
Synthesis and Evaluation of Polyamines as Antimalarial Agents

**A Thesis submitted in part fulfilment
of the requirements of the degree
of Doctor of Philosophy**

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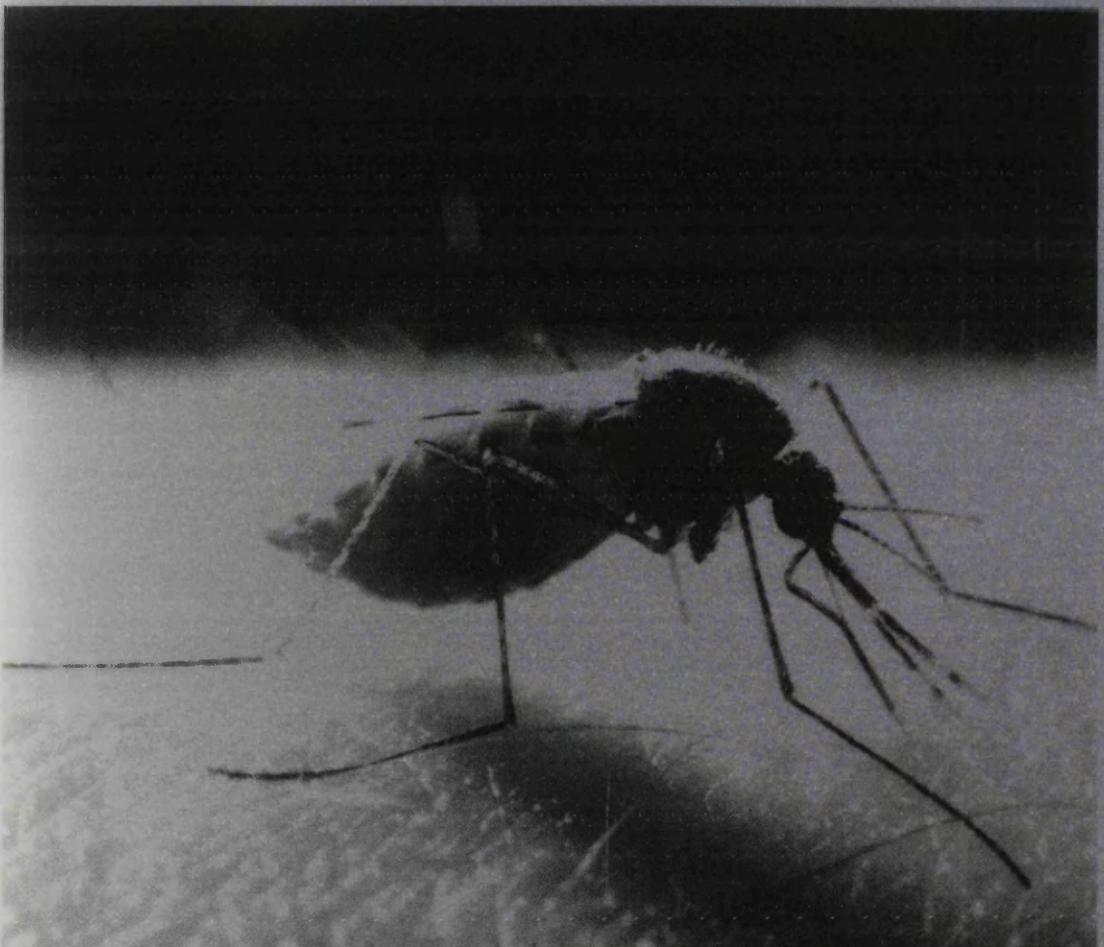
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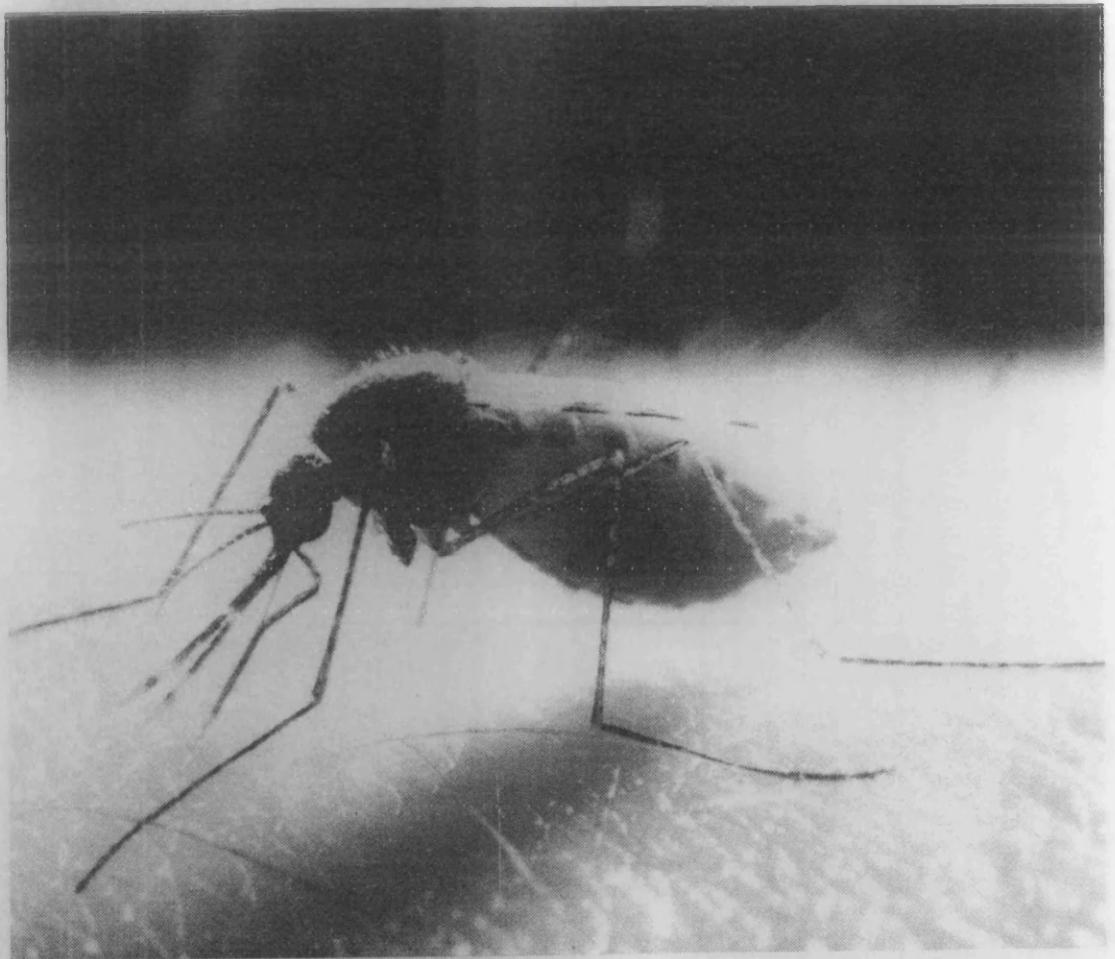
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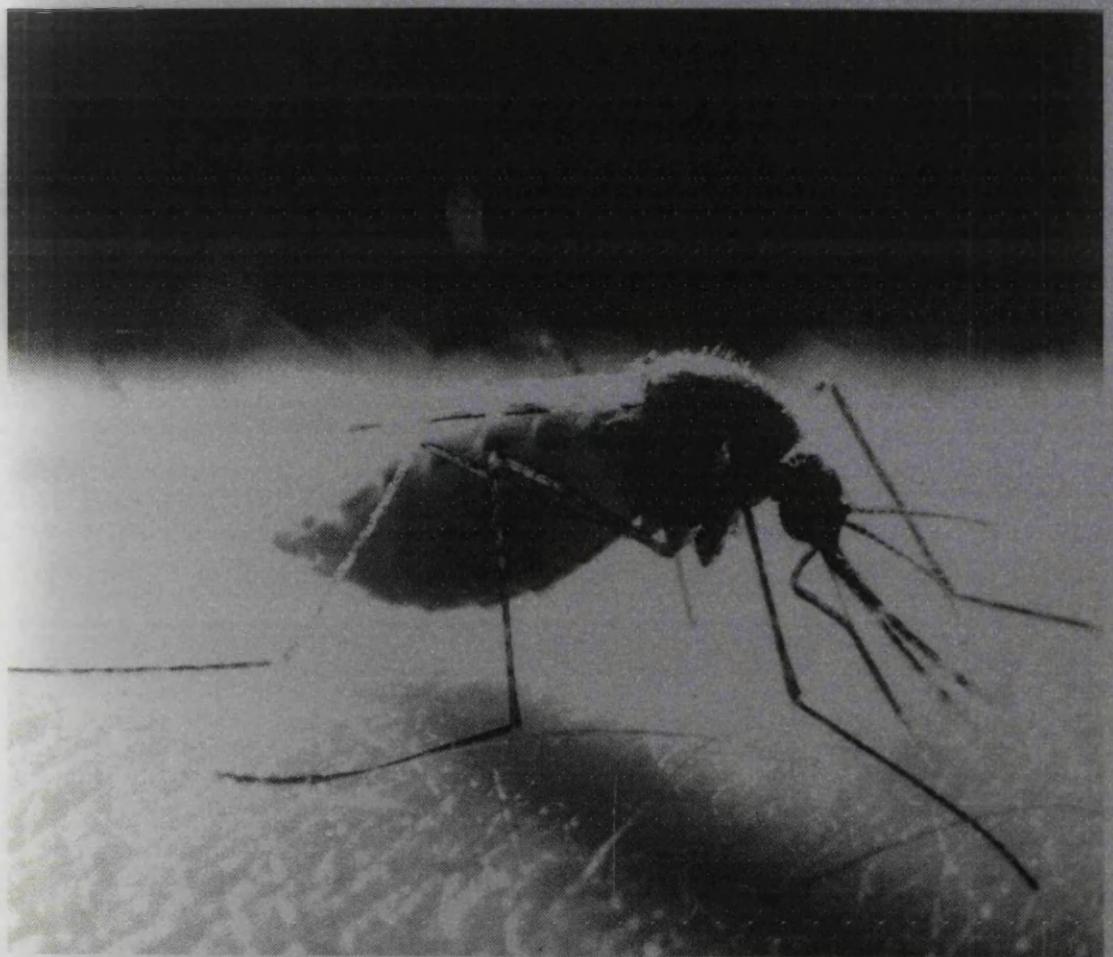


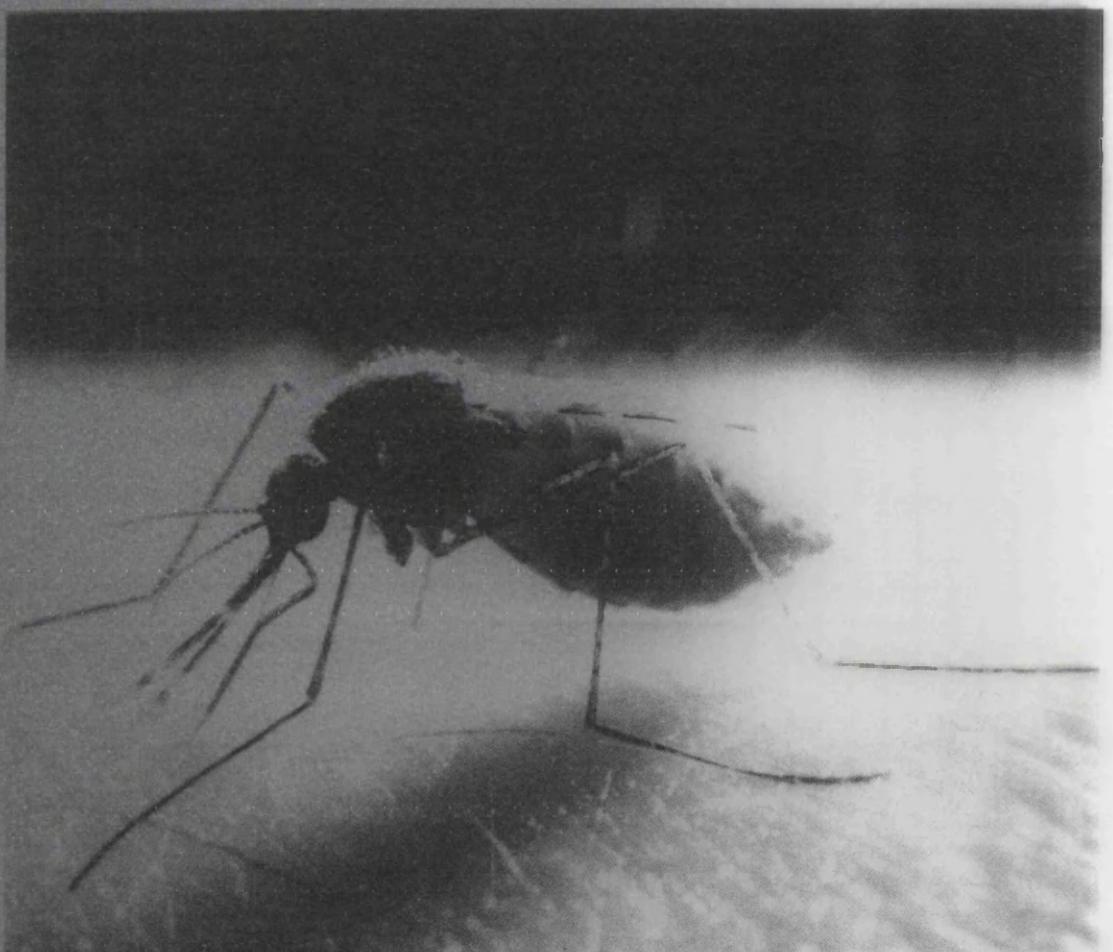




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malaria (mə'lērēə) *n.* an infectious disease characterized by recurring attacks of chills and fever, caused by the bite of an anopheles mosquito infected with any of four protozoans of the genus *Plasmodium* (*P. vivax*, *P. falciparum*, *P. malariae*, or *P. ovale*). [C18: from Italian *mala aria* bad air, from the belief that the disease was caused by the unwholesome air in swampy districts] —*ma'larial*, *ma'larian*, or *ma'larious adj.*

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Throughout the years many people have passed through the chemistry department and my deepest thanks goes to anyone I have had the pleasure of knowing, and for making these years so enjoyable. The Alchemists.

Finally to all those people who have kept me sane when away from chemistry, you know who you are, but especially to everyone at Strathallan.

Why Fall
Man Small
Skies Call
That's All

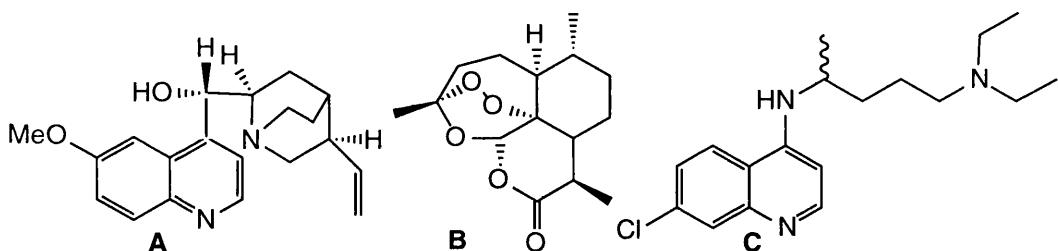
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This is dedicated to my family who have always supported me, but most of all to Danny who has put up with so much, especially when I was writing this thesis, thanks!

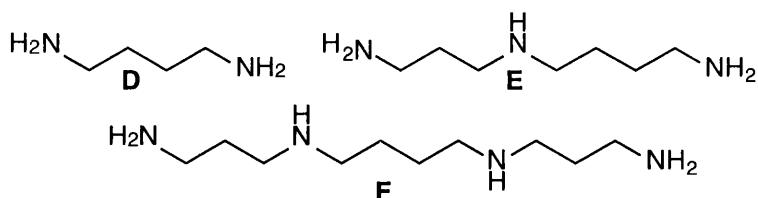
Thanks to the Wellcome Trust for putting their faith and money into this project and for the extra training they provided throughout the PhD.

Summary

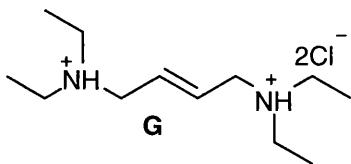
Plasmodium species is transmitted between humans by the female anopheles mosquito. It kills 1.5-2.7 million people every year and 40% of humanity lives in an endemic area. Throughout time numerous attempts have been made to control the disease through natural remedies such as quinine (**A**) and artemisinin (**B**), synthetic drugs e.g. chloroquine (**C**), and also by the use of bed nets and insect repellents.



Polyamines such as putrescine (**D**), spermidine (**E**) and spermine (**F**) are naturally occurring and are widespread in nature. They have been shown to be important in fundamental processes such as cell proliferation and differentiation. The study of these compounds has led to the development of polyamine analogues to treat a wide range of diseases from cancer and parasitic diseases through to their use as anti-fungal agents.

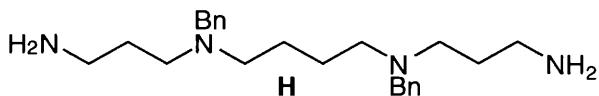


We started work in this area when a simple putrescine analogue (**G**) was shown to have promising antimalarial activity and became the "lead" compound for this project.

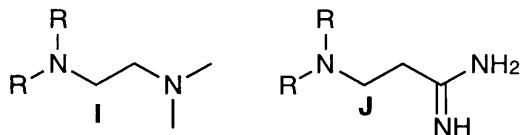


Initially we synthesized a number of analogues of (**G**) varying the carbon chain and the substitution patterns on the nitrogens. We did not see any improvement in activity until a *N,N*-bisbenzyl compound was prepared; however *in vivo* results were disappointing. We decided to investigate analogues of higher polyamines as these had shown better activity than putrescine analogues by workers in the cancer field.

Surprisingly the spermidine analogues that were prepared showed little antimalarial activity, although separate tests showed them to have excellent anti-fungal properties. The spermine analogues synthesized were more promising with the bisbenzyl analogue (**H**) the most active *in vitro* so far.



It was known that the genome of *P. falciparum* is rich in adenine and thymine base pairs compared to the human host so we decided to study some AT specific binding agents to see if this improved the activity. We decided to incorporate the *N,N*-dimethylaminoethyl (**I**) and propioamidino (**J**) moieties into spermine analogues to see if antimalarial activity increased. These functional groups had been used previously in AT binding agents. No test data is available on these compounds.



A number of compounds were prepared and we observed increased activity as we added hydrophobic benzyl groups and also as we increased the number of nitrogens present to four, as in spermine analogues. This is in agreement with the hypothesis that DNA binding is related to the activity of the compounds which itself is controlled by the increased number of nitrogens in the compound. A number of compounds are still awaiting testing.

We have prepared a range of polyamines, both known and novel, and examined their antimalarial activity. Antimalarial testing was carried out by Prof. Stephen Phillips and Fiona McMonagle in the Division of Infection and Immunity and antifungal tests by Dr Dale Walters and Caroline MacIntosh at the Scottish Agricultural College, Auchincruive.

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Abbreviations

ADC	arginine decarboxylase
AdoMet	S-adenosylmethionine
AdoMetDC	S-adenosylmethionine decarboxylase
DCM	dichloromethane
DEET	diethyl- <i>m</i> -toluamide
DFMA	α -difluoromethylarginine
DFMO	α -difluoromethylornithine
DHFR	dihydrofolate reductase
EGBG	ethylglyoxalbis(guanylhydrazone)
L1210	leukaemia cell line
LCLC	large cell lung carcinoma
LEC	lowest effective concentration
MAP	δ -methylacetylenic putrescine
MFMO	α -monofluoromethylornithine
MGBG	methylglyoxalbis(guanylhydrazone)
MTA	5'-methylthioadenosine
N,N-CDI	<i>N,N</i> -carbonyldiimidazole
ODC	ornithine decarboxylase
PAO	polyamine oxidase
PLP	pyridoxal-5'-phosphate
rt	room temperature
SCLC	small cell lung carcinoma
SSAT	spermine/spermidine-N ¹ -acetyl transferases
t_{1/2}	half life
TFA	trifluoroacetyl
TR	trypanothione reductase
TROC	2,2,2-trichloroethyl carbamate
TRT	Triphenylmethyl

1

1 Malaria

1.1 Public Enemy No1

The sun sets - the hunt for human blood begins.

Throughout the dark night the female *Anopheles* mosquito searches for a host. Blood is a necessity. She needs the protein for the development of her eggs which she will lay in a suitable warm, damp place. She enters as quiet as a burglar attracted by your warm body and the carbon dioxide you exhale. Only she is potentially deadly - she carries the malaria parasite and passes this cargo between victims infecting 300-500 million people each year.

Two needle like objects pierce your skin and a local anaesthetic is quickly applied by her saliva. As she searches for a vein two blades saw at your skin until the feed tube sucks your blood and she flies away laden with twice her weight in blood.

Behind her she leaves a deadly legacy. It kills 1.5-2.7 million people every year and 40% of humanity lives in an endemic area. This is by far the world's most important parasitic disease killing more people than any other communicable disease except tuberculosis.

Malaria kills one person every 12 seconds but what is it, how does it survive, what does it do and how may we combat it?

1.2 Malaria - A Potted History

Although it was a long time before the cause of malaria was understood its characteristic symptoms have been documented as far back as the ancient Egyptians followed by Hypocrates who first described the disease in detail. Herodotus (485-425 BC) noticed that in the swampy

regions of Egypt people often slept on raised platforms or under nets out of reach of the mosquitos and from these early times "miasma" or bad air ("mal aria") that emanated from these marshes became associated with this disease.¹

Many ancient treatments for malaria have proven highly effective including an infusion of qinghaosu (*Artemisia annua*) used in China for over 2000 years and recently the subject of much research since the active component Artemisinin was identified. An extract from the cinchona tree was first used successfully in Peru during the fifteenth century and its use was established in India, Ceylon and the East Indies during the nineteenth century. The active ingredient, quinine, was isolated in 1820 by the French chemist Pelletier and was thereafter widely used.

