# Synthesis of Biologically Active Heterocyclic Compounds

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

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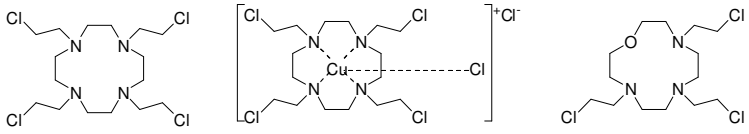
## Abstract

More than 11 million people worldwide are diagnosed with cancer every year. New cancer drugs are required that are more effective and selective. Nitrogen mustard alkylating agents crosslink DNA inhibiting transcription and replication. Use of the mustard pharmacophore as part of a macrocycle allows metal complexation and produces a prodrug. Hypoxic tumour cells have increased concentrations of reductase enzymes which could lead to reduction of the complex *in situ* and release of a cytotoxic drug.

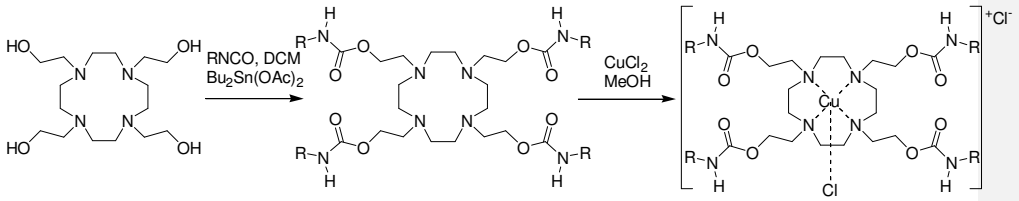
Human African Trypanosomiasis is commonly known as Sleeping Sickness and affects over 36 countries of sub-Saharan Africa. It is transmitted to humans by the tsetse fly which carries the parasitic subspecies *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. Any compounds synthesised would also be tested to assess their potential as anti-parasitic agents.

Parker synthesised a range of polyazamacrocycles. Testing of compound **A** *in vitro* gave highly efficient DNA crosslinking activity. Copper complexes were formed of the macrocycles and **B** was found to be 24 times more toxic against hypoxic cells than oxic cells thus exploiting tumour hypoxia and creating a selective drug. Jones synthesised a range of oxaazamacrocycles such as **C** which when tested *in vitro* exhibited comparable cross-linking activity to the azamacrocycles although it proved impossible to synthesise the corresponding copper complexes.

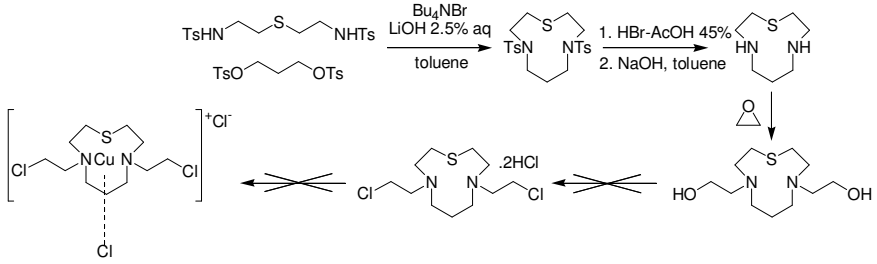



**A** **B** **C**

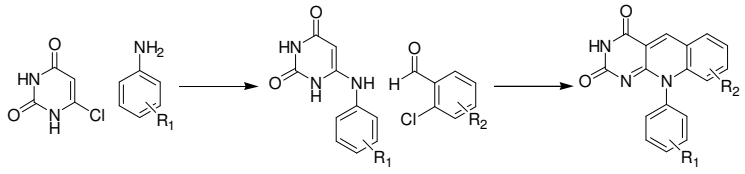
It was decided to vary the leaving group on the alkylating arms to see if the DNA crosslinking results could be improved. Eight carbamates and the corresponding copper complexes were synthesised. The R-groups were alkyl and aromatic. Anti-cancer DNA crosslinking and hypoxia selectivity results were disappointing however, a number of compounds displayed significant activity when tested against *T. brucei*.




A range of thiaazamacrocycles would complete the set of heteroatom-containing macrocycles (N, O, S) and might produce good DNA crosslinking results. It might also be possible to synthesise the corresponding copper complexes producing prodrugs. Six thiaazamacrocycles were synthesised and 2-hydroxyethyl arms were attached. However it proved impossible to isolate the desired alkylating agents with the 2-chloroethyl arms.




In the body, the p53 protein activates the transcription of specific genes. In healthy cells, the levels of p53 have to be kept to a minimum to allow the normal running of the cell, e.g. growth and replication. This function is carried out by the HDM2 protein, which forms an auto-regulatory feedback loop with p53. In some tumours, the p53 function is disrupted due to genetic mutations of p53. However other tumours possess 'wild type' p53 – this type of p53 has lost the ability to respond to oncogenic stress due to over-expression of HDM2. Drugs that inhibit HDM2 should cause stabilisation of p53 and induce apoptosis in cancer cells. A small library of 5-deazaflavins were synthesised and biologically tested producing some interesting biological results.



## Acknowledgement

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A big thanks to Stephen, Kathryn and Caroline from the Robins group who welcomed me into the Henderson Lab with open arms and lots of laughs! I became a Sutherland group member so a big thanks to Andy J, Rosie, Kate, Nicola, Mike and Louise – you know it's going to be quiet without me. I can't leave out all the Hartley group members including Stuart, Carolyn, Louis, Calver, Caroline, Ching and Linsey. I'll remember the lab move to the Loudon Lab for a long time!

Thanks to all my Glagow Uni friends especially the 'girlies' – Gemma, Kate, Katrina and Nicola. It was claimed that I came from the dark side (University of Strathclyde) but you accepted me very quickly. I had a blast and there are photos to prove it!

I need to thank my Mum and Dad for their love and support (emotional and financial!) and my siblings (Val, Lorna and Stewart) who make me laugh a lot. Special thanks to Calum who has put up with fewer tears than he thought he would during the writing of this thesis.

## Author's Declaration

This thesis represents the original work of Jennifer Margaret Wilson unless explicitly stated otherwise in the text. The research was carried out at the University of Glasgow in the Henderson Laboratory and the Loudon Laboratory under the supervision of Prof. David J. Robins and Dr Andrew Sutherland during the period of October 2004 to September 2007. Portions of the work described herein have been published elsewhere as listed below.

Synthesis of 5-deazaflavin derivatives and their activation of p53 in cells – J. M. Wilson, G. Henderson, F. Black, A. Sutherland, R. L. Ludwig, K. H. Vousden and D. J. Robins, *Bioorg. Med. Chem.*, 2007, **15**, 77.

Synthesis, characterisation and anti-protozoal activity of carbamate-derived polyazamacrocycles – J. M. Wilson, F. Giordani, L. J. Farrugia, M. P. Barrett, D. J. Robins and A. Sutherland, *Org. Biomol. Chem.*, 2007, **5**, 3651.

# Abbreviations

| | |
|---|---|
| abs. | absolute |
| AcOH | acetic acid |
| ADEPT | antibody directed enzyme prodrug therapy |
| ADP | adenosine diphosphate |
| AIDS | autoimmune deficiency syndrome |
| ALDH | aldehyde dehydrogenase |
| ARF | auxin reponse factor |
| aq. | aqueous |
| BBB | blood brain barrier |
| BC | before Christ |
| BCEA | *N,N*-bis(2-chloroethyl)amine |
| Boc | *tert*-butoxycarbonyl |
| bs | broad singlet |
| $^{o}C$ | degrees Celsius |
| $CDCl_3$ | deuterated chloroform |
| CI | chemical ionisation |
| CML | chronic myeloid leukaemia |
| CNS | central nervous system |
| conc. | concentrated |
| cyclen | 1,4,7,10-tetraazacyclododecane |
| d | days or doublet |
| dd | doublet of doublets |
| DMF | *N,N*-dimethylformamide |
| DMSO | dimethylsulfoxide |
| DNA | deoxyribose nucleic acid |
| $EC_{50}$ | concentration of a drug to inhibit 50% of growth |
| EI | electron impact ionisation |
| EPO | erythropoietin |
| Et | ethyl |
| EtOH | ethanol |
| eq. | equivalents |
| FAB | fast atom bombardment |
| g | gram |
| GDEPT | gene directed enzyme prodrug therapy |

| | |
|---|---|
| h | hours |
| HAT | Human African Trypanosomiasis |
| HBr | hydrobromic acid |
| HCl | hydrochloric acid |
| HDM2 | human double minute gene 2 |
| HEK | human embryonic kidney |
| HIV | human immunodeficiency virus |
| HPDC | hypoxia activated prodrugs of diffusible cytotoxins |
| HPLC | high performance liquid chromatography |
| HSC | hypoxia selective cytotoxin |
| Hz | hertz |
| $IC_{50}$ | concentration of a drug to inhibit 50% of growth |
| IR | infra-red |
| *J* | NMR spectra coupling constant |
| kDa | kiloDalton |
| kg | kilogram |
| L | litre or ligand |
| µL | microlitre |
| Leu | Leucine |
| lit. | literature |
| m | multiplet |
| M | molar or metal centre of complex |
| $\lambda_{max}$ | maximum UV absorbance |
| MDM2 | mouse double minute gene 2 |
| Me | methyl |
| MeOH | methanol |
| mg | milligram |
| MHz | megaHertz |
| mmol | millimole |
| mM | millimolar |
| µM | micromolar |
| mL | millilitre |
| mp | melting point |
| Ms | methanesulfonyl |
| MSF | Médecins Sans Frontières |
| NMR | nuclear magnetic resonance |

| | |
|---|---|
| ODC | ornithine decarboxylase |
| P | partition coefficient |
| Ph | phenyl |
| Phe | phenylalanine |
| q | quartet |
| RPE | primary human pigment epithelial cells |
| RT | room temperature |
| s | seconds or singlet |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| $S_N1$ | nucleophilic primary substitution |
| t | triplet |
| T | temperature |
| *T.b.g.* | *Trypanosoma brucei gambiense* |
| *T.b.r.* | *Trypanosoma brucei rhodesiense* |
| TEBA | triethylbenzyl ammonium chloride |
| TETA | triethylene tetraamine |
| THF | tetrahydrofuran |
| TLC | thin layer chromatography |
| Try | Tryptophan |
| Ts or tosyl | *para*-toluenesulfonyl |
| UV | ultraviolet |
| WHO | World Health Organisation |

# Table of Contents

# 1 Cancer

Cancer is defined in the Oxford English Dictionary as 'a malignant growth or tumour in different parts of the body, that tends to spread indefinitely and to reproduce itself, as also to return after removal; it eats away or corrodes the part in which it is situated, and generally ends in death.'[1]

Mitosis is the name for the process by which a cell replicates, *i.e.* the division of a cell to produce two identical daughter cells. Cancer occurs when there is uncontrolled cell division and the normal processes of mitosis such as initiation, control and termination cannot occur - generally this is due to genetic mutations. This uncontrolled cell division results in the growth of a tumour.[2] There are two categories of tumour: benign and malignant. A benign tumour will not spread to other parts of the body and rarely causes death. A malignant tumour can metastasise; this means that the cancer can invade other tissues.

One in three people from the western world will contract cancer at some point in their lives and one in four will die as a result of the disease.[2] Although cancer is considered to be a disease of the modern world, the first reference to it appeared in an Egyptian papyrus dating from 3000-1500 BC that mentions breast tumours.[2] Cancer is not just confined to humans, since animals and plants have demonstrated tumorigenesis.[3]

## 1.1 Cancer Treatments

Modern medicine has made huge advances towards the treatment of cancer. There are four main treatments used. Generally a combination of the treatments is given to ensure that the patient receives the best outcome.

### *1.1.1 Surgery*

Surgery was once considered to be the only option, and indeed can completely 'cure' cancer by the removal of cancerous tissue. If the cancer has been diagnosed early then surgery can be the most effective treatment; however the problem is metastasis. Nowadays tissue biopsies are essential for diagnosis and surgery is carried out in 90% of cancer patients.[2]

### *1.1.2 Radiotherapy*

Radiation therapy can stop cell proliferation and disrupts atoms within tumour cells. The cell DNA is damaged beyond repair.[2] However the radiation can't distinguish between healthy and cancer cells – especially rapidly dividing cells such as bone marrow and hair folicles so patients suffer from side-effects. Doses can be given internally or externally and advances in technology mean that the radiation can be targeted more precisely to the desired area increasing dose efficiency and diminishing damage to healthy cells.

### *1.1.3 Biotherapy*

The cancer is controlled by the body's natural defence system. Immunotherapy or biotherapy uses the body's immune system to fight cancer.[2] It is designed to repair, stimulate or enhance the body's immune response. The treatment should stop, control, or suppress processes that permit cancer growth by making cancer cells more recognizable, and therefore more susceptible, to destruction by the immune system. Biotherapy can also block or reverse the process that changes a normal cell or a pre-cancerous cell into a cancerous cell.

### *1.1.4 Chemotherapy*

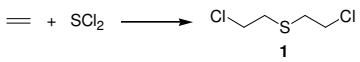
Chemicals are used to kill cancerous cells by damaging the cell proteins or the cell DNA. The aim is to cause apoptosis (cell suicide).[2] Cytotoxic drugs such as antibiotics, antimitotics, hormones, antimetabolites, inorganic compounds and alkylating agents are currently used as chemotherapeutic agents. Chemotherapy cannot distinguish between healthy and cancerous cells so side effects are then experienced.

## 1.2 Alkylating Agents

Alkylating agents are the best known anticancer cytotoxic therapeutic agents available for use in oncology departments today due to their effectiveness in cross-linking DNA, thus inhibiting cell growth and ultimately leading to cell death. Covalent bonds are formed between the DNA strand and the alkylating agent causing intermolecular cross-linking; cell death occurs because the DNA can no longer unwind and replicate.

The anticancer properties of alkylating agents were discovered after the use of mustard gas in World War I – casualties developed a profound fall in their white cell count with damage to marrow and lymphatic tissue.

Sulfur gas **1** was first synthesised in 1860 by Frederick Guthrie using ethylene and sulfur dichloride[4] (**Scheme 1**), although the idea was first developed by M. Depretz in 1822.[5] In its pure form, mustard gas is a colourless, odourless, viscous liquid. However the warfare agent was used impure as a yellow/brown substance with an odour similar to mustard plants (it is not related to culinary mustard), garlic or horseradish.




**1**

**Scheme 1**

Other names for **1** include HD, sengas, sulfur mustard, blister gas, s-lost, Kampstoff LOST, yellow cross liquid and yperite. The LOST part of the name comes from a reference to Lommel and Steinkopf who developed a process for mass producing the gas for war use by reacting thiodiglycol with HCl.[6]

Sulfur gas is a strong vesiccant (blister causing agent). Upon exposure there are no immediate symptoms. Within 4-24 h of exposure any area of skin that comes into contact with the agent will develop deep itchy burning blisters and eyes will become sore and swollen leading to conjunctivitis or blindness. Inhalation of the agent leads to respiratory problems such as mucous membrane damage and pulmonary oedema. If more than 50% of the body surface area is exposed then the result is usually fatal.[7] Sulfur gas was first used effectively in 1917 by the Germans on Canadian soldiers during WW1. It has since been used in several conflicts including the Iraq-Iran war (1981-1988).[5]

The therapeutic effects of mustards were discovered in 1943 in the port of Bari, Italy when a U.S. stockpile of mustard gas was bombed exposing thousands of civilians and 628 Allied troops to the effects of the warfare agent. Medical workers discovered that white cell counts of exposed victims were decreased. Dr Cornelius Packard Rhoads thus investigated the use of mustards as treatment for Hodgkin's lymphoma.[8] Two American pharmacologists (Goodman and Gilman from Yale) were also investigating nitrogen mustards around the same time.[9] This was essentially the birth of anti-cancer chemotherapy.

Alkylating agents are themselves carcinogenic as they damage the genetic material of the cell and the consequences of this genetic change can be the conversion of a normal cell into a cancerous cell. Fortunately the benefits of alkylating agents far outweigh the possibility that they will induce the development of a second cancer.

Nowadays, alkylating agents are designed to form reactive intermediates with certain characteristics such as electron deficiency because these reactive electrophiles will be readily attacked by nucleophilic sites on biological molecules thus forming covalent bonds. Nucleophilic groups such as amino, carboxyl, sulfhydryl or imidazole moieties in proteins and nucleic acids can be alkylated. The intermolecular cross-link (**Figure 1**) generally occurs at the N-7 position on guanine.[10] Two different nucleophiles on separate DNA strands attach to the alkylating agent preventing the unravelling of the DNA.

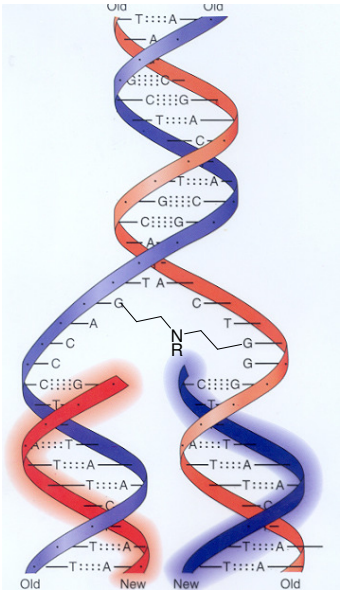


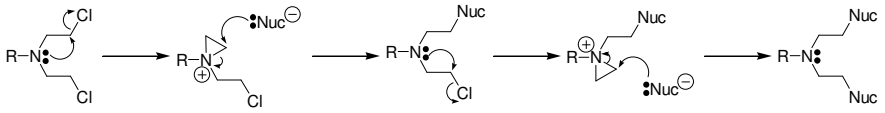

**Figure 1**

The spacing between the nucleophilic sites on each DNA strand and the number of carbon atoms between the two electrophilic sites on the alkylating agent are crucial. Ideally the two DNA strands will become locked in position to prevent replication. Cross-linking can occur on single strands on DNA – unfortunately this scenario is not serious and the DNA will repair itself. Bifunctional alkylating agents are responsible for forming rigid cross-links between the two strands of DNA – these drugs are known as major groove-binders as a link is formed in the major groove of DNA.

## *1.2.1 Classifications of Alkylating Agents*

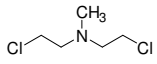
### 1.2.1.1 Nitrogen Mustard Derivatives

There are four main nitrogen mustard derivatives – all four possess the same pharmacophore. The pharmacophore is an *N*-chloroethyl group that can attach itself to DNA. All the nitrogen mustards possess two chloroethyl groups, hence are bifunctional and able to crosslink DNA. This occurs by the release of a chloride ion as the molecule undergoes a first order reaction forming a positively charged reactive intermediate – an aziridinium ion. This intermediate covalently binds to the N-7 group on guanine, which is nucleophilic. The second 'arm' of the drug then undergoes similar reaction with the base on a separate strand of DNA forming the crosslink (**Scheme 2**).[11] Several of the nitrogen mustards are discussed below.



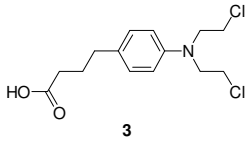
**Scheme 2**

Mechlorethamine[12], commonly known as Mustargen **2**, has a half-life of approximately one minute under physdiological conditions so it must be administered intravenously for the drug to have an effect. This drug is used to treat lymphomas.



**2**

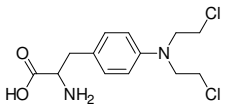
Chlorambucil[13] **3** has a half-life of twenty minutes which means that it can be taken orally in tablet form. Although **3** is insoluble in water, passive diffusion acts as the transport into cells. This drug exists mainly as a zwitterion – this means that the molecule is ionically doubly charged. This is not the reactive form of the drug because the nitrogen is protonated and unable to form the reactive intermediate.



**3**

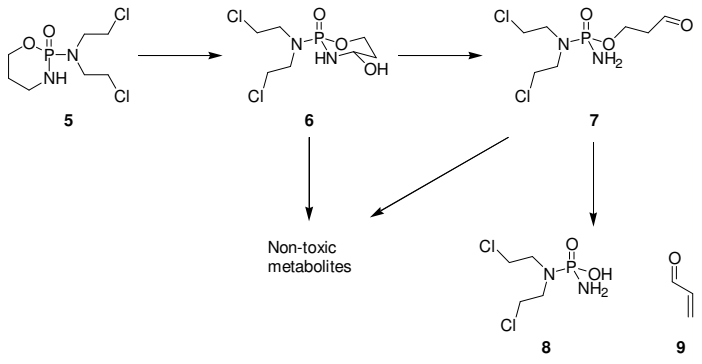
To increase the stability of nitrogen mustards, ring structures were added to the molecule. This increases the stability by decreasing the number of unwanted side reactions as the nucleophilicity of the nitrogen is reduced, for example an aromatic ring withdraws electrons from the nitrogen thus reducing the nitrogen's ability to displace one of the chloride ions. Chlorambucil **3** is used to treat chronic lymphocytic leukaemia, ovarian cancers, trophoblastic neoplasms and Waldenström macroglobulinaemia as well as being used as an immunosuppressant.

Melphalan[13] **4** exists as a highly charged species due to the fact that two nitrogens and one carboxylic acid are present. The uptake of the drug by cancer cells after oral administration is controlled by an amino acid active transport system. A drawback to use of this drug is the build up of resistance due to a mutation that leads to a change in the transport system. Melphalan **4** is used to treat multiple myeloma, malignant myeloma and ovarian cancer. One major drawback to the use of Chlorambucil **3** and Melphalan **4** is their minimal specificity, which means that they can be very toxic to healthy cells in the body, *e.g.* bone marrow cells.




**4**

Cyclophosphamide[14] **5** was one of the first nitrogen mustard prodrugs to be synthesised. It was thought that tumours were rich in phosphoramidase which would convert the drug into its active form.



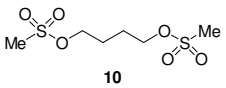

**5** **6** **7** **8** **9**

**Scheme 3**

**Scheme 3** shows what actually happens. The drug **5** is oxidised by enzymes in the liver producing the active metabolite 4-hydroxycyclophosphamide **6** and its tautomer **7** which can react with aldehyde dehydrogenase (ALDH) forming phosphoramide mustard **8** that can crosslink with DNA. A side product is acrolein **9** which is toxic to the bladder epithelium.

### 1.2.1.2 Alkyl Sulfonates

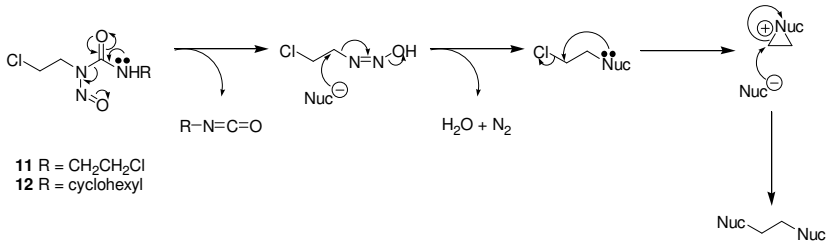
The most common alkyl sulfonate is Busulfan[15] **10** which is also a bifunctional alkylating agent. It is used to treat chronic myeloid leukaemia (CML) and other cancers. It can be taken orally or intravenously.



**10**

### 1.2.1.3 Nitrosoureas

The two main compounds that fall under this category are Carmustine (BCNU) **11** and Lomustine (CCNU) **12**.[16] Decomposition occurs in aqueous conditions.




**11** R = $CH_2CH_2Cl$
**12** R = cyclohexyl

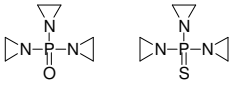
**Scheme 4**

These compounds are used for the treatment of brain tumours as they are extremely lipophilic so can easily pass through the blood-brain barrier (BBB).[17]

### 1.2.1.4 Ethyleneimines

As the classification name suggests, these drugs contain the three-membered ring moiety that appears as an intermediate during the DNA alkylation process. TEPA **13** was the initial compound synthesised although it turned out to be chemically unstable so

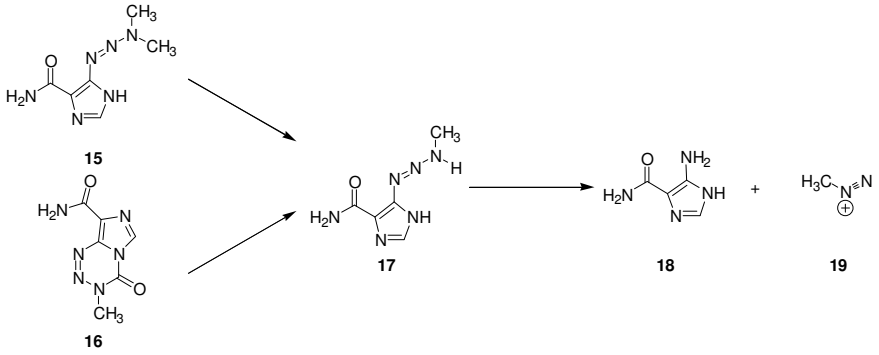
ThioTEPA **14** was produced.[18] ThioTEPA is used to treat breast, ovarian and bladder cancer and is given intravenously.



**13** **14**

### 1.2.1.5 Triazenes

Triazenes are used to treat malignant melanomas and Hodgkin's lymphomas. This group of compounds produces the same intermediates after following different physiological pathways in the body. **Scheme 5** shows two examples, temozolomide **15** and dacarbazine **16** breaking down to form the same intermediate 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) **17** which then breaks down to form amine-imidazole-4-carboxamide (AICA) **18** and the DNA-alkylating methyldiazonium ion **19**.[19]



**15** **16** **17** **18** **19**

**Scheme 5**

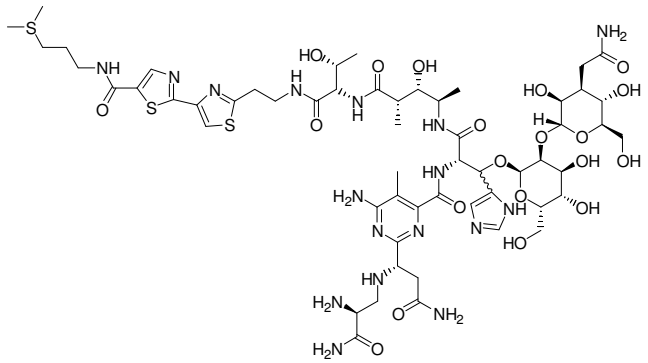
# 2 Hypoxia and Cancer

The majority of solid human tumours contain areas deficient in oxygen and, cells in this condition are referred to as hypoxic.[20] Hypoxia occurs when there is an insufficient amount of oxygen reaching tissues. Hypoxic cells have been found in cervical cancers, squamous cell carcinomas of the head and neck, melanoma, breast and prostate cancers. There is no regularity between individual tumours and there is no way of predicting the levels of hypoxia in a tumour.[21]

Hypoxia is associated with the poor prognosis of cancer. Clinical and experimental evidence show that hypoxia is one of the major causes of resistance to treatment as well as encouraging tumour growth and possibly increasing malignant cell progression. In head and neck cancer there is evidence that the extent of hypoxia is associated with the overall survival of a patient.[20]

The detection of the level of hypoxia present in a cancer patient may be beneficial for designing a patient-specific treatment course. In particular the assessment of early tumour response to treatment as well as examining long-term tumour response is an important area of research. Unfortunately, data on hypoxia in human tumours is scarce due to technical and experimental limitations. The oxygenation pattern of cells and the occurrence of hypoxia does not correlate with the stages of tumour growth.[21] It is impossible to predict the oxygenation status of individual cells in tumours before therapy. Therefore, to design tumour specific treatments the tissue oxygenation of individual tumours must be evaluated.

Hypoxic cells are intrinsically more resistant to treatment. Generally the amount of ionising radiation required for the destruction of hypoxic cells is three times that for oxygenated cancer cells.[22] The resistance to chemotherapy in hypoxic cells is partially caused by reduced toxicity due to the absence of molecular oxygen. Some cancer agents, such as bleomycin[23] **20**, require free radicals for the cell killing mechanism to be activated. Without molecular oxygen this mechanism cannot occur. Other problems occur due to the hypoxia-induced inhibition of the cell cycle. Several chemotherapeutic agents target high-proliferating cells. However the rate of proliferation decreases in hypoxic cells due to the reduction in oxygen concentration.[20] Toxicity of cancer agents also decreases the further the distance the tumour cells are from blood vessels. Simply put, the effective dose of a drug to hypoxic cells will be much less than that to other parts of the tumour.



**20**

## 2.1 The Problems Hypoxia Creates

### *2.1.1 Metastasis*[20]

There is scientific evidence that shows the association of tumour hypoxia with metastasis – this is the spread of cancer from one part of the body to another. The promotion of tumour metastasis by hypoxic cells occurs in two ways: firstly by inducing the expression of certain gene products involved in the metastatic cascade; and secondly by selecting a more aggressive phenotype.

The initiation of metastasis occurs by three major processes.[24] The first step involves the degradation of the basement membrane and extra-cellular matrix of the cell. This is followed by the modulation of cell adhesion molecules resulting in cell migration - the cancer is now 'moving'.

### *2.1.2 Angiogenesis*[20,25]

The formation of new blood vessels, known as angiogenesis is required for tumour progression to occur. The expanding tumour mass requires nutrients and the removal of catabolites, which are produced by the breakdown of complex organic molecules into simpler components, accompanied by the release of energy. The process of angiogenesis is also required for the efficient dissemination of primary tumour cells during metastasis.

The initial stages of both metastasis and angiogenesis are almost identical as both processes involve the degradation of the extra-cellular matrix and the directed migration of cells. Angiogenesis also requires proliferation of migrating endothelial cells. Therefore it

would be logical to think that molecules that aid tumour metastasis are also involved in angiogenesis and thus must also be regulated by hypoxia, *i.e.* matrix metalloproteinases, the urokinase-type plasminogen activator system and cell adhesion molecules.

Tumours require an expansion of the blood supply to provide all nutritional requirements and a sufficient oxygen supply. Tumour cells adjacent to capillaries are adequately oxygenated and are therefore capable of normal aerobic metabolism and cell division. Oxygen must be carried by diffusion to cells not directly adjacent to a capillary and the oxygen tension diminishes with increasing distance from a blood vessel. Hypoxic cells present at the interface between well-oxygenated cells and necrotic tissue rely on anaerobic glycolysis[26] (the process by which the normal pathway of glycolysis is routed to produce lactate) for energy production. These cells are 'dormant', dividing more slowly but remaining viable.

Angiogenesis in a tumour produces vessel architecture that is disproportionate and inadequate as well as structurally and functionally different from normal tissue. A consequence of this is the high rate of endothelial cell proliferation, which offers two ideas for the treatment of the cancer – anti-angiogenesis and vascular targeting. Distorted vasculature is also a problem as the drug delivery to hypoxic areas is challenged.

## 2.2 Strategies for the Treatment of Cancer by Exploiting Hypoxia

It has already been established that hypoxia is detrimental to cancerous cells as it encourages metastasis as well as other processes linked to the worsening of a patient. However, hypoxia can be exploited to our advantage especially if a treatment can be found that will differentiate between hypoxic and normal cells.

### *2.2.1 Anti-angiogenesis*[27]

The idea behind this treatment is to stop the tumour developing new blood vessels. This is a complex biological process where the target population of cells consists of actively dividing and migrating vascular endothelial cells from established host and tumour vessels. Strategies involve the blocking of angiogenic growth factors and the suppression of endothelial cell recruitment through small molecule receptor blockers, specific antibodies, or the use of inhibitors.

In 2004, Bevacizumab[28] (Avastin) became the first anti-angiogenesis drug to be approved for the treatment of cancer. The compound is a monoclonal antibody directed against vascular endothelial growth factor. The clinical trials carried out showed that people who took the drug as well as a course of chemotherapy lived longer (approximately five months) than patients who were just following a course of chemotherapy. However this drug does not seem to be a cure for cancer.

### *2.2.2 Vascular Targeting*[29]

As explained before, solid tumours require a blood supply to provide oxygen and nutrients essential for growth. The growth of a tumour can be described as chaotic and the tumour vasculature results in the formation of subpopulations of hypoxic cells.

Vascular targeting would use agents that exploit vasculature features that are unique within a tumour. There are advantages with this concept such as efficacy against any solid tumour because the endothelial cell lining is targeted, as well as no resistance being built up against the treatment – this is because endothelial cells are genetically stable. There is also easy accessibility of the drug to the target and the potential exists for the death of thousands of tumour cells due to vessel damage and subsequent nutrient deprivation. It is hoped that this technique will result in the death of hypoxic cells at an intermediate level, particularly those resistant to classical treatments.

Generally tumour hypoxia is an independent predictor of the outcome of a tumour, as it does not depend on tumour size, clinical stage, histological type, and extent of necrosis or haemoglobin levels in the patient.[20] Hypoxia has been assigned multiple roles in the whole process of cancer – it can induce angiogenesis, apoptosis and metastasis. The induction of these chemical changes results from changes in the gene expression that accompany hypoxia. The body does try and fight back by expressing genes to counteract hypoxia by increasing oxygenation or by mediating cell death signals and inducing cell death.

### *2.2.3 Improving Oxygenation*

The main interest in this area involves the administration of hyperbaric oxygen, hypoxic cell sensitisers and erythropoietin (EPO - **Figure 2**) to improve the haemoglobin level and avoid repeated blood transfusions.[30]

Mixed results have been achieved from these studies. The best result was in head and neck cancers where analysis of a large number of trials showed that oxygen modification resulted in a significant improvement in local control and survival.

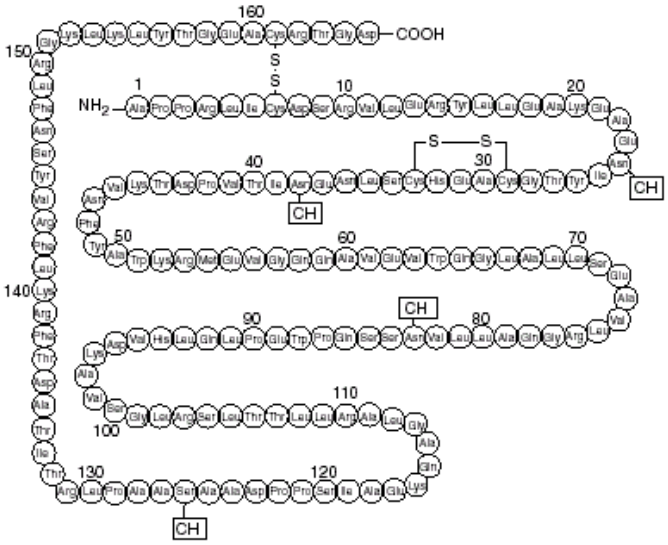


**Figure 2**

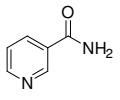
Erythropoietin is a glycoprotein hormone that stimulates red blood cell production. It is produced in the kidneys in response to tissue hypoxia in the bone marrow. The structure is made up of one hundred and sixty five amino acids in a circular form. Two disulfide bonds tether the molecule together between cysteines 29 and 33 and cysteines 6 and 161. Three *N*-linked sugars are present at asparagines 24, 38 and 83, and one *O*-linked sugar is present at serine 126.

This protein can be applied to a tumour model especially in anaemic patients where tumour hypoxia may occur due to low haemoglobin levels.[31] There is active interest in using recombinant human EPO in order to improve tumour oxygenation. Haemoglobin concentration has been shown to be an important factor for the outcome of various cancer types that are conventionally treated by radiotherapy.

### *2.2.4 ARCON*[32]

Accelerated radiotherapy combined with carbogen and nicotinamide is currently undergoing clinical assessment. Both agents have different roles for the treatment of

cancer. Carbogen (95% $O_2$ and 5% $CO_2$) reduces diffusion-limited or chronic hypoxia whereas nicotinamide **21** reduces acute hypoxia from vascular shutdown. Simultaneous use of these agents has increased the radiation damaging effect in rodent models.[33]



**21**

## *2.2.5 Exploitation of the Microenvironment*

There are three main ways that hypoxia can be exploited. Firstly the known molecular and cellular biological responses to hypoxia can be targeted. Secondly, the unique features of tumour vasculature (already discussed) which are responsible for, as well as a consequence of, tumour hypoxia, can also be exploited. Finally the lack of oxygen can be used to our advantage by the administration of bio-reductive drugs.

### 2.2.5.1 Exploitation of Biological Responses[20]

Gene expression is altered by the presence of hypoxic cells. These changes in expression, in turn, cause a cellular and tissue response that affects the processes occurring in the cell, be it angiogenesis, metastasis or sensitivity to treatment. If the activation of the early steps of these processes can be targeted, specific and effective types of gene therapy can be developed. The idea of this concept is to transfer genetic material to the tumour cell in quantities sufficient to obtain a therapeutic level of expression. To date, research carried out has been unsuccessful due to deficiencies in the delivery systems being used. However a current area of research involves the targeting of anaerobic bacteria that will infect the hypoxic/necrotic areas of tumours.

Various biological responses to hypoxia can be seen in a generalised pathway consisting of four steps. Firstly an oxygen sensor is activated – an oxygen sensor is capable of sensing and responding to reduced oxygen levels. Then the activation of this sensor leads to a molecular response, which is quickly followed by a cellular response. The culmination of these steps is a tissue or tumour response.

#### 2.2.5.2 Bio-reductive Drugs[34]

These are inactive compounds that can be reduced by enzymes in the body revealing toxic, active metabolites. They are designed so that this metabolism occurs preferentially in the absence of oxygen making them good agents for targeting hypoxic cancer cells. Used in combination with existing cancer treatments this strategy has potential for improving treatment outcome by targeting the hypoxic fraction of the tumour. Hypoxic cells are also found in a number of different medical conditions including rheumatoid arthritis, diabetes and stroke – thus non-cytotoxic delivery of the therapeutic agent would be preferred.

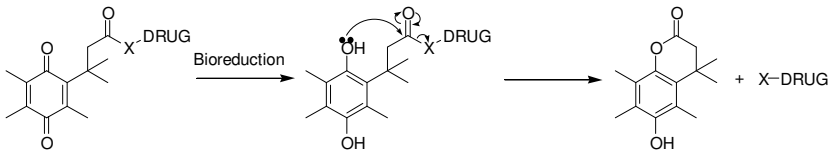
Bio-reductive drugs are also known as hypoxia-selective cytotoxins (HSCs).[35] Conceptually, HSCs consist of three domains: a trigger that undergoes hypoxia selective (oxygen inhibited) reduction; an effector which is activated following reduction of the trigger; and a linker which connects the other two domains and communicates the change.

The trigger must undergo efficient and selective metabolism in the hypoxic cells to generate a toxic effector. Prodrugs are often classified by the nature of the trigger unit. The effector should be potent and able to kill cells in all proliferative states. Ideally it should be able to diffuse back to kill surrounding cells. Therefore, the effector requires good diffusion properties and an appropriate half-life. The linker deactivates the prodrug but must rapidly transmit an activating signal for the metabolism of the trigger.

Bio-reductive drugs should fulfil several requirements. In hypoxic cells the compound should be converted into a stable persistent cytotoxin. The active product should bind non-covalently to DNA with a reasonable affinity to produce cytotoxicity but with enough stability to allow slow diffusion and subsequent cytotoxicity in proximate tumour cells irrespective of the oxygen levels. The HSC must also be easily oxidised back to the parent compound if it comes into contact with an oxygenated cell.

Nitro-aromatic heterocycles and indoloquinones have been studied for use as prodrugs.[36] There is significant interest in the design of a prodrug that when reduced will release, as well as an active drug, a bioreductive agent that itself will show therapeutic efficacy towards tumour cells (perhaps another population of tumour cells). Quinones release their active drug by a mechanism involving bio-reduction followed by intramolecular cyclisation (**Scheme 6**). The benzoquinone acts as the trigger and the propionic moiety acts as the linker.[37] This effectively masks the drug until it is released in hypoxic tissues. The bulky ‘dimethyl lock’ imparts steric hindrance upon the molecule and this aids the

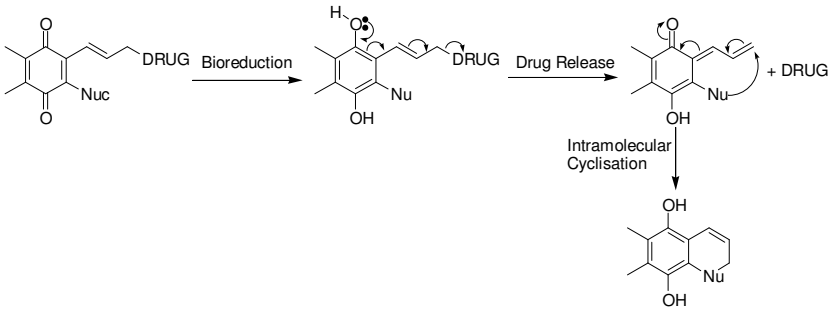
lactonisation (Thorpe-Ingold effect[38]). After reductive activation, intramolecular lactonisation occurs resulting in the elimination of the drug.




