful. These included chloroacetamides as intermediates and the use of reductive alkylation (Scheme 67).[86,210,211,212]



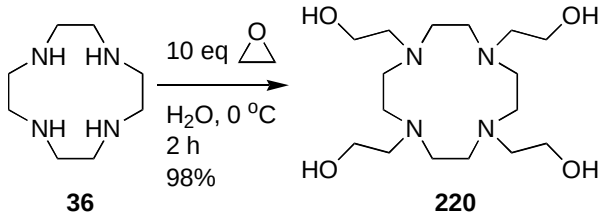
Scheme 67

However, the most successful route involved first synthesising poly-*N*-(2-hydroxyethyl) derivatives using ethylene oxide, before converting the hydroxyl groups into chlorines. Parker used ethanol as the reaction solvent and added a large excess of ethylene oxide. However she produced a number of products with various degrees of alkylation (Scheme 68).



Scheme 68

Although the various products could be separated by Kugelrohr distillation, we adapted a preparation by Buøen and co-workers to use water as the reaction solvent.[213] Previously an uncalculated excess of ethylene oxide had been used, resulting in the formation of a clathrate. Therefore it is essential to use only a small excess (100 - 200%) of the stoichiometric amount of the macrocycle. Ethylene oxide is highly volatile and so controlling the temperature is advantageous. Once the reaction is complete the excess ethylene oxide is simply evaporated *in vacuo*. High temperatures, long reaction times, and large quantities of ethylene oxide can lead to by-products caused by side chain elongation. Ring-opened ethylene oxide (ethylene glycol) can also polymerise to form polyethylene glycols as by-products. Thus, the optimum conditions were achieved when the ethylene oxide was added to a small volume of water at 0 ^{o}C and this solution was dropped slowly into a cooled stirred solution of cyclen **36** in water. The reaction mixture was then stirred for two hours at this temperature before the excess ethylene oxide was removed by rotary evaporation to give the tetrapodand **220** as a yellow oil in 98% yield (Scheme 69).



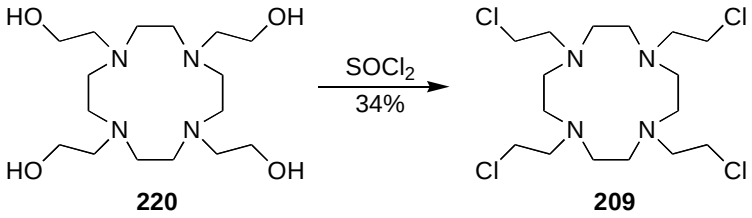
36 **220**

Scheme 69

Many attempts were made to extract and recrystallise **220** using a variety of solvent systems, and a very small amount of pure white crystalline material was collected after continual extraction with hexane.

5.7.2 N-*Mustard Derivatives*

The hydroxyethylated product can then be converted into the active *N*-mustard derivative by stirring and heating in an excess of thionyl chloride overnight (Scheme 70).[214] Removal of any excess thionyl chloride is done *in vacuo*, to isolate the product without need for further purification; however the compound may be precipitated from methanol with diethyl ether if necessary.

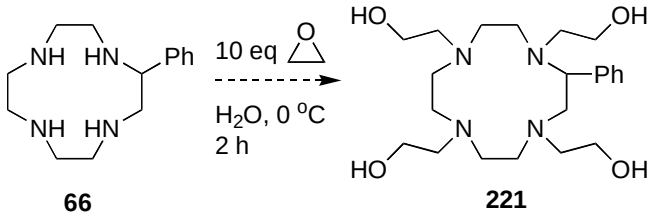


220 **209**

Scheme 70

5.7.3 N-*Hydroxyethylation of Racemic 2-Phenyl-1,4,7,10-Tetraazacyclododecane 66*

N-Hydroxyethylation of substituted tetraazamacrocycle **66** was attempted under the same conditions as before (Scheme 71). However, problems were quickly encountered at this stage.



66 **221**

Scheme 71

Firstly **66** is a viscous oil which did not dissolve well in water, and so it was impossible to stir the reaction mixture smoothly. Despite using the same equivalents of reagents and reaction time, the result was a mixture of products. The hydroxyethyl C-H peaks appear in the same region as the ring C-H protons in the ^{1}H NMR spectrum, making it very difficult to identify the integration of each peak. It was therefore difficult to determine whether the peaks represented the correct four-times hydroxyethylated product, mixtures of partially hydroxyethylated by-products or polyethylene glycol. Attempts were made to crystallise the desired material from a number of different solvent systems with no success. It was thought that the aryl group on the C-2 position may cause steric hindrance around the N-1 site and so the main product may be the tri-hydroxyethylated compound. The computer-generated image in Figure 28 that mimics the ring conformation suggests that the ring sits out of the way, however this is not conclusive evidence.

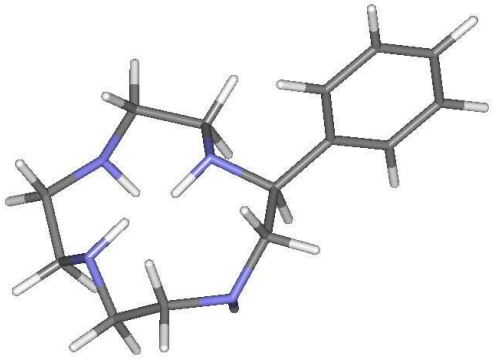
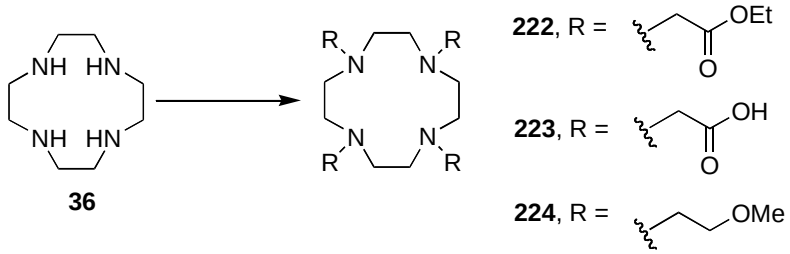


Figure 28

Due to this setback, other methods were tried for the *N*-hydroxyethylation of phenyl-substituted derivatives of cyclen. A number of reactions were investigated using commercially available cyclen as the starting material. Table 11 contains some of the methods, along with the reaction conditions and reagents. In many cases, the product contained a mixture of mono-, bi- and tri-alkylated side products. These mixtures are difficult to separate by any chromatographic method due to the polarity of the compounds, although recrystallisation was attempted in some cases. Routes V, VII and VIII were the most promising.

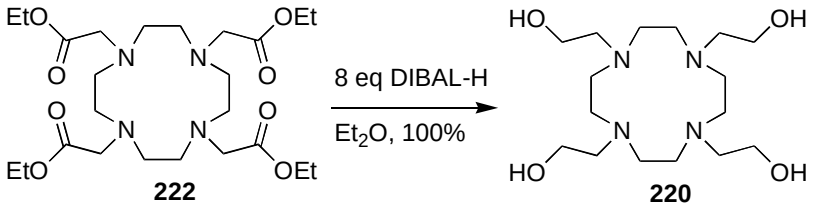


Route	Starting Material	Reagents and Reaction Conditions	Target Material	Result
I	**36**	$BrCH_2CO_2Et$, DMF[215]	**222**	-
II	**36**	$BrCH_2CO_2Et$, KI, DMF	**222**	-
III	**36**	$BrCH_2CO_2Et$, Cs_2CO_3, DMF[216]	**222**	-
IV	**36**	$BrCH_2CO_2Et$, DMF, Δ	**222**	-
V	**36**.4HCl	$ClCH_2CO_2Et$, Na_2CO_3, CH_3CN, Δ[217]	**222**	10%
VI	**36**	$ClCH_2CO_2Et$, Cs_2CO_3, CH_3CN, Δ	**222**	-
VII	**36**	$ClCH_2CO_2H$, NaOH, H_2O, Δ[218]	**223**	23%
VIII	**36**	$ClCH_2CH_2OCH_3$, NaOH, aq EtOH, Δ[219]	**224**	93%

Table 11

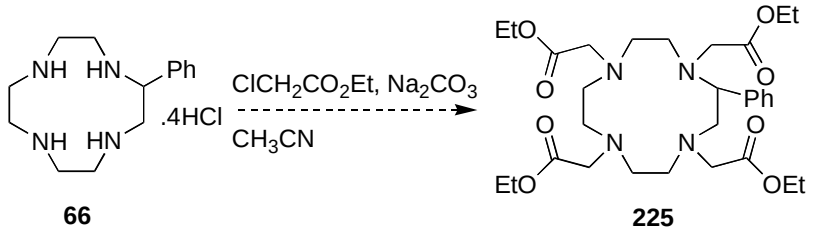
5.7.3.1 Route V

The first promising route towards **220** involved preparing **222** from the tetrahydrochloride salt of cyclen in a low yield, using ethyl chloroacetate and sodium carbonate in acetonitrile.[217] Initially, a brown viscous oil was produced, however recrystallisation was achieved from ethyl acetate. Deprotection and reduction of **222** was carried out successfully using eight equivalents of diisobutyl aluminium hydride to give **220** in quantitative yield (Scheme 72).[220]



Scheme 72

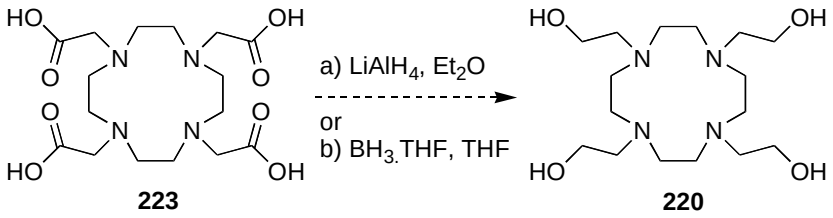
This method was applied to the substituted macrocycle **66**, however, despite many attempts the desired product **225** could not be isolated (Scheme 73).



Scheme 73

5.7.3.2 Route VII

The first attempt *via* route VII produced a white solid in 23% yield, and both the ^{1}H and ^{13}C NMR spectra suggested that the correct product **223** (dota) had been formed.[218] However, an accurate mass measurement could not be obtained. The solid was taken onto the next step regardless, with the aim to reduce the carboxylic acid to the hydroxyl moiety. This was investigated using two methods; the first using lithium aluminium hydride[221] in diethyl ether, and the second using borane.[222] Unfortunately the poor solubility of **223** in the solvents was a problem and therefore no production of target material **220** was obtained (Scheme 74).

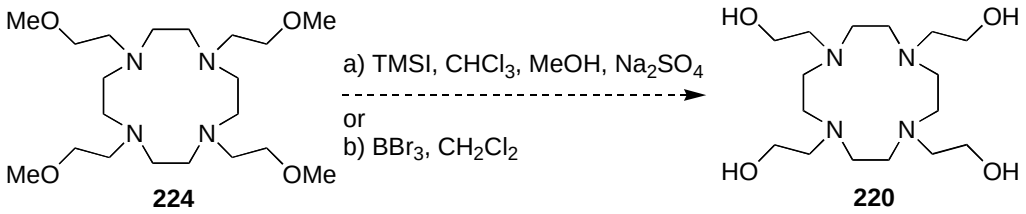


223 **220**

Scheme 74

5.7.3.3 Route VIII

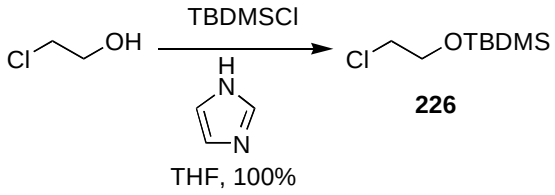
The most successful reaction was route VIII using 2-chloroethyl methyl ether in basic aqueous conditions, yielding yellow-white crystals **224** in 93% yield.[219] The next step is the removal of the methyl ether group to give the tetraol. Literature precedence and experience within the Robins group has shown that methoxy-group deprotection can be carried out using either trimethylsilyl iodide[223] or boron tribromide.[224] Unfortunately, neither of these methods was successful, returning only starting material despite varying the equivalents of reagents or increasing reaction times (Scheme 75).



224 **220**

Scheme 75

It was thought that this route could be adapted by using an alternative protected 2-chloroethanol derivative. Using *tert*-butyl-dimethylsilyl chloride (TBDMSCl) and imidazole in THF, **226** was prepared quantitatively (Scheme 76).[225] The TBDMS protecting group in theory should be easier to remove in later steps.

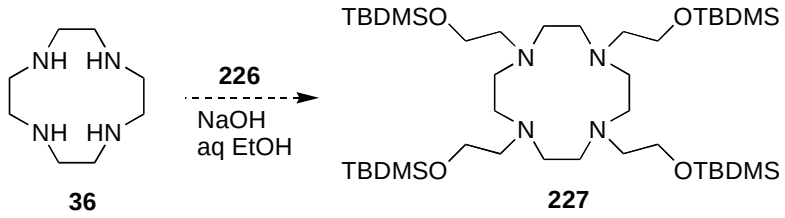


226

Scheme 76

Under the same conditions as before, **226** was reacted with cyclen to produce a yellow-white solid. The ^{1}H NMR spectrum of the crude product showed the absence of the TBDMS peaks, which were expected at around δ 0.8. It was postulated that the basic

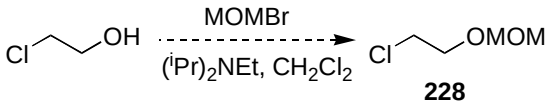
conditions of the reaction had removed the protecting group. Recrystallisation from hexane gave a small amount of white crystals that had the correct mass for deprotected compound **220**, however the ^{1}H and ^{13}C NMR spectra were inconclusive (Scheme 77).



36 **227**

Scheme 77

The methoxymethyl (MOM) group was also used to protect 2-chloroethanol using MOMBr and Hünig's base in dichloromethane (Scheme 78); however we were unable to isolate the target material.[226]



228

Scheme 78

5.8 Conclusions and Future Work

No substituted derivatives of cyclen were successfully converted into their corresponding alkylating agents. A number of problems occurred during this investigation, but these may be overcome in the future. The main point to be taken is the difference in properties between cyclen and its substituted derivatives. In future, the test reactions would be better employed on the substituted derivatives because a number of reactions that were successful on cyclen were not on the aromatic analogues. The solubility of the substituted starting material was problematic, making them difficult to dissolve and stir in a range of starting materials. One possibility would be to attempt the ethylene oxide method again, using ethanol or methanol instead of water.

In the other routes attempted (V, VII, VIII), the recrystallisation step was key. In future, using different solvent systems and longer crystallisation times may increase the yield and quality of product. It would be useful to obtain crystal structures for the products of successful reactions to determine the conformation of the ring in order to confirm whether the substituted analogues with the bulky group could be alkylated at the *N*-1 position. The target material was not isolated from a number of reaction mixtures due to time constraints.

One of the main barriers in the characterisation of compounds was the complicated ^{1}H and ^{13}C NMR spectra of the substituted analogues. Analysis heavily relied on mass spectral data in a number of cases, which often did not give conclusive evidence.

The route presented in Chapter 5.7.3.3 provides an opportunity to investigate different protecting groups of 2-chloroethanol, and in this area of work the possibilities were not exhausted. Other possible protecting groups include substituted ethyl ethers, benzyl ethers and sulfonates. An attractive target material would contain a group such as tosylate, as this often leads to products that are easily crystallised.

6 Synthesis of Pyrazolidine-3,5-diones for the Treatment of Cancer

6.1 Cancer-Causing Genes

It is now understood that cancer is caused and driven by mutations and the abnormal expression of genes that alter cellular pathways, which normally regulate the life and death of healthy cells. These mutations can be the result of many stimuli; some well-documented ones are exposure to cigarette smoke and ultraviolet radiation.[183] There are 291 known cancer-causing genes that are divided into four main groups. [227]

6.1.1 Oncogenes

These are genes that stimulate cell growth rate. When mutated, oncogenes are switched on permanently.[183]

6.1.2 Suicide Genes

Apoptosis (cell suicide) is normal and highly important. Under stress, cells will induce apoptosis to prevent cancer development. If suicide genes are mutated a cell damaged by stress may undergo cell division where the daughter cells are also mutated.[183]

6.1.3 DNA-Repair Genes

DNA is constantly attacked and is often damaged. There are a number of genes that primarily code for DNA-repair proteins. If these genes are damaged, the cell's ability to repair itself is reduced and this results in the accumulation of gene errors leading to cancer formation.[183]

6.1.4 Tumour Suppressor Genes

Tumour suppressor genes perform the opposite role to oncogenes and are active when a cell is at ‘rest’, and they switch off cell division function. One of the most important tumour suppressor genes is called *p53*, which codes for a tetrameric tumour protein p53.[183]

6.2 p53: “Guardian of the Genome”

An article in *Science* states that “it is possible that p53 is absolutely crucial for cancer - that it is always inactivated, directly or indirectly, during all human carcinogenesis”.[228]

p53 was discovered in 1979 by David Lane, Arnold Levine and Lloyd Old, and was voted *molecule of the year* by Science Magazine in 1993.[229] Once described as “the guardian of the genome” due to its role in preventing genomic mutation, p53 is central to a cell's anticancer function.[230] p53 is a DNA binding transcription factor, named on account of its 53 kilodalton (kDa) molecular mass. Its gene *p53* was first cloned in 1983 by Moshe Oren and is located in human chromosome 17.

p53 is 393 amino acids long with three domains, an N-terminal transcription-activator domain, a central DNA-binding core domain, and a C-terminal homo-oligomerisation domain. Mutations of p53 usually occur in the DNA-binding core domain, removing its ability to bind to its target DNA sequences.[231] This results in the loss of the transcriptional activity of these genes. If the gene *p53* is mutated, tumour suppression is greatly reduced. This can be inherited as a disease known as Li-Fraumein syndrome when people inherit only one functional copy of *p53*. People with this disease usually develop tumours in early adulthood.[232] *p53* is directly mutated in ~50% of all human cancers, and in the other 50% of cases, wild type *p53* is inactivated by a variety of other methods.[233]

p53 is normally found in low concentrations in the cell bound to another protein called Human Double Minute-2 (HDM2) and together they form an autoregulatory feedback mechanism to prevent tumour formation. Figure 29 contains the negative feedback loop (however HDM2 is represented by MDM2 (Mouse Double Minute-2)). The presence of p53 stimulates the expression of HDM2 by its gene (*HDM2*), and in turn, HDM2 inhibits p53 activity by blocking its transcriptional activity that induces its degradation by acting as an ubiquitin ligase (E3). Ubiquitinated p53 is transported in the cytoplasm for degradation by cytoplasmic proteasomes. Stress signals, such as DNA damage or oncogene activity, induce p53 activation. This favours p53 phosphorylation, which prevents it binding to HDM2, in turn preventing p53 degradation. p53 can then go on to bind to p53-response elements, inducing the transcription of various genes which results in a number of cellular responses such as DNA repair, differentiation, senescence (cell deterioration), cell-cycle arrest, or apoptosis.[233,234]

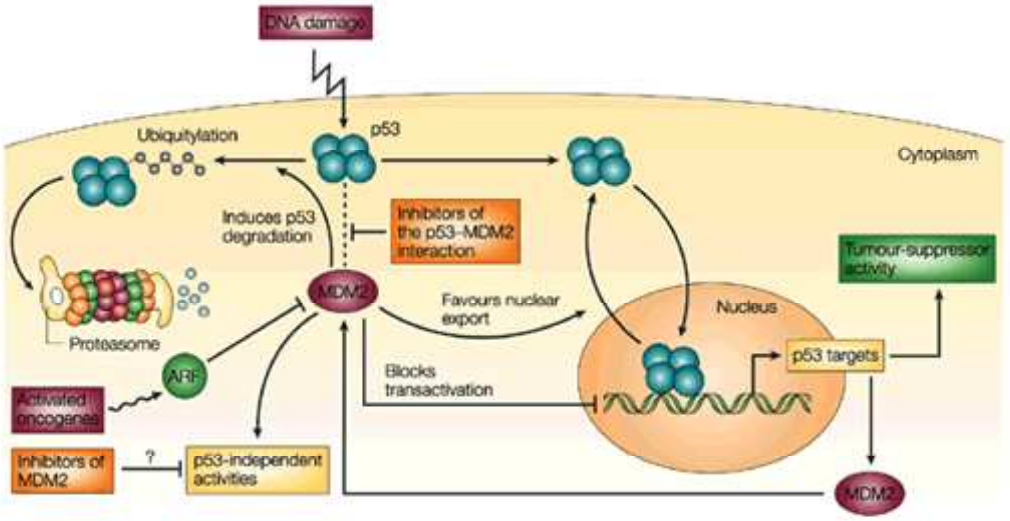


Figure 29

HMD2 requires a closer look; not only is it a p53 binding protein, but it also performs the catalytic function of an E3 ligase, facilitating the covalent attachment of ubiquitin proteins to p53. The ubiquitin-proteasome system is an important general regulatory pathway that controls the levels of key proteins, enzymes and receptors. Substrates that are intended for proteosome-mediated hydrolysis are labelled by the multiple-attachment of ubiquitin to form a poly-ubiquitinated protein.[235]

Ubiquitylation occurs in a cascade of steps (Figure 30). Firstly ubiquitin is activated in two steps by an E1 ubiquitin-activating enzyme in an ATP driven process. The result is a thioester linkage from the E1 cysteine sulfhydryl group to the C-terminal carboxyl group of ubiquitin. The next stage is the transfer of ubiquitin from E1 to the active site of an ubiquitin-conjugating enzyme (E2). The final step of the process occurs when an E3 ubiquitin-protein ligase (HDM2) recognises the E2 and its substrate, and transfers the substrate using the RING (or RING finger) domain. This process is repeated to poly-ubiquitinate the substrate, which is now ready for degradation in the proteasome.[236]

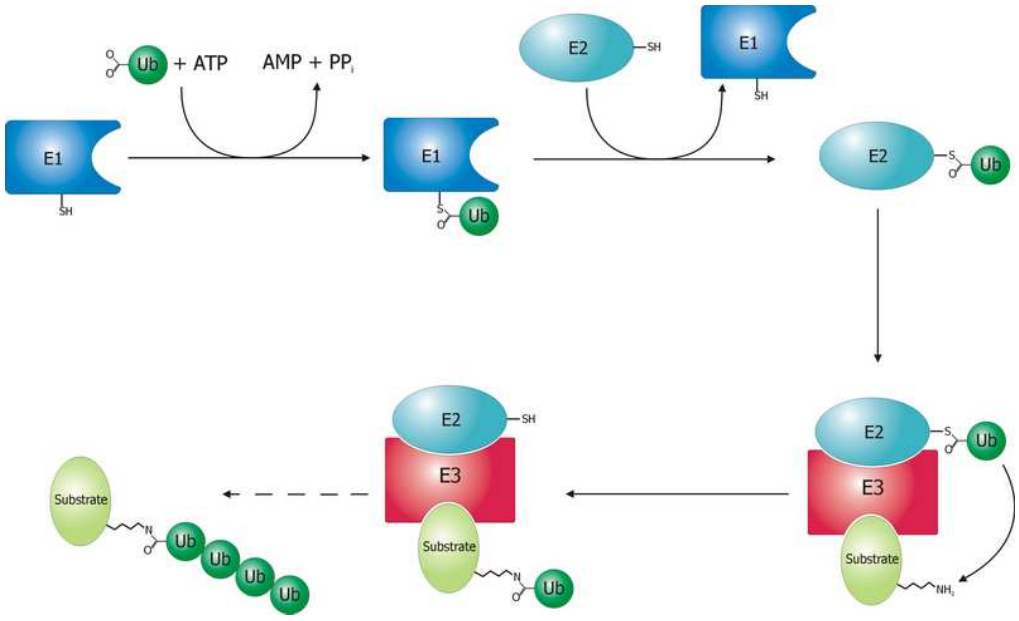


Figure 30

6.3 p53-HDM2 as a Drug Target

The rescue of p53 function in malignant cells would be a desirable anticancer strategy. p53 reactivation in tumour cells could trigger mass cellular suicide, and so there is an urgent need to find HDM2 inhibitors.[237] How do we target E3 ligases with drugs? Protein-protein interactions are awkward targets as proteins usually have large, flat interacting surfaces that are not easily affected by small molecule drug targets. However, the p53-HDM2 interaction site has been mapped to show that when the unstructured p53 protein docks onto HDM2, a conformational change occurs at the p53 transactivation domain to form an amphiphilic α-helix that projects three hydrophobic amino acid residues Phe[19], Trp[23], and Leu[26] into a deep hydrophobic pocket on the HDM2 surface (Figure 31).[238]

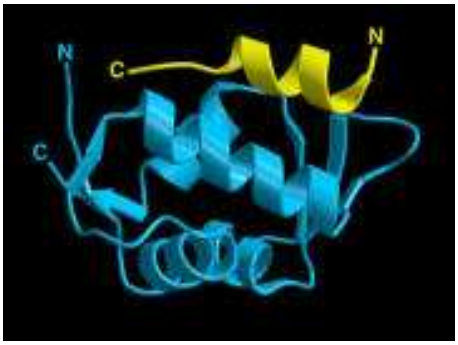
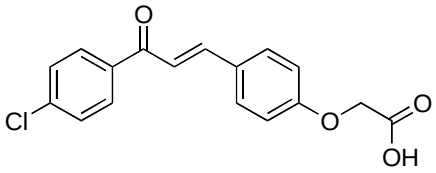


Figure 31

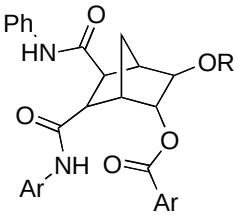
Novel peptides which bound to HDM2 with a higher affinity than p53 were found, allowing biophysical, combinational permutation and crystal structure studies. These, as well as the development of quantitative structure-activity relationship (QSAR) studies, allowed the identification of small molecule HDM2 antagonists.[238]

6.3.1 Small Molecule HDM2 Antagonists

The first small molecule HDM2 antagonists, which were reported in 1999, are derivatives of phenoxy-acetic acid and phenoxymethyl tetrazole, called the chalcones **229**. They have shown IC_{50} values within the μM range by binding in the hydrophobic pocket of HDM2.[239] However, they also inhibit glutathione-*S*-transferase activity as an undesirable side effect. Non-peptidic polycyclic antagonists **230** were also prepared by Zhao and co workers, which show moderate affinity for HDM2 and the initiation of p53-moderated apoptosis in tumour cell lines.[240]

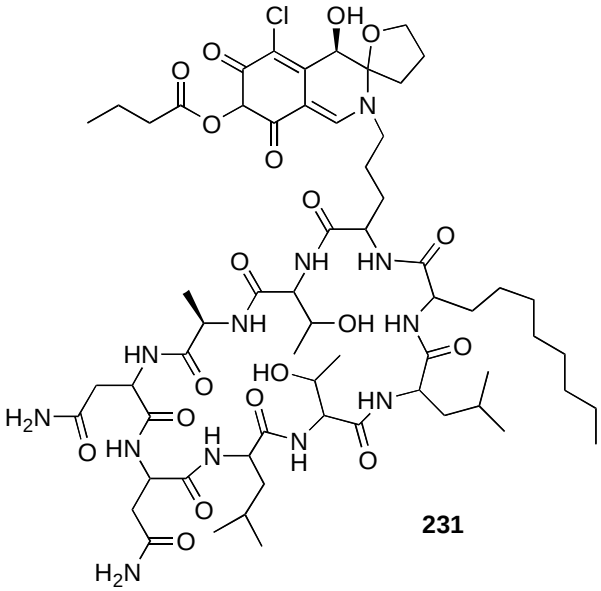


229



230

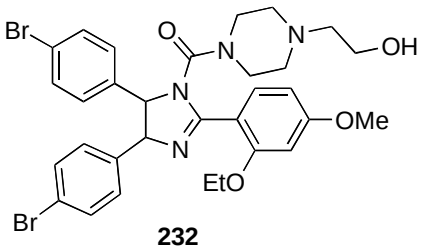
A fungal extract identified from a library screening identified the nonapeptide chlorofusin **231** that inhibits the p53-HDM2 interaction. However due to its complex structure and high molecular mass, chlorofusin is an unsuitable drug candidate but is a lead structure for future drug design.[241]



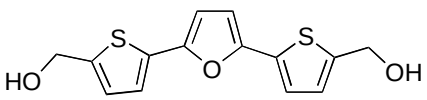
231

The first potent and selective small molecule antagonists were identified by Vassilev and co-workers in 2004. They are *cis*-imidazoline derivatives called the nutlins **232**.[242] Nutlins displace p53 from the hydrophobic pocket of HDM2 and bind tightly with IC_{50} values within the 100-300 nM range. These compounds project functional groups into the binding pocket and mimic the interaction of the three critical amino acids Phe[19], Trp[23], and Leu[26]. Nutlin activity is only observed in cells with wild-type *p53*, but not in mutant or deleted *p53* as the antitumour effect is on the p53 pathway.

In the same year, workers at the Karolinska Institute in Sweden reported a small molecule called Rita **233** (Reactivation of p53 and Induction of Tumour cell Apoptosis), which was identified in a library screen. Like the nutlins, this compound binds to p53 and induces its accumulation in tumour cells and prevents the p53-HDM2 interaction resulting in substantial cell suicide.[237] Rita is 2,5-bis(5-hydroxymethyl-2-thienyl)furan (NSC652287) and may serve as a lead compound for the design of drugs against tumours of wild-type *p53*.



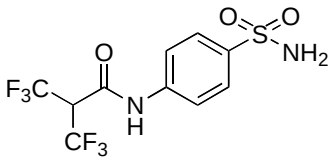
232



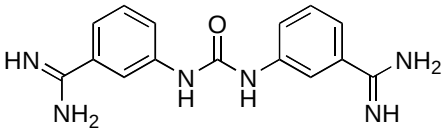
233

6.3.2 Small Molecule HDM2 Ubiquitin Ligase Inhibitors

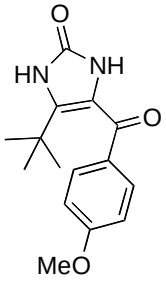
There are also a number of examples of specific targeting of the E3 ubiquitin ligase activity using small molecules. Lai and co workers used an enzyme assay to identify inhibitors by monitoring the HDM2 catalysed ubiquitin-transfer to p53.[235] They reported three chemically distinct types of inhibitor, each with selective action in the μmol range (**234** - **236**). In this case, the compounds behaved as reversible, non-competitive inhibitors with respect to p53 - blocking catalysis by HDM2 *via* a mechanism other than acting in the p53 binding site.



234

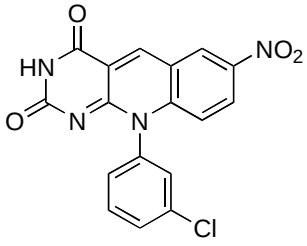


235

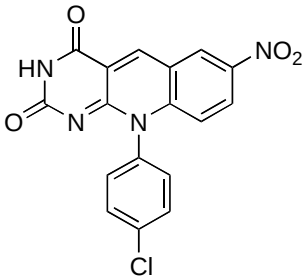


236

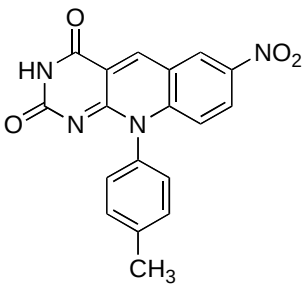
More recently Prof. K. Vousden FRS, director at the Beatson Cancer Institute in Glasgow, in collaboration with Dr Allan Weissman from the National Cancer Institute and Igen Pharmaceuticals, has developed a screen to identify small molecule inhibitors of the E3 activity of HDM2 function.[243] A high throughput assay was carried out in tumours containing wild-type *p53*, and from a library of 10,000 compounds, 40 were found to inhibit HDM2 autoubiquitylation by more than 50%. Additional *in vitro* gel-based assays identified three compounds containing a 5-deazaflavin structural motif (**237** - **239**).[244]



237



238



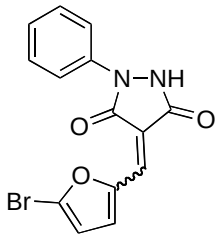
239

These compounds show some specific inhibition of the E3 activity of HDM2 *in vitro* and *in vivo*, allowing the stabilisation of p53 and HDM2, leading to the activation of p53-dependent transcription and apoptosis. Unfortunately p53-independent toxicity was observed. Following this publication, Prof. Robins' group in the University of Glasgow

synthesised a small library of 5-deazaflavin-containing compounds in order for Prof. Vousden to test their inhibitory effects on HDM2 ligase.[245]

6.4 Aim of this Work

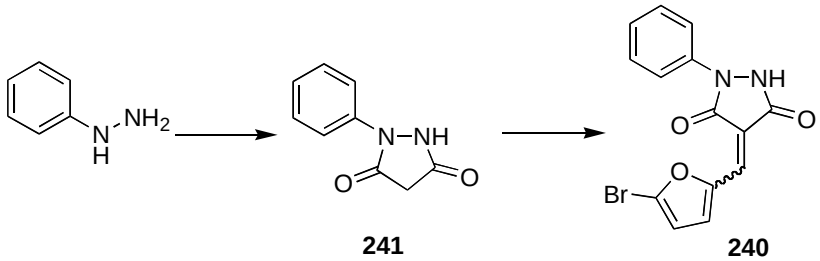
In the high throughput assay carried out by Vousden and co-workers, another compound, 4-(5-bromo-2-furfurylidene)-1-phenyl-pyrazolidine-3,5-dione **240**, was identified as an HDM2 autoubiquitylation inhibitor.[243] Our aim was to extend this work to develop an efficient synthesis of **240**, and to synthesise a small library of analogues, varying the electronic character of the substituents in an attempt to study structure/activity relationships and to develop more potent inhibitors.



240

6.5 Synthesis and Evaluation of Pyrazolidine-3,5-diones

240 can be synthesised over two steps by formation of **241** from phenylhydrazine followed by a Knoevenagel-type condensation with 5-bromo-2-furaldehyde to complete the synthesis (Scheme 79). This route provides a way of making different analogues of **240**, by using differently substituted furfuraldehyde derivatives or phenylhydrazines to build up a library of compounds for investigation of potency, solubility and selectivity of analogues of the lead compound.



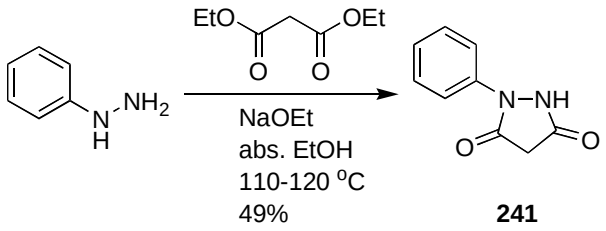
241 **240**

Scheme 79

6.5.1 Preparation of 1-phenylpyrazolidine-3,5-dione 241

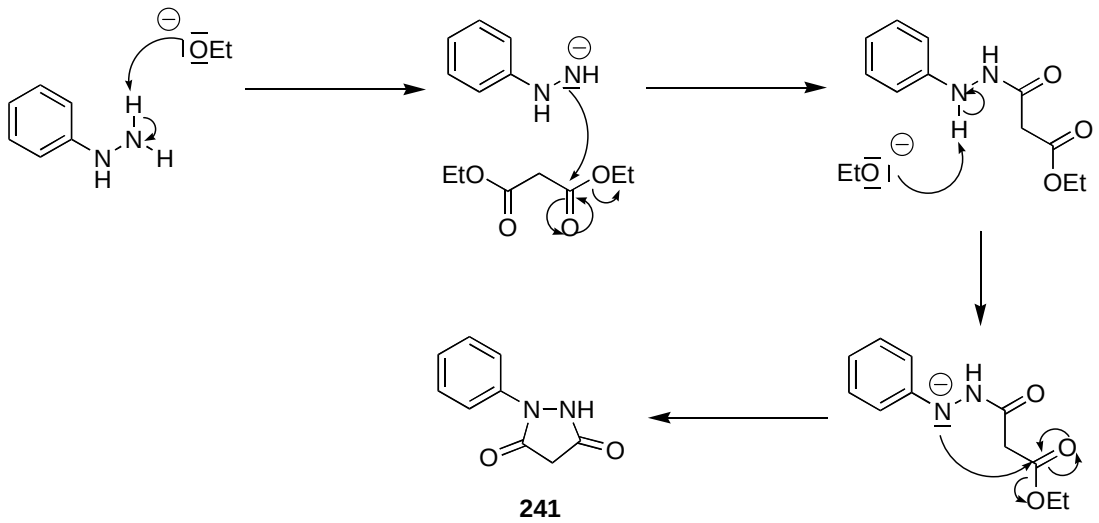
6.5.1.1 Route I

The first route to 1-phenylpyrazolidine-3,5-dione **241** followed a preparation by Conrad *et al.* from 1906.[246] Commercially available phenylhydrazine was reacted with diethyl malonate and sodium ethoxide to give **241** in 49% yield (Scheme 80).