So for many years the disease was described and even treated but no one knew what caused it and how it was transmitted.

The crucial step came on 6th November 1880 when a French army surgeon Charles Louis Alphonse Laveran identified the malaria parasite in the blood of a patient infected with *P. falciparum*.² Unfortunately this was the world of Louis Pasteur, the "germ" was seen as the cause of all disease, and Laveran's observations were not readily accepted.

Throughout the last two decades of the nineteenth century came great advances in this field. An Italian Camillo Golgi identified two human malarias, *P. vivax* and *P. malariae*, and described the asexual multiplication of the parasite in the red blood cell. The recurrent fevers associated with malaria were also linked to this asexual reproductive stage.

The transmission of the disease still remained a mystery.

Patrick Manson, an eminent English physician, and the founder of tropical medicine in England, had discovered that mosquitos could suck up small threadlike worms and believed that maybe they could draw the malaria parasites from human blood. He began an association with Ronald Ross, a doctor in the Indian medical service, and together they planned a series of experiments. Ross was a lone and dedicated researcher despite his wish to be a poet or playwright rather than working in the army. He dissected mosquitos for two years under difficult circumstances; however he was unaware it was only the *Anopheles* mosquitos that transmitted the disease. At last, and by luck, the right mosquitos arrived and after feeding them on an infected patient he saw evidence of the parasite developing in the mosquito on 20th August 1897.² This was the first link between the mosquito and transmission of malaria. On moving to Calcutta he started work on avian malaria parasites and proved that the infective stages of the parasite appear

in the salivary gland of the mosquito and were passed on when the mosquito bit its next victim. Ross was later awarded the second Nobel Prize for medicine. Throughout this time he continued to write poems, one of which he wrote soon after his discovery:

*This day relenting God
 Hath placed within my hand
 A wondrous thing; and God
 Be praised. At His command,
 Seeking His secret deeds
 With tears and toiling breath,
 I find thy cunning seeds,
 O million-murdering death.
 I know this little thing
 A myriad men will save.
 O Death, where is thy sting?
 Thy victory, O Grave?*

A group of Italians were working on the same puzzle and this led to years of controversy between Ross and Giovanni, Battista and Grassi over who first traced the development of the malaria parasite in man. Discovery was rapid and by 1948 elucidation of the life cycle of the malaria parasite in man was complete (1.5). Unfortunately a number of speculations arose. For example it had been noted that Africans were often immune to malaria and in the Journal of Tropical Medicine, 1900, George Nuttal suggested it was "the offensive smell" of Africans that discouraged the mosquitos from approaching and biting them!

1.3 Transmission of Malaria

The malaria parasites are protozoa of the genus *Plasmodium* and these parasites are transmitted by the female *Anopheles* mosquito (Figure 1). "The female of the species is more deadly than the male" and this is certainly true in this case as the humble male feeds only on plant juices and does not spread malaria. Out of a total of 380 species of anopheles mosquito only 60 can transmit malaria between human hosts and there are four generally recognized species of malaria parasite of humans: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*.^{2, 3}

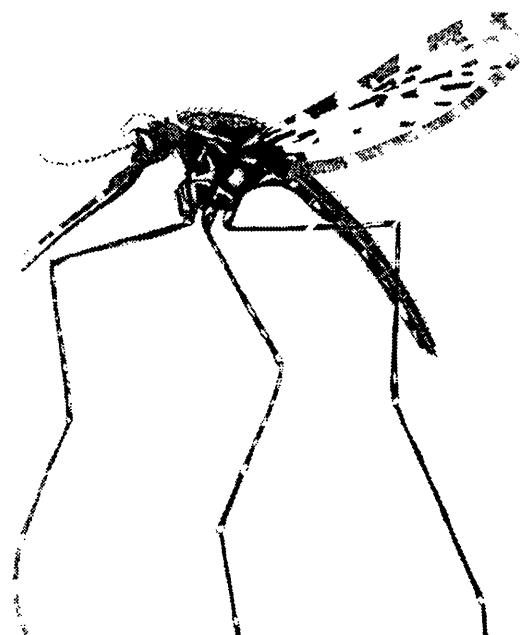


Figure 1 *Anopheles gambiae* - one of the most important vectors for the malaria parasite.

P. falciparum is by far the most lethal form of the parasite and accounts for the majority of fatalities. It is confined to the tropics and sub-tropics as development of the parasite in the mosquito is retarded at temperatures less than 20°C. It kills more people in Africa alone than anything else.

P. vivax was once common in temperate countries, e.g. South England, but it is now found only in the sub-tropics. This leads to a form of malaria where relapses can occur months after initial infection. In some cases there are no symptoms associated with the primary infection and the first clinical symptoms are seen months after initial infection.

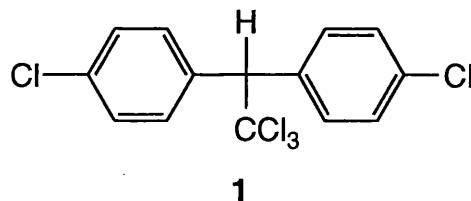
P. malariae is now relatively uncommon since its eradication from temperate climates. This parasite develops relatively slowly in both mosquito and human host, and has long persistence even though the level of parasitaemia is often low. The main threat is the recrudescences which can occur up to 50 years later!

P. ovale is now found only in West Africa and is a rare form of the disease. It is similar to *P. vivax*, but with less chance of relapses and milder symptoms.

Malaria can also be transmitted by blood transfusions, contaminated needles and syringes and very occasionally from mother to baby during pregnancy.

1.4 Prevalence of Malaria

Systematic control of the disease started after the discoveries of Laveran and Ross and led to the idea of total eradication. This gained popularity after World War II when Malaria epidemics had ravaged areas of Southern Europe and the insecticide DDT (**1**) had proven effective in controlling them. Inexpensive and readily available chloroquine-based drugs were also synthesised in this period (**1.7**).^{4, 5}



1955-1969 saw the launch of the WHO Global Eradication Programme aimed at spraying the inside of homes with this insecticide and led to success in large areas of North America, Southern Europe, the former Soviet Union and some areas of Asia and South America. Unfortunately results often varied and in most Asian countries, Latin America and Africa eradication on such a scale was never attempted. Epidemics occurred, notably in Sri Lanka in 1968 where malaria was thought to be eradicated, and the hope for global eradication was abandoned in 1969.³

Control is becoming more difficult as the spread of the disease is linked to activities such as road building, mining, new agricultural projects and logging particularly in regions such as the Amazon. Conditions where the mosquito eggs are laid such as swampy ground should be avoided. In other regions deteriorating social conditions, economic and political pressures lead to migration from non-endemic areas into endemic areas and the loss of health services only worsen the problem.

A Global Malaria Control Strategy was adopted in 1992 by the Ministerial Conference on Malaria in Amsterdam and its plans were updated in 1995.⁴ The overall goal is: "*the prevention of mortality and the reduction of morbidity and social and economic loss due to malaria, through the progressive improvement and strengthening of local and national capabilities for malaria control at national, district and community levels.*"

Within this strategy two main objectives are listed as: "*that by the year 1997 at least 90 % of the countries affected by malaria implement appropriate malaria control programmes.*"

"that by the year 2000 malaria mortality has been reduced by at least 20 % compared to 1995 in at least 75 % of affected countries."

Nowadays malaria is a health problem in 91 countries inhabited by 40 % of the world's population. It kills one person every 12 seconds, mostly children under five years old. Eighty per cent of cases occur in tropical Africa and it is estimated that a bout of malaria costs the equivalent of ten working days-an estimated total of \$1,800 million in 1995.

1.5 Life Cycle of the Malaria Parasite in the Human Host

The malaria parasite is a protozoum (Gr: prot - primitive; zōon - animal) which is a single celled animal. Despite this apparent simplicity the parasite has a complicated life cycle composed of three stages (**Figure 2**).^{2, 6}

1.5.1 The Liver: The Hiding Place of the Parasite

Whilst probing for blood the female mosquito releases, into the human skin, saliva containing sporozoites (Gr: sporá - sowing) which quickly find their way to the liver and invade some liver cells. The cell nucleus divides many times until it forms one large schizont (Gr: schizein - to divide; on, ontos - being) containing thousands of new parasites. Increased pressure in the cell causes it to burst and release all the new parasites, merozoites (Gr: meros - part), which leave the liver and enter the circulating blood stream, where red blood cells rich in nutrients wait. This stage, known as the primary liver stage, usually takes 1-2 weeks, and the human host is unaware there is any infection as no symptoms are associated with this stage of the disease.

1.5.2 The Red Blood Cell: Erythrocytic Stage

The released merozoites must survive this brief extracellular situation until they invade the circulating red blood cells where they feed. They digest the host cytoplasm, i.e. haemoglobin, and grow into ring shaped trophozoites (Gr: trophe - nourishment) which eventually enlarge and lose the vacuolar appearance until they fill the whole cell. A cluster of 8-24 new parasites form and, as before, the cell bursts under increased pressure releasing them into the blood stream. This is the asexual stage in the parasites' life cycle and it

repeats every 2-3 days, depending on the species, resulting in an ever increasing number of parasites in the human's blood stream.

1.5.3 The Mosquito: Sexual Life of a Parasite

Some merozoites grow in the red blood cell without dividing and transform into sexual cells called male and female gametocytes (Gr: gameté - wife; gamétes - husband; kútos - cell) which are necessary for perpetuation of the parasite. These cells can only mature outside the human body and leave with the help of the mosquito as it takes a blood meal. Evolution has come up with a remarkable way of helping both parasite and mosquito - the parasite needs a host to reproduce and the mosquito needs protein from the blood to lay her eggs. Mature gametocytes are taken into the mid gut of the mosquito where they lose the red cell membrane, undergo gametogenesis, and are ready for fertilisation. Fertilisation occurs quickly and the nuclei fuse to form a zygote which grows and eventually moves to the outside wall of the mosquito's gut and secretes a cyst wall around itself. This oocyst grows and gives rise to millions of new sporozoites which migrate to the salivary gland and the cycle starts again.

1.6 Do you have Malaria?

It is the parasites in the blood which cause the illness associated with malaria and the severity of the attack is usually proportional to the number of red blood cells destroyed by the parasites. Symptoms occur 10-16 days after infection and the classical course involves shaking chills which are followed by high fevers, sweating and muscular pain. This similarity with 'flu often leads to mis-diagnosis and is a particular problem with travellers returning home from endemic regions. The spleen becomes enlarged and tender and anaemia develops as red cells are destroyed. This is especially dangerous in children and pregnant women. Other side effects may include jaundice, renal dysfunction and hypoglycaemia among others. The cycle of fever symptoms are associated with the asexual erythrocytic stage in the life cycle of the parasite which releases an estimated quarter of a billion parasites into the blood stream every 1-2 days (**Figure 3**).^{2, 4, 6}

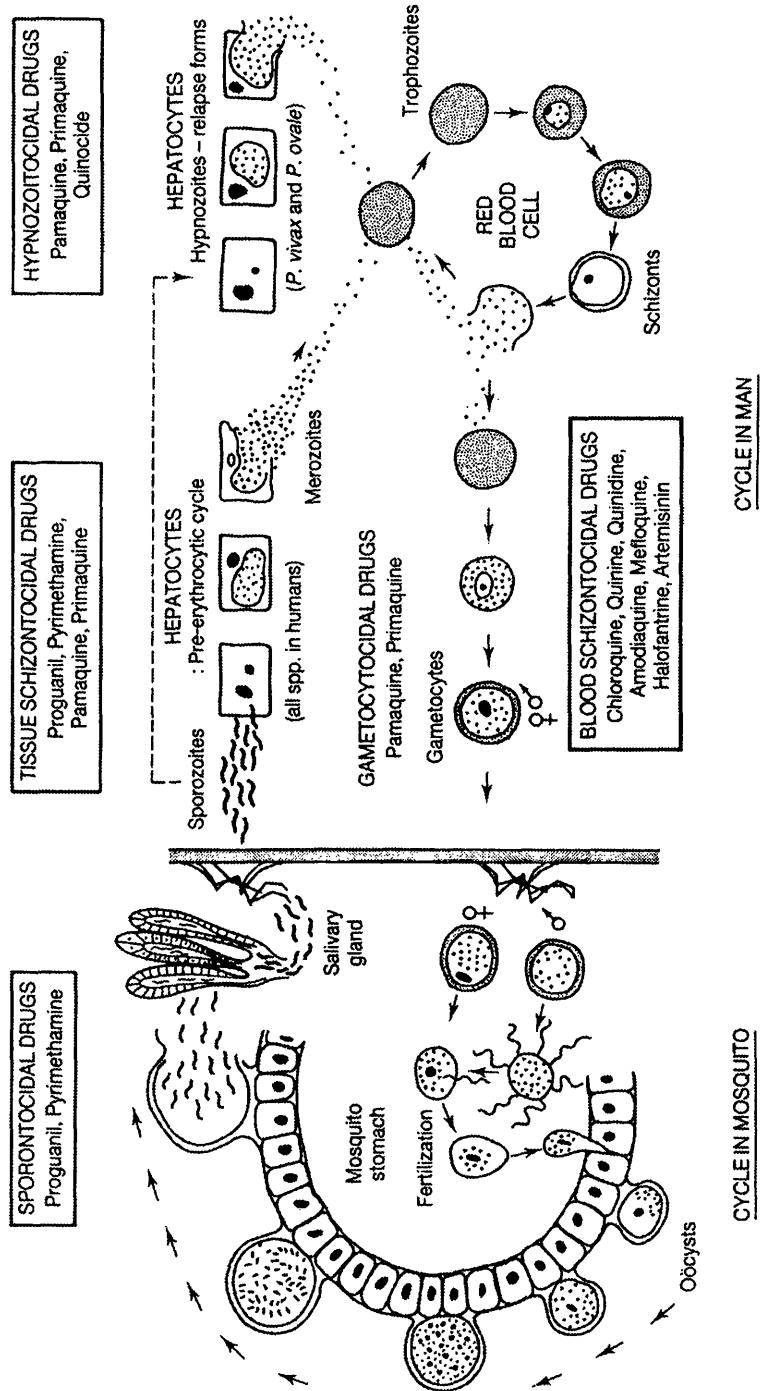


Figure 2 Life cycle of malaria parasite in the *Anopheles* mosquito and human host, including the site of action of antimalarial drugs.³

In *P. falciparum* parasitised cells can block vessels leading to haemorrhages and when they invade the brain they can lead to convulsions and cerebral malaria which is often fatal. Most of the deaths are in children less than 5 years old.

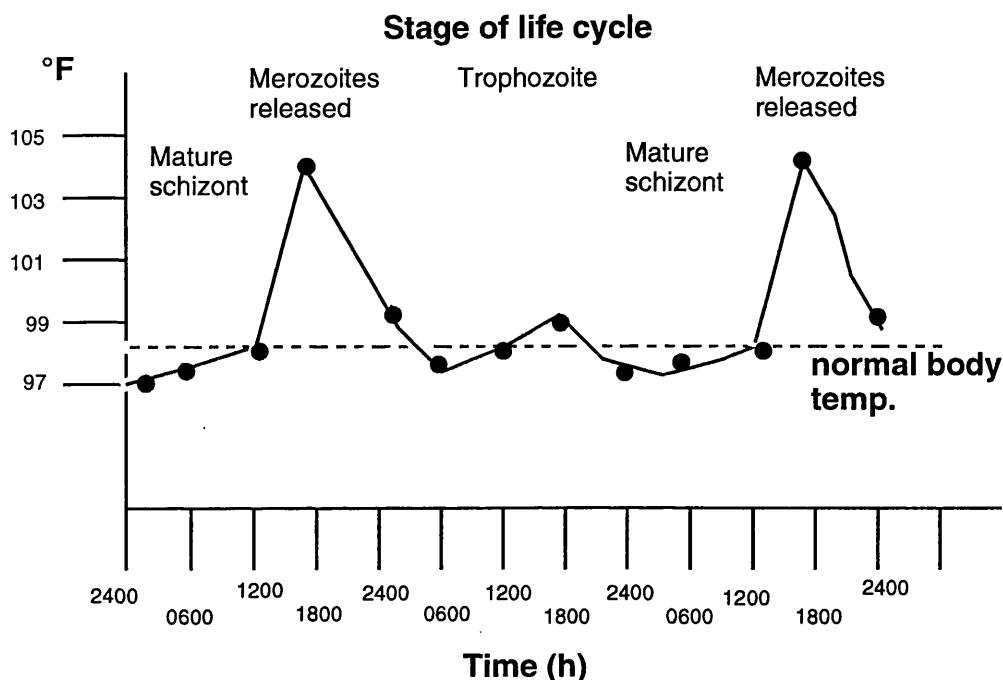


Figure 3 Temperature chart of a patient with *P. vivax*.²

However if humans survive they may build up immunity and many adults who live in endemic areas no longer show symptoms of the disease. Unfortunately effective immunity is quickly lost if you stop living in the malarious area. It is *P. falciparum* which accounts for the high mortality rate of this disease due in part to the high reproduction of the asexual stage of the parasite. Other human malarias are rarely fatal. However with *P. vivax* and *P. ovale* parasites can persist in the liver as dormant stages and later can start new cycles of reproduction in the blood. This persistent infection is the source of recurrent fevers and relapses that may occur months or years after the initial infection. *P. falciparum* and *P. malariae* only have a primary liver phase so if a chemotherapeutic regime is followed which eliminates parasites from the blood stream the patient will be cured.

1.7 Coping with Malaria

Malaria is on the move. Areas previously free of this terror are once again in the front line. Natural immunity is very effective but this only builds up as a result of high and prolonged exposure and does not address the problem of high morbidity and mortality in children under 5 years of age, the main risk group in Africa. A number of possible strategies can be considered:

- 1. Chemoprophylaxis**
- 2. Affordable and effective treatment of the disease**
- 3. Influencing the vector**
- 4. Prevention of insect bites.⁷**

Tropical disease has been hit badly in recent years by the pharmaceutical mergers. The fewer companies now competing concentrate on more profitable areas. Indeed the number of companies researching in tropical medicine can be counted on one hand. Spending on cancer research in the UK alone is twice that spent on malaria world-wide.⁵

1.7.1 Chemotherapy - Preventative and Curative

Chemotherapy has traditionally played an important role in the treatment of malaria, with many synthetic and naturally occurring compounds being used to treat infected patients. Prophylactic drugs reduce the incidence of malaria and if the patient does get an attack it is likely to be less severe. In most countries where *P. falciparum* is endemic there is a varying problem of resistance to a large number of well established and often-used drugs. A drug may be put to several uses both curative and prophylactic, with its efficacy determined by factors such as: species of parasite; drug sensitivity; partial immunity of the patient and side effects.^{8, 9, 10}

Anti-malarials are classed according to the stage in the parasites' life cycle which they attack.⁸ Although some have a specific mode of action many can be classed in more than one category (**Figure 2**).

- 1. Tissue schizontocides** inhibit growth of the pre-erythrocytic stages of the parasite in the liver cell. The less toxic members of this family, e.g. proguanil, are used alone or in combination as prophylactics.
- 2. Hypnozoitocides** kill the dormant liver stages, e.g. primaquine, and are used as anti-relapse drugs for *P. vivax* and *P. ovale*. It is only the 8-aminoquinolines which are effective in man.

3. Blood schizontocides act rapidly on the erythrocytic stage and are generally used for treatment, e.g. chloroquine. The less toxic members of this family are also used as prophylactics.

4. Gametocytocides destroy sexual stages of parasite in the blood, e.g. primaquine.

5. Sporontocides inhibit development of the oocysts and therefore the formation of sporozoites in the mosquito, e.g. pyrimethamine and proguanil.

Chloroquine

Chloroquine (**2**) has been the most popular drug for chemotherapy and prophylaxis of malaria since it was introduced in the mid 1940s. It is cheap, safe, widely available and highly effective against all asexual blood stages of the four *Plasmodium* - the classic characteristics of a successful drug. It is used as a prophylactic and also for treatment; and is sold as the diphosphate salt of the racemic mixture. Unfortunately resistance (7.1.2) by *P. falciparum* to chloroquine has increased over the last 30 years and the effectiveness of this drug has diminished. In many areas, especially in Africa, other drugs are now recommended.