**Scheme 6**

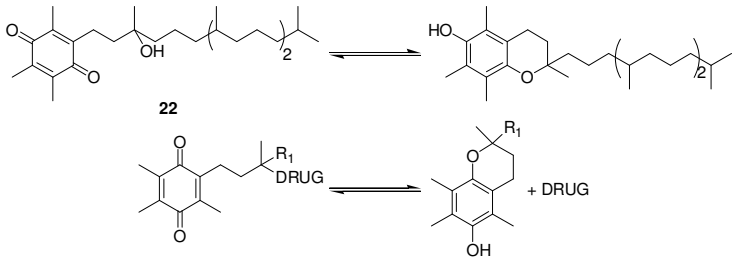
Bio-reductive drugs can also form adducts with DNA via attack by a nucleophilic moiety on DNA itself, *i.e.* the action of alkylating agents. This idea uses a synergistic approach for the killing of tumour cells – delivery of the drug as well as the concomitant activation of another cytotoxic agent.

Another idea is to incorporate a nucleophile within the bio-reductive molecule that will react intramolecularly after bio-reduction and drug release, effectively inactivating the compound (**Scheme 7**).[39]




**Scheme 7**

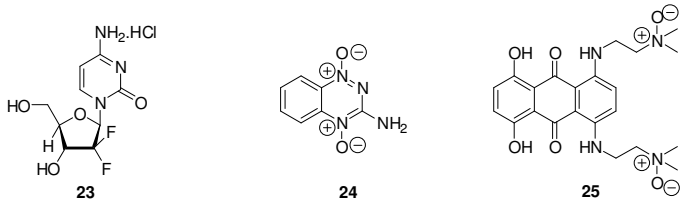
Vitamin E (tocopheryl quinone TQ **22**) can be cyclised in a redox driven manner (**Scheme 8**).[40] The hydroxyl group is ejected upon reduction of TQ **22**. Looking at this mechanism via a prodrug approach, the conjugation of a chemotherapeutic agent via the hydroxyl group would result in the ejection of the drug under favourable conditions.




**Scheme 8**

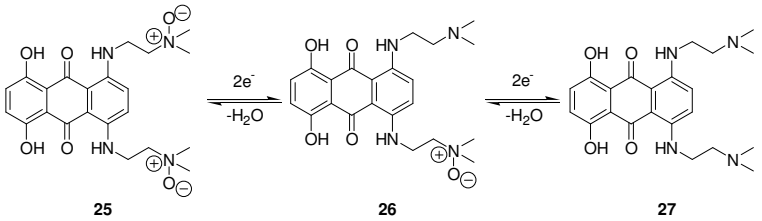
Radio-sensitisers[20] such as gemcitabine **23**,[41] are agents designed to mimic the effects of oxygen during radiation delivery. They perform two tasks – increasing DNA damage as well as restoring radio-sensitivity - and can be effective as this type of cancer agent. Unfortunately the compounds developed thus far could not be administered to patients at effective concentrations with acceptable toxicity.

Two of the best 'prodrugs' are Tirapazamine **24**[42] (TPZ) and AQ4N **25**[43]. TPZ is the leading compound in this class of drug and has been studied in clinical trials, especially the use of the drug in conjunction with cisplatin and/or radiotherapy. In fact a synergistic effect can be seen when the drug is used in conjunction with cisplatin. The release of the toxic moiety occurs by an enzymatically catalysed one-electron reduction of TPZ yielding a reactive hydroxyl radical capable of causing cell death through DNA damage.[44] A strong oxidising species must be formed as TPZ causes double-strand breakage of DNA under anoxic conditions and these effects correlate with the cytotoxicity. In the presence of oxygen, this radical is transformed back into the non-toxic parental compound – thus oxygenated tissues are protected and toxicity is minimised.




TPZ causes several side effects including neutropaenia (an abnormally low level of neutrophils in the blood), fatigue, muscle cramps, nausea and vomiting. The mechanisms of these side effects are not fully understood. They could arise from aerobic redox cycling which leads to a reactive oxygen species or the TPZ radical forming in normal tissues.

In the case of AQ4N **25**,[43] a stable product AQ4 **27** is released from the compound by a reduction process (**Scheme 9**) – this mechanism occurs via an obligate mono-*N*-oxide intermediate, AQ4M **26**. AQ4 intercalates within the DNA strand and blocks topoisomerase II action. The AQ4 released is stable and diffusion to oxygenated cells can occur where it acts producing a 'bystander' effect – this occurs when cells produce damage-response signals that are communicated to their unaffected neighbours.




**25** **26** **27**

**Scheme 9**

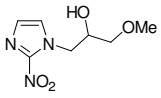
AQ4N **25** is the *N*-oxide of AQ4 **27**. AQ4 **27** has a high DNA affinity constant which stabilises the DNA double helix as measured by a large increase in the DNA melting temperature. The DNA binding of the AQ4 chromophore is greatly facilitated by the electropositive nature of the alkylamino side chains in the protonated form of the drug – this allows for the critical electrostatic interaction with the phosphates of the DNA backbone.

Nitro-heterocycles can act as mimics of molecular oxygen and can be effective as radio-sensitisers of hypoxic cells.[45] Although a correlation between the one-electron reduction potential of such compounds and their efficiency as sensitisers of hypoxic cells does exist, compounds have been recently identified that are markedly more potent than their relatively negative one-electron reduction potentials would suggest.

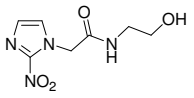
Misonidazole **28**[46] was the first electron-affinic radio-sensitiser to receive extensive clinical study although it was established that it was not possible to administer a sufficient dose to achieve radio-sensitisation of tumours.

The second generation of this class of compounds were 1-substituted-2-nitroimidazoles such as etanidazole **29**,[47] pimonidazole **30**[48] and RSU 1069 **31**,[49] which are selectively toxic towards hypoxic cells because the 2-nitroimidazoles are bio-reduced in cancerous cells to form a specially designed electrophile that will avoid neuro-toxicity by being highly polar and thus less able to cross the 'blood-brain barrier'. Pimonidazole **30** bears a tertiary amine

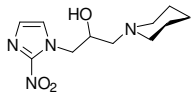
designed to be selectively absorbed by acidic regions of tumour tissue and thus has a higher potency. RSU 1069 **31** is a 'dual-function' agent with electrophilic groups. The compound proved potent as a radio-sensitiser in experimental systems but elicited dose-limiting gastro-intestinal toxicity in clinical trials. RB6145 **32**[50] employs additional DNA-alkylating functionalities in order to produce a toxic bi-functional DNA cross-linking species. At this point it could be seen that general toxicity of the compounds was hindering the development of a selectively bio-reductive cytotoxic species so attention was turned to producing bis(nitroimidazoles) - like compound **33**.[51]



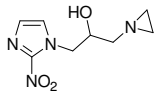

**28**



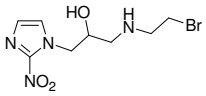

**29**



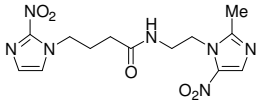

**30**




**31**



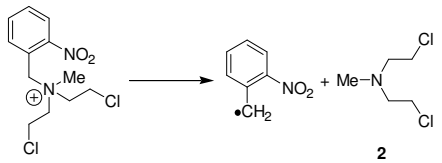

**32**




**33**

Using bis(nitroimidazoles), both of the nitro groups could be reduced providing a bi-functional cytotoxin which was more toxic than the non-reduced species. However the kinetic data collected although consistent with a bi-reduced cytotoxic species, showed that the compound does not act as a DNA cross-linking agent. There were also two important limitations with this class of agent. The dose range could not be evaluated properly due to lack of aqueous solubility and the full hypoxic selectivity was only seen after exposure of several hours. This is presumed to be a consequence of the less electrophilic 5-nitroimidazole moiety.

Nitro-benzyl mustard quaternary salts are a new class of HSCs, which show very high hypoxic selectivities *in vitro*.[52] The proposed products for this class of compounds are shown in **Scheme 10**. A one–electron reduction of the nitro-aromatic compounds is induced by cellular enzymes to give the nitro radical anion. This mechanism is controlled primarily by the reduction potential of the compound and will occur in both oxygenated and hypoxic cells. The following step depends on the nature of the cell. In an oxygenated cell, molecular oxygen will re-oxidise the radical anion producing the parent compound. However, if the cell is hypoxic, fragmentation of the initial radical anion will occur producing a benzyl radical and a reactive nitrogen mustard, such as mechlorethamine **2**.




**Scheme 10**

There is published evidence that closely related nitro-benzyl salts and nitro-benzyl quaternary salts do fragment following a one–electron reduction and that the nitro radical anions of such compounds are readily re-oxidised by oxygen. Other studies involving cyclic voltammetry and direct observation of the absorption spectra of both the transient radical anion and the resulting benzyl radical provide further evidence for the suggested mechanism. The observation that these compounds fragment efficiently using radiolytic reduction also suggests that the drugs could be reduced using radiotherapy rather than enzymatic reduction. The only drawback to this idea would be the need to use more potent cytotoxins for the concept to be therapeutically useful.

Radiation-activated prodrugs use the aquated electron from the radiolysis of water as the reducing agent.[53] The hypoxic selectivity is achieved by the redox cycling of the initial electron adduct in the presence of oxygen. Again it must be reinforced that highly potent cytotoxins are required to compete with endogenous electron acceptors in the body for the radiation-induced reductants.

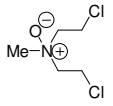
The positive charge on the quaternary salt ensures a high degree of deactivation of the mustard and excellent water solubility of the compound as well as a reduction potential within the range expected to be suitable for metabolic activation. It is now known that the extent to which toxicity is masked by a prodrug is not directly related to the reduction potential. However it is thought that the charge will slow the cellular uptake rate of the drug.[51]

Mechlorethamine **2**[54] is the best mustard so far found to be formed from the fragmentation of the drug. It possesses a half-life long enough for back-diffusion into other tumour cells to occur. The release of mechlorethamine has been confirmed by the trapping of the nitrogen mustard with diethyl dithiocarbamate and HPLC analysis.

The main problem with nitro-benzyl quaternary salts is their aerobic toxicity as this totally negates the idea of a prodrug. Aerobic toxicity is not thought to be caused by the reductive

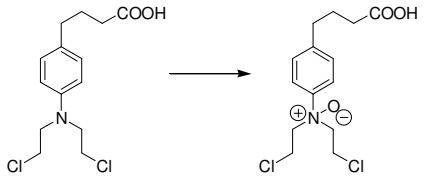
activation of the drug unless the reduced intermediate is not as stable as thought. Other possible theories indicate the slow release of the nitrogen mustard by direct nucleophilic displacement during the drug exposure phase. This potentially could occur chemically or enzymatically. Generation of a reactive oxygen species could also contribute to the aerobic toxicity through redox cycling. The main conclusion to be taken from these studies is that quaternising a mustard does not mask all its toxicity.

Another potential strategy for the design and synthesis of prodrugs involves *N*-oxidation.[55] The electron density on the nitrogen of the mustard is significantly decreased and the $pK_a$ value is lowered by about 5 log units. Molecular oxygen inhibits the metabolic reduction of the tertiary amine *N*-oxides and the *N*-oxide derivative of mechlorethamine, nitromin **34**, has been reported to have modest hypoxia selectivity in cell cultures.




**34**

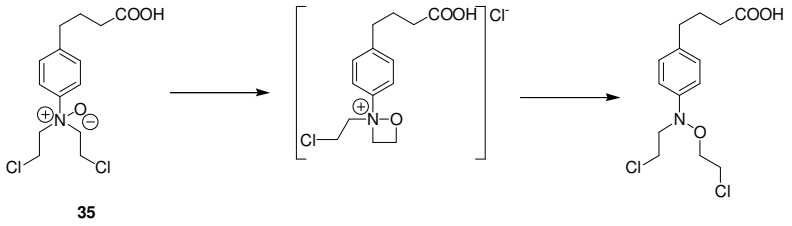
Several different nitrogen mustards that were already used as therapeutic agents were transformed into their *N*-oxides using peracetic acid followed treatment with by sodium bicarbonate (**Scheme 11**).[56]




**3** **35**

**Scheme 11**

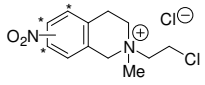
However, aliphatic *N*-oxides can rearrange in aqueous solution via a cyclic intermediate to form tri-substituted hydroxylamines (**Scheme 12**).[57] Comparison of the $^1$H NMR spectra of the product with **35** showed that the chlorambucil *N*-oxide had indeed rearranged. The decomposition products were also tested but chlorambucil *N*-oxide was the most cytotoxic species present.




**35**

**Scheme 12**

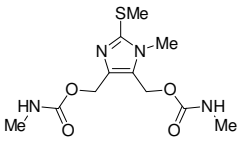
The development of related tetrahydroisoquinoline quaternary salts has also been carried out.[35] The provision of a second, non-cleavable linking chain between the nitro-benzyl chromophore and the quaternary leaving group ensures that the released mustard and the benzyl radical remain linked. A mono-functional mustard will be generated and it is hoped that the simultaneous generation of a linked reactive centre generated from the attached benzyl radical will not hinder the toxicity.



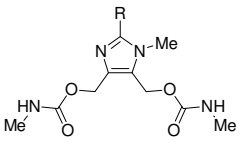

**36**

The three isomers of **36** that were synthesised were less toxic than mechlorethamine – this is due to the powerful deactivating effect of the permanent positive charge of the nitro group. Preliminary studies also show that these compounds inhibit [$^{14}$C]-choline uptake. This strongly suggests that the major mechanism for the uptake of these compounds is via the choline transport pathway instead of passive diffusion.[58] Further work is being carried out to study factors such as radical lifetime or the ability of the drugs to be transported via the choline carrier pathway.

Carmethizole **37** is a novel bis-carbamate alkylating agent, which forms DNA-protein and DNA-DNA crosslinks *in vitro*.[59] It has *in vivo* anti-tumour activity against murine leukaemias, solid tumours and human tumour xenografts.




**37**




**38**

R = $NH_2$, $N_3$, $NHCO_2R_1$

By adding electron-donating substituents at the 2-position the anti-tumour activity was enhanced whereas with electron-withdrawing groups the compounds became inactive. This observation is probably explained by the stabilisation of the transition state of the $S_N1$-type activation of the hydroxymethyl groups that will lead to DNA alkylation. Two different strategies could be employed. Firstly, the bis-hydroxymethylimidazole nucleus derived from carmathizole could be used as the alkylating unit with 2-nitrogen substituents added to deactivate the alkylating units. Secondly the substituted hydroxymethylimidazole ring could be used as a trigger unit rather than an alkylating unit.

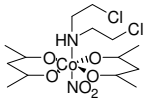
Using the first strategy, **38** (when R = $NH_2$) is expected to be more reactive than carmethizole. This is not useful for a prodrug so by changing the amino group to an azide or a carbamoyl group (*i.e.* considerably less electron donating substituents) a more useful compound would be produced. Azides are known as masked amine functions and the metabolism of aryl azides to amines has been demonstrated.[60]

The second approach involves the reduction of the trigger group leading to activation of a separate alkylating moiety attached via a deactivating carbamoyl linker. The electron-withdrawing substituent on the imidazole ring could be bio-reduced to a more electron-donating moiety that would activate the carbamate unit to nucleophilic attack with the subsequent fragmentation of the resulting carbamic acid, releasing a secondary amine as the cytotoxic effector. The alkylating agent selected was *N,N*-bis(2-chloroethyl)amine (BCEA) and nitro and sulfoxide substituents were selected as suitable electron withdrawing substituents to place in conjugation with the methyl carbamate. The results showed that there is potential in the further study of these compounds especially for the use as trigger units for the release of secondary amino cytotoxins.[59]

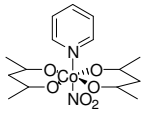
Transition metal complexes have demonstrated cytotoxicity in cell culture and/or anti-tumour activity in tumour-bearing animals.[61] The metals used include Ag(I), Cu(I) and Cu(II), Zn(II), Hg(II), Rh(II), Ru(II) and Ru(III), Pd(II), Pt(II), Co(III) and Fe(III). Platinum complexes are currently in routine clinical use.[62]

The classical inorganic anticancer agent cis-diaminedichloroplatinum(I), cisplatin **39** forms coordination bonds with cellular targets and is classified as an anti-tumour alkylating agent. Other complexes that bind specifically to sites on DNA are chiral Co(III) tris-phenanthroline molecules.


**39**




**40**




**41**

Cobalt (III) complexes are kinetically inert coordination complexes. The inertness is due to the $d^6$ low spin electron configuration of the trivalent cobalt. This inertness is the main reason why cobalt complexes have potential as prodrugs. The biological consequence of the inertness is that the complex will remain intact when added to a culture medium or injected as a drug. The drug should arrive at its cellular targets intact with its original ligand configuration.

The complexes **40** and **41** are interesting as targets for bio-reductive drugs.[60] The bulky pyridine ligand in **41** is similar in size to the BCEA ligand possessed by many alkylating agents such as **40**, although the bifunctional alkylating ability is not present. The Co(III) in both compounds could also be reduced to Co(II) thus releasing BCEA. The lone pairs on the nitrogens in the Co(III) complexes **40** and **41** are deactivated because they are complexed to the metal.

These drugs are known as HPDCs – hypoxia activated prodrugs of diffusible cytotoxins. They are used as a solution to the problem of cells with intermediate oxygen concentrations. Bio-reductive drugs are only activated at very low oxygen concentrations and therefore many tumour cells are sufficiently hypoxic to be radio-resistant even though the prodrug will not be activated. Thus, prodrugs were developed with an appreciable diffusion range – HPDCs. Activation of the drug is confined to regions of very low oxygen tension.

There are other strategies including ADEPT[63] and GDEPT,[64] which use prodrugs. ADEPT is antibody directed enzyme prodrug therapy and GDEPT is gene directed enzyme prodrug therapy.

ADEPT involves a group of drugs called monoclonal antibodies, which recognise certain proteins that are found at the surface of some cancer cells. ADEPT is a targeted therapy – the antibody is attached to a specific enzyme. The monoclonal antibody recognises the protein and locks on to it. A prodrug is then given and cell death should occur.

GDEPT operates in the same way except that a gene for the expression of the prodrug activating enzyme is delivered to the cells. The prodrug becomes activated by the foreign enzymes present in the cancerous cells.

## 2.3 Conclusion

To date, there is no prodrug that has shown sufficient clinical activity to achieve registration for general use. A drug has to be designed that will reach remote hypoxic cells, *i.e.* the diffusion of the drug should be more efficient. The activated effector must possess a better 'bystander' effect so that the 'killing zone' is expanded. A more deliberate design process must be developed allowing the synthesis and biological testing of a drug that will selectively kill cancer cells.

# 3 Human African Trypanosomiasis[65]

## 3.1 Human African Trypanosomiasis

Human African Trypanosomiasis (HAT) is more commonly known as African Sleeping Sickness. The disease is carried by parasites of the *Trypanosoma* genus and is transmitted by the bite of a tsetse fly (*Glossina* genus – **Figure 3**[66]). The tetse flies themselves acquire the infection from mammals (human beings or animals) harbouring the pathogenic parasites. Tsetse flies are found exclusively in Africa in a belt that stretches south of the Sahara and north of the Kalahari desert.

**Figure 3**

The protozoa belong to a family comprised of parasites of insects, plants, fish, amphibians, reptiles, birds and mammals.[67] The parasites generally infect most vertebrate genera and are likened to cancer cells due to their capacity for growth.[68] Only two forms of trypanosomes cause significant human disease. *Trypanosoma brucei* (**Figure 4**[69]) is found in sub-Saharan Africa and causes HAT. The parasites are known as salivaria as the disease is transmitted in tsetse fly saliva. *Trypanosoma cruzi* is found in America, primarily in South America, but it presents a potential hazard in Mexico through blood and organ donations. *T. cruzi* causes Chagas' disease and the parasites are known as stercoraria because transmission is via vector faeces. Both forms of the parasite are single-celled flagellates that are transmitted by insect vectors. The parasites undergo phases of local multiplication followed by dissemination and localisation in target organs where they cause lethal damage. Both vector groups that transmit the diseases are known as K-strategists.[70] They have a low reproductive rate complemented by a high survival rate adapted to exploit their habitats.

It has been shown that humans have been exposed to *T. brucei* during the course of evolution whereas *T. cruzi* evolved independently, only coming into prominence with the appearance of man on the American continent within the past 12,000 years. 4000 year old

mummified remains from South America have been identified as possessing *T. cruzi* DNA.[71]

**Figure 4**

There is one reported case of HAT being transmitted sexually.[72] A 30 year old Portuguese woman presented herself at hospital with HAT symptoms. She had never visited Africa, although her Brazilian partner had been on a military mission to Angola three years previously. He was identified as a symptom free carrier. Both were successfully treated for HAT.

HAT affects 36 countries of sub-Saharan Africa (**Figure 5**[73]) and occurs in remote rural areas where health systems are weak or non-existent and it mainly strikes the active adult population. Epidemics are currently rife in the Democratic Republic of Congo (DRC), Angola and Sudan. Other infected countries include Uganda, Central African Republic, Congo Republic and Tanzania.[74] 60 million people are at risk from HAT.[75] The number of cases is difficult to estimate. In 1998, 40,000 cases were reported but it is believed that the real number of sufferers was in the region of 300,000-500,000 due to cases being undiagnosed and therefore untreated.


**Figure 5**

Surveillance of the disease is being reinforced and the number of cases reported has been substantially reduced. It is still difficult to assess the current situation due to lack of surveillance and diagnostic expertise. The disease was nearly eliminated during the 1960s. However the disease has recently been on the increase due to war, population movements and the collapse of health systems. The disease is the major cause of depopulation of large tracts of Africa. Fear has led to abandonment of fertile land and is an impediment to development.[65]

There are two stages to the disease.[65] After the host has been bitten by the tsetse fly, the parasites proliferate at the site of infection, leading to an inflammatory nodule or ulcer. The parasites then spread to the draining lymph node and reach the bloodstream, initiating the haemolymphatic phase. The sufferer will experience fever, headaches, joint pains and itching. As the disease progresses past the blood-brain barrier, the parasite infects the central nervous system and the second stage known as the neurological phase begins. The sufferer will experience confusion, sensory disturbances, poor coordination and disturbance of the sleep cycle – hence the common name of the disease. How and why the parasites infect the CNS is not clear.

There are two forms of HAT depending on which specific parasite is involved. *Trypanosoma brucei gambiense* (*T.b.g.*) occurs in west and central Africa and accounts for 90% of reported cases. The disease is a chronic infection and the patient can be infected for several months without showing any symptoms. As symptoms do appear the disease will be in the advanced stages with the central nervous system affected. The other form of the disease is *Trypanosoma brucei rhodesiense* (*T.b.r.*) which affects east and southern Africa, although this accounts for less than 10% of the reported cases. This form of the disease is acute and the first symptoms will appear after a few weeks. The disease will develop more rapidly in the later stages. Without treatment HAT is fatal.

The diagnosis relies on detecting parasites in the patient's blood or lymph nodes. If there is a positive test for parasites, a lumbar puncture is performed to identify if the disease has progressed onto the second stage. Current diagnostic methods are invasive, difficult to carry out and require highly trained staff.

## 3.2 Trypanosomes' Life Cycle[76]

The tsetse fly becomes infected when taking a blood meal from an infected mammalian host (**Figure 6**[76]). The parasites transform into procyclic trypomastigotes and multiply. The parasites leave the midgut and transform into epimastigotes that reach the fly's salivary glands and continue mulitiplying.

The infected tsetse fly has a blood meal on a mammalian host and injects metacyclic trypomastigotes into the skin tissue. The parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes and are carried to other sites of the body. Continuous replication occurs.

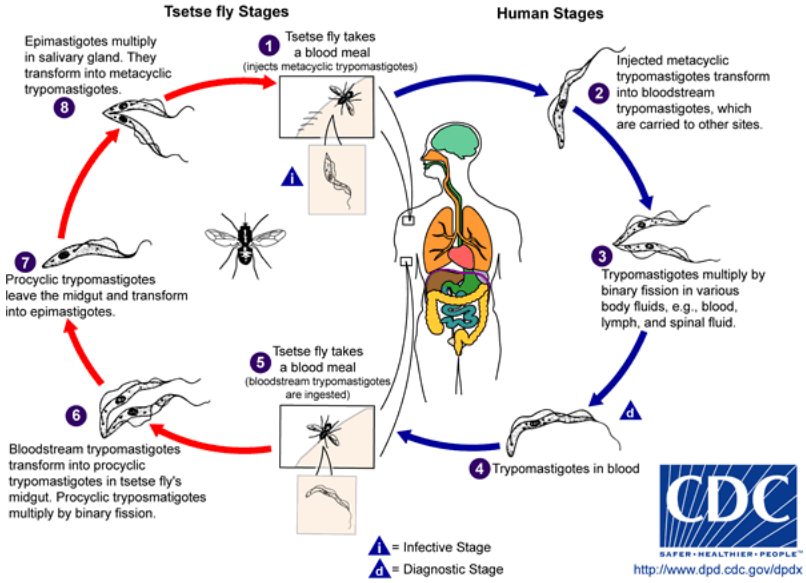


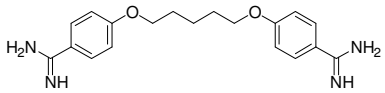

**Figure 6**[76]

## 3.3 Treatment of HAT[77]

The type of treatment a patient receives depends on the stage of the disease. If caught early during the first stage, the drugs used are less toxic, easier to administer and more effective. However, if the disease has developed into the later stages the success of the treatment relies on the drug crossing the blood-brain barrier. Such drugs are toxic and complicated to administer.

### 3.3.1 Pentamidine

Pentamidine **42** was first used in 1941 to treat first stage *T.b.g.* and is well tolerated by patients.[78] The aromatic diamidine must be injected into the muscles. Diamidines work directly against the parasites.

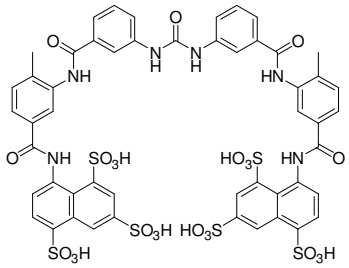


**42**

Pentamidine **42** was developed after the observation that a related compound, synthalin, that induced hypoglycaemia in mammals also displayed trypanocidal activity.[78] The diamidines are dications at physiological pH and they interact with a number of cellular anions. They are known to bind tightly to the minor groove of DNA inhibiting replication.[79]

### 3.3.2 Suramin

Suramin **43** was first developed in 1921 by German chemists and is used to treat *T.b.r.*, although the drug does produce undesirable side effects in the urinary tract and also stimulates allergic reactions.[80] The drug is given to humans and camels. The treatment course takes five weeks which causes problems due to the lack of resources in infected areas.



**43**

The drug is a colourless, polysulfonated symmetrical naphthalene derivative. Other naphthalene dyes were initially developed for their marked trypanocidal activity. At physiological pH, the drug bears six negative charges and it inhibits many enzymes by electrostatic interactions. It has been shown to block the specific cell surface binding of various growth factors[81] and inhibits tyrosine phosphorylation.[82]

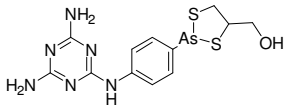
Suramin **43** inhibits reverse transcriptase which is a key enzyme of the human immunodeficiency virus (HIV).[83] The drug had no impact on the progression of AIDS.

Suramin **43** has recently been used in clinical trials against hormone-refractory prostate cancer and other human malignancies as it inhibits angiogenesis.[82,83]

### *3.3.3 Melarsoprol*

Melarsoprol **44** was first used in 1949 and was developed from arsenic. It is known to corrode plastic syringes.[84] The compound **44** is synthesised by complexing melarsen oxide with the metal-chelating drug dimercaptol. In trypanosomiasis, **44** reversibly inhibits the cellular enzyme glutathione reductase and NADPH oxidase.[85]

The melaminophenyl-based organic arsenical **44** induces many different side effects. One of the more serious is encephalopathic syndrome which will kill 3-10% of patients. Statistics show that one in 20 patients treated with melarsoprol will die.




**44**

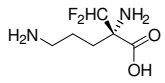
One Médecins Sans Frontières doctor working in Uganda has been quoted as saying '*Melarsoprol is a terrible drug – you don't feel proud injecting it. It is caustic, it burns and you don't know if you are going to save your patient or kill him!'*

The disease can also build up resistance against the drug and around 30% of patients treated will not be cured. This resistance has mainly been observed in central Africa. The drug is accumulated by an unusual amino-purine transporter and loss of this transporter leads to drug resistance. *T. brucei* contain multiple purine transporters at their plasma membranes. Trypanosomes like all protozoan parasites are auxotrophic for purines and rely entirely on salvage from the host environment for their purine supply.[86]

It is generally accepted that the toxic species of the arsenicals is the trivalent oxidation state of the arsenic. Trivalent arsenicals react quickly and reversibly with vicinal thiol groups in proteins such as trypanothione and so many enzymes can be targeted for inhibition. Cell death is rapid.

### 3.3.4 Eflornithine

Eflornithine **45** was first registered in 1990 to treat *T.b.g.* although the drug was initially designed to be an anti-cancer agent. Clinicians are still deliberating about the malignancies for which it is best suited.[87] The compound is a derivative of ornithine. The regimen is extremely strict and difficult to apply as slow drip infusions are administered every six hours for fourteen days.

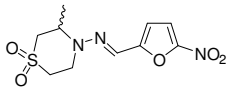



**45**

It is reported that uptake occurs via passive diffusion across the plasma membrane. The compound inhibits ODC.

### 3.3.5 Nifurtimox

Nifurtimox **46** is normally used to treat Chagas' disease and has been used with limited success to treat arsenical-resistant HAT.[88] The drug contains a nitro group that is essential for activity. Uptake is reported to occur via passive diffusion. One electron reduction of the nitro group generates a free radical which in turn generates reduced oxygen metabolites such as superoxide, hydroxyl free radical and hydrogen peroxide all of which can cause the death of the parasite.[89] Mammalian cells have better protection against oxidative damage so the compound is generally parasite selective.




**46**

### 3.3.6 Megazol

1.3.7 Megazol **47** has shown good activity against *T. brucei*.[90] The 5-nitroimidazole possesses the same motif recognised by the transporter responsible for the uptake of several anti-trypanosomal drugs. It was thought that megazol **47** would be of limited use against arsenical-resistant trypanosomes. However it would appear that this drug enters the cell by passive diffusion as opposed to the transporter thus supplying an alternative drug to use

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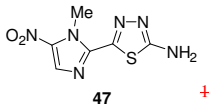
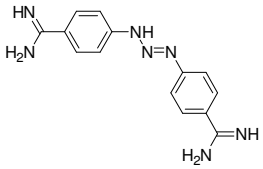
against arsenical-resistant parasites.[91] A combination treatment of megazol **47** and suramin **43** appears to eliminate trypanosomiasis of the CNS.[85]



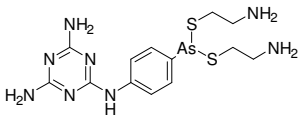
**47**

### 3.3.7 Treatment of Cattle

Domestic cattle can be affected by the disease too although it is referred to as 'Nagana'. This word literally means 'depressed'. There are several drugs that can be used to treat infected livestock such as berenil **48** and cymelarsan **49**.[78] These drugs are based on the human alternatives. Berenil like pentamidine has two positive charges at pH 7.4.



**48**



**49**

Once cured, the cattle must not be slaughtered for human consumption for several months to ensure that the drug is totally excreted.

### ~~1.3.8~~3.3.8 Vector Control

During the colonial era, great efforts were made to eradicate HAT by clearing large areas of land of any vegetation and removing large wild mammals that would act as a reservoir for the disease.[92] This proved very effective but due to the huge resurgence of the disease this is now not practical.

Nowadays, insecticides have proved very successful. The lower parts of trees are targeted for spraying as resting flies can be found there in infested areas.[93] Unfortunately ecological considerations mean that the use of insecticides is becoming more restricted.

Tsetse traps and baits are very useful (**Figure 7**). Trap efficiency depends on the different species of tsetse fly. The traps are baited with odours and impregnated with insecticides. The flies are also attracted to visual aides such as large expanses of black or blue cloth. Acetone is sometimes used as this is the tsetse-atttracting component of cattle breath.

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**Figure 7**

A much publicised approach to controlling tsetse numbers involves the release of sterile male flies that will mate unproductively with females.[94]

## 3.4 Potential Drug Uptake Pathways[95]

Trypanosomes live freely in the bloodstream and cerebral spinal fluid of their mammalian hosts and not intracellularly. They are fully exposed to the host's immune response. The parasite remains protected from the immune response by a dense, highly immunogenic glycoprotein coat.[96] Up to 1000 genes code for these surface glycoproteins and sequential expression of these genes produces antigenically distinct parasite populations allowing survival in the mammalian host.[97] Due to this antigenic variation, the prospects of vaccine development are poor.

Drugs need only cross one membrane in order to reach the trypanosomes' interior which can offer a means of selectively targeting drugs to these cells. Potential drug targets could be designed from biochemical pathways common to the parasites but absent in mammalian hosts.[98] As explained before the only barrier between the parasite and its host is the plasma membrane. Transporters are used for the uptake of nutrients by the parasites. If vital transporters were blocked the parasites would die. Nutrient transporters represent excellent drug targets.[99]

### *3.4.1 Glucose Metabolism*

Trypanosomes are dependent on glycolysis for energy production. The first seven enzymes found in this pathway reside within unusual organelles known as glycosomes.[100] There are several structural and functional features of trypanosome glucose transporters that distinguish them from mammalian counterparts.[101] The transporters possess a relative insensitivity to various pharmacological reagents such as cytochalasin B. The transporters

also have a substrate recognition profile which includes the ability to transport D-fructose.[102]

### *3.4.2 Pentose Phosphate Pathway*

Many enzymes of the pentose phosphate pathway (**Figure 8**) are related to cyanobacterial isoforms rather than to those of eukaryotes.[103] 6-Phosphogluconate dehydrogenase (3rd enzyme of the pathway) is essential for trypanosomal survival.

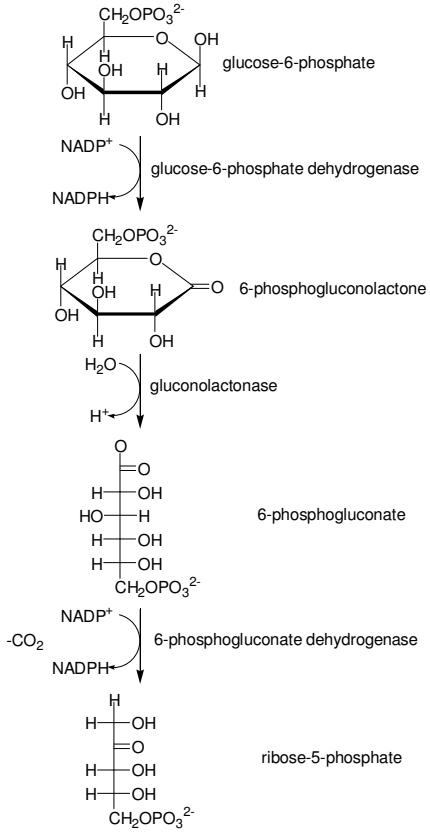
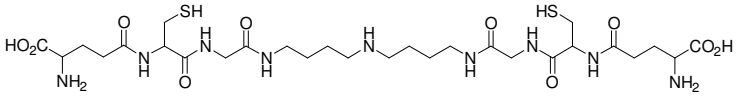



**Figure 8**

There are specific structural differences when compared to the mammalian counterpart and this has been exploited in the design of new selective inhibitors.[104]

### 3.4.3 Thiol Metabolism

The role of glutathione in trypanosomes is undertaken by trypanothione **50** (*N,N*-bis-glutathionylspermidine) which is a low molecular weight thiol consisting of two glutathione molecules linked by spermidine.[105] Enzymes involved in trypanothione metabolism have been identified as good candidate targets.



**50**

Design of new targets should take into account that the interaction between trypanothione **50** and the enzyme appears to occur via a heteroatom of the enzyme that bonds with the free thiol groups of the two cysteines present in the co-factor.[106] Therefore the central atom of any new lead compounds should show a great affinity towards sulfur.

### 3.4.4 Polyamine Metabolism

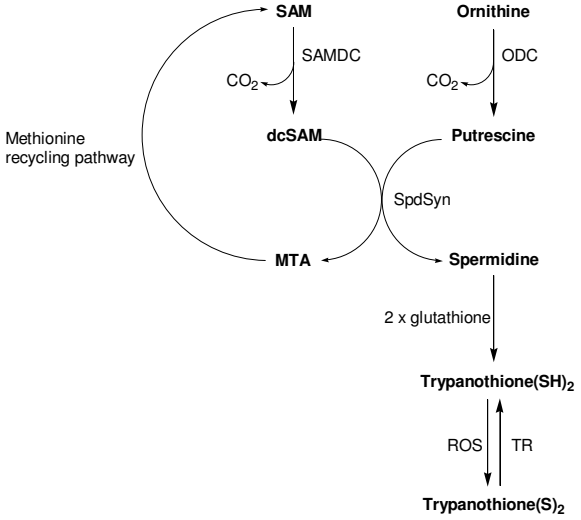



**Figure 9**

By synthesising synthetic analogues of key biosynthetic enzymes, drugs can be directed to specific targets. Ornithine and methionine are essential for the trypanosomes' polyamine biosynthetic pathways (**Figure 9**).[107] Eflornithine **45** is the only drug used to treat HAT for

which a mode of action has been identified. As explained before eflornithine **45** is an inhibitor of ornithine decarboxylase (ODC).

The polyamine metabolic pathway is also a target for anti-cancer chemotherapy due to the central role of these metabolites in cell proliferation.

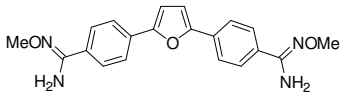
### *3.4.5 Lipid and Sterol Metabolism*

Lipids play a central role in biological membranes as well as participating in cell signalling. Sterol biosynthesis has been targeted for use against fungi which have similar pathways to trypanosomatids. Therefore drugs designed to use against fungi could also be potential anti-parasitic candidates.[108]

## 3.5 Future of HAT Treatments

It is difficult to encourage pharmaceutical companies to carry out research on anti-parasitic agents as there is no monetary incentive for them. Most sufferers live in developing countries. In May 2001, after pressure from several governments and non-governmental organisations, Aventis, MSF and WHO set up a project to produce pentamidine **42**, melarsoprol **44** and eflornithine **45** for at least five years. Bayer and Bristol-Myers Squibb also got involved in the production of sleeping sickness drugs.

A consortium of researchers funded by the Melinda and Bill Gates Foundation have identified prodrug DB289 **51** (2,5-bis[4-amidinophenyl]furan bis-O-methyl amidoximine) as a future HAT treatment.[109] The compound is absorbed across the intestinal epithelia and is converted into a trypanocidal dicationic form.



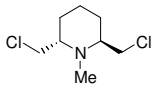

**51**

This is the first 'new' HAT drug for almost 50 years. More research is required to design better drugs. Major pharmaceutical companies must be encouraged to play a greater role in developing new drugs for this developing world disease. Meanwhile it is mainly University researchers who carry out research into the development of new and better anti-parasitic agents.

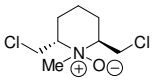
# 4 Previous Work within the Robins Group

## 4.1 Piperidines

The Robins group first started researching alkylating agents in the early 1990s. Henderson synthesised a series of bifunctional mustards based on piperidine such as **52**. The aim of the research was to investigate whether aziridinium ion formation was possible if conformational restrictions were imposed on the molecule. Prodrugs were developed in the form of *N*-oxides **53**. The free bases such as **52** showed good activity in two human carcinoma cell lines ($IC_{50}$ values ~8 μM); however the *N*-oxides such as **53** were inactive in oxic and hypoxic conditions.[110] It is thought that the reduction potentials of the compounds were outside the range needed for bioreduction.



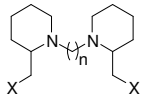

**52**




**53**

## 4.2 Bispiperidines

Henderson then investigated variation of the alkylation selectivity using bispiperidines such as **54**. The piperidines were separated by a chain of varying length to ascertain the optimum distance between the two heterocycles. The derivatives synthesised showed greater selectivity of alkylation at *N*-7 of guanine over melphalan and were cytotoxic in three cisplatin-resistant cell lines.[111] A relationship between linker chain length and compound reactivity was observed. When n = 2, compound **54** was twice as reactive as longer chain analogues.