Scheme 80

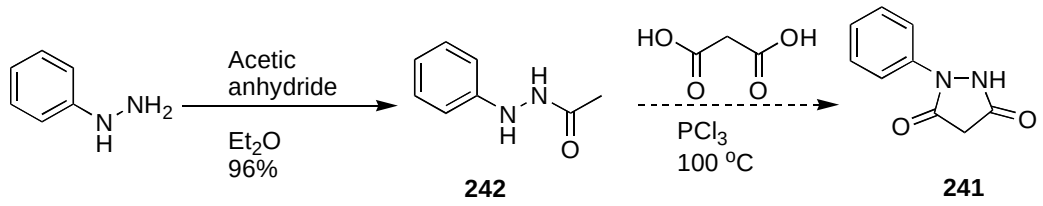
Conrad's 64% yield could not be matched; however their higher yields were possibly due to a mixture of **241** and an inorganic by-product that we had managed to separate. Further purification by recrystallisation from water produced a pure yellow powder, which gave excellent NMR spectroscopic data, but resulted in lower yields. The crude material can be used in the next step of the synthesis. The reaction mechanism for this condensation is given in Scheme 81.



Scheme 81

6.5.1.2 Route II

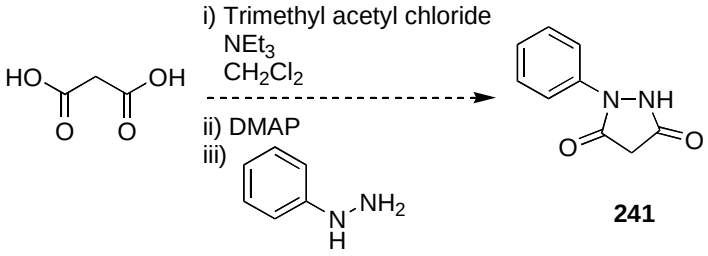
A second route was attempted using a method reported by Duffy and co-workers, which involved reacting phenylhydrazine with acetic anhydride to form **242** in 96% yield (Scheme 82).[247] **242** was then treated with malonic acid and phosphorus trichloride. TLC showed incomplete reaction with little product formed. Flash chromatography could be used to separate the product; however low yields were predicted and so this route was abandoned.



Scheme 82

6.5.1.3 Route III

A route following a preparation by J. Ferguson of the Robins group involved treating trimethylacetyl chloride with triethylamine and malonic acid, before adding DMAP and phenylhydrazine (Scheme 83).[248] Unfortunately TLC of the reaction mixture showed that the reaction had not gone to completion, despite using longer reaction times. The stated work-up using flash chromatography may also have been problematic due to the polar nature of the target material, and so this route was also discarded. Route I was therefore the best method for the synthesis of **241**.

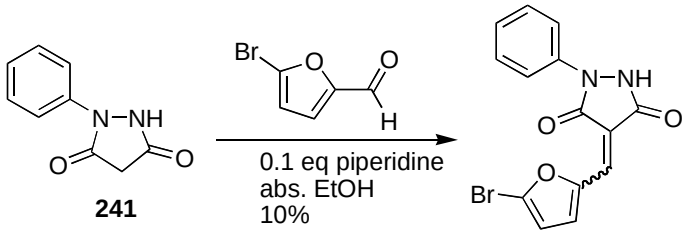


Scheme 83

6.5.2 Preparation of 4-(5-Bromo-2-furfurylidene)-1-phenyl-pyrazolidine-3,5-dione 240

6.5.2.1 Route I

To complete the synthesis of **240** requires only one step, and the first attempt used a method reported by Hassanein which requires heating **241** and 5-bromo-2-furaldehyde under reflux with piperidine (Scheme 84).[249] Filtration of the brown precipitate gave pure compound **240**; however the 10% yield was disappointing.

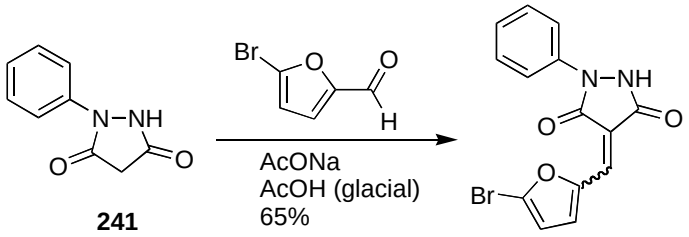


241 **240**

Scheme 84

6.5.2.2 Route II

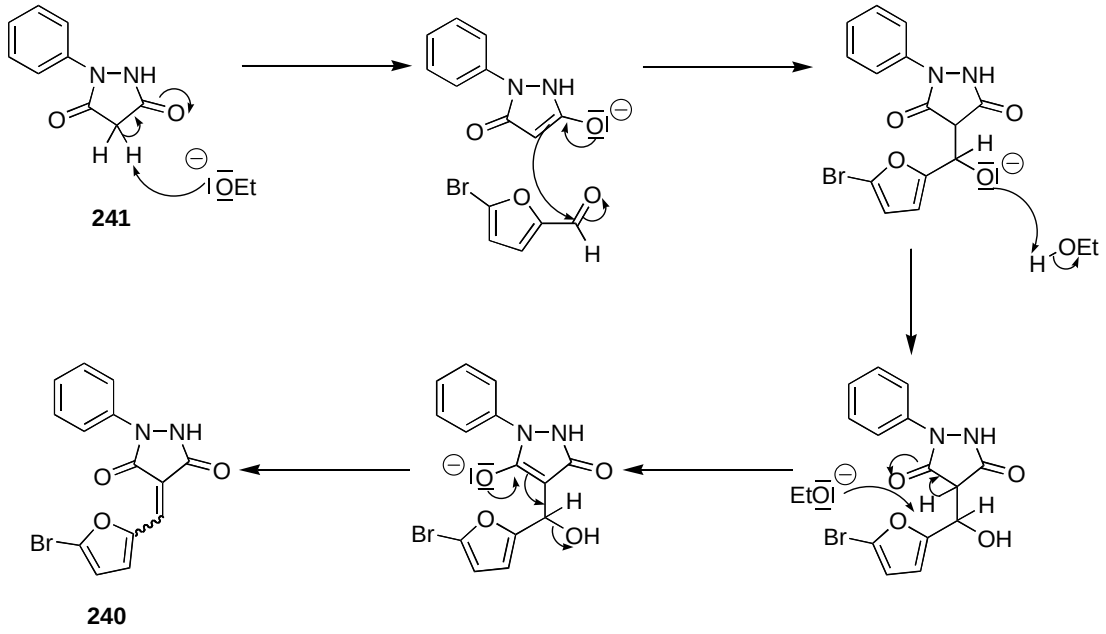
An aldol condensation using a method by Abass provided higher yields of **240**.[250] Equimolar amounts of **241**, 5-bromo-2-furaldehyde and sodium acetate were heated in glacial acetic acid under reflux. Following filtration and rinsing with ethyl acetate, the pure golden brown product was obtained in 65% yield (Scheme 85).



241 **240**

Scheme 85

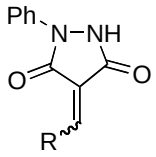
The mechanism of this condensation is shown in Scheme 86, and this route was used to provide analogues of **240**. It must be noted that this reaction results in the production of two different geometric isomers, and the ratio of the isomers can be estimated from the ^{1}H and ^{13}C NMR spectra.



Scheme 86

6.5.3 Preparation of 4-(5-Bromo-2-furfurylidene)-1-phenyl-pyrazolidine-3,5-dione Analogues

Seven analogues of **240** were prepared by condensing **241** with commercially available aldehydes. They included a range of different functional groups on the bottom heterocycles, varying heteroatoms within the five-membered ring, and a larger aromatic moiety (**243** – **249**). A further thirteen analogues were prepared (**250** – **262**) by fourth year project student N. Jobson using the same method and are included here for comparison purposes.[251] Table 12 contains the structures with the yields, log P values and some UV analysis.



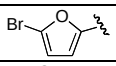
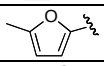
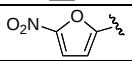
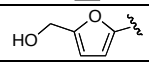
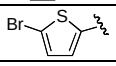
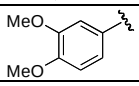
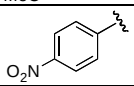
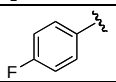
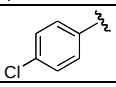
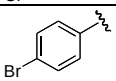
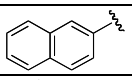
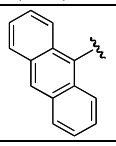
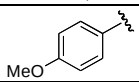
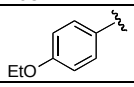
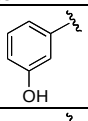
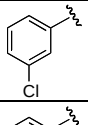
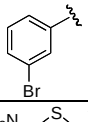
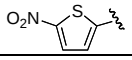
Compound	R	Yield (%)	Est. log P	λ_{max} (nm)
240		65	2.31	-
243		77	1.42	-
244		67	1.97	-
245		86	1.24	-
246		48	0.5	-
247		42	0.94	-
248		82	2.76	-
249		65	2.05	-
250		34	1.69	394
251		22	1.87	326
252		22	2.25	328
253		26	2.69	331
254		23	2.94	334
255		35	3.23	346
256		19	4.40	369
257		24	2.13	-
258		16	2.62	-
259		22	1.57	-
260		14	2.69	-
261		12	2.94	
262		19	1.69	-

Table 12

6.5.4 Biological Evaluation of Library

Compounds **243** – **256** were tested at the Cancer Research UK Beatson Laboratories in Glasgow along with **240** for comparison. The cultured cells were exposed to the compounds and then SDS-PAGE was used to separate the proteins by size. After a Western Blot, the comparison of the intensity of the compound bands with a control band gave an indication of biological activity. A positive control was used to indicate the maximum amount of stabilisation. If the compound band is more intense than the control band, then stabilisation of p53 is taking place. The darker the band appears, the higher the potency of the drug candidate.

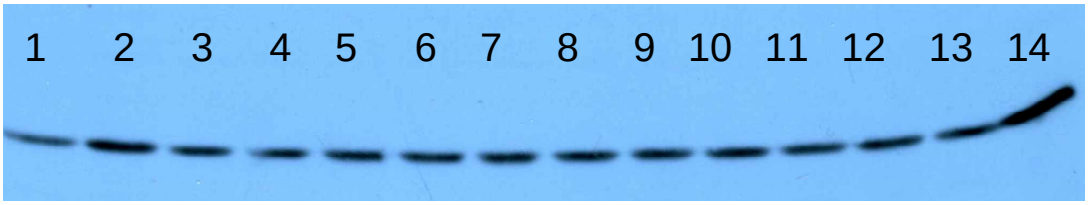


Figure 32

Figure 32 shows an example Western Blot containing a known HDM2 inhibitor (band 2) and a proteosome inhibitor adriamycin (band 14). Bands 3 – 13 are drug candidates. In general it was found that none of the compounds showed significant stabilisation of p53, and although compound **240** was theoretically the positive control, it also displayed little activity.

6.6 Conclusion and Future Work

Overall compounds **243**, **244**, **249**, **250** and **252** displayed activity; however these results were weak and non-reproducible. From the results it is difficult to rank the compounds in order of potency, and no structure-activity relationships can be defined.

7 Experimental

7.1 General Experimental Details

All reactions were carried out under an inert atmosphere unless otherwise stated, using oven-dried or flame-dried glassware. Solutions were added *via* syringe unless otherwise stated. THF and Et_2O were freshly distilled from Na-benzophenone; CH_2Cl_2, toluene, DMF and pyridine were distilled from CaH_2 prior to use. Petroleum ethers refer to the fraction boiling at 40-60 ^oC. Reagents were obtained from Aldrich Chemical Company (Gillingham, Dorset, UK), Alfa Aesar Lancaster (Morecambe, Lancs, UK), or Alfa Aesar Avocado (Heysham, Lancs, UK) and used without further purification unless otherwise stated. Purification by column chromatography was carried out using Fischer Silica 60A silica gel (mesh size 35-70 μm) as the stationary phase. Melting points were measured using Gallenkamp apparatus and are uncorrected. IR spectra were recorded using Golden Gate, nujol or KBr on a JASCO FT/IR 410 spectrometer. NMR spectra were recorded using a Bruker AV400 or DPX/400 spectrometer. Chemical shifts are given in ppm relative to $SiMe_4$. Chemical shifts in ^{13}C NMR spectra are given in ppm relative to $CDCl_3$ as internal standard (77.00 ppm). All NMR *J* values are given in Hz. Mass spectra were recorded on a JEOL JMS700 spectrometer. Optical rotations were determined as solutions irradiating with the sodium D line (λ = 589 nm) using an AA series Automatic polarimeter. $[\alpha]_D$ values are given in units 10^{-1} $degcm^2g^{-1}$.

7.2 Experimental to Chapter 3

7.2.1 General Procedures (3A – 3D)

A. Preparation of tosylated cyclen under phase transfer conditions:[104] A solution of tosylated amine (1 eq) and tosylated diol (1 eq) dissolved in toluene (60 mL per mmol) was added to a mixture of tetrabutylammonium bromide (0.25 eq) and aq LiOH (2.5%) (10 mL per mmol) in toluene (20 mL per mmol) stirred and heated under reflux. The mixture was stirred vigorously at this t for 20 h – 7 d. After this time, the organic layer was separated and evapourated *in vacuo* to leave a solid.

B. Formation of tosylated amines and diols:[113] *p*-Toluenesulfonyl chloride (1 eq per tosylation site) was dissolved in dry pyridine (0.5 mL per mmol) and cooled to 0 ^oC. The amine or diol (1 eq) was added slowly and the solution stirred at this t for 4 h, and then

stirred over night at rt. The solution was then poured into an ice : H_2O mixture and allowed to precipitate over night. After this time, the solid was filtered and washed with H_2O, before recrystallising in EtOH.

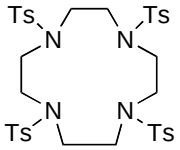
C. Synthesis of glyoxals and monohydrates from acetophenone derivatives:[129] Acetophenone (1 eq) was added to stirring solution of selenium (IV) oxide (1.1 eq) in 1,4-dioxane : H_2O mixture (0.75 mL per mmol : 0.1 mL per mmol). The reaction mixture was stirred and heated under reflux for 18 h -7 d followed by TLC. After this time, the solution was filtered through Celite® to remove selenium residues. The liquid was then concentrated *in vacuo* to give the glyoxal.

The glyoxals in some cases were converted to the monohydrate by dissolving in H_2O (5 mL per mmol) and stirring and heating at 100 ^oC for 2 h.[252] After this time the aq solution was filtered, and left to stand to allow precipitation of the product, which was filtered and dried under reduced pressure.

D. Iron template cyclisation to give substituted tetraazamacrocycles:[128] A solution of triethylenetetramine (TETA) (1 eq), in MeOH (7 mL per mmol) was added dropwise over 10 min to a solution of ferric chloride (1 eq) in MeOH (15 mL per mmol). The mixture was stirred at rt for 2 h. A solution of glyoxal (1 eq) in MeOH (17 mL per mmol) was added and the mixture was stirred for a further 4 h at this t. $NaBH_4$ (10 eq) was added carefully with cooling, and the mixture was heated under reflux for 2-19 h. After this time, the solvent was removed *in vacuo*, and the resulting residue was dissolved in a minimum of H_2O. The pH was adjusted to 1 using conc HCl and the aq layer was extracted with CH_2Cl_2 (x 3). The aqueous layer was adjusted to pH 14 with NaOH pellets then extracted with $CHCl_3$ (x 3). The organic layers were combined, dried over anhydrous K_2CO_3, and concentrated under reduced pressure to give the azamacrocycle.

7.2.2 Experimental Details

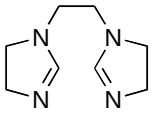
1,4,7,10-Tetrakis-(toluene-4-sulfonyl)-1,4,7,10-tetraaza-cyclododecane (43)



Using general procedure 3A, **43** was synthesised from ditosylated ethylenediamine **45** (737 mg, 2.0 mmol) and *N,N',O,O'*-tetra(*p*-toluenesulfonyl)-*N,N'*-bis(2-hydroxyethyl)-ethylenediamine **44** (1.53 g, 2.0 mmol) after stirring for 20 h under reflux, as a pale solid (1.25 g, 79%). mp: 276-279 ^{o}C (lit.[90] 278-280 ^{o}C); δ_H (400 MHz, $CDCl_3$): 2.38 (12H, s, 4 x CH_3), 3.36 (16H, s, 8 x ring CH_2), 7.26 (8H, d, *J* 8.2 Hz, 8 x Ar-H), 7.62 (8H, d, *J* 8.2 Hz, 8 x Ar-H); δ_C (100 MHz, $CDCl_3$): 21.0 (4 x CH_3), 51.3 (8 x CH_2), 79.2 (4 x ArC), 127.0 (4 x ArCH), 127.4 (4 x ArCH), 130.0 (4 x ArCH), 130.1 (ArCH), 144.0 (4 x ArC); LRMS (FAB): 789.5 ($[M+H]^+$, 16%), 242.4 (100), 142.2 (16), 91.5 (19); HRMS calcd for $C_{36}H_{44}N_4O_8S_4$ 789.2120, found 789.2123.

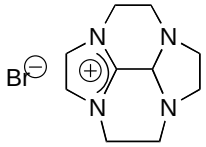
43 was also prepared using general procedure 3A by cyclising **46** (5.0 g, 8.8 mmol) and **47** (5.02 g, 8.8 mmol) to give **43** as a white solid (6.83 g, 98%) after 7 d.

1, 1'-Ethylenedi-2-imidazoline (48)



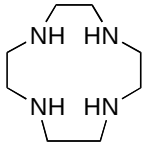
To a stirred solution of TETA (100 mL, 669 mmol), was added *N,N*-dimethylformamide dimethyl acetal (178 mL, 1.34 mol). The solution was heated under reflux for 2 h before the reaction mixture was dried *in vacuo* to produce an off-white solid (111.12 g, 100%). mp: 106.5-108 ^{o}C (lit.[105] 107-109 ^{o}C); ν_{max} (neat)/cm^{-1}: 2935 (CH_2), 2848 (NCH_2, C-H stretch), 1649 (C=N); δ_H (400 MHz, $CDCl_3$): 3.16 (4H, t, *J* 9.6 Hz, 2 x CH_2), 3.20 (4H, s, 2 x CH_2), 3.78 (2H, t, *J* 9.6 Hz, CH_2), 3.78 (2H, t, *J* 9.6 Hz, CH_2), 6.74 (2H, s, 2 x CH); δ_C (100 MHz, $CDCl_3$): 47.1 (2 x CH_2), 49.1 (2 x CH_2), 55.6 (2 x CH_2), 157.8 (2 x CH); LRMS (EI^+): 166.1 ($[M]^+$, 55%), 165.1 (16), 124.1 (7), 84.1 (34), 83.1 (100), 56.1 (100).

2,3,4,5,6,7,8,8*c*-Octahydro-1*H*-4*a*,6*a*,8*a*-triaza-2*a*-azo-niacyclopent[*fg*]acenaphthylene bromide (49)



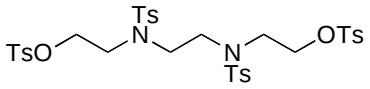
To a solution of CH_3CN (1.4 L) was added **48** (30.0 g, 180 mmol), dibromoethane (21.8 mL, 252.0 mmol), and K_2CO_3 (19.9 g, 144.0 mmol). The mixture was stirred and heated under reflux for 3 h, before the solution was filtered to remove K_2CO_3, and dried *in vacuo*, to give **49** as a dark yellow viscous oil (50 g, 100%). ν_{max} (neat)/cm^{-1}: 2935 (CH_2), 2817 (N-CH) cm^{-1}; NMR spectroscopy agreed with lit. values;[105] δ_H (400 MHz, D_2O): 2.47-2.93 (6H, m, 3 x CH_2), 3.4-3.6 (6H, m, 3 x CH_2), 3.6-3.8 (4H, m, 2 x CH_2), 4.7 (1H, s, CH); δ_C (100 MHz, $CDCl_3$): 41.1 (2 x CH_2), 41.7 (2 x CH_2), 45.7 (2 x CH_2), 48.5 (2 x CH_2), 68.9 (CH), 158.8 (C).

1,4,7,10-Tetraazacyclododecane (cyclen) (36)



49 (105 g, 384.0 mmol) was dissolved in H_2O (420 mL), and the solution was added dropwise to a solution of KOH (172.5 g, 3.08 mol) heated under reflux in H_2O (365 mL). The resulting solution was heated under reflux for a further 30 min. The solution was gravity filtered while hot and the filtrate concentrated *in vacuo* until crystallisation occurred. **36** was produced as pale yellow crystals. Cyclen was extracted into hot toluene and obtained by concentration (47.02 g, 71%); mp: 114-116 ^oC (lit.[106] 110-113 ^oC); ν_{max} (neat)/cm^{-1}: 2927 (CH_2), 2898 (CH_2), 2870 (CH_2), 2812 (NCH_2), 1502 (N-H); δ_H (400 MHz, $CDCl_3$): 1.77 (4H, br s, 4 x NH), 2.60 (16H, s, 8 x CH_2); δ_C (100 MHz, $CDCl_3$): 46.3 (8 x CH_2).

_N,N',O,O'_-Tetra(_p_-toluenesulfonyl)-_N,N'_-bis(2-hydroxyethyl)-ethylenediamine (44)

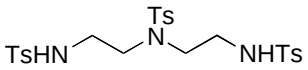


Using *p*-toluenesulfonyl chloride (38.59 g, 202.4 mmol) and *N*,*N'*-bis(2-hydroxyethyl)-ethylenediamine (5 g, 33.74 mmol), **44** was prepared as a bright yellow solid (17.96 g, 70%) *via* general procedure 3B. mp: 127-129 ^{o}C (lit.[253] 144-146 ^{o}C); ν_{max} (neat)/cm^{-1}: 2959 (Ar-CH_3), 1173 (SO_2-O), 1149 (SO_2-O), 1038 (S=O); δ_H (400 MHz, $CDCl_3$): 2.37 (12H, s, 4 x CH_3), 2.23 (4H, s, 2 x NCH_2), 3.29 (4H, t, *J* 5.4 Hz, 2 x NCH_2), 4.07 (4H, t, *J* 5.4 Hz, 2 x OCH_2), 7.27 (8H, d, *J* 8.3 Hz, 8 x Ar-H), 7.64 (4H, d, *J* 8.3 Hz, 4 x Ar-H), 7.7 (4H, d, *J* 8.3 Hz, 4 x Ar-H); δ_C (100 MHz, $CDCl_3$): 22.0 (4 x CH_3), 50.0 (2 x NCH_2), 50.3 (2 x OCH_2), 69.4 (2 x NCH_2), 127.8 (8 x ArCH), 130.4 (8 x ArCH), 132.8 (2 x ArC), 135.4 (2 x ArC), 144.4 (2 x ArC), 145.6 (2 x ArC); LRMS (FAB): 765.5 ($[M+H]^+$, 84%), 593.5 (71), 382.3 (71), 228.2 (37), 154.1 (78), 91.5 (100); HRMS calcd for $C_{34}H_{40}O_{10}N_2S_4$ 765.1644 , found 765.1647.

Another route to prepare **44** involved adding sodium hydroxide pellets (1.34 g, 33.5 mmol) to a cooled stirred mixture of *N*,*N'*-bis(2-hydroxyethyl)-ethylenediamine (1.0 g, 6.7 mmol) in H_2O (4 mL) at 0 ^{o}C. Et_2O (4 mL) was then added with vigorous mixing before *p*-toluenesulfonyl chloride (6.4 g, 33.5 mmol) was added. The solution was stirred at this t for 1 h before warming to rt and stirring for a further 3 h. The mixture was filtered and the precipitate was rinsed with Et_2O (3 x 10 mL) before recrystallising from $CHCl_3$. **44** was produced as white solid (562 mg, 11%).

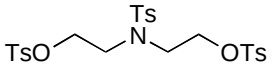
44 was also prepared by adding *N*,*N'*-bis(2-hydroxyethyl)-ethylenediamine (500 mg, 3.4 mmol) in CH_2Cl_2 (5 mL) to a cooled stirred solution of *p*-toluenesulfonyl chloride (3.22 g, 16.9 mmol) in CH_2Cl_2 (5 mL) at 0 ^{o}C. Triethylbenzylammonium chloride (TEBACl) (210 mg, 0.92 mmol) and aq sodium hydroxide (0.68 g, 17.0 mmol in 4 mL H_2O) were then added and the mixture was stirred at rt for 4 h. The solution was then poured into H_2O (10 mL), and the organic phase removed and washed with H_2O (3 x 5 mL) before drying over $MgSO_4$ and concentrating under vacuum. This method gave **44** as a white solid (2.07 g, 80%) which contained some starting material.

N,N',N''-Tris(*p*-tolylsulfonyl)diethylenetriamine (46)



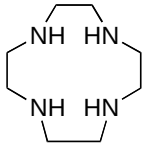
Using general procedure 3B, **46** was prepared from diethylenetriamine (2.0 g, 19.4 mmol) and *p*-toluenesulfonyl chloride (11.1 g, 58.2 mmol) as a white solid (7.7 g, 70%); mp: 174-176 ^{o}C (lit.[254] 174-176 ^{o}C); δ_H (400 MHz, DMSO-d_6): 2.39 (3H, s, CH_3), 2.40 (6H, s, 2 x CH_3), 2.81 (4H, dt, *J* 7.7 Hz, 2 x CH_2-N), 3.02 (4H, t, *J* 7.7 Hz, 2 x CH_2-N), 7.38 (2H, d, *J* 8.2 Hz, 2 x Ar-H), 7.40 (4H, d, *J* 8.2 Hz, 4 x Ar-H), 7.54 (2H, d, *J* 8.2 Hz, 2 x Ar-H), 7.66 (4H, d, *J* 8.2 Hz, 4 x Ar-H); δ_C (100 MHz, DMSO-d_6): 21.3 (3 x CH_3), 48.7 (2 x CH_2N), 48.7 (2 x CH_2N), 126.9 (4 x CH), 127.2 (2 x CH), 130.0 (2 x CH), 130.2 (4 x CH), 135.7 (2 x C), 137.7 (2 x C), 143.1 (2 x C).

N,O,O'-Tri(*p*-toluenesulfonyl)diethanolamine (47)



N,O,O'-Tri(*p*-toluenesulfonyl)diethanolamine **47** was prepared as white needle-like crystals (31.9 g, 59%) from diethanolamine (10 g, 95.1 mmol) and *p*-toluenesulfonyl chloride (81.6 g, 428 mmol) using general procedure 3B. mp: 85-87 ^{o}C (lit.[255] 93-96 ^{o}C); δ_H (400 MHz, $CDCl_3$): 2.45 (3H, s, CH_3), 2.48 (6H, s, 2 x CH_3), 3.40 (4H, t, *J* 6.2 Hz, 2 x CH_2-N), 4.14 (4H, t, *J* 6.2 Hz, 2 x CH_2-O), 7.31 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.38 (4H, d, *J* 8.0 Hz, 4 x Ar-H), 7.64 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.78 (4H, d, *J* 8.0 Hz, 4 x Ar-H); δ_C (100 MHz, $CDCl_3$): 21.6 (CH_3), 21.7 (2 x CH_3), 48.5 (2 x CH_2-N), 68.3 (2 x CH_2-O), 127.3 (4 x CH), 128.0 (4 x CH), 130.0 (4 x CH), 132.5 (2 x C), 135.3 (C), 144.2 (2 x C), 145.3 (C).

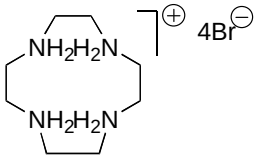
1,4,7,10-Tetraazacyclododecane (36)



43 (1.25 g, 1.58 mmol) and 98% H_2SO_4 (1.6 mL) were stirred and heated at 110 ^{o}C for 40 h. The mixture was then poured into a conical flask and cooled in an ice bath. H_2O (2 mL) was slowly added before KOH pellets were added with stirring to reach pH 14. EtOH (2 mL) was added and the mixture was filtered under reduced pressure. The precipitate was washed with EtOH (4 x 5 mL) and the EtOH extracts combined and dried *in vacuo*. The

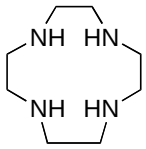
residue produced was dissolved in a minimum of 1M HCl and then extracted with CH_2Cl_2 (3 x 5 mL). The aq phase was then basified to pH 14 with KOH pellets and extracted with $CHCl_3$ (4 x 5 mL). The organic layers were combined, dried over K_2CO_3 and reduced *in vacuo* to give a pale oil. Recrystallisation from acetone gave cyclen **36** as a white solid (240 mg, 88%). δ_H (400 MHz, $CDCl_3$): 1.77 (4H, br s, 4 x NH), 2.60 (16H, s, 8 x CH_2).

Tetrahydrobromide salt of cyclen (53)



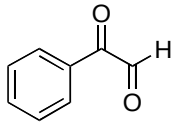
Conc H_2SO_4 (8 mL) was heated to 165 ^oC in a conical flask. **43** (3.0 g, 3.8 mmol) was added and solution stirred rapidly for 6 min. Transferring the solution to a second conical flask cooled in ice H_2O cooled the reaction. The solution was then added dropwise to a stirring flask of EtOH (26 mL). Et_2O (20 mL) was then added slowly and solution cooled to 0 ^oC. The solution was filtered and the solid dissolved in a minimum of hot H_2O (3 mL) and then 48% HBr (3 mL) was added. The solution was then filtered to remove precipitated hydrobromide salt, and the filtrate concentrated *in vacuo* to give the tetrahydrobromide salt **53** as a white powder (798 mg, 42%). δ_H (400 MHz, D_2O): 3.02 (16H, s, 8 x CH_2), 3.36 (4H, s, 4 x NH); δ_C (100 MHz, D_2O): 43.7 (8 x CH_2).

1,4,7,10-Tetraazacyclododecane (36)



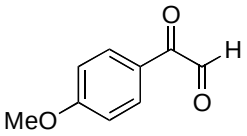
53 (798 mg, 1.6 mmol) was combined with H_2O (10 mL), toluene (3 mL), and NaOH (256 mg, 6.4 mmol) in a round-bottomed flask fitted with Dean-Stark apparatus. The solution was stirred and heated under reflux for 24 h to remove all H_2O, before the toluene solution was filtered and concentrated under vacuum to give cyclen **36** as a cream solid (196 mg, 71%). δ_H (400 MHz, $CDCl_3$): 1.77 (4H, br s, 4 x NH), 2.60 (16H, s, 8 x CH_2).

Phenylglyoxal (59)



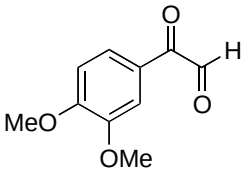
Using acetophenone (700 μL, 5.99 mmol), **59** monohydrate was prepared as a white-green solid (790 mg, 100%) *via* general procedure 3C. mp: 77-78 ^{o}C (lit.[256] 76-78 ^{o}C); δ_H (400 MHz, $CDCl_3$): 4.14 (1.4H, br s, C(O*H*)$_2$H), 5.91 (0.7H, s, C(OH)$_2$*H*), 7.42-7.48 (2H, m, 2 x Ar-H), 7.57-7.63 (1H, m, Ar-H), 8.03-8.11(2H, m, 2 x Ar-H), 9.6 (0.3H, s, C*H*O). δ_C (100 MHz, $CDCl_3$): 89.0 (C(OH)$_2$H), 129.3 (2 x Ar-H), 130.3 (2 x Ar-H), 132.6 (ArC), 135.1 (Ar-H), 193.2 (C=O).

4'-Methoxyphenylglyoxal (60)



Using 4'-methoxyacetophenone (3.5 g, 23.3 mmol), **60** hydrate was prepared as a brown solid (3.82 g, 100%) by general procedure 3C. mp: 93-95 ^{o}C (lit.[257] 126-128 ^{o}C); ν_{max} (neat)/cm^{-1}: 3413 (OH), 2940 (CO-H), 2845 (OCH_3), 1737 (C=O, aldehyde), 1679 (C=O, ketone); δ_H (400 MHz, $CDCl_3$): 3.64 (3H, s, OCH_3), 4.59 (2H, br s, C(O*H*)$_2$H), 6.23 (0.9H, s, C(OH)$_2$*H*), 6.81-6.93 (2H, m, 2 x Ar-H), 8.02-8.05 (2H, m, 2 x Ar-H), 9.57 (0.1H, s, C*H*O); δ_C (100 MHz, $CDCl_3$): 56.0 (CH_3), 89.0 (CH), 114.5 (2 x CH), 125.6 (C), 132.9 (2 x CH), 165.1 (C), 191.6 (C); LRMS (CI$^+$): 165.1 ([M+H]$^+$, 100%), 135.09 (29); HRMS calcd for $C_9H_{10}O_4$ 165.0552, found 165.0553.

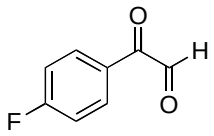
3',4'-Dimethoxyphenylglyoxal (61)



Using 3',4'-dimethoxyacetophenone (4.19 g, 23.3 mmol), **61** was prepared as a dark yellow solid (4.5 g, 100%) using general procedure 3C. mp: 100-103 ^{o}C (hydrate lit.[258] 199-200 ^{o}C); ν_{max} (neat)/cm^{-1}: 3415 (OH), 3114 (ArC-H), 2839 (OCH_3), 1670 (C=O, aldehyde), 1583 (C=O, ketone); δ_H (400 MHz, $CDCl_3$): 6.87 (1H, d, *J* 8.5 Hz, Ar-H), 7.61 (1H, d, *J* 1.9 Hz, Ar-H), 7.91 (1H, dd, *J* 8.5, 1.9 Hz, Ar-H), 9.6 (1H, s, C*H*O); δ_C (100

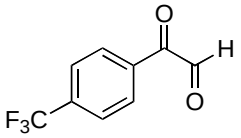
MHz, $CDCl_3$): 56.4 (CH_3), 56.6 (CH_3), 89.2 (ArCH), 110.8 (ArCH), 112.0 (ArCH), 125.4 (ArC), 126.0 (CH), 149.5 (ArC), 155.1 (ArC), 190.6 (C=O); LRMS (CI^+): 194.09 ($[M]^+$, 16%), 165.08 (100), 137.09 (16), 122.06 (13); HRMS calcd for $C_{10}H_{10}O_4$ 194.0579, found 194.0581.

4'-Fluorophenylglyoxal (62)



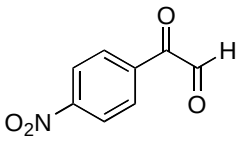
Using general procedure 3C, **62** hydrate was prepared as a brown solid (3.5g, 100%) from 4'-fluoroacetophenone (2.83 mL, 23.3 mmol). mp: 89-91 ^oC (lit.[259] 108-110 ^oC); ν_{max} (neat)/cm^{-1}: 3080 (Ar-H), 2949 (CO-H), 1748 (C=O, aldehyde), 1692 (C=O, ketone), 1101 (C-F); δ_H (400 MHz, $CDCl_3$): 4.95 (1.94H, br s, $C(O\mathit{H})_2\mathit{H}$), 6.24 (0.97H, s, $C(OH)_2\mathit{H}$), 7.06-7.10 (2H, m, 2 x Ar-H), 8.08-8.11 (2H, m, 2 x Ar-H), 9.56 (0.03H, s, C*H*O); δ_C (100 MHz, $CDCl_3$): 88.8 (CH), 116.1 (ArCH), 116.3 (ArCH), 128.7 (ArC), 132.8 (ArCH), 132.9 (ArCH), 165.4 (ArC), 191.2 (C=O); LRMS (CI^+): 153.09 ($[M+H]^+$, 100%), 123.08 (8); HRMS calcd for $C_8H_5O_2F$ 153.0352, found 153.0353.