Chloroquine is a blood schizontocide and acts on the intraerythrocytic stage of the life cycle. *Plasmodium* multiplies by digesting haemoglobin which then discards haem. Haem is toxic to the parasite and is normally eliminated as haemozoin (an insoluble pigment) by oxidative polymerisation. Chloroquine and related antimalarials interrupt this process in some way to produce a toxic substance which kills the parasite (**Figure 4**).^{11, 12}

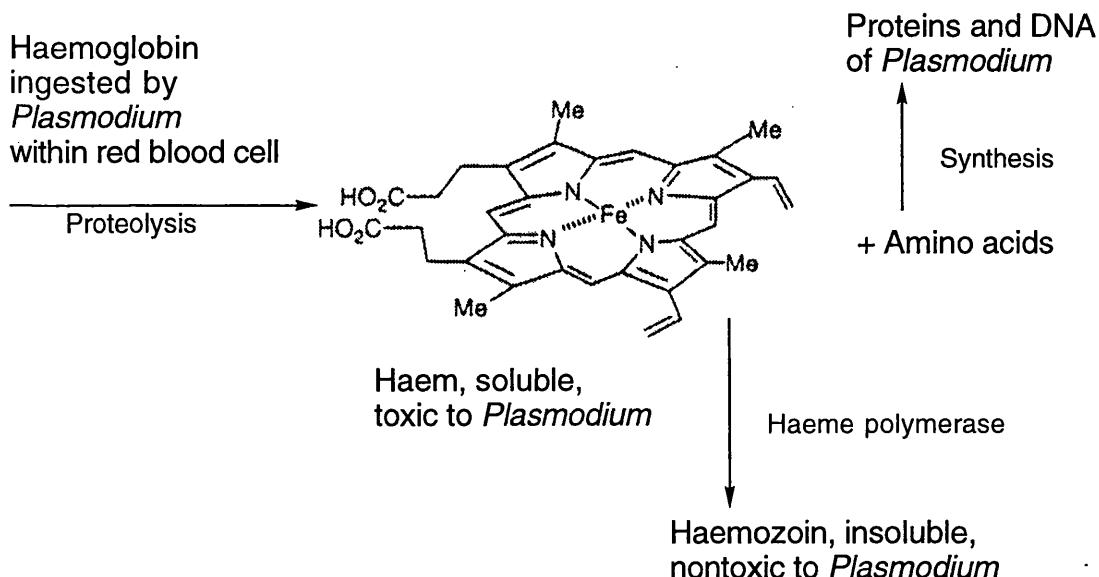
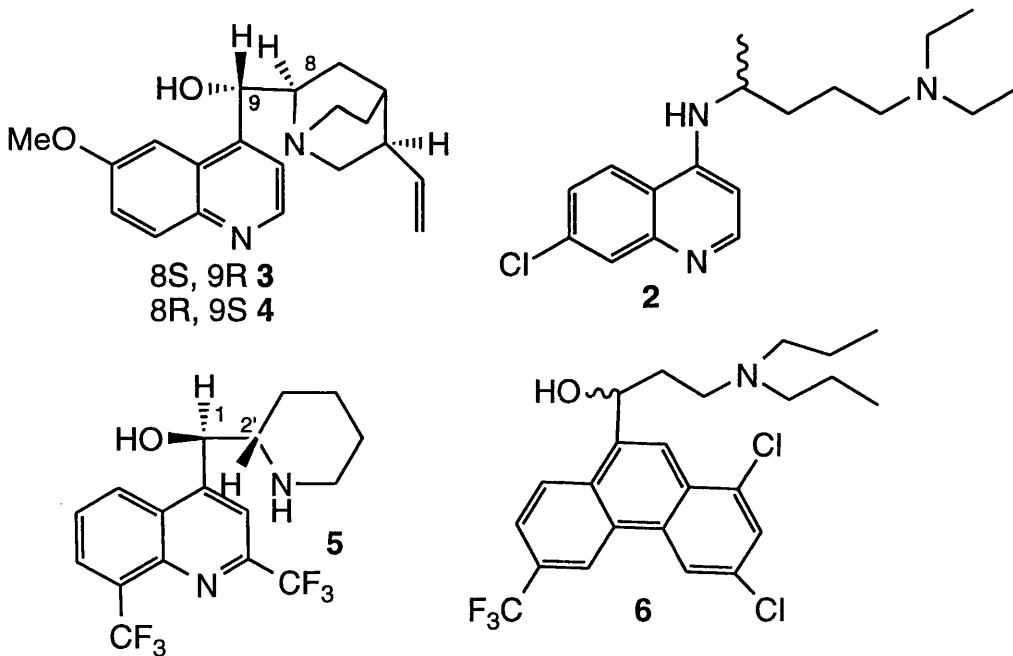


Figure 4 Metabolism of haem by the malaria parasite.¹²

Hoffmann-LaRoche have recently studied chloroquine analogues with shorter side chains that retain their activity against chloroquine resistant *P. falciparum*.¹³

Quinine, Quinidine

The use of quinine (**3**) is well established since its effectiveness was noted by colonials in India taking gin and tonic water, which contains quinine. Both quinine and its diastereoisomer quinidine (**4**) are members of the cinchona alkaloid family, derived from the bark of the cinchona tree of South America and have been used for centuries.¹⁴ Quinine is still regarded as one of the best drugs for termination of acute attacks in non-immune individuals with chloroquine-resistant malaria, but is not used for prophylactic use due to its high toxicity. Quinidine may be superior to quinine for drug resistant infections although both have their use limited due to their cardiotoxicity. Quinine and quinidine are blood schizontocides and inhibit haem polymerase in a manner similar to chloroquine.¹⁵



Mefloquine

Mefloquine (Lariam™) (**5**) was developed by the US Army to prevent chloroquine-resistant malaria encountered during the Vietnam War. Mefloquine is a 4-quinolinemethanol derivative that is extremely well absorbed by the gastrointestinal tract and acts as a blood schizontocide. It is usually sold as the 1RS, 2'SR erythro mixture; however studies have shown that the 1S, 2R is the most potent form. It is active against chloroquine

resistant parasites and is used as a single entity or in combination with other drugs, although for prophylactic use it tends to be used on its own. In recent months controversy has surrounded the use of this drug due to reported undesirable side effects. Hoffman-LaRoche say side effects including psycho-neurotic disorders may occur in 1:10000 cases. However recent evidence suggests figures more like 1:140. WHO tried to restrict the use of mefloquine to treating multiresistant falciparum malaria. However the fact remains this is now the prophylactic drug of choice in a number of areas, e.g. Africa.^{16, 17, 18}

Halofantrine

Halofantrine (**6**) is an orally administered blood schizontocide active against chloroquine-resistant and chloroquine-sensitive malaria, although it may not be effective on *P. falciparum* resistant to mefloquine. It is a phenanthrenemethanol derivative related to mefloquine and quinine and is sold as the racemic mixture of the hydrochloride salt. However it suffers from poor absorption from the gut and high cost as well as possible cardiac toxicity. It has no place as a prophylactic. Its use is limited to areas where resistance to chloroquine and sulfonamide-pyrimethamine combinations is established and hopefully this will extend the useful life of this drug.^{19, 20}

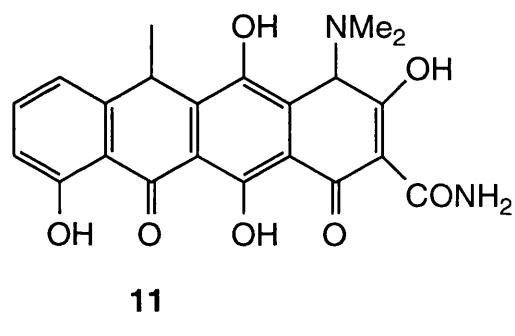
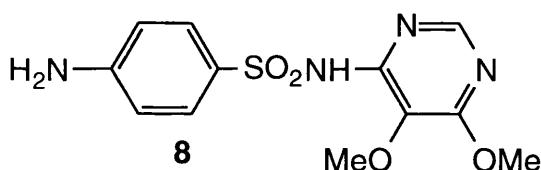
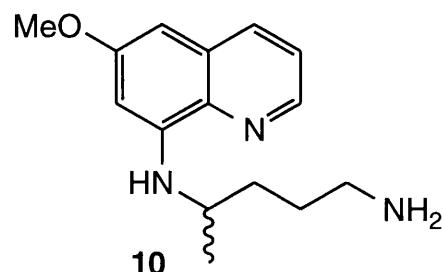
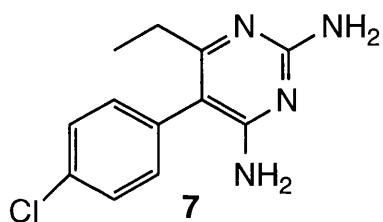
Sulfonamide-pyrimethamine combinations

Combinations of such drugs act at different points along the folate metabolism pathway which increase their effectiveness. Pyrimethamine (**7**) is an inhibitor of dihydrofolate reductase (DHFR), the enzyme which catalyses the reduction of dihydrofolate to tetrahydrofolate. Unfortunately in *P. falciparum* the gene for DHFR mutates leading to resistance. Sulphadoxine (**8**) inhibits dihydropteroate synthase preventing the incorporation of *p*-aminobenzoic acid into a precursor to dihydrofolate. Marketed as Fansidar™ this combination is too slowly-acting to cure acute attacks and is often used intramuscularly with quinine to treat uncomplicated *P.falciparum* cases in chloroquine-resistant areas. It has the benefit of being cheap and is highly effective in much of Africa and Indochina.

Proguanil

Proguanil (Paludrine™) (**9**) is a prodrug and its active metabolite is a triazine, cycloguanil. It is an antifolate which inhibits DHFR. It was developed by the British and Australian scientists during World War II and

when given in proper doses has no side effects. Although it rapidly lost its ability to be useful in treatment, due to resistance, it is still used as a prophylactic. It kills the liver stages of the parasite and therefore prevents blood infection from occurring and also acts as a slow schizontocide. Proguanil now tends to be used in combination with chloroquine for prophylaxis.²¹



Primaquine

Primaquine (**10**) is an 8-aminoquinoline and is currently the only available drug that kills the latent liver stages of *P. vivax* and *P. ovale*. It is sold as the diphosphate salt as a racemic mixture. It is believed to be converted into an active quinone metabolite in the liver and is also an active gametocytocidal drug for *P. falciparum*.²²

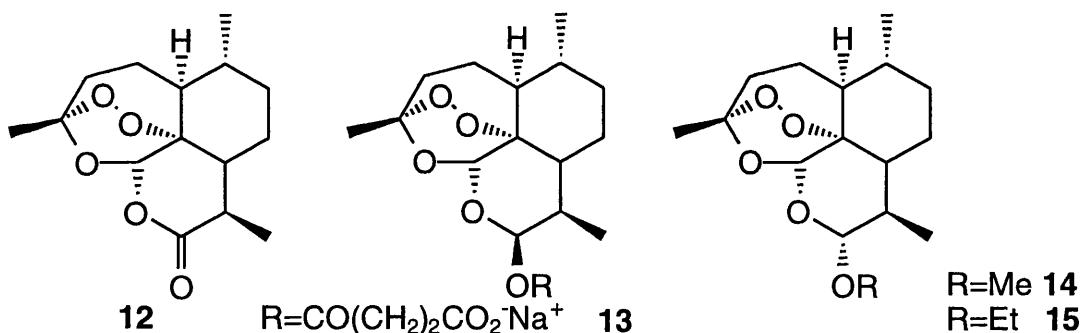
Antibiotics

Tetracyclines were investigated in the 1950s as potential antimalarial drugs with little success, but due to the resistance to some drugs their use was reappraised. They are slow blood schizontocides and are administered with a faster acting blood schizontocide, e.g. quinine. Doxycycline (**11**) is used as a prophylactic in S. E. Asia. However it does have side effects including sun-sensitisation. It does not eliminate the hypnozoite phases of relapsing malaria and primaquine must be given to eradicate this.

Artemisinin (Qinghaosu)

Artemisinin (**12**) is a 15-carbon lactone peroxide, which is naturally occurring, and structurally unrelated to all other antimalarials. It was isolated in 1972 from *Artemisia annua*, a plant used traditionally by the Chinese since 340 AD for the treatment of fever.

Artemisinin shows no cross resistance with known anti-malarials and is used for treating severe malaria although it does need long treatment courses. It is available as the parent compound and also three semi-synthetic derivatives: hemisuccinate salt (**13**) (water soluble), crystalline 10 β -artemether (**14**) (oil soluble) and crystalline 10 β -arteether (**15**) (10 α is an oil). All three are blood schizontocides and are metabolised to dihydroartemisinin which is believed to be an active metabolite. These derivatives have greater potency and stability but are more expensive. Artemisinin is used as a suppository, artemether is introduced by intramuscular injection and artesunate (**13**) is used both intramuscularly and intravenously. None of these compounds are licensed in Europe or the USA although there is intense interest in this area.^{12, 23}



1.7.2 Drug Resistance

Drug resistance is a major problem. It is not restricted to chloroquine but has been described for inhibitors of folate biosynthesis and more recently other important antimalarials including quinine, mefloquine and amodiaquine. Cross resistance within all these aminoquinolines is particularly worrying as the world depends on them for prophylaxis and treatment.

There are at least two possible mechanisms of drug resistance in malaria. First, resistance to inhibitors of folate metabolism which results from specific point mutations of the active site on the dihydroreductase enzyme. This resistance has spread independently and arose quickly after the introduction of such drugs. The second form is resistance to chloroquine

which is likely to involve more than one gene. It is known that chloroquine acts by inhibiting haem polymerase (1.7.1) and it is suggested that the mechanism of resistance is not associated with this, but rather alterations in drug transport.^{24, 25, 26}

As a weak base chloroquine accumulates rapidly in the acid food vacuole of the parasite and it appears that in chloroquine-resistant parasites less drug is accumulated and/or there is an increase in efflux of the drug out of the parasite. Verapamil, a calcium channel blocker, has been shown to reverse chloroquine resistance although quite how this works is not fully understood. One thing for sure is resistance to aminoquinolines is increasing and elucidating the mechanism of action is high on many biologists' agendas.²⁷

1.7.3 Vaccines - Present and Future Prospects.

The issue of vaccine development is extremely wide ranging and complex and as such this thesis is not the place for a review; however a number of the key issues will be covered, in brief.

Residents of malaria endemic areas acquire protective immunity to malaria and the number of clinical episodes declines with increasing age. The idea is that a vaccine could mimic this process and in the 1960s the British scientists Sydney Cohen and Sir Ian McGregor discovered that antibodies from such individuals could reduce parasitaemia and clear the symptoms. Each stage of the life cycle has different antigens that lead to protective immunity. This area is extremely exciting and is generating a lot of financial support. Any vaccine must be easily incorporated into the health programme and give immunity of sufficient duration.²⁸

One of the first approaches was injecting sporozoites from irradiated mosquitos into human volunteers. However this required long exposure times and was impractical. Instead sporozoite proteins were targeted hoping they would reproduce the effect of the whole sporozoite. In the 1980s a gene coding for a sporozoite protein was cloned although vaccines based on this failed in field trials. Pre-erythrocytic vaccines target the sporozoites or the forms in the liver cells. This is ideal as they remain in the cells for several days, although this approach has many problems with the actual vaccine design. All sporozoite vaccines which have undergone trials have had poor results although the latest offering from Smith Kline Beecham and the US Army is exciting as they are given together with compounds that stimulate the immune system.⁵

Anti-aseexual vaccines target antigens expressed on the surface of the red blood cell by the parasites inside. One of the most studied vaccines is based on a surface protein of merozoites and is promising as the protein sequence has no variations. Hopes for a vaccine in this area were raised by Manuel Patarroyo, a Colombian biochemist with trials of SPf66. Design of his trials has been criticised. There have been contradictory results including one that shows that the vaccine has no effect. Trials are still continuing.

Transmission-blocking vaccines interrupt development of the gametes in the mosquito and are aimed at reducing transmission rather than protecting individuals. A yeast-derived polypeptide is likely to undergo trials in the near future although in the long term it will probably be used together with erythrocytic or pre-erythrocytic stage vaccines.

One of the major controversies in the use of vaccines is that decreased transmission is likely to lead to decreasing natural immunity and any breakdown in the vaccines would lead the way for massive epidemics. Malaria parasites are also diverse and can evolve rapidly, especially if the vaccine is based on a single antigenic peptide.

1.7.4 Transgenic Mosquitos

Rather than eradication of the mosquito which has been proven in the past to be nearly impossible, why not influence the vector directly? The idea is to construct a transgenic which is incapable of transmitting the disease and as such can spread natural resistant genes through the wild population. Such genes are currently being studied and a number of scientists are collaborating to map the mosquito genome.

Another approach is to engineer the insects so that they carry foreign genes that confer protection against infection. One researcher at the Liverpool School of Tropical Medicine has cloned a mammalian antibody that attacks an antigen during the ookinete phase of the parasite in the mosquito. Researchers are optimistic that genetically engineered mosquitos will be released into the wild and will spread rapidly through the population.^{5, 29}

1.7.5 Prevention of the Bite.

For many years people have known the benefits of protecting themselves from the mosquito. Amongst the most effective methods are the use of window and door screens made of small mesh and the mosquito bed-net. The netting should be stiff cotton to allow movement and rectangular to

prevent the skin touching it. The *Anopheles* attack between dusk and dawn so adequate prevention is essential when you are asleep. Pyrethrin insecticides kill mosquitos instantly so soaking your bed-net and spraying the room with an appropriate solution also helps. Studies have shown this to reduce childhood mortality by 15-35%. Some scientists argue that the use of such methods will reduce the natural immunity of the population and lead to an eventual increase in mortality.⁴

The most basic precautions such as mosquito repellent will also help. Natural oils such as citronella and eucalyptus have been used; however these are short lived and have been replaced by synthetic compounds. *N,N*-Diethyl-*m*-toluamide (DEET) is one of the newer compounds that is an excellent repellent and is active for 10 hours. When pitching a tent choose an appropriate place away from possible breeding places, e.g. not near damp swampy areas. Wearing beige and yellow long-sleeved clothes and trousers after dark is highly effective but the scent of perfume attracts the mosquito.³

Different strategies are required for different situations, e.g. urban areas are not as high a risk as the African savanna. Altogether a number of simple and cheap options are available to help prevent "the bite". Control depends on elimination of the insect vector by destruction of its breeding areas, killing the larval stages and the adult mosquitos. This is not easy. What is now needed is political will and well-managed resources both at international level and also within the small communities where the risk is highest.

1.8 Summary

Malaria is obviously an extremely complex and problematic disease and for thousands of years people have treated it without knowing exactly what it was. Synthetic compounds and natural remedies have been used and in today's age of genetic engineering transgenic mosquitos are also a possibility to control this disease. Polyamines have been investigated as therapy for a number of diseases, including malaria. In the next chapter aspects of polyamines will be discussed including their biosynthesis and their many chemotherapeutic uses.

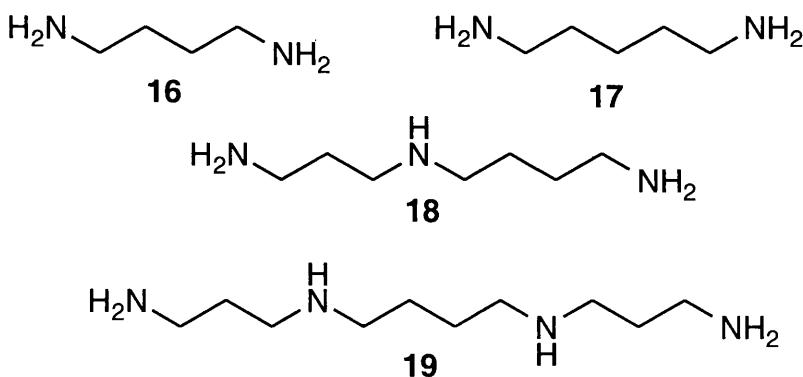
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2 Polyamines

2.1 Introduction

How can compounds so ubiquitous in nature merit so little attention? This was definitely true until the 1960s when research in this challenging area took off and an explosion of literature began to be published.

In 1677 Anton von Leeuwenhoek first observed an unknown crystalline substance, under his microscope, in a sample of human semen. It took a further 250 years to identify it as spermine phosphate and shortly afterwards spermidine was also identified. Putrescine (**16**) and cadaverine (**17**) were discovered in the carcasses of rotting animals and that completed this group of simple aliphatic structures.³⁰

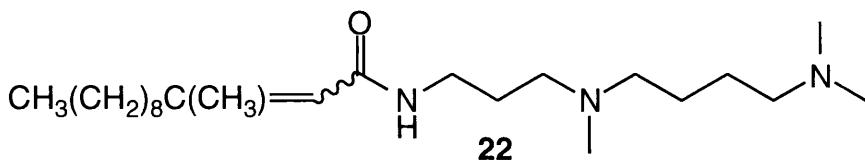
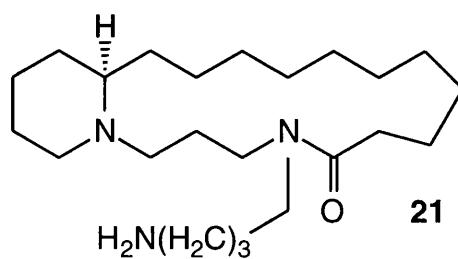
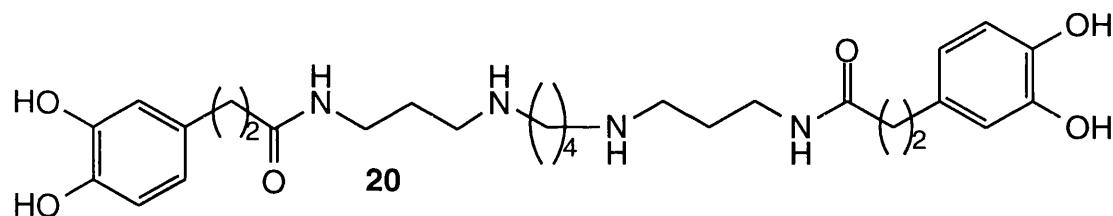


Putrescine, spemidine (**18**) and spermine (**19**) are all found in eukaryotic organisms and have been found to be essential for cell growth and differentiation. It is their importance in such fundamental processes that stimulated research in this area.^{31, 32} The pattern of changes of polyamine content in the cell were related to proliferative processes with a correlation between polyamines, protein synthesis and RNA content. Differentiation can be induced in certain systems by inhibition of polyamine biosynthesis;

spermidine is an essential requirement for milk protein synthesis, a differentiated function.³³

Conformationally mobile polyamines can associate with polyanionic molecules such as DNA, RNA and phospholipids.^{30, 34} This interaction can stabilise polynucleotides against denaturation and can also exert a strong influence on the secondary and tertiary structure of such molecules. Direct *in vitro* effects of polyamines have been studied and they can affect DNA, RNA and thus protein synthesis. The binding is electrostatic although some scientists believe that the polyamines interact in the minor groove of DNA by hydrogen bonding with the edges of the base pairs rather than the phosphate backbone (**2.7**).³⁵

It is worth noting that not only do polyamines exist as these small simple compounds but they may often be found conjugated to sugars, phospholipids, peptides and plant alkaloids. This provides an array of compounds with wide-ranging uses and a staggering variety of complex carbon-nitrogen backbones. Kukoamine A (**20**) is a spermine derivative which exerts a powerful antihypertensive activity and oncinotide (**21**) is a macrocyclic lactam with a spermidine backbone. Cytotoxic agent (**22**) was extracted from coral reef *Sinularia bronngersmai* in 1979 and was the first spermidine metabolite discovered in a marine organism.³⁰