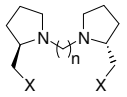



**54**

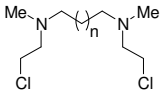
## 4.3 Bispyrrolidines

Anderson carried out further investigation into the length of the linker chain and varied the size of the heterocycle by producing a series of homochiral bispyrrolidines such as **55**.

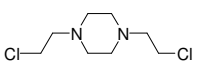
Compound **55** with 2-, 5- and 6- carbon linker chains gave the best alkylating results. Examples of **55** with 3- and 4- carbon linker chains showed no cross-linking activity. A range of alkyl linear **56** and cyclic mustards **57** were also prepared but showed no cytotoxicity in a useful range ($IC_{50}$ values > 100 μM) when tested against human colon carcinoma cell lines.[112]




**55**



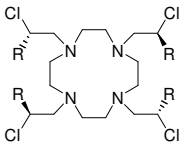

**56**



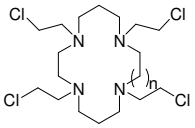

**57**

## 4.4 Azamacrocycles

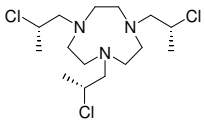
Robins' group research then moved away from heterocyclic mustards towards macrocyclic nitrogen mustards. There were several reasons for this. Firstly the macrocycles would still allow for multiple alkylating arms to be incorporated into the drugs. Secondly the architecture of the compounds would be defined yet chain lengths could still be varied. Finally, and most importantly, bioreducible prodrugs could be synthesised due to metal ion chelation. Inorganic chemists have long studied macrocyclic polyamines as ligands for chelation.[113]




**58** R = H
**59** R = Me




**60** n = 1
**61** n = 2




**62**

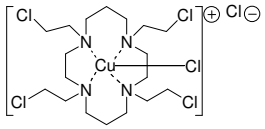
| Compound | % DNA cross-linking at 0.1 μM | $IC_{50}$ μM |
|---|---|---|
| **58** | 100 | 22 |
| **59** | 64 | 10 |
| **60** | 94 | 8 |
| **61** | 100 | 9 |
| **62** | 30 | 13 |

**Table 1**

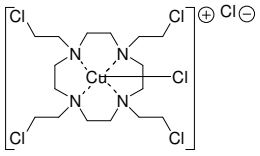
Lacy synthesised a cyclen derivative **58** which showed extremely promising cross-linking results compared to chemotherapeutic agents currently in use.[114] The compound was $10^4$ times more effective at DNA crosslinking than chlorambucil and 2000 times better than melphalan. $IC_{50}$ values for the set of compounds ranged from 6 to 25 μM (chlorambucil – 45 μM and melphalan – 8.5 μM). Anderson synthesised a range of poly-2-chloroethylated

azamacrocycles **59**-**62**.[114] These compounds showed promising biological results. **Table 1** shows a summary of the biological results for this set of compounds.

Due to difficulties experienced during the synthesis of the previously discussed azamacrocycles, Parker investigated the development of a flexible, reliable method for the synthesis of azamacrocycles with varying linker chain lengths. Parker also developed the synthesis of copper(II) complexes of the macrocycles thus producing potentially bioreducible prodrugs.[115] Complex **63** showed no hypoxia selectivity, however complex **64** displayed reversible redox behaviour and showed excellent hypoxia selectivity. When tested against human chronic myeloid leukaemia cell line K562, complex **64** was 24 times more toxic under hypoxic conditions than in oxic conditions. This provided the evidence that further investigation into this area of research would be beneficial.

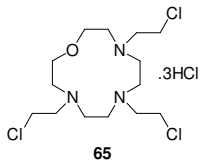



**63**



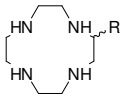

**64**

Jones investigated the synthesis of oxaazamacrocycles and azamacrocycles of varying size and shape to discover if this affects their alkylating ability.[116] Compound **65** proved to be the most toxic ($IC_{50}$ – 4.0 μM) of all the compounds prepared so far. It was unfortunate that it proved impossible to synthesise the corresponding copper complex.

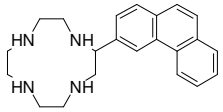



**65**

Reid developed a reliable synthesis of substituted tetraazamacrocycles such as **66**. R groups were aromatic or heteroaromatic producing a range of compounds with varying electronic demand.[117] However, it proved difficult to attach the alkylating arms onto the macrocycles so unfortunately the desired alkylating agents were not prepared. The parent macrocycles were tested against the parasite *Trypanosoma brucei*. Analogue **67** was the most toxic and underwent *in vivo* testing although a dose of 20 mg/kg did not cure trypanosomiasis.



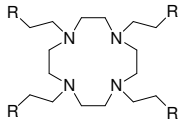

66




67

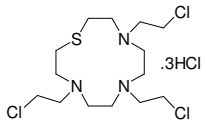
## 4.5 Aims of the Project

It was thought that the favourable results from compounds **64** and **65** could be improved. So far only mustards bearing a 2-chloroethyl alkylating arm have been produced. It would be interesting to investigate varying the leaving group R (see **68**). The aziridinium ion formation could turn out to be more efficient producing better biological activity. A selection of R groups attached to the cyclen skeleton would be desirable. These compounds would then be sent for biological testing to assess DNA cross-linking ability, hypoxia selectivity and anti-parasite activity.




68

It would be sensible to complete the 'set' of heteroatom-containing macrocycles by synthesising thiaazamacrocycles containing N, O, and S such as **69**. Multiple alkylating sites would still be available and there is literature evidence that sulfur binds to copper(II) more strongly than oxygen which may overcome the problems experienced during the synthesis of the copper complexes of the oxaazamacrocycles.

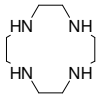



69

# 5 Synthesis of Azamacrocyclic Mustard Derivatives

## 5.1 Synthesis of Cyclen

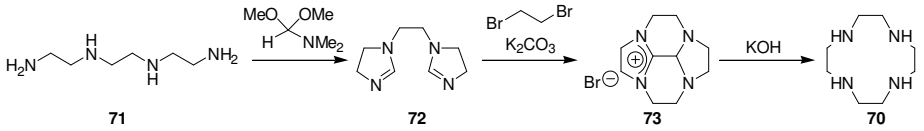
The first aim of the project was to investigate a quick and reliable synthesis for compound **70** – commonly known as cyclen. Cyclen has been used as an intermediate for the synthesis of diagnostic and therapeutic agents.[118] The tetraazamacrocycle is involved in the development of magnetic resonance imaging contrast agents[119] and more recently as a chelating agent for use against targeted cancer agents.[120] Cyclen **70** is commercially available but is expensive. There are several ways of synthesising cyclen but in each case reaction conditions favour intramolecular cyclisation over intermolecular cyclisation.




**70**

### *5.1.1 Template Synthesis*[121]

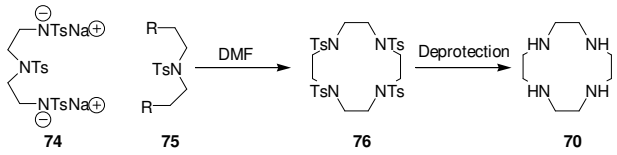
This synthesis is an established procedure within the Robins Group, based on published work and can be carried out on a multigram scale (**Scheme 13**). The three step synthesis uses a carbon template around which the macrocycle is built and the two carbon infrastructure is then removed leaving the tetraazamacrocycle. Starting from triethylenetetraamine (TETA) **71**, this synthesis exploits the reactivity of formamide acetals forming bis-imidazoline **72**. Cyclisation occurred by reacting **72** with 1,2-dibromoethane to give the monoimidazolinium compound **73**. The desired macrocycle **70** was obtained after alkaline hydrolysis with potassium hydroxide. The disadvantage to template synthesis is that it cannot be used to synthesise a range of macrocycles with varying ring size and heteroatom content. It was decided to attempt other cyclen syntheses that could provide the opportunity to vary the range of compounds synthesised.




**Scheme 13**

## *5.1.2 Richman Atkins Cyclisation*

Before the 1970s, the most efficient method for the synthesis of polyazamacrocycles was by Stetter and Roos.[122] By combining terminal dihalides and bis-sulfonamide sodium salts under high dilution conditions, the protected product was isolated in poor to moderate yields. Although this procedure was versatile with respect to ring size, there were disadvantages such as excessive use of solvent, poor yields and long reaction times. In 1974 Richman and Atkins published a letter detailing a procedure with improved cyclisation yields without high dilution conditions (**Scheme 14**). Nine to 21 membered macrocycles could be prepared using DMF as a dipolar aprotic solvent.[123]




**Scheme 14**

More recently, this reaction was carried out between the dianion of bis-toluenesulfonamide and bis-tosylated derivatives in DMF. The dianion was created either by addition of sodium hydride or more recently *in situ* by addition of caesium carbonate.[124]

The tosyl groups are essential for the success of the reaction. Not only do they render the amine hydrogens more acidic to aid deprotonation but their steric bulk encourages the intermediate to undergo intramolecular cyclisation rather than oligomerisation by adopting a *pseudo*-Thorpe Ingold effect (**Figure 10**).[125] The distance between the reacting termini is decreased by the bulk of the tosyl groups pushing the termini closer together.

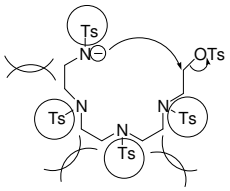
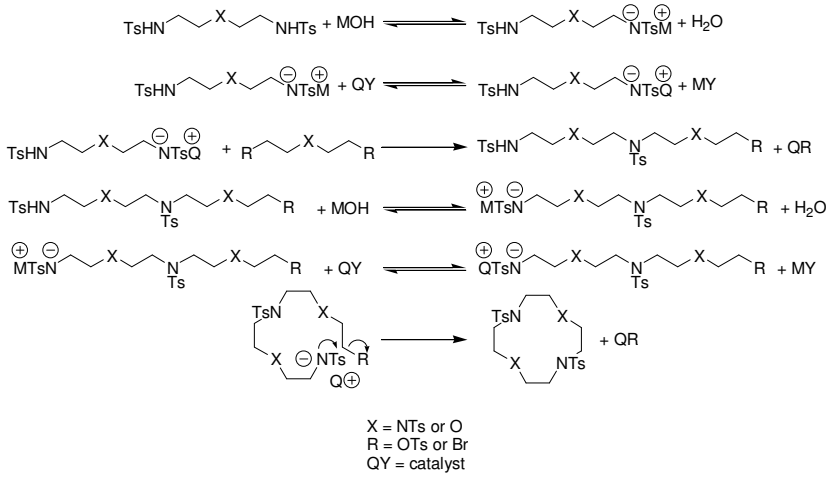



**Figure 10**

### *5.1.3 Phase Transfer Cyclisation*

Lukyanenko published work describing the synthesis of azamacrocycles and oxaazamacrocycles using a biphasic system.[126] Bis-sulfonamides were reacted with dibromides or ethylene glycol bis-(toluene-4-sulfonyl) derivatives in a toluene and aqueous alkali mixture.

Quaternary ammonium salts were used as phase transfer catalysts. The charged centre of the salt allows the compound to dissolve in an aqueous environment and the butyl groups permit the compound to solubilise in an organic medium. **Scheme 15** shows the mechanism for the cyclisation. The alkali deprotonates a sulfonamide producing an anion that exchanges cations with the catalyst. The ionic species formed undergoes alkylation in the organic phase. The alkylated species then undergoes deprotonation once more forming another ionic precursor that undergoes intramolecular cyclisation completing the process.



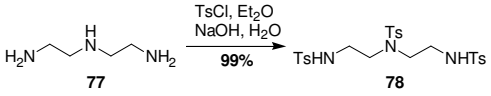

**Scheme 15**

## *5.1.4 Synthesis of Tosylated Cyclen*

### 5.1.4.1 Tosylation of Precursors

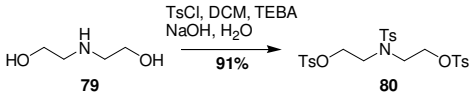
The starting materials for both the Richman-Atkins cyclisation and the phase-transfer cyclisation require the corresponding amine or diol to be tosylated. Well known procedures previously used by members of the Robins group were applied successfully.

**Scheme 16** shows the reaction of diethylene triamine **77** with tosyl choride – these reaction conditions require vigorous stirring and product **78** was produced in very high yield.[127]




**Scheme 16**

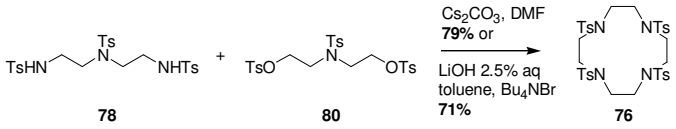
**Scheme 17** shows that different conditions were required when tosylating diethanolamine **79**.[128] Triethylbenzylammonium chloride (TEBA) was used as a phase transfer catalyst. Product **80** was prepared in good yield.




**Scheme 17**

### 5.1.4.2 Cyclisation

Both the Richman Atkins and the phase-transfer reactions were carried out to compare yields. As **Scheme 18** shows both reactions produced the desired macrocycle **76** in good yield. The phase transfer procedure does have advantages over the Richman Atkins cyclisation. The main one is the length of time required for the reaction to go to completion. To achieve optimum yields with the Richman Atkins procedure the reaction mixture must be left stirring for two weeks as opposed to an overnight reaction using the phase transfer procedure.




**Scheme 18**

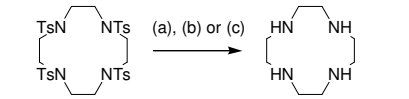
## *5.1.5 Deprotection of Tosyl Groups*

The final step required to achieve cyclen **70** is the removal of the tosyl groups. There are many examples in the literature that include conc. sulfuric acid,[129] sodium/mercury amalgam,[130] potassium fluoride on alumina,[131] lithium aluminium hydride[132] and hydrobromic acid in varying concentrations of acetic acid solution.[125,133] All of these conditions are harsh and generally low yielding.

Several procedures were investigated. The first reaction involved reacting the tosylated macrocycle with HBr-AcOH 45% and phenol (**Scheme 19**).[133] The protected amine becomes protonated resulting in attack by a bromide ion producing tosyl bromide. In order to prevent this by-product from re-attaching to the macrocycle, phenol was added to 'mop up' any by-product. The intermediate formed at this point is the hydrobromide salt. The free base **70** was isolated either by using ion exchange chromatography or by using sodium hydroxide and a Dean-Stark apparatus. Although this reaction was successful, there were impurities present which could affect future reactions.

The second method applied involved heating the protected macrocycle under reflux in conc. sulfuric acid for 40 hours (**Scheme 19**).[133] Pure **70** was isolated, however the yield was poor.

The final procedure attempted involved heating the macrocycle in conc. sulphuric acid at 165 $^{o}$C for 10 minutes (**Scheme 19**). This black solution was cooled and then added dropwise to an ethanol/diethyl ether mixture from which a solid precipitated. This solid was dissolved in a minimum volume of water and an equivalent volume of hydrobromic acid was added dropwise until crystals were observed. This hydrobromide salt was heated under reflux overnight with sodium hydroxide solution in toluene using a Dean-Stark apparatus producing the pure free base **70** in good yield.[134]



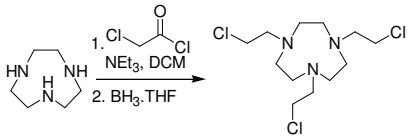

**76** **70**

(a) HBr-AcOH, PhOH, 80 $^{o}$C, 3 d, **46%**
(b) $H_2SO_4$, 110 $^{o}$C, 40 h, **13%**
(c) $H_2SO_4$, EtOH, $Et_2O$, HBr then NaOH, toluene, **88%**

**Scheme 19**

## 5.2 Alkylation of Cyclen

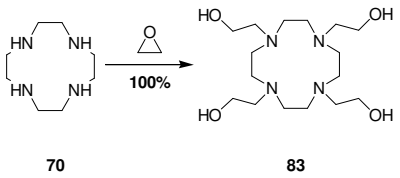
In order to synthesise the desired mustard pharmacophore, a two carbon chain must be added to the amine groups. Parker initially synthesised chloroacetamides which were subsequently reduced (**Scheme 20**) and this method was particularly effective for triazamacrocycles.[135] However a more efficient route was then discovered using ethylene oxide.[115]




**81** **82**

**Scheme 20**

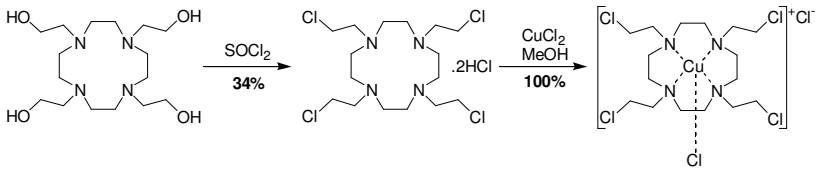
Alkylation using ethylene oxide is clean and high yielding although a high yield is dependent on the purity of the parent macrocycle. Reaction conditions must be carefully monitored to prevent the formation of unwanted side-products such as over-alkylated and polymerised species. Optimum reaction conditions involve the use of water as the reaction solvent and the reaction temperature must be maintained for three hours at 0 $^{o}$C. Gaseous ethylene oxide was added to the reaction mixture with the use of a cold finger condenser and addition funnel to ensure accurate volumes of reactant were added. Once the reaction was complete excess ethylene oxide was removed by rotary evaporator yielding pure **83** – no further purification was required (**Scheme 21**). The 2-hydroxyethylated compound **83** provides the precursor required to attempt the synthesis of a range of compounds with different leaving groups present on the mustard functionality.




70 83

**Scheme 21**

In order to synthesise the chlorinated mustard **58**, the 2-hydroxyethylated macrocycle **83** was reacted with thionyl chloride (**Scheme 22**).[115] The mustard **58** was isolated as the corresponding stable dihydrochloride salt which prevents immediate activation as the lone pairs on the nitrogens are protonated and aziridinium ion formation cannot occur.




83 58 64

**Scheme 22**

Copper complexation occurred quantitatively by treating the hydrochloride salt **58** with anhydrous copper (II) chloride in hot methanol.[115] The complex **64** precipitated from the solution upon cooling, producing the prodrug.

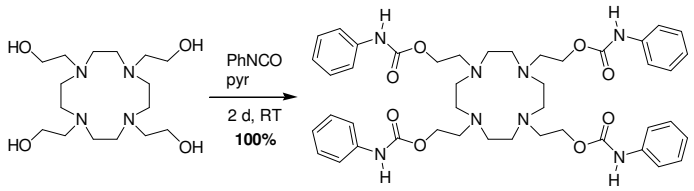
## 5.3 Synthesis of Carbamates

It was decided to form carbamate groups on the macrocyclic skeleton in the same way as the Robins group had previously synthesised carbamate derivatives that showed cytotoxic activity.[111] Carbamates are also generally crystalline so purification is easier and they are reasonably stable although it is hoped that they will be displaced under physiological conditions.

### *5.3.1 Route I*

The phenyl carbamate **84** was synthesised in quantitative yield using a procedure involving the 2-hydroxylethylated macrocycle **83**, pyridine and phenyl isocyanate (**Scheme 23**).[136] This compound was crystallised and an X-ray structure was obtained (**Figure 11**).

Compound **84** crystallises in the monoclinic space group *P*21/a and the structure clearly shows the formation of the macrocyclic ring and four carbamate side-chains.




**83** **84**

**Scheme 23**

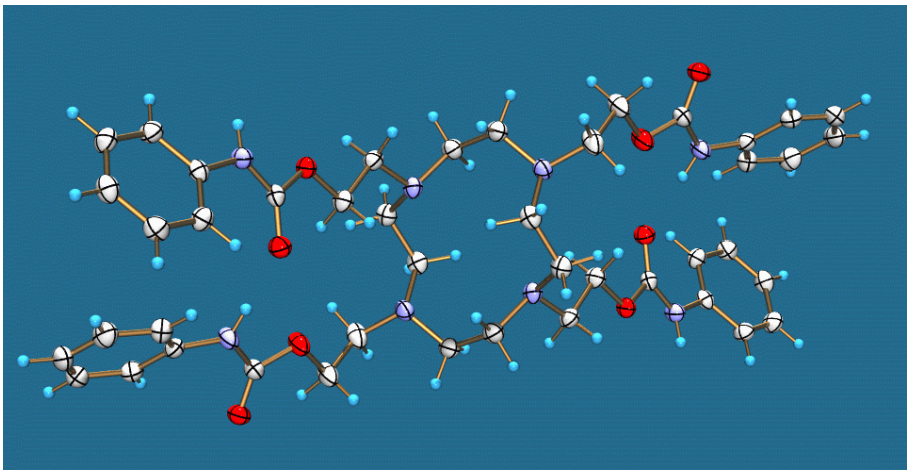


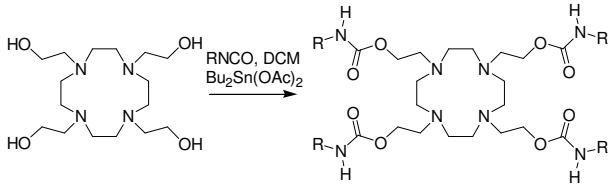
**Figure 11**

Due to the success of this reaction, substituted phenyl carbamates were targeted, *e.g.* 4-nitrophenyl, 4-bromophenyl and 4-methoxyphenyl. However it was impossible to isolate the desired compounds and the only products observed in the $^{1}$H NMR spectra were the corresponding aromatic amines produced after hydrolysis of the isocyanates.

The synthesis of alkyl carbamates was also attempted using the same procedure. The isopropyl derivative **85** was isolated in a poor yield (11%), however it proved impossible to synthesise the ethyl derivative.

### *5.3.2 Route II*

A different procedure was used to try and synthesise a range of carbamates. A method using the 2-hydroxyethyl precursor **83**, an isocyanate and dibutyl tin diacetate in dichloromethane was attempted (**Scheme 24**).[137]




**Scheme 24**

The two previously obtained macrocyclic carbamates were synthesised again by stirring the reaction mixtures at room temperature for two days. The phenyl derivative **84** was again produced quantitatively and the isopropyl derivative **85** was obtained in a good 60% yield. It was also possible to crystallise **85** and an X-ray crystal structure was obtained (**Figure 12**). As before this compound crystallises in the monoclinic space group *P*21/a and shows successful formation of the macrocycle and four side-arms.

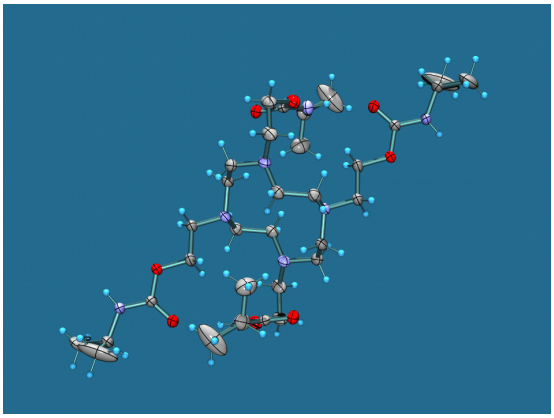


**Figure 12**

Using the same reaction conditions, it proved impossible to synthesise the propyl **86** and the 4-methoxyphenyl **87** analogues. The reactions needed to be heated under reflux conditions overnight and these two compounds were successfully isolated in moderate yields. By studying reaction conditions and the number of equivalents of isocyanate used,

it proved possible to synthesise a range of substituted carbamates. The reaction conditions required and yields are summarised in **Table 2**.

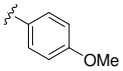
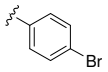
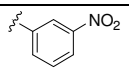
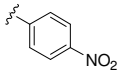
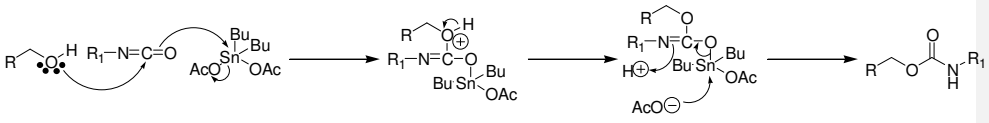
| Cpd No. | R | Eq. RNCO | T | Yield |
|---|---|---|---|---|
| **84** |  | 40 | RT | 100% |
| **85** |  | 40 | RT | 60% |
| **86** |  | 40 | reflux | 41% |
| **87** |  | 40 | reflux | 56% |
| **88** |  | 40 | reflux | 64% |
| **89** |  | 10 | reflux | 64% |
| **90** |  | 10 | reflux | 46% |
| **91** |  | 10 | reflux | 60% |

**Table 2**

The mechanism of the reaction is assumed to proceed as shown in **Scheme 25**.[137] A lone pair of electrons from the alcohol attacks the carbonyl of the isocyanate which in turn attaches itself onto the dibutyl tin diacetate with loss of an acetate group. The acetate group reattaches onto the tin moiety reforming the catalyst and resulting in the formation of the desired compound.

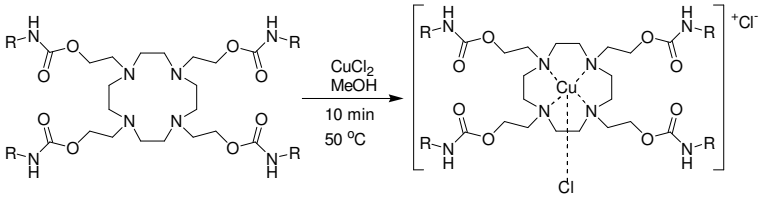


**Scheme 25**

## 5.4 Synthesis of Copper Complexes

In order to transform the macrocycles into prodrugs, copper complexes had to be formed. Using the same procedure previously described in **Scheme 22**,[115] eight copper complexes

were synthesised from the corresponding macrocyclic carbamates using one equivalent of copper(II) chloride (**Scheme 26**). Yields are summarised in **Table 3**.




**84-91** **92-99**

**Scheme 26**

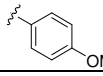
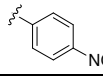
| Cpd No. | R | Yield |
|---|---|---|
| **92** |  | 100% |
| **93** |  | 58% |
| **94** |  | 64% |
| **95** |  | 23% |
| **96** |  | 39% |
| **97** |  | 18% |
| **98** |  | 74% |
| **99** |  | 80% |

**Table 3**

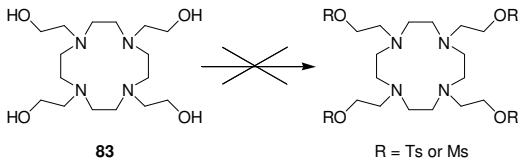
Characterisation using mass spectrometry and UV-vis spectrometry proved that the complexes had been successfully formed. It has been reported that the $\lambda_{max}$ for the d to d transition of $CuCl_2$ occurs at 810 nm. Upon complexation of amine ligands to the copper, the $\lambda_{max}$ for the d to d transition dropped to 690 nm.[138] Parker obtained a $\lambda_{max}$ of 622 nm for mustard **64**. Similar results were obtained for the macrocyclic copper-complexed carbamates **92-99** and are detailed in the Experimental chapter. It was unfortunate that crystals of the complexes could not be grown as X-ray crystal structures would have been desirable.

## 5.5 Unsuccessful Attempts at Formation of Other Leaving Groups

Although the carbamate synthesis was successful, many unsuccessful attempts were made to attach different leaving groups onto the cyclen macrocycle.

### *5.5.1 Sulfonamides*

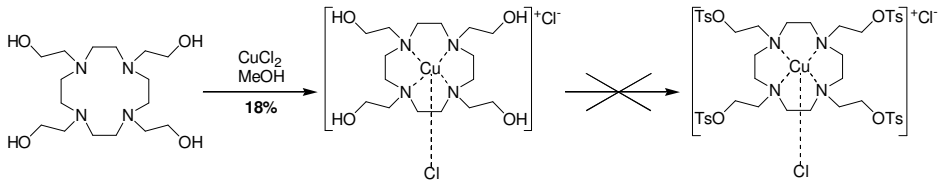
It was desirable to synthesise a mustard derivative with tosyl groups attached to the alkylating arms as this functional group is considered to be a good leaving group. Several procedures were attempted using different solvents, bases and reaction conditions (**Scheme 27**).[139] It proved difficult to produce the tetratosylated macrocycle. A mesylation was also attempted but again isolation of the desired compound was unsuccessful.[140]




**83** R = Ts or Ms

**Scheme 27**

It is thought that the product is so reactive that aziridinium ion formation occurs and the molecule then breaks itself apart. In order to prevent this happening it was decided to form the copper complex of the 2-hydroxyethylated macrocycle **83** as the nitrogen lone pairs would then be bound to the copper and aziridinium ion formation should not occur (**Scheme 16**).




**83** **100**

**Scheme 28**

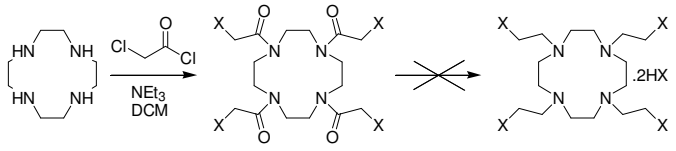
The copper complex **100** was successfully synthesised using the procedure previously discussed. Tosylation was then attempted using tosyl chloride and pyridine (**Scheme 28**).[141] Only starting material was recovered although there had been solubility issues with the

reaction. The reaction was modified to use DMF as a co-solvent which resulted in complete dissolution of the starting material. Unfortunately the desired product was not produced and it was not possible to obtain starting material from the reaction mixture.

### 5.5.2 Chloroacetamides and Bromoacetamides

Parker had used a successful strategy for the alkylation of triazamacrocycles using chloroacetyl chloride (**Scheme 20**).[142] The carbonyl groups could then be reduced leaving the desired alkylating agent. By using bromoacetyl bromide it was hoped that a bromosubstituent could be synthesised as previous work carried out on the bromo-derivative had been unsuccessful.[143]

Both the chloro- and the bromo-derivatives were synthesised using a mixture of cyclen **70** in dichloromethane with triethylamine and the corresponding haloacetyl halide (**Scheme 29**). The yields for both reactions were poor but the desired precursors were produced.




**70**

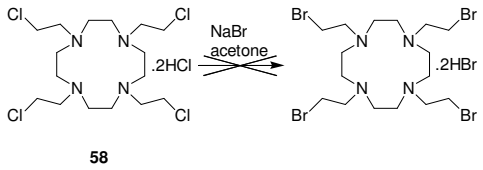
**101** X = Cl **10%**
**102** X = Br **11%**

**Scheme 29**

Reduction of the carbonyl groups was attempted using $BH_3$.THF.[144] However the desired compounds appeared to have degraded and could not be isolated. A procedure involving lithium aluminium hydride was then tried although this resulted in the same conclusions that the product, if formed at all, had degraded.[145]

### 5.5.3 Finkelstein Reaction

A commonly used reaction for the exchange of halides, a Finkelstein reaction, was attempted using 2-chloroethylated mustard **58**, sodium bromide and acetone.[146] Again it appeared that there was degradation of the product. Perhaps the molecule is once again too reactive and aziridinium ion formation occurs.



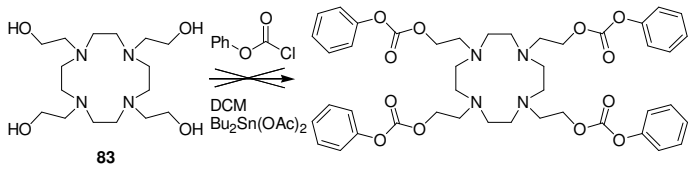

58

**Scheme 30**

## *5.5.4 Carbonates*

As it had been possible to synthesise a range of carbamates **84-91**, it was thought that it would be possible to synthesise a range of carbonates. These should be easier to cleave under physiological conditions to produce aziridinium ions. The precursor is the 2-hydroxylethylated macrocycle **83**.

The first attempt involved the procedure used to synthesise the carbamates with phenyl chloroformate used instead of phenyl isocyanate (**Scheme 31**).[137] However the desired compound could not be isolated and it looked as though the starting material had degraded as seen before during the development of the carbamate synthesis.




83

**Scheme 31**

The next protocol tried, used alkyl bromide, phase transfer catalyst, potassium carbonate as base and DMF under a carbon dioxide atmosphere that was obtained by dissolving dry ice in the reaction mixture and sealing the vessel.[147] It proved impossible to extract anything resembling the desired product.

Several other attempts were made to synthesise a carbonate derivative. The first involved using phenyl chloroformate with pyridine as solvent and base.[148] The second used DCM as the solvent and this time no extra additives were added. The only reagents were the alcohol precursor and the phenyl chloroformate and the reaction was carried out at -40 $^{o}$C.[149] A final attempt used sodium methoxide in toluene with phenyl chloroformate.[150] Unfortunately the product was not obtained using any of these methods.

# 5.6 Biological Results

## *5.6.1 Anti-Cancer Test Results*

The series of carbamates **84-91** were sent to Prof. John Hartley at University College, London to establish the DNA-crosslinking and cytotoxicity of the uncomplexed mustards against the human chronic myeloid leukaemia cell line K562.[151] The copper complexes **92-99** were sent to Prof. Ian Stratford at the University of Manchester for aerobic and anaerobic testing against the lung-derived tumour cell line A549.[152]

Unfortunately the range of compounds **84-91** proved to be too stable to be efficient DNA cross-linkers. Aziridinium ion formation did not occur. There are no quantitative data for this range of compounds ($XL_{50}$ > 1 mM and $IC_{50}$ > 100 μM) but **Figures 13** and **14** show that after one hour or two hour incubation with the drug, there is no significant cross-linking even at high concentrations. No hypoxia selectivity was thus observed with the corresponding copper complexes due to the lack of activation of the prodrugs. The data for **84-86** is not shown but similar results were obtained.

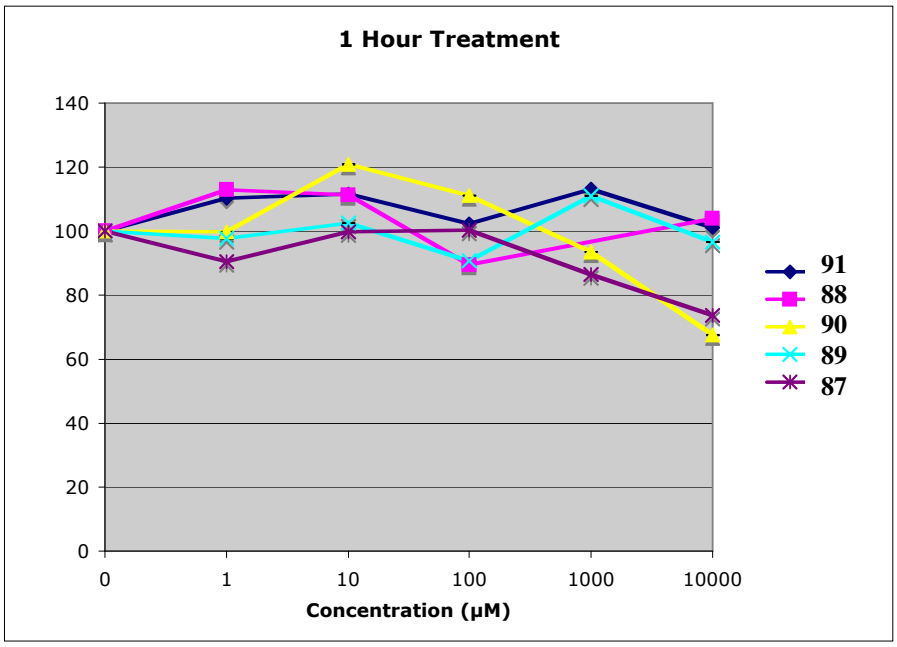



**Figure 13**

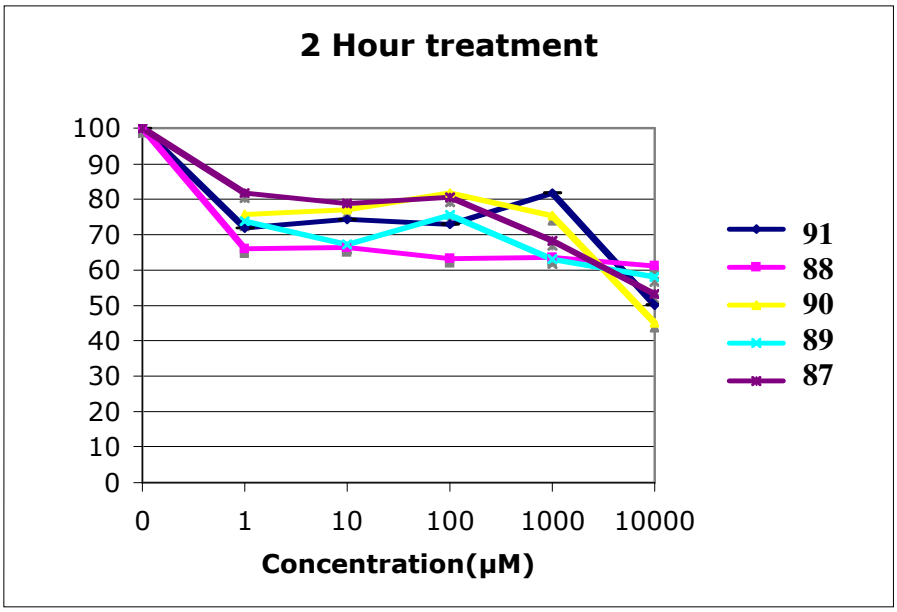



**Figure 14**

### *5.6.2 Anti-Parasitic Test Results*

The carbamates **84**-**91** were sent to Dr Mike Barrett in the Department of Parasitology at the University of Glasgow for anti-parasitic testing against *Trypanosoma brucei* (Alamar Blue assay).[153] Polyamine analogues have shown the ability to disrupt natural polyamine metabolism and transport, thus inhibiting parasitic growth. As explained before Reid had synthesised a range of azamacrocycles which displayed good results ($EC_{50}$ values as low as 1.26 μM).[117]

The compounds were also tested against HEK cells which should show if the compound is selective towards the parasites.[154] A selective drug would show $EC_{50}$ values of 1-2 μM against *Trypanosoma brucei* but would be inactive against the HEK cell lines.

The Alamar blue results (**Table 4**) show that the *ortho*-, *meta*- and *para*-nitrocarbamates are the best trypanocides. The *meta*-derivative is also significantly less active against the HEK cells which is required for the drug to be useful as an anti-parasitic agent. Fluorescence was measured for the three best compounds. However none of the compounds seemed to fluoresce. The compounds kill the cells but don't rupture the cellular membrane.

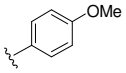
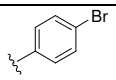
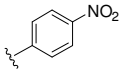
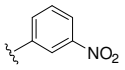
| Cpd no. | R | Est. log P | *T. brucei* $EC_{50}$ (μM) | HEK $EC_{50}$ (μM) |
|---|---|---|---|---|
| **84** |  | 4.66 | 9.22 | 56.6 |
| **85** |  | 1.11 | 16.9 | 152 |
| **86** |  | 1.40 | 10.1 | ≥200 |
| **87** |  | 4.98 | 4.00 | 20.4 |
| **88** |  | 8.22 | 5.81 | 26.0 |
| **89** |  | 6.24 | 0.463 | * |
| **90** |  | 6.24 | 2.00 | 79.1 |
| **91** |  | 6.24 | 0.916 | 23.0 |

**Table 4**

* shows a gradual decreasing of fluorescence with a biphasic curve: at lower drug concentrations (0.1-0.78 μM) there is a slight cytostatic effect, while at the higher doses there is a toxic effect.

Log P is the ratio of concentrations of a compound in the two phases of a mixture of two immiscible solvents at equilibrium (octanol and water).[155] The estimated log P values are included in the table (obtained from an online programme[156]) as Lipinski's rules state that the optimum log P values for a drug should be between 1 and 3.[157] The compounds synthesised by Reid that showed good activity all possessed log P values that fell into this category.[117] It should be noted that the compounds **89**-**91** that showed good activity all possess log P values greater then 3. These log P values mean that the drug structures would have to be modified for optimum uptake of the drug by the body. These compounds are quite insoluble and it was difficult to dissolve the compounds in DMSO to perform the assays. It is therefore unrealistic to expect that these compounds would be easily taken-up into the body and would cross the blood brain barrier.

## 5.7 Synthesis of Carbamates to Investigate Structure-Activity Relationships

It is unknown whether the mechanism of action of the drugs on the *T. brucei* involves the cyclen moiety or the carbamate side chain. In order to assess the structure-activity relationships, it was decided to synthesise a set of carbamates without the cyclen macrocycle. These contained the functional groups that had given the best biological results from the macrocycles that had been tested – *e.g. ortho-*, *meta-* and *para*-nitrophenyl and *para*-methoxyphenyl derivatives.