4'-(Trifluoromethyl)-phenylglyoxal (63)



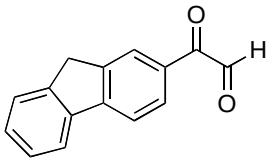
Using 4'-(trifluoromethyl)acetophenone (2.0 g, 10.6 mmol), **63** hydrate was prepared as a brown solid (2.15 g, 100%) *via* general procedure 3C. mp: 88-90 ^oC (lit.[260] 90-105 ^oC); ν_{max} (neat)/cm^{-1}: 3326 (OH), 3227 (OH), 2975 (Ar-H), 2895 (CH_2), 1701 (C=O, aldehyde), 1582 (C=O, ketone), 1323 (C-F); δ_H (400 MHz, $CDCl_3$): 6.29 (1H, s, $C(OH)_2\mathit{H}$), 7.69 (2H, d, *J* 8.3 Hz, 2 x Ar-H), 8.16 (2H, d, *J* 8.3 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 89.3 (CH), 126.3 (2 x ArCH), 130.6 (2 x ArCH), 135.2 (ArC), 136.0 (ArC), 136.2 (CF_3, q, *J* 33.0 Hz), 192.4 (C=O); LRMS (CI^+): 203.1 ($[M+H]^+$, 100%), 173.1 (25); HRMS calcd for $C_9H_5O_2F_3$ 203.032, found 203.0318.

4'-Nitrophenylglyoxal (64)



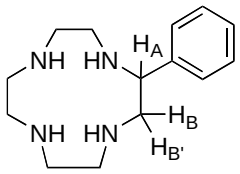
Using 4'-nitroacetophenone (5.0 g, 30.2 mmol), **64** was prepared using general procedure 3C as a yellow solid (4.34 g, 80%); mp: 67-72 ^{o}C (lit.[257] 131-132 ^{o}C); ν_{max} (neat)/cm^{-1}: 3415 (OH), 3114 (Ar-H), 2857 (CO-H), 1705 (C=O, aldehyde), 1604 (C=O, ketone), 1518 (NO_2); δ_H (400 MHz, $CDCl_3$): 7.19 (0.36H, s, C(OH)$_2$*H*), 8.22-8.36 (4H, m, 4 x Ar-H), 9.59 (0.64H, s, C*H*O); δ_C (100 MHz, $CDCl_3$): 90.0 (CH), 123.5 (ArCH), 123.6 (ArCH), 130.5 (ArCH), 130.8 (ArCH), 138.5 (ArC), 149.8 (ArC), 195.3 (C=O); LRMS (CI$^+$): 180.1 ([M+H]$^+$, 100%), 166.1 (5), 150.1 (22); HRMS calcd for $C_8H_5O_4N$ 180.0297, found 180.0297.

2-Fluorenylglyoxal (65)



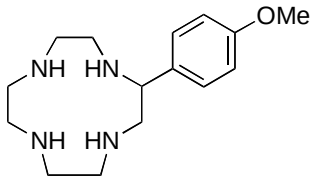
By general procedure 3C, **65** hydrate was prepared as a white solid (892 mg, 42%) from 2-acetylfluorene (2 g, 9.6 mmol). mp: 125-127 ^{o}C (lit.[260] 127-129 ^{o}C); ν_{max} (neat)/cm^{-1}: 3404 (OH), 3352 (OH), 3051 (Ar-H), 2957 (CH_2), 1685 (C=O, aldehyde), 1608 (C=O, ketone); δ_H (400 MHz, $CDCl_3$): 3.9 (2H, s, CH_2), 7.32-7.39 (2H, m, 2 x Ar-H), 7.53-7.55 (1H, m, Ar-H), 7.78-7.84 (2H, m, 2 x Ar-H), 8.19 (1H, dd, *J* 8, 0.8 Hz, Ar-H), 8.33 (1H, d, *J* 0.8 Hz, Ar-H), 9.66 (1H, s, C*H*O); δ_C (100 MHz, $CDCl_3$): 36.4 (CH_2), 89.2 (CH), 119.8 (ArCH), 121.1 (ArCH), 125.4 (ArCH), 126.2 (ArCH), 127.0 (ArCH), 128.1 (ArCH), 128.6 (ArCH), 132.0 (ArC), 140.0 (ArC), 142.9 (ArC), 144.5 (ArC), 145.8 (ArC), 195.9 (C=O); LRMS (EI$^+$): 222.1 ([M]$^+$, 5%), 210 (33), 193.1 (73), 165.1 (100); HRMS calcd for $C_{15}H_{10}O_2$ 222.0681, found 222.0682.

2-(Phenyl)-1,4,7,10-tetraazacyclododecane (66)



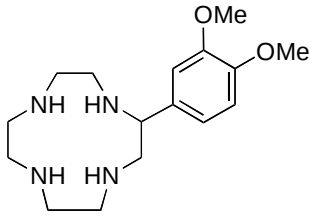
Using phenylglyoxal (3.0 g, 19.2 mmol) **66** was afforded as a viscous brown oil (4.12 g, 84%) *via* general procedure 3D; ν_{max} (neat)/cm^{-1}: 3273 (NH), 3055 (Ar-H), 2936 (CH_2), 2804 (NCH_2); NMR spectroscopy agreed with lit. values;[128] δ_H (400 MHz, $CDCl_3$): 1.65 (4H, s, 4 x NH), 1.97 (1H, t, *J* 10.4 Hz, $H_{B'}$), 2.11 (1H, dt, *J* 11.2, 3.2 Hz, CH), 2.46 (2H, t, *J* 6.0 Hz, CH_2), 2.62 (2H, t, *J* 6.4 Hz, 2 x CH) 2.66 (2H, t, *J* 6.4 Hz, 2 x CH), 2.74 (2H, t, *J* 6.0 Hz, 2 x CH), 2.81 (2H, br t, *J* 10.8 Hz, CH, CH_B), 2.96 (1H, dt, *J* 10.8, 2.4 Hz, CH), 3.0 (1H, dt, *J* 11.6, 2.4 Hz, CH), 3.79 (1H, dd, *J* 10.0, 2.4 Hz, CH_A), 7.21 (1H, t, *J* 7.2 Hz, Ar-H), 7.25 (2H, t, *J* 7.2 Hz, 2 x Ar-H), 7.31 (2H, t, *J* 6.8 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 41.8 (CH_2), 46.3 (CH_2), 46.3 (CH_2), 52.8 (CH_2), 53.4 (CH_2), 58.3 (CH_2), 60.4 (CH), 61.7 (CH_2), 127.0 (2 x ArCH), 127.5 (ArCH), 128.4 (ArCH), 142.6 (ArC); LRMS (CI^+): 249.3 ($[M+H]^+$, 100%), 243.2 (8), 206.2 (6), 163.2 (9); HRMS calcd for $C_{14}H_{24}N_4$ 249.2079, found 249.2077.

2-(4'-Methoxyphenyl)-1,4,7,10-tetraazacyclododecane (67)



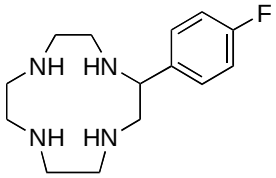
Using **60** (372 mg, 2.73 mmol) **67** was produced as a dark brown solid (634 mg, 83%) *via* general procedure 3D; ν_{max} (neat)/cm^{-1}: 3249 (NH), 2936 (CH_2), 2804 (NCH_2, OCH_3); δ_H (400 MHz, $CDCl_3$): 1.42 (4H, s, 4 x NH), 1.95 (1H, t, *J* 10.8 Hz, $CH_{B'}$), 2.09 (1H, dt, *J* 11.2, 3.6 Hz, CH), 2.45 (2H, t, *J* 6.4 Hz, 2 x CH), 2.61 (2H, t, *J* 5.6 Hz, 2 x CH), 2.66 (2H, t, *J* 6.4 Hz, 2 x CH), 2.74 (2H, t, *J* 6.4 Hz, 2 x CH), 2.77-2.79 (2H, m, CH_B, CH), 2.97 (1H, dt, *J* 11.2, 2.4 Hz, CH), 3.0 (1H, br t, *J* 10.8 Hz, CH), 3.72 (3H, s, OCH_3), 3.74 (1H, br d, *J* 2.4 Hz, CH_A), 6.80 (2H, d, *J* 8.4 Hz, 2 x Ar-H), 7.23 (2H, d, *J* 8.4 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 40.8 (CH_2), 45.3 (2 x CH_2), 51.8 (CH_2), 52.4 (CH_2), 54.2 (CH_3), 57.3 (CH_2), 58.8 (CH), 60.7 (CH_2), 112.7 (2 x ArCH), 127.0 (ArCH), 128.4 (ArCH), 133.9 (ArC), 157.9 (ArC); LRMS (CI^+): 267.3 ($[M+H]^+$, 100%), 261.2 (12), 224.2 (4); HRMS calcd for $C_{14}H_{23}FN_4$ 267.1985, found 267.1989.

2-(3',4'-Dimethoxyphenyl)-1,4,7,10-tetraazacyclododecane (68)



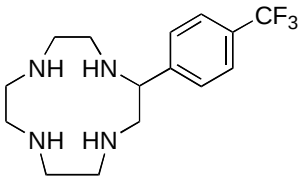
Using **61** (1.59 g, 8.19 mmol) **68** was synthesised as a brown oil (2.04 g, 81%) by general procedure 3D; ν_{max} (neat)/cm^{-1}: 3304 (NH), 2939 (CH_2), 2834 (NCH_2, OMe); δ_H (400 MHz, $CDCl_3$): 1.89 (4H, br s, 4 x NH), 1.97 (1H, t, *J* 10.4 Hz, $CH_{B'}$), 2.10 (1H, dt, *J* 11.2, 2.8 Hz, CH), 2.46 (2H, t, *J* 6.4 Hz, 2 x CH), 2.63 (2H, t, *J* 5.6 Hz, 2 x CH), 2.67 (2H, t, *J* 6.0 Hz, 2 x CH), 2.74-2.81 (4H, m, CH_B, 3 x CH), 2.96 (1H, dt, *J* 11.2, 2.4 Hz, CH), 3.01 (1H, br t, *J* 11.2 Hz, CH), 3.73 (1H, dd, *J* 10.0, 2.0 Hz, CH_A), 3.79 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 6.75 (1H, d, *J* 8.0 Hz, Ar-H), 6.84 (1H, dd, *J* 8.0, 2.0 Hz, Ar-H), 6.89 (1H, s, Ar-H); δ_C (100 MHz, $CDCl_3$): 41.8 (CH_2), 46.5 (CH_2), 46.5 (CH_2), 52.7 (CH_2), 54.0 (CH_2), 56.3 (2 x CH_3), 58.5 (CH_2), 60.5 (CH), 62.1 (CH_2), 110.5 (ArCH), 111.3 (ArCH), 119.4 (ArCH), 135.6 (ArC), 148.7 (ArC), 149.2 (ArC); LRMS (CI^+): 309.3 ($[M+H]^+$, 100%), 266.2 (23), 223.2 (10), 181.1 (12); HRMS calcd for $C_{16}H_{28}O_2N_4$ 309.2291, found 309.2289.

2-(4'-Fluorophenyl)-1,4,7,10-tetraazacyclododecane (69)



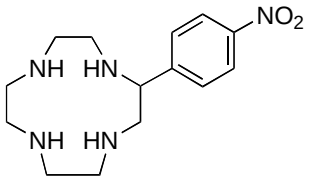
Using **62** (831 mg, 5.46 mmol) gave **69** as a dark brown solid (1.17 g, 80%) *via* general procedure 3D; ν_{max} (neat)/cm^{-1}: 3262 (NH), 3006 (Ar-H), 2939 (CH_2), 2809 (NCH_2), 1219 (C-F); δ_H (400 MHz, $CDCl_3$): 1.55 (4H, br s, 4 x NH), 1.93 (1H, t, *J* 10.4 Hz, $CH_{B'}$), 2.08 (1H, dt, *J* 10.8, 3.2 Hz, CH), 2.45 (2H, t, *J* 6.4 Hz, 2 x CH), 2.61 (2H, t, *J* 6.0 Hz, 2 x CH), 2.65 (2H, t, *J* 6.0 Hz, 2 x CH), 2.72-2.79 (4H, m, CH_B, 3 x CH), 2.95 (1H, dt, *J* 11.2, 2.4 Hz, CH), 3.0 (1H, br t, *J* 11.2 Hz, CH), 3.76 (1H, dd, *J* 10.4, 2.4Hz, CH_A), 6.93 (2H, t, *J* 8.8 Hz, 2 x Ar-H), 7.26-7.30 (2H, m, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 42.2 (CH_2), 46.6 (CH_2), 46.6 (CH_2), 53.1 (CH_2), 53.6 (CH_2), 58.6 (CH_2), 60.1 (CH), 62.1 (CH_2), 115.4 (ArCH), 115.6 (ArCH), 128.8 (ArCH), 128.9 (ArCH), 138.8 (ArC), 157.9 (ArC-F, d, *J* 243.5 Hz); LRMS (EI^+): 278.3 ($[M+H]^+$, 12%), 205.2 (100), 193.2 (8), 162.1 (15), 121.1 (48); HRMS calcd for $C_{15}H_{26}ON_4$ 278.2107, found 278.2108.

2-(4'-Trifluoromethylphenyl)-1,4,7,10-tetraazacyclododecane (70)



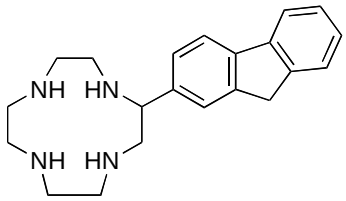
Using **63** (205 mg, 1.01 mmol) **70** was made as a yellow-brown oil (203 mg, 64%) using general procedure 3D; ν_{max} (neat)/cm^{-1}: 3305 (NH), 2925 (CH_2), 2815 (NCH_2), 1161 (C-F) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.67 (4H, br s, 4 x NH), 1.96 (1H, t, *J* 10.0 Hz, $CH_{B'}$), 2.15 (1H, m, CH), 2.46 (2H, t, *J* 6.4 Hz, 2 x CH), 2.62 (2H, t, *J* 6.0 Hz, 2 x CH), 2.66 (2H, t, *J* 6.8 Hz, 2 x CH), 2.73 (2H, t, *J* 6.0 Hz, 2 x CH), 2.81 (2H, m, CH_B, CH), 2.90-2.93 (1H, m, CH), 2.98-3.06 (1H, m, CH), 3.87 (1H, br d, *J* 10.0 Hz, CH_A), 7.45 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.51 (2H, d, *J* 8.0 Hz, 2 x Ar-H); HCl salt δ_C (100 MHz, D_2O): 35.9 (CH_2), 43.8 (CH_2), 44.5 (CH_2), 48.9 (CH_2), 51.6 (CH_2), 53.7 (CH_2), 55.2 (CH_2), 58.2 (CH), 123.2 (ArC), 126.2 (2 x ArCH), 128.0 (2 x ArCH), 138.5 (ArC), 145.5 (CF_3, m); LRMS (CI^+): 317.2 ($[M+H]^+$, 100%), 274.2 (51), 254.2 (20), 231.2 (32), 173.2 (42), 79.1 (69); HRMS calcd for $C_{15}H_{24}N_4F_3$ 317.1953, found 317.1952.

2-(4'-Nitrophenyl)-1,4,7,10-tetraazacyclododecane (71)



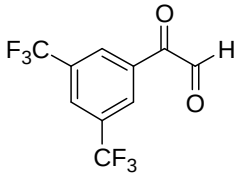
Using **64** (978 mg, 5.46 mmol) **71** was yielded as a brown oil (1.08 g, 67%) using general procedure 3D; ν_{max} (neat)/cm^{-1}: 3285 (NH), 2936 (CH_2), 2815 (NCH_2), 1513 (NO_2); NMR spectroscopy agreed with lit. values;[128] δ_H (400 MHz, $CDCl_3$): 1.86 (4H, br s, 4 x NH), 1.95 (1H, br t, *J* 10 Hz, $CH_{B'}$), 2.12 (1H, dt, *J* 11.2, 3.2 Hz, CH), 2.48 (2H, t, *J* 6.0 Hz, CH), 2.63-2.67 (4H, m, 4 x CH), 2.76-2.83 (4H, m, CH_B, 3 x CH), 2.98 (1H, dt, *J* 11.6, 2.8 Hz, CH), 3.04 (1H, br t, *J* 11.6 Hz, CH), 3.92 (1H, dd, *J* 10.0, 2.4 Hz, CH_A), 7.51 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 8.11 (2H, d, *J* 8.8 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 41.9 (CH_2), 46.3 (CH_2), 46.4 (CH_2), 52.6 (CH_2), 53.2 (CH_2), 58.3 (CH_2), 60.1 (CH), 61.6 (CH_2), 123.7 (ArCH), 124.0 (ArCH), 128.3 (2 x ArCH), 147.7 (ArC), 150.4 (ArC); LRMS (EI^+): 293.41 ($[M+H]^+$, 5%), 276.2 (19), 220.1 (100); HRMS calcd for $C_{14}H_{23}O_2N_5$ 293.1852, found 293.1854.

2-(Fluorenyl)-1,4,7,10-tetraazacyclododecane (72)



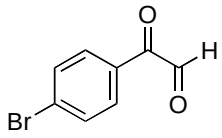
Using **65** (892 mg, 4.0 mmol) **72** was produced as a brown oil (1.35 g, 100%) *via* general procedure 3D; ν_{max} (neat)/cm^{-1}: 2796 (NCH_2, CH_2), 1645 (ArC=C), 1298 (C-N); δ_H (400 MHz, $CDCl_3$): 1.8 (4H, br s, 4 x NH), 2.04 (1H, t, *J* 10.8 Hz, $CH_{B'}$), 2.14 (1H, br t, *J* 10.8 Hz, CH), 2.48 (2H, t, *J* 6.0 Hz, 2 x CH), 2.63 (2H, t, *J* 6.0 Hz, 2 x CH), 2.67 (2H, t, *J* 6.0 Hz, 2 x CH), 2.74 (1H, t, *J* 10.8 Hz, CH_B), 2.75 (2H, t, *J* 5.6 Hz, 2 x CH), 2.85 (1H, t, 10.8 Hz, CH), 3.00 (1H, br t, *J* 11.6 Hz, CH), 3.04 (1H, br t, *J* 10.8 Hz, CH), 3.8 (2H, s, CH_2), 3.86 (1H, br d, *J* 7.6 Hz, CH_A), 7.22 (1H, d, *J* 7.2 Hz, Ar-H), 7.29 (2H, m, 2 x Ar-H), 7.46 (1H, d, *J* 7.2 Hz, Ar-H), 7.52 (1H, s, Ar-H), 7.64-7.70 (2H, m, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 37.2 (CH_2), 43.2 (CH_2) 46.6 (2 x CH_2), 52.9 (CH_2), 53.8 (CH_2), 58.6 (CH_2), 61.0 (CH), 62.1 (CH_2), 120.1 (ArCH), 120.2 (ArCH), 124.0 (ArCH), 125.4 (ArCH), 126.2 (ArCH), 127.0 (ARCH), 127.1 (ArCH), 138.5 (ArC), 141.6 (2 x ArC), 143.7 (ArC), 143.9 (ArC); LRMS (CI^+): 337.3 ($[M+H]^+$, 14%), 294.2 (100), 251.2 (22); HRMS calcd for $C_{21}H_{28}N_4$ 337.2392, found 337.2393.

3,5-Bis-trifluoromethyl-phenyl-oxo-acetaldehyde 75



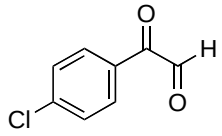
Using 3',5'-bis(trifluoromethyl)acetophenone (1.8 mL, 10 mmol), hydrate **75** was prepared as a green oil (3.2 g, 100%) *via* general procedure 3C; ν_{max} (neat)/cm^{-1}: 3417 (O-H), 1703 (C(O)H), 1617 (Ar-H), 1278 (C-F); δ_H (400 MHz, DMSO-d_6): 8.00 (1H, s, Ar-H), 8.31 (2H, s, 2 x Ar-H), 9.59 (1H, s, C(O)H); δ_C (100 MHz, DMSO-d_6): 120.5 (ArC), 123.2 (ArC), 125.4 (ArCH), 127.3 (2 x ArCH), 131.4 (2 x CF_3, q, *J* 33.8 Hz), 137.4 (ArC), 187.2 (C(O)H), 194.1 (C=O); LRMS (FAB^+): 271.1 ($[M+H]^+$, 98%), 241.1 (100), 155.0 (84), 137.2 (49), 90.9 (31); HRMS calcd for $C_{10}H_5O_2F_6$ 271.0194, found 271.0199.

4'-Bromophenylglyoxal (76)



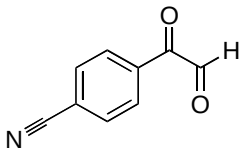
76 was synthesised *via* general procedure 3C from 4'-bromoacetophenone (10 g, 50 mmol) as a yellow solid (10.65 g, 100%) and then converted to the monohydrate to give a pale brown solid. mp: 51-53 ^{o}C (lit.[261] 51-52 ^{o}C); δ_H (400 MHz, $CDCl_3$): 3.98 (2H, s, C(O*H*)$_2$H), 5.84 (1H, s, C(OH)$_2$*H*), 7.60 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 7.93 (2H, d, *J* 8.8 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 128.3 (CH), 129.9 (2 x ArCH), 131.3 (ArC), 131.9 (2 x ArCH), 135.8 (ArC), 197.1 (C=O); LRMS (CI^+): 215.0 ($[M+H]^+$, 100%), 213.0 (97), 199.0 (13), 185.0 (9), 135.1 (24), 121.1 (5), 85.1 (15); HRMS calcd for $C_8H_6O_2{}^{79}Br$ 212.9551, found 212.9556.

4'-Chlorophenylglyoxal (77)



77 was synthesised *via* general procedure 3C from 4'-chloroacetophenone (26 mL, 200 mmol) to give the glyoxal as a green-orange solid (33.7 g, 100%) and then converted to the monohydrate to give a white-pink solid. mp: 40-41 ^{o}C (lit.[261] 40-42 ^{o}C); δ_H (400 MHz, $CDCl_3$) 4.94 (1H, s, C(OH)$_2$*H*), 5.85 (2H, s, C(O*H*)$_2$H), 7.44 (2H, dd, *J* 8.8, 1.0 Hz, 2 x Ar-H), 8.02 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 9.57 (0.5H, s, C(O)*H*); δ_C (100 MHz, $CDCl_3$): 88.7 (C(OH)$_2$H), 129.3 (2 x ArCH), 130.48 (ArC), 131.3 (2 x ArCH), 141.5 (ArC), 191.7 (C(O)H), 197.0 (C=O); LRMS (CI^+): 169.06 ($[M+H]^+$, 100%), 155.08 (5), 139.05 (8), 135.10 (4), 113.19 (3), 73.08 (12); HRMS calcd for $C_8H_6O_2{}^{35}Cl$ 169.0056, found 169.0057.

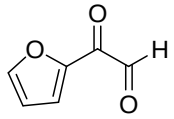
4-Oxoacetyl-benzonitrile (78)



Using general procedure 3C, **78** was synthesised from 4'-acetyl benzonitrile (2.0 g, 14 mmol). The resulting residue was then separated on a silica column eluted with 1 : 1 hexane : EtOAc to give a green oil (2.2 g, 100%). R_f = 0.13 (hexane : EtOAc 1 : 1) ; ν_{max}

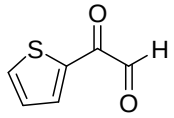
(KBr)/cm^{-1}: 3034 (ArC-H), 2238 (C≡N), 1697 (C=O), 1657 (C=O), 1609 (ArC=C); δ_H (400 MHz, Acetone-d_6): 5.82 (2H, s, C(O*H*)$_2$H), 7.84 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 8.20 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 9.5 (trace 1H, s, C(O)H); δ_C (100 MHz, Acetone-d_6): 89.7 (CH), 118.3 (CN), 130.7 (2 x ArCH), 132.9 (2 x ArCH), 137.5 (ArC), 161.9 (ArC), 189.3 (C=O); LRMS (CI$^+$): 160.1 ([M+H]$^+$, 100%), 103.1 (60); HRMS calcd for $C_9H_5NO_2$ 160.0399, found 160.0397.

Furan-2-yl-oxo-acetaldehyde (79)



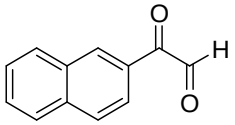
Using general procedure 3C, hydrate **79** was prepared as a dark brown oil (7.5 g, 100%) from 2-acetylfuran (5.0 g, 45 mmol). Kugelröhr distillation afforded the product as a yellow-white solid (4.2 g, 75%); mp: 64-66 ^{o}C (lit.[262] 68-69 ^{o}C); ν_{max} (neat)/cm^{-1}: 3414 (O-H), 3346 (O-H), 3134 (C-H), 1665 (C=O); δ_H (400 MHz, DMSO-d_6): 5.49 (1H, s, C(O)H), 6.73-6.74 (1H, m, Ar-H), 7.53 (1H, d, *J* 3.2, Ar-H), 8.04 (1H, s, Ar-H); δ_C (100 MHz, DMSO-d_6): 88.6 (C(O)H), 112.4 (ArCH), 120.8 (ArCH), 148.1 (ArCH), 149.4 (ArC), 185.3 (C=O); LRMS (CI$^+$): 139.01 (36%), 125.07 ([M+H]$^+$, 100%), 95.06 (39), 81.09 (3); HRMS calcd for $C_6H_4O_3$ 125.0239, found 125.0240.

Thiophen-2-yl-oxo-acetaldehyde (80)



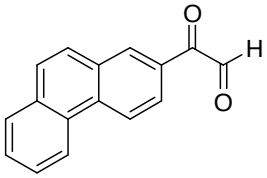
Using 2-acetylthiophene (4.9 mL, 45 mmol), hydrate **80** was prepared as a brown oil (6.31 g, 100%) *via* general procedure 3C. Kugelröhr distillation afforded the product as a cream solid (6 g, 95%). mp: 89-92 ^{o}C (lit.[263] 88-89 ^{o}C); ν_{max} (neat)/cm^{-1}: 3444 (O-H), 3093 (C-H), 1646 (C=O); δ_H (400 MHz, DMSO-d_6): 5.87 (1H, s, OH), 5.89 (1H, s, OH), 7.27 (1H, dd, *J* 5.2, 4.0 Hz, Ar-H), 7.55 (1H, d, *J* 9.2 Hz, Ar-H), 8.07-8.09 (1H, m, Ar-H); δ_C (100 MHz, DMSO-d_6): 91.1 (C(OH)$_2$H), 128.8 (ArCH), 135.5 (ArCH), 135.9 (ArCH), 139.6 (ArC), 187.2 (C=O); LRMS (CI$^+$): 141.1 ([M + H]$^+$, 100%), 111.1 (12), 85.2 (5); HRMS calcd for $C_6H_4O_2S$ 141.0010, found 141.0011.

Naphthalen-2-yl-oxo-acetaldehyde (81)



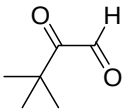
Using 2-acetylnaphthalene (5 g, 29 mmol), **81** was prepared as pale yellow crystals (4.5 g, 83%) *via* general procedure 3C. mp: 108-111 ^{o}C (lit.[264] 108 ^{o}C); ν_{max} (neat)/cm^{-1}: 3446 (O-H), 3430 (O-H), 3099 (C-H), 2896 (C=O), 1682 (C=O); δ_H (400 MHz, DMSO-d$_6$): 5.87 (1H, s, C(OH)$_2$*H*), 6.87 (1H, s, OH), 6.88 (1H, s, OH), 7.65 (2H, ddt, *J* 24, 6.8, 1.2 Hz, 2 x Ar-H), 7.98-8.12 (4H, m, 4 x Ar-H), 8.80 (1H, s, Ar-H); δ_C (100 MHz, DMSO-d$_6$): 89.2 (C(OH)$_2$H), 124.7 (ArCH), 126.9(ArCH), 127.7 (ArCH), 128.0 (ArCH), 128.7 (ArCH), 129.6 (ArCH), 130.9 (ArC), 131.4 (ArCH), 132.0 (ArC), 135.1 (ArC), 196.2 (C=O); LRMS (EI$^+$): 184.03 (M, 12%), 155.03 (100), 127.05 (88), 101.03 (7), 82.94 (12), 77.04 (10); HRMS calcd for $C_{12}H_8O_2$ 184.0524, found 184.0523.

Phenanthren-2-yl-oxo-acetaldehyde (82)



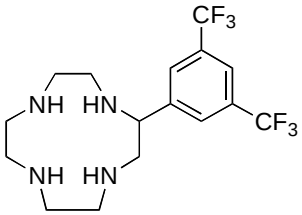
Via general procedure 3C, **82** hydrate was prepared as a white solid (525 mg, 99%) from 2-acetylphenanthrene (0.5 g, 2.3 mmol). Trituration with benzene gave the product as a pale yellow powder (0.23 g, 43%); mp 130-133 ^{o}C (lit.[260] 136-138 ^{o}C); ν_{max} (neat)/cm^{-1}: 3307 (O-H), 3048 (C-H), 1694 (C=O); δ_H (400 MHz, DMSO-d$_6$): 6.28 (1H, d, *J* 9.6 Hz, C(OH)$_2$*H*), 7.21 (1H, dt, *J* 35.2, 7.2 Hz, OH), 7.71 (1H, s, OH), 7.72-7.76 (2H, m, 2 x Ar-H), 7.84 (2H, q, *J* 12.4, 9.2 Hz, 2 x Ar-H), 8.0-8.04 (1H, m, Ar-H), 8.29 (1H, dd, *J* 8.8, 2.0 Hz, Ar-H), 8.77 (1H, d, *J* 1.6 Hz, Ar-H), 8.83-8.85 (1H, m, Ar-H), 8.90 (1H, d, *J* 9.2 Hz, Ar-H); δ_C (100 MHz, DMSO-d$_6$): 91.2 (C(OH)$_2$H), 123.3 (ArCH), 123.7 (ArCH), 126.1 (ArCH), 127.3 (2 x ArCH), 127.6 (ArCH), 128.1 (ArCH), 128.6 (ArCH), 129.0 (ArC), 130.8 (ArC), 131.2 (ArCH), 131.3 (ArC), 132.6 (ArC), 133.0 (ArC), 193.6 (C=O); LRMS (EI$^+$): 234 (M, 30%), 205 (100), 177 (85), 176 (62), 151 (25), 150 (14), 88 (25), 84 (43), 66 (46); HRMS calcd for $C_{16}H_{10}O_2$ 234.0681, found 234.0682.

3,3-Dimethyl-2-oxo-butyraldehyde (83)



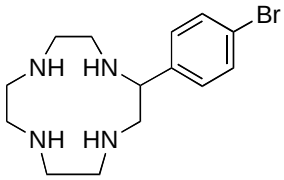
Using general procedure 3C, hydrate **83** was produced from pinacolone (2 mL, 14.5 mmol) as a pale green oil (1.16 g, 100%) which turned into a white solid after a few hours. mp: 90-91 ^{o}C (lit.[265] 91-92 ^{o}C); ν_{max} (neat)/cm^{-1}: 3382 (O-H), 2973 (CH_3); δ_H (400 MHz, $CDCl_3$): 1.21 (9H, s, 3 x CH_3), 9.26 (1H, s, C(O)H); δ_C (100 MHz, $CDCl_3$): 24.5 (3 x CH_3), 25.3 (C(O)H), 40.9 (C), 187.9 (C).

2-(3,5-Bis-trifluoromethyl-phenyl)-1,4,7,10-tetraazacyclododecane (84)



Using **75** (0.88 g, 5.4 mmol), **84** was produced as a brown oil (1.3 g, 54%) *via* general procedure 3D; ν_{max} (NaCl)/cm^{-1}: 3303 (N-H), 2933 (ArC-H), 1624 (ArC=C), 1339 (C-N), 1177 (C-F), 1140 (C-F); δ_H (400 MHz, $CDCl_3$): 1.92-1.99 (1H, m, $CH_{B'}$), 2.10-2.9 (1H, m, CH), 2.35-2.37 (2H, m, 2 x CH), 2.44-2.46 (2H, m, 2 x CH), 2.61-2.88 (6H, m, 6 x CH), 2.96 (1H, br t, *J* 11.6 Hz, CH_B), 3.01 (1H, br t, *J* 11.2 Hz, CH), 3.92 (1H, br d, *J* 10.0 Hz, CH_A), 7.69 (1H, s, Ar-H), 7.80 (2H, s, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 38.3 (CH_2), 45.7 (CH_2),45.8 (CH_2), 53.3 (CH_2), 57.7 (CH_2), 59.5 (CH), 60.5 (CH_2), 61.2 (CH_2), 119.3 (ArC), 120.9 (ArCH), 122.0 (ArC), 124.7 (ArC), 125.6 (ArCH), 127.4 (ArCH), 131.5 (2 x CF_3, q, *J* 33.0 Hz); LRMS (CI$^+$): 385.3 ([M+H]$^+$, 100%), 381.2 (69), 338.2 (15), 241.2 (41), 173.3 (55); HRMS calcd for $C_{16}H_{23}N_4F_6$ 385.1827, found 385.1825.

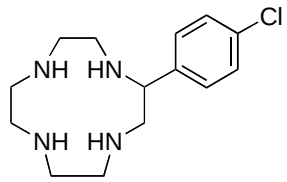
2-(4'-Bromophenyl)-1,4,7,10-tetraazacyclododecane (85)



Using 4'-bromophenyl glyoxal monohydrate **76** (2.70 g, 11.7 mmol) the substituted azamacrocycle **85** was synthesised *via* general procedure 3D as a brown oil (2.70 g, 71%). ν_{max} (NaCl)/cm^{-1}: 2943 (Ar), 2895 (Ar), 1460 (-NH-), 822 (C-Br); δ_H (400 MHz, $CDCl_3$):

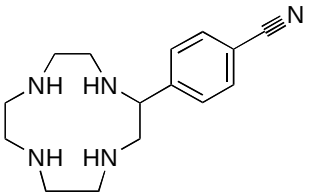
1.05-3.00 (18H, m, 14 x CH, 4 x NH), 3.75 (1H, d, *J* 8.4 Hz, CH_A), 7.20 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.37 (2H, d, *J* 8.0 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 38.8 (CH_2), 41.6 (CH_2), 46.1 (CH_2), 52.5 (CH_2), 53.2 (CH_2), 58.1 (CH_2), 59.8 (CH), 61.5 (CH_2) 121.2 (ArC), 128.8 (2 x ArCH), 131.4 (2 x ArCH), 141.6 (ArC); LRMS (CI^+): 327.2 ($[M+H]^+$, 58%), 284.1 (72), 241.1 (57), 173.3 (100), 130.2 (31); HRMS calcd for $C_{14}H_{24}N_4{}^{79}Br$ 327.1184, found 327.1180.

2-(4'-Chlorophenyl)-1,4,7,10-tetraazacyclododecane (86)



86 was synthesised using 4'-chlorophenylglyoxal monohydrate **77** (855 mg, 4.6 mmol) *via* general procedure 3D as an orange oil (1.02 g, 78%). ν_{max} (NaCl)/cm^{-1}: 3283 (N-H), 2938 (ArC-H), 1658 (ArC=C), 1315 (C-N), 755 (C-Cl); δ_H (400 MHz, $CDCl_3$): 1.92 (2H, t, *J* 10.4 Hz, 2 x CH), 2.08 (2H, t, *J* 10.4 Hz, 2 x CH), 2.34-3.08 (10H, m, 10 x CH), 3.76 (1H, d, *J* 9.6 Hz, CH_A), 7.20-7.24 (4H, m, 4 x Ar-H); δ_C (100 MHz, $CDCl_3$): 41.7 (CH_2), 46.1 (CH_2), 46.2 (CH_2), 52.6 (CH_2), 53.2 (CH_2), 58.2 (CH_2), 59.7 (CH), 61.6 (CH_2), 128.4 (2 x ArCH), 128.5 (2 x ArCH), 133.1 (ArC), 141.1 (ArC); LRMS (CI^+): 283.3 ($[M+H]^+$, 100%), 240.2 (29), 197.2 (18), 173.3 (49); HRMS calcd for $C_{14}H_{24}N_4{}^{35}Cl$ 283.1689, found 283.1687.