2.2 Polyamines: Formation and Degradation

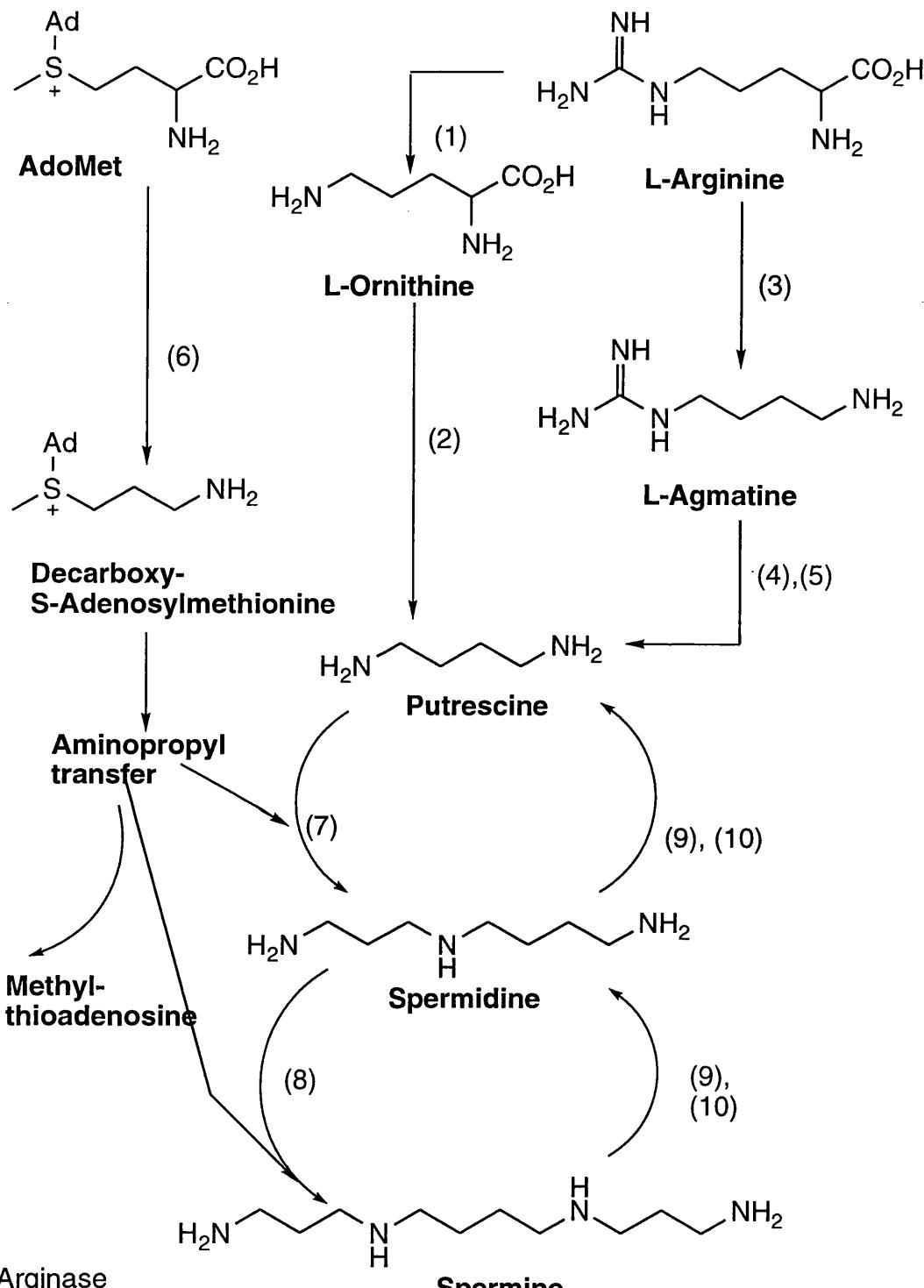
2.2.1 Biosynthesis

Polyamines are distinct from metal ions whose circulation depends on diffusion or transport to pass through cell membranes. Polyamine levels are controlled carefully and precisely according to cell needs. Collective effects from the biosynthetic pathway, a catabolic pathway and an uptake mechanism combine to keep the intracellular levels of these compounds within fairly tight restraints.^{36, 37}

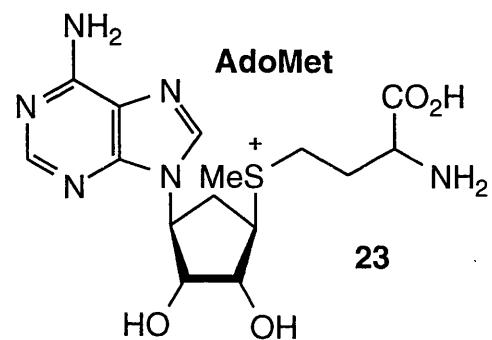
In mammalian cells, protozoa, and fungi, the first step in polyamine biosynthesis is decarboxylation of ornithine to putrescine, using an enzyme, ornithine decarboxylase (ODC).³⁹ Higher polyamines such as spermidine and spermine are formed by the successive addition of aminopropyl groups and this is catalysed by the aminopropyl transferases. *S*-Adenosylmethionine (**23**) (AdoMet) is converted into decarboxylated *S*-adenosylmethionine, which contains the desired three carbon chain, by the enzyme *S*-adenosylmethionine decarboxylase (AdoMetDC) and the aminopropyl group is then transferred.^{31, 32, 34, 38} The other product of the aminopropyl transferase reaction is 5'-methylthioadenosine (MTA) produced in stoichiometric amounts with the polyamines. It is rapidly degraded to adenine and 5'-methylthioribose-1-phosphate by a phosphorylase enzyme. 5'-Methylthioribose-1-phosphate is converted back into methionine and adenine goes to the nucleotide pool (**Scheme 1**).³³

In plants and some fungi putrescine can also be formed indirectly from arginine. This route utilises arginine decarboxylase (ADC) to give agmatine which is converted into *N*-carbamoylputrescine by agmatine iminohydrolase.⁴⁰ The last stage is conversion into putrescine via the enzyme *N*-carbamoylputrescine amidohydrolase. The simple diamine cadaverine is formed by decarboxylation of lysine which in plants is catalysed by lysine decarboxylase, but in animal cells ODC is used, albeit inefficiently.^{34, 38}

ODC is an enzyme with a known turnover, $t_{1/2}$ 15-20 min, which is the shortest $t_{1/2}$ of any mammalian enzyme studied so far.³³ All ODC enzymes identified require pyridoxal-5'-phosphate (PLP) for activity and show high selectivity for *L*-ornithine as a substrate.⁴¹ PLP is the co-factor for ODC and probably sits in a "pocket" of the enzyme where ornithine can approach and be decarboxylated.

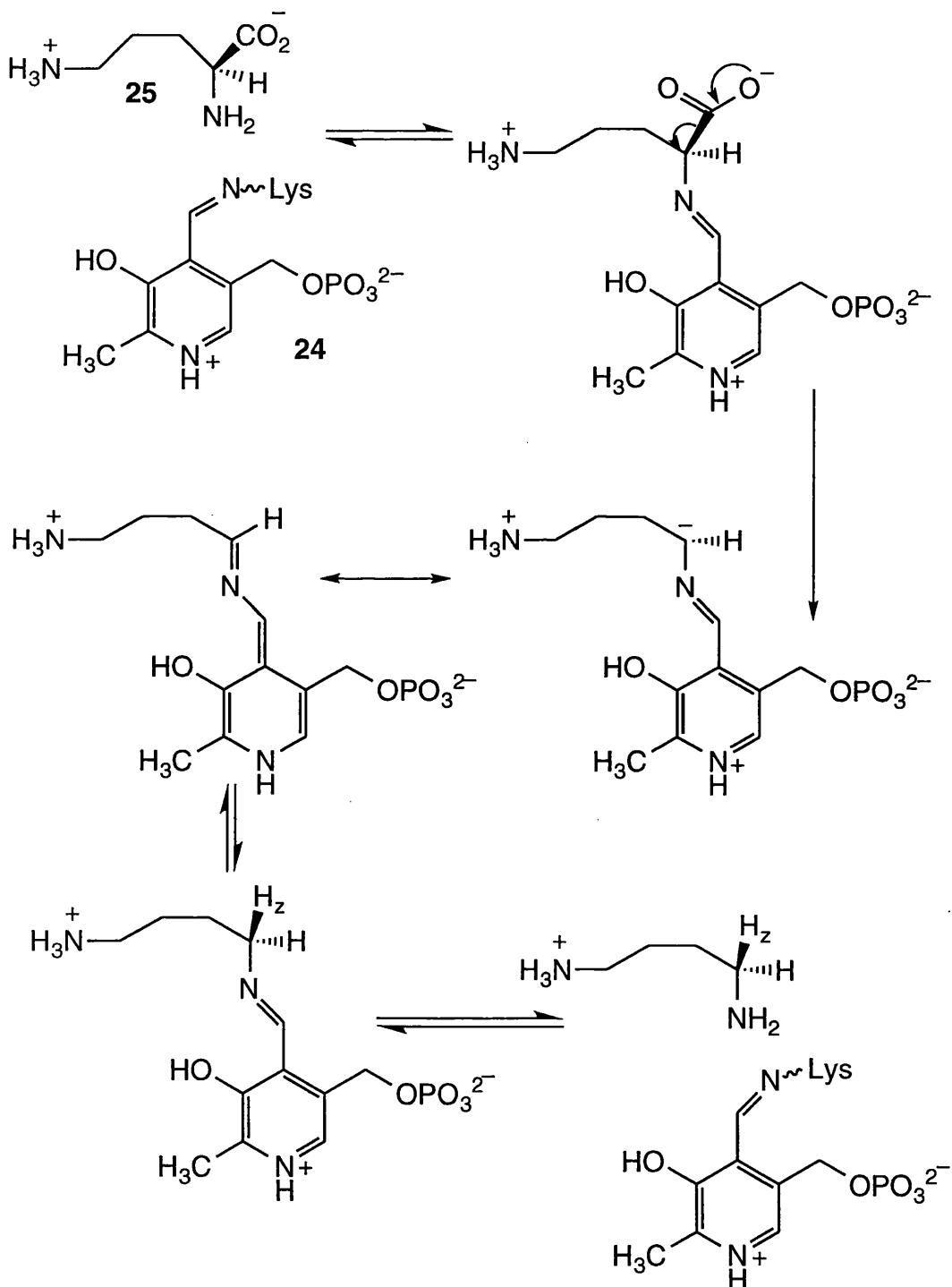


- (1) Arginase
- (2) Ornithine decarboxylase (ODC)
- (3) Arginine decarboxylase (ADC)
- (4) Agmatine iminohydrolase
- (5) N-Carbamoylputrescine amidohydrolase
- (6) S-Adenosylmethionine decarboxylase (AdoMetDC)
- (7) Spermidine synthase
- (8) Spermine synthase
- (9) Polyamine acetylase
- (10) Polyamine oxidase



Scheme 1 Biosynthesis and catabolism of polyamines

It is worth noting that PLP cofactor decarboxylates ornithine with retention of configuration, i.e. the new hydrogen atom H_z has the same stereochemical orientation as the leaving carboxyl group. PLP is shown here linked to a lysine residue of the apoenzyme (24).

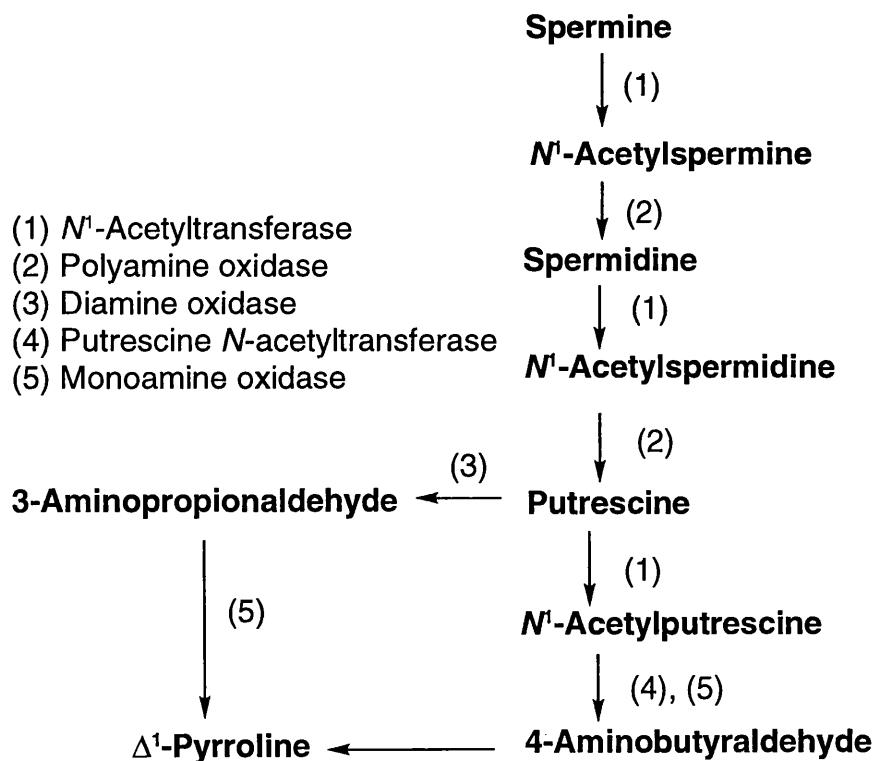


Scheme 2 Decarboxylation of ornithine to putrescine

As soon as a substrate appears the lysine detaches and leaves an aldehyde which quickly forms a Schiff base adduct with the α -amino group of ornithine (**25**) and the carboxyl group leaves in an irreversible reaction. The intermediate is stabilised by conjugation and protonation occurs to form an imine which hydrolyses to the product putrescine. The original PLP-lysine apoenzyme is regenerated (**Scheme 2**).^{32, 38} Mammalian AdoMetDC has a pyruvate residue covalently linked to the enzyme as a prosthetic group.³³

2.2.2 Catabolism

The main enzymes critical to the breakdown of polyamines in mammalian cells are N^1 -acetyltransferases and polyamine oxidases. Spermine and spermidine are converted into N^1 -acetylspermine and N^1 -acetylspermidine respectively by spermine/spermidine- N^1 -acetyl transferase (SSAT) in the presence of acetyl-CoA and the products are excellent substrates for polyamine oxidase (PAO). The PAO breaks down the substrates into N -acetylpropionaldehyde and spermidine or putrescine depending on the substrate. Putrescine is degraded in two ways: oxidative deamination catalysed by PAO or via N -acetylputrescine. The final product is γ -aminobutyraldehyde which is cyclised to Δ^1 -pyrroline (**Scheme 3**).³³



Scheme 3 Catabolism of polyamines

2.2.3 Polyamine Transport Apparatus

If the biosynthetic pathway to polyamines breaks down or is inhibited by chemotherapy how will the cell sustain normal growth? The cell has a mechanism it can start up which will ensure polyamine pools are maintained by scavenging exogenous polyamines and bringing them into the cell. To avoid toxicity it is also possible to down-regulate this mechanism in response to a flood of extra polyamines.³⁶

The mammalian transport system is believed to be protein in nature and highly specific for polyamines and it can be affected by a variety of agents including protein kinase C inhibitors. As well as moving polyamines the system can also carry synthetic compounds such as the herbicide paraquat and polyamine analogues. It has also been utilised to transport established therapeutic moieties into the cell where their site of action is and hence increase their potency (2.5.3).

Recent work on regulation of the polyamine transport system has shown that lowering polyamine pools through biosynthesis inhibition will lead to an increase in polyamine uptake. This occurs rather slowly and in conjunction with polyamine depletion. Exogeneously supplied polyamines and/or polyamine analogues are capable of down-regulating the transport system. In contrast to the above response this is very sensitive and occurs rapidly resulting in only minor changes in the intracellular polyamine pools.³⁶

2.3 Polyamine Inhibitors

The ubiquitous occurrence of polyamines and their association with nucleic acids heralded a considerable amount of research into specific inhibition of polyamine biosynthetic enzymes. A decrease in the polyamine pool causes a response from the two main controlling enzymes in the biosynthetic pathway: ODC and AdoMetDC. A brief consideration of early developments in this field will be considered, followed by advances made in individual therapeutic areas.

2.3.1 Ornithine Decarboxylase (ODC) Inhibitors

ODC is one of the most highly regulated enzymes in eukaryotic organisms.³⁴ The first paper dealing with ODC inhibition appeared in 1972⁴²

and since then three main approaches for the development of inhibitors have been considered.

1. Synthesis of ornithine and putrescine analogues as competitive inhibitors.

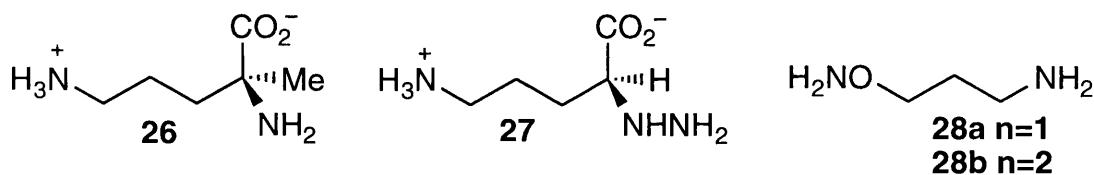
2. Synthesis of compounds capable of interacting with the PLP co-factor.

3. Design of enzyme activated inhibitors.

The first two approaches are concerned with competitive/reversible inhibition of the enzyme and in the third approach irreversible inhibitors are designed.³⁸

1. Analogues of ornithine and putrescine.

α -Methylornithine (**26**) was one of the first reversible inhibitors reported. Decarboxylation occurred 6000 times slower than ornithine and a gradual decrease in activity of ODC was observed.^{43, 44} (*E*)-Dehydropatrescine analogues, α -hydrazinoornithine (**27**) and 1-aminoxy-4-aminobutane (**28a**) all had inhibitory effects but were not potent enough to decrease intracellular spemidine significantly. These compounds often had problems with selectivity and increased the $t_{1/2}$ of ODC when tested *in vivo*.^{31, 34, 45}



2. Reversible inhibitors that interact with PLP.

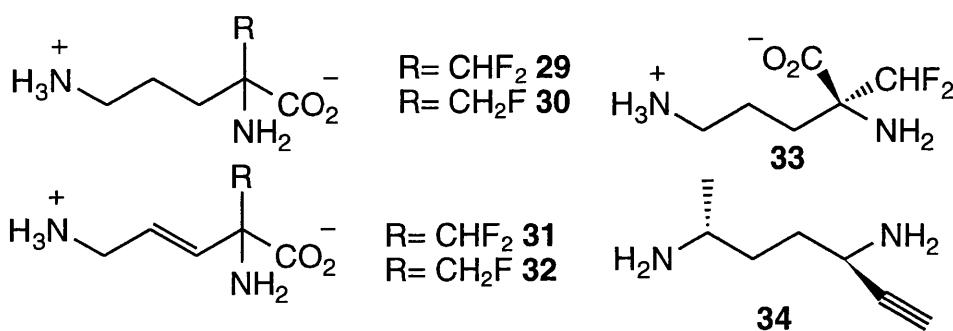
The first step of interaction with the PLP co-factor is formation of the Schiff base. A variety of substrates could be envisaged that would replace the natural substrate and form a stable adduct with PLP hence inhibiting the enzyme. It is thought that the inhibitory effects of such compounds occur as they mimic transition states along the reaction pathway. 1-Aminoxy-3-aminopropane (**28b**) was shown to be an extremely potent inhibitor of mouse kidney ODC and rat liver ODC; however it also inactivated AdoMetDC and spemidine synthase showing a lack of the desired specificity. More recent work in this area by Khomutov *et al.* has looked at more analogues of (**28**).³² α -Hydrazinoornithine as described above also had the same problems with selectivity.^{46, 47}

A main problem with reversible inhibitors is lack of activity *in vivo* compared to promising *in vitro* results. They also appear to increase levels of ODC. When the inhibitor is metabolised and its concentration drops the enzyme is reactivated and putrescine production goes through the roof. The focus turned towards irreversible inhibitors.

3. Irreversible inhibition of ODC.

This area involves the design of a chemically inert pseudo-substrate that contains a functional group that will eventually be transformed to leave a species that inactivates the enzyme. PLP enzymes are good targets for this approach and should lead to selectivity *in vivo*. A huge amount of manpower was spent working in this area by scientists at Merrell Dow.^{43, 48} This led to the development of α -difluoromethylornithine (DFMO) (**29**) which became a drug tested on almost every disease. The chemotherapeutic uses of DFMO will be discussed in detail later (**2.6**). DFMO acts by binding to the enzyme and then decarboxylation occurs. The enzyme is inactivated by alkylation of a postulated nucleophilic residue at or close to the active site.³³ Both enantiomers of DFMO have been shown to inhibit mammalian ODC, although (-)-DFMO (**33**) is the more active enantiomer.⁴⁹

Although DFMO is nontoxic it needs to be given in large doses and more longer acting and potent inhibitors were sought. Numerous ornithine and putrescine analogues were prepared. To summarise a lot of work in this area, for ornithine analogues an increase in the size of the leaving group on the α -methyl substituent reduced activity and the most active analogues contained an α -fluorinated methyl or acetylenic group. The vinylic analogue was inactive *in vitro*. Introduction of a 3,4-*trans*-double bond increased the activity of DFMO and α -fluoromethylornithine (MFMO) (**30**) and this led to two new compounds (*E*)-3,4-dehydro-(α -difluoromethyl)-ornithine (**31**) and (*E*)-3,4-dehydro-(α -fluoromethyl)-ornithine (**32**). Similar results were found with comparable putrescine analogues although when tested *in vivo* they were converted into γ -aminobutyric acid (GABA) analogues, hence blocking GABA transaminase, which was undesirable. One problem of such analogues was oxidation of the terminal amino group by amine oxidases. This was overcome by synthesising analogues with δ -methyl substituents such as δ -methylacetylenicputrescine (MAP). MAP is a mixture of four diastereoisomers but studies showed that the *R,R*-isomer (**34**) was responsible for most of the activity.^{31, 50}



In vivo studies showed that none of the new analogues was superior to DFMO but Δ -MFMO when given as its methyl ester (which is inactive *in vitro*) has promising activity against parasites and *R,R*-MAP is also being investigated.