### *5.7.1 Piperazine Based Bis-Carbamates*

Henderson had previously synthesised a range of piperazine-based carbamates.[137] In fact it was a procedure developed by Henderson that had been used to synthesise the range of macrocyclic carbamates **84**-**91**. It was decided to synthesise a range of piperazine-based carbamates. The compounds would still incorporate a heterocycle. They should be useful for comparison purposes with the macrocyclic carbamates. As the precursor to the desired compounds was commercial, only one synthetic step would be necessary (**Scheme 32**).

Using the same general procedure described for the synthesis of the other carbamates, four piperazine derivatives **103**-**106** were successfully produced and the yields are summarised in **Table 5**.


**Scheme 32**

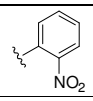
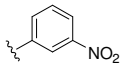
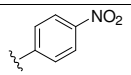
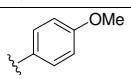
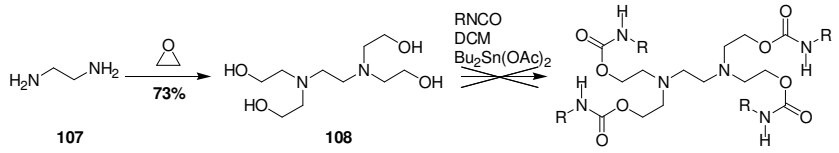
| Cpd no. | R | Yield |
|---|---|---|
| **103** |  | 68% |
| **104** |  | 55% |
| **105** |  | 90% |
| **106** |  | 99% |

**Table 5**

### *5.7.2 Linear Carbamates*

It was also decided to synthesise a linear compound that would have four arms, similar to the tetraazamacrocyclic cyclen moiety. 2-Hydroxyethyl arms were added to ethylene diamine **107** producing the tetraol **108** required for carbamate formation (**Scheme 33**). The next step was the addition of the carbamate functionality to the four side arms. The same procedure and reaction conditions were attempted as for the macrocyclic carbamates, unfortunately it proved impossible to form the desired compounds. It was decided to test biologically the four piperazine derivatives that had been successfully produced.




**Scheme 33**

## 5.8 Biological Results

The four piperazine based derivatives **103**-**106** were tested as before against *Trypanasoma brucei* and HEK cells. The results are summarised below (**Table 6**).

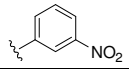
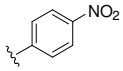
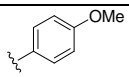
| Cpd no. | R | Est. log P | *T. brucei* $EC_{50}$ (µM) | HEK $EC_{50}$ (µM) |
|---|---|---|---|---|
| **103** |  | 3.23 | >100 | >200 |
| **104** |  | 3.23 | 16.6 | 14.1 |
| **105** |  | 3.23 | 1.31 | >200 |
| **106** |  | 2.61 | >100 | >200 |

**Table 6**

The biological results show that **103** and **106** have no trypanocidal activity or any activity against the HEK cells. However the corresponding cyclen derivatives **87** and **89** with the same functional groups in the same positions do show activity against trypanosomes, therefore the cyclen ring of the macrocycles is responsible for some of the activity. The

derivative **104** with the nitro group in the *meta* position displays low trypanocidal activity, however this compound seems to be toxic to HEK cells. It appears to act more as a cytostatic than a toxic drug. The best results occur with the *para*-nitro derivative **105**. Strong trypanocidal activity is displayed however the compound does not seem toxic to HEK cells.

It is not understood why moving the nitro group around the aromatic ring displays such an array of results. The results do not help explain the structure-activity relationship for the azamacrocycles in any more detail.

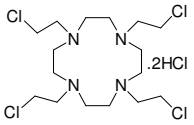
## 5.9 Conclusions

A short efficient synthesis of a range of eight macrocyclic carbamates was developed and the corresponding copper complexes were successfully synthesised as alkylating agents disguised as pro-drugs. Unfortunately the compounds synthesised proved to be too stable to be activated as aziridinium ions and the corresponding copper complexes were therefore unselective towards hypoxic cells. Further work is necessary to design successful anti-cancer prodrugs.

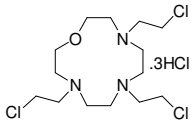
It would appear that the eight macrocyclic carbamates provide a good model for the development of anti-parasitic drugs as they gave promising results when tested against *Trypanosoma brucei*. The structure-activity relationship for the eight macrocyclic carbamates was unclear so a range of piperazine carbamates were synthesised to investigate whether the activity of the compounds is due to the cyclen moiety or the carbamate functional group. Initial biological results show that the nitro group in the *para*-position of the piperazine carbamates produces the best anti-parasitic activity for these compounds. Futhermore, it is evident that the macrocyclic cyclen ring does confer significant anti-parasitic activity. Futher work is required to determine the mode of action of the macrocyclic compounds. In addition, the structures of the compounds needs modified to enhance their solubility. It is hoped that the transport of these compounds through the parasite plasma membrane will be better understood by studying the different carbamates that have been synthesised. There is a realisitic prospect of developing more potent and selective analogues.

# 6 Synthesis of Thiaazamacrocycles

As previous members of the Robins group had synthesised a range of azamacrocycles[115] and oxaazamacrocycles[116] such as **58** and **65**, it was decided to synthesise a range of thiaazamacrocycles to complete the set of macrocycles containing the common heteratoms of N, O and S.

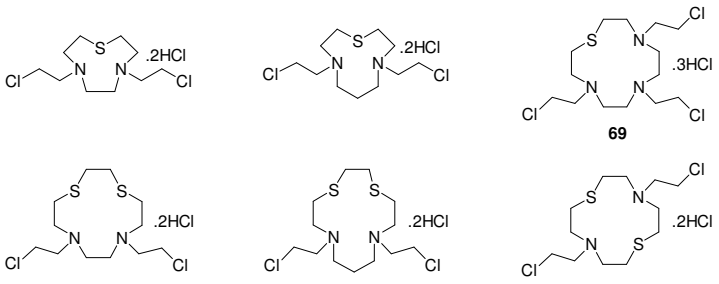



**58**




**65**

The previously synthesised oxaazamacrocycles were promising alkylating agents, and compound **65** displayed the most potent cytotoxicity of any macrocycle synthesised to date.[116] It was therefore planned to make a range of thiaazamacrocycles (**Figure 15**). They might display different biological results to the oxaazamacrocycles. It should be noted that it was impossible to synthesise the corresponding pro-drug of **65** by complexing the macrocycle to copper, although there is literature precedent for the complexation of copper to ligands containing oxygen and nitrogen.[158] It is well-documented that thiaazamacrocycles complex to copper[159] and the proposed thiaazamacrocycles should form the desired pro-drugs with copper(II) and might produce hypoxia selective compounds.




**69**

**Figure 15**

Extreme caution would have to be exercised during the synthesis of the thiaazamacrocycles to ensure that at no time would a sulfur mustard be formed as an intermediate. If the pathway used to synthesise the azamacrocycles[115] and oxaazamacrocycles[116] was modified to synthesise the sulfur derivatives, several sulfur mustards might be required as precursors or intermediates. It was decided that it would be prudent to find a synthesis of a

thiaazamacrocycle that was designed in such a way that epi-sulfonium ion formation could not occur – *i.e.* the C-2 of the 2-chloroethyl arm (or corresponding leaving group) was protected so that the lone pairs on the sulfur could not activate the mustard mechanism (**Figure 16**).

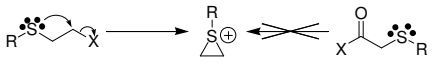


**Figure 16**

## 6.1 Amide Strategy

A literature search provided the necessary solution to the problem. There are multiple examples in the literature of the synthesis of thiaazamacrocycles by forming amide bonds.[160] The carbonyl group present on the β-carbon to the sulfur provides protection against epi-sulfonium ion activation. Once the macrocycle is formed, the carbonyl groups would be reduced leaving the desired parent macrocycle. At this stage epi-sulfonium ion formation cannot occur. There were many literature precedents available for the reduction of the carbonyl groups.[159,161] Not only would this pathway (**Figure 17**) to new macrocycles produce the desired compounds safely, it would also eliminate the difficult low yielding de-tosylation step and reduce the number of synthetic steps required to synthesise the compounds. Once the reduced macrocycle was obtained, the alkylating arms of the compound would be added using ethylene oxide. Again, there are literature examples detailing the 2-hydroxyethylation of thiaazamacrocycles.[162] Chlorination would then take place producing the desired mustard. Copper complexation would be the final synthetic step required to produce the prodrug.

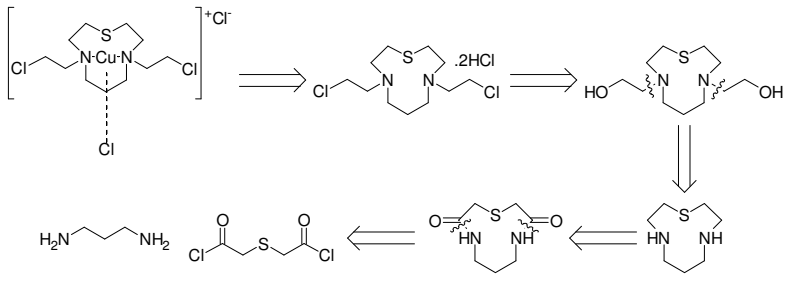
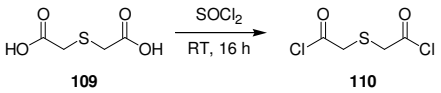


**Figure 17**

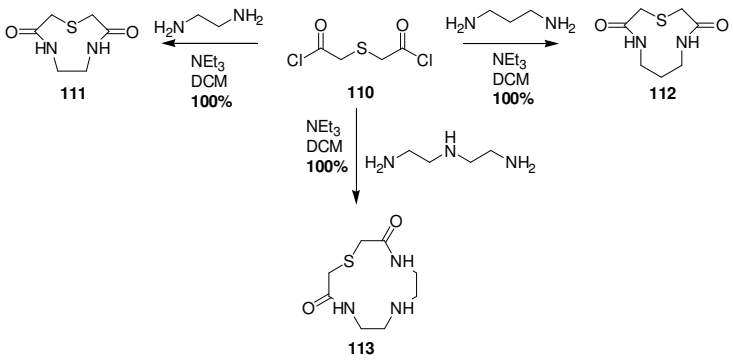
### *6.1.1 Formation of Amide-Protected Macrocycles*

The first step of the reaction involves the synthesis of the sulfur-containing acid chloride that is the precursor for the amide bond formation. This was simply prepared by stirring thiodiglycolic acid **109** in thionyl chloride at room temperature overnight (**Scheme 34**).[160c] The acid chloride **110** was isolated by removing the excess thionyl chloride under vacuum. This product was not purified but used directly in the next reaction. Quantitative conversion was assumed.



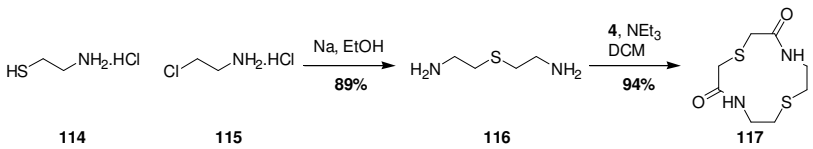

**Scheme 34**

Formation of the macrocycles occurred using a high dilution simultaneous drop-wise addition of thiodiglycolic acid chloride **110** and a diamine.[160c] The product precipitated from the reaction mixture. Three macrocycles **111**-**113** were synthesised using commercial diamines (**Scheme 35**). These reactions work very well and quantitative yields were achieved for the three macrocycles **111**-**113**.




**Scheme 35**

In one case, it was necessary to synthesise the diamine precursor **116** required to carry out the cyclisation. There are several procedures in the literature to synthesise sulfur-containing diamines.[163] It was decided to use a process that involved reacting cysteamine hydrochloride **114** with 2-chloroethylamine hydrochloride **115** in a sodium ethoxide solution.[164] The desired amine **116** was successfully formed and was subsequently reacted with the thiodiglycolic acid chloride **110** producing the desired macrocycle **117** in excellent yield (**Scheme 36**).



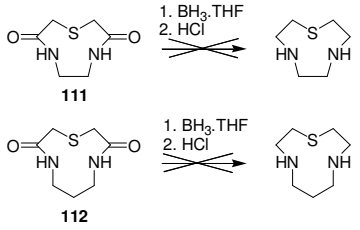

114 115 116 117

**Scheme 36**

It should be noted that NMR spectroscopic characterisation carried out on the macrocycles corresponded to literature data. However it proved very difficult to obtain any mass spectrometry data for any of these macrocyclic amides (**111-113**, **117**).

## *6.1.2 Reduction of Carbonyl Groups*

Four amide-protected macrocycles (**111-113**, **117**) had been successfully synthesised. The next step involved reducing the amide functional groups. The use of 1M borane in tetrahydrofuran solution as the reducing agent was reported in the literature.[160] Macrocycles **111** and **112** were heated under reflux in borane solution for four hours (**Scheme 37**). The compounds did not dissolve and grey suspensions were observed. The excess borane was quenched and the reaction mixtures were concentrated. The resulting residues were heated under reflux in 6M hydrochloric acid for two hours. However, after basification and extraction according to literature procedures, only trace amounts of material were isolated. The aqueous phases were concentrated and re-extracted but nothing was recovered. It was assumed that the borane solution had degraded or that the starting materials had broken apart due to harsh reaction conditions.




**Scheme 37**

The reactions were repeated using new borane reagent and the reaction mixtures were vigorously stirred to aid dissolution of the starting materials. Again only small amounts of crude material were recovered. Perhaps heating the compound under reflux in acid during the work-up was too harsh and the macrocycle was degrading.

The solubility of the starting materials could be an issue. The reaction mixture was sonicated in an attempt to encourage the starting material to dissolve in the tetrahydrofuran solution. The macrocycles **111** and **112** are extremely insoluble so that no common organic solvents can be used ruling out the use of other well-known reducing agents such as lithium aluminium hydride.

It was decided to continue developing the borane reduction and instead of heating under reflux in 6M hydrochloric acid as a work-up, the corresponding hydrobromide salt was formed. No basic work-up was performed as it was felt that the free base could be isolated by carrying out anion exchange column chromatography. Preliminary results looked promising but the isolated material was a mixture of compounds which proved difficult to purify. In certain cases the recovered products were the diamines used to form the macrocycle. Thus, the skeleton of the macrocycle had broken apart into several fragments. Dean-Stark conditions were also applied to isolate the free base from the hydrobromic salt but no product was isolated.

Different extraction conditions involving heating the quenched mixture under reflux in a methanol, water and hydrochloric acid mixture were attempted but again the desired products were not isolated. Different work-up conditions were tried including concentrating the quenched reaction mixtures and extracting the product into chloroform and ethyl acetate but nothing was recovered.

When the reaction was carried out on macrocycles **113** and **117,** complete decomposition of the starting materials was observed.

It was decided that the best course of action could be to do no work-up at all. After quenching the excess borane, the reaction mixture was concentrated. $^{1}$H NMR spectra for the desired products showed the expected peaks although not necessarily at the expected frequency (**Figures 18** and **19** – some impurities are present as these are crude reaction mixtures). For compound **111,** the expected $^{1}$H NMR signals of the corresponding amine would be a singlet corresponding to a $CH_2N$ peak and two triplets corresponding to a $CH_2N$ peak and a $CH_2S$ peak. For compound **112,** the expected $^{1}$H NMR signals for the corresponding amine would be three triplets corresponding to two $CH_2N$ peaks and a $CH_2S$ peak as well as a quintuplet/multiplet signifying the $CH_2$. It should be pointed out that it was impossible to obtain mass spectra for these supposedly reduced macrocycles.

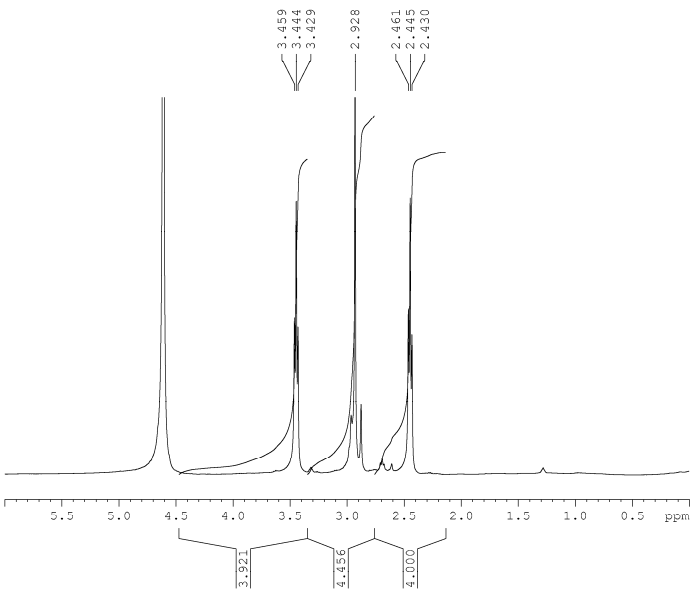



**Figure 18**

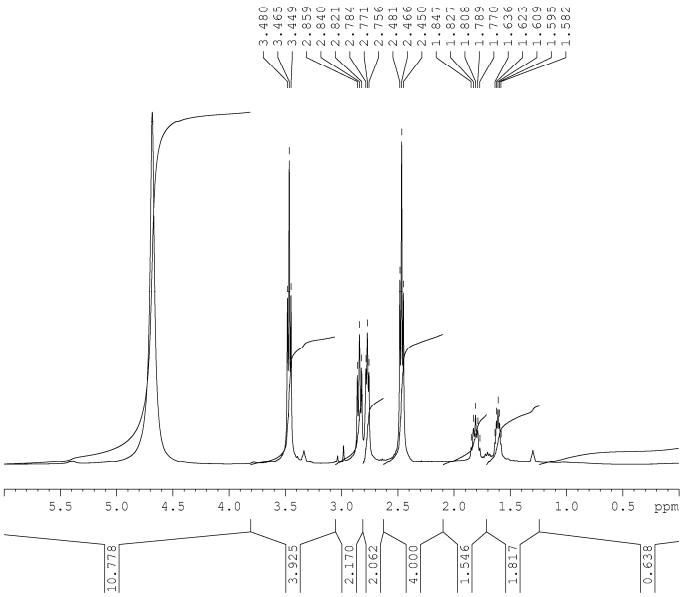



**Figure 19**

The next step of the reaction sequence was carried out. Using ethylene oxide, the addition of the 2-hydroxyethyl arms was attempted.[115] Again $^{1}$H NMR spectra suggested that the

desired 2-hydroxyethylated compounds may have been synthesised yet it was still impossible to obtain mass spectra. It was decided to try and chlorinate the 2-hydroxyethyl arms using thionyl chloride – this should produce a solid that could be purified by recrystallisation. However when this procedure was carried out on the supposed 2-hydroxyethylated thiaazamacrocycles, no product was isolated. It appeared that the reaction components had completely degraded.

At this point it was decided to change the synthetic pathway. There was no proof that the intermediates were being produced. It was disappointing that this pathway resulted in failure as there does appear to be literature precedent.

## 6.2 Tosyl-protected Macrocycle Strategy

It was decided to try and synthesise some tosyl-protected thiaazamacrocycles as this synthesis pathway had been highly successful for the production of the azamacrocycles[115] and the oxaazamacrocycles.[116] Characterisation of intermediates should confirm or exclude the presence of the desired compounds from the previous pathway. As explained previously, extreme care would need to be exercised to ensure that no sulfur mustard intermediates were formed (**Figure 20**).

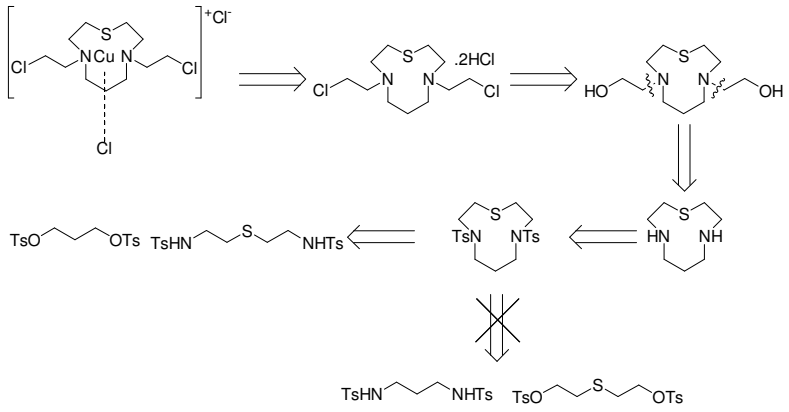
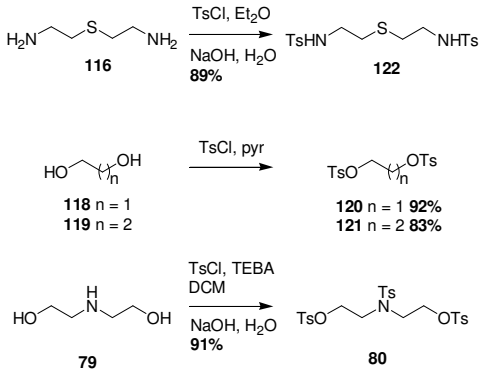



**Figure 20**

### *6.2.1 Formation of Tosyl-protected Macrocycles*

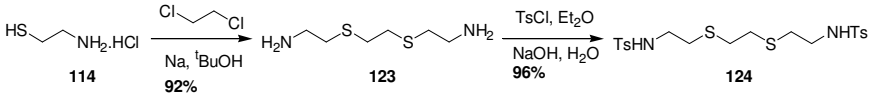
In order to synthesise the tosyl-protected macrocycles the precursors needed to be prepared. To produce the two thia-diazamacrocycles (*S,N,N*), the diaminothiol **116** which was synthesised for the amide synthetic pathway and the necessary diols were tosyl-

protected using standard conditions (**Scheme 38**). 1,2-Ethanediol **118** and 1,3-propanediol **119** were protected using tosyl chloride and pyridine.[128] The protection of diaminothiol **116** required a vigorously stirred reaction mixture of toluene-4-sulfonyl chloride in diethyl ether and sodium hydroxide solution.[165] For the thia-triazamacrocycle (*S,N,N,N*), the tosyl protected diethanolamine **80** synthesised in Chapter 5 was used as well as the tosyl protected diaminothiol **122**.



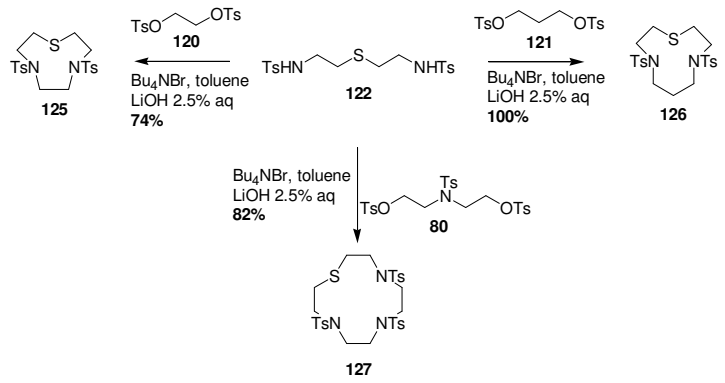

**Scheme 38**

For the '*pseudo*-cis'-dithia-diazamacrocycles (*S,S,N,N*), a new diaminodithiol **123** required to be synthesised but there was literature precedent for the synthesis of this compound.[166] Cysteamine hydrochloride **114** was reacted with 1,2-dichloroethane in a sodium butoxide solution (**Scheme 39**). This dithiadiamine **123** was then tosyl protected using the same conditions described for **122**.[165]

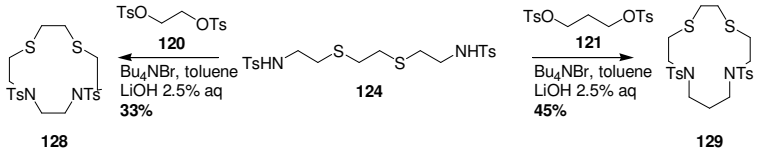



**Scheme 39**

The cyclisations took place under the phase-transfer conditions previously described in Chapter 5 (**Scheme 40** and **41**).[126]

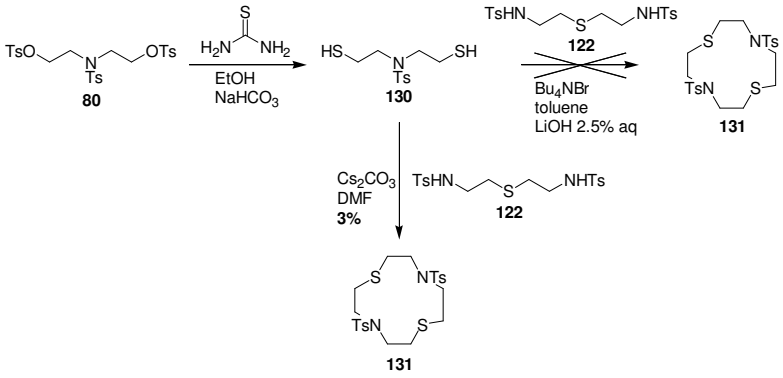



**Scheme 40**




**Scheme 41**

For the '*pseudo*-trans'-dithia-diazamacrocycle (*S,N,S,N*), a different approach was required to avoid using a sulfur mustard. The precursor was prepared by reacting the corresponding tosyl protected diol **80** with thiourea producing the dithiol **130**[160a] that reacted with the tosyl protected diaminothiol **122** (**Scheme 42**). The cyclisation was unsuccessful using phase-transfer conditions[126] but a trace amount of desired product **131** was obtained using standard Richman-Atkins conditions.[123]



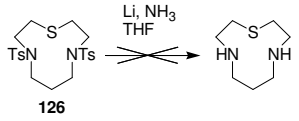

**Scheme 42**

Therefore it was decided to carry on with the other five macrocycles (**125-129**) to complete the synthesis pathway. It should be noted that the yields for cyclisation to **128** and **129**

were disappointing when compared to those for the azamacrocycles and oxaazamacrocycles.

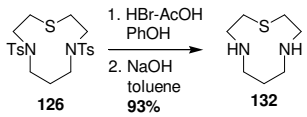
### *6.2.2 Tosyl Deprotection of Macrocycles*

As explained before, the procedures used to de-tosylate macrocycles are harsh and low yielding. It was decided to investigate the reaction conditions for de-tosylating thiaazamacrocycles. There was literature precedent for the removal of tosyl groups from thiaazamacrocycles.[133] The first method attempted used Birch reduction conditions. The tosyl-protected macrocycle **126** was reacted with a lithium and ammonia mixture in tetrahydrofuran (**Scheme 43**). Unfortunately no product was isolated.




**Scheme 43**

It was then decided to use the HBr-AcOH mixture discussed previously.[133] The tosyl-protected macrocycle **126** was heated under reflux for two days in an HBr-AcOH and phenol mixture (**Scheme 44**). The free amine was isolated in a number of ways. The first involved an ion-exchange column, however this was extremely low yielding and the product was not pure. The product could not be separated from the crude mixture by Kugelrohr distillation. Dean-Stark conditions were employed and the hydrobromide salt and sodium hydroxide were heated under reflux overnight in toluene. Upon concentration of the solvent the product **132** was obtained.




**Scheme 44**

However the crude mixture appeared to contain more than one product. As the compound is very polar, purification is difficult. Compound **132** is so polar that it would not pass through a silica column. Alumina column chromatography would be a good alternative as the concept of reverse phase chromatography is used. The product was separated quickly

and efficiently providing the desired compound. This technique proved extremely successful for obtaining the pure free amine macrocycle **132**.

**Figure 21** shows the yields achieved for the five substrates (**132**-**136**). This reaction is generally not high yielding and starting materials (tosylated macrocycle) were sometimes recovered. However it was felt that as long as the desired free bases were obtained in pure form, the yields were sufficient.

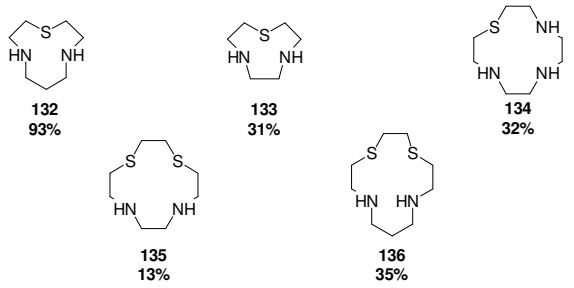
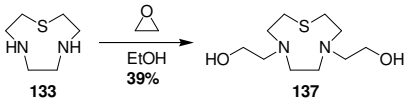



**Figure 21**

It should be pointed out that $^{1}$H NMRs collected for the free bases (**132** and **133**) were compared with the $^{1}$H NMRs shown in **Figures 18** and **19**. It was determined that the desired compounds had not been synthesised using the amide synthetic pathway and wny further research hwould involve the tosyl-protected macrocycles.

### *6.2.3 Addition of 2-Hydroxyethyl Arms*

The next step of the synthesis involved the addition of the carbon chain that would make up the 2-carbon link required for optimum DNA cross-linking. This was achieved by reacting the free amines (**132**-**136**) with ethylene oxide as described before although it was necessary to change the reaction conditions required for the azamacrocycles and oxaazamacrocycles.[115] Instead of carrying out the reaction in water, ethanol was used as the reaction solvent (**Scheme 45**). Although the reaction mixture was initially cooled to 0 $^{o}$C, it was allowed to return to room temperature and the mixture was stirred overnight in a sealed reaction vessel.




133 137

**Scheme 45**

When this reaction was carried out for the synthesis of the azamacrocycles and the oxaazamacrocycles, no purification was required as the desired pure compounds were isolated with no by-products present. In the case of the thiaazamacrocycles, $^{1}$H NMR spectra showed the presence of other peaks. It is likely these are polymerised or over-alkylated products. Alumina column chromatography was used once again to purify the compounds and all five substrates (**137-141**) were successfully isolated (**Figure 22**).

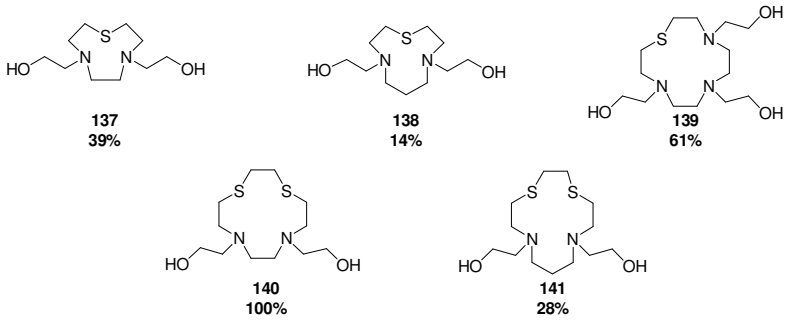
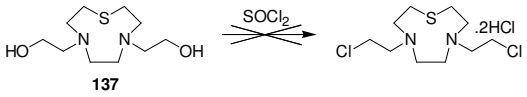



**Figure 22**

## *6.2.4 Chlorination of Macrocycles – Mustard Formation*

All that remained was the final chlorination step that would produce the desired alkylating agents as hydrochloride salts. The protonated nitrogens should prevent aziridinium ion formation.

The 2-hydroxyethylated macrocycles (**137**-**141**) were reacted overnight in thionyl chloride at 50 $^{o}$C (**Scheme 46**).[115] Normally a white solid is isolated after extraction and purification but with every reaction attempted, a brown viscous oil was recovered. Numerous attempts were made to try and isolate a pure product but nothing was successful. $^{1}$H NMR spectra showed that no expected peaks were present, and it appeared that degradation had occurred.



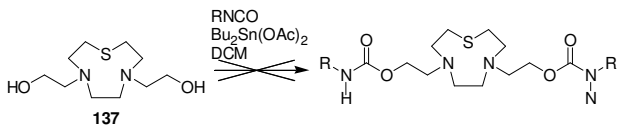

**Scheme 46**

It was extremely disappointing to discover that the chlorinations were unsuccessful, particularly as the synthesis pathway to the 2-hydroxyethylated thiaazamacrocycles had been arduous and difficult throughout with unexpected purification required at each step. The synthesis of the azamacrocycles and the oxaazamacrocycles had been less complicated. Without the chlorinated mustards it was clearly impossible to synthesise the desired alkylating agents and corresponding prodrugs.

### *6.2.5 Carbamate Formation*

It was decided to try and synthesise a selection of carbamates from the range of 2-hydroxyethylated thiaazamacrocycles (**137-141**) that had been successfully synthesised.[137] Although it was highly probable that the carbamates would be too stable to be efficient alkylating agents due to biological testing carried out previously, synthesis of copper(II) complexes could be attempted to see if the sulfur coordinates to the copper better than with the oxygen-containing macrocycles. Anti-parasite testing would also be carried out to determine whether the thiaazamacrocycles (**137**-**141**) are more cytotoxic than the azamacrocyclic carbamates (**84-91**) thus providing the prospect of a new molecular skeleton to develop anti-parasitic drugs.

The 2-hydroxyethylated macrocycle **137** was heated under reflux with 4-nitrophenyl isocyanate and dibutyl tin diacetate in DCM overnight (**Scheme 47**).[137] Unfortunately the only product that was isolated was the corresponding hydrolysed isocyanate as seen before during the azamacrocyclic carbamate (**84-91**) synthesis. Several different reaction conditions were investigated as had been required during the synthesis of azamacrocyclic carbamates but nothing proved successful.

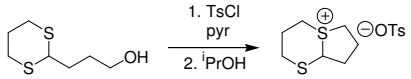



**Scheme 47**

It was decided to leave this area of the project and concentrate on the synthesis of compounds that would prove to be biologically active.

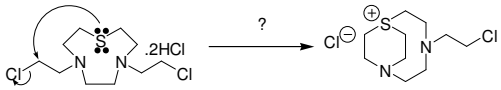
## 6.3 Conclusions

It was extremely frustrating that none of the desired final compounds could be synthesised. A possible explanation to the problem was discovered; Hartley and co-workers had investigated the synthesis of thioacetals as pathway intermediates due to the fact that carbonyl groups can be successfully masked to allow synthetic change to other parts of the molecule.[167] It was discovered that a 1,3-dithiane displaces tosylate by a 5-*exo*-tet cyclisation to give a bicyclic sulfonium salt which was isolable (**Scheme 48**).[168] This was verified by X-ray crystallography.




**Scheme 48**

It was suggested that a similar mechanism could be occurring with the chlorinated thiaazamacrocyclic mustards. A favoured 6-*exo*-tet cyclisation mechanism could happen forming a strained structure that then broke apart resulting in the degradation of the macrocycle.




**Scheme 49**

Despite not achieving the aims of the project, a reliable synthetic pathway has been developed that allows the preparation of pure thiaazamacrocycles as free bases. Functionalisation has also been added to the thiaazamacrocycles in the form of 2-hydroxyethyl arms.

# 7 Synthesis of Substituted Macrocycles

As stated before, Reid had investigated the synthesis of aromatic-substituted azamacrocycles.[117] By using a two-step iron templated synthesis (**Figure 23**),[169] a range of 20 compounds was produced and several of these compounds displayed excellent results when tested against *Trypanosoma brucei*.

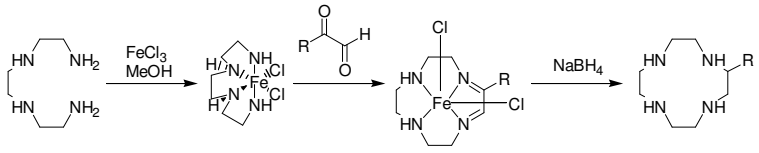
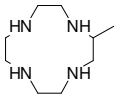



**Figure 23**

## 7.1 Synthesis of an Alkyl Substituted Azamacrocycle

It would be beneficial to synthesise an alkyl substituted macrocycle to discover whether it possesses different biological activity as this would aid the design and development of new anti-parasite drugs. It was decided to attempt the synthesis of a macrocycle **142** with a methyl group attached as this is the simplest alkyl group and if the synthesis was successful it would provide a pathway for the synthesis of a range of compounds with different alkyl substituents.



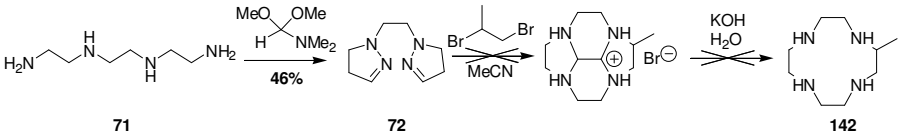

**142**

### *7.1.1 Template Synthesis*

It was decided to attempt a template synthesis as discussed previously in Chapter 5 for the synthesis of cyclen **70**.[121] If successful this would provide a short route for the multigram preparation of the susbstituted macrocycle.

The first step of the reaction was the synthesis of bisimidazoline **72** by treating triethylenetetraamine **71** (TETA) with dimethylformamide dimethyl acetal (**Scheme 50**). The desired product **72** was obtained in crystalline form in moderate yield and was reacted with 1,2-dibromopropane to form the carbon skeleton of the macrocycle. The $^{1}$H NMR

spectrum showed the disappearance of the bisimidazoline so the reaction was carried onto the next step. Alkaline hydrolysis should remove the two carbon infrastructure leaving the desired product **142**. Unfortunately no product **142** or any starting material was recovered; the harsh reaction conditions must have broken down the reagents. It is unknown whether the macrocyclic skeleton was formed at all.

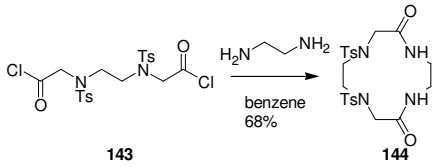



**Scheme 50**

## *7.1.2 High Dilution Amide Synthesis*

### 7.1.2.1 Formation of Macrocycle

Reid also carried out some research into the synthesis of enantiomerically pure substituted macrocycles.[117] By adapting this line of research, the synthesis of the methyl substituted macrocycle **142** should be possible. Stetter and Mayer in 1961 had synthesised dione **144** by reacting diacid chloride **143** with ethylenediamine (**Scheme 51**).[170]




**Scheme 51**

By substituting 1,2-diaminopropane for the ethylenediamine, it would be possible to synthesise a compound with the molecular skeleton of the desired target compound. Reduction of the carbonyl groups and removal of the tosyl groups would produce the methyl-susbtituted macrocycle **142** as a free base (**Figure 24**).

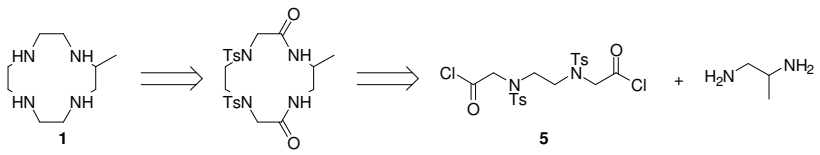
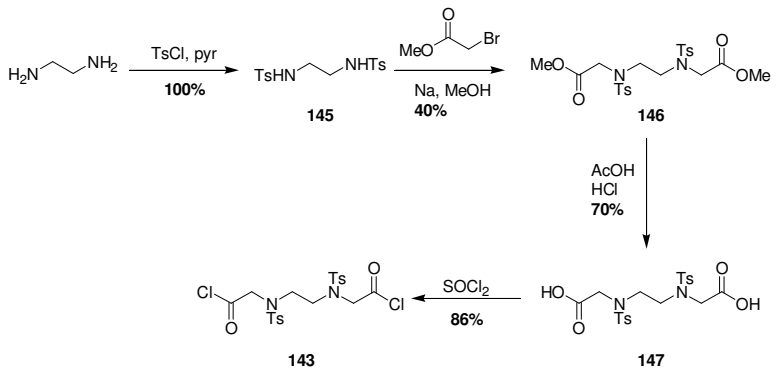


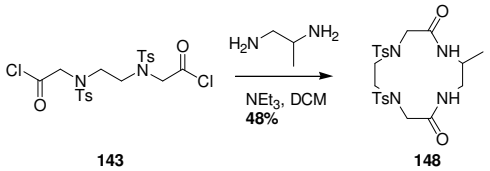
**Figure 24**

The first stage of the reaction pathway was the synthesis of the diacid chloride **143**.[170] This was achieved in a four step process (**Scheme 52**). Tosyl-protected ethylenediamine **145** (prepared quantitatively from ethylenediamine using toluene-4-sulfonyl chloride and pyridine), was reacted with methyl bromoacetate in a sodium methoxide solution to give diester **146**. The diacid **147** was obtained by heating **146** under reflux with acetic acid and hydrochloric acid. The desired intermediate **143** was obtained by heating diacid **147** under reflux in thionyl chloride.



**Scheme 52**

The next step of the pathway was the key cyclisation reaction.[170] The original procedure used benzene as the solvent for this reaction. However this reaction requires high dilution conditions so for health and safety reasons dichloromethane was substituted for the benzene. The diacid chloride **143** and 1,2-diaminopropane were added dropwise simultaneously and the desired product **148** precipitated from the reaction mixture (**Scheme 53**).