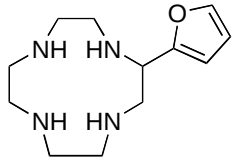
2-(Benzonitrile)-1,4,7,10-tetraazacyclododecane (87)



87 was synthesised from 4-oxoacetyl-benzonitrile (2.16 g, 13.6 mmol) using general procedure 3D as a brown solid (2.66 g, 72%). mp: 51-55 ^oC; ν_{max} (NaCl)/cm^{-1}: 3260 (N-H), 2226 (C≡N), 1652 (N-H), 1608 (ArC=C), 1311 (C-N), 1133 (C-N); δ_H (400 MHz, $CDCl_3$): 1.73 (4H, br s, 4 x NH), 1.97 (1H, t, *J* 10.8 Hz, $CH_{B'}$), 2.14 (1H, dt, *J* 10.8, 3.2 Hz, CH), 2.38 (2H, t, *J* 6.0 Hz, 2 x CH), 2.49 (2H, t, *J* 6.4 Hz, 2 x CH), 2.64-2.86 (7H, m, 7 x CH), 3.00 (1H, dt, *J* 6.4, 2.4 Hz, CH), 3.90 (1H, dd, *J* 10.0, 2.4 Hz, CH_A), 7.48 (2H, d, *J* 8.2 Hz, 2 x Ar-H), 7.58 (2H, d, *J* 8.2 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 38.8 (CH_2), 41.7 (CH_2), 46.1 (CH_2), 51.3 (CH_2), 53.3 (CH_2), 58.2 (CH_2), 60.0 (CH), 61.3 (CH_2), 111.2

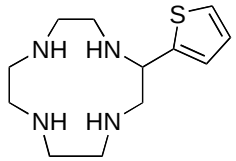
(CN), 118.8 (ArC), 127.8 (2 x ArCH), 132.2 (2 x ArCH), 148.0 (ArC); LRMS (FAB): 274.2 ($[M+H]^+$, 95%), 214.5 (35), 200.6 (60), 173.9 (100), 169.9 (77), 143.2 (32), 114.6 (27), 98.8 (59), 87.0 (35); HRMS calcd for $C_{15}H_{24}N_5$ 274.2032, found 274.2027.

2-(Furan-2-yl)-1,4,7,10-tetraazacyclododecane (88)



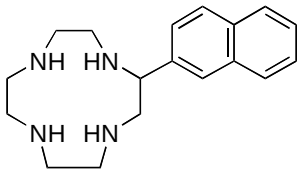
Using **79** (0.6 g, 4.8 mmol) **88** was produced as a dark brown oil (0.62 g, 54%) *via* general procedure 3D; ν_{max} (neat)/cm^{-1}: 3289 (N-H), 2942 (C-H), 2815 (-CH_2-), 1650 (C=C); δ_H (400 MHz, $CDCl_3$): 1.87 (4H, br s, 4 x NH), 2.11 (1H, dt, *J* 10.8, 3.3 Hz, $CH_{B'}$), 2.15 (1H, br t, *J* 11.0 Hz, CH), 2.35 (2H, t, *J* 6.4 Hz, 2 x CH), 2.47 (2H, t, *J* 5.2 Hz, 2 x CH), 2.62 (2H, t, *J* 5.6 Hz, 2 x CH), 2.66 (2H, t, *J* 6.0 Hz, 2 x CH), 2.79-2.83 (2H, m, CH, CH_B), 2.88-3.00 (2H, m, 2 x CH), 3.91 (1H, dd, *J* 9.6, 2.8 Hz, CH_A), 6.12 (1H, d, *J* 3.2 Hz, Ar-H), 6.23-6.25 (1H, m, Ar-H), 7.27 (1H, s, Ar-H); δ_C (100 MHz, $CDCl_3$): 41.7 (CH_2), 46.1 (CH_2), 46.1 (CH_2), 52.6 (CH_2), 53.4 (CH_2), 53.7 (CH), 58.2 (CH_2), 61.1 (CH_2), 104.2 (ArCH), 109.0 (ArCH), 140.5 (ArCH), 154.4 (ArC); LRMS (CI^+): 239.2 ($[M+H]^+$, 100%), 233.2 (62), 196.2 (18), 173.2 (23), 153.1 (14); HRMS calcd for $C_{12}H_{22}N_4O$ 239.1872, found 239.1870.

2-(Thiophene-2-yl)-1,4,7,10-tetraazacyclododecane(89)



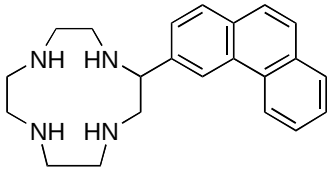
Using **80** (0.73 g, 5.2 mmol) **89** was produced as a viscous dark brown oil (1.04 g, 79%) *via* general procedure 3D; ν_{max} (neat)/cm^{-1}: 3402 (N-H), 3231 (ArC-H), 2807 (-CH_2-), 1639 (C=C); δ_H (400 MHz, $CDCl_3$): 2.09 (1H, t, *J* 10.0 Hz, $CH_{B'}$), 2.34-2.42 (1H, m, CH), 2.47 (2H, t, *J* 6.0 Hz, 2 x CH), 2.58-2.82 (8H, m, 8 x CH), 2.91 (1H, br t, *J* 11.6 Hz, CH_B), 3.00 (1H, br t, *J* 11.6 Hz, CH), 4.13 (1H, dd, *J* 10.0, 3.2 Hz, CH_A), 6.88-6.90 (2H, m, 2 x Ar-H), 7.14 (1H, dd, *J* 4.8, 0.8, Ar-H); δ_C (100 MHz, $CDCl_3$): 41.5 (CH_2), 46.0 (2 x CH_2), 52.2 (CH_2), 53.3 (CH_2), 55.6 (CH), 57.9 (CH_2), 61.9 (CH_2), 123.7 (ArCH), 124.1 (ArCH), 126.4 (ArCH), 146.2 (ArC); LRMS (CI^+): 255.2 ($[M+H]^+$, 100%), 249.1 (46), 212.1 (29), 169.1 (42), 144.1 (9), 126.1 (23), 71.1 (43); HRMS calcd for $C_{12}H_{22}N_4S$ 255.1643, found 255.1641.

2-(Naphthalen-2-yl)-1,4,7,10-tetraazacyclododecane (90)



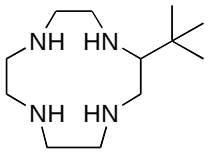
Using **81** (1.01 g, 5.5 mmol), **90** was produced as a viscous dark brown oil (1.26 g, 77%) *via* general procedure 3D; ν_{max} (neat)/cm^{-1}: 3426 (N-H), 2944 (C-H), 2830 (-CH_2-), 1653 (C=C); δ_H (400 MHz, $CDCl_3$): 1.97 (4H, br s, 4 x NH), 2.06 (1H, t, *J* 10.4 Hz, $CH_{B'}$), 2.15 (1H, dt, *J* 10.8, 3.2 Hz, CH), 2.48 (2H, t, *J* 6.0 Hz, 2 x CH), 2.59-2.63 (4H, m, 4 x CH), 2.67 (2H, t, *J* 6.0 Hz, 2 x CH), 2.76 (1H, m, CH), 2.82 (1H, br t, *J* 10.4 Hz, CH_B), 2.89 (1H, m, CH), 3.02 (1H, br t, *J* 10.8 Hz, CH), 3.95 (1H, br d, *J* 8.0 Hz, CH_A), 7.35-7.39 (2H, m, 2 x Ar-H), 7.72-7.75 (4H, m, 4 x Ar-H), 7.78 (1H, s, Ar-H); δ_C (100 MHz, $CDCl_3$): 41.6 (CH_2), 46.2 (CH_2), 46.3 (CH_2), 52.4 (CH_2), 53.4 (CH_2), 58.1 (CH_2), 60.4 (CH), 61.6 (CH_2), 125.4 (ArCH), 125.5 (ArCH), 125.8 (ArCH), 126.1 (ArCH), 127.6 (ArCH), 127.9 (ArCH), 128.0 (ArCH), 133.0 (ArC), 133.4 (ArC), 140.0 (ArC); LRMS (CI^+): 299.2 ($[M+H]^+$, 17%), 256.2 (100), 213.1 (63), 188.1 (27), 170.1 (31), 161.1 (19), 143.1 (19), 81.1 (33); HRMS calcd for $C_{18}H_{27}N_4$ 299.2236, found 299.2234.

2-(Phenanthrene-3-yl)-1,4,7,10-tetraazacyclododecane (91)



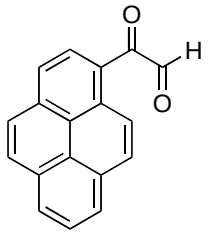
Using **82** (0.18 g, 0.77 mmol), **91** was produced as a viscous brown oil (1.04 g, 79%) *via* general procedure 3D; ν_{max} (neat)/cm^{-1}: 3257 (N-H), 2940 (C-H), 2860 (-CH_2-), 1633 (C=C); δ_H (400 MHz, $CDCl_3$): 2.10 (1H, t, *J* 10.4 Hz, $CH_{B'}$), 2.13 (1H, br t, *J* 9.2 Hz, CH), 2.34 (2H, t, *J* 6.4 Hz, 2 x CH), 2.49 (2H, t, *J* 6.0 Hz, 2 x CH), 2.64 (2H, t, *J* 5.6 Hz, 2 x CH), 2.67-2.96 (5H, m, 4 x CH, CH_B), 3.09 (1H, br t, *J* 11.6 Hz, CH), 4.02 (1H, br d, *J* 10.0 Hz, CH_A), 7.50-7.61 (6H, m, 6 x Ar-H), 7.65 (1H, s, Ar-H), 7.80-7.84 (1H, m, Ar-H), 8.55-8.60 (1H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 37.7 (CH_2), 40.6 (CH_2), 45.2 (CH_2), 52.2 (CH_2), 57.1 (CH_2), 59.2 (CH), 60.0 (CH_2), 60.6 (CH_2), 121.6 (ArCH), 121.9 (ArCH), 124.8 (ArCH), 125.3 (ArCH), 125.5 (ArCH), 125.6 (ArCH), 125.9 (ArCH), 126.1 (ArCH), 127.5 (ArCH), 128.6 (ArC), 129.1 (ArC), 130.9 (ArC), 131.0 (ArC), 139.8 (ArC); LRMS (FAB): 349.3 ($[M+H]^+$, 27%), 306.2 (11), 205.4 (10), 154.9 (100), 137.1 (72), 75.1 (31); HRMS calcd for $C_{22}H_{29}N_4$ 349.2392, found 349.2399.

2-(*tert*-Butyl)-1,4,7,10-tetraazacyclododecane (92)



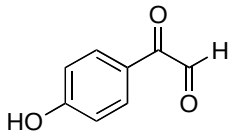
Using general method 3D azamacrocycle **92** was synthesised as a brown oil (1.15 g, 63%). ν_{max} (NaCl)/cm^{-1}: 3281 (N-H), 2810 (C-H), 1666 (N-H), 1315 (C-N); δ_H (400 MHz, $CDCl_3$): 0.85 (9H, s, 3 x CH_3), 1.52 (4H, br s, 4 x NH), 1.69 (1H, t, *J* 10.5 Hz, CH), 1.87 (1H, dt, *J* 10.5, 3.5 Hz, $CH_{B'}$), 2.33-2.47 (4H, m, 4 x CH), 2.59-2.68 (4H, m, 4 x CH), 2.70-2.81 (4H, m, 4 x CH), 3.59 (1H, br t, *J* 9.6 Hz, CH_A); δ_C (100 MHz, $CDCl_3$): 38.8 (CH_2), 43.2 (CH_2), 46.1 (CH_2), 47.3 (C), 48.5 (CH_2), 51.2 (CH_2), 53.3 (CH_2), 61.2 (CH_2), 65.9 (CH), 79.8 (3 x CH_3); LRMS (FAB): 229.4 ($[M+H]^+$, 100%), 186.7 (27), 144.1 (26), 129.3 (19), 100.7 (14); HRMS calcd for $C_{12}H_{28}N_4$, 229.2392 found 229.2396.

Oxo-pyrene-2-yl-acetaldehyde (103)



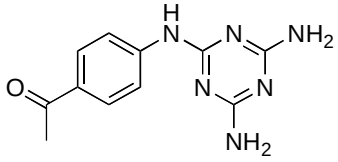
103 was synthesised from 1-acetylpyrene (1.5 g, 6 mmol) using general procedure 3C. The resulting residue was then separated on a silica column eluted with 1 : 1 hexane : EtOAc to give a yellow powder (1.59 g, 100%). R_f = 0.23 (hexane : EtOAc 1 : 1); mp: 107-111 ^oC; ν_{max} (NaCl)/cm^{-1}: 3041 (ArC-H), 1671 (C=O), 1594 (ArC=C), 1508 (ArC=C); δ_H (400 MHz, $CDCl_3$: 7.68-8.17 (9H, m, 9 x Ar-H), 9.81 (1H, s, C(O)H); δ_C (100 MHz, $CDCl_3$): 123 8 (ArCH), 124.2 (ArCH), 124.7 (ArCH), 126.4 (ArCH), 126.7 (ArCH), 127.0 (ArCH), 127.3 (ArCH), 129.3 (ArC), 129.6 (ArC), 130.4 (ArCH), 130.5 (ArC), 131.1 (ArCH), 131.9 (ArC), 134.0 (ArC), 135.4 (ArC), 135.7 (ArC), 190.5 (C=O); LRMS (EI^+): 258.1 (M, 15%), 229.1 (96), 201.1 (78), 100.0 (17), 82.9 (100), 47.0 (21); HRMS calcd for $C_{18}H_{10}O_2$ 258.0681, found 258.0680.

(4-Hydroxy-phenyl)-oxo-acetaldehyde (105)



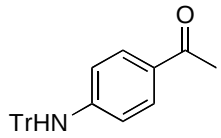
105 was prepared from 4'-hydroxyacetophenone (3 g, 22 mmol) using general procedure 3C. The resulting liquid was separated on a silica column eluted with 1 : 1 hexane : EtOAc to give a pale yellow foam (2.54 g, 77%). R_f = 0.12 (hexane : EtOAc 1 : 1); mp: 85-86 ^{o}C (lit.[257] 86.5-87.5 ^{o}C); δ_H (400 MHz, Acetone-d_6): 6.3 (1H, s, OH), 6.93 (2H, d, *J* 10.0 Hz, 2 x Ar-H), 8.09 (2H, d, *J* 10.0 Hz, 2 x Ar-H), 9.64 (1H, s, C(O)H); δ_C (100 MHz, Acetone-d_6): 90.0 ($C(OH)_2H$), 116.5 (2 x ArCH), 126.1 (ArC), 133.3 (2 x ArCH), 163.7 (ArC), 192.2 (C=O); LRMS (EI^+): 150.0 (M, 16%), 121.0 (100), 93.0 (100), 65 (100), 46 (100); HRMS calcd for $C_8H_6O_3$ 150.0317, found 150.0315.

1-[4-(4,6-Diamino-[1,3,5]-triazin-2-ylamino)-phenyl]-ethanone (109)



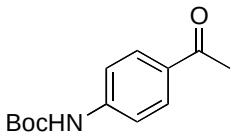
A mixture of 4'-aminoacetophenone (1 g, 7.4 mmol) in H_2O (26 mL) was heated under reflux. A solution of aq NaOH (7.4 mM) was then added dropwise over 1 h. Heating and stirring was continued for a further 3 h and then the mixture was cooled to rt. The solid was filtered and washed with H_2O (10 mL) before drying under vacuum to **109** as a white solid (1.31 g, 72%). mp: >250 ^{o}C (lit.[266] >300 ^{o}C); ν_{max} (KBr)/cm^{-1}: 3488 (N-H), 3344 (N-H), 3120 (ArC-H), 1683 (C=O), 1637 (C=N), 1566 (ArC=C), 1527 (ArC=C); δ_H (400 MHz, DMSO-d_6): 2.52 (3H, s, CH_3), 6.92 (1H, br s, NH), 7.15 (2H, br s, NH_2), 7.23 (2H, br s, NH_2), 7.89 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 7.98 (2H, d, *J* 8.8 Hz, 2 x Ar-H); LRMS (EI^+): 243.1 (M, 2%), 145.0 (100), 110.0 (44), 84.0 (78), 66.0 (90); HRMS calcd for $C_{11}H_{12}N_6O$, 244.0994, found 244.0991.

1-[4-(Trityl-amino)-phenyl]-ethanone (110)



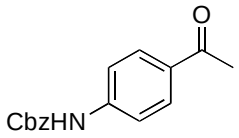
Triphenylmethyl chloride (1.06 g, 3.8 mmol) was added to a solution of 4'-aminoacetophenone (460 mg, 3.4 mmol) in dry pyridine (12 mL). The solution was stirred at rt for 4 d before the reaction mixture was diluted with EtOAc (25 mL) and then washed with aq $CuSO_4$ solution (0.5 M, 50 mL) and brine (3 x 20 mL). The organic layers were dried over $MgSO_4$, and concentrated *in vacuo* to give a white solid (938 mg, 73%). R_f = 0.74 (20% THF in CH_2Cl_2); mp: 154-157 ^oC; ν_{max} (KBr)/cm^{-1}: 3055 (ArC-H), 1658 (C=O), 1594 (ArC=C), 1281 (C-N); δ_H (400 MHz, $CDCl_3$): 2.19 (3H, s, CH_3), 5.60 (1H, s, NH), 6.22 (2H, d, *J* 8.4 Hz, 2 x Ar-H), 6.98-7.18 (15H, m, 15 x Ar-H), 7.40 (2H, d, *J* 8.4 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 26.0 (CH_3), 71.4 (C), 127.3 (2 x ArCH), 128.1 (2 x ArCH), 128.3 (6 x ArCH), 129.2 (6 x ArCH), 129.8 (3 x ArCH), 144.7 (3 x ArC), 150.9 (2 x ArC), 196.5 (C); LRMS (EI^+): 377.0 (M, 16%), 300.1 (19), 244 (100), 215 (32), 165 (100), 106.1 (28), 77 (73), 43 (46); HRMS calcd for $C_{27}H_{23}NO$ 377.1780, found 377.1779.

4-[(1,1-Dimethylethoxy)carbonylamino]acetophenone (111)



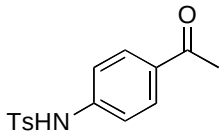
Di-*tert*-butyl dicarbonate (4.8 g, 22 mmol) was added to a solution of 4'-aminoacetophenone (3 g, 22 mmol) in dry THF (130 mL). The mixture was stirred and heated at reflux for 5 d, before pouring onto H_2O (50 mL), and extracting with EtOAc (100 mL). The organic layer was then washed with H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4, and then dried under rotary evaporation to give a yellow solid. This was separated on a silica column eluted with 1 : 1 hexane : EtOAc to yield **111** as a white solid (3.53 g, 75%). R_f = 0.44 (hexane : EtOAc 1 : 1); mp: 134-136 ^oC; NMR spectroscopy agreed with lit. values;[267] δ_H (400 MHz, $CDCl_3$): 1.45 (9H, s, 3 x CH_3), 2.49 (3H, s, CH_3), 6.81 (1H, br s, NH), 7.40 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 7.83 (2H, d, *J* 8.8 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 26.4 (CH_3) 28.3 (3 x CH_3) 81.2 (C), 117.4 (2 x ArCH), 129.9 (2 x ArCH), 131.7 (ArC), 143.1 (ArC), 152.3 (C=O), 197.1 (C=O); LRMS (EI^+): 235.1 (M, 37%), 179.1 (78), 164.0 (65), 120.0 (76), 57.1 (100); HRMS calcd for $C_{13}H_{17}NO_3$ 235.1208, found 235.1210.

(4-Acetyl-phenyl)-carbamic acid benzyl ester (112)



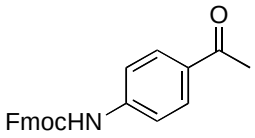
Benzylchloroformate (1.14 mL, 8 mmol) was added dropwise to a solution of 4'-aminoacetophenone (1.08 g, 8 mmol) and NaOH (320 mg, 8 mmol) in 1,4-dioxane : H_2O mixture (8mL : 3 mL) cooled to 0 ^oC. The reaction mixture was then stirred at this temperature for 30 min, and then for a further 13 h at rt. The solvent was then removed under reduced pressure, and resulting solid dissolved into a EtOAc : H_2O mixture (40 mL : 40 mL). The organic layer was separated and concentrated *in vacuo* to give a bright yellow solid (2.15 g, 100%). R_f = 0.43 (hexane : EtOAc 1 : 1); mp: 112-115 ^oC (lit.[268] 118-119 ^oC); δ_H (400 MHz, $CDCl_3$): 2.48 (3H, s, CH_3), 5.13 (2H, s, CH_2), 7.08 (1H, br s, NH), 7.26-7.32 (5H, m, 5 x Ar-H), 7.42 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 7.84 (2H, d, *J* 8.8 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 26.4 (CH_3), 67.4 (CH_2), 117.7 (ArCH), 128.4 (2 x ArCH), 128.6 (2 x ArCH), 128.7 (2 x ArCH), 129.9 (2 x ArCH), 132.2 (ArC), 135.7 (ArC), 142.4 (ArC), 152.9 (C=O), 197.1 (C=O); LRMS (EI^+): 269.1 (M, 15%), 225.1 (7), 210.1 (6), 167.0 (6), 149.0 (14), 107.0 (18), 91.0 (100), 69.1 (41); HRMS calcd for $C_{16}H_{15}NO_3$ 269.1052, found 269.1049.

***p*-(*N*-*p*-Tolylsulfonylamino)acetophenone (113)**



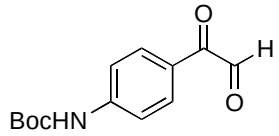
A solution of 4'-aminoacetophenone (2.0 g, 15 mmol) in pyridine (35 mL) was added dropwise to a stirring solution of *p*-toluenesulfonyl chloride (3.39 g, 18 mmol) in pyridine (100 mL) cooled to 0 ^oC. The mixture was then stirred at rt for 24 h before pouring onto an ice : H_2O mixture (300 mL). The solution was left to stand for 4 h to allow product to precipitate, which was then filtered and dried *in vacuo* yielding **113** as white fluffy crystals (3.32 g, 78%). mp: 202-204 ^oC (lit.[269] 204-205 ^oC); δ_H (400 MHz, $CDCl_3$): 2.32 (3H, s, CH_3), 2.47 (3H, s, CH_3), 6.69 (1H, s, NH), 7.08 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 7.19 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.66 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.78 (2H, d, *J* 8.8 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 21.6 (CH_3), 26.4 (CH_3), 119.0 (2 x ArCH), 127.3 (2 x ArCH), 129.9 (2 x ArCH), 130.0 (2 x ArCH), 141.1 (2 x ArC), 144.5 (2 x ArC), 196.8 (C=O).

(4-Acetyl-phenyl)-carbamic acid 9*H*-fluoren-9-yl ester (114)



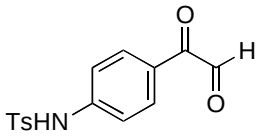
4'-Aminoacetophenone (523 mg, 4 mmol) was added to a stirring solution of 9-fluorenylmethylchloroformate (1.0 g, 4 mmol) in Et_2O (35 mL) cooled to 0 ^oC. The mixture was stirred for 20 min at this temperature, and then at rt for 1 h. After this time, H_2O (250 μL) was added and solution stirred for 10 min before filtering to give **114** as a white powder (731 mg, 53%). mp: 140-145 ^oC; ν_{max} (KBr)/cm^{-1}: 3350 (N-H), 3057 (Ar), 2891 (C-H), 1608 (ArC=C), 1693 (NCO), 1520 (Ar), 1244 (COO); δ_H (400 MHz, $CDCl_3$): 2.49 (3H, s, CH_3), 4.20 (1H, t, *J* 6.4 Hz, CH), 4.51 (2H, d, *J* 6.4 Hz, CH_2), 6.89 (1H, s, NH), 7.25 (2H, dt, *J* 7.6, 1.2 Hz, 2 x Ar-H), 7.35 (2H, t, *J* 7.6 Hz, 2 x Ar-H), 7.54 (2H, d, *J* 7.4 Hz, 2 x Ar-H), 7.71 (2H, d, *J* 7.4 Hz, 2 x Ar-H), 7.84 (2H, d, *J* 8.4 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 26.4 (CH_3), 47.1 (CH), 67.1 (CH_2), 117.7 (2 x ArCH), 120.2 (2 x ArCH), 124.9 (2 x ArCH), 127.2 (2 x ArCH), 127.9 (2 x ArCH), 129.9 (2 x ArCH), 132.3 (ArC), 141.4 (2 x ArC), 142.2 (ArC), 143.6 (2 x ArC), 152.9 (C=O), 196.9 (C=O).

[4-(2-Oxo-acetyl)-phenyl]-carbamic acid *tert*-butyl ester (116)



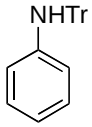
Using general procedure 3C, **116** was synthesised from **111** (801 mg, 3.4 mmol). The resulting residue was separated on a silica column eluted with 1 : 1 hexane : EtOAc to give a yellow foamy solid (847 mg, 100%). R_f = 0.25 (hexane : EtOAc 1 : 1); ν_{max} (KBr)/cm^{-1}: 3344 (N-H), 2978 (ArC-H), 1708 (C=O), 1602 (ArC=C), 1154 (C-O); δ_H (400 MHz, $CDCl_3$): 1.45 (9H, s, 3 x CH_3) 5.06 (1H, br s, $C(OH)_2H$), 6.23 (1H, br s, OH), 6.97 (1H, br s, OH), 7.39 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 7.99 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 9.57 (trace 1H, s, C(O)H); δ_C (100 MHz, $CDCl_3$): 28.2 (3 x CH_3), 81.6 (C), 88.6 ($C(OH)_2H$), 117.6 (ArCH), 117.6 (ArCH), 126.6 (ArC), 131.6 (ArCH), 132.3 (ArCH), 144.5 (ArC), 190.2 (C=O), 191.3 (C=O); LRMS (EI^+): 249.2 (M, 5%), 220.1 (76), 164.1 (98), 120.1 (47), 85.0 (100), 47.0 (100); HRMS calcd for $C_{13}H_{15}NO_4$ 249.1001, found 249.1004.

4-Methyl-*N*-[4-(2-oxo-acetyl)-phenyl]-benzenesulfonamide (118)



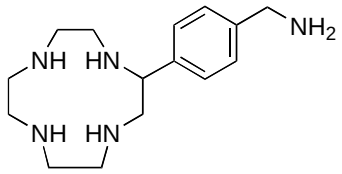
118 was prepared using general procedure 3C from **113** (1.0 g, 3.46 mmol). The crude residue was then chromatographed on a silica column eluted with hexane : EtOAc (1 : 1) to give a green oil (36 mg, 3%). R_f = 0.1 (hexane : EtOAc 1 : 1); δ_H (400 MHz, $CDCl_3$): 2.28 (3H, s, CH_3), 5.01 (1H, s, $C(OH)_2H$), 7.11 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 7.16 (2H, d, *J* 8.4 Hz, 2 x Ar-H), 7.69 (2H, d, *J* 8.6 Hz, 2 x Ar-H), 7.75 (2H, d, *J* 8.6 Hz, 2 x Ar-H), 8.11 (1H, s, NH); δ_C (100 MHz, $CDCl_3$): 20.6 (CH_3), 87.6 ($C(OH)_2H$), 117.2 (2 x ArCH), 117.3 (2 x ArCH), 126.2 (ArCH), 126.3 (ArCH), 129.0 (2 x ArCH), 134.6 (ArC), 141.8 (ArC), 143.7 (ArC), 188.8 (ArC), 190.4 (C=O); LRMS (FAB): 304.0 ($[M+H]^+$, 89%), 274.0 (100), 84.9 (75), 75.1 (24); HRMS calcd for $C_{15}H_{14}NO_4S$ 304.0644, found 304.0649.

***N*-Tritylaniline (120)**



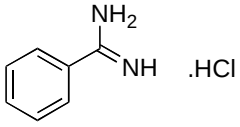
Triphenylmethyl chloride (3.35 g, 12 mmol) was added to a solution of aniline (1 mL, 11 mmol) in dry pyridine (45 mL). The solution was stirred at rt for 4 d. The reaction mixture was then diluted with EtOAc (100 mL) and then washed with aq $CuSO_4$ solution (0.5 M) (150 mL) and brine (3 x 50 mL). The organic layers were dried over $MgSO_4$, and concentrated *in vacuo* to give a pale yellow solid (3.51 g, 95%). mp: 150-152 ^oC (lit.[270] 151-152 ^oC); δ_H (400 MHz, $CDCl_3$): 4.82 (1H, s, NH), 6.26 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 6.44 (1H, t, *J* 14.2 Hz, Ar-H), 6.61 (2H, dd, *J* 14.2, 8.0 Hz, 2 x Ar-H), 7.09-7.29 (15H, m, 15 x Ar-H); δ_C (100 MHz, $CDCl_3$): 71.5 (C), 116.2 (2 x ArCH), 117.4 (ArCH), 126.9 (3 x ArCH), 128.0 (6 x ArCH), 228.3 (2 x ArCH), 129.3 (6 x ArCH), 145.5 (3 x ArC) 146.3 (ArC); LRMS (EI^+): 335.2 (M, 10%), 243.1 (100), 228.1 (18), 183.1 (13), 165.1 (96), 84.0 (40), 77.1 (35); HRMS calcd for $C_{25}H_{21}N$ 335.1674, found 335.1672.

2-(4'-Benzylamine)-1,4,7,10-tetraazacyclododecane (124)



A solution of BH_3.THF (1M) (10 mL) was added slowly to a flask of stirring **87** (600 mg) under Ar. The solution was then stirred at rt for a further 19 h before the excess borane was quenched with MeOH (3 mL). The solvent was removed under vacuum and the residue was treated with boiling HCl (6M) (20 mL) for 2 h. After removal of the solvents *in vacuo*, the residue was basified to pH 12 using KOH (7M, 10 mL), and the aq solution extracted with CH_2Cl_2 (3 x 20 mL) which was then dried under reduced pressure to give **124** as a white viscous oil (391 mg, 64%). ν_{max} (NaCl)/cm^{-1}: 3466 (N-H), 2971 (ArC-H), 1615 (ArC=C), 1072 (C-N); δ_H (400 MHz, $CDCl_3$): 1.91 (2H, t, *J* 10.0 Hz, $CH_{B'}$), 2.07-2.97 (15H, m, 14 x CH, CH_2, CH_A), 7.17 (2H, d, *J* 7.0 Hz, 2 x Ar-H), 7.27 (2H, d, *J* 7.0 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 38.5 (CH_2), 41.4 (CH_2), 45.8 (CH_2), 52.5 (CH_2), 53.1 (CH_2), 58.1 (CH_2), 59.8 (CH), 61.0 (CH_2), 61.5 (CH_2), 126.7 (2 x ArCH), 126.9 (2 x ArCH), 141.0 (ArC), 142.4 (ArC); LRMS (CI^+): 277.2 (M, 9%), 260.2 (12), 204.1 (100), 187.1 (74), 132.1 (21), 99.1 (37), 58.1 (62); HRMS calcd for $C_{15}H_{27}N_5$ 277.2266, found 277.2264.

Benzamidine (129)

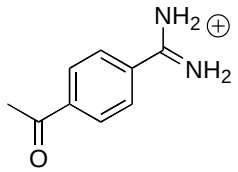


129 was prepared as the hydrochloride salt under Pinner conditions:[153] Benzonitrile (4.08 mL, 40 mmol) was dissolved in dry CH_2Cl_2 (88 mL) and abs. EtOH (4.7 mL) added and cooled to -8 ^oC in a NaCl ice bath. The solution was saturated with dry HCl at this t for 20 min, and then stirred at rt under an atmosphere of HCl for 18 h. The solvent was then removed under vacuum and Et_2O (100 mL) was added. The precipitate was filtered, dissolved in ice cold H_2O (30 mL) and 10% aq K_2CO_3 (75 mL) was added. The mixture was stirred at rt for 5 min before it was extracted with EtOAc (150 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give a clear oil. This oil was combined with NH_4Cl (2.35 g, 44 mmol) and MeOH : H_2O (70 mL : 9 mL) and stirred and heated under reflux for 6 h. The solvent was then removed under vacuum and acetone (75 mL) was added. The precipitate was collected by filtration to achieve **129** as a white

solid (4.78 g, 76%). mp: 176-178 ^{o}C (lit.[271] 174-179 ^{o}C); δ_H (400 MHz, DMSO-d_6): 7.55 (2H, t, *J* 6.8 Hz, 2 x Ar-H), 7.63 (1H, t, *J* 7.4 Hz, Ar-H), 7.85 (2H, d, *J* 7.4 Hz, 2 x Ar-H); δ_C (100 MHz, DMSO-d_6): 127.8 (2 x ArCH), 129.1 (2 x ArCH), 131.9 (ArCH), 135.2 (ArC), 165.6 (CNH_2).

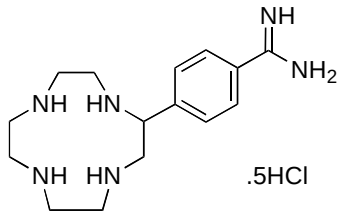
Benzamidine **129** was also prepared using lithium hexamethyl disilazane (LiHMDS): Benzonitrile (0.1 mL, 1 mmol) was dissolved in dry THF (4 mL) and cooled to -78^{o}C. LiHMDS. THF (2 mL, 2 mmol) was added dropwise, and mixture stirred at this t for 2 h. The reaction mixture was then stirred for a further 2 h while warming to 0 ^{o}C. H_2O (2 mL) was then added carefully, and solution extracted with CH_2Cl_2 (30 mL). The organic layer was then washed with brine (10 mL) and dried over $MgSO_4$ before filtering and concentrating *in vacuo* to give a green oil (77 mg, 64%). LRMS (CI^+): 121.2 ($[M+H]^+$, 100%), 85.1 (73); HRMS calcd for $C_7H_8N_2$ 121.0766, 121.0765.

4-Acetyl-benzamidine (132)



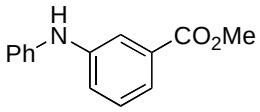
4'-Acetylbenzonitrile (0.5 g, 3.4 mmol) was dissolved in dry THF (14 mL) and cooled to -78^{o}C. LiHMDS.THF (7 mL, 7 mmol) was added dropwise, and mixture stirred at this temperature for 2 h. The reaction mixture was then stirred for a further 2 h while warming to 0 ^{o}C. H_2O (15 mL) was then added carefully, and solution extracted with CH_2Cl_2 (50 mL). The aq layer was acidified with conc HCl and extracted with EtOAc (25 mL), and then basified with NaOH and extracted with EtOAc (25mL). The organic layers were all combined and washed with brine (40 mL) before drying over $MgSO_4$. Filtration and concentrating *in vacuo* gave **132** as a white solid (360 mg, 65%). mp: 54-55 ^{o}C; ν_{max} (KBr)/cm^{-1}: 3354 (N-H), 3096 (ArC-H), 1695 (C=N), 1685 (C=O), 1604 (ArC=C); δ_H (400 MHz, DMSO-d_6): 2.69 (3H, s, CH_3), 3.44 (4H, br s, 4 x NH), 8.09-8.10 (4H, m, 4 x Ar-H); δ_C (100 MHz, DMSO-d_6): 27.0 (CH_3), 128.3 (2 x ArCH), 129.5 (2 x ArCH), 134.5 (ArC), 139.8 (ArC) 166.6 (CNH_2), 197.7 (C=O); LRMS (EI^+): 164.0 ($[M+H]^+$, 6%), 149.0 (22), 121.0 (6), 84.0 (100), 66.0 (99), 46.0 (37); HRMS calcd for $C_9H_{12}N_2O$ 164.0950, found 164.0944.

2-(Benzamidine)-1,4,7,10-tetraazacyclododecane.pentahydrochloride (131)



87 (637 mg, 2.3 mmol) was dissolved in THF (9 mL) under Ar and cooled to -78 ^{o}C. LiHMDS.THF (11.7 mL, 11.7 mmol) was then added dropwise with stirring. The mixture was stirred and warmed to rt and stirred at this t for 24 h. The solution was then cooled to 0 ^{o}C and HCl.Et_2O (10 mL) added. The brown precipitate was filtered and recrystallised from EtOH and Et_2O, and the hygroscopic solid filtered and washed quickly with cold EtOH and Et_2O a number of times to leave a fine pale brown powder (1.04g, 95%). ν_{max} (KBr)/cm^{-1}: 3150 (N-H), 1679 (C=N), 1546 (ArC=C), 1377 (C-N); δ_H (400 MHz, DMSO-d_6): 2.60-3.40 (21H, m, 14 x CH, 7 x NH), 3.85-3.88 (1H, m, CH_A), 7.89-8.00 (4H, m, 4 x Ar-H); δ_C (100 MHz, DMSO-d_6): 35.3 (CH_2), 42.5 (CH_2), 43.4 (CH_2), 48.2 (CH_2), 48.7 (CH_2), 53.8 (CH_2), 55.0 (CH_2), 57.6 (CH), 126.1 (2 x ArCH), 126.5 (2 x ArCH), 139.5 (ArC), 163.3 (C=N).