2.3.2 S-Adenosylmethionine Inhibition

Due to the rapid turnover of this enzyme effective inhibition is not a simple task. Almost all inhibitors of AdoMetDC contain a bis(guanylhydrazone) moiety and methylglyoxalbis(guanylhydrazone) MGBG (35) was the first inhibitor of polyamine biosynthesis to be discovered. It is an extremely potent inhibitor, however it causes massive damage to mitochondria and inhibits respiration.³⁴ Substitution at C-4 has a major effect on the inhibition of AdoMetDC with an ethyl substituent producing a more potent analogue (EGBG). **Figure 5** shows the structure-activity relationship for MGBG. The length of the molecule (A) is critical for utilisation of the polyamine carrier system and the aminoguanidine portions (B) are responsible for inhibiting the action of diamine oxidase. These analogues also stimulate ODC, maybe due to stabilisation of the enzyme protein against intracellular degradation. This is a problem with all these types of inhibition as deactivating one enzyme sets off compensatory measures in another.⁵¹

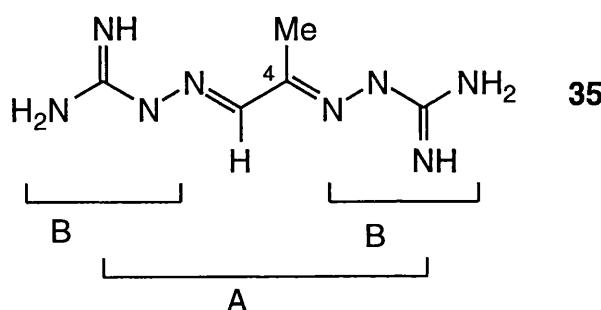


Figure 5 Structure-activity relationships of MGBG

One compound which has proven successful in inhibiting only AdoMetDC is 6-spermyne. Initial activity of both ODC and AdoMetDC decreased over 2 hours then the level of ODC activity recovered to normal levels over 48 hours. The AdoMetDC enzyme remained suppressed, presumably acting through a regulatory mechanism inherent to the biosynthetic pathway. This result suggests that the two enzymes, so closely related, are regulated through two distinct mechanisms.⁵²

2.3.3 Inhibitors of the Spermidine/Spermine *N*¹-Acetyltransferases (SSAT)

Cyclohexylamine is one of the most powerful inhibitors of spermidine synthase in mammals, trypanosomes, plants and some bacteria. Cyclohexylamine is rapidly taken up by mammalian cells and leads to rapid depletion in spermidine levels, although no information is available on its effect on other biosynthetic enzymes. Further investigations led to *S*-adenosyl-1,8-diamino-3-thiooctane being identified as a powerful inhibitor.³⁴ It is extremely effective on spermidine synthase from many sources but has no effect on spermine synthase. The structural requirements for the active site of SSAT have not been examined in detail; however a free aminopropyl is essential for a compound to be a substrate. This is easily proven as spermidine and spermine are substrates whereas *N*¹-acetylspermidine and *N*¹-acetylspermine are not.⁵³ It has also been shown that SSAT has a greater affinity for H₂N(CH₂)₃NH(CH₂)₃NH(CH₂)₃NH₂ (denoted 3,3,3) and H₂N(CH₂)₃NH(CH₂)₄NH(CH₂)₃NH₂ (denoted 3,4,3) carbon chains than H₂N(CH₂)₃NH(CH₂)₄NH₂ (denoted 3,4) or H₂N(CH₂)₃NH(CH₂)₃NH₂ (denoted 3,3).⁵⁴ It is thought that in some cases the ability to superinduce SSAT leads to the growth inhibitory properties of a compound (**2.4**)

2.3.4 Arginine Decarboxylase (ADC) Inhibitors.

Due to the related mechanism of PLP dependent decarboxylases a similar approach to ODC inhibition was used for ADC. A range of α -fluoromethyl analogues of arginine were prepared and were excellent inhibitors of bacterial ADC. One inhibitor, α -difluoromethylarginine (DFMA), was used in studies of putrescine biosynthesis. It was discovered that DFMA is metabolised to DFMO by arginase and it is DFMO that is thought to be the active inhibitor.³⁴

2.4 Polyamines as Potential Anti-fungal Agents

Plants are attacked by a wide range of fungi which cause considerable loss of crops and the economic consequences that go with this. There is constant need for the development of novel fungicides with new modes of action and in today's world they must be environmentally friendly!

In fungi the major polyamine is spermidine with putrescine and spermine present in less than 10% the quantities of spermidine. Little was known about the role of polyamines in pathogenic fungi; however research was sparked off as the realisation dawned that a specific inhibitor of polyamine biosynthesis can lead to powerful fungicidal effects.³⁴

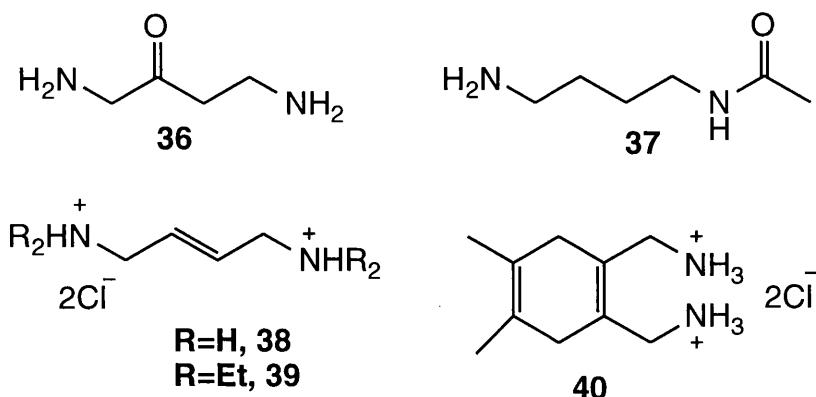
Polyamines varying slightly in structure might be capable of reducing cell growth by the following.

- 1 Inhibition of polyamine biosynthesis.**
- 2. Regulation of polyamine biosynthetic enzymes.**
- 3. Competing for polyamine binding sites.**
- 4. Binding at polyamine sites and therefore disrupting the structure and/or function.**

Initial studies in this area really began with the design of ornithine and putrescine analogues as described previously. In 1985 it was shown that DFMO inhibited several fungi on artificial media and this was extended to the control of rust infection of pinto beans. It was shown to be active if applied before or after inoculation with the fungus and systemic action was also observed. 1,4-Diaminobutan-2-one (**36**) has been shown to control a number of important plant fungi and interestingly *N*-acetylputrescine (**37**) was inactive, probably due to the deacetylases present.⁵⁵

Research workers in our group investigated a number of putrescine analogues as potential fungicidal agents.^{56, 57, 58, 38} Synthetic routes were investigated and a number of unsaturated analogues, both alicyclic and cyclic, were investigated and (**38**), (**39**) and (**40**) were particularly active. (**38**) and (**39**) controlled five important plant pathogens and also powdery mildew on spring barley. (**38**) reduced ODC and AdoMetDC levels; however levels of putrescine increased seven fold, spermine increased 60% and spermidine decreased by only 32%. (**39**) reduced putrescine by 58%, spermidine by 35% and increased ODC. The powerful anti-fungal activity of these analogues is undoubtedly due to their reduction of polyamine pools but their reduction of polyamine pools is not enough to account for their activity. These compounds do increase AdoMet levels and it is speculated that this might account for their activity. The work of Byers *et al.* suggests that the increase in AdoMet levels may account for

the activity of DFMO. The excess of AdoMet may lead to aberrant methylation which would disrupt cell growth.^{34, 59}



More recent work in our group has looked at spermidine analogues and their activity and this will be discussed later.

2.5 Evaluation of Polyamines as Anti-cancer Agents

2.5.1 Introduction

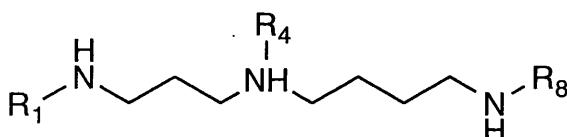
The level of polyamine biosynthesis is very high in transformed and rapidly dividing cells. This is reflected in higher activity of the polyamine enzymes and also increased concentrations of polyamines in extracellular fluids. Urinary excretion of polyamines was greater in patients suffering from leukaemia or lymphoma and in patients with solid tumours when compared to patients with non-malignant diseases.⁵⁹ The association of polyamines with nucleic acids and their accumulation stimulated research into the use of biosynthetic inhibitors to treat proliferative diseases such as cancer.^{33, 60, 61}

2.5.2 Cancer Chemotherapy

Unsurprisingly DFMO and MGBG were among the first inhibitors to be tested both *in vitro* and *in vivo* against tumours and both were found to be effective. MGBG inhibits AdoMetDC and SSAT; however it is not clear which of these roles is important in inhibiting the cell growth. Clinical trials were not as good as hoped, MGBG was too toxic and DFMO didn't show significant impact on tumours. This maybe due to the short t_{1/2} of these enzymes which means patients have to be exposed for long periods of time.^{60, 62}

Polyamine analogues were designed which are enough like the natural polyamine to shut down the enzymes when cells are exposed to exogenous spermine. They will be transported into the cell via the transport apparatus, find their way to the same subcellular sites as natural polyamines and hopefully be unable to be further metabolised. A polyamine analogue which can regulate the activities of the biosynthetic enzymes and can't substitute for the natural polyamines in cell growth may be a good antiproliferative agent.⁶⁰

Initial studies by Bergeron and co-workers⁶³ looked at spermidine analogues which were substituted on N^1 , N^8 - or N^4 . Their ability to compete with [3 H]spermidine for cellular uptake, inhibit cell growth, affect polyamine biosynthesis and suppress enzyme activity were assessed.



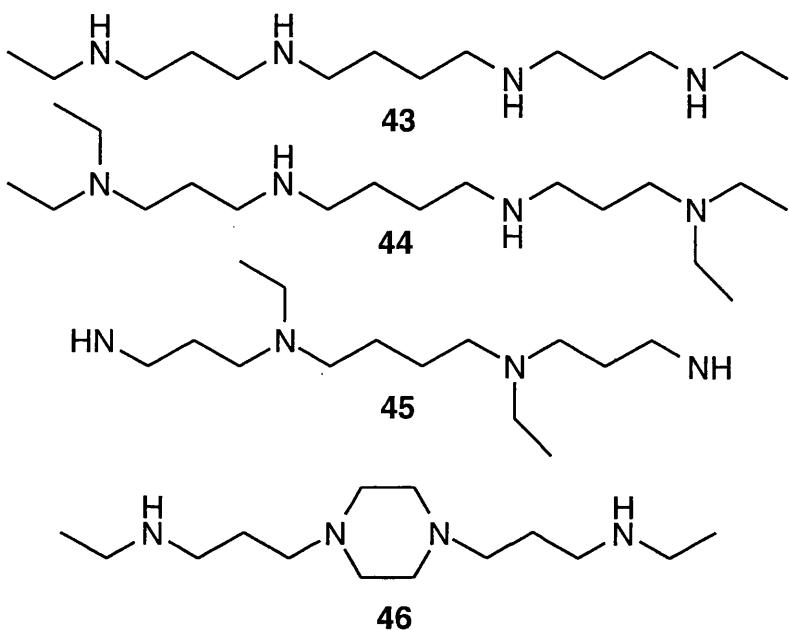
$R_1=H$, $R_4=Me$, $R_8=H$	
$R_1=H$, $R_4=Et$, $R_8=H$	$R_1=Et$, $R_4=H$, $R_8=Et$, 41
$R_1=H$, $R_4=acetyl$, $R_8=H$	$R_1=acetyl$, $R_4=H$, $R_8=acetyl$
$R_1=H$, $R_4=hexyl$, $R_8=H$	$R_1=propyl$, $R_4=H$, $R_8=propyl$, 42
$R_1=H$, $R_4=hexanoyl$, $R_8=H$	$R_1=propionyl$, $R_4=H$, $R_8=propionyl$
$R_1=H$, $R_4=benzyl$, $R_8=H$	
$R_1=H$, $R_4=benzoyl$, $R_8=H$	

$R_1=Et$, $R_4=H$, $R_8=Et$, 41
$R_1=acetyl$, $R_4=H$, $R_8=acetyl$
$R_1=propyl$, $R_4=H$, $R_8=propyl$, 42
$R_1=propionyl$, $R_4=H$, $R_8=propionyl$

The diethyl (**41**) and dipropyl (**42**) derivatives had reasonable antiproliferative properties. The N^4 -derivatives were generally inactive and this is surprising as they have better uptake but are less effective at inhibiting ODC. Growth inhibition would be expected when the analogue is incapable of substituting for spermidine. The N^1,N^8 - derivative (**41**) inhibits spermidine uptake, inhibits growth, decreases all polyamine pools, suppresses ODC and does not substitute for spermidine. This is in contrast to DMFO which depletes only putrescine and spermidine as (**41**) also depletes spermine by 50%. The terminal nitrogens are critical for cellular uptake and cell proliferation and the central nitrogen seems more involved in regulatory activities.

Further work by Bergeron and co-workers⁶² established that (**43**) had improved anti-proliferative activity when compared to (**41**), testing on cultured L1210 cells. *In vitro* results suggested that mono-alkylation of the terminal nitrogens gave the best activity as (**43**) was more active than either the tetra-alkylated analogue (**44**) or the internally alkylated analogue (**45**). *In vitro* structure-activity results showed activity decreased as alkyl substitution went

ethyl-propyl-methyl and also decreased if the internal nitrogens were incorporated in a 1,4-piperazine system (**46**). It is clear that spermine analogues were more active than spermidine analogues and this agrees with the studies by Porter *et al.*⁶⁴ They found that the order of activity for diethyl analogues was putrescine < spermidine < spermine.



(**43**) cleared putrescine and spermidine from within the cell and depleted spermine by 74%. In untreated cells it only affected ODC not AdoMetDC so its mechanism of action was due to some regulatory mechanism. More analogues were prepared to establish definitive structure-activity relationships.

1. Symmetrical methylene backbones, same alkyl substituent.
2. Symmetrical methylene backbones, different alkyl substituent.
3. Unsymmetrical methylene backbones, same alkyl substituent.
4. Unsymmetrical methylene backbones, different alkyl substituent.

This gave a range of analogues based on norspermine (3,3,3), spermine (3,4,3), homospermine (4,4,4) and other homospermine homologues. All were then tested to evaluate IC₅₀ values on a murine L1210 cell line, plus affects on ODC, AdoMetDC and SSAT and on polyamine pools. For those tetraamines that showed IC₅₀<25μM structural parameters were established for activity (**Figure 6**).⁶⁵

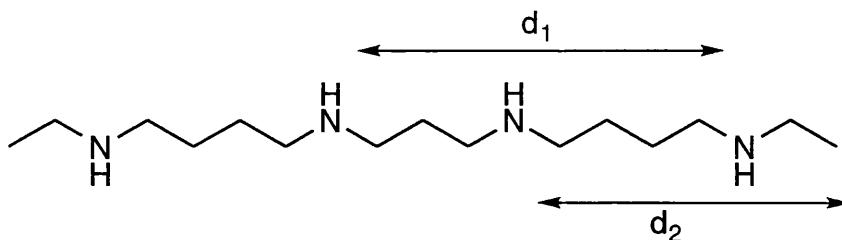
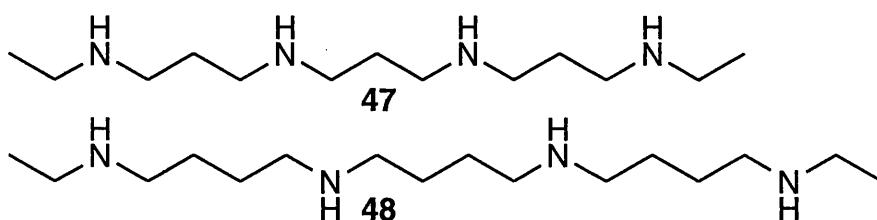


Figure 6 Structure-activity relationship for polyamines

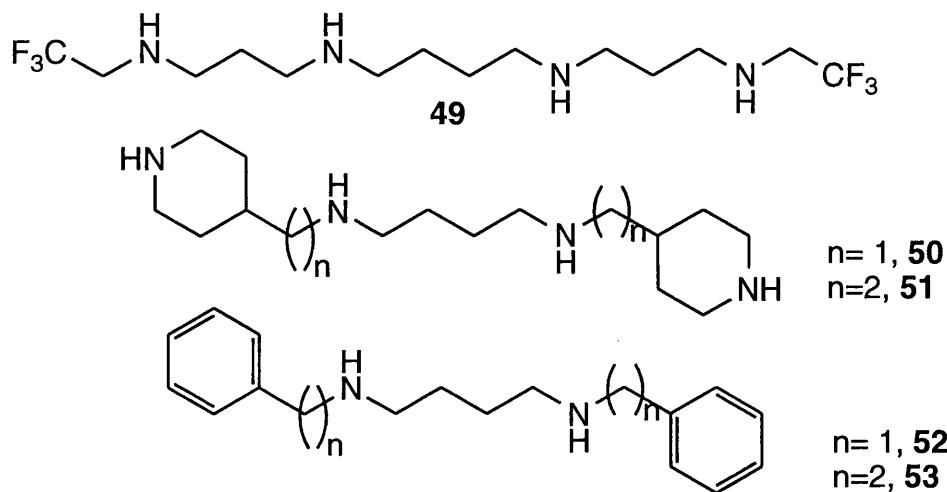
d_1 is the number of chain atoms between a terminal nitrogen and the nitrogen second closest to it and it must be greater than or equal to eight. d_2 is the distance from the terminal carbon to the second nitrogen (including terminal carbon) and this must be greater than or equal to seven. Bulky substituents decrease activity and this may be due to the ability of the molecule to interact with e.g. nucleic acids, as although the molecule is a tetracation the bulkiness could compromise electrostatic interaction. (47) was the most active analogue, superinducing SSAT. The level of accumulation of the analogues did not reflect their activity and neither did the ability of an analogue to deplete the polyamine pools. These results suggested that the analogues owe their activity to something other than just affecting polyamine metabolism, e.g. protein synthesis.

The role of charge in polyamine recognition was also studied by the very prolific Bergeron and co-workers⁶⁰ as extremely similar compounds often behaved very differently. (43), (47) and (48) all suppress ODC and AdoMetDC to the same levels and yet (47) up-regulates SSAT by 1200 fold compared to 250 and 30 fold for (43) and (48) respectively.

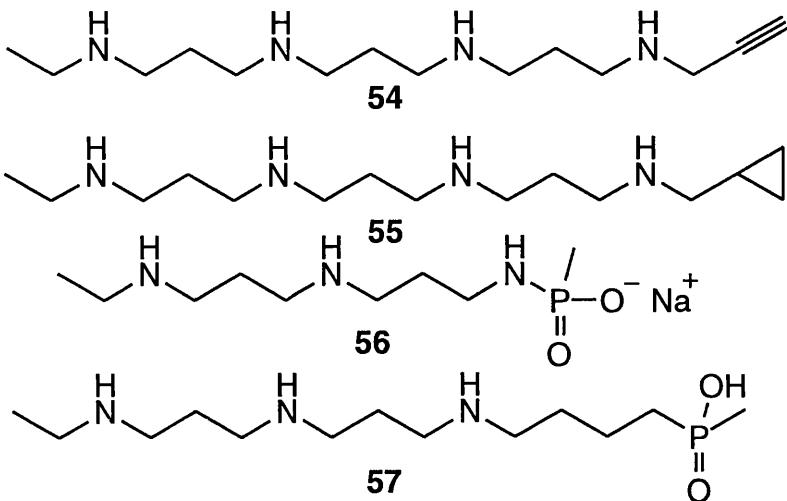


All of those molecules are highly protonated at physiological pH and compare well with spermine and homospermine. Compounds were prepared that would appear to the cell as differently charged molecules and the ability to impact on cell growth was measured. Those such as (47) (50) and (51) which would appear as tetracations competed well with spermidine for uptake and were effective at controlling cell growth. Those that would appear as dications such as (52) and (53) competed poorly and were inactive when tested against LC1210 cell lines. The same results were

observed when the analogues were tested on ODC, AdoMetDC and SSAT with the dication having little or no effect on the enzymes.