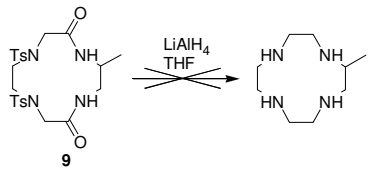



143 148

**Scheme 53**

### 7.1.2.2 Formation of Free Base

The next step of the pathway required the removal of the tosyl groups and the reduction of the carbonyl groups. A procedure was found that claimed to use lithium aluminium hydride to remove tosyl protecting groups from cyclen **70**.[171] It was thought that this method could be used to reduce the carbonyl groups and remove the tosyl groups in one step. The reaction was carried out by heating the macrocycle **148** under reflux in THF with lithium aluminium hydride for three days (**Scheme 54**). However the conditions were too harsh and the macrocycle was destroyed.

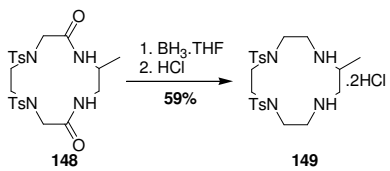



9

**Scheme 54**

It was decided to use milder conditions to reduce the carbonyl groups and then the tosyl groups would be removed using de-tosylation methods well known in the Robins group.

The carbonyl groups were successfully reduced using a 1M borane in tetrahydrofuran solution followed by hydrolysis using conc. hydrochloric acid yielding the desired compound **149** (**Scheme 55**).[172]




148 149

**Scheme 55**

Only one step remained to complete the synthesis of the methyl-substituted macrocycle **142**. The de-tosylation step used to obtain cyclen **70** was attempted. The hydrobromide salt was formed followed by formation of the free base under Dean-Stark conditions but attempts to isolate the desired compound were unsuccessful.[173] Use of other established de-tosylation techniques were also unsuccessful (*e.g.* HBr-AcOH 45%).[133]

It was decided to abandon this synthesis as the desired alkylated compound had not been produced and other areas of research were producing better results. It was hoped that more success would be had synthesising substituted thiaazamacrocycles.

## 7.2 Synthesis of Aromatic-Substituted Thiaaza-macrocycles

Reid had synthesised a range of azamacrocycles based on the cyclen skeleton.[117] It would be interesting to investigate whether the cytotoxicity of the compounds changes depending on the presence of different heteroatoms in the macrocyclic structure. As work was already being carried out trying to synthesise thiaazamacrocycles for use as alkylating agents, it seemed prudent to synthesise a range of substituted thiaazamacrocycles (**Figure 25**).

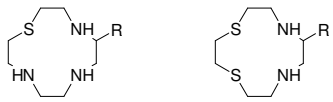



**Figure 25**

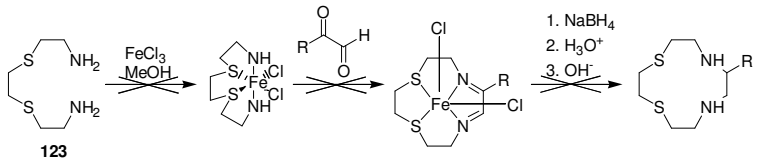
It was decided to attempt the synthesis of the substituted thiaazamacrocycles using the iron templated synthesis that had been successful for Reid.[169] It is well-documented that triethylene tetraamine (TETA) **71** can form complexes (*cis*-[$MLCl_2$]) with first-row transition metals.[174] It was unknown whether the iron would complex to the ligand if any sulfur atoms were present.

### *7.2.1 Synthesis of a Substituted Dithia-diazamacrocycle*

#### 7.2.1.1 Iron Templated Synthesis

The reaction was attempted using the dithiadiamine **123** synthesised previously (Chapter 6). Diamine **123** was reacted with ferric chloride and methanol to form the diimine

(**Scheme 56**). Commercial phenyl glyoxal monohydrate was added. In order to isolate the desired compound, sodium borohydride was added to reduce the iron(III) to iron(II). An acidic work-up removed the coordinated iron leaving the free base.[169] When Reid synthesised the azamacrocycles, there were specific colour changes associated with the different stages of the reaction, however these were not observed when attempting the synthesis of the thiaazamacrocycle. The $^{1}$H NMR spectrum of the crude product looked promising. However it was soon discovered that cyclisation had not occurred and reduced starting materials were obtained from the mixture, thus explaining the 'positive' $^{1}$H NMR spectrum.



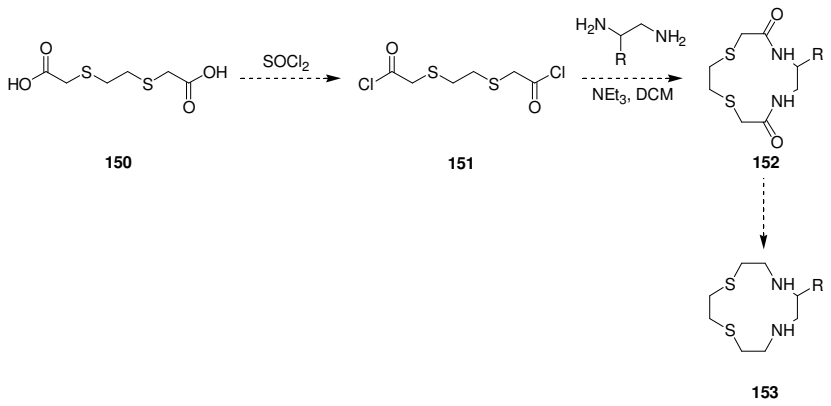

**Scheme 56**

### 7.2.1.2 Future Work - High Dilution Amide Synthesis

It was disappointing that the iron templated synthesis was unsuccessful and due to time constraints more research was not carried out in this area of the project. However upon reflection, there is another route that could be followed to synthesise a substituted dithia-diazamacrocycle.

The high dilution route described previously to synthesise the methyl-substituted azamacrocycle **142** failed due to the presence of the tosyl-protecting groups which could not be removed under standard conditions. However if the same pathway was applied in the instance of the dithia-diazamacrocycles, there would be no tosyl groups present (**Scheme 57**).

The dithia-diacid **150** is commercially available. This would be transformed to the diacid chloride **151** which should then undergo cyclisation under high dilution conditions to form the macrocycle **152**.[170] Reduction conditions have already been investigated during the synthesis of the methyl-substituted azamacrocycle,[172] so the carbonyl groups should be reduced leaving the desired substituted thiaazamacrocycle **153** as the free base.

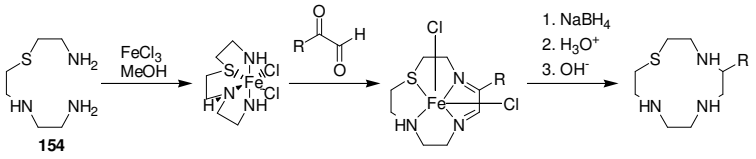



**Scheme 57**

This three step synthesis if successful would provide a means to synthesise a range of substituted dithia-diazamacrocycles simply and quickly, allowing an investigation into the development of better and more potent anti-parasitic drugs.

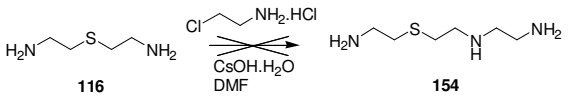
## *7.2.2 Synthesis of a Substituted Thia-triazamacrocycle*

It is thought that one of the reasons the iron templated synthesis was unsuccessful for the dithia-diaazamacrocycles was that the two sulfur atoms did not coordinate strongly to the iron centre. It was hoped that if only one sulfur was present, coordination would still occur with the three nitrogen atoms (**Scheme 58**).




**Scheme 58**

In order to test this theory, the sulfur-containing triamine **154** needed to be synthesised. There is literature precedent for the synthesis of this compound.[163c,175] A procedure was attempted using the thia-diamine **116** already synthesised (**Scheme 59**). 2-Chloroethylamine hydrochloride and caesium hydroxide monohydrate were reacted with the thia-diamine **116** in DMF – the literature quoted good yields for this reaction.[175]




116 154

**Scheme 59**

Several attempts were made to reproduce the literature results but unfortunately it was impossible to synthesise the thia-triamine **154**. Without this intermediate the reaction pathway could not be continued.

## 7.3 Conclusions

The desired substituted macrocycles could not be obtained by the routes and procedures tried. Further research needs to be carried out to obtain the desired target compounds. As discussed previsouly it is thought that the substituted dithiadiazamacrocycle (S,S,N,N) would be synthesised using the pathway discussed. These compounds would allow an investigation into cytotoxicity and a structure-activity relationship could be established leading to a better understanding of the drug's interactions in the body as well as allowing the design and development of more potent and efficient anti-parasitic agents.

# 8 Synthesis of HDM2 Inhibitors

## 8.1 Introduction – p53: 'Guardian of the Genome'

p53 was discovered in 1979[176] and voted '*molecule of the year 1993*' by *Science* magazine.[177] It has been identified as an important tumour suppressor gene, hence the description 'guardian of the genome'.[178] 50% of all tumours possess a mutated form of the p53 protein – it is the most frequently altered gene in cancers. The other 50% of tumours possess 'wild-type' p53 which is inactivated by a variety of methods. p53 stands for the 53 kDa gene product found in cells that are infected with a virus.[179]

In the body, the p53 protein activates the transcription of specific genes by forming a tetramer that binds to the DNA sequence. p53 uses two different mechanisms for tumour suppression although both processes produce the same result which is the prevention of harmful gene mutations being passed onto daughter cells. Firstly, p53 responds to DNA-damaging environmental stresses by stopping the cell cycle; DNA replication is halted allowing the damaged DNA to be repaired. Secondly, p53 can also initiate apoptosis (programmed cell death) as shown in **Figure 26**.[180]

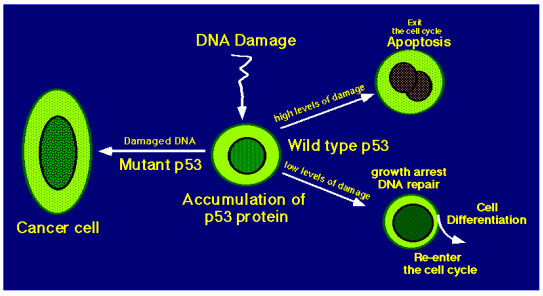



**Figure 26 - Roles of p53**[180]

Cancer can occur in cells where mutated p53 exists and apoptosis cannot occur – thus the damaged DNA replication occurs resulting in the development of a tumour. p53 mutations are largely found in the hydrophobic region of the protein which is the site of DNA binding. Due to the loss of active p53, the cancer is also less responsive to radiotherapy or chemotherapy. This genetic mutation can be inherited as a disease known as Li-Fraumeni syndrome – the sufferer inherits only one functional copy of p53.[181]

However, in healthy cells, the levels of p53 have to be kept to a minimum to allow the normal running of the cell, *e.g.* growth and replication. This function is carried out by the

HDM2 protein (Human Double Minute-2), which forms an auto-regulatory feedback loop with p53. HDM2 is the product of a p53 inducible gene.[182] p53 initiates HDM2 transcription and HDM2 inhibits p53 in three ways. HDM2 can bind to the transactivation domain of p53, which blocks the transcription of genes. Also HDM2 contains a sequence of amino acids that is similar to the nuclear export 'signal' that exists in vital proteins. Therefore binding the HDM2 to p53 induces the eviction of the complex from the nucleus to the cytoplasm where normal cell functions cannot be carried out. Finally, HDM2 is also an ubiquitin ligase protein – p53 becomes 'tagged' with a ubiquitin molecule and this targets p53 for degradation by proteasomes in the cell. It can be seen that HDM2 regulates the stability of p53 as in **Figure 27** (HDM2 is represented by MDM2 – Mouse Double Minute-2).[183]

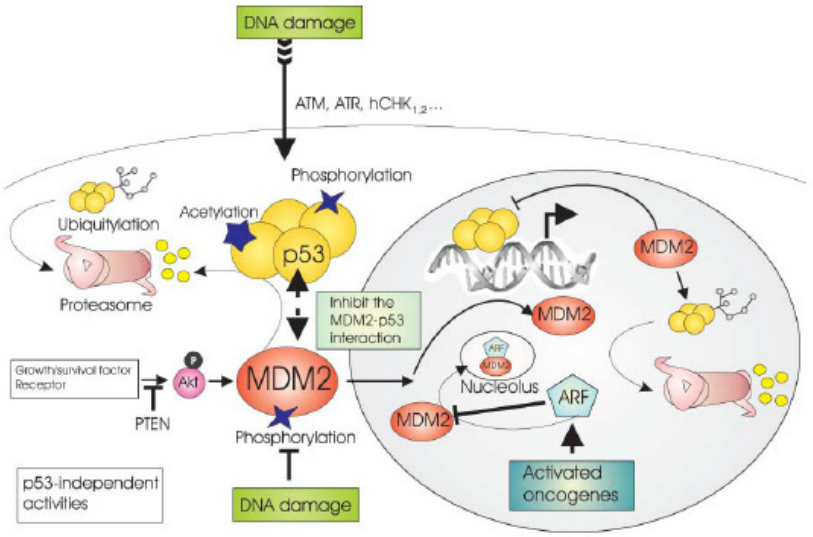



**Figure 27 - Regulation of p53 by MDM2**

However in certain circumstances, *e.g.* when a cell is put under oncogenic stress, HDM2 activity must be inhibited to allow p53 levels to increase and the cell will undergo DNA repair or apoptosis. This can be achieved in several ways. The DNA damage can initiate phosphorylation of several amino acids in p53, which prevents binding with HDM2. Secondly, the activation of oncogenes (cancer causing cells) results in the expression of a protein known as Auxin Response Factor (ARF). This ADP ribosylation factor protein binds to HDM2 using a site remote from the p53 binding site resulting in a conformational change in the HDM2 protein preventing binding and ubiquitylation on p53.[182]

### *8.1.1 Using HDM2 As A Therapeutic Target*

In some tumours, the p53 function is disrupted due to genetic mutations of p53. However other tumours possess 'wild type' p53 – this type of p53 has lost the ability to respond to oncogenic stress due to over-expression of HDM2. Thus the chance of forming a tumour is increased by the inhibition of p53 stabilisation. Generally soft tissue tumours, osteocarcinomas and oesophagal carcinomas demonstrate the highest frequency of HDM2 amplification.[184] In fact it is thought that these types of cancers require HDM2 over-expression for tumour survival. Therefore, HDM2-inhibiting drugs should cause stabilisation of p53 and induce apoptosis in cancer cells.

Past investigation into inhibitors of non-enzyme protein-protein interactions has not proved successful.[185] An investigation into the p53-HDM2 complex had to be carried out before inhibitors of HDM2 could be developed.[186] Protein crystallography was used to determine the structure of the complex of HDM2 with peptides of the p53-binding domain. The results showed that HDM2 has a deep hydrophobic cleft constructed of $\alpha$-helices and $\beta$-sheets. The HDM2 cleft is filled with three amino acid side chains from the hydrophobic face of p53 (Phe 19, Try 23 and Leu 26) – the p53 forms an amphipathic α-helix with one side hydrophobic and one hydrophilic. Van der Waals interactions and steric complementarity constitute the main sources of attraction between p53 and HDM2.

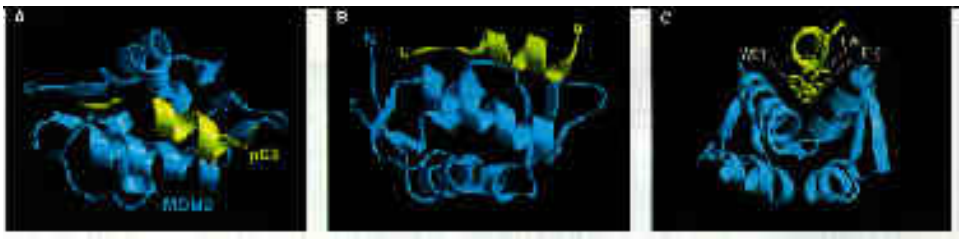


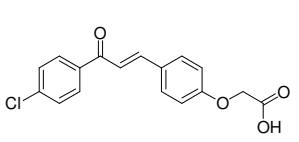

**Figure 28 - Hydrophobic Pocket in HDM2**

**Figure 28** shows the HDM2 $NH_2$ terminal in blue with its twisted 'trough' type structure. The p53 peptide, shown in yellow, may bind to the HDM2 as an $\alpha$-helix. **A** shows the HDM2-p53 complex with the floor of the HDM2 cleft in the plane of the picture. **B** shows the complex rotated $90^o$, looking down the helical axis of p53. **C** shows the complex rotated $90^o$ from the vertical axis of **B**.[185]

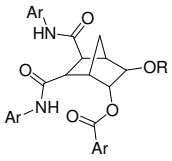
### *8.1.2 Inhibitors Of The HDM2-p53 Complex*

p53 derived peptides inhibit the interaction between p53 and HDM2. However peptides are not useful as drugs as stomach acid and hydrolytic enzymes hydrolyse the peptides too easily.[187]

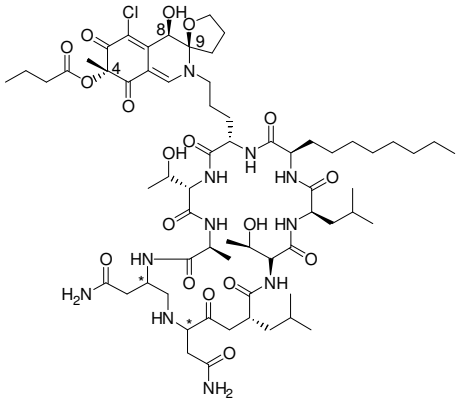
Inhibitors should be designed to mimic p53. The reason for this is that only HDM2 has structurally well-defined binding sites whereas p53 can undergo structural change, *e.g.* phosphorylation or change of conformation can occur due to stress and p53 is composed of a single short stretch of amino acids. The interactions between p53 and HDM2 consist of three hydrogen bonds and the contact surface area is small. Therefore a low molecular weight inhibitor may work which also favours an oral administration for the drug.



**155**



**156**



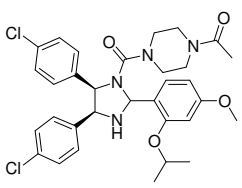
**157**

Several compounds have been identified as small molecule HDM2 antagonists. These include the chalcones **155** that are based on phenoxy-acetic acid derivatives and phenoxymethyl tetrazole derivatives.[188] The chalcones were first reported in 1999 and bind in the hydrophobic pocket of HDM2 showing $IC_{50}$ values within the μM range. Zhao and co-workers prepared non-peptidic polycyclic antagonists **156** which have shown moderate affinity for HDM2 and initiate p53-controlled apoptosis in tumour cell lines.[189] During a library screening, the nonapeptide chlorofusin **157** was identified as an inhibitor but due to the high molecular mass and complex structure it is an unsuitable drug candidate.[190]

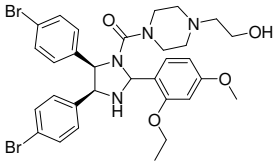
However, the molecule could prove useful for future drug design. The stereochemistry of C-4, C-8 and C-9 is as shown or inverted and the asterisks denote two stereocentres where one is *R* and the other is *S*.

Three years ago, Roche published the structure of three *cis*-imidazoline based compounds **158-160**, known as the Nutlins – named after the Nutley inhibitor.[185] The compounds were synthesised as racemic mixtures and were separated using chiral columns. One enantiomer of the products formed had potent binding activity, whereas the other enantiomer was found to be 150 times less active.

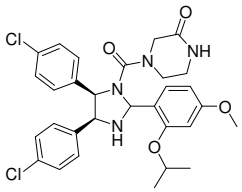
Bindings of these compounds to the p53 binding site were proven by X-ray crystal structures. An *in vivo* study of these compounds on mice with human tumour xenografts showed 90% inhibition of tumour growth after 20 days compared to the control. More importantly no side effects such as weight loss or abnormalities were observed.[185]




**158**

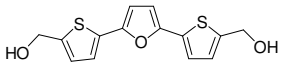



**159**




**160**

At the same time a small molecule called RITA **161** (**R**eactivation of p53 and **I**nduction of **T**umour cell **A**poptosis) was discovered by workers at the Karolinska Institute in Sweden during a library screen.[191] Again this compound could serve as a lead compound for future drug design.

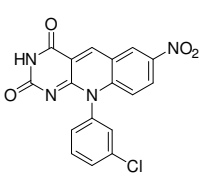



**161**

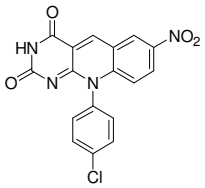
## 8.2 Aims Of The Project

A high throughput assay to identify low molecular weight inhibitors of HDM2 was developed by Professor Karen Vousden FRS and her team from the Cancer Research UK Beatson laboratories together with Dr Allan Weissman from the National Cancer Institute and Igen Pharmaceuticals in the USA.[192] The screen was carried out in tumours containing wild-type p53 and a library of 10,000 compounds was tested. Forty compounds were found

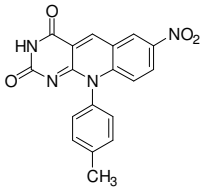
to inhibit HDM2 autoubiquitylation by more than 50%. The compounds **162-164** were identified after additional *in vitro* gel-based assays.[193] The compounds all contain a 5-deazaflavin structural motif.



**162**



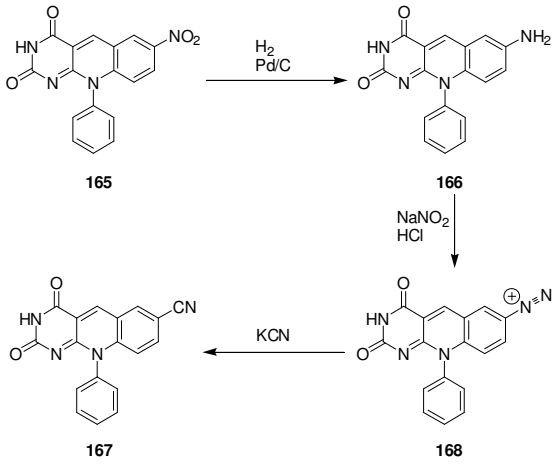
**163**



**164**

A 'library' of new analogues of these compounds has already been synthesised and tested.[194] The biological results showed that the *para*-substituted compounds are favoured over the *meta*-substituted.

As work has been carried out concerning the phenyl substituent,[194] it was decided to focus on the 7-position of the heterocycle (**Scheme 60**). An amino group instead of the nitro group at C-7 on compound **165** would produce a more water-soluble compound **166**, which should be more suited for a drug that has to be taken orally or intravenously. This amino group could then be treated with sodium nitrite and acid to form a diazonium salt **167** which should lead to the synthesis of further compounds such as the cyano derivative **168**.



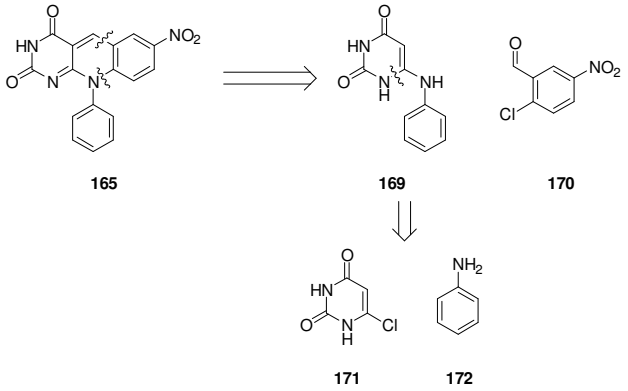
**Scheme 60**

All compounds synthesised up to this point had contained a 7-nitro group.[194] It could not be ruled out that part of the mechanism of action of these compounds involved the nitro group and oxidative stress processes.[195] In order to investigate the structure-activity relationship

of the 5-deazaflavins, compounds containing different functional groups would be prepared. The size of the binding pocket could also be investigated by synthesising deazaflavins with different aromatic rings such as quinoline.

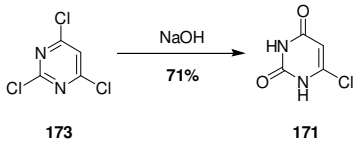
## 8.3 Synthesis of Deazaflavins

Retrosynthesis of the 5-deazaflavin skeleton **165** by disconnection of the central ring produces two fragments consisting of a 6-anilinouracil compound **169** and a substituted nitrobenzaldehyde **170**. Further disconnection of **169** produces 6-chlorouracil **171** and aniline **172** (**Scheme 61**). Analogues are easily prepared by using substituted derivatives of these synthons.




**Scheme 61**

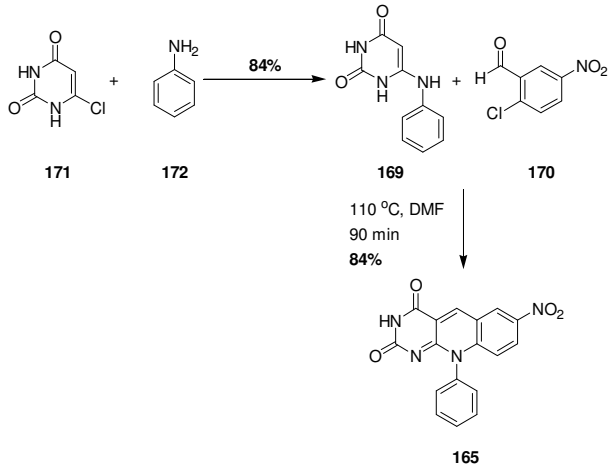
6-Chlorouracil **171** is a commercial compound, however due to problems with the supplier it was necessary to synthesise the starting material. This was successfully achieved from 2,4,6-trichloropyrimidine **173** as shown in **Scheme 62**.[196]




**Scheme 62**

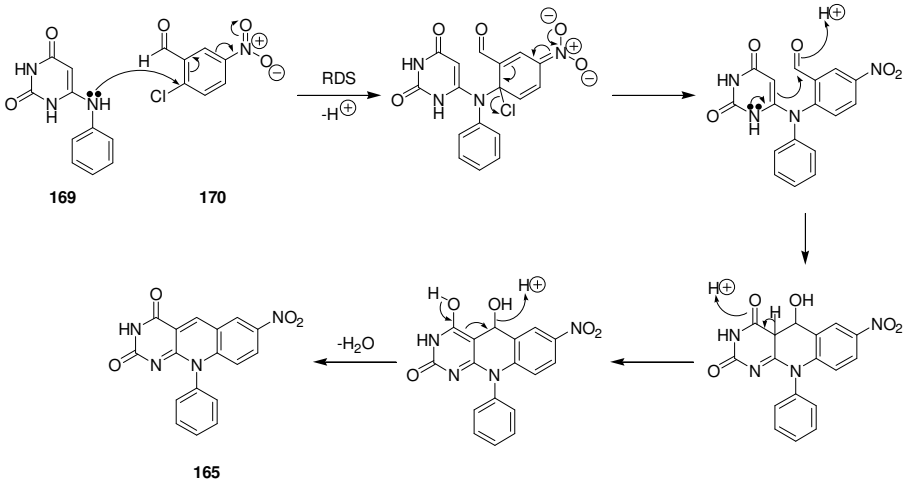
The first coupling reaction involved reacting 6-chlorouracil **171** with aniline **172** (**Scheme 63**). Diversity can be added to the synthesis by using substituted anilines. The two reactants were melted together at 170 $^{o}$C for 20 minutes producing a white solid in good yield.[197] Nucleophilic substitution occurs at the 6-position of the uracil – the presence of the

chlorine encourages this reaction. The reaction is similar to nucleophilic aromatic substitution as the intermediate formed is stabilised by the presence of the electronegative element. However the reaction is more like a 1,4-carbonyl addition. The second step of the synthesis involved the condensation of the secondary amine **169** with 2-chloro-5-nitrobenzaldehyde **170** (**Scheme 63**).[197] Water was then added resulting in the precipitation of the desired product **165**.




**Scheme 63**

The mechanism of the reaction is shown in **Scheme 64**. Nucleophilic aromatic substitution occurs before the condensation reaction takes place.[198] $S_NAr$ occurs because $S_N2$ will not occur at $sp^2$ hybridised orbitals and it highly unlikely that an $S_N1$ reaction would occur as it would involve unaided loss of the leaving group and the formation of an aryl cation with an empty $sp^2$ hybridised orbital outside of the benzene ring and a full p-orbital inside the ring. $S_NAr$ involves addition of the nucleophile followed by loss of the leaving group. The presence of an electron withdrawing group positioned *ortho-* or *para-* to the ring aids the reaction as electrons can be delocalised around the aromatic ring. 2-Chloro-5-nitrobenzaldehyde possesses two electron withdrawing groups, therefore it reacts readily with the secondary amine.




**Scheme 64**

A small library of derivatives was synthesised using the previously discussed method.[197] The results are summarised in **Figure 29**. The quoted yields are for the two successive steps.

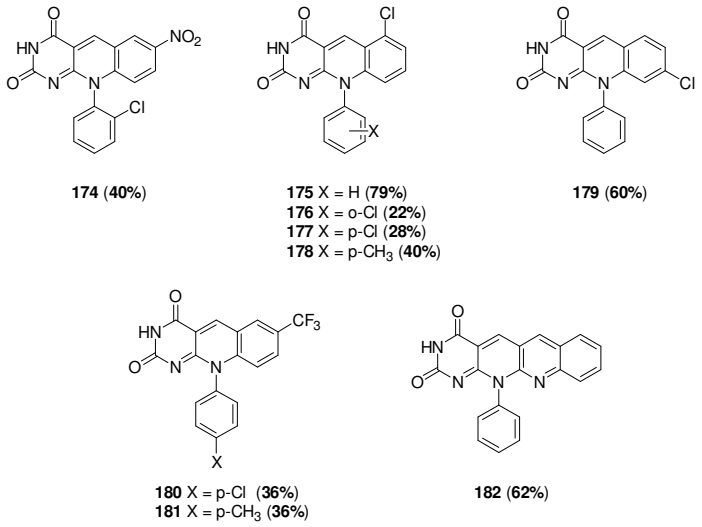



**Figure 29**

It was possible to grow crystals of **181** and an X-ray crystal structure was obtained (**Figure 30**). As expected the lower aromatic ring is twisted out of the plane of the deazaflavin system.

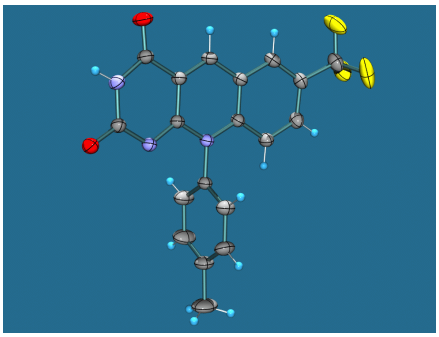
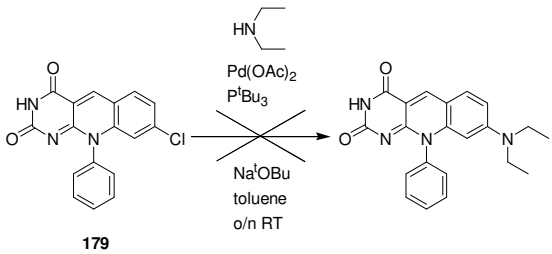


**Figure 30**

As discussed previously it was desirable to synthesise an amino derivative **166** as this would produce a precursor to many different analogues. Unfortunately it was not possible to reduce the nitro compound **165** although several different methods were tried. Firstly a reduction using iron powder and hydrochloric acid[199] was attempted but the desired product was undetectable in the $^{1}$H NMR spectrum. A radical method using samarium and 1,1'-dioctadecyl-4,4'-bipyridinium dibromide[200] also failed – the starting material was recovered. A method using formic acid, triethylamine and 10% palladium on carbon[201] also proved unsuccessful. It was thought that these reactions were unsuccessful due to the limited solubility of the nitro compound **165**.

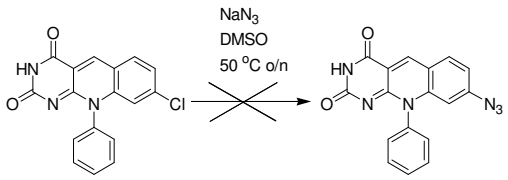
It was then decided to use Buchwald-Hartwig aminations[202] to introduce the amino moiety using compound **179** which had been synthesised using the established pathway previously discussed. Compound **179** was reacted with diethylamine, palladium diacetate, tri-*tert*-butylphosphine, sodium *tert*-butoxide and toluene (**Scheme 65**).




**Scheme 65**

Unfortunately only starting material was recovered. It was decided to attempt a microwave technique using diethylamine, potassium *tert*-butoxide and DMSO.[203] Again only starting material was recovered.

It was then thought that the chlorine atom of **179** could be substituted with an azide anion (**Scheme 66**). This involves nucleophilic aromatic substitution as discussed previously.[198] The azide would provide the opportunity to add functionality onto the molecule. Unfortunately this reaction was not successful.[204]



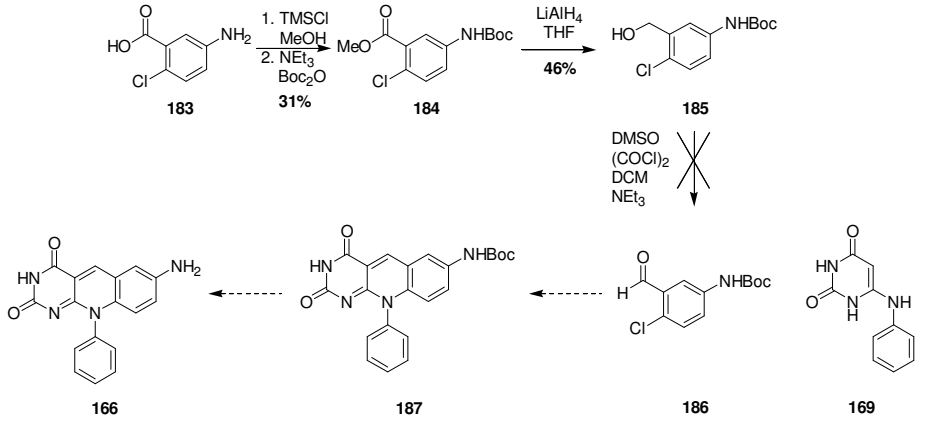

**179**

**Scheme 66**

As attempts to reduce the nitro group on the 5-deazaflavin had proved unsuccessful it was decided to use a preformed amino-chlorobenzaldehyde (**Scheme 67**). This compound should react with the anilinouracil to produce the desired compound although it would have to be prepared as there are no commercial amino-chlorobenzaldehydes available.

5-Amino-2-chlorobenzoic acid **183** was transformed into **184** using a one-pot procedure to add the methyl ester group and Boc protect the amine.[205] Conditions for the reduction of **184** to **185** were investigated.[206] A procedure using lithium aluminium hydride and THF was successful.

At first it had been assumed that the methyl ester **184** could be directly reduced to form the aldehyde compound **186**, however it was impossible to isolate the pure aldehyde.[205] Therefore it was decided to form the alcohol **185** and then oxidise the primary alcohol to the corresponding aldehyde **186**.




**Scheme 67**

Unfortunately, it proved impossible to oxidise the alcohol **185** to aldehyde **186**. Numerous attempts were made, none of which produced the desired compound.[207] This was disappointing as there only remained two steps to produce the desired amino compound – the coupling of **168** and **186** followed by Boc-deprotection should produce **166**.

## 8.4 Biological Test Results

All synthesised compounds were tested at the Beatson Oncology Laboratories, Glasgow. All the compounds were analysed for their ability to stabilise endogenous HDM2 and p53 in primary human pigment epithelial cells (RPE). Several of the compounds showed activity in the stabilisation of p53 comparable to compound **162**. Three of the analogues, **174**, **177** and **178** demonstrated stronger p53 stabilisation and activity at lower concentrations than compound **162**. Generally stabilisation of p53 is accompanied by the activation of DNA damage-induced kinases – p53 becomes phosphorylated at serine 15. However in this case there was no clear indication that phosphorylation had occurred on the p53 stabilised by the 5-deazaflavins. This shows that the compounds stabilise p53 by inhibition of HDM2 as opposed to the activation of a DNA damage response.

The original lead compounds **162-164** contain a nitro group that could be reduced to a nitro anion radical in biological systems. This radical then interacts with DNA to induce DNA damage. However the results from compounds **174**, **177** and **178** argue against this notion. More interesting was the fact that analogues without the nitro group retained biological activity. The results are summarised in **Table 7**. These compounds **176-178** contained a 6-chloro group instead of the 7-nitro group.

| Compound | 1 µM | 5-10 µM |
|---|---|---|
| **165** | ND | - |
| **174** | + | ++ |
| **175** | ND | - |
| **176** | - | + |
| **177** | + | ++ |
| **178** | + | ++ |
| **179** | ND | - |
| **180** | - | - |
| **181** | - | - |
| **182** | ND | - |

**Table 7.** Stabilisation of p53 measured by Western blotting in RPE cells treated with the analogues at the indicated concentrations for 24 hours. Results of the three experiments are summarised to indicate no elevation of p53 levels (-); increase in p53 with that seen with 5 µM compound **162** (+); or increase in p53 levels in excess of that seen with 5 µM compound **162** (++). ND, not determined.

## 8.5 Conclusions

By consideration of the test results obtained, it has been determined that the compounds must have certain structural characteristics in order to be good inhibitors of HDM2. For example there must be a substituent on the aromatic ring of the fused cyclic system.

The most important new finding is that the nitro group present in the three original compounds **162-164** is not essential for stabilisation and activation of p53.

The R substituent on the phenyl ring must be in the *ortho* or *para* position and requires some bulk, e.g. a methyl group or a chlorine atom is adequate although a bromine atom appears to be too big. This work has been published as part of a wider study of these types of compounds.[208]

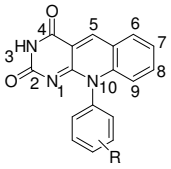



**Figure 31**

If any further work is carried out on this project it would be beneficial to complete the synthesis of the amino-derivative as this compound would allow an investigation into the mode of action of the deaazaflavins.

# 9 Experimental

## 9.1 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. Tetrahydrofuran and diethyl ether were freshly distilled from sodium-benzophenone; dichloromethane, toluene, dimethylformamide and pyridine were distilled from calcium hydride prior to use. Petroleum ethers refer to the fraction boiling at 40-60 $^{o}$C. Brine refers to a saturated sodium chloride solution. 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 trimethylsilane. Chemical shifts in $^{13}$C NMR spectra are given in ppm relative to $CDCl_3$ as internal standard (77.0 ppm). All NMR *J* values are given in Hz. Mass spectra were recorded on a JEOL JMS700 spectrometer.

## 9.2 Experimental to Chapter 5

### *9.2.1 General Procedures (A-C)*

**General procedure A** – Copper complex formation: A stirred solution of macrocycle (1 eq) and methanol/water mixture (5:1) was added to a round-bottomed flask fitted with a water-cooled condenser. Anhydrous copper (II) chloride (1 eq) was added and the reaction mixture was heated to 50 $^{o}$C for 10 min. A blue or green solid precipitated on cooling and was collected by filtration.

**General procedure B** – Method 1 for synthesis of carbamates: A stirred solution of 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (1 eq) and pyridine (5 mL) was prepared. Isocyanate (eq) was added and the reaction mixture was stirred for 18 h at RT. The pyridine was removed by rotary evaporator and the beige solid was filtered and

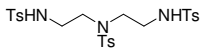
washed with diethyl ether (20 mL). White crystals were obtained by recrystallising from hot methanol.

**General procedure C** – Method 2 for synthesis of carbamates: A stirred solution of alcohol (1 eq), dibutyl tin diacetate (3 drops) and dichloromethane (10 mL) was prepared. Isocyanate (0.88 mL, 9.28 mmol) was added and the reaction mixture was stirred and heated under reflux for 24 h. The solvent was concentrated and the residue was filtered and washed with diethyl ether (20 mL). The crude product was recrystallised from ethyl acetate and hexane.

**General procedure D** – Acylation of azamacrocycles: A stirred solution of cyclen (1 eq) and dichloromethane (5 mL) was prepared and cooled to 0 $^{o}$C in an ice bath. Triethylamine (4 eq) was added. Once all the reactants had dissolved, chloroacetyl chloride or bromoacetyl bromide (4 eq) was added. The reaction mixture was stirred for 20 min at 0 $^{o}$C then allowed to return to RT and stirred for 16 h. The dark brown solution was washed with water (2 x 25 mL). The organic layer was dried ($MgSO_4$) and concentrated leaving a dark brown oil that was purified by column chromatography (eluant: dichloromethane/methanol 97/3).