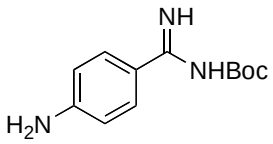
Methyl 3-phenylaminobenzoate (134)



A screw-cap Schlenk tube equipped with a stirrer bar was charged with $Pd_2(dba)_3$ (9.2 mg, 0.01 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (XPhos) (24 mg, 0.05 mmol) and sodium *t*-butoxide (135 mg, 1.4 mmol). The tube was evacuated and refilled with argon before methyl 3-chlorobenzoate (170 mg, 1.0 mmol), aniline (109 μL, 1.2 mmol) and *t*-BuOH (1 mL) were added. The tube was then capped and reaction mixture heated and stirred at 100 ^{o}C for 30 min. After this time, the mixture was cooled to rt, diluted with EtOAc (5 mL) and suction filtered. The filtrate was reduced under vacuum, and residue separated by flash chromatography (1 : 1 hexane : Et_2O) to give **134** as a yellow solid (57 mg, 25%). mp: 110-112 ^{o}C (lit.[159] 111-112.5 ^{o}C); δ_H (400 MHz, $CDCl_3$): 3.81 (3H, s, OCH_3), 5.79 (1H, s, NH), 6.89 (1H, t, *J* 7.6 Hz, Ar-H), 7.00 (2H, dd, *J* 8.4, 0.8 Hz, 2 x Ar-H), 7.14-7.24 (4H, m, 4 x Ar-H), 7.48 (1H, dt, *J* 7.6, 1.4 Hz, Ar-H), 7.63 (1H, t, *J* 1.4 Hz, Ar-H); δ_C (100 MHz, $CDCl_3$): 52.2 (CH_3), 118.2 (ArCH), 118.4 (ArCH), 121.5

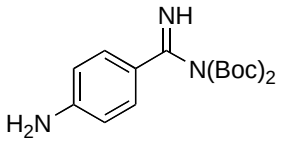
(ArCH), 121.8 (ArCH), 129.2 (ArCH), 129.4 (2 x ArCH), 129.5 (2 x ArCH), 131.3 (ArC), 142.4 (ArC), 143.6 (ArC), 167.2 (C=O).

4-Amino-1-(2-*tert*butoxycarbonyl)-benzamidine (137)



Di-*tert*-butyl dicarbonate (524 mg, 2.4 mmol) was added slowly to a stirring solution of 4-aminobenzamidine dihydrochloride (500 mg, 2.4 mmol), aq NaOH (1M) (4.8 mL), THF (2.4 mL) and H_2O (2.4 mL). The mixture was stirred at rt for 3 d before extraction with EtOAc (30 mL). The organic layer was dried over Na_2SO_4 and dried under reduced pressure. The residue was then separated on a silica column eluted with 1 : 1 hexane : EtOAc to give a white solid (370 mg, 66%). R_f = 0.07 (hexane : EtOAc 1 : 1); mp: decomposition >220 ^oC (lit.[161] no characterisation); ν_{max} (KBr)/cm^{-1}: 3386 (-NH-), 1711 (COO), 1610 (Ar), 1496 (NCO); δ_H (400 MHz, DMSO-d_6): 1.42 (9H, s, 3 x CH_3), 5.79 (2H, s, NH_2), 6.54 (2H, d, *J* 8.8 Hz, Ar-H), 7.71 (2H, d, *J* 8.8 Hz, 2 x Ar-H); δ_C (100 MHz, DMSO-d_6): 28.0 (3 x CH_3), 77.1 ($C(CH_3)_3$), 112.5 (2 x ArCH), 120.3 (ArC), 129.2 (2 x ArCH), 152.5 (ArC), 163.9 (C=N), 166.2 (C=O); LRMS (EI^+): 235.1 ($[M]^+$, 45%), 180.1 (83), 162.0 (65), 135.1 (82), 119.0 (100), 92.0 (23), 57.1 (95), 41.1 (33); HRMS calcd for $C_{12}H_{17}N_3O_2$ 235.1321, found 235.1322.

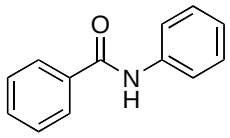
4-Amino-1-(2-di-*tert*butoxycarbonyl)-benzamidine (138)



Di-*tert*-butyl dicarbonate (26 g, 119 mmol) was added slowly to a stirring solution of 4-aminobenzamidine dihydrochloride (10 g, 48 mmol), aq NaOH (1 M) (96 mL), THF (48 mL) and H_2O (48 mL). The mixture was stirred at rt for 7 d before extraction with EtOAc (300 mL). The organic layer was dried over Na_2SO_4 and dried under reduced pressure. The residue was then separated on a silica column eluted with 1 : 1 hexane : EtOAc to give a white solid (10.4 g, 65%). R_f = 0.33 (hexane : EtOAc 1 : 1); mp: 175-177 ^oC; ν_{max} (KBr)/cm^{-1}: 3386 (-NH-), 1711 (COO), 1610 (Ar), 1496 (NCO); δ_H (400 MHz, CD_3OD): 1.51 (9H, s, 3 x CH_3), 1.52 (9H, s, 3 x CH_3), 4.92 (2H, s, NH_2), 7.51 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 7.75 (2H, d, *J* 8.8 Hz, 2 x Ar-H); δ_C (100 MHz, CD_3OD): 28.6 (3 x CH_3), 28.7 (3 x

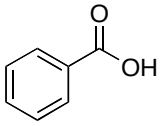
CH_3), 80.3 ($C(CH_3)_3$), 81.3 ($C(CH_3)_3$), 118.8 (2 x ArCH), 129.5 (ArC), 129.7 (2 x ArCH), 144.6 (ArC), 164.8 (C=N), 169.7 (2 x C=O); LRMS (EI^+): 335.1 ($[M]^+$, 8%), 280.1 (18), 206.0 (14), 179.1 (31), 135.1 (16), 57.1 (100); HRMS calcd for $C_{17}H_{25}N_3O_4$ 335.1845, found 335.1847.

Benzanilide (143)



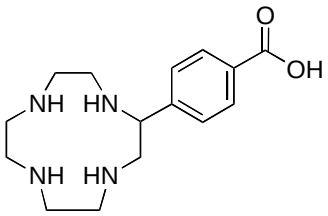
Benzoyl chloride (1.0 g, 7 mmol) was added to a stirring solution of aniline (532 μL, 5.8 mmol), 4-(dimethylamino)pyridine (992 mg, 8.12 mmol) and pyridine (695 μL, 8.58 mmol) in CH_2Cl_2 (70 mL). The mixture was stirred at rt for 19 h before it was diluted with CH_2Cl_2 (50 mL) and washed with saturated aq Na_2CO_3 solution. The organic phase was then reduced *in vacuo*. The residue was then separated on a silica column eluted with 1 : 1 hexane : EtOAc to give an off white solid (1.04 g, 90%). R_f = 0.5 (hexane : EtOAc 1 : 1); mp: 161-163 ^oC (lit.[272] 163 ^oC); δ_H (400 MHz, $CDCl_3$): 7.09 (1H, tt, *J* 7.2, 1.4 Hz, Ar-H), 7.31 (2H, t, *J* 8.4 Hz, 2 x Ar-H), 7.42 (2H, tt, *J* 6.8, 1.6 Hz, 2 x Ar-H), 7.49 (1H, tt, *J* 7.2, 1.4 Hz, Ar-H), 7.58 (2H, d, *J* 7.4 Hz, 2 x Ar-H), 7.75 (1H, s, NH), 7.81 (2H, d, *J* 7.4 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 120.2 (2 x ArCH), 124.6 (ArCH), 127.0 (2 x ArCH), 128.9 (2 x ArCH), 129.2 (2 x ArCH), 131.9 (ArCH), 135.0 (ArC), 137.9 (ArC), 170.5 (C=O).

Benzoic acid (144)



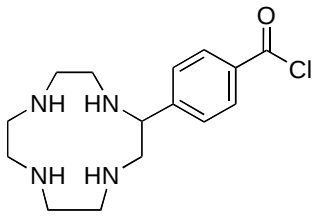
To a solution of benzonitrile (2 mL, 19.6 mmol) in EtOH (20 mL) and H_2O (20 mL) was added KOH (40 g, 713 mmol). The mixture was heated and stirred under reflux for 24 h before Et_2O (15 mL) was added and the aq layer separated. The organic phase was washed with H_2O (2 x 20 mL) and the aq layers combined and acidified with conc HCl to pH1. The aq phase was then extracted with Et_2O (50 mL), which was washed with H_2O (10 mL), dried over $MgSO_4$ and the solvent removed *in vacuo*. **114** was achieved as white needle-like crystals (1.85 g, 77%). mp: 121-122 ^oC (lit.[273] 123-124 ^oC); δ_C (100 MHz, $CDCl_3$): 128.5 (2 x ArCH), 129.4 (ArC), 130.3 (2 x ArCH), 133.9 (ArCH), 172.8 (C=O).

2-(Benzoic acid)-1,4,7,10-tetraazacyclododecane (142)



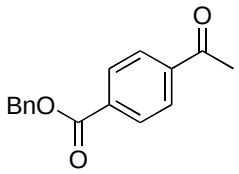
KOH pellets (10.0 g, 183 mmol) were slowly added to a stirring solution of **87** (1.0 g, 3.7 mmol), EtOH (4 mL) and H_2O (4 mL). The mixture was then stirred and heated under reflux for 3 d before Et_2O (3 mL) was added, and the organic layer separated and washed with H_2O (2 x 5 mL). The aq layers were combined and acidified with conc HCl before extraction with Et_2O (75 mL). The organic phase was separated, washed with H_2O (20 mL), dried over $MgSO_4$ and reduced *in vacuo* to give **142** as a pale brown solid (953 mg, 88%). The mp is unavailable as compound is hygroscopic; ν_{max} (KBr)/cm^{-1}: 3632 (-NH-), 3255 (COO-H), 1669 (Ar), 1558.0 (Ar); δ_H (400 MHz, CD_3OD): 3.17-4.09 (15H, m, 14 x CH, CH_A), 7.77 (2H, d, *J* 8.2 Hz, 2 x Ar-H), 8.13 (2H, d, *J* 8.2 Hz, 2 x Ar-H); δ_C (100 MHz, CD_3OD): 37.1 (CH_2), 44.41 (CH_2), 44.9 (CH_2), 46.0 (CH_2), 50.1 (CH_2), 54.0 (CH_2), 55.5 (CH_2), 59.1 (CH), 129.2 (2 x ArCH), 131.7 (2 x ArCH), 133.7 (ArC), 138.9 (ArC), 168.7 (C=O); LRMS (FAB): 293.2 ($[M+H]^+$, 22%), 192.5 (100), 176.7 (45), 131.4 (5); HRMS calcd for $C_{15}H_{25}O_2N_4$ 293.1978, found 293.1980.

2-(Benzoyl chloride)-1,4,7,10-tetraazacyclododecane (145)



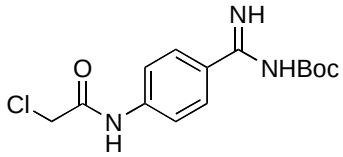
142 (50 mg, 0.17 mmol) and $SOCl_2$ (0.5 mL, 5.95 mmol) were stirred and heated at 70 ^oC for 2 h until HCl production stopped. The excess $SOCl_2$ was removed *in vacuo* to give **145** as a yellow solid (53 mg, 100%). **145** was taken onto next step without further purification or characterisation.

4-Acetyl-benzoic acid benzyl ester (146)



Benzyl bromide (362 μL, 3 mmol) was added to a stirring solution of 4'-acetyl benzoic acid (500 mg, 3 mmol) and triethylamine (425 μL, 3 mmol) in THF (6 mL). The solution was stirred at rt for 48 h after which the solvent was removed under reduced pressure. The residue was then extracted with Et_2O (75 mL) and H_2O (30 mL). The organic layer was separated, washed with brine and dried over $MgSO_4$ before reducing *in vacuo* to give a white solid (562 mg, 72%). mp: 32-36 ^oC (lit.[166] no characterisation); ν_{max} (KBr)/cm^{-1}: 1214 (COO), 1710 (C=O ketone), 3034 (Ar), 2962 (CH_2), 1499 (Ar); δ_H (400 MHz, $CDCl_3$): 2.57 (3H, s, CH_3), 5.32 (2H, s, CH_2), 7.29-7.40 (5H, m, 5 x Ar-H), 7.93 (2H, d, *J* 8.6 Hz, 2 x Ar-H), 8.09 (2H, d, *J* 8.6 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 26.9 (CH_3), 67.2 (CH_2), 128.2 (2 x ArCH), 128.3 (ArCH), 128.5 (2 x ArCH), 128.7 (2 x ArCH), 130.0 (2 x ArCH), 133.9 (2 x ArC), 140.3 (ArC), 165.6 (C=O), 197.5 (C=O); LRMS (EI^+): 254.0 ($[M]^+$, 73%), 239.0 (19), 147.0 (100), 91.0 (93); HRMS calcd for $C_{16}H_{14}O_3$ 254.0943, found 254.0947.

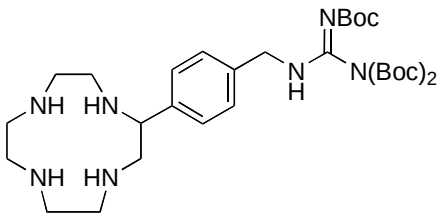
4-(1-Chloromethyl-1-formylamino)-1-(2-tertbutoxycarbonyl)-benzamidine (150)



To a solution of 4-amino-1-(2-tertbutoxycarbonyl)-benzamidine (220 mg, 0.94 mmol), and triethylamine (195 μL, 1.4 mmol) in CH_2Cl_2 (2.4 mL) was added dropwise a solution of chloroacetyl chloride (85 μL, 0.98 mmol) in CH_2Cl_2 (1 mL). The reaction mixture was stirred at rt for 19 h before washing with aq HCl (1M) (3 mL) and H_2O (2 x 3 mL). The organic layer was then dried over Na_2SO_4 and reduced *in vacuo*. The residue was then separated on a silica column eluted with 1 : 1 hexane : EtOAc to give **150** as a green oil (293 mg, 59%). R_f = 0.1 (hexane : EtOAc 1 : 1); (lit.[161] no characterisation); δ_H (400 MHz, $CDCl_3$): 1.45 (9H, s, 3 x CH_3), 4.13 (2H, s, CH_2), 7.52 (1H, d, *J* 8.8 Hz, Ar-H), 7.62 (1H, d, *J* 8.8 Hz, Ar-H), 7.75 (2H, dd, *J* 8.8, 3.2 Hz, 2 x Ar-H), 8.19 (NH), 8.64 (NH), 8.70 (NH); δ_C (100 MHz, $CDCl_3$): 28.1 (3 x CH_3), 43.0 (CH_2), 82.9 (C), 119.6 (2 x ArCH),

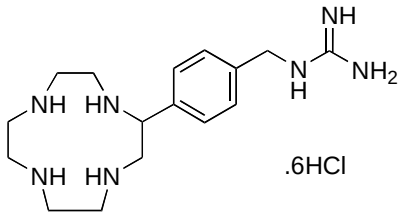
129.0 (2 x ArCH), 129.3 (ArC), 141.1 (ArC), 149.8 (C=N), 164.5 (C=O), 164.5 (C=O); LRMS (EI^+): 312.0 ($[M]^+$, 4%), 238.0 (15), 212.0 (28), 196.0 (23), 162.0 (9), 120.0 (23), 59.0 (95), 41.1 (100); HRMS calcd for $C_{14}H_{19}N_3O_3{}^{35}Cl$ 312.1115, found 312.1113.

N-[4-(1,4,7,10-Tetraazacyclododec-2-yl)-benzyl]-*N*-(*tert*-butoxycarbonyl)-*N*-bis(*tert*-butoxycarbonyl)-carboxamidine (151)



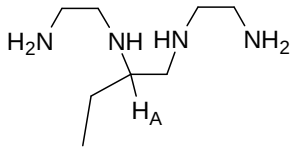
124 (305 mg, 1.1 mmol) was dissolved in abs MeOH (17 mL) under Ar at rt. *N*,*N*'-Di-Boc-1*H*-pyrazole-1-carboxamidine (170 mg, 0.55 mmol) and then *N*,*N*-diisopropylethylamine (96 μL, 0.55 mmol) were added and the mixture stirred for 24 h at rt. The reaction mixture was then concentrated *in vacuo* to give a yellow oil (488 mg, 100%), which was then taken onto the Boc-deprotection step without further purification.

2-(N-Benzylguanidine)-1,4,7,10-tetraazacyclododecane.hexahydrochloride (152)



151 (488 mg, 1.1 mmol) was stirred in aq HCl (6M) (10 mL) for 5 h at rt. The solution was concentrated under reduced pressure, and the yellow residue recrystallised from EtOH and Et_2O to give the hexahydrochloride salt **152** as a white solid (1.4 g, 99%). ν_{max} (KBr)/cm^{-1}: 3397 (N-H), 2900 (ArC-H), 2957 (ArC-H), 1670 (C=N), 1446 (C-N); δ_H (400 MHz, CD_3OD): 3.26-3.87 (16H, m, 14 x CH, CH_2), 4.48 (1H, d, *J* 6.4 Hz, CH_A), 6.80 (3H, br s, 3 x NH), 7.45 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.58 (2H, br s, 2 x NH), 7.73 (2H, d, *J* 8.0 Hz, 2 x Ar-H); δ_C (100 MHz, CD_3OD): 35.6 (CH_2), 39.7 (CH_2), 40.7 (CH_2), 43.35 (CH_2), 47.4 (2 x CH_2), 51.2 (CH_2), 53.2 (CH), 56.0 (CH_2), 127.7 (ArCH), 128.4 (ArCH), 131.7 (ArC), 133.3 (2 x ArCH), 139.3 (ArC), 157.2 (C=NH, rotomer), 158.7 (C=NH, rotomer).

***N*[1],*N*[2]-Bis-(2-aminoethyl)-butane-1,2-diamine (155)**



1,2-Dibromobutane (1.81 mL, 15 mmol) was added over 5 min to a stirring solution of 1,2-diaminoethane (2 mL, 30 mmol) in EtOH (10 mL). The mixture was heated under reflux for 18 h. KOH (4.2 g, 8.6 mol) was added and solution stirred at rt for 10 min. The solution was reduced by rotary evaporation until most of the solvent had been removed, before filtering through sintered glass, and rinsing with aq KOH solution (50%, 10 mL). The filtrate was recovered and another volume of aq KOH solution added (10 mL) before filtering again. The yellow organic layer was separated from the aq phase, and dried over KOH pellets. Filtering the solution and removal of solvent *in vacuo* gave the tetraamine **155** as a racemic yellow solid (1.9 g, 36%). ν_{max} (neat)/cm^{-1}: 1599 (-NH-), 1560 (-NH$_2$); δ_H (400 MHz, $CDCl_3$): 0.69 (3H, t, *J* 7.6 Hz, C*H*$_3$-CH$_2$), 1.28 (2H, m, CH$_3$-C*H*$_2$), 2.40-2.53 (10H, m, 5 x CH$_2$), 2.87 (1H, t, *J* 6.4 Hz, H$_A$); δ_C (100 MHz, $CDCl_3$): 10.0 (CH_3), 25.2 (CH_2), 41.5 (CH_2), 42.0 (CH_2), 49.5 (CH_2), 52.4 (CH_2), 52.5 (CH_2), 60.1 (CH); LRMS (CI$^+$) 175.2 ([M+H]$^+$, 100%), 160.2 (5), 142.2 (26), 132.2 (16); HRMS calcd for $C_8H_{22}N_4$ 175.1923, found 175.1920.

7.2.3 Biological Materials and Methods for Chapter 3

7.2.3.1 Trypanosome Cell Line

Trypanosoma brucei brucei 427

T. b. brucei strain EATRO (East African Trypanosomiasis Research Organisation) 427 was used routinely for uptake assays. These stocks were supplied by Dr M. P. Barrett, University of Glasgow.

T. b. brucei *427 Δ*Tbat1 *Mutant Clone*

The *ΔTbat1* (P2) null mutant clone was constructed by sequential replacement of *TbAT1* with the neomycin and puromycin resistance markers in *T. b. brucei* 427. This knockout cell line was four-fold less sensitive to melarsoprol and melarsen oxide. These stocks were originally supplied by E. Matovu and are held at the University of Glasgow.

7.2.3.2 Growth and Maintenance of Bloodstream-Form Trypanosome Stocks

In vitro *culture*

Cultures were incubated in sterile culture flasks at 37 ^{o}C and 5% CO_2 in HMI-9 medium containing 20% heat-inactivated foetal calf serum. 5 mL cultures were seeded with 200 μL of culture at 2 x 10^6 cells/mL. Cells were allowed to grow for 3 d after which they had reached maximum density (approximately 2 x 10^6 cells/mL) and were passaged again in the same way.

In vivo *culture*

Trypanosomes were stored in capillary tubes, in liquid nitrogen storage facilities, in blood containing 10.5% DMSO. Wistar rats or ICR mice (depending on the number of trypanosomes required) were infected by intraperitoneal injection of the parasite stabilates. Parasitaemias were checked daily by examination of a tail blood smear under phase contrast. Parasitaemia was scored using the rapid matching method. When the parasitaemia reached 1 x 10^9 cells/mL the animal was sacrificed using CO_2 and the blood collected by cardiac puncture in a syringe containing 100 U/mL heparin in CBSS.

7.2.3.3 Trypanotoxicity

In vitro *Toxicity Assay (Alamar Blue Assay)*

The Alamar Blue assay was used to assess toxicity *in vitro*.[274] Doubling serial dilutions of test compound were set out in a 96 well plate in duplicate, in a volume of 100 μL HMI-9 medium.[275] 100 μL of trypanosomes at 2 x 10^5 cells/mL were added to each well. After 48 h incubation at 37 ^{o}C and 5% CO_2, 10% Alamar Blue (20 μL) was added to each well and the plates incubated for a further 24 h. Unspecified enzymes in the live cells reduce the Alamar Blue to the colourless form. The concomitant change in absorption at 600 nm can be measured spectrophotometrically, or fluorometric measurements can be taken at 530 nm excitation and 590 nm emission as the amount of fluorescence detectable at these wavelengths increases in the reduced dye. All measurements were taken fluorometrically. The IC_{50} value was calculated by analysing the data with the IC_{50} value algorithm of the Grafit 4.0 (Erithracus Software) or using Prism 3.0 (GraphPad, San Diego, CA, USA). Values were checked for accuracy by visually examining cell viability and motility. Each experiment was performed in duplicate and replicated at least once.

In the investigation of polyamine effect on toxicity, the drug compound was dissolved in a 0.1 M solution of putrescine, spermidine or spermine in HMI-9 before serial dilutions were made.

In vivo *Toxicity Assay*

Female NMRI mice weighting 22-25 g were infected with cryopreserved stabilates of *T. b. brucei* STIB 795 (derivate of strain 427). Each mouse was infected intraperitoneally with (2-4) x 10^4 bloodstream forms. Melarsoprol (arsobal; Aventis) acted as standard drug and was diluted with sterile distilled water to an appropriate concentration. Groups of four mice were treated on days three, four, five and six intraperitoneally with 20 mg/kg of **91**. A control group remained untreated. The parasitaemia of all animals were checked on day seven post-infection and every second day until visible, at which point, mice were euthanased.

7.2.3.4 Human Embryonic Kidney Cells

Human Embryonic Kidney Cells (HEK), strain 293T, were used as the mammalian cell line for comparative analyses. HEK cells were cultured in Dulbecco's Modified Eagle's Medium (Sigma) with penicillin/streptomycin (10 mg/mL), L-Glutamax (200 nM), and 10% newborn calf serum in vented culture flasks at 37 ^oC in 5-10% CO_2 atmosphere, passaging when cells on the monolayer are 80-85% confluent. The Alamar Blue assay protocol was modified from the one used for live trypanosomes; 100 μL of a 3 x 10^5 cells/mL suspension was added to each well of a 96 well plate and incubated at 37 ^oC for 3 h to allow cells to adhere to the bottom of the wells. Preparation of drug stocks in doubling dilution was added after the incubation period, incubated for a further 12-16 h before the addition of 10% Alamar Blue (20 μL). After 24 h the plates were read fluorometrically and visually as above.

7.2.3.5 Fluorescence Assays

Wet Slides

A culture of the cells in HMI-9 medium was centrifuged and the excess medium poured off. 5.0 mL of CBSS solution was used to re-suspend the cells before centrifuging and pouring off excess CBSS. This process was repeated twice to wash the cells and remove soluble blood components. 2.0 μL of 10.0 mM fluorescent drug compounds was added to 0.5 mL cells in CBSS solution in a 0.5 mL vial. After five min the vial was centrifuged, the excess solution removed, and 0. 2 μL solution removed to a glass slide and a cover slip

added. This was viewed directly through the Zeiss Axioscope FL fluorescence microscope using a Zeiss 02 filter at an excitation wavelength of 330 nm and an emission wavelength of 400 nm. Images were obtained by the same method but using an Axiovert 200M Fluorescence microscope. This technique was repeated to cover a 5-90 min time period

Blood Smear

Blood was collected as previously described from ICR mice presenting peak parasitaemias. 0.5 μL of 10.0 mM fluorescent drug compounds was added to 500 μL of whole blood at rt to give a starting concentration of 10.0 μM. This was thoroughly mixed and approximately 2.0 μL removed to a glass slide and covered to produce a wet blood smear that was viewed over a 5-90 min time period. This was viewed, and images obtained directly as before.

7.2.3.6 Malarial Assays

The parasitaemia of a *Plasmodium falciparum* (3D7 strain) culture was determined by counting Giemsa stained blood smears and diluted to 1% parasitaemia in 2% haematocrit. The compounds tested were diluted to 400 μM in medium and filter sterilised. 96 well plates were prepared by pipetting 100 μL medium (RPMI 1640) into each well. To the first well 100 μL of drug was added and doubling dilutions were carried out across the plate. As a control no drug was added to the last well. Into each well, 100 μL culture was added. The plates were incubated at 37 ^{o}C in a sealed culture chamber gassed with 5% CO_2, 1% O_2 and 94% N_2. After 24 h, 100 μL media was removed and 100 μL fresh medium containing 0.5 μCi/well [^{3}H]-labelled hypoxanthine (Amersham, 37 MBq/mL (1mCi/mL) radioactive concentration, 925 GBq/mmol (25 Ci/mmol) specific activity) was added. The plates were incubated as before 24 h. Afterwards the plates were frozen at -20 ^{o}C until harvested.

After thawing at rt, the cells were harvested onto a filter mat (Wallac) using a Tomec cell harvester. The filter mats were dried and sealed in sample bags with Wallac Betaplate Scint scintillation liquid (Perkin Ellmer). The mats were counted using a Wallac Trilux 1450 microbeta liquid scintillation and luminescence counter.

7.2.3.7 Enzyme Assays

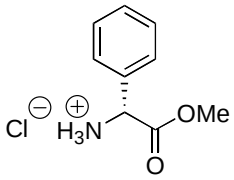
Recombitant *Leishmania major* oligopeptidase B (5.0 ng) was incubated in 100 mM Tris (pH 8) with varying concentrations of polyamines (**72**, **88**, **90**, **91** and **131**) for 10 mins prior to the addition of the substrate, z-RR-amc (Bachem, Nottingham, UK), at a saturating

concentration (10 μM). Activity was determined by the change in fluorescence (λ_{ex} = 355 nm, λ_{ex} = 460 nm) at 21 ^{o}C using an EnVision 2102 plate reader (Perkin Elmer, Bucks, UK). The relative activity was calculated from the ratio of initial velocity in the presence of inhibitor to that of uninhibited controls and IC_{50} values were calculated using FigP (Fig.P Software Corporation, Hamilton, Canada).

7.3 Experimental to Chapter 4

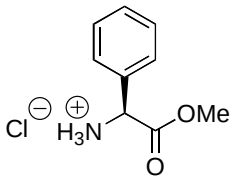
7.3.1 Experimental Details

Methyl (*R*)-phenylglycinatehydrochloride (160)



The hydrochloride salt was obtained by forming a suspension of (*R*)-phenylglycine (9.6 g, 64 mmol) in dry MeOH (50 mL), and conc HCl (116 mmol, 10 mL) was added. The mixture was then stirred and heated at reflux for 8 h. The solution was concentrated *in vacuo* to leave a white powder (12.8 g, 100%); mp: 228-230 ^{o}C (lit.[180] 202 ^{o}C); δ_H (400 MHz, $CDCl_3$): 3.74 (3H, s, OCH_3), 5.22 (1H, s, CH), 7.38-7.48 (5H, m, 5 x Ar-H); δ_C (100 MHz, $CDCl_3$): 54.3 (CH_3), 56.7 (CH), 128.4 (2 x ArCH), 130.1 (2 x ArCH), 130.82 (ArCH), 139.1 (ArC), 170.0 (C=O); LRMS (CI^+): 349.3 ($[M+H]^+$, 100%), 347.3 (7), 287.3 (44), 271.2 (8), 243.2 (6), 216.2 (10); HRMS calcd for $C_{16}H_{36}O_4N_4$ 349.2815, found 349.2815.

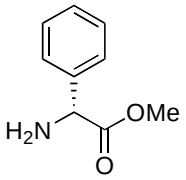
Methyl (*S*)-phenylglycinatehydrochloride (161)



The hydrochloride salt was obtained by forming a suspension of (*S*)-phenylglycine (10 g, 66 mmol) in dry MeOH (50 mL), and conc HCl (166 mmol, 10 mL) was added. The mixture was then stirred and heated at reflux for 8 h. The solution was concentrated *in vacuo* to leave a white powder (10.3 g, 100%); mp: 214-216 ^{o}C (lit.[180] 202 ^{o}C); δ_H (400 MHz, $CDCl_3$): 3.76 (3H, s, OCH_3), 5.22 (1H, s, CH), 7.39-7.47 (5H, m, 5 x Ar-H); δ_C (100

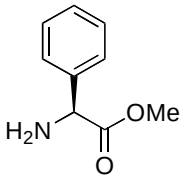
MHz, $CDCl_3$): 54.3 (CH_3), 56.7 (CH), 128.4 (2 x ArCH), 130.1 (2 x ArCH), 130.82 (ArCH), 139.7 (ArC), 170.0 (C=O).

Methyl (*R*)-phenylglycinate (162)



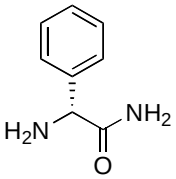
Liquid NH_3 was bubbled through a suspension of hydrochloride **160** (12.90 g, 64 mmol), in $CHCl_3$ (55 mL) for 10 min. The precipitated ammonium chloride was filtered off, and washed with $CHCl_3$ (10 mL). The organic solution was concentrated *in vacuo* to give **162** as a pale yellow oil (8.84 g, 84%).$[\alpha]_D^{21}$ -23.2 (*c* 1.0, $CHCl_3$) (lit.[178] $[\alpha]_D$ -180 (c 2.0, C_6H_6); ν_{max} (neat)/cm^{-1}: 3028 (Ar-H), 1604 (NH_2), 1221 (COO), 1168 (COO) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.94 (3H, s, OCH_3), 4.61 (1H, s, CH), 7.27-7.39 (5H, m, 5 x Ar-H); δ_C (100 MHz, $CDCl_3$): 52.8 (CH_3), 59.1 (CH), 127.2 (2 x ArCH), 128.4 (ArCH), 129.4 (2 x ArCH), 140.7 (ArC), 174.9 (C=O); LRMS (CI^+): 166.1 ($[M+H]^+$, 100%), 149.1 (5), 106.1 (6); HRMS calcd for $C_9H_{11}O_2N$ 166.0868, found 166.0867.

Methyl (*S*)-phenylglycinate (163)



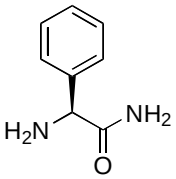
Liquid NH_3 was bubbled through a suspension of hydrochloride **161** (10.28 g, 51 mmol), in $CHCl_3$ (40 mL) for 10 min. The precipitated NH_4Cl was filtered off, and washed with $CHCl_3$ (10 mL). The organic solution was concentrated *in vacuo* to give **163** as a pale yellow oil (7.94 g, 94%). $[\alpha]_D^{21}$ 23.4 (*c* 1.0, $CHCl_3$) (lit.[178] $[\alpha]_D$ 170 (c 2.0, C_6H_6); ν_{max} (neat)/cm^{-1}: 3031 (Ar-H), 1599 (NH_2), 1220 (COO), 1168 (COO) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.94 (3H, s, OCH_3), 4.62 (1H, s, CH), 7.29-7.40 (5H, m, 5 x Ar-H); δ_C (100 MHz, $CDCl_3$): 52.8 (CH_3), 59.2 (CH), 127.2 (2 x ArCH), 128.4 (ArCH), 129.2 (2 x ArCH), 140.7 (ArC), 174.9 (C=O); LRMS (CI^+): 166.1 ($[M+H]^+$, 100%), 149.1 (5), 106.1 (7); HRMS calcd for $C_9H_{11}O_2N$ 166.0868, found 166.0865.

(*R*)-Phenylglycinamide (164)



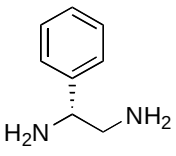
A solution of **162** (8.843 g, 54 mmol) in conc aq NH_3 (7 mL, 35%) was stirred at rt for 2 d, before evaporating to dryness to give **164** as a pale yellow solid (7.49 g, 92%). mp: 119-123 ^oC (lit.[178] 130-136 ^oC); ν_{max} (neat)/cm^{-1}: 3370 ($CONH_2$), 3338 ($CONH_2$), 3054 (Ar-H), 1604 (NH_2); δ_H (400 MHz, $CDCl_3$): 4.47 (1H, s, CH), 5.57 (br s, NH_2), 6.77 (br s, NH_2), 7.22-7.36 (5H, m, 5 x Ar-H); δ_C (100 MHz, $CDCl_3$): 60.3 (CH), 127.3 (2 x ArCH), 128.6 (ArCH), 129.3 (2 x ArCH), 141.2 (ArC), 176.2 (C=O); LRMS (CI^+): 151.1 ($[M+H]^+$, 100%), 134.12 (4), 106.13 (11); HRMS calcd for $C_8H_{10}ON_2$ 151.0871, found 151.0872.

(*S*)-Phenylglycinamide (165)



A solution of **163** (7.94 g, 48 mmol) in conc aq NH_3 (7.94 mL, 35%) was stirred at rt for 2 d, before evaporating to dryness to give **165** as a pale yellow solid (5.85 g, 81%). mp: 111-114 ^oC (lit.[178] 130-136 ^oC); ν_{max} (neat)/cm^{-1}: 3370 ($CONH_2$), 3339 ($CONH_2$), 3053 (Ar-H), 1604 (NH_2) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 4.46 (1H, s, CH), 5.71 (br s, NH_2), 6.83 (br s, NH_2), 7.22-7.36 (5H, m, 5 x Ar-H); δ_C (100 MHz, $CDCl_3$): 60.3 (CH), 127.3 (2 x ArCH), 128.6 (ArCH), 129.3 (2 x ArCH), 141.1 (ArC), 176.3 (C=O); LRMS (CI^+): 151.1 ($[M+H]^+$, 100%), 134.1 (3), 106.1 (11); HRMS calcd for $C_8H_{10}ON_2$ 151.0871, found 151.0871.

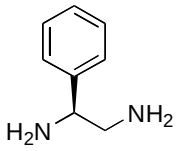
(*R*)-1-Phenyl-ethylenediamine (158)



To a suspension of $LiAlH_4$ (18.94 g, 499 mmol) in THF (300 mL), was added **164** (7.49 g, 49.9 mmol) portionwise with stirring and cooling with ice. The mixture was then stirred and heated at reflux for 5 d. With ice cooling, sodium sulfate decahydrate and Et_2O (300 mL) were added, and the solution was filtered through Celite® and rinsed with Et_2O (100

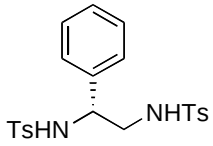
mL). Organics were combined and dried over $MgSO_4$, then concentrated *in vacuo* to give **158** as a yellow oil (2.11 g, 35%); $[\alpha]_D^{24}$ -3.1 (*c* 1.0, $CHCl_3$) (lit.[178] $[\alpha]_D$ -29.7); δ_H (400 MHz, $CDCl_3$): 2.72 (1H, dd, *J* 12.8, 6.8 Hz, CH), 2.81 (1H, dd, *J* 12.8, 5.2 Hz, CH), 3.79-3.82 (1H, m, CH), 7.16-7.28 (5H, m, 5 x Ar-H); δ_C (100 MHz, $CDCl_3$): 48.9 (NCH_2), 57.3 (NCH), 125.5 (2 x ArCH), 126.2 (2 x ArCH), 127.5 (ArCH), 142.0 (ArC).