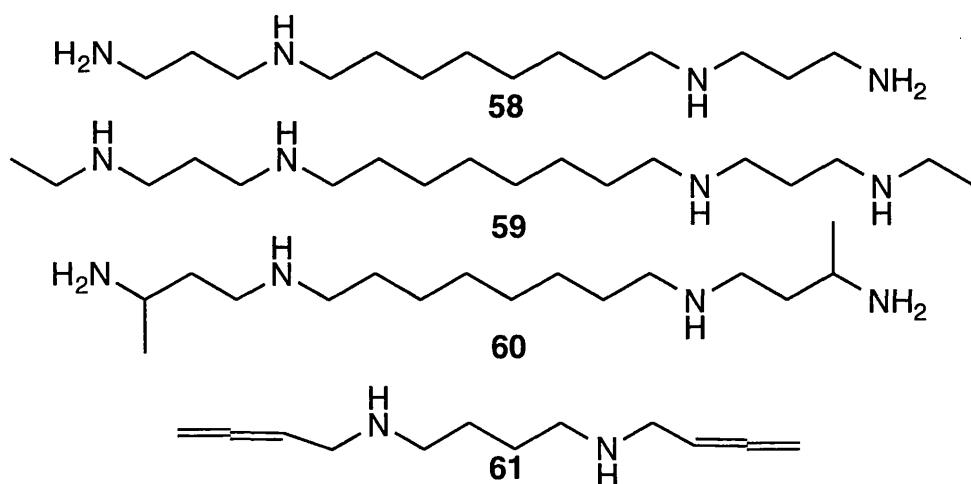


The induction of SSAT in two human lung cancer cell lines which respond differently to inhibitors of polyamine biosynthesis has been studied. The diethylspermine analogue (**47**) was cytotoxic against large cell lung carcinoma (LCLC) causing massive depletion of polyamines and ODC activity. However it minimally affected small cell lung carcinoma (SCLC). In the LCLC line SSAT was induced 1000 fold and Saab and co-workers suggested SSAT plays an important role in the specificity of such agents. The ability to superinduce SSAT is postulated to play a role in the toxic action of such drugs. To investigate SSAT further they prepared two unsymmetrically substituted polyamines (**54**) and (**55**) which were shown to have similar potency and activity on SSAT to (**47**).⁵³



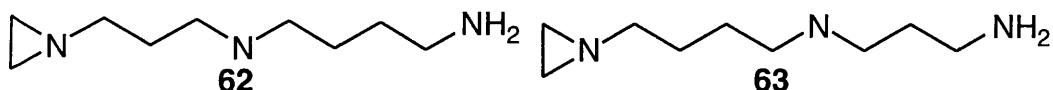
Other work by Saab *et al.*⁵⁴ looked at the evidence that (54) and (55) superinduce SSAT in LCLC cell lines. Reactions catalysed by SSAT have been shown to proceed by a bi-bi mechanism where the polyamine is the first to bind to the surface of the enzyme.⁶⁶ They prepared phosphonamide and phosphinate analogues which have been used in other work to mimic the tetrahedral transition state.^{67, 68} (56) was too labile for their studies and (57) was ineffective as a growth inhibitor, maybe due to destabilization of SSAT.

Other research in this area by Edwards, Bitonti and co-workers⁶⁹ had also concluded that spermine analogues were giving the best activity against tumours. They investigated the effect of increasing the carbon chain length between the central nitrogens and the most active analogue was attained when eight carbon atoms connected them (58). *In vivo* studies showed rapid metabolism of this compound so methyl groups were placed α - to the terminal nitrogen (60) or α - to the central nitrogen but this decreased activity. Alkyl groups were introduced on the terminal nitrogens also to inhibit amine oxidases (59) and results were similar to those of Bergeron as activity decreased as the alkyl group size increased. One analogue (61) was found to be a potent irreversible inhibitor of PAO. *In vivo* tests were carried out against the L1210 model and the activity of the compounds was found to increase when co-administered with bisallene PAO inhibitor (61), suggesting these types of compounds are metabolised by PAO.



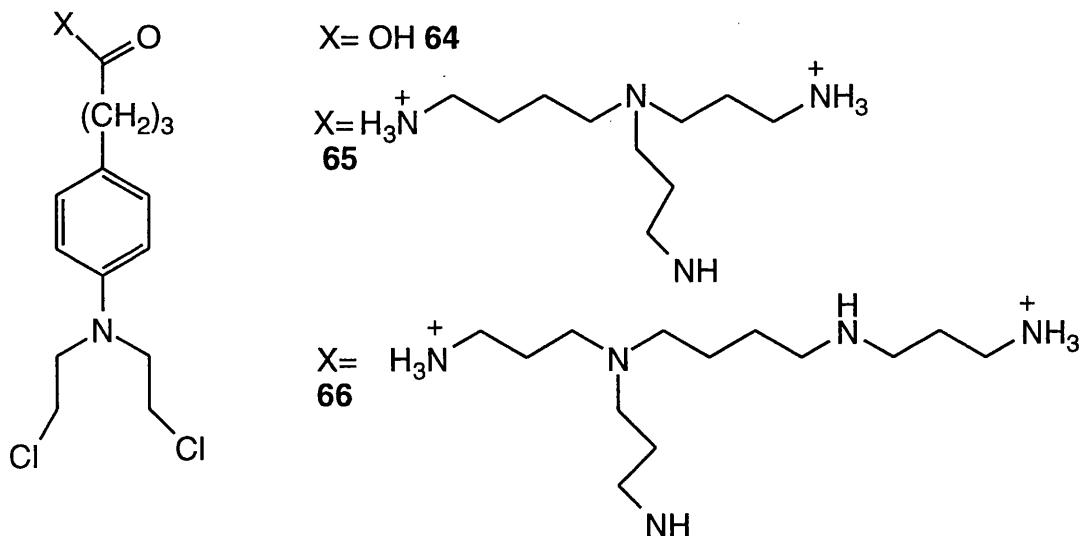
Two aziridine-containing spermidine analogues were prepared by Yuan and co-workers,⁷⁰ containing the aziridine at N^1 - (62) or N^8 - (63). An amino group was replaced with the ring strained alkylating aziridine ring that is known to react covalently with DNA causing massive damage. N -(4-

Aminobutyl)aziridine has been shown to be cytotoxic against prostatic carcinoma cells and is a potent irreversible inhibitor of DAO. Both the new spermidine analogues were also shown to be cytotoxic against L1210 cells and the *N*¹-substituted compound was more active than the *N*⁸-. They also studied the incorporation of [³H]thymidine and [³H]uridine into DNA and RNA but from the results it was unclear whether DNA/RNA was the target of these compounds.



2.5.3 Transport of Therapeutic Moieties via Polyamines

The site of many cytotoxic agents is intracellular so for a drug to be active it must pass many hurdles. Firstly it must cross the cell membrane and then recognise and interact selectively with cellular targets. If a drug can be helped to pass these hurdles it would probably improve on its cytotoxicity and activity. Cullis and co-workers hoped to exploit the polyamine uptake mechanism and the high affinity of polyamines for DNA by preparing a spermidine-chlorambucil conjugate. Chlorambucil (**64**) is a proven anti-cancer drug used for treating lung and ovarian cancer. It is believed that this bifunctional alkylating agent owes its activity to its ability to crosslink DNA and it has been shown that cytotoxicity relates well to the ability to crosslink. By preparing this conjugate (**65**) it was hoped to improve on the activity of chlorambucil.^{71, 72, 73}



Initial studies showed an increase in activity with naked DNA by a factor of 10^4 . *In vitro* cytotoxicity of the conjugate in polyamine-depleted cells was increased 200 fold compared to chlorambucil but this fell to only four fold *in vivo*. The spermine conjugate (**66**) is even more active than the spermidine one (**65**) although they both show identical crosslinking specificities.

2.6 Polyamines to Fight Parasitic Diseases

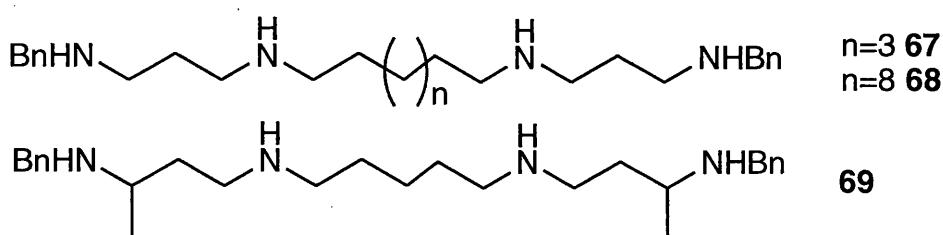
The biosynthesis of polyamines has been a target for cancer chemotherapy and then moved on into parasitic diseases.⁷⁴

The group of Edwards and co-workers has shown that parasitic protozoa are sensitive to inhibitors of polyamine biosynthesis, with DFMO effective against African trypanosomes both in the laboratory and in the clinic. DFMO also inhibits the blood stages of the human parasite *P. falciparum* *in vitro* and of *P. berghei* in mice (**Figure 7**).^{75, 76},

Potent Activity	Some Activity	No Activity
<i>T. brucei brucei</i>	<i>P. falciparum</i>	<i>T. cruzi</i>
<i>T. brucei rhodesiense</i>	<i>P. berghei</i>	<i>Leishmania</i>
<i>T. brucei gambiense</i>	(exoerythrocytic only)	<i>P. gallinaceum</i> (erythrocytic only)

Figure 7 Activity of DFMO on parasitic diseases

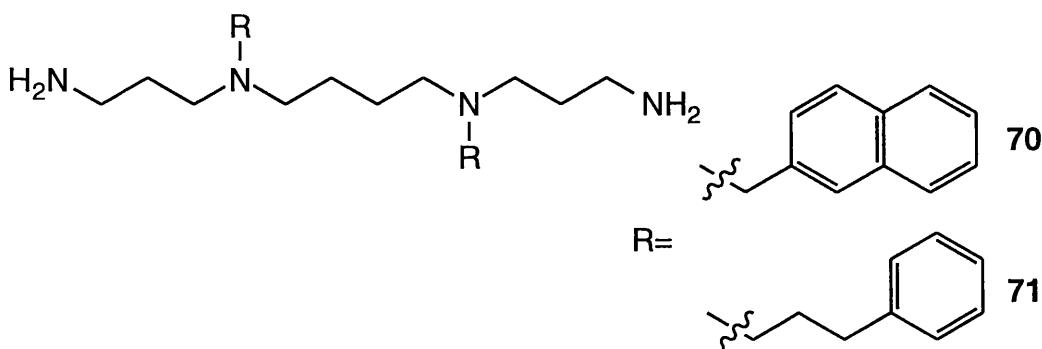
A series of tetraamines were prepared and examined for activity against *P. falciparum*.⁷⁷



Compounds with benzyl groups on the terminal nitrogens were the most active followed by those with one benzyl and those with no alkylation were the least active. The central carbon chain was extended to twelve methylene units (**68**) and this had the optimum *in vitro* activity (IC_{50} 0.2 μ M).

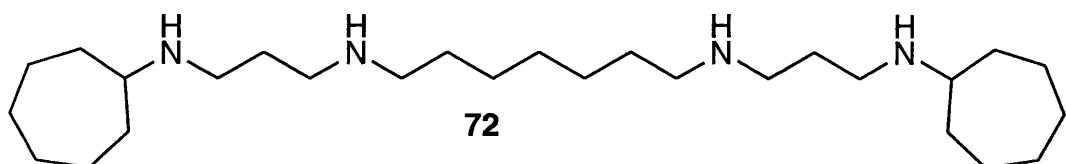
Alkylation on the terminal nitrogen was varied; however benzyl remained the best group with activity decreasing as substitution varied from benzyl > thienylmethyl > cyclohexylmethyl > n-butyl. The most active dibenzyl analogues were found to debenzylate when incubated with PAO although compounds such as (69) prepared to overcome this had no extra activity. The most active compound *in vivo* was (67) and when given in combination with DFMO was curative. Increasing the central chain past seven carbons led to an increase in toxicity. It was noted that such compounds accumulated to high concentrations in the cell due to greater accumulation of these bisbenzyl analogues compared to the free amine equivalents.⁷⁸ Although no evidence was obtained that showed (67) inhibited polyamine biosynthesis other studies showed (67) decreased ODC and AdoMetDC in cultured rat hepatoma cells.⁷⁹ The debenzylation of the compound was thought to be a contributing factor to its activity, as it is metabolised to the free amine which is then capable of repressing polyamine biosynthesis. Activity was less than chloroquine (IC_{50} 0.014 μM) but on a par with tetracycline (IC_{50} 62 μM) which has been used to treat multiply resistant malaria infections. It is worth noting that (67) also had activity against *Leishmania donovani* which is another parasitic infection.⁸⁰

Trypanosoma cruzi is a protozoan parasite that causes Chagas disease, mainly affecting people in Central and South America. Trypanosomes contain a unique enzyme trypanothione reductase (TR) which has been targeted by the group of O'Sullivan.⁸¹ A number of spermidine and spermine analogues were prepared and (70) and (71) were found to be competitive inhibitors of TR. It is hoped that these compounds will be modified to produce irreversible inhibitors.

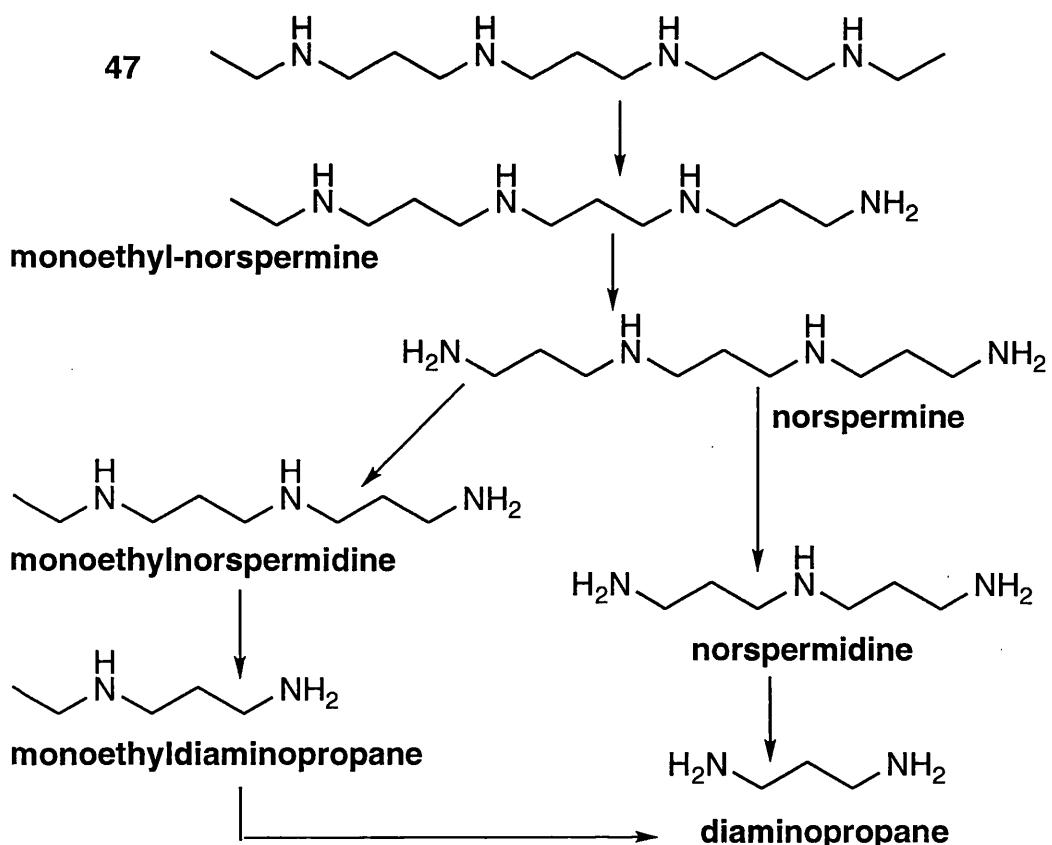


Bellevue and co-workers⁸⁰ have prepared compounds and tested them for antitumour activity or antiparasitic activity. Two compounds (54) and (55), described earlier, had excellent antitumour activity and they

extended the range of analogues by preparing symmetrical and unsymmetrical compounds. Analogues with a 3,3,3 carbon skeleton had excellent antitumour activity but little antitrypanosomal activity *in vitro*. In contrast the 3,7,3 skeleton was more effective against parasites than tumours. The most active analogue was (**72**) and it is also being evaluated against *P. falciparum* and *Leishmania donovani*.

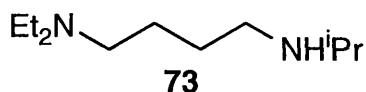


The mechanism of action of (**72**) is as yet unknown; however the activity of bisbenzyl analogue (**67**) is thought to be related to its rapid metabolism by PAO. The metabolism of diethyl analogue (**47**) has been shown to proceed via *N*-dealkylation followed by SSAT acetylation and oxidation by PAO (**Scheme 4**).⁸²



Scheme 4 Metabolism of diethylnorspermine analogue (**47**)

De *et al.* had looked at diaminobutanes and alkanolamines for reversal of chloroquine resistance. Their most active compound was (73) although further studies were halted due to problems with photosensitivity of the active compound.⁸³

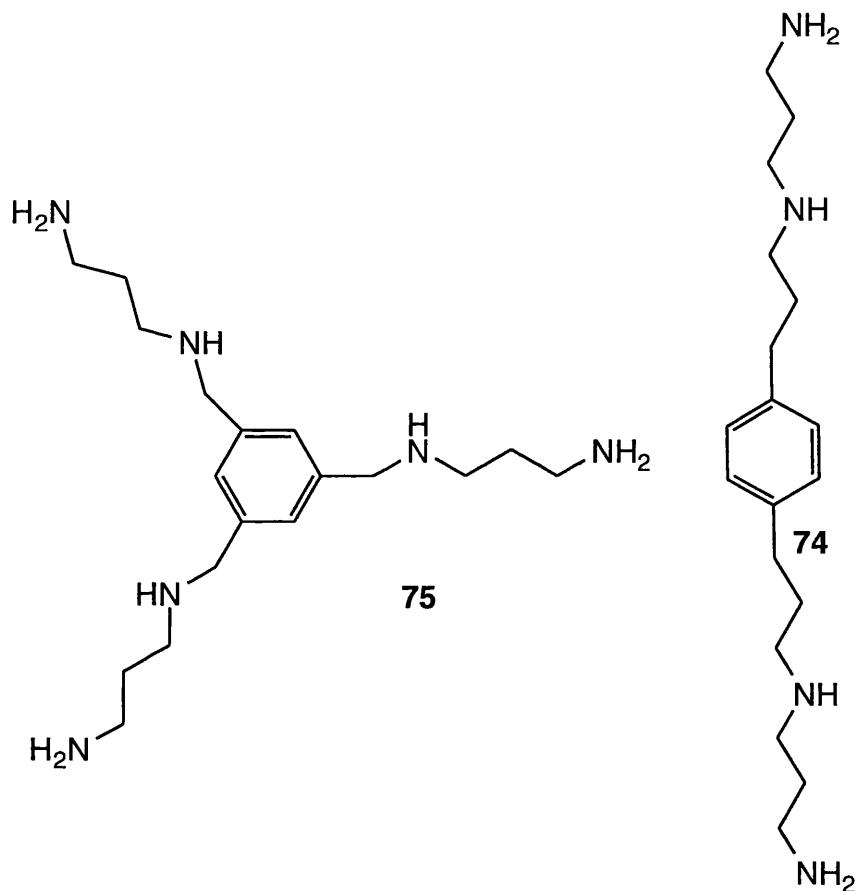


2.7 DNA Binding Properties of Polyamine Analogues

Polyammonium salts bind to DNA through an electrostatic interaction which is non-sequence specific, with high affinities when the charge is +3 or +4. Whilst remaining close to the DNA the polycation retains a high degree of freedom within the polyamine-DNA complex.^{30, 34} Cullis and co-workers favour the "bind and slide" mechanism where the polyamine slides up and down the phosphate backbone of DNA.⁷¹ In contrast, the group of Burrows and co-workers³⁵ favour the minor groove with the polyamine hydrogen bonding to the edges of the base pairs. Studies have shown that three to four carbon spacing is nearly ideal to ensure full protonation of the nitrogens and hence maximum interaction. The exact position of the binding interaction is not known (e.g. major groove, minor groove or phosphate backbone); however there are some crystal structure of polyamines bound to oligomers.^{84, 85, 86} In one case spermine was found to span the major groove.⁸⁴

Edwards and co-workers have synthesized a number of polyamine analogues with both antitumour and antiprotozoal activity.^{69, 77} The mechanism of action is not clearly understood, although one hypothesis is that they may act through displacement of the naturally occurring polyamines from DNA binding sites. If such an action is relevant to the antitumour activity of such compounds correlation might be expected between DNA binding ability and growth inhibition. A large range of compounds were prepared which enabled the researchers to look at the effects of: length of the central chain; terminal N-alkyl substitution; branching of the carbon chain; and the distance between the nitrogen atoms on DNA binding. The polyamines showed a wide tolerance for functional groups in the aminopropyl moiety and although increased alkyl substitution lowered DNA binding the effect was minimal. The order of ability to interact with DNA follows the

pattern diamines < triamines < tetraamines and those compounds with terminal benzyl groups showed a preference for AT base pairs in DNA compared to GC base pairs in DNA. Polyamines with a central aromatic core (**74**) were comparable or superior to spermine for DNA binding.⁸⁷



This finding compares well with the work of Stewart and Gray⁸⁸ who looked at a wide range of such compounds. Their most active analogue for DNA binding was (**75**). They studied polyamine-DNA interactions to try and establish a set of rules for binding that might be used for the design of complexation agents in the future.

2.8 Summary

Polyamines have been used in the treatment of a wide range of diseases and therapeutic uses are being discovered for them all the time. It seems hard to draw on all the examples given, which are so wide ranging, and come up with definitive structure-activity relationships. In cancer cases a simple ethyl group on a 3,3,3 carbon skeleton seems the best, whereas in parasitic diseases benzyl groups on a 3,7,3 skeleton are active. Links have been drawn between the ability of compounds to superinduce SSAT activity and their antitumour activity, whereas the ability of a compound to be metabolised may be important in parasitic cases. These simple compounds also have a remarkable affinity for DNA and RNA which may lead to interrupting protein synthesis. No link for activity can be drawn between therapy of different diseases but one thing remains true; there is every reason to investigate these types of compounds. In the next chapter we will discuss a number of putrescine analogues that were the lead into this project on polyamines as prospective antimalarials.