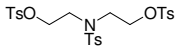
## *9.2.2 Experimental Details*

***N,N',N''*-Tri(toluene-4-sulfonyl)diethylene triamine 78**[209]



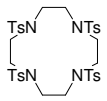

A stirred solution of diethylene triamine (21.7 mL, 0.20 mol) and distilled water (125 mL) was prepared. Sodium hydroxide pellets (24.0 g, 0.6 mol) were added and the temperature was kept below 40 °C. Diethyl ether (125 mL) was added and the reaction mixture was stirred vigorously. Toluene-4-sulfonyl chloride (114.5 g, 0.6 mol) was added, the temperature was kept below 20 °C during addition. The reaction mixture was cooled to 0 °C and stirred for one hour. The white precipitate was filtered and washed with diethyl ether (250 mL). Recrystallisation from chloroform gave the desired product (112.6 g, 99%). mp 176-178 °C (lit. 177-179 °C); $^{1}$H NMR (400 MHz, $CDCl_3$): 2.50 (9H, s, $CH_3$), 2.63 (2H, t, *J* 6.0 Hz, 2 x NH), 3.14-3.22 (8H, m, 4 x $CH_2$), 7.29-7.38 (6H, m, 6 x ArH), 7.64 (2H, d, *J* 8.4 Hz, 2 x ArH), 7.77-7.87 (4H, m, 4 x ArH); *m/z* (FAB): 566 ($MH^+$, 99%), 412 (71), 227 (53), 154 (50), 136 (35), 92 (31).

**_N,O,O'_-Tri(toluene-4-sulfonyl)diethanolamine 80**[210]




A stirred solution of toluene-4-sulfonyl chloride (114.5 g, 0.6 mol) and dichloromethane (140 mL) was prepared and cooled to 0 °C. Diethanolamine (19.2 mL, 0.2 mol), benzyltriethylammonium chloride (18.2 g, 80.0 mmol) and 30% NaOH solution (24.0 g in 150 mL distilled water) were added whilst stirring vigorously. The reaction mixture was allowed to return to room temperature and was stirred for another hour. The reaction mixture was poured onto distilled water (300 mL). The organic phase was separated and washed with distilled water (3 x 150 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated. The viscous oil was placed on a vacuum line to eliminate any presence of solvent. The product crystallised over a period of 2 weeks (102.9 g, 91%). mp 97-99 °C (lit. 101-103 °C); $^1$H NMR (400 MHz, $CDCl_3$): 2.45 (3H, s, $CH_3$), 2.49 (6H, s, 2 x $CH_3$), 3.40 (4H, t, *J* 6.0 Hz, 2 x $CH_2N$), 4.14 (4H, t, *J* 6.0 Hz, 2 x $CH_2O$), 7.31-7.34 (2H, m, 2 x ArH), 7.38 (4H, d, *J* 8.0 Hz, 4 x ArH), 7.63 (2H, d, *J* 8.4 Hz, 2 x ArH), 7.78 (4H, d, *J* 8.0 Hz, 4 x ArH); *m/z* (CI): 568 ($MH^+$, 13%), 432 (12), 396 (12), 242 (100), 157 (35).

**1,4,7,10-Tetra(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane 76**[123]




Method 1: A mixture of toluene (200 mL), tetrabutylammonium bromide (0.81 g, 2.5 mmol) and 2.5% LiOH solution (2.56 g in 100 mL distilled water) was heated under reflux conditions. *N,N',N''*-Tri(toluene-4-sulfonyl)diethylene triamine (5.65 g, 10 mmol), *N,O,O'*-tri(toluene-4-sulfonyl)diethanolamine (5.68 g, 10 mmol) and toluene (400 mL) were added in small portions. The reaction mixture was heated under reflux overnight. The reaction mixture was cooled and the white precipitate was filtered and washed with methanol (100 mL) giving a white solid. (5.42 g, 69%). mp 276-279 $^o$C (lit. 278-280 $^o$C); $^1$H NMR (400 MHz, $CDCl_3$): 2.47 (12H, s, 4 x $CH_3$), 3.45 (16H, bs, 8 x $CH_2$), 7.34 (8H, d, *J* 7.0 Hz, 8 x ArH), 7.66 (8H, d, *J* 7.0 Hz, 8 x ArH); *m/z* (FAB): 789 ($MH^+$, 99%), 633 (40), 477 (16), 323 (20), 253 (37), 154 (38), 92 (54).

Method 2: A stirred solution of *N,N',N''*-tri(toluene-4-sulfonyl)diethylene triamine (8.0 g, 11 mmol), caesium carbonate (13.68 g, 42 mmol) and DMF (300 mL) was prepared.

*N,O,O'*-Tri(toluene-4-sulfonyl)diethanolamine (5.68 g, 11 mmol) was dissolved in DMF (125 mL) and added to a dropping funnel. The contents of the dropping funnel were added dropwise over a period of 3 h. The reaction mixture was stirred for 5 d at room temperature. The DMF was removed using a pump-assisted rotary evaporator. The residue was taken up in dichloromethane (150 mL) and distilled water (150 mL). The aqueous layer was extracted with dichloromethane (100 mL). The combined organic phases were washed with brine (150 mL), dried ($MgSO_4$) and concentrated. The light brown residue was recrystallised from methanol (5.25 g, 47%). Spectroscopic data as described above.

**1,4,7,10-Tetraazacyclododecane 70 – Cyclen**[211]

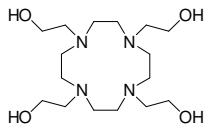

Method 1: A stirred solution of 1,4,7,10-tetra(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane (5.42 g, 6.87 mmol), phenol (12.80 g, 0.14 mol) and HBr-AcOH 45% (270 mL) was prepared. The round bottomed flask was fitted with a water-filled condenser and an air condenser. This allows the evolving HBr gas to escape to the top of the fume hood. The reaction mixture was heated under reflux for 3 d. The reaction mixture was cooled and the acetic acid was removed under vacuum. The residue was taken up in toluene (4 x 30 mL) to azeotrope off all traces of acetic acid on the rotary evaoporator. The dark purple residue was dissolved in distilled water (150 mL) and dichloromethane (75 mL) was added. The layers were separated and the aqueous layer was washed with dichloromethane (4 x 75 mL). The aqueous layer was concentrated yielding a brown residue which was purified by an anion exchange column. The column was prepared using Amberlite IRA-400 resin. Distilled water (200 mL), potassium hydroxide (50 mL, 10 % solution) and distilled water (350 mL) were passed down the column until pH 7 was achieved. The crude residue was dissolved in a minimum volume of distilled water and loaded onto the column. Water was eluted through the column until the pH dropped from 11 to 7. The aqueous eluant was concentrated. Methanol (4 x 25 mL) was added to azeotrope off all traces of water. The product was recrystallised from hot toluene yielding a white powder (0.54 g, 46%). mp 98-100 $^{o}$C (lit. 110-113 °C); $^{1}$H NMR (400 MHz, $D_2O$): 2.48 (16H, bs, 8 x $CH_2$); *m/z* (CI): 173 ($MH^+$, 61%), 113 (8), 97 (9), 79 (100).

Method 2: A stirred solution of 1,4,7,10-tetrakis-(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane (9.66 g, 12.66 mmol) and conc. sulfuric acid (25 mL) was prepared

and stirred at 110 $^{o}$C for 40 h. The brown/black solution was poured into a conical flask and cooled in an ice bath. Distilled water (20 mL) was added slowly. Potassium hydroxide (45 g) pellets were added until the pH was 13. Ethanol (150 mL) was added and the mixture was filtered. The solid residue was washed with ethanol (5 x 20 mL) and the filtrate was concentrated. The residue was taken up in the minimum volume of 1 M hydrochloric acid (40 mL) and dichloromethane (30 mL) was added. The layers were separated and the aqueous layer was washed with dichloromethane (4 x 30 mL). The pH was raised to 13 by adding potassium hydroxide pellets. This was extracted with chloroform (4 x 25 mL). The organic layers were combined, dried ($K_2CO_3$) and concentrated yielding a yellow solid (0.28 g, 13%). Spectroscopic data as described above.

Method 3 : Conc. sulphuric acid (11 mL) was heated to 165 °C. 1,4,7,10-Tetra(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane (1.20 g, 2.42 mmol) was added in a single portion and the solution was stirred until the reaction mixture had turned black. The reaction mixture was cooled by transferring the mixture into a Buchner flask and submersing this flask in cold water. This mixture was added dropwise to ethanol (36 mL) that was stirred. The precipitate was the polyhydrosulfate salt. Diethyl ether (27 mL) was added and the solution was cooled to 0 °C in an ice bath. The solid was filtered and dissolved in the minimum volume of hot water (7 mL), and an equivalent volume of HBr (48% aq, 7 mL) was added. Overnight the tetrahydrobromide salt crystallised. This was filtered and washed with hydrobromic acid (5 mL) and ethanol (5 mL). The white crystals were dried under high vaccuum. The crystals were then added to a round-bottomed flask charged with toluene (20 mL), distilled water (5 mL) and sodium hydroxide pellets (0.4 g, 10 mmol). Dean-Stark apparatus was fitted and the reaction mixture was heated under reflux for 24 h. The toluene solution was then filtered and concentrated yielding white crystals. (0.97 g, 88%). Spectroscopic data as described above.

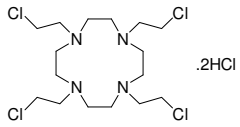
**1,4,7,10-Tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane 83**[115]




1,4,7,10-Tetraazacyclododecane (0.97 g, 5.63 mmol) and distilled water (9.7 mL) were added to a round-bottomed flask fitted with an dropping funnel and a cooling finger. Ethylene oxide (2.48 g, 56.3 mmol) and distilled water (1.9 mL) were added to the reaction

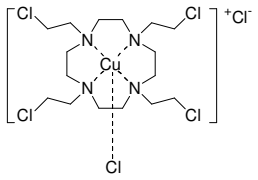
mixture dropwise. The reaction mixture was stirred for 2 h and the temperature was kept below 5 °C. The water and excess ethylene oxide were removed by rotary evaporator, yielding white crystals (2.10 g, 100%). mp 67-69 °C (no lit. mp available); $^1$H NMR (400 MHz, $CDCl_3$): 2.42 (8H, t, *J* 4.8 Hz, 4 x $CH_2N$), 2.46 (16H, bs, 8 x $CH_2N$), 3.55 (8H, t, *J* 4.8 Hz, 4 x $CH_2O$); *m/z* (FAB): 349 ($MH^+$, 99%), 305 (18), 248 (4), 175 (9), 154 (14), 89 (15).

**1,4,7,10-Tetra(2-chloroethyl)-1,4,7,10-tetraazacyclododecane dihydrochloride 58**[115]



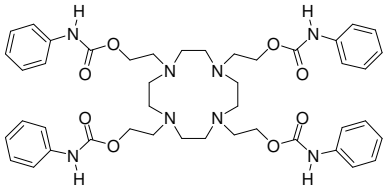

1,4,7,10-Tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (100 mg, 1.43 mol) and thionyl chloride (25 mL, 5 mL/100 mg tetraol) were added to a round-bottomed flask fitted with a water-cooled condenser and this was heated at 50 $^o$C overnight. The excess thionyl chloride was removed by rotary evaporator. The desired product was recrystallised from hot propan-2-ol (48 mg, 34%). mp 206-208 $^o$C (no lit. mp available); $^1$H NMR (400 MHz, $D_2O$): 3.28 (16H, bs, 8 x $CH_2N$), 3.38 (8H, bs, 4 x $CH_2N$), 3.84-3.90 (8H, m, 4 x $CH_2Cl$); *m/z* (FAB): 423 [(M-2Cl)$H^+$, $^{35}$Cl, 100%], 421 (79), 387 (22), 359 (17), 211 (26), 106 (38).

**1,4,7,10-Tetra(2-chloroethyl)-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 64**[115]



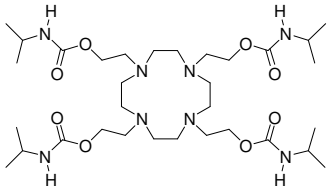

Using general procedure A, **64** was synthesised from 1,4,7,10-tetra(2-chloroethyl)-1,4,7,10-tetraazacyclododecane dihydrochloride **58** (20 mg, 40 mmol), as blue crystals (21 mg, 100%), *m/z* (FAB): 483 [(M-2Cl)$^+$, $^{35}$Cl, 60%].

**1,4,7,10-Tetra(phenylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane 84**



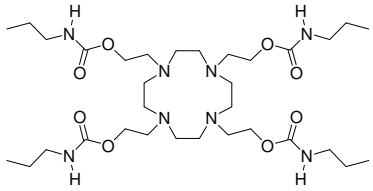
Using general procedure B, **84** was synthesised from 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **83** (0.1 g, 0.29 mmol) and phenyl isocyanate (1.09 mL, 10 mmol), as white crystals (0.24 g, 100%). mp 150-152 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3296 (NH), 2944 (CH), 1696 (CO), 1228, 797; $^{1}$H NMR (400 MHz, D$_6$-DMSO): 2.62 (24H, bs, 12 x $CH_2N$), 4.10 (8H, t, *J* 6.0 Hz, 4 x $CH_2O$), 6.50 (4H, t, *J* 8.0 Hz, 4 x ArH), 7.24 (8H, t, *J* 8.0 Hz, 8 x ArH), 7.45 (8H, d, *J* 8.0 Hz, 8 x ArH), 9.56 (4H, bs, 4 x NH); $^{13}$C NMR (100 MHz, D$_6$-DMSO): 52.4 ($CH_2$), 53.8 ($CH_2$), 62.1 ($CH_2$), 122.3 (CH), 128.6 (CH), 128.7 (CH), 128.8 (CH), 129.0 (CH), 134.8 (C), 153.5 (C); *m/z* (FAB): 825.4296 ($MH^+$, $C_{44}H_{57}N_8O_8$ requires 825.4299), 511 (4), 358 (95), 307 (11), 155 (100), 109 (29).

**1,4,7,10-Tetra(isopropylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane 85**



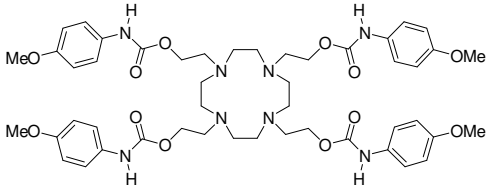
Using general procedure B, **85** was synthesised from 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **83** (0.2 g, 0.58 mmol) and isopropyl isocyanate (0.91 mL, 9.28 mmol), as white crystals (0.24 g, 60%). Found: C, 55.8; H, 9.4; N, 16.3. $C_{36}H_{64}N_8O_8$ requires C, 55.8; H, 9.5; N, 16.1%; mp 141-143 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3279 (NH), 2969 (CH) 1684 (CO), 1539; $^{1}$H NMR (400 MHz, D$_6$-Acetone): 1.00 (24H, d, *J* 6.8 Hz, 8 x $CH_3$), 2.45 (24H, bs, 12 x $CH_2N$), 3.59-3.64 (4H, m, 4 x CH), 3.94 (8H, t, *J* 6.0 Hz, 4 x $CH_2O$); $^{13}$C NMR (100 MHz, D$_6$-DMSO): 22.5 ($CH_3$), 42.2 (CH), 52.7 ($CH_2$), 54.0 ($CH_2$), 61.6 ($CH_2$), 155.3 (C); *m/z* (FAB): 689 ($MH^+$, 100%), 586 (10), 503 (18), 331 (85), 174 (54), 131 (100), 90 (79), 72 (37).

**1,4,7,10-Tetra(propylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane 86**



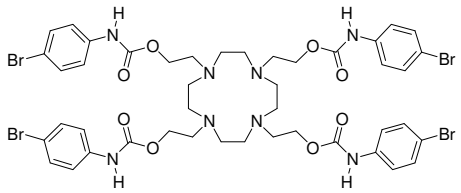
Using general procedure C, **86** was synthesised from 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **83** (0.2 g, 0.58 mmol) and propyl isocyanate (0.88 mL, 9.28 mmol), as white crystals (0.16 g, 41%). mp 142-144 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3312 (NH), 2960 (CH), 1686 (CO), 1548, 1272, 1008, 668; $^{1}$H NMR (400 MHz, $D_6$-Acetone): 0.91 (12H, t, *J* 7.4 Hz, 4 x $CH_3$), 1.48-1.57 (8H, m, 4 x $CH_2$), 2.65 (24H, bs, 12 x $CH_2N$), 3.09 (8H, t, *J* 7.0 Hz, 4 x $CH_2N$), 4.10 (8H, t, *J* 6.0 Hz, 4 x $CH_2O$); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 11.2 ($CH_3$), 22.7 ($CH_2$), 42.0 ($CH_2$), 52.6 ($CH_2$), 54.0 ($CH_2$), 61.8 ($CH_2$) 156.2 (C); *m/z* (FAB): 689.4922 ($MH^+$, $C_{32}H_{65}N_8O_8$ requires 689.4925), 433 (4%), 331 (2), 289 (20), 146 (100).

**1,4,7,10-Tetra(4-methoxyphenyl)aminocarbonyloxyethyl-1,4,7,10-tetraazacyclododecane 87**



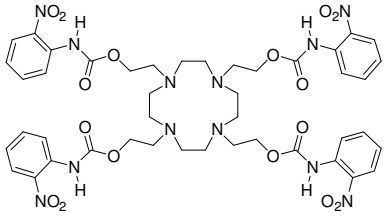
Using general procedure C, **87** was synthesised from 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **83** (0.2 g, 0.58 mmol) and 4-methoxyphenyl isocyanate (3.0 mL, 23.2 mmol), as white crystals (0.55 g, 56%). mp 166-168 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3294 (NH), 2957 (CH), 1698 (CO), 1509, 1240, 827; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.06 (24H, bs, 12 x $CH_2N$), 3.24 (12H, bs, 4 x $OCH_3$), 3.65 (8H, bs, 4 x $CH_2O$), 6.38 (8H, d, *J* 9.0 Hz, 8 x ArH), 6.90 (8H, d, *J* 9.0 Hz, 8 x ArH), 8.89 (4H, bs, 4 x NH); $^{13}$C NMR (400 MHz, $D_6$-DMSO): 52.5 ($CH_2$), 53.8 ($CH_2$), 55.1 ($CH_3$), 62.0 ($CH_2$), 113.8 (CH), 119.9 (CH), 132.2 (C), 153.7 (C), 154.7 (C); *m/z* (FAB): 945.4724 ($MH^+$, $C_{48}H_{65}N_8O_{12}$ requires 945.4722), 779 (4%), 695 (3), 459 (4) 338 (5), 238 (22), 170 (51), 87 (100).

**1,4,7,10-Tetra(4-bromophenyl)aminocarbonyloxyethyl-1,4,7,10-tetraazacyclododecane 88**



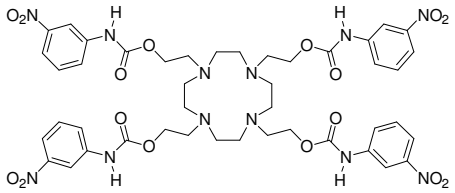
Using general procedure C, **88** was synthesised from 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **83** (0.1 g, 0.29 mmol) and 4-bromophenyl isocyanate (2.30 g, 11.6 mmol), as white crystals (0.21 g, 64%). mp 212-214 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3303 (NH), 2833 (CH), 1709 (CO), 1393, 1227, 820; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.50 (24H, bs, 12 x $CH_2N$), 4.11 (8H, t, *J* 6.0 Hz, 4 x $CH_2O$), 7.41-7.46 (16H, m, 16 x ArH), 8.86 (4H, s, 4 x NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 52.4 ($CH_2$), 53.8 ($CH_2$), 62.3 ($CH_2$) 113.4 (C), 120.2 (CH), 131.5 (CH), 138.9 (C), 153.4 (C); *m/z* (FAB): 1143 [MH$^+$ ($^{81}$Br), 4%], 1141 [MH$^+$ ($^{79}$Br), 5%], 371 (5), 253 (9), 170 (100), 87 (95).

**1,4,7,10-Tetra(2-nitrophenyl)aminocarbonyloxyethyl-1,4,7,10-tetraazacyclododecane 89**



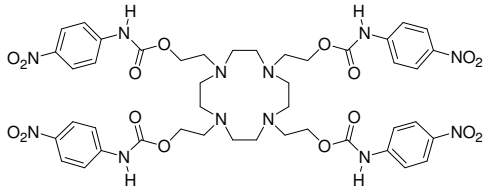
Using general procedure C, **89** was synthesised from 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **83** (0.1 g, 0.29 mmol) and 2-nitrophenyl isocyanate (0.48 g, 2.90 mmol), as a pale yellow solid (0.18 g, 64%). mp 82-84 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3350 (NH), 2359 (CH), 1718 (CO), 1428, 1237, 743; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.59 (24H, bs, 12 x $CH_2N$), 4.11 (8H, bs, 4 x $CH_2O$), 7.28 (4H, t, *J* 8.0 Hz, 4 x ArH), 7.66 (4H, t, *J* 8.0 Hz, 4 x ArH), 7.73 (4H, d, *J* 8.0 Hz, 4 x ArH), 7.96 (4H, d, *J* 8.0 Hz, 4 x ArH), 9.77 (4H, bs, 4 x NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 52.6 ($CH_2$), 53.6 ($CH_2$), 63.2 ($CH_2$), 123.8 (CH), 124.2 (CH), 125,7 (CH), 132.5 (C), 134.4 (CH), 140.7 (C), 153.4 (C); *m/z* (FAB): 1006 (MH$^+$, 97%), 842 (100), 826 (13), 678 (43), 660 (16), 490 (18), 326 (12), 232 (32), 158 (48), 81 (100).

**1,4,7,10-Tetra(3-nitrophenyl)aminocarbonyloxyethyl-1,4,7,10-tetraazacyclododecane 90**



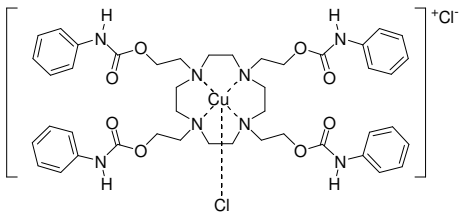
Using general procedure C, **90** was synthesised from 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **83** (0.1 g, 0.29 mmol) and 3-nitrophenyl isocyanate (0.48 g, 2.9 mmol), as a pale yellow solid (0.14 g, 46%). mp 151-153 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3389 (NH), 2797 (CH), 1720 (CO), 1526, 1081, 735; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.61 (24H, bs, 12 x $CH_2N$), 4.14 (8H, t, *J* 6.0 Hz, 4 x $CH_2O$), 7.47-7.56 (4H, m, 4 x ArH), 7.80-7.96 (12H, m, 12 x ArH), 10.09 (4H, bs, 4 x NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 52.6 ($CH_2$), 53.7 ($CH_2$), 62.6 ($CH_2$), 121.9 (CH), 122.3 (CH), 128.9 (CH), 133.7 (CH), 146.4 (C), 146.7 (C), 151.4 (C); *m/z* (FAB): 1005.3709 ($MH^+$, $C_{44}H_{53}N_{12}O_{16}$ requires 1005.3702), 391 (3), 322 (3), 238 (30), 170 (64), 87 (100).

**1,4,7,10-Tetra(4-nitrophenyl)aminocarbonyloxyethyl-1,4,7,10-tetraazacyclododecane 91**



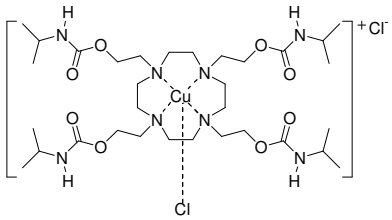
Using general procedure C, **91** was synthesised from 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **83** (0.1 g, 0.29 mmol) and 4-nitrophenyl isocyanate (0.48 g, 2.9 mmol), as a bright yellow solid (0.18 g, 60%). mp 179-181 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3323 (NH), 2831 (CH), 1728 (CO), 1329, 855; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.60 (24H, bs, 12 x $CH_2N$), 4.14 (8H, bs, 4 x $CH_2O$), 7.64 (8H, d, *J* 8.8 Hz, 8 x ArH), 8.12 (8H, d, *J* 8.8 Hz, 8 x ArH), 10.30 (4H, s, 4 x NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 52.6 ($CH_2$), 53.7 ($CH_2$), 62.6 ($CH_2$), 117.5 (CH), 124.9 (CH), 141.5 (C), 145.7 (C), 153.1 (C); *m/z* (FAB): 1005.3697 ($MH^+$, $C_{44}H_{53}N_{12}O_{16}$ requires 1005.3702), 990 (3), 824 (2), 782 (1), 628 (1), 560 (1), 475 (1), 322 (3), 238 (17), 170 (100), 87 (100).

**1,4,7,10-Tetra(phenylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 92**



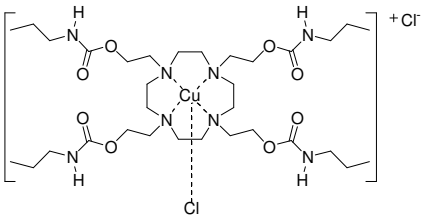
Using general procedure A, **92** was synthesised from 1,4,7,10-tetra(phenylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane **84** (0.24 g, 0.29 mmol), as light blue crystals (0.28, 100%). mp 176-178 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3185 (NH), 2291, 1716 (CO), 1270, 1086; *m/z* (FAB): 887 ([M-2Cl]$^{+}$, 100%), 826 (8), 768 (18), 750 (8), 410 (14), 367 (11), 185 (33), 94 (89), 71 (22). UV($H_2O$): $\lambda_{max}$ - 616 nm, $\varepsilon_{coeff}$ - 202 L cm$^{-1}$ mol$^{-1}$.

**1,4,7,10-Tetra(isopropylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 93**



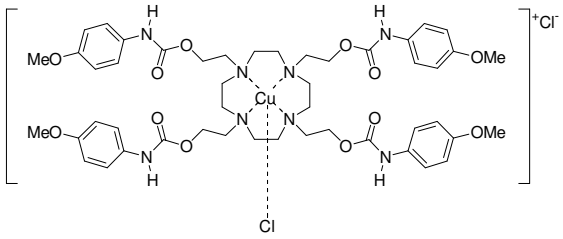
Using general procedure A, **93** was synthesised from 1,4,7,10-tetra(isopropylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane **85** (0.24 g, 0.35 mmol), as green crystals (0.17 mg, 58%). mp 201-203 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3341 (NH), 2968 (CH), 1699 (CO), 1250, 1094; *m/z* (FAB): 751.4142 ([M-2Cl]$^{+}$, $C_{32}H_{64}N_8O_8Cu$ requires 751.4143), 688 (15%), 331 (13), 221 (15), 174 (17), 131 (100), 90 (38), 72 (20); UV($H_2O$): $\lambda_{max}$ - 614 nm, $\varepsilon_{coeff}$ - 375 L cm$^{-1}$ mol$^{-1}$.

**1,4,7,10-Tetra(propylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 94**



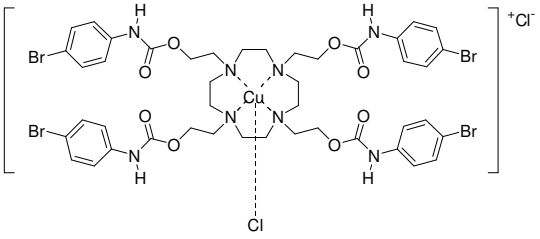
Using general procedure A, **94** was synthesised from 1,4,7,10-tetra(propylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane **86** (0.16 g, 0.24 mmol), as green crystals (0.13 g, 64%). mp 66-67 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3336 (NH), 2964 (CH), 1706 (CO), 1539, 1263; *m/z* (FAB): 752 ([M-2Cl]$^{+}$, 100%), 688 (10), 667 (8), 221 (12), 131 (20); UV($H_2O$): $\lambda_{max}$ - 614 nm, $\varepsilon_{coeff}$ - 375 L cm$^{-1}$ mol$^{-1}$.

**1,4,7,10-Tetra(4-methoxyphenylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 95**



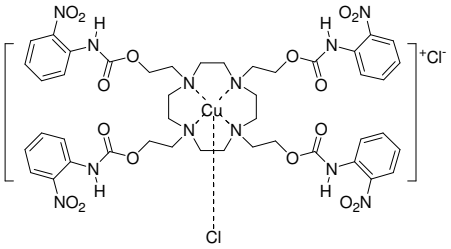
Using general procedure A, **95** was synthesised from 1,4,7,10-tetra(4-methoxyphenylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane **87** (0.3 g, 0.32 mmol), as green crystals (78.0 mg, 23%). mp 186-188 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3234 (NH), 2942 (CH), 1721 (CO), 1511, 1221, 827; *m/z* (FAB): 1042.2632 ([M-Cl]$^{+}$, $C_{48}H_{64}N_8O_{12}Cu^{35}Cl$ requires 1042.2628), 307 (22%), 155 (100), 109 (20); UV($H_2O$): $\lambda_{max}$ - 604 nm, $\varepsilon_{coeff}$ - 381 L cm$^{-1}$ mol$^{-1}$.

**1,4,7,10-Tetra(4-bromophenyl)aminocarbonyloxyethyl-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 96**



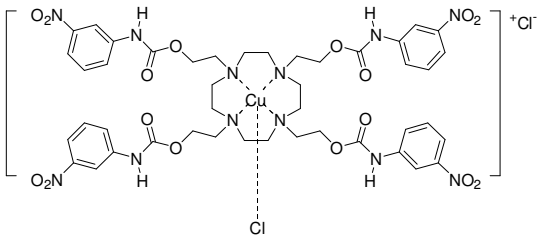
Using general procedure A, **96** was synthesised from 1,4,7,10-tetra(4-bromophenylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane **88** (0.1 g, 80 µmol), as blue crystals (40 mg, 39%). mp 212-214 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3333 (NH), 3102 (CH), 1723 (CO), 1537, 1219, 881; *m/z* (FAB): 1238 [(M-2Cl)$^{+}$, $^{79}Br_2$, $^{81}Br_2$, 100%], 1002 (5), 557 (10), 336 (15); UV($H_2O$): $\lambda_{max}$: 786 nm, $\varepsilon_{coeff}$: 1793 L cm$^{-1}$ mol$^{-1}$.

**1,4,7,10-Tetra(2-nitrophenyl)aminocarbonyloxyethyl-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 97**



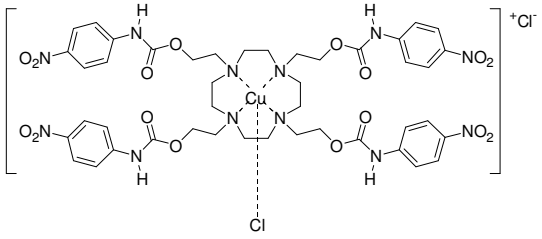
Using general procedure A, **97** was synthesised from 1,4,7,10-tetra(2-nitrophenylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane **89** (0.1 g, 90 µmol), as green crystals (40 mg, 39%). mp 149-151 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3363 (NH), 1735 (CO), 1512, 1340, 739; *m/z* (FAB): 1102 [(M-Cl)$^{+}$, $^{35}$Cl, 3%], 903 (5), 209 (20), 158 (100), 81 (55); UV($H_2O$): $\lambda_{max}$ - 623 nm, $\varepsilon_{coeff}$ - 1026 L cm$^{-1}$ mol$^{-1}$.

**1,4,7,10-Tetra(3-nitrophenyl)aminocarbonyloxyethyl-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 98**



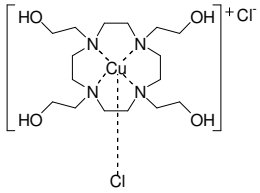
Using general procedure A, **98** was synthesised from 1,4,7,10-tetra(3-nitrophenylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane **90** (0.1 g, 90 µmol), as turquoise crystals (76 mg, 74%). mp 222-223 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 2959 (NH), 1718 (CO), 1427, 1089, 735; *m/z* (FAB): 1102 [(M-Cl)$^{+}$, $^{35}$Cl, 1.7%] 235 (8), 158 (100), 81 (100); UV($H_2O$): $\lambda_{max}$ - 623 nm, $\varepsilon_{coeff}$ - 935 L cm$^{-1}$ mol$^{-1}$.

**[1,4,7,10-Tetra(4-nitrophenyl)aminocarbonyloxyethyl-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 99**



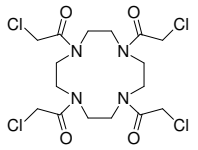
Using general procedure A, **99** was synthesised from 1,4,7,10-tetra(4-nitrophenylaminocarbonyloxyethyl)-1,4,7,10-tetraazacyclododecane **91** (0.1 g, 90 µmol), as green crystals (81 mg, 80%). mp 156-157 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 2448, 1734 (CO), 1508, 1111, 853. *m/z* (FAB): 1102 [(M-Cl)$^{+}$, $^{35}$Cl, 1.3%], 232 (22), 81 (100); UV($H_2O$): $\lambda_{max}$ - 623 nm, $\varepsilon_{coeff}$ - 935 L cm$^{-1}$ mol$^{-1}$.

**1,4,7,10-Tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane [Cu(II)]$Cl_2$ 100**



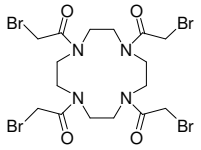
Using general procedure A, **100** was synthesised from 1,4,7,10-tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **83** (0.2 g, 0.57 mmol), as bright blue crystals (49.0 mg, 18%) mp 234-236 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3554, 3235, 1621, 1440, 1085, 1015; *m/z* (FAB): 411.2036 [(M-2Cl)$^{+}$, $C_{16}H_{36}N_4O_4Cu$ requires 411.2033], 349 (6%), 247 (5), 90 (5); UV($H_2O$): $\lambda_{max}$ – 602 nm, $\varepsilon_{coeff}$ – 371 L cm$^{-1}$ mol$^{-1}$.

**1,4,7,10-Tetrachloroacetyl-1,4,7,10-tetraazacyclododecane 101**



Using general procedure D, **101** was synthesised from 1,4,7,10-tetraazacyclododecane (0.50 g, 2.9 mmol) and chloroacetyl chloride (1.39 mL, 17 mmol), as a beige solid (0.11 g, 10%). mp 72-74 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3450, 1786, 1653 (CO), 1423, 1131, 791. $^{1}$H NMR (400 MHz, $CDCl_3$): 3.74 (8H, s, 4 x $CH_2$), 4.26 (16H, s, 8 x $CH_2N$); $^{13}$C NMR (100 MHz, $CDCl_3$): 39.7 ($CH_2$), 52.5 ($CH_2$), 170.5 (C); *m/z* (FAB): 477.0612 (MH$^{+}$, $^{35}$Cl, $C_{16}H_{25}N_4O_4Cl_6$ requires 477.0630), 441 (22), 401 (7), 281 (12), 256 (8), 207 (11), 179 (66), 148 (15), 75 (100).

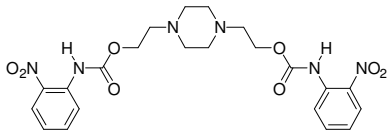
**1,4,7,10-Tetrabromoacetyl-1,4,7,10-tetraazacyclododecane 102**



Using general procedure D, **102** was synthesised from 1,4,7,10-tetraazacyclododecane (0.50 g, 2.9 mmol) and bromoacetyl bromide (1.51 mL, 17 mmol), as a brown oil (0.18 g,

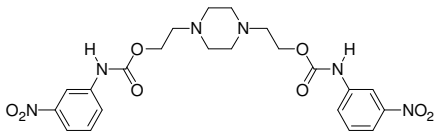
11%). $\nu_{max}$ (KBr)/cm$^{-1}$: 3444, 2957 (CH), 1732, 1645 (CO), 1174; $^{1}$H NMR (400 MHz, $CDCl_3$): 3.64 (8H, s, 4 x $CH_2$), 3.88 (16H, s, 8 x $CH_2N$). $^{13}$C NMR (100 MHz, $CDCl_3$): 25.7 ($CH_2$), 53.5 ($CH_2$), 171.3 (C); *m/z* (FAB): 652.8601 ($MH^+$, $C_{16}H_{25}{}^{79}Br_4N_4O_4$ requires 652.8609), 577 (20%), 329 (15), 219 (20), 165 (28), 71 (47).

**1,4-Bis(2-*o*-nitrophenylaminocarbonyloxyethyl)piperazine 103**



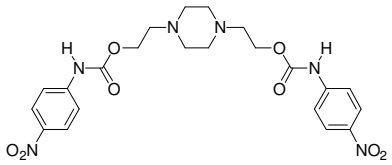
Using general procedure C, **103** was synthesised from 1,4-bis(2-hydroxyethyl)piperazine (0.2 g, 1.15 mmol) and 2-nitrophenyl isocyanate (0.57 g, 3.45 mmol), as white crystals (0.36 g, 68%). mp 134-136 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3072 (NH), 2826 (CH), 1742 (CO), 1236, 785; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.50 (8H, s, 4 x $CH_2N$), 2.55 (4H, t, *J* 5.5 Hz, 2 x $CH_2N$), 4.17 (4H, t, *J* 5.5 Hz, 2 x $CH_2O$), 7.30-7.34 (2H, m, 2 x ArH), 7.62-7.71 (4H, m, 4 x ArH), 7.95 (2H, dd, *J* 8.2, 1.4 Hz, 2 x ArH), 9.91 (2H, bs, 2 x NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 52.8 ($CH_2$), 56.3 ($CH_2$), 62.3 ($CH_2$), 124.3 (CH), 124.5 (CH), 125.2 (CH), 132.0 (C), 134.1 (CH), 141.5 (C), 153.5 (C); *m/z* (FAB): 503.1886 ($MH^+$, $C_{22}H_{27}N_6O_8$ requires 503.1890), 238 (9%), 170 (71), 87 (100).

**1,4-Bis(2-*m*-nitrophenylaminocarbonyloxyethyl)piperazine 104**



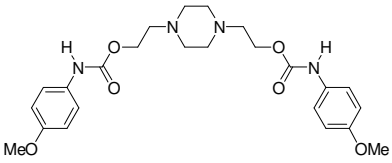
Using general procedure C, **104** was synthesised from 1,4-bis(2-hydroxyethyl)piperazine (0.20 g, 1.15 mmol) and 3-nitrophenyl isocyanate (0.57 g, 3.45 mmol), as a yellow solid (0.30 g, 55%). mp 152-154 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3385 (NH), 2821 (CH), 1742 (CO), 1277, 779; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.47 (8H, s, 4 x $CH_2N$), 2.59 (4H, t, *J* 5.4 Hz, 2 x $CH_2N$), 4.23 (4H, t, *J* 5.4 Hz, 2 x $CH_2O$), 7.58 (2H, t, *J* 8.2 Hz, 2 x ArH), 7.80-7.88 (4H, m, 4 x ArH), 8.47 (2H, s, 2 x ArH), 10.26 (2H, bs, 2 x NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 52.8 ($CH_2$), 56.4 ($CH_2$), 61.7 ($CH_2$), 112.0 (CH), 116.9 (CH), 124.1 (CH), 130.2 (CH), 140.5 (C), 148.1 (C), 153.5 (C); *m/z* (FAB): 503.1891 ($MH^+$, $C_{22}H_{27}N_6O_8$ requires 503.1890), 238 (14%), 170 (78), 87 (100).

**1,4-Bis(2-*p*-nitrophenylaminocarbonyloxyethyl)piperazine 105**[137]



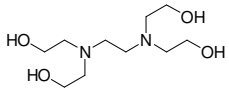
Using general procedure C, **105** was synthesised from 1,4-bis(2-hydroxyethyl)piperazine (0.20 g, 1.15 mmol) and 4-nitrophenyl isocyanate (0.57 g, 3.45 mmol), as a yellow solid (0.49 g, 90%). mp 212-214 $^{o}$C (lit. m.p. 215-217 $^{o}$C); $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.33-2.37 (8H, m, 4 x $CH_2N$), 3.46 (4H, t, *J* 5.2 Hz, 2 x $CH_2N$), 4.37 (4H, t, *J* 5.2 Hz, 2 x $CH_2O$), 7.73 (4H, d, *J* 9.4 Hz, 4 x ArH), 8.22 (4H, d, *J* 9.4 Hz, 4 x ArH), 9.74 (2H, bs, 2 x NH); *m/z* (FAB): 503 ($MH^+$, 20%), 238 (11), 170 (75), 87 (100).