(*S*)-1-Phenyl-ethylenediamine (159)



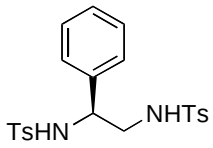
159 was prepared in the same way as (*R*)-isomer **158** starting with **165** (4.76 g, 31.7 mmol) to give a yellow oil (389 mg, 9%). $[\alpha]_D^{24}$ 11.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 2.75 (1H, dd, *J* 12.8, 6.8 Hz, CH), 2.85 (1H, dd, *J* 12.8, 5.2 Hz, CH), 3.82-3.85 (1H, m, CH), 7.12-7.30 (5H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 49.1 (NCH_2), 57.5 (NCH), 125.7 (2 x ArCH), 126.4 (2 x ArCH) 127.7 (ArCH), 142.2 (ArC).

1,2-Bis(toluol-4-sulfonylamino)-phenyl-ethamine (*R*) (166)



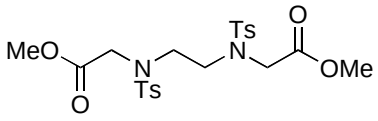
NaOH (1.24 g, 31.0 mmol), was added slowly to an ice-cooled solution of **158** (2.11 g, 15.5 mmol), in H_2O (10 mL). Et_2O (10 mL), then *p*-toluenesulfonyl chloride (5.9 g, 31.0 mmol) were added and reaction mixture stirred vigorously for 1 h at this temp. The solid formed was filtered and rinsed with Et_2O, then dried *in vacuo*. **166** was obtained as white crystals (4.18 g, 61%). mp: 138-142 ^oC (lit.[276] 160-161 ^oC); $[\alpha]_D^{21}$ -21.2 (*c* 0.5, CH_2Cl_2); δ_H (400 MHz, $CDCl_3$): 2.30 (3H, s, CH_3), 2.34 (3H, s, CH_3), 3.04-3.16 (2H, m, CH_2), 4.25 (1H, br d, *J* 5.0 Hz, CH), 4.92 (1H, br s, NH), 5.49 (1H, br d, *J* 5.5 Hz, NH), 6.89-6.91 (2H, m, 2 x Ar-H), 7.06-7.11 (5H, m, 5 x Ar-H), 7.20 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.50 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.60 (2H, d, *J* 8.2 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 21.9 (CH_3), 21.9 (CH_3), 48.6 (CH_2), 57.7 (CH), 127.0 (2 x ArCH), 127.5 (2 x ArCH), 127.6 (2 x ArCH), 128.6 (ArCH), 129.2 (2 x ArCH), 130.0 (2 x ArCH), 130.2 (2 x ArCH), 136.9 (ArC), 137.18 (ArC), 137.9 (ArC), 143.9 (ArC), 144.1 (ArC); LRMS (CI^+): 445.1 ($[M+H]^+$, 100%), 274.1 (69), 260.1 (35), 157.1 (39), 106.1 (27); HRMS calcd for $C_{22}H_{24}O_4N_2S_2$ 445.1256, found 445.1258.

1,2-Bis(toluol-4-sulfonylamino)-phenyl-ethamine (*S*) (167)



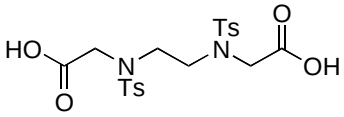
NaOH (237 mg, 5.92 mmol), was added slowly to an ice-cooled solution of **159** (403 mg, 2.96 mmol), in H_2O (1.85 mL). Et_2O (1.85 mL), then *p*-toluenesulfonyl chloride (1.13 g, 5.92 mmol) were added and reaction mixture stirred vigorously for 1 h at this temp. The solid formed was filtered and rinsed with Et_2O, then dried *in vacuo*. **167** was obtained as white crystals (461 mg, 35%). mp: 138-142 ^oC (lit.[276] 160-161 ^oC); $[\alpha]_D^{21}$ 19.0 (*c* 0.5, CH_2Cl_2); δ_H (400 MHz, $CDCl_3$): 2.31 (3H, s, CH_3), 2.35 (3H, s, CH_3), 3.06-3.12 (2H, m, CH_2), 4.24 (1H, br d, *J* 5.0 Hz, CH), 4.81 (1H, br s, NH), 5.37 (1H, br d, *J* 5.5 Hz, NH), 6.89-6.91 (2H, m, 2 x Ar-H), 7.09-7.11 (5H, m, 5 x Ar-H), 7.21 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.51 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.61 (2H, d, *J* 8.4 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 21.9 (CH_3), 21.9 (CH_3), 48.6 (CH_2), 57.6 (CH), 127.0 (2 x ArCH), 127.5 (2 x ArCH), 127.6 (2 x ArCH), 128.6 (ArCH), 129.2 (2 x ArCH), 129.2 (2 x ArCH), 130.2 (2 x ArCH), 136.9 (ArC), 137.2 (ArC), 137.9 (ArC), 143.99 (ArC), 144.19 (ArC).

***N,N'*-Ditosyl-ethylenediamine-*N,N*'-diacetic acid-dimethyl ester (173)**



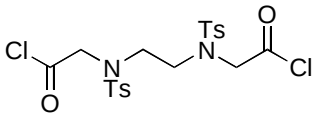
In a 3-necked flask was dissolved Na (250.6 mg, 10.9 mmol) in MeOH (14 mL) and *N,N'*-ditosyl-ethylenediamine **45** added (2.0 g, 5.4 mmol). The mixture was then stirred and heated at reflux for 2 h. The MeOH was then distilled, and the remaining residue dried *in vacuo*. The powder was then added portionwise over 30 min to a stirring, ice-cooled flask of methylbromoacetate (4.11 mL, 43.4 mmol). After stirring at rt for 30 min, the excess methylbromoacetate was removed *in vacuo*. A mixture of H_2O (10 mL) and $CHCl_3$ (10 mL) was added to the residue. The $CHCl_3$ layer was washed with H_2O (2 x 5 mL) before drying over $CaCl_2$. The solution was then filtered and reduced *in vacuo*. The product was then crystallised from MeOH (1.14 g, 41%). mp: 108-112 ^oC (lit.[165] 124.3-126.8 ^oC); δ_H (400 MHz, $CDCl_3$): 2.36 (6H, s, 2 x CH_3), 3.14 (4H, s, 2 x CH_2), 3.56 (6H, s, 2 x OCH_3), 4.01 (4H, s, 2 x CH_2), 7.24 (4H, d, *J* 8.0 Hz, 4 x Ar-H), 7.63 (4H, d, *J* 8.0 Hz, 4 x Ar-H); δ_C (100 MHz, $CDCl_3$): 21.6 (2 x CH_3), 48.6 (2 x CH_2), 50.1 (2 x CH_2), 52.2 (CH_3), 127.5 (4 x ARCH), 129.7 (4 x ArCH), 135.7 (2 x ArC), 143.9 (2 x ArC), 169.3 (2 x C=O).

N,N'-Ditosyl-ethylenediamine-*N,N*'-diacetic acid (174)



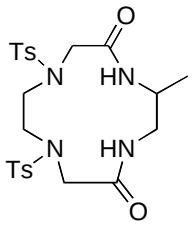
A mixture of **173** (1.1g, 2.15 mmol), AcOH (5.16 mL) and conc HCl (1.29 mL) was stirred and heated under reflux for 12 h. The resulting white crystals were filtered and rinsed with acetic acid (2 x 2 mL) before drying *in vacuo* (574 mg, 55%). mp: 206-208 ^{o}C (lit.[165] 228.9-229.8 ^{o}C); δ_H (400 MHz, DMSO-d_6): 2.42 (6H, s, 2 x CH_3), 3.27 (4H, s, 2 x CH_2), 3.98 (4H, s, 2 x CH_2), 7.41 (4H, d, *J* 8.2 Hz, 4 x Ar-H), 7.66 (4H, d, *J* 8.2 Hz, 4 x Ar-H); δ_C (100 MHz, DMSO-d_6): 21.0 (2 x CH_3), 47.0 (2 x CH_2), 49.3 (2 x CH_2), 126.9 (4 x ArCH), 129.7 (4 x ArCH), 136.3 (2 x ArC), 143.3 (2 x ArC), 170.4 (2 x C=O).

N,N'-Bis-(toluene-4-sulfonyl)-*N,N'*-ethanediyl-bis-glycyl chloride (169)



174 (531 mg, 1.1 mmol) and $SOCl_2$ (5.6 mL, 76.8 mmol) were stirred and heated under reflux for 5 h, heating no higher than 80 ^{o}C. After HCl production had ceased, excess thionyl chloride was removed *in vacuo*. The pale brown solid **169** was crystallised from benzene (536.3 mg, 94%); mp: 134-136 ^{o}C (lit.[165] 223-224 ^{o}C); ν_{max} (neat)/cm^{-1}: 1799 (COCl), 1345 (SO_2N), 1159 (SO_2N); δ_H (400 MHz, DMSO-d_6): 2.47 (6H, s, 2 x CH_3), 3.31 (4H, s, 2 x CH_2), 4.04 (4H, s, 2 x CH_2), 7.46 (4H, d, *J* 8.4 Hz, 4 x Ar-H), 7.72 (4H, d, *J* 8.4 Hz, 4 x Ar-H); δ_C (100 MHz, DMSO-d_6): 21.0 (2 x CH_3), 49.3 (2 x CH_2), 126.9 (4 x ArCH), 129.7 (4 x ArCH), 136.3 (2 x ArC), 143.3 (2 x ArC), 170.4 (2 x C=O).

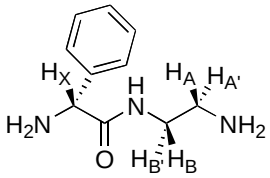
11-Methyl-4,7-bis-(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane-2,9-dione (176)



In a dry flask was added dry CH_2Cl_2 (27 mL) and NEt_3 (0.053 mL, 0.38 mmol). To this was added simultaneously *via* two separate syringe pumps, **169** (100 mg, 0.19 mmol) in CH_2Cl_2 (7 mL), and 1,2-diaminopropane (0.016 mL, 0.19 mmol) in CH_2Cl_2 (7 mL), over 7

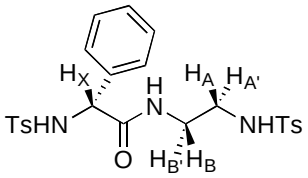
h while stirring at rt. After a further 30 min stirring at rt, the mixture was reduced under pressure to yield **176** as a white solid (163.0 mg, 100%); ν_{max} (neat)/cm^{-1}: 3256 (C(O)N), 1346 (SO_2N), 1160 (SO_2N);LRMS (CI$^+$): 523.1 ([M+H]$^+$, 3%), 157.1 (45), 85.1 (100); HRMS calc for $C_{23}H_{31}O_6N_4S_2$, 523.1685, found 523.1682.

2-Amino-*N*-(2-aminoethyl)-2-phenyl acetamide (179)



160 (12.9 g, 64 mmol) was dissolved in neat ethylenediamine (30 mL) at 0-5 ^oC, and reaction mixture stirred at rt for 4 d. After this time the excess ethylenediamine was removed *in vacuo*. The residue was taken up in $CHCl_3$ (250 mL) and precipitate filtered off. The organic solution was concentrated under reduced pressure to give a dark brown viscous oil (10.43 g, 84%); $[\alpha]_D^{21}$ -47.4 (*c* 0.5, CH_2Cl_2); ν_{max} (neat)/cm^{-1}: 3286 (NH), 3028 (NH), 2925 (NH), 2862 (NH), 1519 (CO-N); NMR spectroscopy agreed with lit. values;[277] δ_H (400 MHz, D_2O): 2.53 (1H, d, *J* 6.4 Hz, CH_A), 2.55 (1H, d, *J* 6.4 Hz, $CH_{A'}$), 3.10 (1H, d, *J* 6.4 Hz, CH_B), 3.12 (1H, d, *J* 6.4 Hz, $CH_{B'}$), 4.40 (1H, s, CH_X), 7.23-7.32 (5H, m, 5 x Ar-H); δ_C (100 MHz, D_2O): 39.7 (CH_2), 41.7 (CH_2), 58.8 (CH), 126.6 (2 x ArCH), 128.2 (ArCH), 129.6 (2 x ArCH), 140.1 (ArC), 176.0 (C=O); LRMS (CI$^+$): 194.1 ([M+H]$^+$, 100%), 176.1 (25), 106.1 (17); HRMS calcd for $C_{10}H_{15}N_3O$ 194.1293, found 194.1295.

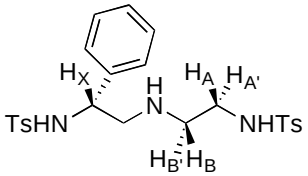
2-Phenyl-2-(toluene-4-sulfonylamino)-*N*-[2-(toluene-4-sulfonylamino)-ethyl]-acetamide (180)



To a solution of *p*-toluenesulfonyl chloride (670 mg, 3.5 mmol) in dry pyridine (11 mL) at 0 ^oC, was added drop-wise a solution of **179** in dry pyridine (2 mL). The solution was then stirred at 0 ^oC for 3 h, then at rt for 3 d. Solution added to ice and H_2O, and left overnight to allow precipitation of product as a yellow solid (645 mg, 96%). mp: 138-139 ^oC; $[\alpha]_D^{20}$ -3.4 (*c* 1.0, CH_2Cl_2); ν_{max} (neat)/cm^{-1}: 2359 (NH), 2341 (NH), 1541 (CO-N), 1353 (SO_2-N), 1160 (SO_2-N); δ_H (400 MHz, $CDCl_3$): 2.31 (3H, s, CH_3), 2.35 (3H, s, CH_3), 2.93-2.96 (2H, m, CH_2), 3.19-3.31 (2H, m, CH_2), 4.71 (1H, d, *J* 6.0 Hz, NH), 5.51-5.52 (1H, m, NH), 6.10

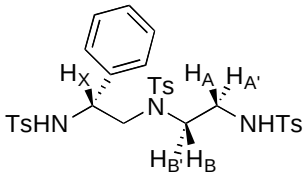
(1H, dd, *J* 8.8, 6.0 Hz, NH), 7.01 (2H, m, 2 x Ar-H), 7.08-7.16 (5H, m, 5 x Ar-H), 7.19-7.22 (2H, m, 2 x Ar-H), 7.56 (2H, d, *J* 7.2 Hz, 2 x Ar-H), 7.63 (2H, d, *J* 8.4 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 20.5 (2 x CH_3), 38.8 (CH_2), 41.4 (CH_2), 60.0 (CH), 126.0 (2 x ArCH), 126.3 (2 x ArCH), 126.5 (2 x ArCH), 127.6 (ArCH), 127.9 (2 x ArCH), 128.6 (2 x ArCH), 128.8 (2 x ArCH), 134.9 (ArC), 135.0 (ArC), 135.7 (ArC), 142.5 (ArC), 142.8 (ArC), 169.2 (C=O); LRMS (FAB^+): 502.2 ($[M+H]^+$, 94%), 369.2 (18), 331.2 (37), 303.2 (33), 260.1 (47), 215.1 (18), 147.1 (22), 106.3 (41), 73.7 (100); HRMS calcd for $C_{24}H_{27}N_3O_5S_2$ 502.1470, found 502.1474.

(2*R*)-2-Phenyl-2-(toluenesulfonylamino)-*N*-[2-(toluenesulfonylamino)ethyl]-aminoethane (181)



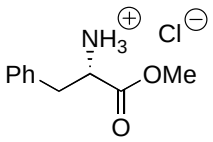
To a cooled solution of **180** (5.16 g, 10.3 mmol) suspended in THF (20 mL), was added BH_3.THF (1M) (50 mL, 52.0 mmol) slowly. The mixture was then stirred and heated at reflux for 7 d. The excess borane was quenched by careful addition of MeOH (20 mL). The reaction mixture was then concentrated under reduced pressure. Dry EtOH (40 mL) was then added, and solution saturated with HCl gas at 0 ^oC. The mixture was then heated at reflux overnight, after which the solution was cooled in an ice bath. The resulting yellow solid was filtered, washed with Et_2O (100 mL) and dried *in vacuo* (2.41 g, 48%). ν_{max} (neat)/cm^{-1} 3059 (ArH), 2361 (NH), 2337 (NH), 1323 (SO_2-N), 1156 (SO_2-N); δ_H (400 MHz, CD_3OD): 2.34 (3H, s, CH_3), 2.46 (3H, s, CH_3), 3.20-3.22 (2H, m, 2 x CH), 3.33-3.42 (4H, m, 4 x CH), 4.68-4.71 (1H, m, H_X), 7.02 (2H, d, *J* 8.2 Hz, 2 x Ar-H), 7.14-7.21 (5H, m, 5 x Ar-H), 7.44 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.54 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.80 (2H, d, *J* 8.0 Hz, 2 x Ar-H); δ_C (100 MHz, CD_3OD): 21.4 (CH_3), 21.5 (CH_3), 39.9 (CH_2), 49.2 (CH_2), 52.9 (CH_2), 56.2 (CH), 128.0 (4 x ArCH), 128.3 (4 x ArCH), 129.6 (ArCH), 129.9 (2 x ArCH), 130.0 (2 x ArCH), 131.0 (2 x ArCH), 137.2 (ArC), 137.5 (ArC), 138.7 (ArC), 144.9 (ArC), 145.4 (ArC); LRMS (FAB): 488.1 ($[M+H]^+$, 90%), 317.2 (7), 227.3 (9), 75.1 (100); HRMS calcd for $C_{24}H_{30}O_4N_3S_2$ 488.1678, found 488.1674.

(2*R*)-*N*-[2-[(Toluenesulfonylamino)ethyl]-*N*-[2-(toluenesulfonyl)amino]-2-phenyl-ethyl-toluenesulfonamide (182)



To a solution of *p*-toluenesulfonyl chloride (1.13 g, 5.9 mmol), in dry pyridine (10 mL), at 0 ^{o}C, was added a solution of **181** (2.41 g, 4.9 mmol) in dry pyridine (20 mL) dropwise. The solution was then stirred at this temp for 3 h, then at rt for 2 d. The resulting yellow solution was added to a mixture of ice and H_2O, and left for 48 h to allow precipitation of a yellow product. Filtration followed by drying *in vacuo* yielded **182** as pale yellow fluffy crystals (2.83 g, 90%). mp: 70-73 ^{o}C; $[\alpha]_D^{24}$ -5.3 (*c* 1.0, $CHCl_3$); ν_{max} (neat)/cm^{-1}: 3461 (N-H), 3022 (C-H), 2724 (N-CH_2), 1153 (N-SO_2); δ_H (400 MHz, CD_3OD): 2.34 (3H, s, CH_3), 2.42 (3H, s, CH_3), 2.43 (3H, s, CH_3), 2.46-2.51 (1H, m, CH), 2.60-2.67 (1H, m, CH), 2.85-3.00 (2H, m, 2 x CH), 3.10 (1H, dd, *J* 14.8, 7.6 Hz, CH_B), 4.51 (1H, t, *J* 7.6 Hz, CH_A), 7.02-7.05 (2H, m, 2 x Ar-H), 7.11-7.12 (3H, m, 3 x Ar-H), 7.17 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.33 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.37 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.57 (4H, d, *J* 8.0 Hz, 4 x Ar-H), 7.66 (2 H, d, *J* 8.0 Hz, 2 x Ar-H); δ_C (100 MHz, CD_3OD): 21.4 (CH_3), 21.5 (CH_3), 21.6 (CH_3), 42.2 (CH_2), 50.8 (CH_2), 55.9 (CH_2), 58.2 (CH) 124.0 (ArCH), 127.0 (ArCH), 127.1 (ArCH), 127.3 (ArCH), 127.4 (ArCH), 127.6 (ArCH), 127.8 (ArCH), 128.6 (ArCH), 129.1 (ArCH), 129.2 (ArCH), 129.4 (ArCH), 129.8 (ArCH), 130.0 (ArCH), 134.9 (ArC), 136.4 (ArCH), 136.8 (ArC), 137.0 (ArC), 138.1 (ArC), 143.2 (ArC), 143.5 (ArC), 144.1 (ArC), 149.5 (ArCH), 2 missing; LRMS (FAB): 642.3 ($[M+H]^+$, 75%), 471.3 (51), 381.2 (41), 316.2 (100), 260.1 (20), 227.1 (25), 154.1 (48); HRMS calcd for $C_{31}H_{36}O_6N_3S_3$ 642.1766, found 642.1765.

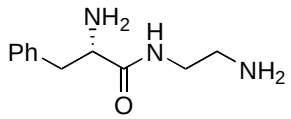
L-Phenylalanine methyl ester hydrochloride (185)



Conc HCl (5 mL) was added to a solution of *L*-phenyl alanine (5 g, 30 mmol) in MeOH (25 mL) and mixture stirred at rt for 19 h. Excess reagents and solvent were then removed under vacuum to give **185** as a pure white solid (6.07 g, 93%). mp: 213-216 ^{o}C; $[\alpha]_D^{24}$ 12.5 (*c* 1.0, H_2O); NMR spectroscopy agreed with lit. values;[278] δ_H (400 MHz, D_2O): 3.06 (1H, dd, *J* 14.8, 7.6 Hz, CH), 3.20 (1H, dd, *J* 14.8, 5.6 Hz, CH), 3.69 (3H, s, CH_3), 4.18 (1H, dd,

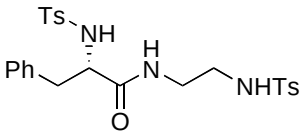
J 7.6, 5.6 Hz, C(NH_3)*H*), 7.13-7.30 (5H, m, 5 x ArH); δ_C (100 MHz, D_2O): 35.6 (CH_2), 53.4 (CH), 54.2 (CH_3), 128.0 (ArCH), 129.2 (2 x ArCH), 129.4 (2 x ArCH), 134.0 (ArC), 171.5 (C=O); LRMS (CI^+): 180.23 (M, 71%), 166.18 (100), 120.16 (20), 71.1 (37); HRMS calcd for $C_{10}H_{14}NO_2$ 180.1025, found 180.1026.

N'-(2-Aminoethyl)-*L*-phenylalaninamide (186)



185 (6.0 g, 28 mmol) was dissolved in neat 1,2-diaminoethane (10 mL) at 0 °C. The mixture was then stirred at rt for 4 d. Excess 1,2-diaminoethane was then removed under reduced pressure. The resultant solid was dissolved in $CHCl_3$ (200 mL) and filtered. The filtrate was concentrated *in vacuo* to give a dark yellow oil (1.58 g, 27%). $[\alpha]_D^{24}$ 21.9 (*c* 1.0, H_2O); NMR spectroscopy agreed with lit. values;[279] δ_H (400 MHz, D_2O): 2.30 (2H, dd, *J* 9.6, 6.0 Hz, CH_2), 2.67 (1H, dd, *J* 13.2, 8.8 Hz, CH), 2.85 (2H, dd, *J* 13.6, 6.0 Hz, CH_2), 3.05 (1H, dt, *J* 13.6, 6.0 Hz, C(NH_2)*H*), 3.44 (1H, dd, *J* 8.8, 6.0 Hz, CH), 7.08-7.25 (5H, m, 5 x Ar-H); δ_C (100 MHz, D_2O): 39.6 (CH_2), 40.8 (CH_2), 41.6 (CH_2), 56.6 (CH), 127.0 (ArCH), 128.7 (2 x ArCH), 129.3 (2 x ArCH), 137.1 (ArC), 176.6 (C=O); LRMS (CI^+): 208.3 ($[M+H]^+$, 100%), 139.2 (13), 101.2 (46); HRMS calcd for $C_{11}H_{17}N_3O$ 208.1450, found 208.1447.

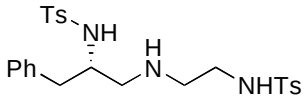
3-Phenyl-2-(toluene-4-sulfonylamino)-*N*-[2-(toluene-4-sulfonylamino)-ethyl]-propionamide (187)



To a solution of *p*-toluenesulfonyl chloride (3.08 g, 16 mmol) in dry pyridine (9 mL) cooled to 0 °C, was added **186** (1.4 g, 6.7 mmol) dropwise. The solution was stirred at this temperature for 3 h, and then for a further 3 d at rt. The mixture was then poured onto an ice : H_2O mixture (300 mL) and left overnight to precipitate which was then filtered off and dried *in vacuo*. The product was recrystallised from EtOH as white crystals (3.75 g, 50%). mp: 169-171 °C; $[\alpha]_D^{20}$ 25.6 (*c* 0.5, CH_2Cl_2); ν_{max} (KBr)/cm^{-1}: 3334 (N-H), 3208 (N-H), 3252 (N-H), 3029 (ArC-H), 1650 (C=O), 1596 (ArC=C), 1329 (SO_2-N), 1307 (C-N), 1162 (SO_2-N); δ_H (400 MHz, $CDCl_3$): 2.35 (3H, s, CH_3), 2.36 (3H, s, CH_3), 2.73 (1H, dd, *J* 14, 8.8 Hz, CH), 2.90-3.00 (3H, m, 3 x CH), 3.13-3.20 (1H, m, CH), 3.29-3.37 (1H,

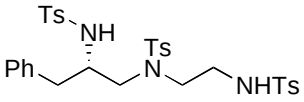
m, CH), 3.66 (1H, dt, *J* 8.4, 5.6 Hz, CH), 4.85 (1H, d, *J* 5.2 Hz, NH), 5.12 (1H, t, *J* 6.4 Hz, NH), 6.69 (1H, t, *J* 6.0 Hz, NH) 6.82 (2H, d, *J* 7.2 Hz, 2 x Ar-H), 7.06-7.13 (5H, m, 5 x Ar-H), 7.25 (2H, d, *J* 8.4 Hz, 2 x Ar-H), 7.37 (2H, d, *J* 8.4 Hz, 2 x Ar-H), 7.69 (2H, d, *J* 8.4 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 21.6 (2 xCH_3), 38.2 (CH_2), 39.7 (CH_2), 42.6 (CH_2), 58.2 (CH), 127.1 (2 x ArCH), 127.3 (2 x ArCH), 127.4 (ArCH), 129.0 (4 x ArCH), 129.8 (2 x ArCH), 129.9 (2 x ArCH), 134.8 (ArC), 135.1 (ArC), 137.0 (ArC), 143.6 (ArC), 144.1 (ArC), 171.1 (C=O); LRMS (FAB); 516.2 ($[M+H]^+$, 100%), 360.2 (7), 274.1 (24), 154.9 (40), 138.1 (32), 92.8 (20); HRMS calcd for $C_{25}H_{29}N_3O_5S_2$ 516.1627, found 516.1630.

(2*S*)-2-Benzyl-2-(toluenesulfonylamino)-*N*-[2-(toluenesulfonylamino)ethyl]-aminoethane (188)



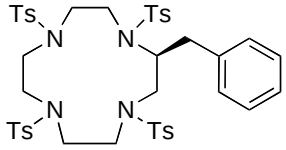
187 (500 mg, 0.97 mmol) was suspended in dry THF (7 mL) and cooled to 0 ^oC. BH_3.THF (1M) (1 mL) was added dropwise, and then mixture stirred and heated at reflux for 6 d. The excess borane was then quenched by careful addition of MeOH (4 mL). Reaction mixture was then concentrated *in vacuo*. Conc HCl (7 mL) was added to the residue, and mixture stirred and heated under reflux overnight. The solution was then cooled using an ice bath, and product filtered, washed with Et_2O (10 mL), and dried by rotary evaporation to give **188** as a white powder (375 mg, 77%). mp: 193-195 ^oC; ν_{max} (KBr)/cm^{-1}: 3434 (N-H), 3063 (ArC-H), 3030 (ArC-H), 1599 (ArC=C), 1325 (SO_2-N), 1157 (SO_2-N), 1092 (C-N); $[\alpha]_D^{21}$ -17.32 (*c* 1.0, MeOH); δ_H (400 MHz, CD_3OD): 2.56 (3H, s, CH_3), 2.34 (3H, s, CH_3), 2.48-2.65 (3H, m, 3 x CH), 2.76-2.93 (4H, m, 4 x CH), 4.54 (1H, br s, NH), 6.92-6.94 (2H, m, 2 x Ar-H), 7.01-7.03 (3H, m, 3 x Ar-H), 7.10 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.30 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 7.43 (2H, d, *J* 8.4 Hz, 2 x Ar-H), 7.61 (2H, d, *J* 8.4 Hz, 2 x Ar-H). δ_C (100 MHz, CD_3OD): 21.5 (2 x CH_3), 39.5 (CH_2), 40.0 (CH_2), 48.9 (CH_2), 52.7 (CH_2), 53.7 (CH), 128.0 (ArCH), 128.2 (2 x ArCH), 128.3 (2 x ArCH), 129.8 (2 x ArCH), 130.1 (2 x ArCH), 131.0 (4 x ArCH), 137.2 (ArC), 137.7 (ArC), 138.4 (ArC), 145.2 (ArC), 145.4 (ArC). LRMS (FAB): 502.3 ($[M+H]^+$, 100%), 348.3 (9), 227.3 (13), 92.8 (42); HRMS calcd for $C_{25}H_{32}O_4N_3S_2$ 502.1834, found 502.1838.

(2*S*)-*N*-[2-[(toluenesulfonylamino)ethyl]-*N*-[2-(toluenesulfonyl)amino]-3-phenyl-propyl-toluenesulfonamide (189)



A solution of **188** (1.89 g, 3.8 mmol) in pyridine (10 mL) was added dropwise to a stirring solution of *p*-toluenesulfonyl chloride (869 mg, 4.56 mmol) in pyridine (30 mL) cooled to 0 ^{o}C. The mixture was then stirred at rt for 7 d before being poured onto an ice : H_2O mixture (150 mL), and left to precipitate overnight. The yellow solid **189** was then filtered and dried (2.17 g, 87%). mp: 73-76 ^{o}C (lit.[120] no characterisation); $[\alpha]_D^{21}$ 23.9 (*c* 1.0, $CHCl_3$); ν_{max} (KBr)/cm^{-1}: 3287 (-NH-), 1334 (SO_2-N), 1160 (SO_2-N); δ_H (400 MHz, $CDCl_3$): 2.30 (3H, s, CH_3), 2.32 (3H, s, CH_3), 2.34 (3H, s, CH_3), 2.49 (1H, dd, *J* 14.0, 7.8 Hz, CH), 2.75 (1H, dd, *J* 14.0, 6.2 Hz, CH), 2.95-3.00 (4H, m, 4 x CH), 3.05-3.14 (1H, m, CH), 3.19 (1H, dd, *J* 14.0, 6.6 Hz, CH), 3.51-3.57 (1H, m, CH), 5.05 (1H, d, *J* 6.0 Hz, NH), 5.34 (1H, t, *J* 5.8 Hz, NH), 6.83 (2H, d, *J* 8.2 Hz, 2 x Ar-H), 7.01-7.08 (5H, m, 5 x Ar-H), 7.20 (4H, dd, *J* 8.2, 2.2 Hz, 4 x Ar-H), 7.47 (4H, dd, *J* 8.2, 3.8 Hz, 4 x Ar-H), 7.66 (2H, d, *J* 8.2 Hz, 2 x Ar-H); δ_C (100 MHz, $CDCl_3$): 21.6 (3 x CH_3), 38.8 (CH_2), 42.6 (CH_2), 51.3 (CH_2), 54.4 (CH), 54.9 (CH_2), 126.7 (ArCH), 127.1 (2 x ArCH), 127.2 (2 x ArCH), 127.5 (2 x ArCH), 128.7 (2 x ArCH), 129.1 (2 x ArCH), 129.8 (4 x ArCH), 130.0 (4 x ArCH), 134.3 (ArC), 136.3 (2 x ArC), 136.8 (ArC), 143.4 (ArC), 143.5 (ArC), 144.2 (ArC); LRMS (FAB): 656.1 ([M+H]$^+$, 100%), 502.1 (6), 381.1 (11), 274.1 (13), 227.3 (31), 92.8 (47); HRMS calcd for $C_{32}H_{38}N_3O_6S_3$ 656.1923, found 656.1921.

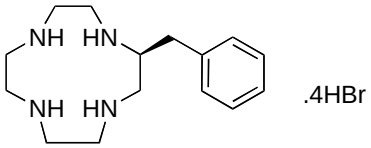
2-(Benzyl)-1,4,7,10-tetrakis-(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane (190)



A solution of *N*,*O*,*O'*-Tri(*p*-toluenesulfonyl)diethanolamine **47** (433 mg, 0.76 mmol) in DMF (5 mL) added dropwise to a stirring solution of **189** (500 mg, 0.76 mmol) and caesium carbonate (743 mg, 2.3 mmol) in DMF (15 mL). The mixture was then stirred at rt for 14 d, before solvent was removed *in vacuo* and residue transferred to a separating funnel with CH_2Cl_2 (100 mL) and H_2O (30 mL). The organic layer was separated, and the aq layer extracted with CH_2Cl_2 (2 x 25 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure to yield a yellow solid (668 mg, 100%).

mp: 72-78 ^{o}C (lit.[120] no characterisation); $[\alpha]_D^{21}$ 16.38 (*c* 1.0, $CHCl_3$); ν_{max} (KBr)/cm^{-1}: 1597.7 (Ar), 1494.6 (Ar), 1339.3 (SO_2-N), 1160.0 (SO_2-N); δ_H (400 MHz, $CDCl_3$): 2.25 (3H, s, CH_3), 2.60 (3H, s, CH_3), 2.29 (3H, s, CH_3), 2.32 (3H, s, CH_3), 2.43-4.23 (15H, m, 14 x CH, CH_A), 6.79-7.72 (21H, m, 21 x Ar-H); δ_C (100 MHz, $CDCl_3$): 21.5 (3 x CH_3) 21.7 (CH_3), 38.8 (CH_2), 48.5 (CH_2), 51.0 (CH_2), 54.5 (CH_2), 54.7 (CH), 62.0 (CH_2), 66.1 (CH_2), 68.4 (CH_2), 127.1 (2 x ArCH), 127.1 (2 x ArCH), 127.2 (2 x ArCH), 127.5 (2 x ArCH), 128.0 (2 x ArCH), 128.5 (ArCH), 129.0 (2 x ArCH), 129.2 (2 x ArCH), 129.7 (2 x ArCH), 129.9 (2 x ArCH), 130.1 (2 x ArCH), 132.4 (ArC), 134.4 (2 x ArC), 136.7 (2 x ArC), 143.2 (2 x ArC), 143.4 (2 x ArC), 144.0 (2 x ArC), 145.3 (2 x ArC), 160.6 (2 x ArC), 162.7 (2 x ArC); LRMS (FAB): 900.9 ([M+Na]$^+$, 24%), 787.8 (15), 678.0 (40), 656.(12), 396.0 (7), 317.1 (7), 239.2 (15), 92.9 (100); HRMS calcd for $C_{43}H_{50}N_4O_8S_4Na$ 901.2409, found 901.2412.

2-Benzyl-1,4,7,10-tetraazacyclododecane (191)

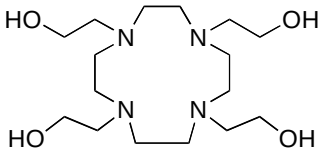


190 (205 mg, 0.23 mmol) was added to H_2SO_4 (98%) (250 μL) heated to 165 ^{o}C in a conical flask. The solution was stirred vigorously at this t for six minutes. The mixture was then cooled in an ice bath before the liquid was added dropwise to a stirring flask of EtOH (2 mL). Et_2O (1.2 mL) was then added and the solution cooled to 0 ^{o}C before the precipitate was collected by filtration. The solid was dissolved in hot H_2O (200 μL) and HBr (48%) (200 μL) added. The tetrahydrobromide salts formed was filtered and dried *in vacuo* (111 mg, 83%). Due to time constraints, **191** was not converted to the free base and requires full characterisation. (lit.[120] no characterisation).