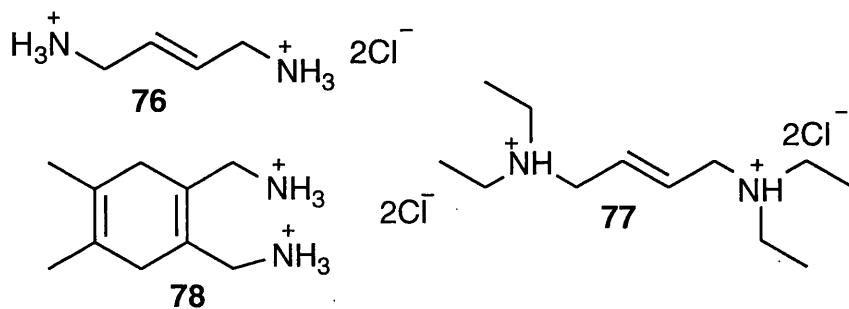
3

3 Synthesis and Evaluation of Putrescine Analogues

3.1 Introduction and Rationale

The widespread occurrence of polyamines led to a large amount of research in this area. Of special interest was the synthesis of inhibitors of the polyamine biosynthetic pathway which led to the development of DFMO (2.3.1). A number of fungi only possess the ODC pathway to synthesise putrescine so if an inhibitor could block ODC the fungi would die. Plants have the alternative method of synthesising putrescine via the arginine pathway and would remain unaffected.³⁸

A wide range of ornithine and putrescine analogues had been prepared in the search for inhibitors of ODC and AdoMetDC and also to evaluate their affect on polyamine pools.^{43, 48} In our group previous work had focused on the design and synthesis of putrescine analogues for anti-fungal testing. It was found from these studies that the 1,4-diaminobut-2-ene moiety was especially interesting and synthesis of compounds continued. Initially (**76**) was identified as having good fungicidal properties and a series of *N*-alkylated analogues were prepared which led to the antifungal compound (**77**).^{89, 90} Alicyclic derivatives were also prepared utilising well known Diels-Alder chemistry to synthesise a number of structures such as (**78**).⁹¹



(77) was shown to control a wide range of fungi including powdery mildew on barley and (78) was even more effective. Tests were done to see the effect these compounds had on the enzymes ODC and AdoMetDC and also on the levels of putrescine, spermidine and spermine in plants.^{38, 89} These results are briefly summarised in **Figure 8**.

	ODC	AdoMet DC	put	spd	spm	powdery mildew control
76	- 87%	- 82%	+ >100%	- 32%	+ 61%	75%
77	+ 81%	- 62%	- 49%	- 19%	- 4%	80%
78	- 18%	+ >100%	- 68%	no effect	- 12%	93%

Figure 8 Variation in polyamines and enzyme levels due to antifungal compounds

These analogues do not deplete intracellular polyamines in plants sufficiently to account for their activity. Both (76) and (77) deplete AdoMetDC and it has been speculated that this may be related to their activity.³⁸

As shown in 2.6 polyamines have been evaluated against a range of parasitic diseases, including malaria. Our group branched into the malaria area when (77) was screened against an *in vitro* culture of *P. falciparum* and showed promising activity. So from the anti-fungal work had come our "lead" compound and we decided to examine a wider range of polyamine analogues and evaluate their activity against malaria. Many of the compounds discussed in this chapter have been prepared before, either within our group or by other researchers; however little work had been done on their antimalarial activity.

3.2 Evaluation of Antimalarial Activity

It seems sensible at this point to discuss briefly the testing processes carried out on our compounds to investigate their activity. This work was carried out by Professor Stephen Phillips and Fiona McMonagle in the Division of Infection and Immunity, University of Glasgow. Initial tests were carried out *in vitro* and if interesting activity was found *in vivo* tests were used.

3.2.1 *In vitro* Testing against *P. falciparum* Asexual Erythrocytic Stages

- S. R. Phillips and F. McMonagle, Division of Infection and Immunity

P. falciparum culture adapted strains FCR (Gambia) and JS (Zimbabwe) were used. Both these strains are chloroquine sensitive and were grown as stock cultures *in vitro* in petri dishes by a modification of the candle jar technique of Trager and Jensen.⁹² In culture the parasites were asynchronous in their growth and therefore all stages in the 48 hour asexual erythrocytic cycle would be represented at any one time. The assay system for screening compounds for activity against *P. falciparum* was that described by Desjardins *et al.*⁹³ Each test concentration was carried out as a minimum in triplicate. After 24 hours incubation in a candle jar 100 millilitres were removed from each well and replaced with 100 ml fresh medium containing 1 µCi ^3H -hypoxanthine.⁹⁴ The plate was incubated for a further 18 hours before the parasites were harvested and parasite growth was measured as the incorporation of the isotope, using conventional liquid scintillation counting. The mean and standard deviation of the counts per minute for each dilution of the test compounds or the control cultures were calculated and the results displayed using Cricket graph. The results were analysed using a standard t-test.

The activity of the compounds was shown as the lowest concentration in µg ml⁻¹ where the test compound showed any reasonable activity. For a further comparison this lowest activity was converted into a lowest effective concentration (LEC) which, in µM, takes into account the molecular weight of the compounds. Although not an accurate IC₅₀ value the LEC does represent a good guide to a compound's activity. We analysed the data using an activity scale (**Figure 9**) designed to assess whether certain analogues were worth pursuing with *in vivo* testing and/or further chemical modifications. Chloroquine is >9 on this scale and any compound with activity less than 4 was not pursued.

Activity Scale	1	2	3	4	5	6	7	8	9
Lowest Activity (µg ml ⁻¹)	1000	500	100	50	10	5	1	0.5	0.05

Figure 9 Activity Scale for *in vitro* test results

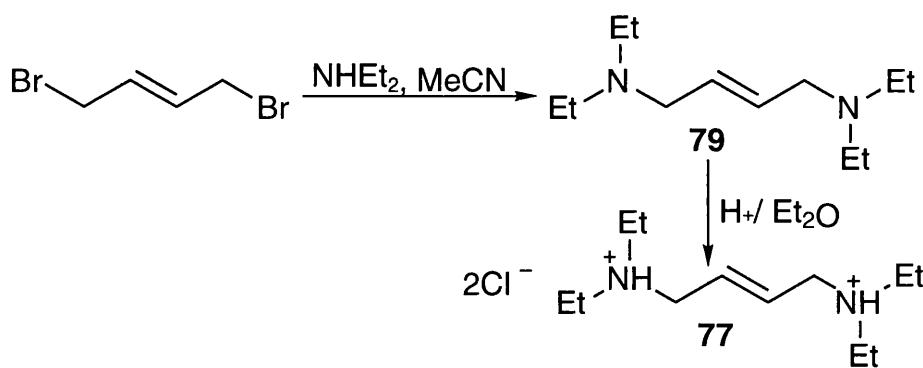
3.2.2 *In vivo Testing against P. chabaudi* - S. R. Phillips and F. McMonagle, Division of Infection and Immunity

P. chabaudi AS strain, in inbred NIH mice, was handled as described by McDonald and Phillips.⁹⁵ In brief three months old male inbred NIH mice were infected intravenously with 1×10^6 *P. chabaudi* parasitized erythrocytes and groups of five mice immediately afterwards were injected with either chloroquine at 50 mg/Kg body weight or the test compounds. The compounds were dissolved in physiological saline and injected intraperitoneally. A control group of mice received saline only.

3.3 Synthesis of Lead Compound (77)

The synthesis of (**79**) had been reported previously.⁵⁶ An S_N2 displacement of (*E*)-1,4-dibromobut-2-ene with diethylamine yielded the free base (**79**). Dissolving the free base in ether and carefully adding ethereal hydrogen chloride (see 7, procedure A1) precipitated the hydrochloride salt (74%) (**77**). The resulting salt could be purified, if needed, by re-dissolving the salt in ethanol and precipitating with ether. This is a reliable way of producing dihydrochloride salts for this compound (**Scheme 5**).

Significant *in vitro* activity of (**77**) was present at 18-38 μM which was promising (c.f. IC₅₀ chloroquine 0.014 μM ; IC₅₀ tetracycline 62 μM) but how could we try and improve on this?



Scheme 5 Synthesis of lead compound (**77**)

By looking at (**77**) we can establish a number of features that may be altered to see how they affect the activity. The development of further analogues by this route would contribute to the elucidation of structure-activity relationships (**Figure 10**).

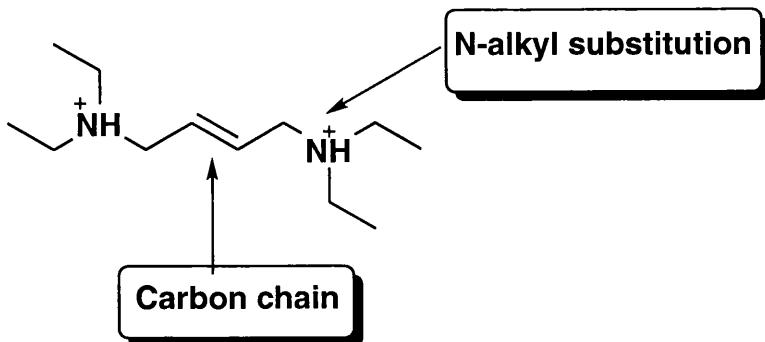


Figure 10 Structural considerations for variation of (77)

- 1. Carbon chain** - prepare unsaturated and saturated analogues to establish the importance of the trans-double bond and chain length.
- 2. N-Substitution** - vary the alkyl group to see the affect of size and form both tetra-substituted, disubstituted, symmetrical or unsymmetrical compounds.

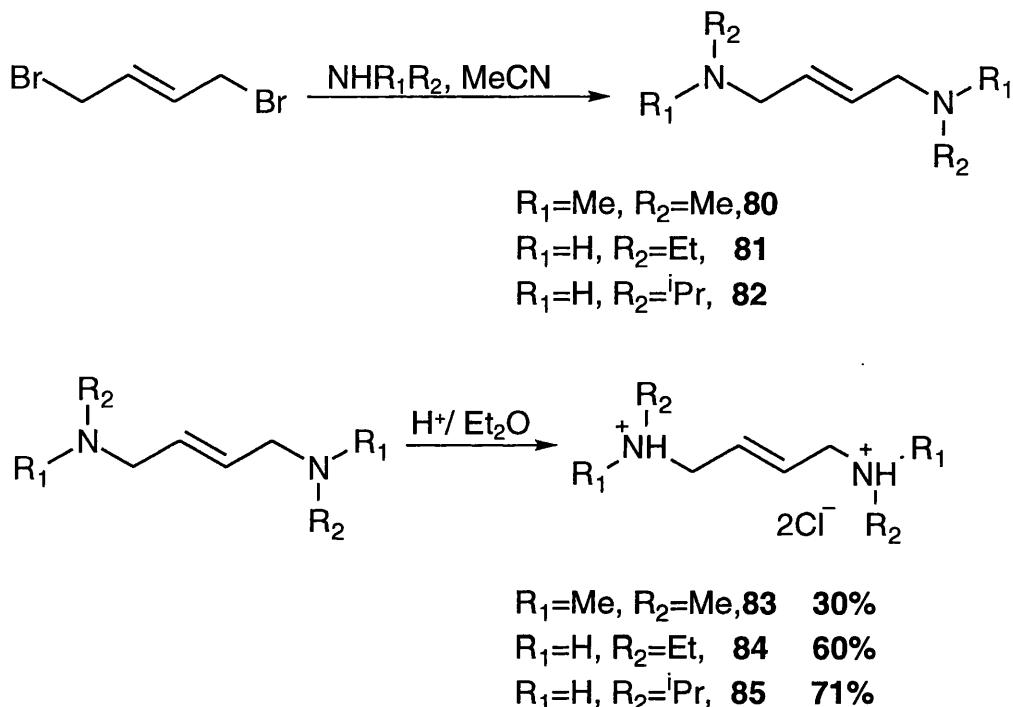
3.4 Alkyl Analogues of (77)

3.4.1 Synthesis of Compounds (83), (84), (85), (89), (90) and (92)

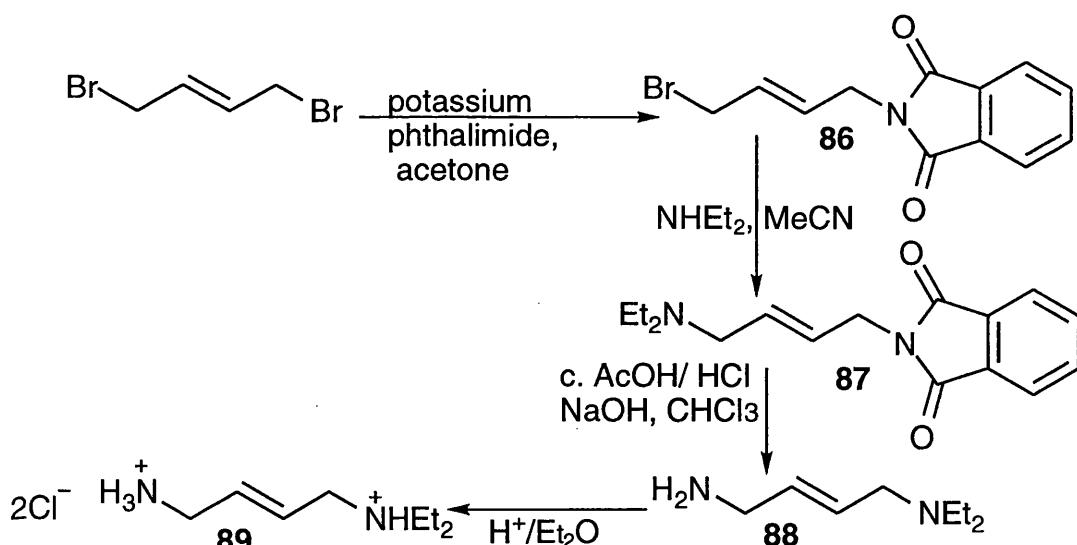
The free bases (**80**) and (**81**) had been prepared previously in our group.^{38, 57} (**82**) was prepared by a similar route.⁹⁶ The free bases were all prepared utilising the well known $\text{S}_{\text{N}}2$ displacement of an alkyl halide with an amine quickly producing the desired compounds in good yields (**Scheme 6**). In previous work many of these compounds had been prepared as the dihydrobromide salts and we prepared the dihydrochloride salts (**83**), (**84**), (**85**). The yield of (**80**) was lower than expected, maybe due to a lower boiling point of the product. It was important when isolating the free base by concentrating *in vacuo* to keep the water bath temperature to a minimum so less product is lost. The dihydrochloride salts were precipitated by the addition of ethereal hydrogen chloride as described for (**77**).

In order to extend the series the unsymmetrically substituted compound (**89**) was prepared following the procedure reported (**Scheme 7**).⁵⁷ One equiv. of potassium phthalimide was heated with (*E*)-1,4-dibromobut-2-ene to yield (**86**), although some di-phthalimide was formed as a by-product. The phthalimide (**86**) formed white needle-like crystals which were purified easily by recrystallisation from acetone. $\text{S}_{\text{N}}2$ displacement as

above yielded the protected diamine (**87**) which was not purified but hydrolysed in acid conditions. This method of removal of the phthalimide group is one of many and is used because of the good yields obtained. A basic work up yielded the free base and the hydrochloride salt was isolated as usual.

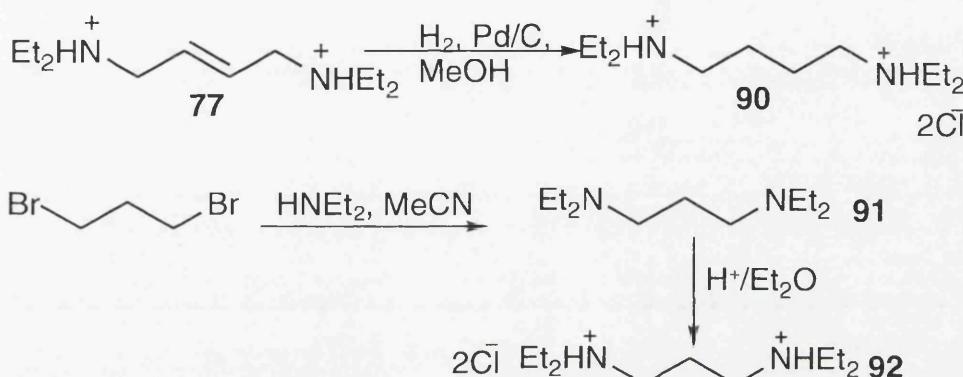


Scheme 6 Synthesis of *N*-alkylated derivatives (**83**), (**84**) and (**85**)



Scheme 7 Use of the phthalimide protecting group to produce unsymmetrical analogue (**89**)

The 1,4-diaminobut-2-ene moiety had been shown to give good activity in the anti-fungal tests.³⁴ This had also been true in the earlier work by the scientists at Merrell Dow on ornithine and putrescine analogues (**2.3.1**).^{43, 48, 31} In the malaria work the importance of the *trans* carbon-carbon double bond was investigated. The unsaturated analogue (**77**) was hydrogenated over palladium on carbon to yield (**90**) (compound prepared by 4th year project student). The three carbon analogue (**92**) had been prepared by Ingold and Rothstein and we also prepared it (**Scheme 8**).⁹⁷



Scheme 8 Synthesis of saturated putrescine analogues (**90**) and (**92**)

3.4.2 Biological Evaluation

The activity of (**83**), (**84**), (**85**), (**89**), (**90**) and (**92**) against an *in vitro* culture of *P. falciparum* was assessed and compared with (**77**) with chloroquine as a control (**Figure 11**).

	activity (μg ml ⁻¹)	LEC (μM)
tetraethyl 77	5-10	18-36
tetramethyl 83	50	232
<i>N,N</i> '-diethyl 84	50-100	232-465
<i>N,N</i> '-diisopropyl 85	100	411
<i>N,N</i> -diethyl 89	50	232
tetraethyl (sat. 4C) 90	100	366
tetraethyl (sat. 3C) 92	10-50	38-190
chloroquine 2	<1	<1.9

Figure 11 *In vitro* activity of aliphatic putrescine analogues

The tetraethyl analogue (**77**) was the most active compound of this group. Keeping the tetrasubstituted pattern but decreasing the size of the

alkyl group to methyl decreased the activity dramatically. Going from tetraethyl to *N,N'*-diethyl also decreased the activity. Within the group (84), (85) and (89) the compounds were fairly similar, despite the change in substitution patterns and alkyl group size. The *trans*-carbon carbon double bond seemed to aid activity as the saturated compounds were both less active than (77). Surprisingly (92) with three carbons linking the nitrogens was more active than the four carbon saturated analogue (90).

These results should be compared with other testing on polyamines. Investigating polyamine analogues as anti-tumour agents (2.3.1). Porter⁶³ showed that monoalkylation of the terminal nitrogens in spermidine derivatives produced compounds with the best activity. It was also shown by Bergeron that tetrasubstitution on spermine analogues decreased activity.⁶² In our *in vitro* evaluation of putrescine analogues against *P. falciparum* we found the opposite to be true as our tetrasubstituted compound was the most active. Increasing the size of the alkyl group decreased the activity which corresponds to the work of Bergeron⁶⁵ where the order of activity was ethyl > isopropyl. We observed the same results from (77) to (85); however the compounds (84) and (85) do not show such a dramatic difference in activity. Finally the unsaturated compounds gave the best activity. This corresponds well with the results on ornithine and putrescine analogues for ODC inhibition (2.3.1).

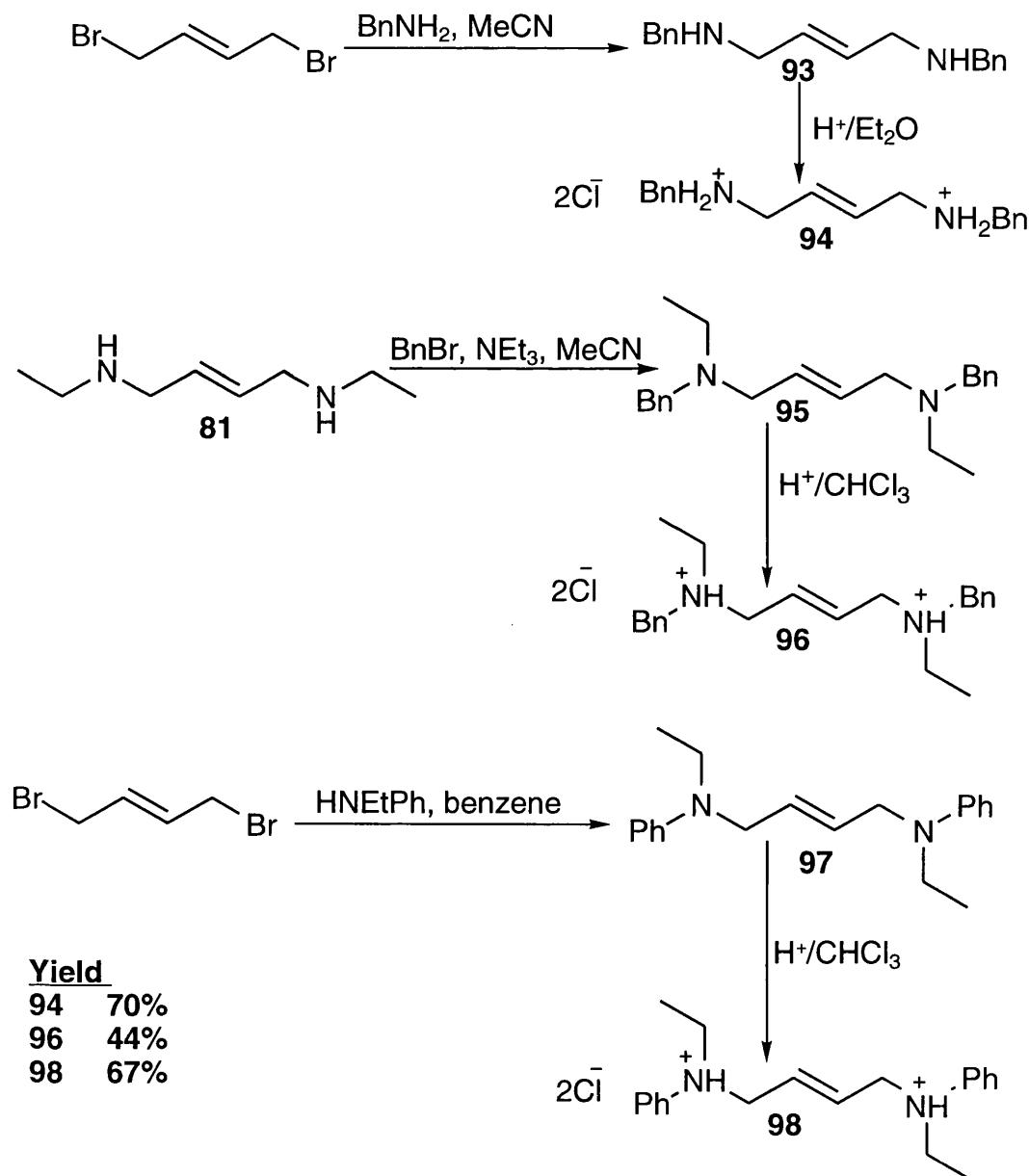
We prepared a number of aliphatic compounds but none had improved on the activity of our initial lead compound. We decided to produce a number of aromatic compounds and see the effect the hydrophobic group had on activity. This is an attractive idea as the work of Edwards⁷⁷ on polyamines with antimalarial activity showed that terminal monoalkylation with aromatic groups greatly improved the activity of their compounds (2.6).