**1,4-Bis(2-*p*-methoxyphenylaminocarbonyloxyethyl)piperazine 106**



Using general procedure C, **106** was synthesised from 1,4-bis(2-hydroxyethyl)piperazine (0.20 g, 1.15 mmol) and 4-methoxyphenyl isocyanate (0.44 mL, 3.45 mmol) as a white solid (0.51 g, 99%). mp 155-157 $^{o}$C; $\nu_{max}$ (KBr)/$cm^{-1}$: 3323 (NH), 2812 (CH), 1690 (CO), 1219, 826; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.44 (8H, bs, 4 x $CH_2N$), 2.55 (4H, t, *J* 5.6 Hz, 2 x $CH_2N$), 3.69 (6H, s, 2 x $OCH_3$), 4.15 (4H, t, *J* 5.6 Hz, 2 x $CH_2O$), 6.85 (4H, d, *J* 9.0 Hz, 4 x ArH), 7.35 (4H, d, *J* 9.0 Hz, 4 x ArH), 9.48 (2H, bs, 2 x NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 52.9 ($CH_2$), 55.1 ($CH_3$), 56.6 ($CH_2$), 61.1 ($CH_2$), 113.9 (CH), 119.8 (CH), 132.2 (C), 132.9 (C), 153.6 (C); *m/z* (FAB): 473.2398 ($MH^+$, $C_{24}H_{33}N_4O_6$ requires 473.2400), 238 (11%), 170 (68), 87 (100).

**1,2-Tetra(2-hydroxyethyl)ethylenediamine 108**[212]



Ethylenediamine (0.56 mL, 8.32 mmol) and water (50 mL) were added to a round-bottomed flask fitted with an dropping funnel and a cooling finger. Ethylene oxide (9.11 mL, 0.17 mol) and water (20 mL) were added to the reaction mixture dropwise. The reaction stirred for two hours with the temperature kept under 5 $^{o}$C. The water and excess ethylene oxide were removed by rotary evaporator, yielding a colourless oil (1.43 g, 73%). $^{1}$H NMR ($CDCl_3$, 400 MHz): 2.64 (8H, t, *J* 4.7 Hz, 4 x $CH_2N$), 2.68 (4H, s, 2 x $CH_2N$), 3.68 (8H, t, *J* 4.7 Hz, 4 x $CH_2N$), 3.76 (4H, s, 4 x OH); *m/z* (FAB): 237 ($MH^+$, 100%), 177 (5), 155 (32), 133 (40), 119 (22), 90 (8).

## 9.3 Experimental to Chapter 6

### *9.3.1 General Procedures (A-F)*

**General procedure A** – Synthesis of macrocycles (amide bond formation): A stirred solution of triethylamine (2 eq) and dichloromethane (100 mL) was prepared. Diamine (1 eq) in dichloromethane (50 mL) and thiodiglycolic acid chloride (1 eq) in dichloromethane (50 mL) were added dropwise using syringe pumps over a period of 5 h. The beige precipitate was filtered off, washed with dichloromethane (100 mL) and recrystallised from methanol.

**General procedure B** – Tosylation of diols: A stirred solution of toluene-4-sulfonyl chloride (2 eq) and dry pyridine (10 mL) was prepared in an ice bath at 0 $^{o}$C. Diol (1 eq) and dry pyridine (25 mL) were added dropwise. The reaction mixture was stirred for 1 h at 0 $^{o}$C then allowed to return to RT and stirred for 3 h. The reaction mixture was poured onto distilled water (150 mL). The white precipitate was filtered off and yielded white crystals from hot ethanol.

**General procedure C** – Tosylation of diamines: A solution of diamine (1 eq), sodium hydroxide (2.1 eq) and distilled water (100 mL) was prepared. A solution of toluene-4-sulfonyl chloride (2.1 eq) and diethyl ether (100 mL) was added. The reaction mixture was stirred at room temperature for 24 h. The layers were separated and the aqueous layer was washed with chloroform (2 x 75 mL). The organic layers were combined, dried and concentrated yielding a brown viscous oil which was purified using column chromatography (eluant: dichloromethane/methanol 95/5).

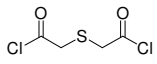
**General prodecure D** – Synthesis of tosyl-protected macrocycles (phase transfer cyclisation conditions): A mixture of toluene (200 mL), tetrabutylammonium bromide (0.25 eq) and 2.5% lithium hydroxide solution solution (50 mL) was heated under reflux. Tosyl-protected diamine (1 eq) and tosyl-protected diol (1 eq) and toluene (400 mL) were added in small portions. The reaction mixture was heated under reflux overnight. After cooling, the organic layer was separated and concentrated and the white solid was filtered and recrystallised from methanol.

**General procedure E** – Detosylation of macrocycles: A solution of tosyl-protected macrocycle (1 eq), phenol (10 eq) and HBr-AcOH 45% (5 mL per 100 mg) was prepared and stirred at 80 $^{o}$C for 3 d. An air condenser was placed on top of the water condenser to ensure safe passage of the evolved HBr to the top of the fumehood. The reaction mixture was cooled and concentrated. The dark brown residue was azeotroped using toluene (4 x 15 mL) to remove all traces of acetic acid. The dark brown residue was dissolved in dichloromethane (30 mL) and distilled water (30 mL) was added. The layers were separated and the aqueous layer was washed with dichloromethane (4 x 15 mL). The aqueous layer was concentrated. The resulting orange residue was dissolved in distilled water (5 mL) and sodium hydroxide (10 eq) was added. The flask was topped up with toluene and the resulting mixture was azeotroped using Dean-Stark apparatus until all the water had been removed. The toluene was decanted off and concentrated. The crude product was purified using alumina column chromatography (eluant: dichloromethane/methanol 95/5) yielding a transparent oil.

**General procedure F** – Hydroxyethylation of macrocycles: A stirred solution of macrocycle (1 eq) and ethanol (5 mL) was prepared and cooled to 0 $^{o}$C using an ice bath. Ethylene oxide (5 eq per NH) was added dropwise using a cooling finger and addition funnel. The reaction mixture was sealed, allowed to return to room temperature and stirred overnight. The reaction mixture was concentrated. The crude product was purified using alumina column chromatography (eluant: dichloromethane/methanol 95/5).

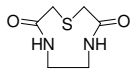
## *9.3.2 Experimental Details*

**Thiodiglycolic acid chloride 110**



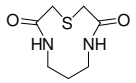
A mixture of thiodiglycolic acid (15.0 g, 0.1 mol) and thionyl chloride (150 mL, 2.1 mol), was stirred overnight at RT. Excess thionyl chloride was removed under vacuum. The cream coloured solid was used in the next reaction without purification assuming 100% conversion.

**1-Thia-4,7-diazacyclononane-3,7-dione 111**



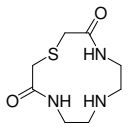
Using general procedure A, **111** was synthesised from ethylene diamine (1.78 mL, 26.7 mmol) and thiodiglycolic acid chloride **110** (5.0 g, 26.7 mmol), as a beige solid (4.65 g, 100%). mp 162-164 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 2930 (NH), 1661 (CO), 1249, 794; $^{1}$H NMR (400 MHz, $D_2O$): 3.08 (4H, s, 2 x $CH_2N$), 3.19 (4H, s, $CH_2S$); $^{13}$C NMR (100 MHz, $D_2O$): 36.6 ($CH_2$), 37.3 ($CH_2$), 177.8 (C).

**1-Thia-4,8-diazacyclodecane-3,9-dione 112**[160c]



Using general procedure A, **112** was synthesised from 1,3-diaminopropane (2.23 mL, 26.7 mmol) and thiodiglycolic acid chloride **110** (5.0 g, 26.7 mmol), as a beige solid (5.03 g, 100%). mp 188-189 $^{o}$C (lit. > 200 $^{o}$C); $\nu_{max}$ (KBr)/cm$^{-1}$: 2365 (NH), 1700 (CO), 1206, 814; $^{1}$H NMR (400 MHz, $D_2O$): 1.87-1.95 (2H, m, $CH_2$), 2.95 (4H, t, *J* 7.8 Hz, 2 x $CH_2N$), 3.19 (4H, s, $CH_2S$); $^{13}$C NMR (100 MHz, $D_2O$): 24.9 ($CH_2$), 36.6 ($CH_2$), 37.3 ($CH_2$), 177.8 (C).

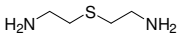
**1-Thia-4,7,10-triazacyclododecane-3,11-dione 113**



Using general procedure A, **113** was synthesised from diethylene triamine (1.73 mL, 16.0 mmol) and thiodiglycolic acid chloride **110** (3.0 g, 16.0 mmol), as white crystals (3.49 g, 100%). mp 49-51 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 2922 (NH), 2121, 1601 (CO), 1218, 872; $^{1}$H NMR (400 MHz, $D_2O$): 2.76 (4H, t, *J* 6.2 Hz, 2 x $CH_2N$), 2.96 (4H, t, *J* 6.2 Hz, 2 x $CH_2N$), 3.10

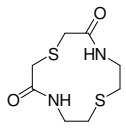
(4H, s, 2 x $CH_2S$); $^{13}C$ NMR (100 MHz, $D_2O$): 37.3 ($CH_2$), 38.8 ($CH_2$), 45.1 ($CH_2$), 177.7 (C).

**Bis(2-aminoethyl)sulfide 116**[164]



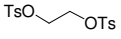

A stirred solution of sodium (0.60 g, 25.9 mmol) and ethanol (100 mL) was prepared. Cysteamine hydrochloride (1.0 g, 8.62 mmol) was added and the reaction mixture was heated under reflux for 30 min. 2-Chloroethylamine hydrochloride (0.98 g, 8.62 mmol) was added. The reaction mixture was heated under reflux for a further 2 h. The reaction mixture was cooled and the precipitated sodium chloride was filtered off and the filtrate was concentrated yielding the product as a pale yellow oil (0.92 g, 89%). $^1H$ NMR (400 MHz, $CDCl_3$): 1.21 (4H, bs, 2 x $NH_2$), 2.55 (4H, t, *J* 6.5 Hz, 2 x $CH_2S$), 2.82 (4H, t, *J* 6.5 Hz, $CH_2N$); *m/z* (EI): 121 ($MH^+$, 100%), 104 (60), 71 (33).

**1,7-Dithia-4,10-diazacyclododecane-3,11-dione 117**




Using general procedure A, **117** was synthesised from bis(2-aminoethyl)sulfide (1.29 g, 10.7 mmol) and thiodiglycolic acid chloride **110** (2.0 g, 10.7 mmol), as white crystals (2.37 g, 94%). mp 131-133 $^oC$; $\nu_{max}$ (KBr)/$cm^{-1}$: 3567 (NH), 1595 (CO), 1388 (CH); $^1H$ NMR (400 MHz, $D_2O$): 2.65 (4H, t, *J* 6.6 Hz, 2 x $CH_2N$), 2.99-3.02 (8H, m, 4 x $CH_2S$); $^{13}C$ NMR (100 MHz, $D_2O$): 27.8 ($CH_2$), 37.3 ($CH_2$), 38.1 ($CH_2$), 177.6 (CO).

**1,2-Ethanediol ditosylate 120**[213]

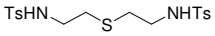



Using general procedure B, **120** was synthesised from 1,2-ethanediol (3.14 mL, 56 mmol), as white crystals (19.16 g, 92%). mp 122-124 $^oC$ (lit. 123-125 $^oC$); $^1H$ NMR (400 MHz, $CDCl_3$): 2.45 (6H, s, 2 x $CH_3$), 4.18 (4H, s, 2 x $CH_2$), 7.33 (4H, d, *J* 8.4 Hz, 4 x ArH), 7.72 (4H, d, *J* 8.4 Hz, 4 x ArH); *m/z* (FAB): 371 ($MH^+$, 57%).

**1,3-Propanediol ditosylate 121**[214]

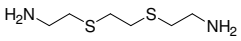

Using general procedure B, **121** was synthesised from 1,3-propanediol (4.75 mL, 65.7 mmol), as white crystals (19.3 g, 83%). mp 90-92 $^{o}$C (lit. 91-93 $^{o}$C); $^{1}$H NMR (400 MHz, $CDCl_3$): 2.01 (2H, quin, *J* 6.0 Hz, $CH_2$), 2.47 (6H, s, 2 x $CH_3$), 4.07 (4H, t, *J* 6.0 Hz, 2 x $CH_2N$), 7.36 (4H, d, *J* 8.0 Hz, 4 x ArH), 7.76 (4H, d, *J* 8.0 Hz, 4 x ArH); *m/z* (FAB): 385 ($MH^+$, 100%).

***N,N'*-Bis(toluene-4-sulfonyl)-bis(2-aminoethylsulfide) 122**[165]



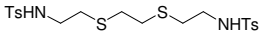

Using general procedure C, **120** was synthesised from bis(2-aminoethylsulfide) **116** (4.0 g, 33 mmol), as a brown viscous oil (12.7 g, 89%). $^{1}$H NMR (400 MHz, $CDCl_3$): 2.45 (6H, s, 2 x $CH_3$), 2.57 (4H, t, *J* 6.4 Hz, 2 x $CH_2S$), 3.09 (4H, q, *J* 6.4 Hz, 2 x $CH_2N$), 5.28 (2H, t, *J* 6.4 Hz, 2 x NH), 7.37 (4H, d, *J* 6.2 Hz, 4 x ArH), 7.76 (4H, d, *J* 6.2 Hz, 4 x ArH); *m/z* (FAB): 429 ($MH^+$, 100%), 386 (5), 258 (4), 199 (35), 156 (25), 140 (15), 93 (12).

**1,8-Diamino-3,6-dithiaoctane 123**[166]




A stirred solution of sodium (4.14 g, 0.18 mol) and *tert*-butanol (100 mL) was prepared and heated under reflux. Cysteamine hydrochloride (10.00 g, 88.0 mmol) was added and the reaction mixture was stirred for 30 min. 1,2-Dichloroethane (3.47 mL, 44.0 mmol) and *tert*-butanol (25 mL) were added dropwise and the reaction mixture was stirred for 3 h. After cooling, the precipitated NaCl was filtered off and the filtrate was concentrated. Kugelrohr distillation isolated the pure product as a pale yellow oil that solidified upon standing (7.31 g, 92%). $^{1}$H NMR (400 MHz, $CDCl_3$): 1.33 (4H, bs, 4 x NH), 2.65 (4H, t, *J* 6.5 Hz, 2 x $CH_2N$), 2.74 (4H, s, 2 x $CH_2S$), 2.87 (4H, t, *J* 6.5 Hz, 2 x $CH_2S$). *m/z* (CI): 181 ($MH^+$, 100%), 164 (9), 136 (33), 104 (70), 76 (7).

**1,8-Bis(toluene-4-sulfonyl)-1-8-diamino-3,6-dithiaoctane 124**



Using general procedure C, **124** was synthesised from 1,8-diamino-3,6-dithiaoctane **123** (5.0 g, 27.7 mmol), as a brown solid (13.0 g, 96%). mp 87-88 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3239 (NH), 2922 (CH), 1596, 1424; $^{1}$H NMR (400 MHz, $CDCl_3$): 2.41 (6H, s, 2 x $CH_3$), 2.66-2.99 (8H, m, 2 x $CH_2S$, 2 x $CH_2S$), 3.14 (4H, q, *J* 6.2 Hz, 2 x $CH_2N$), 5.28 (2H, t, *J* 6.2 Hz, 2 x NH), 7.34 (4H, d, *J* 8.1 Hz, 4 x ArH), 7.78 (4H, d, *J* 8.1 Hz, 4 x ArH); $^{13}$C NMR (100 MHz, $CDCl_3$): 21.6 ($CH_3$), 31.6 ($CH_2$), 32.1 ($CH_2$), 42.5 ($CH_2$), 127.1 (CH), 129.8 (CH), 126.8 (C), 143.7 (C); *m/z* (FAB): 489.1006 ($MH^+$, $C_{20}H_{29}N_2O_4S_4$ requires 489.1010), 258 (100%), 199 (40).

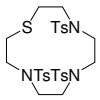
**4,7-Bis(toluene-4-sulfonyl)-1-thia-4,7-diazacyclononane 125**[165]


Using general procedure D, **125** was synthesised from *N,N'*-bis(toluene-4-sulfonyl)-bis(2-aminoethyl)sulfide (2.0 g, 4.67 mmol) **122** and 1,3-ethanediol ditosylate **120** (1.8 g, 4.67 mmol), as a white solid (1.6 g, 74%). mp 270-272 $^{o}$C (lit. 276-279 $^{o}$C); $^{1}$H NMR (400 MHz, $CDCl_3$): 2.43 (6H, s, 2 x $CH_3$), 3.13 (4H, bs, $CH_2N$), 3.37 (4H, s, $CH_2N$), 3.51 (4H, bs $CH_2S$), 7.53 (4H, d, *J* 8.4 Hz, 4 x ArH), 7.73 (4H, d, *J* 8.4 Hz, 4 x ArH); *m/z* (FAB): 455 ($MH^+$, 100%), 395 (3), 299 (35), 199 (4), 155 (32), 137 (32), 93 (35), 90 (34).

**4,8-Bis(toluene-4-sulfonyl)-1-thia-4,8-diazacyclodecane 126**[160a]

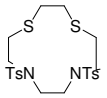

Using general procedure D, **126** was synthesised from *N,N'*-bis(toluene-4-sulfonyl)-bis(2-aminoethylsulfide) **122** (2.0 g, 4.67 mmol) and 1,3-propanediol ditosylate **121** (1.8 g, 4.67 mmol), as a white solid (2.2 g, 100%). mp 204-205 $^{o}$C (lit. 285-287 $^{o}$C – large difference could be due to different polymorph); $^{1}$H NMR (400 MHz, $CDCl_3$): 2.36-2.42 (2H, m, $CH_2$), 2.44 (6H, s, 2 x $CH_3$), 3.08 (4H, t, *J* 4.8 Hz, 2 x $CH_2N$), 3.14 (4H, t, *J* 6.0 Hz, 2 x $CH_2N$), 3.33 (4H, t, *J* 4.8 Hz, 2 x $CH_2S$), 7.34 (4H, d, *J* 8.0 Hz, 4 x ArH), 7.67 (4H, d, *J* 8.0 Hz, 4 x ArH); *m/z* (FAB): 469 ($MH^+$, 100%), 313 (55), 242 (100), 199 (14), 156 (20), 143 (14), 93 (27).

**4,7,10-Tris(toluene-4-sulfonyl)-1-thia-4,7,10-triazacyclododecane 127**



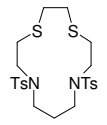

Using general procedure D, **127** was synthesised from *N,N'*-bis(toluene-4-sulfonyl)-bis(2-aminoethyl)sulfide **122** (2.0 g, 4.67 mmol) and *N,O,O'*-tris(toluene-4-sulfonyl)diethanolamine **80** (2.7 g, 4.67 mmol), as white crystals (2.5 g, 82%). mp 184-186 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3433, 2924 ($CH_2$), 1334, 1150; $^{1}$H NMR (400 NMR, $CDCl_3$): 2.45 (9H, s, 3 x $CH_3$), 3.00 (4H, t, *J* 5.2 Hz, 2 x $CH_2S$), 3.43 (12H, bs, 6 x $CH_2N$), 7.34 (6H, d, *J* 8.0 Hz, 4 x ArH), 7.68 (6H, d, *J* 8.0 Hz, 6 x ArH); $^{13}$C NMR (100 MHz, $CDCl_3$): 21.6 ($CH_3$), 31.7 ($CH_2$), 50.9 ($CH_2$), 52.2 ($CH_2$), 127.6 (CH), 129.9 (CH), 134.2 (C), 144.1 (C); *m/z* (FAB): 652.1647 ($MH^+$, $C_{29}H_{38}N_3O_6S_4$ requires 652.1643), 592 (20%), 496 (40), 253 (73), 156 (40), 93.0 (85).

**7,10-Bis(toluene-4-sulfonyl)-1,4-dithia,7,10-diaazacyclododecane 128**



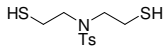

Using general procedure D, **128** was synthesised from 1,8-bis(toluene-4-sulfonyl)-1,8-diamino-3,6-dithiaoctane **124** (2.0 g, 4.09 mmol) and 1,2-ethanediol ditosylate **120** (1.52 g, 4.09 mmol), as white crystals (0.70 g, 33%). mp 176-178 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3088, 2893 (CH), 1536, 1333, 1166; $^{1}$H NMR (400 MHz, $CDCl_3$): 2.38 (6H, s, 2 x $CH_3$), 2.78 (4H, s, 2 x $CH_2S$), 2.83 (4H, t, *J* 6.2 Hz, 2 x $CH_2S$), 3.33 (4H, t, *J* 6.2 Hz, 2 x $CH_2N$), 3.42 (4H, s, 2 x $CH_2N$), 7.24 (4H, d, *J* 8.0 Hz, 4 x ArH), 7.63 (4H, d, *J* 8.0 Hz, 4 x ArH); $^{13}$C NMR (100 MHz, $CDCl_3$): 21.6 ($CH_3$), 31.8 ($CH_2$), 43.9 ($CH_2$), 50.7 ($CH_2$), 52.2 ($CH_2$), 127.6 (CH), 129.9 (CH), 134.4 (C), 143.9 (C); *m/z* (FAB): 515.1159 ($MH^+$, $C_{22}H_{31}N_2O_4S_4$ requires 515.1167), 487 (84%), 455 (100), 395 (28), 359 (28), 116 (37), 88 (94).

**7,11-Bis(toluene-4-sulfonyl)-1,4-dithia-7,11-diazacyclotridecane 129**



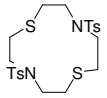
Using general procedure D, **129** was synthesised from 1,8-bis(toluene-4-sulfonyl)-1,8-diamino-3,6-dithiaoctane **124** (2.0 g, 4.09 mmol) and 1,3-propanediol ditosylate **121** (1.57 g, 4.09 mmol), as a white solid (0.97 g, 45%). mp 52-54 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3435, 2924 (CH), 1597, 1339, 1157; $^{1}$H NMR (400 MHz, $CDCl_3$): 1.89-1.95 (2H, m, $CH_2$), 2.38 (6H, s, 2 x $CH_3$), 2.68 (4H, s, 2 x $CH_2S$), 2.76-2.80 (4H, m, 2 x $CH_2S$), 3.10-3.18 (8H, m, 4 x $CH_2N$), 7.27 (4H, d, *J* 8.2 Hz, 4 x ArH), 7.61 (4H, d, *J* 8.2 Hz, 4 x ArH); $^{13}$C NMR (100 MHz, $CDCl_3$): 19.7 ($CH_3$), 26.6 ($CH_2$), 28.5 ($CH_2$), 29.3 ($CH_2$), 46.5 ($CH_2$), 47.5 ($CH_2$), 125.2 (CH), 128.0 (CH), 133.1 (C), 141.2 (C); *m/z* (FAB): 529.1326 ($MH^+$, $C_{23}H_{33}N_2O_4S_4$ requires 529.1323), 469 (44%), 409 (25), 373 (40), 214 (50), 156 (35), 93 (100).

**_N_-(Toluene-4-sulfonyl)-3-azapentane-1,5-dithiol 130**[160a]




A stirred solution of *N,O,O'*-tris(p-tolylsulfonyl)diethanolamine (5.85 g, 10.3 mmol) and anhydrous ethanol (40 mL) was prepared. Thiourea (1.72 g, 22.6 mmol) was added and the mixture was heated under reflux conditions overnight. The reaction mixture was concentrated and taken up in saturated sodium hydrogen cabronate solution (20 mL) and heated under reflux conditions for 3 h. After cooling, 6 N hydrochloric acid was added dropwise until the pH of the reaction mixture was 7. The aqueous solution was extracted with dichloromethane (3 x 50 cm$^3$). The organic layers were combined, dried ($MgSO_4$) and concentrated. The crude product was purified using flash column chromatography (eluant: dichloromethane/methanol 99:1) yielding a transparent oil (1.98 g, 66%). $^{1}$H NMR (400 MHz, $CDCl_3$): 1.36 (2H, t, *J* 8.5 Hz, 2 x SH), 2.37 (3H, s, $CH_3$), 2.64-2.69 (4H, m, 2 x $CH_2S$), 3.21 (4H, t, *J* 7.5 Hz, 2 x $CH_2N$), 7.26 (2H, d, *J* 8.1 Hz, 2 x ArH), 7.63 (2H, d, *J* 8.1 Hz, 2 x ArH); *m/z* (FAB): 292 ($MH^+$, 100%), 258 (95), 244 (47), 232 (22), 199 (13), 173 (36), 156 (47), 140 (15), 104 (25), 93 (91).

**4,10-Bis(toluene-4-sulfonyl)-1,7-dithia-4,10-diaazacyclododecane 131**




Using general procedure D, **131** was synthesised from *N,N'*-bis(toluene-4-sulfonyl)-bis(2-aminoethyl)sulfide **122** (3.41 g, 6.0 mmol) and *N*-(toluene-4-sulfonyl)-3-azapentane-1,5-dithiol **130** (1.75g, 6.0 mmol), as white crystals (0.07 g, 3%). mp 164-166 $^{o}$C; $\nu_{max}$

(KBr)/cm$^{-1}$: 3417, 1667, 1327, 1077; $^{1}$H NMR (400 MHz, $CDCl_3$): 2.36 (6H, s, 2 x $CH_3$), 2.86 (8H, t, *J* 6.6 Hz, 4 x $CH_2S$), 3.28 (8H, t, *J* 6.6 Hz, 4 x $CH_2N$), 7.25 (8H, d, *J* 8.2 Hz, 8 x ArH), 7.61 (8H, d, *J* 8.2 Hz, 8 x ArH); $^{13}$C NMR (100 MHz, $CDCl_3$): 21.6 ($CH_3$), 31.5 ($CH_2$), 50.6 ($CH_2$), 127.3 (CH), 129.9 (CH), 135.1 (C), 143.9 (C); *m/z* (FAB): 515.1178 ($MH^+$, $C_{22}H_{31}N_2O_4S_4$ requires 515.1167), 359 (13%), 258 (17), 199 (28), 155 (85), 137 (58).

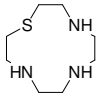
**1-Thia-4,8-diazacyclodecane 132**[215]


Using general procedure E, **132** was synthesised from 4,8-bis(toluene-4-sulfonyl)-1-thia-4,8-diazacyclodenane **126** (0.5 g, 1.07 mmol), as a transparent oil (25.3 mg, 93%). $^{1}$H NMR (400 NMR, $CDCl_3$): 1.51-1.62 (2H, m, $CH_2$), 2.59-2.81 (12H, m, 4 x $CH_2N$, 2 x $CH_2S$); *m/z* (CI): 161 ($MH^+$, 100%), 135 (22), 127 (18), 101 (17), 87 (10), 75 (5).

**1-Thia-4,7-diazacyclononane 133**[162]


Using general procedure E, **133** was synthesised from 4,7-bis(toluene-4-sulfonyl)-1-thia-4,7-diazacyclononane **125** (0.5 g, 1.10 mmol), as a transparent oil (0.04 g, 31%). $^{1}$H NMR (400 NMR, $CDCl_3$): 1.96 (2H, bs, 2 x NH), 2.67-2.71 (8H, m, 2 x $CH_2N$, 2 x $CH_2S$), 2.87 (4H, t, *J* 5.2 Hz 2 x $CH_2N$); *m/z* (CI): 147 ($MH^+$, 100%), 145 (7), 113 (4), 85 (4), 69 (4).

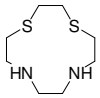
**1-Thia-4,7,10-triazacyclododecane 134**




Using general procedure E, **134** was synthesised from 4,7,10-tris(toluene-4-sulfonyl)-1-thia-4,7,10-triazacyclododecane **127** (0.5 g, 0.77 mmol), as a white solid (0.04 g, 32%). mp 58-60 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3405 (NH), 2923 (CH), 1576, 1457; $^{1}$H NMR (400 NMR, $CDCl_3$): 2.62-2.65 (4H, m, 2 x $CH_2$), 2.68-2.71 (4H, m, 2 x $CH_2$), 2.74-2.80 (8H, m, 4 x

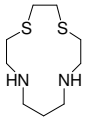
$CH_2$); $^{13}C$ NMR (100 MHz, $CDCl_3$): 32.4 ($CH_2$), 45.7 ($CH_2$), 45.9 ($CH_2$), 47.4 ($CH_2$); *m/z* (FAB): 190.1376 ($MH^+$, $C_8H_{19}N_3S$ requires 190.1378), 113 (12%), 85 (50), 69 (72).

**1,4-Dithia-7,10-diazacyclododecane 135**[159]



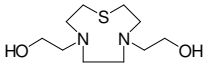

Using general procedure E, **135** was synthesised from 7,10-bis(toluene-4-sulfonyl)-1,4-thia-7,10-diazacyclododecane **128** (0.5 g, 0.97 mmol), as a yellow oil (0.025 g, 13%). $^1H$ NMR (400 NMR, $CDCl_3$): 2.68-2.72 (4H, m, 2 x $CH_2S$), 2.75 (4H, s, 2 x $CH_2S$), 2.81 (4H, s, 2 x $CH_2N$), 2.82-2.85 (4H, m, 2 x $CH_2N$); *m/z* (CI): 207 ($MH^+$, 100%), 179 (2), 145 (2), 113 (9), 103 (3), 85 (3), 69 (3).

**1,4-Dithia-7,11-diazacyclotridecane 136**[159]



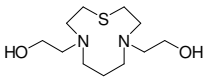

Using general procedure E, **136** was synthesised from 7,11-bis(toluene-4-sulfonyl)-1,4-thia-7,11-diazacyclotridecane **129** (0.5 g, 0.94 mmol), as a transparent oil (0.07 mg, 35%). $^1H$ NMR (400 NMR, $CDCl_3$): 1.77 (2H, quin, *J* 5.4 Hz, $CH_2$), 2.72-2.75 (4H, m, 2 x $CH_2S$), 2.78-2.81 (8H, m, 2 x $CH_2S$, 2 x $CH_2N$), 2.83-2.86 (4H, m, 2 x $CH_2N$), 3.77 (2H, bs, 2 x NH); *m/z* (CI): 221 ($MH^+$, 100%), 193 (2), 157 (2), 127 (9), 99 (4), 85 (6), 71 (5).

**4,7-Bis(2-hydroxyethyl)-1-thia-4,7-diazacyclononane 137**[162]



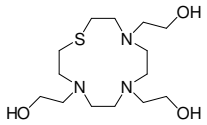

Using general procedure E, **137** was synthesised from 1-thia-4,7-diazacyclononane **133** (40.0 mg, 0.27 mmol), as a transparent oil (24.2 mg, 39%). $^1H$ NMR (400 NMR, $CDCl_3$): 2.66-2.99 (8H, m, 2 x $CH_2S$, 2 x $CH_2N$), 2.71-2.74 (4H, m, 2 x $CH_2N$), 2.78-2.81 (4H, m, 2 x $CH_2N$), 3.55 (4H, t, *J* 5.0 Hz, 2 x $CH_2O$); *m/z* (CI): 235 ($MH^+$, 100%), 190 (1), 176 (2), 135 (3), 90 (10), 76 (7).

**4,8-Bis(2-hydroxyethyl)-1-thia-4,8-diazacyclodecane 138**



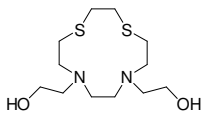
Using general procedure E, **138** was synthesised from 1-thia-4,8-diazacyclodenane **132** (83.1 mg, 0.52 mmo), as a transparent oil (17.5 mg, 14%). $\nu_{max}$ (KBr)/$cm^{-1}$: 3381 (OH), 2946, 1655, 1458, 1049; $^{1}H$ NMR (400 NMR, $CDCl_3$): 1.56 (2H, quin, *J* 6.5 Hz, $CH_2$), 2.48-2.51 (8H, m, 2 x $CH_2S$, 2 x $CH_2N$), 2.63 (8H, m, 4x $CH_2N$), 3.50 (4H, t, *J* 5.0 Hz, 2 x $CH_2O$); $^{13}C$ NMR (100 MHz, $CDCl_3$): 25.2 ($CH_2$), 30.8 ($CH_2$), 52.1 ($CH_2$), 53.5 ($CH_2$), 55.8 ($CH_2$), 59.2 ($CH_2$); *m/z* (CI): 249 ($MH^+$, 7%), 243 (4), 216 (10), 90 (65).

**4,7,10-Tris(2-hydroxyethyl)-1-thia-4,7,10-triazacyclododecane 139**



Using general procedure E, **139** was synthesised from 1-thia-4,7,10-triazacyclododecane **134** (0.14 g, 0.74 mmol), as a transparent oil (0.14 g, 61%). $\nu_{max}$ (KBr)/$cm^{-1}$: 3360 (OH), 2812, 1654, 1452, 1070; $^{1}H$ NMR (400 NMR, $CDCl_3$): 2.45 (4H, t, *J* 4.6 Hz, 2 x $CH_2S$), 2.54 (8H, bs, 4 x $CH_2N$) 2.66 (10H, bs, 5 x $CH_2N$), 3.56 (4H, t, *J* 5.0 Hz, 2 x $CH_2O$); $^{13}C$ NMR (100 MHz, $CDCl_3$): 28.5 ($CH_2$), 50.9 ($CH_2$), 51.8 ($CH_2$), 54.2 ($CH_2$), 56.8 ($CH_2$), 59.4 ($CH_2$); *m/z* (CI): 322.2159 ($MH^+$, $C_{14}H_{32}N_3O_3S$ requires 322.4922), 221 (5%), 154 (10), 88 (15).

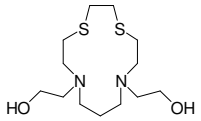
**7,10-Bis(2-hydroxyethyl)-1,4-dithia-7,10-diazacyclododecane 140**



Using general procedure E, **140** was synthesised from 1,4-dithia-7,10-diazacyclododecane **135** (25.0 mg, 0.12 mmol), as a transparent oil (38.6 mg, 100%). $\nu_{max}$ (KBr)/$cm^{-1}$: 3386 (OH), 2809, 1451 ($CH_2$), 1059; $^{1}H$ NMR (400 NMR, $CDCl_3$): 2.49 (4H, t, *J* 4.8 Hz, 2 x $CH_2S$), 2.60 (4H, s, 2 x $CH_2S$) 2.67-2.73 (8H, m, 4 x $CH_2N$), 2.86 (4H, s, 2 x $CH_2N$), 3.56 (4H, t, *J* 4.8 Hz, 2 x $CH_2O$); $^{13}C$ NMR (100 MHz, $CDCl_3$): 28.6 ($CH_2$), 29.9 ($CH_2$), 50.8

($CH_2$), 54.2 ($CH_2$), 56.4 ($CH_2$), 59.5 ($CH_2$); *m/z* (CI): 295 ($MH^+$, 100%), 201 (10), 172 (35), 71 (10).

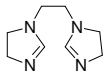
**7,11-Bis(2-hydroxyethyl)-1,4,-dithia-7,11-diazacyclotridecane 141**




Using general procedure E, **141** was synthesised from 1,4-dithia-7,11-diazacyclotridecane **136** (70.0 mg, 0.32 mmol), as a transparent oil (27.5 mg, 28%). $^1$H NMR (400 NMR, $CDCl_3$): 1.65 (2H, quin, *J* 6.5 Hz, $CH_2$), 2.50-2.56 (8H, m 4 x $CH_2S$), 2.68 (8H, bs, 4 x $CH_2N$), 2.76 (4H, bs, 2 x $CH_2N$), 3.52 (4H, t, *J* 5.2 Hz, 2 x $CH_2O$); $^{13}$C NMR (100 MHz, $CDCl_3$): 25.2 ($CH_2$), 30.6 ($CH_2$), 32.6 ($CH_2$), 52.2 ($CH_2$), 53.9 ($CH_2$), 56.3 ($CH_2$), 58.8 ($CH_2$); *m/z* (CI): 309.1668 ($MH^+$, $C_{13}H_{29}N_2O_2S_2$ requires 309.1670), 215 (25%).

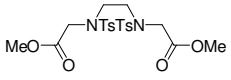
## 9.4 Experimental to Chapter 7

**1,1-Ethylenedi-2-imidazoline 72**[121]



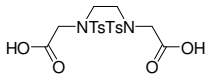

*N,N'*-Dimethylformamide dimethyl acetal (10.9 mL, 82 mmol) was added to triethylenetetraamine (6.1 mL, 41 mmol) and the mixture was heated under reflux for 1 h. Excess solvent was removed under vacuum and the white/yellow residue was recrystallised from tetrahydrofuran to give the desired product as white crystals (3.15 g, 46%). m.p. 107-108 $^o$C (lit. 107-109 $^o$C); $^1$H NMR ($CDCl_3$, 400 MHz): 3.16 (4H, t, *J* 9.6 Hz, 2 x $CH_2N$), 3.20 (4H, s, 2 x $CH_2$), 3.78 (4H, t, *J* 9.6 Hz, 2 x $CH_2N$) 6.74 (2H, s, 2 x CH); *m/z* (EI): 166 ($M^+$, 55%), 165 (16), 124 (7), 84 (34), 83 (100), 56 (100).

***N,N'*-Bis(toluene-4-sulfonyl)-ethylenediamine-*N,N'*-diacetic acid-dimethyl ester 146**[170]



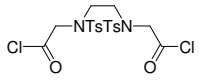
Sodium (0.25 g, 10.9 mmol) was dissolved in absolute methanol (14 mL) in a 3-necked flask. *N,N'*-Bis(toluene-4-sulfonyl)-ethylenediamine (2.0 g, 5.4 mmol) was added. The mixture was stirred and heated under reflux for 2 h. The methanol was removed by distillation and the residue was dried under vacuum. The powder was added portionwise over 30 min to a stirred ice-cooled flask of methyl bromoacetate (4.1 mL, 43.4 mmol). After stirring for 30 min at RT, the excess methyl bromoacetate was removed under vacuum. A mixture of chloroform (10 mL) and water (10 mL) was added to the residue. The chloroform layer was washed with water (2 x 10 mL) before drying over calcium chloride. The solution was filtered and concentrated. The product was recrystallised from methanol (1.05 g, 40%). mp 123-125 $^{o}$C (lit. 124-126 $^{o}$C); $^{1}$H NMR (400 MHz, $CDCl_3$): 2.36 (6H, s, 2 x $CH_3$), 3.41 (4H, s, 2 x $CH_2$), 3.55 (6H, s, 2 x $OCH_3$), 4.00 (4H, s, 2 x $CH_2$), 7.24 (8H, d, *J* 8.0 Hz, 4 x ArH), 7.63 (8H, d, *J* 8.0 Hz, 4 x ArH); *m/z* (CI): 513 ($MH^+$, 98%), 453 (35), 357 (21), 270 (13), 256 (35), 156 (23), 93 (30).

**_N, N'_-Bis(toluene-4-sulfonyl)-ethylenediamine-_N,N'_-diacetic acid 147**[170]




A mixture of *N,N'*-bis(toluene-4-sulfonyl)-ethylenediamine-*N,N'*-diacetic acid-dimethyl ester (0.64 g, 1.24 mmol), acetic acid (5.2 mL) and conc. hydrochloric acid (1.3 mL) was stirred and heated under reflux for 12 h. The resulting white crystals were filtered and rinsed with acetic acid (2 x 3 mL) before drying under vacuum (0.80 g, 70%). mp 240-242 $^{o}$C (lit. 228-229 $^{o}$C); $^{1}$H NMR (400 MHz, DMSO): 2.41 (6H, s, 2 x $CH_3$), 3.27 (4H, s, 2 x $CH_2$), 3.98 (4H, s, 2 x $CH_2$), 7.41 (8H, d, *J* 8.0 Hz, 4 x ArH), 7.66 (8H, d, *J* 8.0 Hz, 4 x ArH), 12.77 (2H, s, 2 x OH); *m/z* (FAB): 485 ($MH^+$, 17%), 439 (5), 285 (6), 238 (24), 170 (21), 87 (100).

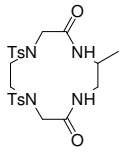
**_N,N'_-Bis(toluene-4-sulfonyl)-_N,N'_-ethanediyl-bis-glycyl chloride 143**[170]




*N,N'*-Bis(toluene-4-sulfonyl)-ethylenediamine-*N,N'*-diacetic acid (0.51 g, 1.06 mmol) and thionyl chloride (5.4 mL, 74.2 mmol) were stirred and heated using a water bath at 80 $^{o}$C for 5 h. The reaction mixture was concentrated and the crude product was recrystallised from benzene (special permission was given to use benzene) yielding fluffy white crystals

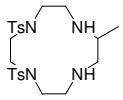
(0.55 g, 86%). mp 127-129 °C (lit. 223-224 °C); $^1$H NMR ($D_6$-DMSO, 400 MHz): 2.40 (6H, s, 2 x $CH_3$), 3.25 (4H, s, 2 x $CH_2$), 3.98 (4H, s, 2 x $CH_2$), 7.39 (4H, d, *J* 8.1 Hz, 4 x ArH), 7.65 (4H, d, *J* 8.1 Hz, 4 x ArH).