7.4 Experimental to Chapter 5

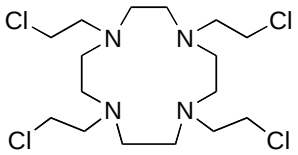
7.4.1 Experimental Details

Tetrakis-(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (220)



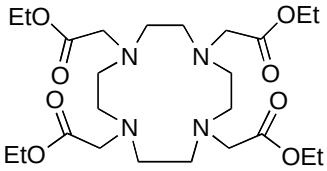
36 (970 mg, 5.6 mmoles) was dissolved in H_2O (10 mL) and the solution cooled to 0 ^oC. Ethylene oxide (2.66 mL, 560.0 mmol) was added *via* a cooling finger to a dropping funnel containing H_2O (2 mL). The ethylene oxide solution was added to the cyclen solution, and the resulting mixture stirred for 2 h at 0 ^oC before the reaction was stopped by removal of excess ethylene oxide under vacuum. The product was dried *in vacuo* to leave a viscous yellow oil (1.92 g, 98%). This crude product was continually extracted with hexane for 30 min to get **220** as white crystals (<1%). mp: 89-91 ^oC (lit.[213] 90-92 ^oC); ν_{max} (neat)/cm^{-1}: 3350 (O-H stretching), 2811 (NCH_2, CH_2) cm^{-1}; δ_H (400 MHz, D_2O): 2.58 (8H, t, *J* 6.0 Hz, 4 side chain NCH_2), 2.69 (16H, s, 8 ring NCH_2), 3.61 (8H, t, *J* 6.0 Hz, 4 side chain OCH_2); δ_C (100 MHz, D_2O): 49.9 (8 ring NCH_2), 56.3 (4 side chain NCH_2), 59.0 (4 side chain OCH_2); LRMS (CI^+): 349.3 ($[M+H]^+$, 100%), 347.3 (7), 287.3 (44), 271.2 (8), 243.2 (6), 216.2 (10); HRMS calcd for $C_{16}H_{36}O_4N_4$ 349.2815, found 349.2815.

1,4,7,10-Tetra-(2-chloroethyl)-1,4,7,10-tetraazacyclododecane dihydrochloride (209)



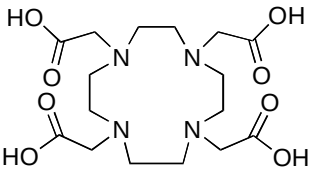
220 (500 mg, 1.43 mmol) and $SOCl_2$ (343 mmol, 25 mL) were added to a round-bottomed flask fitted with a water-cooled condenser and this was heated at 50 ^oC for 18 h. After this time, the excess thionyl chloride was removed by rotary evaporation. The product was recrystallised using hot propan-2-ol as white crystals. (47.7 mg, 34%). mp: 206-208 ^oC; NMR spectroscopy agreed with lit. values;[209] δ_H (400 MHz, D_2O): 3.28 (16H, s, ring 8 x CH_2), 3.38 (8H, s, 4 x N-CH_2), 3.84 - 3.90 (8H, m, 4 x Cl-CH_2).; LRMS (FAB, glycerol): 423.2 ($[M+H]^+$, 100%), 421.2 (79), 387.3 (21), 359.2 (19), 211.2 (26), 197.1 (12), 161.1 (9), 106.4 (39), 57.9 (12), 57.0 (11).

1,4,7,10-Tetrakis[(ethoxycarbonyl)methyl]-1,4,7,10-tetraazacyclododecane (222)



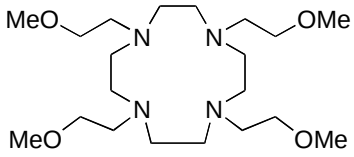
A mixture of cyclen tetrahydrochloride **36** (364 mg, 1.14 mmol), ethyl chloroacetate (0.6 mL, 5.7 mmol) and Na_2CO_3 (1.8 g, 17 mmol) in CH_3CN (45 mL) was stirred and heated under reflux for 18 h. The solution was then cooled to rt before filtering, and the filtrate was concentrated *in vacuo*. The resulting brown residue was washed with hexane, then gave white hexagonal crystals (7 mg, 10%) from dichloromethane and hexane. mp: decomposition >150 ^oC (lit.[217] decomposition 81 ^oC); ν_{max} (neat)/cm^{-1}: 2980 (N-CH_2), 2826 (N-CH_2), 1724 (C=O), 1203 (CO-O), 1104 (CO-O); δ_H (400 MHz, D_2O): 1.17 (16H, t, J 7.1 Hz, 4 x CH_3), 2.29 (16H, br s, 8 x ring CH_2), 1.96 (8H, br s, 4 x arm CH_2), 4.12 (8H, q, *J* 7.1 Hz, 4 x C*H*$_2$-CH_3); δ_C (100 MHz, D_2O): 13.4 (4 x CH_3), 55.2 (4 x CH_2-O), 62.1 (12 x CH_2-N), 175.0 (4 x C); LRMS $(EI)^+$: 516.2 ($[M+H]^+$, 5%), 443.2 (74), 374.2 (25), 271.1 (46), 245.1 (100), 185.1 (52), 130.1 (80), 98.1 (34); HRMS calcd for $C_{24}H_{44}N_4O_8$, 516.3159, found 516.3162.

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (dota) (223)



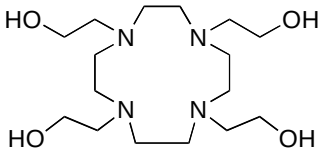
A solution of chloroacetic acid (876 mg, 9.3 mmol) in H_2O (2 mL) was added to a stirring solution of cyclen **36** (0.2 g, 1 mmol) in H_2O (3 mL), and the pH was adjusted to 9 with NaOH (5M). The t was raised to 70 ^oC and the pH maintained between 8.5 and 9 by the addition of NaOH (5M) as necessary. The mixture was stirred at this t for 48 h before cooling to rt and concentrating *in vacuo*. The residue was acidified with HCl (1M) to pH 2.5 and the solution was passed through an cation exchange coloumn (Dowex 50W-8X (H^+)). The column was washed with H_2O until the eluent was neutral, and then the ligand was brought off the column using NH_3 : H_2O (20 mL : 100 mL). The basic eluent was reduced under reduced pressure to leave **223** as a white solid (143 mg, 23%). mp: 263-266 ^oC (lit.[280] 265-267 ^oC); δ_H (400 MHz, D_2O): 3.17 (16H, s, 8 x ring CH_2), 3.51 (8H, s, 4 x arm CH_2); δ_C (100 MHz, D_2O): 49.7 (4 x CH_2), 55.9 (8 x CH_2), 171.82 (4 x C=O).

1,4,7,10-Tetrakis-(2-methoxyethyl)-1,4,7,10-tetraazacyclododecane (224)



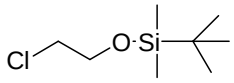
36 (1.0 g, 5.8 mmol), 2-chloroethyl-methyl ether (12 mL, 232 mmol), NaOH pellets (0.93 g, 23 mmol), and aq EtOH (50%, 50 mL) were stirred and heated under reflux for 9 d. A pH greater than 11 was maintained by regular addition of NaOH pellets. After this time, the solvent was removed under vacuum. The solid residue was dissolved in H_2O (30 mL) and made basic with conc NaOH (15 mL), before exhaustively extracting with $CHCl_3$. The organic layer was then dried with Na_2SO_4 and concentrated, yielding product as yellow-white crystals (3.44 g, 100%). ν_{max} (neat)/cm^{-1}: 2956 (CH), 2879 (CH), 2809 (O-CH_3); NMR spectroscopy agreed with lit. values;[218] δ_H (400 MHz, D_2O): 2.56 (24H, m; 16 x ring N-CH_2, 8 x side chain N-CH_2), 3.34 (12H, s, 4 x O-CH_3), 3.51 (8H, t, *J* 5.2, 4 x O-CH_2); δ_C (100 MHz, D_2O): 49.2 (8 x side chain CH_2), 52.2 (8 x side chain CH_2), 58.6 (4 x CH_3), 68.2 (16 x ring CH_2); LRMS (CI^+): 405.3 ($[M+H]^+$, 51%), 373.4 (26), 316.3 (9), 290.3 (31), 189.2 (59), 157.2 (19), 102.1 (100), 71.1 (58); HRMS calcd for $C_{20}H_{44}N_4O_4$ 405.3441, found 405.3440.

Tetrakis-(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (220)



222 (185 mg, 0.36 mmol) was dissolved in Et_2O (5 mL) and the solution stirred and cooled to -78 ^oC. Di-isobutyl aluminium hydride (3.2 mL, 3.2 mmol) was added drop wise, and then the mixture was stirred at this t for one h, before warming to rt and stirring for a further 24 h. The reaction was quenched by addition of a saturated ammonium chloride solution (5 mL) and the solution was stirred until a white precipitate formed. The solution was filtered through a pad of celite and washed with Et_2O (30 mL). The organic phase was dried over $MgSO_4$ before concentrating under reduced pressure to give **220** as a white solid (120 mg, 96%). mp: 89-91 ^oC (lit.[213] 90-92 ^oC); δ_H (400 MHz, D_2O): 3.20 (8H, s, 4 side chain NCH_2), 3.35 (16H, s, 8 ring NCH_2), 3.92 (8H, t, *J* 6.0 Hz, 4 side chain OCH_2).

tert-Butyl-(2-chloroethoxy)-dimethyl-silane (226)



To a stirred solution of dry THF (4 mL), was added *tert*-butyldimethylsilyl chloride (4.1 g, 27 mmol) and imidazole (3.81 g, 56 mmol) at 0 ^{o}C. 2-Chloroethanol (1.5 mL, 22 mmol) was added dropwise at this t, and the solution warmed to rt and stirred for 6 d. A small amount of ice was added to quench the reaction and the solution was extracted with Et_2O (5 x 30 mL). The ether layer was washed with H_2O (10 mL) and brine (10 mL) before drying over $MgSO_4$. The solvent was removed by rotary evaporation to leave the product as a clear colourless liquid (5 g, 100%). δ_H (400 MHz, $CDCl_3$): 0.08 (6H, s, 2 x CH_3-Si), 0.82 (9H, s, 3 x CH_3), 3.43 (2H, t, *J* 6.4 Hz, CH_2-O), 3.75 (2H, t, *J* 6.4 Hz CH_2-Cl); δ_C (100 MHz, $CDCl_3$): –5.33 (2 x CH_3-Si), 18.4 (C), 25.8 (3 x CH_3), 45.1 (CH_2), 63.6 (CH_2); LRMS (CI^+): 195.14 ($[M+H]^+$, 100%), 137.07 (10); HRMS calcd for $C_8H_{19}OSi^{35}Cl$ 195.0972, found 195.0976.

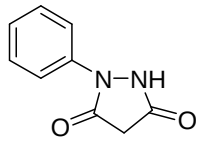
7.5 Experimental to Chapter 6

7.5.1 General Procedure (6A)

A. Preparation of pyrazolidine derivatives.[250] Phenyl-pyrazolidine-3,5-dione (1 eq), aldehyde (1 eq) and freshly fused sodium acetate (1 eq) were dissolved in glacial acetic acid (5 ml per mmol phenyl-pyrazolidine-3,5-dione). The solution was heated at reflux for 2-19 h then cooled and filtered. The precipitate was rinsed with EtOAc (2 x 30 ml) and dried *in vacuo* to give the pyrazolidine as a mixture of geometric isomers.

7.5.2 Experimental Details

Phenyl-pyrazolidine-3,5-dione (241)

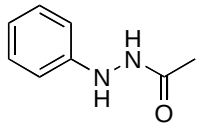


This method is adapted from the literature.[246]

Sodium ethoxide (5.44 g, 81.6 mmol) was dissolved in dry EtOH (60 ml). Phenylhydrazine (4.02 ml, 40.8 mmol), and diethyl malonate (6.2 ml, 40.8 mmol) were added at rt with

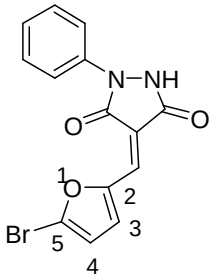
stirring. The reaction mixture was heated at 110-120 ^{o}C for 1 h before the EtOH was removed by distillation. The residue was heated at this t for a further 2 h. The flask was cooled to 0 ^{o}C, and a small volume of distilled H_2O (30 mL) was added. The flask was sonicated to dissolve all residues, and then the aq phase was extracted with Et_2O (5 x 20 ml) to remove any remaining starting material. The aq phase was acidified with HCl (6 M) to pH 1-2. The product precipitated as a yellow solid. The aq layer was then extracted with EtOAc (10 x 30 ml) to extract more of the product. The organic extracts were concentrated *in vacuo* to leave a yellow-brown residue. EtOH (200 ml) was added to dissolve all residues, and the flask was sonicated, before heating under reflux for 4 h, filtering occasionally to remove inorganic white salt. The product dissolved in EtOH was left to stand for 3 weeks to allow formation of any remaining salt, before filtering a final time. The organic extracts were concentrated *in vacuo* to leave phenyl-pyrazolidine-3,5-dione as a yellow solid. Re-crystallisation from EtOH gave **241** as yellow needles (3.55 g, 49%). mp: 190-191 ^{o}C (lit.[246] 192 ^{o}C); ν_{max}(neat)/cm^{-1}: 2910 (Ar), 1744 (amide), 1672 (amide); δ_H (400 MHz, DMSO-d_6): 3.59 (2H, s, CH_2), 7.18 (1H, t, *J* 7.4 Hz, Ar-H), 7.42 (2H, t, *J* 8.0 Hz, 2 x Ar-H), 7.66 (2H, d, *J* 8.0 Hz, 2 x Ar-H), 11.46 (1H, br s, NH); δ_C (100 MHz, DMSO-d_6): 38.1 (CH_2), 118.8 (2 x ArCH), 124.87 (ArCH), 129.2 (2 x ArCH), 137.2 (C), 166.4 (C=O), 167.9 (C=O); LRMS (EI$^+$): 176.09 ([M]$^+$, 100%), 108.09 (54), 77.05 (54); HRMS calcd for $C_9H_8N_2O_2$ 176.0586, found 176.0587.

1-Acetyl-1-phenylhydrazine (242)



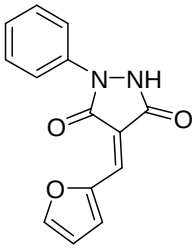
Phenyl hydrazine (3.0 mL, 0.03 mmol) was suspended in Et_2O (30 mL) at 0 ^{o}C, and a solution of acetic anhydride (6.4 mL, 0.07 mmol) in Et_2O (15 mL) was added dropwise over 10 min. The mixture was then stirred at this t for a further 10 min. The solution was then diluted with petroleum ether (30 mL) and filtered. The solid was washed with petroleum ether and Et_2O, to give **242** as a white powder (4.43 g, 96%). mp: 127-129 ^{o}C (lit. [281] 129 ^{o}C); δ_H (400 MHz, DMSO-d_6): 1.91 (3H, s, CH_3), 6.70-6.74 (3H, m, 3 x Ar-H), 7.11-7.16 (2H, m, 2 x Ar-H), 7.64 (1H, s, NH), 9.61 (1H, s, NH); δ_C (100 MHz, DMSO-d_6): 21.0 (CH_3), 112.4 (2 x ArCH), 118.7 (ArCH), 129.4 (2 x ArCH), 149.7 (ArC), 169.4 (C=O).

4-(5-Bromo-2-furfurylidene)-1-phenyl-pyrazolidine-3,5-dione (240)



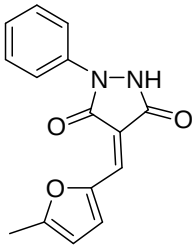
4-5-Bromo-2-furfurylidene was synthesised as a 2:3 mixture of geometric isomers *via* general method 6A using phenyl-pyrazolidine-3,5-dione (250 mg, 1.4 mmol), 5-bromo-2-furaldehyde (248 mg, 1.4 mmol) and sodium acetate (115 mg, 1.4 mmol). The reaction gave a brown/black precipitate (302.4 mg, 65%). mp: decomposition > 210 ^{o}C; δ_H (400 MHz, DMSO-d_6): 7.06 (1H, d, *J* 3.6 Hz, furan 4-H), 7.21 (1H, t, *J* 7.2 Hz, Ar-H), 7.45 (2H, dt, *J* 7.6, 2.4 Hz, 2 x Ar-H), 7.56 (0.4 H, s, vinylic CH), 7.58 (0.6 H, s, vinylic CH), 7.74 (2H, dd, *J* 14.4, 8 Hz, 2 x Ar-H), 8.37 (0.6 H, d, *J* 3.2 Hz, furan 3-H), 8.45 (0.4 H, d, *J* 3.2 Hz, furan 3-H); δ_C (100 MHz, DMSO-d_6): 115.1 (C), 117.6 (CH-4), 118.6 (2 x ArCH), 118.9 (CH), 125.1 (CH-3), 127.6 (ArCH), 129.3 (2 x ArCH), 131.7 (ArC), 152.4 (CH-2), 152.3 (CH-5), 159.3 (C=O), 160.8 (C=O); LRMS (EI$^+$): 332.0 ([M+H]$^+$, 7%), 78.0 (80), 63.0 (100); HRMS calcd for $C_{14}H_9O_3N_2{}^{79}Br$ 331.9797, found 331.9800.

4-(2-Furfurylidene)-1-phenyl-pyrazolidine-3,5-dione (243)



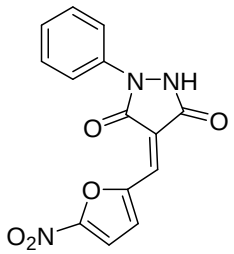
243 was synthesised as a 1:1 mixture of geometrical isomers using general method 6A, by reacting **241** (250 mg, 1.4 mmol) with furfuraldehyde (0.12 ml, 1.4 mmol) and sodium acetate (0.12 g, 1.4 mmol). **243** was produced as a gold/brown precipitate (276 mg, 77%). mp: decomposition >210 ^{o}C; ν_{max} (neat)/cm^{-1}: 3126 (Ar), 3011 (Ar), 1709 (C(O)N), 1652 (C(O)N); δ_H (400 MHz, DMSO-d_6): 6.93 (1H, dd, *J* 3.5, 1.21 Hz, CH-4), 7.20 (1H, dt, *J* 7.4, 1.1 Hz, Ar-H), 7.45 (2H, dt, *J* 7.5. 1.9 Hz, 2 x Ar-H), 7.63 (0.5 H, s, vinylic CH), 7.65 (0.5 H, s, vinylic CH), 7.75 (2H, dd, *J* 14.6, 8.0 Hz, 2 x Ar-H), 8.25 (1H, t, *J* 1.9 Hz, CH-5), 8.43 (0.5 H, d, *J* 3.0 Hz, CH-3), 8.53 (0.5 H, d, *J* 3.7 Hz, CH-3), 11.5 (1H, br s, NH); δ_C (100 MHz, DMSO-d_6): 114.8 (C), 115.4 (CH-4), 118.6 (2 x ArCH), 118.9 (CH), 125.0 (CH-3), 125.8 (ArCH), 129.3 (2 x ArCH), 138.0 (ArC), 150.2 (CH-2), 151.1 (CH-5), 159.3 (C=O), 160.8 (C=O).

4-(5-Methyl-2-furfurylidene)-1-phenyl-pyrazolidine-3,5-dione (244)



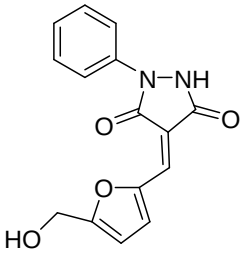
Analogue **244** was synthesised as a 1:1 mixture of geometric isomers by general method 6A using **241** (150 mg, 0.85 mmol), 5-methyl-2-furaldehyde (0.08 ml, 0.85 mmol) and sodium acetate (70 mg, 0.85 mmol). **244** was produced as an orange solid (152 mg, 67%). mp: 201-203 ^{o}C; ν_{max} (neat)/cm^{-1}: 3126 (Ar), 3020 (CH_3), 1709 (C(O)N), 1653 (C(O)N); δ_H (400 MHz, DMSO-d_6): 2.53 (3H, s, CH_3), 6.7 (1H, d, *J* 3.1 Hz, CH-4), 7.25 (1H, t, *J* 7.2 Hz, Ar-H), 7.5 (2H, t, *J* 7.2, 2 x Ar-H), 7.6 (0.5H, s, CH), 7.62 (0.5H, s, CH), 7.79 (2H, dd, *J* 14.4, 8 Hz, 2 x Ar-H), 8.51 (0.5H, br s, CH-3), 8.57 (0.5H, d, *J* 3.6 Hz, CH-3), 11.3 (1H, br s, NH); δ_C (100 MHz, DMSO-d_6): 14.5 (CH_3), 112.5 (C), 113.0 (CH-4), 118.5 (2 x ArCH), 118.8 (CH), 124.9 (CH-3), 128.3 (ArCH), 129.2 (2 x ArCH), 138.0 (ArC), 149.2 (CH-2), 149.3 (CH-5), 159.8 (C=O), 162.0 (C=O); LRMS (CI$^+$): 269.1 ([M+H]$^+$, 6%), 85.1 (8), 79.1 (100); HRMS calcd for $C_{15}H_{13}O_3N_2$ 269.0926, found 269.0929.

4-(5-Nitro-2-furfurylidene)-1-phenyl-pyrazolidine-3,5-dione (245)



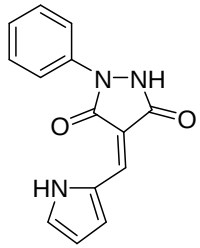
245 was prepared as a mixture of geometric isomers *via* general method 6A using **241** (150 mg, 0.85 mmol), 5-nitro-2-furaldehyde (0.09 ml, 0.85 mmol) and sodium acetate (70 mg, 0.85 mmol). **245** was produced as a dark brown solid (218 mg, 86%). mp: decomposition > 230 ^{o}C; ν_{max} (neat)/cm^{-1}: 3138 (Ar), 1672 (amide), 1623 (amide), 1527 (C-NO_2), 1344 (C-NO_2); δ_H (400 MHz, DMSO-d_6): 7.24 (1H, t, *J* 7.6 Hz, Ar-H), 7.48 (2H, dt, *J* 8.8, 2.0 Hz, 2 x Ar-H), 7.61-7.63 (1H, m, CH), 7.73-7.75 (2H, m, 2 x Ar-H), 7.90 (1H, t, *J* 4.0 Hz, CH-3), 8.52 (1H, d, *J* 4.0 Hz, CH-4), 11.70 (1H, br s, NH).

4-(5-Hydroxymethyl-2-furfurylidene)-1-phenyl-pyrazolidine-3,5-dione (246)



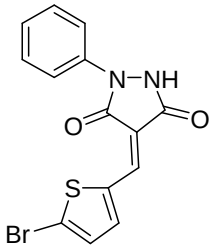
246 was synthesised as a 1:3 mixture of geometric isomers by general method 6A by reacting **241** (120 mg, 0.68 mmol), 5-hydroxymethyl-2-furaldehyde (86 mg, 0.68 mmol) and sodium acetate (70 mg, 0.68 mmol) to give **246** as a brown solid (93 mg, 48%). mp: 175-179 ^{o}C; ν_{max} (neat)/cm^{-1}: 3070 (Ar), 1498.42 (C(O)N), 1344 (O-H), 1063 (C-O); δ_H (400 MHz, DMSO-d_6): 4.62 (2H, s, CH_2O), 5.24 (1H, s, OH), 6.83 (1H, d, *J* 3.7 Hz, CH-4), 7.25 (1H, t, *J* 7.4 Hz, Ar-H), 7.5 (2H, t, *J* 8.6 Hz, 2 x Ar-H), 7.61 (0.25H, s, CH), 7.64 (0.75H, s, CH), 7.79 (2H, dd, *J* 15.2, 8.2 Hz, 2 x Ar-H), 8.53 (0.75H, br s, CH-3), 8.58 (0.25H, d, *J* 2.8 Hz, CH-3), 11.3 (1H, br s, NH); δ_C (100 MHz, DMSO-d_6): 56.5 (CH_2O), 113.8 (C), 115.3 (CH-4), 118.6 (2 x ArCH), 118.8 (CH), 125.0 (CH-3), 127.2 (ArCH), 129.2 (2 x ArCH), 138.0 (ArC), 149.5 (CH-2), 151.1 (CH-5), 161.0 (C=O), 164.6 (C=O); LRMS (EI$^+$): 284.1 ([M+H]$^+$, 9%), 150.0 (5), 44.0 (50), 28.1 (100); HRMS calcd for $C_{15}H_{12}O_4N_2$ 284.0797, found 284.0799.

4-(2-Pyrrolylidene)-1-phenyl-pyrazolidine-3,5-dione (247)



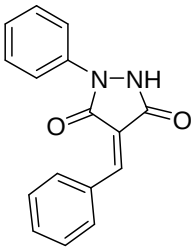
Analogue **247** was prepared as a mixture of geometric isomers *via* general method 6A. **241** (150 mg, 0.85 mmol), 2-pyrrolecarboxaldehyde (86 mg, 0.85 mmol) and sodium acetate (70 mg, 0.85 mmol) were reacted to give **247** as a brown solid (89 mg, 42%). mp: decomposition > 230 ^{o}C; ν_{max} (neat)/cm^{-1}: 3059 (Ar), 1653 (C(O)N), 1595 (C(O)N), 1496 (NH); δ_H (400 MHz, DMSO-d_6): 7.21 (1H, dd, *J* 15.6, 7.6 Hz, CH), 7.31 (1H, br s, NH), 7.43-7.49 (2H, m, 2 x ArH), 7.68-7.70 (4H, m, 3 x ArH, CH), 7.74 (0.5H, s, CH), 7.78 (0.5H, s, CH), 7.80 (1H, s, H) 11.45 (1H, br s, NH); δ_C (100 MHz, DMSO-d_6): 107.9 (C), 113.8 (CH), 113.9 (CH), 118.3 (2 x ArCH), 118.9 (CH), 124.2 (ArCH), 128.9 (2 x ArCH), 129.4 (C), 136.9 (ArC), 161.8 (C=O), 1 x missing C=O; LRMS (EI$^+$): 253.1 ([M+H]$^+$, 100%), 146.0 (44), 119.0 (38), 63.0 (41); HRMS calcd for $C_{14}H_{11}O_2N_3$ 253.0851, found 253.0849.

4-(5-Bromo-2-thiophenylidene)-1-phenyl-pyrazolidine-3,5-dione (248)



248 was synthesised as a mixture of geometric isomers by general method 6A. **241** (150 mg, 0.85 mmol), 5-bromothiophene-2-carboxaldehyde (0.1 ml, 0.85 mmol) and sodium acetate (70 mg, 0.85 mmol) were reacted to give **248** as a brown solid (243 mg, 82%). mp: > 250 ^{o}C; ν_{max} (neat)/cm^{-1}: 2958 (Ar), 1713 (C(O)N), 1659 (C(O)N), 741 (C-Br); δ_H (400 MHz, DMSO-d$_6$): 7.22 (1H, t, Ar-H), 7.46 (2H, t, *J* 8.0 Hz, 2 x Ar-H), 7.53 (1H, d, *J* 4.0 Hz, CH-4), 7.72 (2H, dd, *J* 12.9, 8.1 Hz, 2 x Ar-H), 8.03 (0.5H, d, *J* 4.1 Hz, CH-3), 8.07 (0.5H, d, *J* 4.1 Hz, CH-3), 8.13 (0.5H, s, vinylic CH), 8.16 (0.5H, s, vinylic CH), 11.3 (1H, br s, NH); δ_C (100 MHz, DMSO-d$_6$): 118.5 (CH), 128.9 (2 x ArCH), 129.0 (2 x ArCH), 131.9 (CH), 132.0 (CH), 137.4 (ArC), 137.7 (ArC), 2 x C=O missing; LRMS (EI$^+$): 349.92 ([M+H]$^+$, 15%), 255.1 (24), 78.0 (85), 63.0 (100); HRMS calcd for $C_{14}H_9O_2N_2{}^{81}BrS$ 349.9548, found 349.9550.

4-Benzylidene-1-phenyl-pyrazolidine-3,5-dione (249)



Analogue **249** was synthesised as a 2:1 mixture of geometric isomers using general method 6A. **241** (150 mg, 0.85 mmol), benzaldehyde (0.09 ml, 0.85 mmol) and sodium acetate (70 mg, 0.85 mmol) were reacted to give **249** as a vivid orange solid (145 mg, 65%). mp: 251-253 ^{o}C; ν_{max} (neat)/cm^{-1}: 3068 (Ar), 1683 (C(O)N), 1658 (C(O)N); δ_H (400 MHz, DMSO-d$_6$): 7.22 (1H, t, *J* 7.2 Hz, Ar-H), 7.44-7.49 (2H, m, 2 x Ar-H), 7.55-7.59 (2H, m, 2 x Ar-H), 7.62-7.66 (1H, m, Ar-H), 7.74-7.76 (2H, m, 2 x Ar-H), 7.9 (0.3H, br s, CH), 7.95 (0.6H, s, CH), 8.56-8.61 (2H, m, 2 x Ar-H); δ_C (100 MHz, DMSO-d$_6$): 118.3 (CH), 128.7 (2 x ArCH), 128.8 (2 x ArCH), 128.9 (2 x ArCH), 129.0 (2 x ArCH), 132.3 (ArC), 133.4 (ArCH), 134.0 (ArC), 134.1 (ArCH), 2 missing C=O; LRMS (EI$^+$): 264.1 ([M+H]$^+$, 100%), 195.1 (26), 130.0 (33), 78.0 (71), 63.0 (85); HRMS calcd for $C_{16}H_{12}O_2N_2$ 264.0899, found 264.0898.

7.5.3 Biological Materials and Methods for Chapter 6

7.5.3.1 Western Blotting

Primary human pigment epithelial (RPE) cells were incubated with 1-20 μM of each drug compound for 24 h. The protein from whole cell extracts were separated by sodium dodecyl sulfate 12% poly-acrylamide gel electrophoresis (SDS-PAGE) and analysed by Western blotting with anti-p53 DO-1 (pharmingen), anti-HDM-2 AB1/AB2 (Oncogene Science), anti-phospho-p53 (serine 15) (Cell Signaling), anti-$21^{WAF1CIP1}$ (Santa Cruz Biotechnology) antibodies. Blots were also probed with an anti-Cdk4 antibody (Santa Cruz Biotechnology) to monitor protein loading. Visual comparison of the intensity of the compound bands with the control bands gave an indication of biological activity.

8 References

1. WHO Media Centre, **2001**, "*Factsheet No. 259: African Trypanosomiasis or Sleeping Sickness* (http://www.who.int/mediacentre/factsheets/fs259/en/index.html).
2. UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, *TDR News*, **2006**, *76*, 8.
3. Médecins Sans Frontièrs (MSF), *Access to Essential Medicines Campaign, Sleeping Sickness Factsheet*, **2004** (http://www.accessmed-msf.org).
4. Rocha, G.; Martins, A.; Gama, G.; Brandão, F.; Atouguia, J., *Lancet*, **2004**, *363*, 247.
5. Barrett, M. P.; Burchmore, R. J. S.; Stich, A.; Lazzari, J. O.; Frasch, A. C.; Cazzulo, J. J.; Krishna, S., *Lancet*, **2003**, *362*, 1469.
6. http://www.who.int/emc/diseases/tryp/trypanodat.html, September **2006**.
7. WHO (Geneva) "*World Health Report 2000: Health Systems Improving Performance*, **2000** (http://www.who.int/tdr/diseases/tryp/direction.htm#Refs)".
8. Seed, J. R., Current status of African trypanosomiasis. *ASM News*, **2000**, *66*, 395.
9. Report of a WHO Expert Committee. Control and Surveillance of African Trypanosomes. WHO Technical Report Series 881, **1998**, (http://www.who.int/tdr/diseases/tryp/direction.htm#Refs).
10. Centre for Disease Control and Prevention (CDC), http://www.dpd.cdc.gov/dpdx/HTML/TrypanosomiasisAfrican.htm, September **2006**.
11. Cross, G. A. M., *Science*, **2005**, *309*, 355.
12. Denise, H.; Barratt, M. P., *Biochem. Pharmacol.*, **2001**, *61*, 1.
13. Rudenko, G., *Curr. Opin. Microbiol.*, **1999**, *2*, 651.
14. Hasne, M. –P.; Barratt, M. P., *J. Applied Microbiology*, **2000**, *89*, 697.
15. Voogd, T. E.; Vansterkenburg, E. L.; Wilting, J.; Jansen, L. H., *Pharmacol. Rev.*, **1993**, *45*, 177.
16. Mitsuya, A. H.; Popovic, M.; Yarchoan, R., *Science*, **1984**, *226*, 172.
17. Kehinde, E. O., *Cancer Surv.*, **1995**, *23*, 281.
18. Gagliardi, A.; Hadd, H.; Collins, D. C., *Cancer Res.*, **1992**, *52*, 5073.
19. Cardinali, M.; Sartor, O.; Robbins, K. C., *J. Clin. Invest.*, **1992**, *89*, 1242.
20. Barrett, M. P.; Fairlamb, A. H., *Parasitology Today*, **1999**, *15*, 136.
21. Lykknes, A.; Kuittingen, L., *J. Chem. Ed.*, **2003**, *80*, 497.
22. Barrett, S. V.; Barrett, M. P., *Parasitology Today*, **2000**, *16*, 7.
23. Ehrlich, P., *The Collected Papers of Paul Ehrlich, Histology, Biochemistry and Pathology*, Vol. 2, Pergamon Press, **1956**.

24. Friedheim, E. A. H., *Am. J. Trop. Med. Hyg*., **1949**, *29*, 173.
25. Carter, N. S.; Fairlamb, A. H., *Nature*, **1993**, *361*, 173.
26. Konig, A.; Wrazel, L.; Warrell, R. P.; Rivi, R.; Pandolfi, P. P.; Jakugowski, A.; Gabrilove, J. L., *Blood*, **1997**, *90*, 562.
27. Rousselot, P.; Labaume, S.; Marrolleau, S.; Larghero, J.; Noguera, M. H.; Brouet, J. C.; Fermand, J. P., *Cancer Res*., **1999**, *59*, 1041.
28. Wickware, P., *Nature Medicine*, **2002**, *8*, 908.
29. Docampo, R.; Moreno, S. N. J.; Stoppani, A. O. M.; Leon, W.; Cruz, F. S.; Villalta, F.; Muniz, R. F. A., *Biochem. Pharmacol*., **1981**, *30*, 1947.
30. Decampo, R., *Chem. Biol. Interact*., **1990**, *73*, 1.
31. Pepin, J.; Milord, F.; Meurice, F.; Ethier, L.; Loko, L.; Mpia, B., *Trans. R. Soc. Trop. Med. Hyg.*, **1992**, *86*, 254.
32. Filardi, L. S.; Brener, Z., *Ann. Trop. Med. Parasitol*., **1982**, *76*, 293.
33. Enanga, B.; Keita, M.; Chauvière, G.; Dumas, M.; Bouteille, B., *Trop. Med. Int. Health*, **1998**, *3*, 736.
34. Hawking, F., *Ann. Trop. Med. Parasitol*., **1938**, *32*, 313.
35. Williamson, J., *Br. J. Pharmacol*., **1959**, *14*, 431.
36. Borst, P.; Fairlamb, A. H., *Annu. Rev. Microbiol.*, **1998**, *52*, 745.
37. De Koning, H. P.; Jarvis, S. M., *Mol. Pharmacol.*, **1999**, *56*, 1162.
38. Mäser, P.; Sutterlin, C.; Kralli, A.; Kaminsky, R., *Science*, **1999**, *285*, 242.
39. Sanchez, M. A.; Ullman, B.; Landfear, S. M.; Carter, N. S., *J. Biol. Chem.*, **1999**, *274*, 30244.
40. Susperregui, J.; Petsom, A.; Bayle, M.; Lain, G.; Giroud, C.; Baltz, T.; Délèris, G., *Eur. J. Med. Chem.*, **1997**, *32*, 123.
41. Soulère, L.; Hoffman, P.; Bringaud, F.; Périé, J., *Braz. J. Med. Biol. Res.*, **1999**, *32*, 1447.
42. De Koning, H. P.; Jarvis, S. M., *Eur. J. Biochem.*, **1997**, *247*, 1102.
43. De Koning, H. P.; Jarvis, S. M., *Mol. Biochem. Parasitol.*, **1997**, *89*, 245.
44. Bringaud, F.; Baltz, T., *Mol. Biochem. Parasitol.*, **1992**, *52*, 111.
45. Tetaud, E.; Bringaud, F.; Chabas, S.; Barrett, M. P.; Baltz, T., *Proceedings of the National Academy of Sciences USA*, **1994**, *91*, 8278.
46. Barrett, M. P.; Tetaud, E.; Seyfang, A.; Bringaud, F. Baltz, T., *Mol. Biochem. Parasitol.*, **1998**, *91*, 195.
47. Fry, A. J.; Towner, P.; Holman, G. D.; Eisenthal, R., *Mol. Biochem. Parasitol.*, **1993**, *60*, 9.
48. Eisenthal, R.; Game, S.; Holman, G. D., *Biochim. Biophys. Acta*, **1989**, *985*, 81.

49. Walmsley, A. R.; Barrett, M. P.; Bringaud, F.; Gould, G. W., *Trends Biochem. Sci.*, **1998**, *23*, 476.
50. Brody, T., *Nutr. Biochem.*, **1994**, 336.
51. Bouteille, B.; Marie-Daragon, A.; Chauvière, G.; De Albuquerque, C.; Enanga, B.; Darde, M.-L.; Vallat, J.-M.; Périé, J.; Dumas, M., *Acta Trop.*, **1995**, *60*, 73.
52. Barrett, M. P.; Fairlamb, A. H.; Rousseau, B.; Chauvière, G.; Périé, J., *Biochem. Pharmacol.*, **2000**, *59*, 615.
53. Legros, D.; Evans, S.; Maiso, F.; Enyaru, J. C.; Mbulamberi, D., *Trans. R. Soc. Trop. Med. Hyg.*, **1999**, *93*, 439.
54. Stanghellini, A.; Josenando, J., *Trop. Med. Int. Health*, **2001**, *6*, 330.
55. Iten, M.; Mett, H.; Evans, A.; Enyaru, J. C.; Brun, R.; Kaminsky, R., *Antimicrob. Agents Chemother.*,**1997**, *41*, 1922.
56. Carter, N. S.; Berger, B. J.; Fairlamb, A. H., *J. Biol. Chem.*, **1995**, *270*, 28153.
57. Matovu, E.; Geiser, F.; Schneider, V.; Mäser, P.; Enyaru, J. C.; Kaminsky, R.; Gallati, S.; Seebeck, T., *Mol. Biochem. Parasitol.*, **2001**, *117*, 73.
58. Fevre, E. M.; Coleman, P. G.; Odiit, M.; Magona, J. W.; Welburn, S. C.; Woolhouse, M. E., *Lancet*, **2001**, 625.
59. Tetty, J.; Atsriku, C.; Chizyuka, G.; Slingenberg, J., *Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vectors*, No. 5, **2002**, 24 (http://www.ictv.org/Newsletters/Newsletter5/page24-25.pdf).
60. The University of North Carolina at Chapel Hill News Release, May **2006**, No. 275 (http://www.unc.edu/news/archives/may06/gatesgiftDB289052206.htm).
61. *DB289 versus TMP-SMX for Treatment of Acute Pneumocystis Jiroveci Pneumonia*, Identifier No. NCT00302341 (www.clinicaltrials.gov), September **2006**.
62. Jannin, J. A.; Cattand, P. B., *Curr. Opin. Infect. Dis.*, **2004**, *17*, 565.
63. McCann, P. P.; Pegg, A. E.; Sjoerdsma, A., *Inhibition of Polyamine Metabolism: Biological Significance and Basis for New Therapies*, Academic Press Inc., **1987**.
64. Bacchi, C. J.; Yartlett, N., *Acta Trop.*, **1993**, *54*, 225.
65. Yerlikaya, A., *Turk. J. Biochem.*, **2004**, *29*, 208.
66. Nishioka, K., *Polyamines in Cancer: Basic Mechanisms and Clinical Approaches*, Springer-Verlag, **1996**.
67. Fairlamb, A. H.; Cerami, A., *Ann. Rev. Microbiol.*, **1992**, *46*, 695.
68. Fairlamb, A. H.; Henderson, G. B.; Bacchi, C. J.; Cerami, A., *Mol. Biochem. Parasitol.*, **1987**, *24*, 185.
69. Marton, L. J.; Pegg, A. E., *Annu, Rev. Pharmacol. Toxicol.*, **1995**, *35*, 55.
70. Criss, W. E., *Turk. J. Med. Sci.*, **2003**, *33*, 195.

71. Morris, D. R.; Marton, L. J., *Polyamines in Biology and Medicine*, Marcel Dekker, New York, **1981**.
72. Tabor, C. W.; Tabor, H., *Ann. Rev. Biochem*., **1984**, *53*, 749.
73. McCann, P. P.; Pegg, A. E., *Pharmac. Ther*., **1992**, *54*, 195.
74. Pegg, A. E.; Williams-Ashman, H. G., *Biochem. J*., **1969**, *115*, 241.
75. Casara, P.; Marchal, P.; Wagner, J.; Danzin, C., *J. Am. Chem. Soc*., **1989**, *111*, 9111.
76. Pegg, A. E., *Cancer Res*., **1988**, *48*, 759.
77. Casero, R. A.; Celano, P.; Ervin, S. J.; Porter, C. W.; Bergeron, R. J.; Libby, P. R., *Cancer Res*., **1989**, *49*, 3829.
78. Wallace, H. M.; Fraser, A. V., *Biochem. Soc. Trans*., **2003**, *31*, 293.
79. Porter, C. W.; Bernacki, R. J.; Miller, J.; Bergeron, R. J., *Cancer Res*., **1993**, *53*, 581.
80. Müller, S.; Coombs, G. H.; Walter, R. D., *Trends Parasitol.*, **2001**, *17*, 242.
81. Bacchi, C. J.; Nathan, H. C.; Hutner, S. H.; McCann, P. P.; Sjoerdsma, A., *Science*, **1980**, *210*, 332.
82. Bacchi, C. J.; McCann, P. P; Nathan, H. C.; Hutner, S. H; Sjoerdsma, A., *Adv. Polyamine Res.*, **1982**, *4*, 221.
83. Bitonti, A. J.; Dumont, J. A.; McCann, P. P., *Biochem. J*., **1986**, *237*, 685.
84. Sufrin, J. R.; Speiss, A. J.; Kramer, D. L.; Libby, P. R.; Miller, J. T.; Bernacki, R. J.; Lee, Y.; Borchardt, T.; Porter, C. W., *J. Med. Chem*., **1991**, *34*, 2600.
85. Slater, L. A., *PhD Thesis*, **1998**, University of Glasgow.
86. Parker, L. L., *PhD Thesis*, **2003**, University of Glasgow.
87. Girault, S.; Grellier, P.; Berecibar, A.; Maes, L.; Lemiere, P.; Mouray, E.; Davioud-Charvet, E.; Sergheraert, C., *J. Med. Chem.*, **2001**, *44*, 1658.
88. Sibert, J. W.; Cory, A. H.; Cory, J. G., *Chem. Commun.*, **2002**, 154.
89. Jones, S. W.; *PhD Thesis*, **2006**, University of Glasgow.
90. Richman, J. E.; Atkins, T. J., *J. Am. Chem. Soc*., **1974**, *96*, 2268.
91. Atkins, T. J.; Richman, J. E.; Oettle, W. F., *Org. Synth*., **1978**, *58*, 86.
92. Stetter, H.; Roos, E. E., *Chem. Ber*., **1954**, *87*, 566.
93. Krakoviak, K. E.; Bradshaw, J. S.; Zamecka-Krakoviak, D. J., *Chem. Rev*., **1989**, *89*, 929.
94. Bradshaw, J. S.; Krakoviak, K. E.; Izatt, R. M., *Aza-Crown Macrocycles: The Chemistry of Heterocyclic Compounds*, Wiley and Sons, New York, **1993**.
95. Kumar, K.; Tweedle, M., *Pure Appl. Chem.*, **1993**, *65*, 51.
96. Caravan, P.; Ellison, J. J.; McMurray, T. J.; Lauffer, R. B., *Chem. Rev*., **1999**, *99*, 2293.
97. Lui, S.; Edwards, S. D., *Bioconjugate Chem*., **2001**, *12*, 7.

98. Stetter, H.; Roos, E. E., *Chem. Ber.*, **1955**, *88*, 1390.
99. Vriesema, B. K.; Buter, J.; Kellogg, R. M., *J. Org. Chem.*, **1984**, *49*, 110.
100. Dijkstra, G.; Kruizinga, W. H.; Kellogg, R. M., *J. Org. Chem.*, **1987**, *52*, 4230.
101. Parker, D., *Macrocycle Synthesis: a Practical Approach*, Oxford University Press, Oxford, **1996**.
102. Beesley, R. M.; Ingold, C. K.; Thorpe, J. F., *J. Chem. Soc.*, **1915**, *107*, 1080.
103. Ingold, C. K.; Beesley, R. M.; Thorpe, J. F., *J. Chem. Soc.*, **1921**, *119*, 305.
104. Lukyanenko, N. G.; Basok, S. S.; Filonova, L. K., *J. Chem. Soc. Perkin Trans. I*, **1988**, 3141.
105. Athey, P. S.; Kiefer, G. E., *J. Org. Chem.*, **2002**, *67*, 4081.
106. Weisman, G. R.; Reed, D. P., *J. Org. Chem.*, **1996**, *61*, 5186.
107. Weisman, G. R.; Reed, D. P.; *Org. Synth.*, **2001**, *78*, 73.
108. Ferrari, M.; Giovenzana, G.; Palmisano, G.; Sisti, M., *Synth. Commun.*, **2000**, *30*, 15.
109. Herve, G.; Bernard, H.; Toupet, L.; Handel, H., *Eur. J. Org. Chem.*, **2000**, *1*, 33.
110. Herve, G.; Bernard, H.; Le Bris, N.; Le Baccon, M.; Yaouane, J. J.; Handel, H., *Tetrahedron Lett.*, **1999**, *40*, 2517.
111. Patai, S., *The Chemistry of Amidines and Imidates*, Vol. 1, Wiley and Sons, New York, **1975**.
112. Anderson, F. M., *PhD Thesis*, **1999**, University of Glasgow.
113. Dale, J.; Kristiansen, P. O., *Acta Chem. Scand.*, **1972**, *26*, 1471.
114. Ciampolini, M.; Fabbrizzi, L.; Licchelli, M.; Perotti, A.; Poggi, A.; Pezzin, F., *Inorg. Chem.*, **1986**, *25*, 4131.
115. Clegg, C.; Iveson, P. B.; Lockhart, J. C., *J. Chem. Soc. Dalton Trans.*, **1992**, *2*, 3291.
116. Sessler, J. L.; Sibert, J. W., *Tetrahedron*, **1993**, *49*, 8727.
117. Sabitha, G.; Abraham, S.; Reddy, B. V. S.; Yadav, J. S., *Synlett.*, **1999**, *11*, 1745.
118. Petraskiewicz, M.; Jurczak, J., *Tetrahedron*, **1984**, *40*, 2967.
119. Tundo, P., *Tetrahedron Lett.*, **1978**, *47*, 4693.
120. Garrity, M. L.; Brown, G. M.; Elbert, J. E.; Sachleben, R. A., *Tetrahedron Lett.*, **1993**, *34*, 5531.
121. Cox, J. P. L.; Craig, A. S.; Helps, I. M.; Janowski, K. J.; Parker, D.; Eaton, M. A. W.; Millican, A. T.; Miller, K.; Beeley, N. R. A.; Boyce, B. A., *J. Chem. Soc. Perkin Trans. I*, **1990**, 2567.
122. Moi, M. K.; Meares, C. F., *J. Am. Chem. Soc.*, **1988**, *110*, 6266.
123. Takenouchi, K.; Tabe, M.; Watanabe, K.; Hazato, A.; Kato, Y.; Shionoya, M.; Koike, T.; Kimura, E., *J. Org. Chem.*, **1993**, *58*, 6895.

124. McMurry, T. J.; Brechbiel, M.; Kumar, K.; Gansow, O. A., *Bioconjugate Chem.*, **1992**, *3*, 108.
125. Renn, O.; Meares, C. F., *Bioconjugate Chem.*, **1992**, *3*, 563.
126. Edlin, C. D.; Faulkner, S.; Parker, D.; Wilkinson, M. P., *Chem. Commun.*, **1996**, 1249.
127. Rai, P. K.; Prasad, R. N., *Synth. React. Inorg. Met. Org. Chem.*, **1994**, *24*, 749.
128. Edlin, C. D.; Faulkner, S.; Parker, D.; Wilkinson, M. P.; Woods, M.; Lin, J.; Lasri, E.; Neth, F.; Port, M., *New J. Chem.*, **1998**, 1359.
129. Marchand, N. J.; Grée, D. M.; Martelli, J. T.; Grée, R. L., *J. Org. Chem.*, **1996**, *61*, 6063.
130. Paul, S.; Gupta, M.; Gupta, R.; Loupy, A., *Synthesis*, **2002**, *1*, 75.
131. Paulmier, C., *Selenium Reagents and Intermediates in Organic Synthesis*, Vol. 4, Pergamon Press, **1986**.
132. Fuson, R. C.; Gray, H.; Gouza, J. J., *J. Am. Chem. Soc.*, **1939**, *61*, 1937.
133. http://www.syracuseresearch.com/esc/est_kowdemo.htm, September **2006**.
134. Hansch, C.; Fujita, T., *J. Am. Chem. Soc.*, **1964**, *86*, 1616.
135. Earll, M., *A guide to Log P and pKa measurements and their use* (http://www.raell.demon.co.uk/chem/logp/logpka.html), September **2006**.
136. O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F., *Eur. J. Biochem.*, **2000**, *267*, 5421.
137. Klenke, B.; Stewart, M. L.; Barrett, M. P.; Brun, R.; Gilbert, I., *J. Med. Chem.*, **2001**, *44*, 3440.
138. Baliani, A.; Bueno, G. J.; Stewart, M. L.; Yardley, V.; Brun, R.; Barrett, M. P.; Gilbert, I. H., *J. Med. Chem.*, **2005**, *48*, 5570..
139. Ireland, R. E.; Anderson, R. C.; Badoud, R.; Fitzsimmons, B. J.; McGarvey, G. J.; Thaisrivongs, S.; Wilcox, C. S., *J. Am. Chem. Soc.*, **1983**, *105*, 1988.
140. Kondo, Y.; Kojima, S.; Sakamoto, T., *J. Org. Chem.*, **1997**, *62*, 6507.
141. Park, C,-H.; Givens, R. S., *J. Am. Chem. Soc*, **1997**, *119*, 2453.
142. Carpino, L. A.; Han, G. Y.; *J. Org. Chem.*, **1972**, *37*, 3404.
143. Helps, I. M.; Morphy, J. R.; Chapman, J., *Tetrahedron*, **1989**, *45*, 219.
144. Patai, S., *The Chemistry of Amidines and Imidates*, Vol. 2, Wiley and Sons, New York, **1991**.
145. Partridge, M. W.; Smith, A., *J. Chem. Soc., Perkin Trans. 1*, **1973**, 453.
146. Weintraub, L.; Oles, S. R.; Kalish, N., *J. Org. Chem.*, **1968**, *33*, 1679.
147. Kikimoto, M.; Ogata, S.; Mochizuki, A.; Imai, Y., *Chem. Lett.*, **1984**, 821.
148. Nii, Y.; Okano, K.; Kobayashi, S.; Ohno, M., *Tetrahedron Lett.*, **1979**, *27*, 2517.

149. Zablocki, J. A.; Miyano, M.; Garland, R. B.; Pireh, D.; Schretzman, L.; Rao, S. N.; Lindmark, R. J.; Panzer-Knodle, S. G.; Nicholson, N. S.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P., *J. Med. Chem*., **1993**, *36*, 1811.
150. Roger, R.; Neilson, D. G., *Chem. Rev.*, **1961**, *61*, 179.
151. Pinner, A.; *Ber*., **1883**, *16*, 1654.
152. Pinner, A., *Ber*., **1884**, *17*, 178.
153. Dabak, K., *Turk. J. Chem*., **2002**, *26*, 547.
154. Baksheev, A. N.; Gavirov, N. I., *Chem. Abstracts*, **1953**, *47*, 8641.
155. Štefanič, P.; Simončič, Z.; Breznik, M.; Plavec, J.; Anderluh, M.; Addicks, E.; Giannis, A.; Kikelj, D., *Org. Biol. Chem*., **2004**, *2*, 1511.
156. Bacchi, C. J.; Weiss, L. M.; Lane, S.; Frydman, B.; Valasinas, A.; Reddy, V.; Sun, J. S.; Marton, L. J., Khan, I. A.; Moretto, M.; Yarlett, N.; Wittner, M., *Antimicrob. Agents Chemother*., **2002**, *46*, 55.
157. Boussard, C.; Klimkait, T.; Mahmood, N.; Pritchard, M.; Gilbert, I. H., *Bioorg. Med. Chem. Lett*., **2004**, *14*, 2673.
158. Iorga, B.; Ricard, L.; Savignac, P., *J. Chem. Soc., Perkin Trans. 1*, **2000**, 3311.
159. Huang, X.; Anderson, K. W.; Zim, D.; Jiang, L.; Klapars, A.; Buchwald, S. L., *J. Am. Chem. Soc*., **2003**, *125*, 6653.
160. Wolfe, J. P.; Tomori, H.; Sadighi, J. P.; Yin, J.; Buchwald, S. L., *J. Org. Chem*., **2000**, *65*, 1158.
161. Stewart, M. L.; Bueno, G. J.; Baliani, A.; Klenke, B.; Brun, R.; Brock, J. M.; Gilbert, I. H.; Barrett, M. P., *Antimicrob. Agents Chemother*., **2004**, *48*, 1733.
162. Keenan, R. M.; Callahan, J. F.; Samanen, J. M.; Bondinell, W. E.; Calvo, R. R.; Chen, L.; DeBrosse, C.; Eggleston, D. S.; Haltiwanger, R. C.; Hwang, S. M.; Jakas, D. R.; Ku, T. W.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M. F.; Southall, L. S.; Uzinskas, I.; Vasko-Moser, J. A.; Venslavsky, J. W.; Wong, A. S.; Huffman, W. F., *J. Med. Chem*., **1999**, *42*, 545.
163. Yee, Y. K.; Tebbe, A. L.; Linebarger, J. H.; Beight, D. W.; Craft, T. J.; Gifford-Moore, D.; Goodson, T.; Herron, D. K.; Klimkowski, V. J.; Kyle, J. A.; Sawyer, J. S.; Smith, G. F.; Tinsley, J. M.; Towner, R. D.; Weir, L.; Wiley, M. R., *J. Med. Chem*., **2000**, *43*, 873.
164. Xia, W.; Scheffer, J. R.; Botoshansky, M.; Kaftory, M., *Org. Lett*., **2005**, *7*, 1315.
165. Stetter, H.; Mayer, K. H., *Chem. Ber*., **1961**, 1410.
166. Goossen, L. J.; Döhring, A., *Synlett*, **2004**, *2*, 263.
167. Hamilton, D. J.; Sutherland, A., *Tetrahedron Lett*., **2004**, *45*, 5739.
168. http://www-micro.msb.le.ac.uk/224/Malaria.html, October **2006**.

169. Barrett, A. J.; Rawlings, N. D.; Woessner, J. F., *Handbook of Proteolytic Enzymes*, Elsevier Academic Press, San Diego, CA, **2004**.
170. http://probes.invitrogen.com/servlets/spectraviewer?fileid1=143iggp8, October **2006**.
171. http://www.cdc.gov/NCIDOD/DPD/parasites/leishmania/factsht_leishmaniasis.htm, October **2006**.
172. Jadassohn, W.; Fierz, H. E.; Vollenweider, H., *Helv. Chim. Acta.*, **1944**, *27*, 1384.
173. Collins, A. N.; Sheldrake, G. N.; Crosby, J., *Chirality in Industry*, Wiley, New York, **1992**.
174. Wainer, I. W., *Drug Stereochemistry: Analytical Methods and Pharmacology*, 2nd Edition, Marcel Dekker, New York, **1993**.
175. Aboul-Enein, H. Y.; Wainer, I. W., *The Impact of Stereochemistry on Drug Development and Use*, Wiley, New York, **1997**.
176. Smith, D. F., *Handbook of Stereoisomers: Therapeutic Drugs*, Boca Raton, CRC Press, **1989**.
177. Agranat, I.; Caner, H.; Caldwell, J., *Nature Reviews: Drug Discovery 1*, **2002**, 753.
178. Belokon, Y. N.; Pritula, L. K.; Tararov, V. I.; Bakhmutov, V. I.; Struchkov, Y. T.; Timofeeva, T. V.; Belikov, V. M., *J. Chem. Soc., Dalton Trans.*, **1990**, 1867.
179. Malkov, A. V.; Spoor, P.; Vinader, V.; Kočovský, P., *Tetrahedron Lett.*, **2001**, *42*, 509.
180. Reihlen, H.; Knöpfle, L., *Leibigs Ann. Chem.*, **1936**, *523*, 199.
181. Mishra, A. K.; Panwar, P.; Chopra, M.; Sharma, R. K.; Chatal, J.-F., *New. J. Chem.*, **2003**, *27*, 1054.
182. Thomas, J. B.; Atkinson, R. N.; Vinson, N. A.; Catanzaro, J. L.; Perretta, C. L.; Fix, S. E.; Mascarella, S. W.; Rothman, R. B.; Xu, H.; Dersch, C. M.; Cantrell, B. E.; Zimmerman, D. M.; Carroll, F. I., *J. Med. Chem.*, **2003**, *46*, 3127.
183. www.cancerresearchuk.org, October **2006**.
184. www.cancerhelp.org.uk, October **2006**.
185. *Alkylating Agents: The Janus Effect* http://www.chemheritage.org/EducationalServices/pharm/chemo/readings/alkyl.htm, September **2006**.
186. Jacques, J., *New J. Chem.*, **1991**, *15*, 3.
187. Substance Profile: Mustard Gas, CAS No. 505-60-2, *Report on Carcinogens*, 11th Edition, **1980**.
188. Tallet, K., *Mustard Gas Poisoning*, http://ourworld.compuserve.com/homepages/kylet1/gas.htm, October **2006**.

189. Sidell, F. R.; Urbanetti, J. S.; Smith, W. J.; Hurst, C. G., *Textbook of Military Medicine*: Chapter 7 http://www.bordeninstitute.army.mil/cwbw/default_index.htm, October **2006**.
190. Ritchie, M., *Biographical Memoirs: Alfred Gilman*, http://www.nap.edu/html/biomems/agilman.html, October **2006**.
191. Sunters, A.; Springer, C. J.; Bagshawe, K.; Souhami, R. L.; Hartley, J. A., *Biochem. Pharmacol*., **1992**, *44*, 59.
192. Mattes, W. B.; Hartley, J. A.; Kohn, K. W., *Nucleic Acids Res.*, **1986**, *14*, 2971.
193. Kohn, K. W.; Hartley, J. A.; Mattes, W. B., *Nucleic Acids Res.*, **1987**, *15*, 10531.
194. http://pharmacology.unmc.edu/cancer/alkylate.htm, September **2006**.
195. Chabner, B. A.; Longo, D. L., *Cancer Chemotherapy and Biotherapy: Principles and Practice*, Lippincott-Raven, Philadelphia, PA, **1996**.
196. Grando, S. A., *Life Sci.*, **2003**, *72*, 2135.
197. Birdsall, N. J.; Burgen, A. S.; Hulme, E. C., *Mol. Pharmacol*., **1978**, *14*, 723.
198. Remers, W. A., *Antineoplastic Agents*, Vol. 3, Wiley and Sons, New York, **1984**.
199. Henderson, N. D., *PhD Thesis*, University of Glasgow, **1994**.
200. Henderson, N. D.; Plumb, J. A.; Robins, D. J.; Workman, P., *Anti-Cancer Drug Des.*, **1996**, *11*, 421.
201. Henderson, N. D.; Lacy, S. M.; O'Hare, C. C.; Hartley, J. A.; McClean, S.; Wakelin, L. P. G.; Kelland, L. R.; Robins, D. J., *Anti-Cancer Drug Des.*, **1998**, *13*, 749.
202. Anderson, F. M.; O'Hare, C. C.; Hartley, J. A.; Robins, D. J., *Anti-Cancer Drug Des.*, **2000**, *15*, 119.
203. Alexander, V., *Chem. Rev.*, **1995**, *95*, 273.
204. Hancock, R. D.; Maumela, H.; Desousa, A. S., *Coord. Chem. Rev.*, **1996**, *148*, 315.
205. Motekaitis, R. J.; Rogers, B. E.; Reichert, D. E.; Martell, A. E.; Welch, M. J., *Inorg. Chem.*, **1996**, *35*, 3821.
206. Weitl, F. L.; Raymond, K. N., *J. Am. Chem. Soc*., **1979**, *101*, 2728.
207. Parker, L. L.; Anderson, F. M.; O'Hare, C. C.; Lacy, S. M.; Bingham, J. P.; Robins, D. J.; Hartley, J. A., *Bioorg. Med. Chem.*, **2005**, *13*, 2389.
208. Parker, L. L.; Gowans, N. D.; Jones, S. W.; Robins, D. J., *Tetrahedron*, **2003**, *59*, 10165.
209. Parker, L. L.; Lacy, S. M.; Farrugia, L. J.; Evans, C.; Robins, D. J.; O'Hare, C. C.; Hartley, J. A.; Jaffar, M.; Stratford, I. J., *J. Med. Chem*., **2004**, *47*, 5683.
210. Boyce, B. A.; Carroy, A.; Lehn, J. M.; Parker, D., *J. Chem. Soc., Chem. Commun*., **1984**, 1546.
211. Lee, M.; Garbiras, B. J., *Synth. Commun*., **1994**, *24*, 3129.

212. Watanabe, T.; Kinoyama, I.; Kakefuda, A.; Okazaki, T.; Takizawa, K.; Hirano, S.; Shibata, H.; Yanagisawa, I., *Chem. Pharm. Bull*., **1997**, *45*, 996.
213. BuØen, S.; Dale, J.; Krane, J., *Acta Chem. Scand. B*, **1984**, *38*, 773.
214. Rejzek, M.; Wimmer, Z.; Saman, D.; Ricankova, M.; Nemec, V., *Helv. Chim. Acta*, **1994**, *77*, 1241.
215. Koike, T.; Kajitani, S.; Nakamura, I.; Kimura, E.; Shiro, M., *J. Am. Chem. Soc*., **1995**, *117*, 1210.
216. Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M., *J. Chem. Soc., Perkin Trans. 2*, **1999**, 493.
217. Shinoda, S.; Nishimura, T.; Tadokoro, M.; Tsukube, H., *J. Org. Chem*., **2001**, *66*, 6104.
218. Stephens, A. K. W.; Lincoln, S. F., *J. Chem. Soc., Dalton Trans*., **1993**, 2123.
219. Baker, W. C.; Choi, M. J.; Hill, D. C.; Thompson, J. L.; Petillo, P. A., *J. Org. Chem*., **1999**, *64*, 2683.
220. Jamieson, A. G.; Sutherland, A., *Org. Biomol. Chem*., **2005**, *3*, 735.
221. Lambert, T. N.; Dasaradhi, L.; Hubert, V. J.; Gopalan, A. S.; *J. Org. Chem*., **1999**, *64*, 6097.
222. Menger, F. M.; Lee, J.-J., *J. Org. Chem*., **1993**, *58*, 1909.
223. Focher, F.; Ubiali, D.; Pregnolato, M.; Zhi, C.; Gambino, J.; Wright, G. E.; Spadari, S., *J. Med. Chem*., **2000**, *43*, 2601.
224. Epple, R.; Wallenborn, E.-U.; Carell, T., *J. Am. Chem. Soc.*, **1997**, *119*, 7440.
225. Itoh, T.; Inoue, H.; Emoto, S., *Bull. Chem. Soc. Jpn*, **2000**, *73*, 409.
226. Stork, G.; Takahashi, T., *J. Am. Chem. Soc.*, **1977**, *99*, 1275.
227. Workman, P., *Mol. Biosyst.*, **2005**, *1*, 17.
228. Kaelin, W. G., *Science*, **1998**, *281*, 57.
229. Koshland, D. E., *Science Magazine*, **1993**, 1953.
230. Strachan, T.; Read, A. P., *Human Molecular Genetics 2.*, *Cancer Genetics*, **1999**.
231. Bell, S.; Klein, C.; Muller, L.; Hansen, S.; Buchner, J., *J. Mol. Biol*., **2002**, *322*, 917.
232. Blagosklonny, M. V., *Int. J. Cancer*, **2002**, *98*, 161.
233. Chène, P., *Nature*, **2003**, *3*, 102.
234. Michael, D.; Oren, M., *Curr. Opin. Genet. Dev.*, **2002**, *12*, 53.
235. Lai, Z.; Yang, T.; Kim, Y. B.; Sielecki, T. M.; Diamond, M. A.; Strack, P.; Rolfe, M.; Caligiuri, M.; Benfield, P. A.; Auger, K. R.; Copeland, R. A., *Proc. Natl Acad. Sci. USA*, **2002**, *99*, 14734.
236. http://en.wikipedia.org/wiki/Ubiquitin, October **2006**.

237. Issaeva, N.; Bozko, P.; Enge, M.; Protopopova, M.; Verhoef, L. G. G. C.; Masucci, M.; Pramanik, A.; Selivanova, G., *Nat. Med.*, **2004**, *10*, 1321.
238. Klein, C.; Vassilev, L. T., *Br. J. Cancer*, **2004**, *91*, 1415.
239. Stoll, R.; Renner, C.; Hansen, S.; Palme, S.; Klein, C.; Belling, A.; Zeslawski, W.; Kamionka, M.; Rehm, T.; Muhlhahn, P.; Schumacher, R.; Hesse, F.; Kaluza, B.; Voelter, W.; Engh, R. A.; Holak, T. A., *Biochem.*, **2001**, *40*, 336.
240. Zhao, J.; Wang, M.; Chen, J.; Luo, A.; Wang, X.; Wu, M.; Yin, D.; Liu, Z., *Cancer Lett.*, **2002**, *183*, 69.
241. Duncan, S. J.; Gruschow, S.; Williams, D. H.; McNicholas, C.; Purewal, R.; Hajek, M.; Gerlitz, M.; Martin, S.; Wrigley, S. K.; Moore, M., *J. Am. Chem. Soc.*, **2001**, *123*, 554.
242. Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovic, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E. A., *Science*, **2004**, *303*, 844.
243. Davydov, I. V.; Woods, D.; Safiran, Y. J.; Oberoi, P.; Fearnhead, H. O.; Fang, S.; Jensen, J. P.; Weissman, A. M.; Kenten, A. M.; Vousden, K. H., *J. Biomol. Screen*, **2004**, *9*, 695.
244. Yang, Y.; Ludwig, R. L.; Jensen, J. P.; Pierre, S. A.; Medaglia, M. V.; Davydov, I. V.; Safiran, Y. J.; Oberoi, P.; Kenten, A. M.; Phillips, A. C.; Weissman, A. M.; Vousden, K. H., *Cancer Cell*, **2005**, *7*, 547.
245. Wilson, J. M.; Henderson, G.; Black, F.; Sutherland, A.; Ludwig, R. L.; Vousden, K. H.; Robins, D. J., *Bioorg. Med. Chem*. in Press.
246. Conrad, M.; Zart, A. *Chem. Ber.*, **1906**, *39*, 2283.
247. Duffy, K. J.; Darcy, M. G.; Delorme, E.; Dillon, S. B.; Epplley, D. F.; Erickson-Miller, C.; Giampa, L.; Hopson, C. B.; Huang, Y.; Keenan, R. M.; Lamp, P.; Leong, L.; Liu, N.; Miller, S. G.; Price, A. T.; Rosen, J.; Shah, R.; Shaw, T. N.; Smith, H.; Stark, K. C.; Tian, S. S.; Tyree, C.; Wiggall, K. J.; Zhang, L.; Luengo, J., *J. Med. Chem.*, **2001**, *44*, 3720.
248. Fergusson, J., *Fourth Year Project Report*, University of Glasgow, **2001**.
249. Abd El Basat Hassanein, A. Z., *Synth. Commun*., **2000**, *30*, 3883.
250. Abass, M., *Synth. Commun*., **2000**, *30*, 2735.
251. Jobson, N. K., *Fourth Year Project Report*, University of Glasgow, **2004**.
252. Fodor, G.; Kovács, Ö., *J. Am. Chem. Soc.*, **1949**, *71*, 1045.
253. Pulacchini, S.; Watkinson, M., *Eur. J. Org. Chem.*, **2001**, *22*, 4233.
254. Hoye, R. C.; Richman, J. E.; Dantas, G. A.; Lightbourne, M. F.; Shinneman, L. S., *J. Org. Chem*., **2001**, *66*, 2722.

255. Clark, A. J.; Echerique, J.; Haddleton, D. M.; Straw, T. A.; Taylor, P. C., *J. Org. Chem.*, **2001**, *66*, 8687.
256. Liu, Z.; Chen, Z.-C.; Zheng, Q.-G., *Org. Lett.*, **2003**, *5*, 3321.
257. Jagt, D. L. V.; Han, L.-P. B.; Lehman, C. H., *J. Org. Chem.*, **1972**, *37*, 4100.
258. Härter, H. P.; Liisberg, S., *Acta Chem. Scand.*, **1968**, *22*, 2685.
259. Venien, F.; Brault, A.; Kerfanto, M., *Hebd. Seances Acad. Sci. Ser. C*, **1968**, *266*, 1650.
260. Anderson, E. L.; Casey, J. E.; Emas, M.; Force, E. E.; Jensen, E. M.; Matz, R. S.; Rivard, D. E., *J. Med. Chem.*, **1963**, *6*, 787.
261. Arnold, R. T.; Fuson, R. C., *J. Am. Chem. Soc.*, **1936**, 58, 1295.
262. Kipnis, F.; Ornfelt, J., *J. Am. Chem. Soc.*, **1948**, *70*, 3948.
263. Krönke, B., *Chem. Ber.*, **1936**, *69*, 2006.
264. Howe, R.; Crowther, A. F.; Stephenson, J. S.; Rao, B. S.; Smith, L. H., *J. Med. Chem.*, **1968**, *11*, 1000.
265. Fuson, R. C.; Gray, H.; Gouza, J. J., *J. Am. Chem. Soc.*, **1939**, *61*, 1937.
266. Walker, D. F.; L'Italien, Y. J.; Pearlman, W. M, Banks, C. K., *J. Am. Pharm. Assoc.*, **1950**, *39*, 393.
267. Kodaka, M.; Yli-Kauhaluoma, J. T.; Hase, A., *Acta Chem. Scand.*, **1999**, *53*, 493.
268. Park, C.-H.; Givens, R. S., *J. Am. Chem. Soc.*, **1997**, *119*, 2453.
269. Southam, R. M.; Whiting, M. C., *J. Chem. Soc., Perkin Trans. 2*, **1982**, 579.
270. Maender, O. W.; Janzen, E. G.; *J. Org. Chem.*, **1969**, *34*, 4072.
271. Eisen, M. S.; Kapon, M., *J. Chem. Soc., Dalton Trans.*, **1994**, 3507.
272. Badr, M. Z. A.; Aly, M. M.; Mahgoub, S. A.; Fahmy, A. M.; Atallah, A. A., *Bull. Chem. Soc. Jpn*, **1988**, *61*, 1779.
273. Tiecco, M.; Testaferri, L.; Tingoli, M.; Chianelli, D.; Montanucci, M., *Synth. Commun.*, **1983**, *13*, 617.
274. Raz, B.; Iten, M.; Grether-Buhler, Y.; Kaminsky, R.; Brun, R., *Acta Trop.*, **1997**, *68*, 139.
275. Hirumi, H.; Hirumi, K., *J. Parasitol.*, **1989**, *75*, 985.
276. Neilson, D. G.; Ewing, D. F., *J. Chem. Soc.*, **1966**, 393.
277. Huang, X.; Rickman, B. H.; Borhan, B.; Berova, N.; Nakanishi, K., *J. Am. Chem. Soc.*, **1998**, *120*, 6185.
278. Rivero, I. A.; Heredia, S.; Ochoa, A., *Synth. Commun.*, **2001**, *31*, 2169.
279. Laurent, S.; Elst, L. V.; Houzé, S.; Guérit, N.; Muller, R. N., *Helv. Chim. Acta*, **2000**, *83*, 394.

280. Broan, C. J.; Cox, J. P. L.; Craig, A. S.; Kataky, R.; Parker, D.; Harrison, A.; Randall, A. M.; Ferguson, G., *J. Chem. Soc., Perkin Trans. 2*, **1991**, 87.

281. Verardo, G.; Toniutti, N.; Giumanini, A. G., *Can. J. Chem.*, **1998**, *76*, 1180.