**4,7-Bis(toluene-4-sulfonyl)-2-methyl-1,4,7,10-tetraazacylcododecane -2,9-dione 148**




A stirred solution of triethylamine (0.86 mL, 6.17 mmol) and dichloromethane (140 mL) was prepared. Over a period of 6 h, *N,N'*-bis(toluene-4-sulfonyl)-*N,N'*-ethanediyl-bis-glycyl chloride (1.61 g, 3.1 mmol) in dichloromethane (37.5 mL) and 1,2-diaminopropane (0.26 mL, 3.09 mmol) in dichloromethane (37.5 mL) were added using 2 syringe pumps. The reaction mixture was then stirred for an additional 2 h. The white precipitate was filtered off (0.78 g, 48%). mp ≥360 °C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3212 (NH), 3090 (CH), 1644 (CO), 1350, 1162, 822, 550. $^1$H NMR ($D_6$-DMSO, 400 MHz): 0.98 (3H, d, *J* 6.8 Hz, $CH_3$), 2.42 (6H, s, 2 x $CH_3$), 2.66-2.77 (4H, m, 2 x $CH_2N$), 3.21-3.47 (4H, m, 2 x $CH_2$), 3.97-4.02 (3H, m, CH, $CH_2$), 7.47 (4H, d, *J* 7.6 Hz, 4 x ArH), 7.65 (4H, d, *J* 7.6 Hz, 4 x ArH); $^{13}$C NMR ($D_6$-DMSO, 100 MHz): 16.5 ($CH_3$), 19.7 ($CH_3$), 42.9 ($CH_2$), 43.4 (CH), 45.9 ($CH_2$), 51.9 ($CH_2$), 52.1 ($CH_2$), 125.8 (CH), 128.6 (CH), 133.3 (C), 142.3 (C), 164.7 (C), 165.3 (C). *m/z* (FAB): 523.1679 ($MH^+$, $C_{23}H_{29}N_4O_6S_2$ requires 523.1685), 367 (14%), 239 (22) 170 (45), 87 (100).

**4,7-Bis(toluene-4-sulfonyl)-2-methyl-1,4,7,10-tetraazacyclododecane 149**




A stirred solution of 4,7-bis(toluene-4-sulfonyl)-2-methyl-1,4,7,10-tetraazacyclododecane -2,9-dione (0.2 g, 0.38 mmol) and tetrahydrofuran (10 mL) was prepared and cooled using an ice bath. Borane in tetrahydrofuran (3.64 mL, 38.0 mmol) was added dropwise. The reaction mixture was heated under reflux for 3 d. Methanol was added dropwise to quench the reaction. The reaction mixture was concentrated. The minimum volume of ethanol was added to dissolve the brown residue. 6M Hydrochloric acid (2 mL) was added and the reaction mixture was heated under reflux overnight. The mixture was cooled using an ice

bath and the resulting white precipitate was filtered off and dried (0.11 g, 59%). mp 216-218 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3612 (NH), 2974 (CH), 2369, 1596 (CO), 1332, 1155; $^{1}$H NMR ($D_6$-DMSO, 400 MHz): 1.57 (3H, bs, $CH_3$), 2.44 (6H, s, 2 x $CH_3$), 2.88-2.91 (2H, m, $CH_2$), 2.97-3.01 (2H, m, $CH_2$), 3.19-3.38 (3H, m, CH, $CH_2$), 3.90 (1H, bs, CH), 4.02 (2H, bs, $CH_2$), 4.24 (1H, bs, $CH_2$), 7.53 (4H, d, *J* 7.6 Hz, 4 x ArH), 7.72 (4H, d, *J* 7.6 Hz, 4 x ArH), 9.46 (1H, bs, NH), 10.02 (1H, bs, NH); $^{13}$C NMR ($D_6$-DMSO, 100 MHz): 15.1 (CH), 19.0 ($CH_3$), 38.7 ($CH_2$), 39.3 ($CH_2$), 41.8 ($CH_2$), 43.9 ($CH_3$), 46.7 ($CH_2$), 47.0 ($CH_2$), 49.9 ($CH_2$), 125.7 (CH), 128.1 (CH), 129.2 (C), 142.6 (C); *m/z* (EI): 494.2018 ($M^+$, $C_{23}H_{34}N_4O_4S_2$ requires 494.2021), 424 (30%), 339 (28), 296 (44), 91 (100).

## 9.5 Experimental to Chapter 8

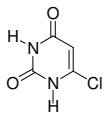
### *9.5.1 General Procedures (A & B)*

**General procedure A** – Synthesis of aminouracils: 6-Chlorouracil (1 eq) and aniline (3 eq) were heated at 170 °C under nitrogen for 20 min. The mixture was cooled and diethyl ether (15 mL) was added. The mixture was sonicated for 15 min. The white suspension was filtered and washed with distilled water (15 mL), methanol (15 mL) and diethyl ether (15 mL). The white solid was placed in a desiccator and dried overnight using silica and $P_2O_5$.

**General procedure B** – Synthesis of deazaflavins: A suspension of aminouracil (0.30 g, 1.40 mmol) and DMF (15 mL) was prepared. A 2-chloro-5-nitrobenzaldehyde (0.33 g, 1.7 mmol) was added and the reaction mixture was heated at 110 °C for 90 min under nitrogen. The yellow transparent solution was cooled and distilled water (25 mL) was added. The resulting precipitate was filtered and washed with water (15 mL). The yellow solid was placed in a desiccator and dried overnight using silica and $P_2O_5$

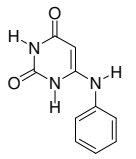
### *9.5.2 Experimental Details*

**6-Chlorouracil 171**[196]



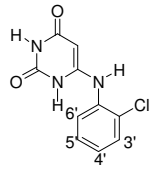
2,4,6-Trichloropyrimidine (15 g, 8.2 mmol) was added to a stirring solution of sodium hydroxide (13.2 g, 0.328 mol) and distilled water (135 mL). The reaction mixture was heated under reflux for one hour. The solution was cooled and the pH was adjusted to ~2-3 using conc. hydrochloric acid (18 mL). The mixture was stored at 0 °C overnight. The white precipitate was filtered and recrystallised from water (9.1g, 71%). mp 287-289 °C (lit. 300 °C); $^{1}$H NMR (400 MHz, $D_6$-DMSO): 5.76 (1H, s, 5-H), 11.33 (1H, s, NH), 12.09 (1H, s, NH); *m/z* (FAB): 147 ($MH^+$, $^{35}$Cl, 86%), 119 (2), 113 (12), 79 (100).

**6-Phenylamino-*1H*-pyrimidine-2,4-dione 169**



Using general procedure A, **169** was synthesised from 6-chlorouracil **171** (1.0 g, 6.82 mmol) and aniline (1.8 mL, 20 mmol), as a white solid (1.2 g, 84%). mp 332-333 °C; Found: C, 59.1; H, 4.4; N, 20.4. $C_{10}H_9N_3O_2$ requires C, 59.1; H, 4.5; N, 20.7.; $\nu_{max}$ (neat)/cm$^{-1}$: 3414 (NH), 1818 (CO), 1807 (CO); $^{1}$H NMR (400 MHz, $D_6$-DMSO): 4.70 (1H, s, 5-H), 7.15-7.23 (3H, m, 3 x ArH), 7.40 (2H, t, *J* 7.8 Hz, 2 x ArH), 8.28 (1H, s, NH), 10.20 (1H, s, NH), 10.50 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 75.8 (CH), 122.7 (CH), 124.7 (CH), 129.4 (CH), 137.9 (C), 150.8 (C), 152.2 (C), 164.4 (C). *m/z* (FAB): 204 ($MH^+$, 20%), 158 (40), 81 (100).

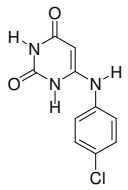
**6-(2-Chlorophenylamino)-*1H*-pyrimidine-2,4-dione**



Using general procedure A, 6-(2-chlorophenylamino)-*1H*-pyrimidine-2,4-dione was synthesised from 6-chlorouracil **171** (1.0 g, 6.82 mmol) and 2-chloroaniline (2.15 mL, 20.5 mmol), as a white solid. mp 321-323 $^{o}$C (decomp); Found: C, 50.4; H, 3.3; N, 17.5, $C_{10}H_8ClN_3O_2$ requires C, 50.5; H, 3.4; N, 17.7; $\nu_{max}$ (neat)/cm$^{-1}$: 3202 (NH), 1731 (CO), 1632 (C=C), 1540, 1480, 1224, 1078, 796, 736 ; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 4.37 (1H, s, 5-H), 7.29 (1H, ddd, *J* 8.0, 8.0, 1.6 Hz, 4'-H), 7.41 (1H, ddd, *J* 8.0, 8.0, 1.3 Hz, 5'-

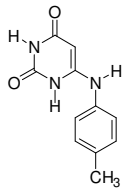
H), 7.46 (1H, dd, *J* 8.0, 1.6 Hz, 6'-H), 7.59 (1H, dd, *J* 8.0, 1.3 Hz, 3'-H), 8.10 (1H, s, NH) 10.38 (1H, s, NH), 10.55 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 76.1 (CH), 126.8 (CH), 127.4 (CH), 128.2 (CH), 128.4 (C), 130.1 (CH), 134.4 (C), 150.7 (C), 152.1 (C), 164.2 (C); *m/z* (CI): 238 ($MH^+$, $^{35}$Cl, 91%) 204 (8), 147 (10), 79 (100).

**6-(4-Chlorophenylamino)-*1H*-pyrimidine-2,4-dione**



Using general procedure A, 6-(4-chloro-phenylamino)-*1H*-pyrimidine-2,4,dione was synthesised from 6-chlorouracil **171** (1.0 g, 6.82 mmol) and 4-chloroaniline (2.61 g, 20.5 mmol), as a white solid (0.97 g, 60%). mp 344-346 $^{o}$C (decomp); Found: C, 50.3; H, 3.2; N, 17.4, $C_{10}H_8ClN_3O_2$ requires C, 50.5; H, 3.4; N, 17.7; $\nu_{max}$ (KBr)/cm$^{-1}$: 3195 (NH), 1754 (CO), 1616 (C=C), 1488, 1298, 788, 752 ; $^1$H NMR (400 MHz, $D_6$-DMSO): 4.73 (1H, s, 5-H), 7.23 (2H, d, *J* 8.6 Hz, 2 x ArH), 7.43 (2H, d, *J* 8.6 Hz, 2 x ArH), 8.42 (1H, s, NH), 10.32 (1H, s, NH), 10.55 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 76.6 (CH), 124.1 (CH), 128.2 (C), 129.3 (CH), 137.1 (C), 150.9 (C), 151.9 (C), 164.4 (C); *m/z* (CI): 238 ($MH^+$, $^{35}$Cl, 4%), 157 (60), 79 (100).

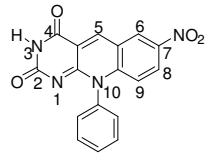
**6-(4-Methylphenyl)-*1H*-pyrimidine-2,4-dione**



Using general procedure A, 6-(4-methylphenyl)-*1H*-pyrimidine-2,4-dione was synthesised from 6-chlorouracil **171** (1.0 g, 6.82 mmol) and 4-tolylaniline (2.19 g, 20.0 mmol), as a white solid (1.35 g, 91%) mp 321-323 $^{o}$C; Found: C, 60.7; H, 5.1; N, 19.1, $C_{11}H_{11}N_3O_2$ requires C, 60.8; H, 5.1; N, 19.3; $\nu_{max}$ (KBr)/cm$^{-1}$: 3273 (NH), 2764, 1772 (CO), 1634 (C=C) 1446, 822, 786; $^1$H NMR (400 MHz, $D_6$-DMSO): 2.29 (3H, s, $CH_3$), 4.61 (1H, s, 5-H), 7.10 (2H, d, *J* 8.4 Hz, 2 x ArH), 7.20 (2H, d, *J* 8.4 Hz, 2 x ArH), 8.23 (1H, s, NH), 10.18 (1H, s, NH), 10.45 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 20.4 ($CH_3$), 75.2

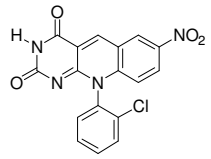
(CH), 123.1 (CH), 129.8 (CH), 134.1 (C), 135.1 (C), 150.8 (C), 152.5 (C), 164.3 (C); *m/z* (EI): 217 ($M^+$, 10%), 154 (8), 78 (90), 63 (100), 45 (26).

**10-Phenyl-7-nitro-*10H*-pyrimido[4,5-b]quinoline-2,4-dione 165**



Using general procedure B, **165** was synthesised from 6-phenylamino-*1H*-pyrimidine-2,4-dione (0.3 g, 1.47 mmol), and 2-chloro-5-nitrobenzaldehyde (0.33 g, 1.78 mmol), as a yellow solid (413 mg, 84%). mp >380 °C; Found: C, 60.7; H, 2.8; N, 16.4, $C_{17}H_{10}N_4O_4$ requires C, 61.1; H, 3.0; N, 16.7; $\nu_{max}$ (neat)/$cm^{-1}$: 3220 (NH), 1722 (CO), 1610; $^1$H NMR (400 MHz, $D_6$-DMSO): 6.87 (1H, d, *J* 9.6 Hz, 9-H), 7.47-7.75 (2H, m, 2 x ArH), 7.65-7.75 (3H, m, 3 x ArH), 8.45 (1H, dd, *J* 9.2, 2.4 Hz, 8-H), 9.23 (1H, d, *J* 2.4 Hz, 6-H), 9.31 (1H, s, 5-H), 11.32 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 117.5 (C), 118.5 (CH), 120.4 (C), 127.2 (CH), 128.3 (2 CH), 128.4 (2 CH), 129.7 (CH), 130.4 (CH), 137.2 (C), 142.1 (C), 145.1 (C), 156.3 (C), 159.5 (C), 161.4 (C); *m/z* (FAB): 335 ($MH^+$, 13%), 232 (15), 157 (80), 80 (100).

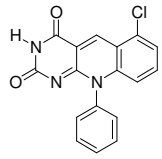
**10-(2-Chlorophenyl)-7-nitro-*10H*-pyrimido[4,5-b]quinoline-2,4-dione 174**



Using general procedure B, **174** was synthesised from 6-(2-chlorophenylamino)-*1H*-pyrimidine-2,4-dione (0.1 g, 0.42 mmol) and 2-chloro-5-nitrobenzaldehyde (94 mg, 0.5 mmol), as a yellow solid (101 mg, 66%). mp 326-328 $^o$C; $\nu_{max}$ (KBr)/$cm^{-1}$: 3144 (NH), 2826, 1683 (CO), 1617, 1340, 1218, 825, 796; $^1$H NMR (400 MHz, $D_6$-DMSO): 6.88 (1H, d, *J* 9.6 Hz, 9-H), 7.56-7.70 (3H, m, 3 x ArH), 7.81-7.89 (1H, m, ArH), 8.48 (1H, dd, *J* 9.6, 2.8 Hz, 8-H), 9.27 (1H, s, 5-H), 9.32 (1H, d, *J* 2.8 Hz, 6-H), 11.43 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 117.2 (C), 117.7 (CH), 120.4 (C), 127.5 (CH), 129.0 (CH), 129.5 (CH), 130.4 (CH), 130.9 (CH), 131.6 (C), 131.9 (CH), 134.2 (C), 142.7 (CH), 143.3 (C).

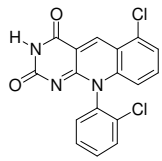
143.8 (C), 156.2 (C), 158.8 (C), 161.1 (C); *m/z* (FAB): 369.0391 ($MH^+$, $C_{17}H_{10}{}^{35}ClN_4O_4$ requires 369.0388), 232 (20%), 158 (40), 81 (100).

**6-Chloro-10-phenyl-*10H*-pyrimido[[4,5-b]quinoline-2,4-dione 175**



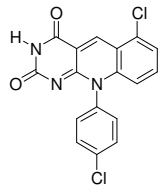
Using general procedure B, **175** was synthesised from 6-phenylamino-*1H*-pyrimidine-2,4-dione (0.15 g, 0.74 mmol) and 2,6-dichlorobenzaldehyde (0.26 g, 1.50 mmol), as a yellow solid (0.22 g, 92%). mp (decomp) 338-340 °C; $\nu_{max}$ (neat)/$cm^{-1}$: 3381 (NH), 3234 (NH), 1702 (CO), 1669 (CO); $^1$H NMR (400 MHz, $D_6$-DMSO): 6.70 (1H, dd, *J* 8.0, 1.2 Hz, 8-H), 7.43-7.45 (2H, m, 7-H, 9-H), 7.63-7.71 (5H, m, 5 x ArH), 9.04 (1H, s, 5-H), 11.27 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 116.7 (C), 116.8 (CH), 118.4 (C), 124.9 (2 CH), 128.3 (CH), 129.5 (2 CH), 130.3 (CH), 133.7 (C), 135.2 (CH), 136.5 (CH), 137.6 (C), 143.1 (C), 156.3 (C), 158.5 (C), 161.6 (C); *m/z* (FAB): 324.0536 ($MH^+$, $C_{17}H_{11}{}^{35}ClN_3O_2$ requires 324.0540), 292 (20%), 80 (100).

**6-Chloro-10-(2-chlorophenyl)-*10H*-pyrimido[4,5-b]quinoline-2,4-dione 176**



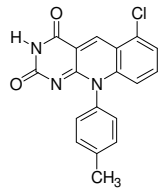
Using general procedure B, **176** was synthesised from 6-(2-chlorophenylamino)-*1H*-pyrimidine-2,4-dione (0.3 g, 1.26 mmol) and 2-chloro-6-fluorobenzaldehyde (0.24 g, 1.51 mmol), as a yellow solid (55 mg, 36%). mp 305-307 °C; $\nu_{max}$ (KBr)/$cm^{-1}$: 3142 (NH), 2801, 1709 (CO), 1685 (C=C), 1607, 1219, 794, 769; $^1$H NMR (400 MHz, $D_6$-DMSO): 6.72 (1H, d, *J* 10.8 Hz, 9-H), 7.62-7.66 (1H, m, 8-H), 7.67-7.79 (4H, m, 4 x ArH), 7.85-7.96 (1H, m, 7-H), 9.89 (1H, s, 5-H), 10.33 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 115.9 (CH), 116.5 (C), 118.4 (C), 125.4 (CH), 129.5 (CH), 130.4 (CH), 130.8 (CH), 131.0 (C), 131.7 (C), 134.1 (C), 134.5 (C), 135.9 (CH), 137.2 (C), 141.9 (C), 156.2 (C), 157.9 (C), 161.3 (C); *m/z* (CI): 358.0151 ($MH^+$, $C_{17}H_{10}{}^{35}Cl_2N_3O_2$ requires 358.0150), 128 (10%), 79 (100).

**6-Chloro-10-(4-chlorophenyl)-*10H*-pyrimido[4,5-b]quinoline-2,4-dione 177**



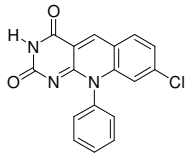
Using general procedure B, **177** was synthesised from 6-(4-chlorophenylamino)-*1H*-pyrimidine-2,4,dione (0.3 g, 1.26 mmol) and 2-chloro-6-fluorobenzaldehyde (0.24 g, 1.51 mmol), as a yellow solid (0.28 g, 63%). mp 220-222 $^{o}$C (decomp); $\nu_{max}$ (KBr)/cm$^{-1}$: 3254 (NH), 1667 (C=C), 1602 (C=C), 1363, 1245, 797, 747; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 6.55 (1H, d, *J* 8.8 Hz, 9-H), 7.50 (2H, d, *J* 8.4 Hz, 2 x ArH), 7.59-7.65 (2H, m, 7-H, 8-H), 7.79 (2H, d, *J* 8.4 Hz, 2 x ArH), 9.07 (1H, s, 5-H), 11.29 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 116.7 (C), 116.8 (CH), 118.5 (C), 124.9 (CH), 130.3 (CH), 130.4 (CH), 133.8 (C), 134.2 (C), 135.3 (CH), 136.3 (C), 136.7 (CH), 142.9 (C). 156.2 (C), 158.6 (C), 161.5 (C); *m/z* (FAB): 358.0152 ($MH^+$, $C_{17}H_{10}{}^{35}Cl_2N_3O_2$ requires 358.0150), 232 (8%), 158 (100), 81 (100).

**5-Chloro-10-(4-methylphenyl)-*10H*-pyrimido[4,5-b]quinoline-2,4-dione 178**



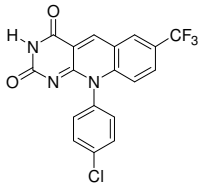
Using general procedure B, **178** was synthesised from 6-*p*-tolylamino-*1H*-pyrimidine-2,4-dione (0.1 g, 0.46 mmol) and 2-chloro-6-fluorobenzaldehyde (87 mg, 0.55 mmol), as a yellow solid (69 mg, 44%). mp >360 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3131 (NH), 3000, 2803, 1707 (CO), 1656 (C=C), 1221, 796, 740; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.49 (3H, s, $CH_3$), 6.75 (1H, d, *J* 9.2 Hz, 9-H), 7.30 (2H, d, *J* 8.2 Hz, 2 x ArH), 7.50 (2H, d, *J* 8.2 Hz, 2 x ArH), 7.66-7.73 (2H, m, 7-H, 8-H), 9.02 (1H, s, 5-H), 11.24 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 20.8 ($CH_3$), 116.9 (CH), 118.4 (C), 124.8 (CH), 125.3 (C), 127.9 (CH), 130.7 (CH), 133.7 (C), 134.9 (C), 135.1 (CH), 136.4 (CH), 139.1 (C) 143.3 (C), 156.3 (C), 158.6 (C), 161.6 (C); *m/z* (FAB): 338.0700 ($MH^+$, $C_{18}H_{13}{}^{35}ClN_3O_2$ requires 338.0696), 232 (20%), 158 (65), 81 (100).

**8-Chloro-10-phenyl-*10H*-pyrimido[4,5-b]quinoline-2,4-dione 179**



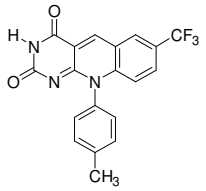
Using general procedure B, **179** was synthesised from 6-phenylamino-*1H*-pyrimidine-2,4-dione (0.15 g, 0.74 mmol) and 2,4-dichlorobenzaldehyde (0.26 g, 1.50 mmol), as a yellow solid (0.17 g, 71%). mp (decomp) 370-372 °C; Found: C, 62.8; H, 3.1; N, 12.8, $C_{17}H_{11}ClN_3O_2$ requires C, 63.1; H, 3.1; N, 13.0; $\nu_{max}$ (neat)/$cm^{-1}$: 3335 (NH), 3145 (NH), 1700 (CO), 1659 (CO); $^1$H NMR (400 MHz, $D_6$-DMSO): 6.50 (1H, d, *J* 1.6 Hz, 9-H), 7.38-7.40 (2H, m, 2 x ArH), 7.52 (1H, dd, *J* 8.8, 2 Hz, 7-H), 7.57-7.65 (3H, m, 3 x ArH), 8.22 (1H, d, *J* 8.8 Hz, 6-H) 9.08 (1H, s, 5-H), 11.10 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 115.9 (C), 116.1 (CH), 119.8 (C), 124.7 (CH), 128.3 (CH), 129.6 (C), 130.4 (CH), 133.2 (CH), 137.1 (C), 139.3 (C), 141.7 (CH), 142.5 (C), 156.3 (C), 158.5 (C), 158.8 (C), 161.7 (C); *m/z* (FAB): 324 ($MH^+$, $^{35}$Cl, 100%) 281 (7), 232 (12), 157 (25), 80 (50).

**10-(4-Chlorophenyl)-7-trifluoromethyl-*10H*-pyrimido[4,5-b]quinoline-2,4-dione 180**



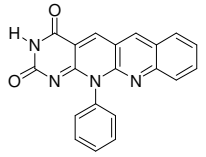
Using general procedure B, **180** was synthesised from 6-(4-chlorophenylamino)-*1H*-pyrimidine-2,4,dione (0.3 g, 1.26 mmol) and 2-chloro-5-(trifluoromethyl)benzaldehyde (0.22 mL, 1.51 mmol), as a yellow solid (0.40 g, 83%). mp 342-343 $^o$C; $\nu_{max}$ (KBr)/$cm^{-1}$: 3155 (NH), 2825, 1690 (C=C), 1618, 1337, 1276, 831, 797; $^1$H NMR (400 MHz, $D_6$-DMSO): 6.97 (1H, d, *J* 9.2 Hz, 9-H), 7.54 (2H, d, *J* 8.6 Hz, 2 x ArH), 7.79 (2H, d, *J* 8.6 Hz, 2 x ArH), 8.01 (1H, dd, *J* 9.2, 2.0 Hz, 8-H), 8.54 (1H, d, *J* 1.2 Hz, 6-H), 9.22 (1H, s, 5-H), 11.27 (1H, s, NH); $^{13}$C NMR (400 MHz, $D_6$-DMSO): 117.0 (C), 118.4 (CH), 120.6 (C), 123.3 (C), 124.9 ($CF_3$, *J* 33 Hz), 128.9 (CH), 130.5 (CH), 131.2 (CH), 132.8 (CH), 134.3 (C), 136.1 (C), 142.1 (CH), 143.6 (C). 156.3 (C), 159.8 (C), 161.5 (C); $^{19}$F NMR (376 MHz, $D_6$-DMSO): -60.7 ($CF_3$); *m/z* (FAB): 392.0413 ($MH^+$, $C_{18}H_{10}{}^{35}ClF_3N_3O_2$ requires 392.0414), 349 (15%), 81 (30).

**10-*p*-tolyl-6-trifluoromethyl-*10H*-pyrimido-[4,5-b]quinoline-2,4-dione 181**



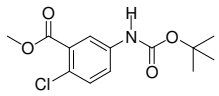
Using general procedure B, **181** was synthesised from 6-*p*-tolylamino-*1H*-pyrimidine-2,4-dione (0.1 g, 0.46 mmol) and 2-chloro-5-(trifluoromethyl)benzaldehyde (80 µmol, 5.52 mmol), as a yellow solid (67 mg, 40%). mp >360 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3145 (NH), 3006, 1714 (CO), 1670 (C=C), 1333, 1212, 796; $^{1}$H NMR (400 MHz, $D_6$-DMSO): 2.47 (3H, s, $CH_3$), 6.90-6.93 (1H, d, *J* 9.2 Hz, 9-H), 7.32 (2H, d, *J* 8.2 Hz, 2 x ArH), 7.50 (2H, d, *J* 8.2 Hz, 2 x ArH), 7.99-8.02 (1H, dd, *J* 9.2, 2.0 Hz, 8-H), 9.22 (1H, bs, 6-H), 10.33 (1H, s, 5-H), 11.23 (1H, s, NH); $^{13}$C NMR (100 MHz, $D_6$-DMSO): 20.8 (CH), 117.0 (CH), 118.5 (CH), 120.5 (C), 124.4 ($CF_3$, *J* 33 Hz), 128.0 (CH), 128.8 (CH), 130.3 (CH), 130.8 (CH), 134.7 (C), 139.1 (C), 141.9 (CH), 143.9 (C). 156.4 (C), 159.3 (C), 161.6 (C); $^{19}$F NMR (367 MHz, $D_6$-DMSO): -60.7 ($CF_3$); *m/z* (FAB): 372.0966 ($MH^+$, $C_{19}H_{13}F_3N_3O_2$ requires 372.0960), 232 (20%), 158 (40), 81 (100).

**12-Phenyl-*12H*-1,3,11,12-tetraaza-naphthacene-2,4-dione 182**



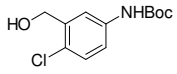
Using general procedure B, **182** was synthesised from 6-phenylamino-*1H*-pyrimidine-2,4-dione (0.2 g, 0.98 mmol) and 2-chloro-3-quinolinecarboxaldehyde (0.23 g, 1.18 mmol), as a yellow solid (0.24 g, 74%). mp (decomp) 353–355 °C; Found: C, 70.5; H, 3.5; N, 16.3, $C_{20}H_{12}N_4O_2$ requires C, 70.6; H, 3.5; N, 16.5; $\nu_{max}$ (neat)/cm$^{-1}$: 3368 (NH), 3230 (H), 1713 (CO), 1628 (C=C aromatic); $^{1}$H NMR (400 MHz, $D_6$-DMSO): 7.40-7.42 (2H, m, 7-H, 10-H), 7.56-7.58 (1H, m, ArH), 7.62-7.68 (4H, m, 4 x ArH), 7.85-7.87 (1H, m, 8-H), 8.31 (1H, d, *J* 7.6 Hz, 9-H), 9.21 (1H, s, 6-H), 9.34 (1H, s, 5-H), 11.3 (1H, s, NH). *m/z* (FAB): 341 ($MH^+$, 1%) 232 (30), 157 (100), 80 (100).

**5-*tert*-Butoxycarbonylamino-2-chlorobenzoic acid methyl ester 184**



2-Chloro-5-amino-benzoic acid (1.97 g, 11.5 mmol) was dissolved in methanol (22 mL) under a nitrogen atmosphere and cooled to 0 $^{o}$C using an ice bath. Trimethylsilyl chloride (2.93 mL, 22.9 mmol) was added dropwise to the lilac coloured solution. After 1 h, the reaction mixture was returned to RT and stirred overnight. Triethylamine (3.19 mL, 22.9 mmol) and di-*tert*-butyl dicarbonate (2.75 mL, 12.6 mmol) were added dropwise to the grey reaction mixture and this was stirred for 2 h. The brown reaction mixture was concentrated. The greenish residue was dissolved in diethyl ether (30 mL) and filtered to remove the precipitate (triethylamine salt). The filtrate was concentrated and purified using column chromatography (eluant: pet ether/ethyl acetate 60/40) yielding a white solid (3.30 g, 51%). mp 99-100 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3356, 2969 (NH), 1725, 1699 (CO), 1528, 1310, 1231, 1060, 972; $^{1}$H NMR (400 MHz, $CDCl_3$): 1.45 (9H, s, 3 x $CH_3$), 3.90 (3H, s, $OCH_3$), 6.51 (1H, s, NH), 7.28 (1H, d, *J* 8.8 Hz, ArH), 7.41 (1H, dd, *J* 8.8, 2.8 Hz, ArH), 7.76 (1H, d, *J* 2.8 Hz, ArH). $^{13}$C NMR (100 MHz, $CDCl_3$): 27.4 ($CH_3$), 28.3 ($CH_3$), 52.5 (CH), 120.9 (CH), 122.4 (CH), 127.3 (C), 130.2 (C), 131.5 (CH), 137.1 (C), 152.4 (C), 165.8 (C); *m/z* (EI): 285.0767 ($M^+$, $C_{13}H_{16}{}^{35}ClNO_4$ requires 285.0768), 229 (35%), 185 (30), 83 (20), 57 (100).

**(1-Chloro-2-hydroxymethyl-4-*tert*-butoxycarbonylamino)benzene 185**



A suspension of lithium aluminium hydride (0.177 g, 4.62 mmol) in tetrahydrofuran (10 mL) was prepared and cooled to 0 $^{o}$C using an ice bath. 5-*tert*-Butoxycarbonylamino-2-chlorobenzoic acid methyl ester was added in small portions. The reaction mixture was heated at 40 $^{o}$C for 2 d. The reaction mixture was quenched by the careful addition of water. Diethyl ether was added and the layers were separated. The aqueous layer was washed with diethyl ether (3 x 20 mL). The combined organic layers were washed with water (3 x 20 mL), dried ($MgSO_4$), concentrated and purified using column chromatography eluant: petroleum ether/ethyl acetate 70/30 yielding a pale yellow solid (1.18 g, 42%). mp 66-68 $^{o}$C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3287, 2812 (NH), 1608 (CO), 1238, 1056,

873; $^{1}$H NMR (400 MHz, $CDCl_3$): 2.86 (9H, s, 3 x $CH_3$), 4.68 (2H, s, $CH_2$), 6.49 (1H, dd, *J* 8.4, 2.8 Hz, 6-ArH), 6.72 (1H, d, *J* 2.8 Hz, 2-ArH), 7.05 (1H, d, *J* 8.4 Hz, 5-ArH), 7.25 (1H, s, NH); $^{13}$C NMR (100 MHz, $CDCl_3$) 30.8 ($CH_3$), 63.2 ($CH_2$), 77.3 (C), 112.5 (CH), 112.6 (CH), 120.2 (C), 129.8 (CH), 138.6 (C), 148.3 (C); *m/z* (EI): 257.0816 ($M^+$, $C_{12}H_{16}{}^{35}ClNO_3$ requires 257.0819), 201 (100%), 157 (92), 57 (100).

# 9.6 Biological Testing

## *9.6.1 Biological Testing Chapter 5*

### 9.6.1.1 DNA Cross-linking Activity of Macrocyclic Mustards

The method used was the agarose gel cross-link assay.[151a] pBR322 plasmid DNA was linearised by digestion with HinDIII and dephosphorylated by treatment with bacterial alkaline phosphatase. The DNA was 5'-end labeled using T4 polynucleotide kinase and [$\gamma$-$^{32}$P] ATP (5000 Ci/mmol, Amersham, UK). Following precipitation and removal of unincorporated ATP, the DNA was resuspended in sterile double-distilled water at 1 mg/mL. Approximately 10 ng of labeled DNA were used for each experimental point. Reactions with drug were performed in 25 mM triethanolamine, 1 mM EDTA (pH 7.2) at 37 $^{o}$C for 2 h. Reactions were terminated by the addition of a greater than equal volume of stop solution (0.6 M sodium acetate, 20 mM EDTA, 100 µg/mL tRNA) and the DNA was precipitated by the addition of 3 vols 95% ethanol. Following centrifugation and removal of supernatant, the DNA pellet was dried by lyophilisation. Samples were dissolved in 10 µL strand separation buffer (30% DMSO, 1mM EDTA, 0.04% bromophenol blue, 0.04% xylene cyanol), heated at 90 $^{o}$C for 2 min and chilled immediately in an ice-water bath prior to loading. Control undenatured samples were dissolved in 10 µL 6% sucrose, 0.04% bromophenol blue and loaded directly. Electrophoresis was performed on 20 cm long 0.8% submerged horizontal agarose gels at 40 V for 16 h. The gel and running buffer were 40 mM Tris, 20 mM acetic acid and 2 mM EDTA (pH 8.1). Gels were dried at 80 $^{o}$C onto one layer of Whatman 3 MM and one layer of DE81 filter papers on a BioRad Model 583 gel drier connected to a vacuum. Autoradiography was performed with Hyperfilm MP (Amersham, UK) at -70 $^{o}$C using a DuPont-Cronex Lightening-plus intensifying screen. Sharper images were obtained by overnight exposure without the intensifying screen. The percentage double stranded (cross-linked) DNA was obtained in each lane using a BioRad Imaging Densitometer.

### 9.6.1.2 Cytotoxicity of Macrocyclic Mustards

The cytotoxic effects of the free ligands studied were measured against the human chronic myeloid leukaemia cell line K562. Cells were maintained as a suspension in RPMI 1640 medium supplemented with 10% foetal calf serum and 2 mM glutamine at 37 $^{o}$C in a humidified atmosphere containing 5% $CO_2$. Following a 1 h exposure to the sample, the

corresponding $IC_{50}$ value was determined using the MTT assay.[151b,151c] This is based on the ability of viable tumour cells to convert a yellow tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolyium bromide, MTT] into mauve formazan crystals.

#### 9.6.1.3 Trypanosome Cell Line

##### *9.6.1.3.1 Trypanosoma brucei brucei 427*

*T. b. brucei* 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.

##### *9.6.1.3.2 T. b. brucei 427 ΔTbat1 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 melasoprol and melarsen oxide. These stocks are held at the University of Glasgow.

#### 9.6.1.4 Growth and Maintenance of Bloodstream-Form Trypanosome Stocks

##### *9.6.1.4.1 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.

#### 9.6.1.5 Trypanotoxicity

##### *9.6.1.5.1 In vitro Toxicity Assay (Alamar Blue Assay)*

The Alamar Blue assay was used to assess toxicity *in vitro*.[153] 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.[154] 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 $EC_{50}$ value was calculated by analysing the data with the $EC_{50}$ 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.

#### 9.6.1.6 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 $^{o}$C 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 $^{o}$C 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.

#### 9.6.1.7 Fluorescence Assays

##### *9.6.1.7.1 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. The 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.

### *9.6.2 Biological Testing Chapter 8*

Protein expression levels were assessed by Western Blotting, after incubation of primary human pigment epithelial (RPE) cells with 1-20 µM of each compound for 24 h. Proteins

from whole cell extracts were separated by SDS 12% polyacrylamide gel electrophoresis and analysed by Western blotting with anti-p53 DO-1 (Pharmingen), anti-HDM2 AB1/AB2 (Oncogene Science), anti-phospho-p53 (serine-15) (Cell Signaling) and anti-$21^{WAF1/CIP1}$ (Santa Cruz Biotechnology) antibodies. Blots were also probed with an anti-Cdk4 antibody (Santa Cruz Biotechnology) to monitor protein loading. The effects of some of the compounds were assessed by flow cytometry.

## 9.7 Experimental Crystallography Details

Details of data collection procedures and structure refinement are given in the following table. CIF data can be accessed online at http://www.chem.gla.ac.uk/~louis/data/. Single crystals of suitable size were attached to glass fibres using acrylic resin, and mounted on a goniometer head in a general position. Data was collected on an Enraf-Nonius KappaCCD diffractometer, running under Nonius Collect software, and using graphite monochromated X-radiation (λ=0.71073 Å). All data sets were collected at a temperature of 150 K using an Oxford Instruments Cryostream. Typically scan angles of 1-2 $^{o}$ were used, with integration times of 50-100 s per image. Precise unit cell dimensions were determined by post-refinement of the setting angles of a large proportion of the data set. The frame images were integrated using Denzo(SMN)[216] and the resultant raw intensity files processed using a locally modified version of DENZOX.[217] Absorption corrections, either by Gaussian quadrature,[218] based on the measured crystal faces, or by a semi-empirical correction[219] were applied to all data sets. Data were then sorted and merged using SORTAV.[220] Structures were solved either by Patterson interpretation (DIRDIF-99)[221] or by direct methods (SIR92).[222] Refinement with SHELXL97-2 using full-matrix least squares on $F^2$ and all the unique data. Neutral atom scattering forces, coefficients of anomalous dispersion and absorption coefficients were obtained from reference. All calculations were carried out using the WinGX package of crystallographic programs.[223]

| Compound number | **84** | **85** | **181** |
|---|---|---|---|
| Compound formula | $C_{44}H_{56}N_8O_8$ | $C_{32}H_{64}N_8O_8$ | $C_{19}H_{12}F_3N_3O_2$ |
| Compound colour | colourless | colourless | yellow |
| $M_r$ | 824.97 | 688.91 | 371.32 |
| Space group | $P2_1/a$ | $P2_1/a$ | $P_{-1}$ |
| Crystal system | monoclinic | monoclinic | triclinic |
| a/Å | 11.0274 (5) | 12.8699 (5) | 10.0933 (4) |
| b/Å | 8.9869 (5) | 9.5994 (4) | 12.4331 (5) |
| c/Å | 21.2550 (8) | 17.0767 (8) | 14.8188 (6) |
| β/deg | 91.304 (3) | 111.043 (2) | 93.650 (2) |
| V/Å$^{-3}$ | 2105.87 (17) | 1969.01 (15) | 1736.60 (12) |
| Z | 2 | 2 | 4 |
| $D_{calc}$/ g cm$^{-3}$ | 1.301 | 1.162 | 1.42 |
| F(000) | 880 | 752 | 760 |
| μ(Mo-$K_a$)/mm$^{-1}$ | 0.091 | 0.084 | 0.116 |
| Crystal size/mm | 0.48 x 0.41 x 0.13 | 0.39 x 0.35 x 0.25 | 0.6 x 0.4 x 0.2 |
| Transmission coefficients (range) | 1.000-0.798 | n/a | n/a |
| θ range/deg | 2.46-26.45 | 1.28-26.14 | 1.46-26.51 |
| No. of data measured | 21987 | 17875 | 33364 |
| No. of unique data | 4331 | 3920 | 7137 |
| $R_{int}$ | 0.0758 | n/a | 0.0102 |
| No. of data in refinement | 4331 | 3920 | 7137 |
| No. of refined parameters | 280 | 218 | 501 |
| Final R[I>2σ(*l*)] (all data) | 0.0589 | 0.0631 | 0.047 |
| $R_w^2$[I>2σ(*l*)] (all data) | 0.1194 | 0.0998 | 0.0759 |
| Flack parameter | n/a | n/a | n/a |
| Goodness of fit S | 1.056 | 1.086 | 1.052 |
| Largest residuals/eÅ$^{-3}$ | 0.31, -0.323 | 0.444, -0.323 | 0.188, -0.19 |
| Max shift/esd in last cycle | 0.001 | 0.004 | 0.008 |

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