3.5 Aromatic Analogues of (77)

3.5.1 Synthesis of Compounds (94), (96) and (98)

(94) was prepared as shown in **Scheme 9** using freshly distilled benzylamine and excess amine was removed by distillation. The free base (93) was converted into the dihydrochloride salt as usual to yield (94) in good yield (70%). In our hands tetrasubstituted compounds gave the best *in vitro* activity so an analogue was prepared that was substituted with diethyl and the hydrophobic benzyl group. (81) was stirred in acetonitrile and

triethylamine and benzyl bromide was added dropwise, in an attempt to minimise side reactions of the extremely reactive benzyl bromide. After work up the yield of the desired product was fairly poor and there was an intense UV active spot on the baseline of the TLC plate. This may be due to over-alkylation of the nitrogen by benzyl bromide to give the quaternary salt. If this compound was to be prepared again a few changes could be made to try and improve the yield. Benzyl chloride might be tried as an alternative alkylating source or Hünig's base could be used instead of triethylamine. Hünig's base is often used to help prevent formation of quaternary salts in such reactions.



Scheme 9 Synthesis of aromatic putrescine analogues (**94**), (**96**) and (**98**)

The other compound of this aromatic series to be prepared was (**96**). S_N2 displacement of the dibromide by *N*-ethylaniline gave the free base as a white solid. This had been previously prepared by Roberts and Ross⁹⁸ using the (*E*)-dichloroalkene. We had the dibromoalkene handy and obtained a 67% yield compared to the reported 57%. The analytical data were expanded to include NMR and MS. The dihydrochloride salt was formed by bubbling dry HCl through a solution of the free base in chloroform, due to the insolubility of (**97**) in ether (**Scheme 9**).

3.5.2 NMR Studies on (**95**) and (**97**) and their Dihydrochloride Salts

Nuclei in regions of high electron density experience a field proportionally weaker than those in a region of low electron density. They are said to be shielded by the electrons and it takes a higher field to bring them into resonance (i.e. low δ). If the nuclei are in a region of low electron density it takes less field to bring them into resonance (i.e. higher δ). These principles can be applied to most compounds when analysing and explaining their NMR data.⁹⁹

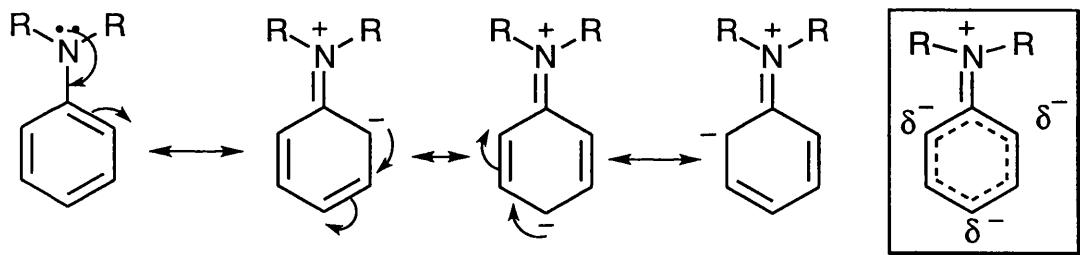
Going from the free base of an amine to the hydrochloride salt (**95**)-(**96**) we tend to see an increase in δ values. The explanation for this is covered in **3.9**. The nitrogen atoms in polyamines exert strong effects due to their electronegative behaviour. They will pull electrons towards themselves and away from any neighbouring atoms, hence deshielding them (**Figure 12**).

When comparing the chemical shifts of 6-H₃ and 5-H₂ the methylene protons come at higher δ than the methyl protons. The methylene protons are α - to the nitrogen atom which exerts a strong effect, however this decreases with distance. Typical vicinal coupling constants for the ethyl groups are 7 Hz, $^3J_{HH}$ usually in the range 6-8 Hz. Our values for all ethyl-substituted compounds fall within the range 6.5-7.5 Hz. The allylic protons appear downfield as do the methylene protons "sandwiched" between a nitrogen and the benzyl ring (7-H₂). In (**95**) and (**96**) the aromatic protons all have similar chemical shifts forming a close multiplet (**Figure 12**).

95 (base)	δ	96 (salt)	δ	97 (base)	δ	98 (salt)	δ
6-H ₃	1.03	6-H ₃	1.08	6-H ₃	1.12	6-H ₃	0.84
5-H ₂	2.50	5-H ₂	2.93	5-H ₂	3.33	5H ₂	3.26
1,4-H ₂	3.06	1,4-H ₂	3.56	1,4-H ₂	3.85	1,4-H ₂	3.99
7-H ₂	3.54	7-H ₂	4.05	-----	-----	-----	-----
2,3-H	5.68	2,3-H	5.82	2,3-H	5.62	2,3-H	5.55
9,10,11-H	7.24	9,10,11-H	7.26	9,11-H	6.65	10-H	7.15
				10-H	7.20	9,11-H	7.38

Figure 12 Comparison of NMR data for aromatic amines

The aromatic amines (**97**) and (**98**) show an extra effect. In (**97**) the nitrogen lone pair is delocalised into the aromatic ring to form a number of resonance structures as shown below.



The nitrogen is now even more electron withdrawing due to the partial positive charge. All the methyl and methylene protons in the rest of the structure are at higher δ than in (**95**) due to this effect, with the vinylic protons least affected. Due to the resonance structures protons 9-H and 11-H are shielded compared to 10-H with 9-H and 11-H therefore appearing at lower δ . The integral ratio for the aromatic region is 3:2. As (**98**) is formed the lone pair is now a bonding pair of electrons, there are no resonance effects, and the chemical shift values are more comparable with (**96**). 9-H and 11-H increase in chemical shift by δ 0.78 whereas 10-H is virtually unaffected.

The ^{13}C NMR spectra of these compounds does not show such large effects of aromaticity as the ^1H NMR although carbons 9-H and 11-H are shielded in (**97**).

3.5.3 Biological Evaluation

As expected the addition of the dibenzyl moiety to give (**94**) greatly improved on the *in vitro* results so far. Again the tetrasubstituted analogue was the most active with (**96**) having greater activity than (**94**). The aniline analogue (**98**) was comparatively inactive (**Figure 13**).

	activity $\mu\text{g ml}^{-1}$	$\text{LEC}_{50} \mu\text{M}$
tetraethyl 77	5-10	18-36
dibenzyl 94	1-5	3-15
dibenzyldiethyl 96	0.5-1.0	1.3-2.5
diphenyldiethyl 98	>100	>272
chloroquine 2	<1	<1.9

Figure 13 *In vitro* results for the aromatic compounds

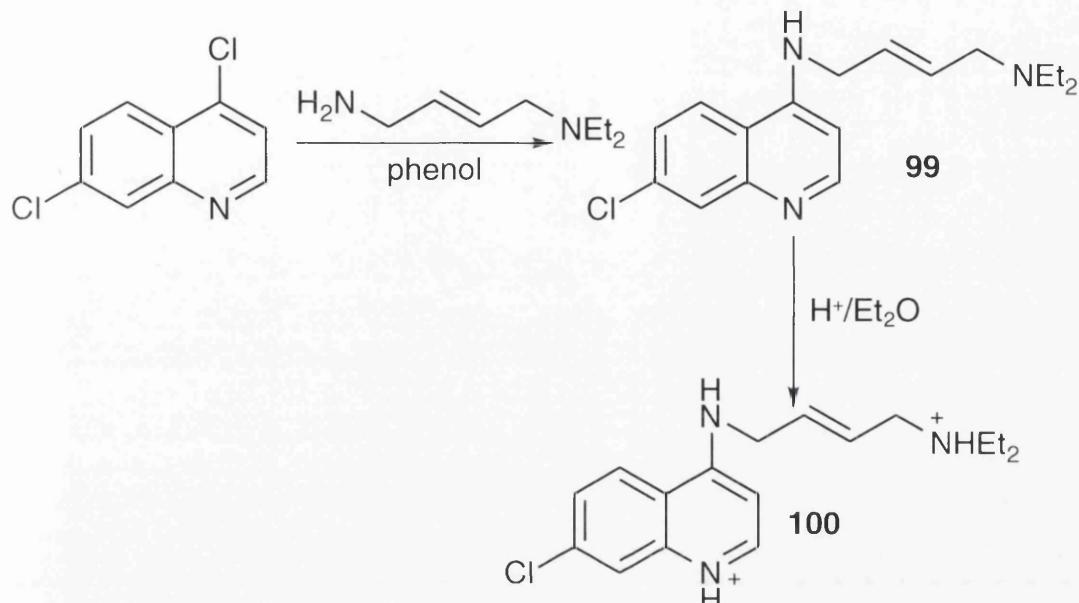
These results correspond well with those of Edwards⁷⁷ where the most active compounds reported were monoalkylated with benzyl groups on the terminal nitrogens. The result of the phenyl derivative was surprising as Edwards had found the benzyl and phenyl analogues to have very little variance in activities. It is tetrasubstituted but in our hands those analogues have always given the better activity. One consideration is how these compounds appear to the cell. Work on the importance of charge in cell recognition and its relation to antiproliferative activity proved that tetracationic species were transported better than dicationic species (**2.5.2**). With the nitrogen lone pair delocalised into the aromatic ring the nitrogen will not be as highly charged as aliphatic analogues and may not appear totally dicationic. This may result in lower uptake and therefore lower activity.⁶⁰

3.6 Quinoline-Polyamine Derivatives

3.6.1 Synthesis of Compound (**100**)

The final compound of this series was the quinoline derivative (**100**). This had been prepared in 1969 by the group of Singh¹⁰⁰ however the analytical data were incomplete. Synthesis is easy although not in great yield (25%) taking 4,7-dichloroquinoline and heating in phenol with diamine (**88**). The reported yield was only 32% to (**99**) so our yield to (**100**) was

reasonable (**Scheme 10**). This route is analogous to the preparation of chloroquine which may also be prepared by this route.¹⁰¹



Scheme 10 Preparation of the quinoline derivative

3.6.2 Biological Evaluation

Unsurprisingly the quinoline analogue had the best activity out of this range of putrescine analogues as shown in **Figure 14**.

	activity $\mu\text{g ml}^{-1}$	LEC μM
tetraethyl 77	5-10	18-36
dibenzyl 96	0.5-1.0	1.3-2.5
quinoline 100	0.05-0.005	0.13-0.013
chloroquine 2	<0.005	<0.009

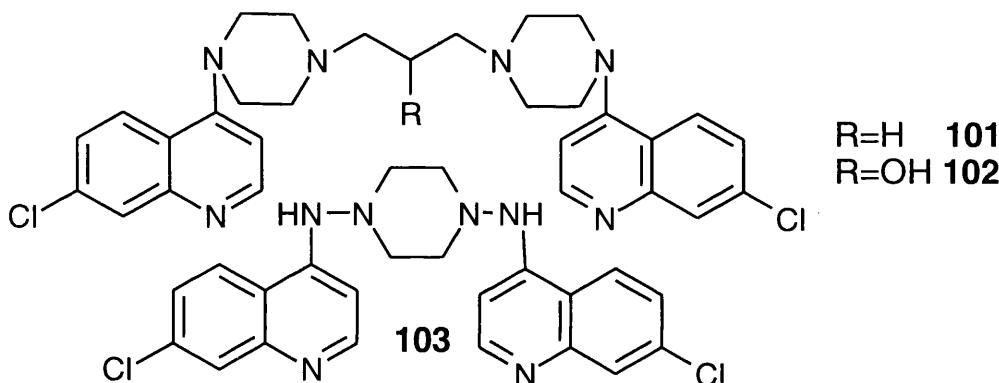
Figure 14 Comparison of *in vitro* activity

The group of Singh had tested (**99**) *in vivo* against *P. berghei* and found it to be active when administered at 40 and 80 mg kg^{-1} and curative at 160 mg kg^{-1} and higher. Our initial tests were on the dihydrochloride salt (**100**) carried out *in vitro* on *P. falciparum* where it had activity comparable with chloroquine.¹⁰⁰

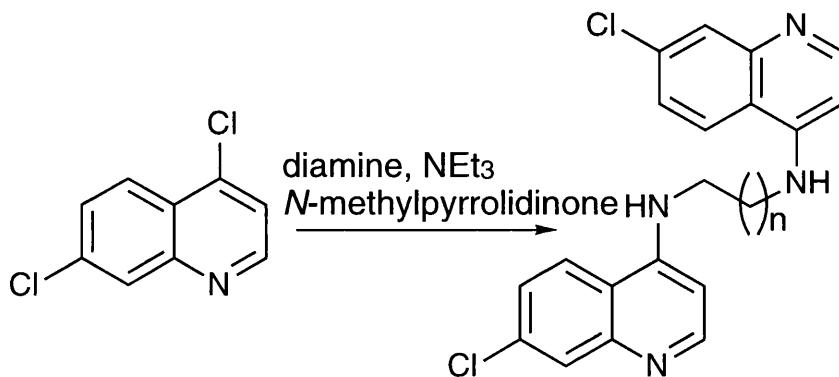
3.7 Attempted Preparation of Bisquinolines

3.7.1 Introduction

Quinolinemethanols such as quinine and mefloquine have some activity against chloroquine-resistant parasites; however there is a significant amount of cross resistance among quinoline-containing antimalarial drugs. A promising lead was reports of several bisquinolines that are active against chloroquine-resistant malaria. These include piperaquine (**101**), hydroxypiperaquine (**102**) and 1,4-bis(7-chloro-4-quinolylamino)piperazine (**103**).¹⁰²



A systematic study of bisquinolines had not been reported and a range of simple bisquinolines was prepared by Vennerstrom (**Scheme 11**). In the straight chain series the carbon bridge with two methylene units was the most active; however the greatest *in vivo* activity was observed with a central chain of *trans*-1,2-cyclohexyl.¹⁰² The diamine was heated with 4,7-dichloroquinoline in *N*-methylpyrrolidinone, a solvent which has been shown to improve the yields of these types of reactions.¹⁰³



Scheme 11 Synthesis of bisquinolines

Raynes *et al.* have also looked at some bisquinolines; however these were joined by bisamide links from the quinoline ring leaving the diethylaminobutyl side chain of quinoline intact (**Figure 15**). Two series of compounds were produced linked through the 6- or 8-position of the quinoline ring with the most active compounds being those linked through the 8-position. When n=4 and the substitution was in the 8-position the compound had 2-3 times greater activity than chloroquine against chloroquine-resistant strains of the parasite.¹⁰⁴

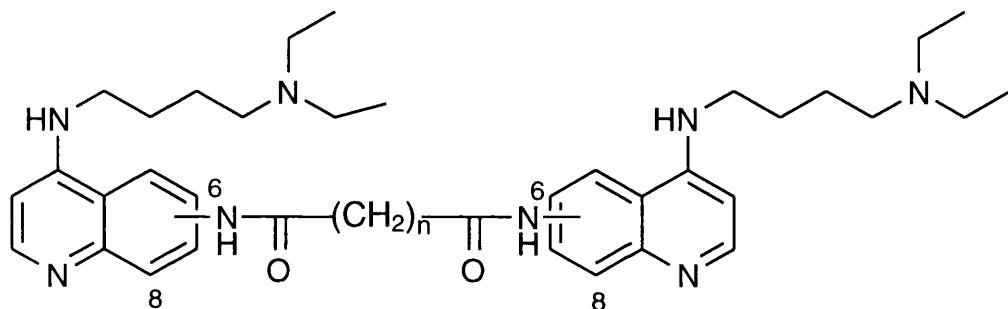
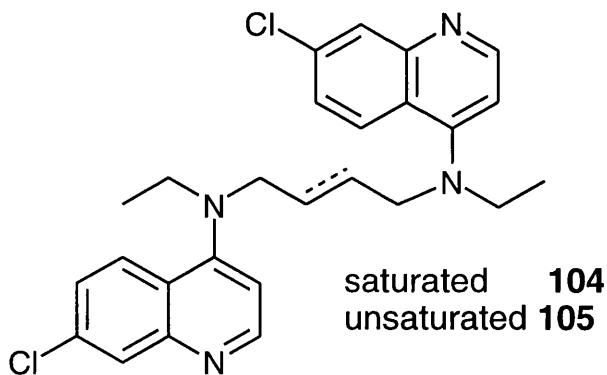


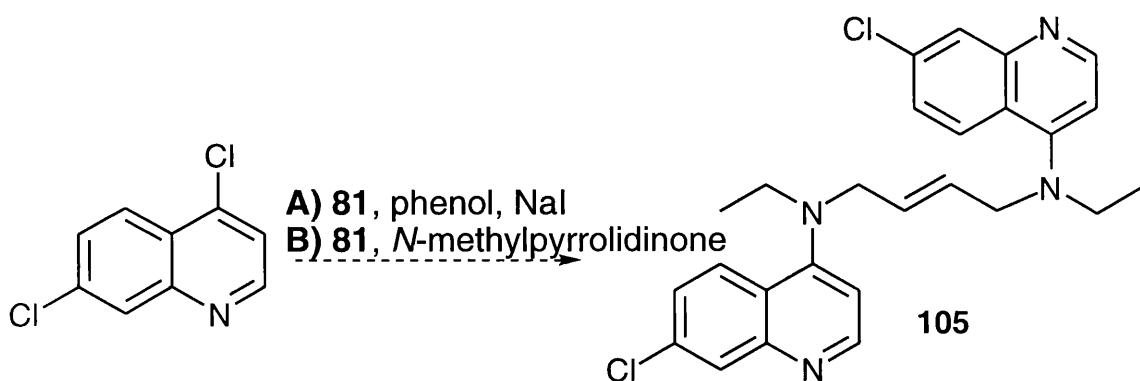
Figure 15 Bisquinolines linked from the 6- or 8-position

Our work was concerned with putrescine analogues so we decided to prepare two analogues (**104**) and (**105**).



3.7.2 Attempted Preparation of (**105**)

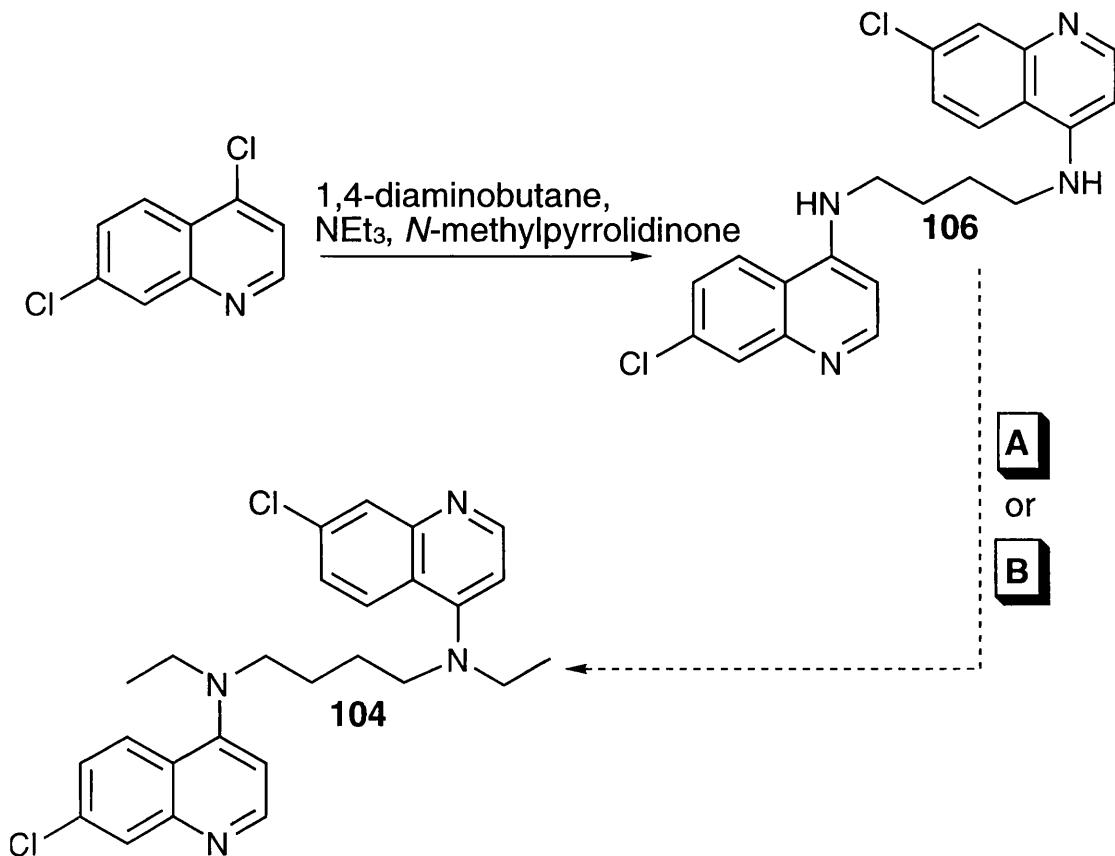
4,7-Dichloroquinoline was heated in phenol with (**81**) and sodium iodide crystals at 150-160 °C for 24 hours; however no product formation was observed. The reaction was also tried in *N*-methyl-2-pyrrolidinone which is reported as increasing the facility of these reactions; however only impure black product was obtained (**Scheme 12**).



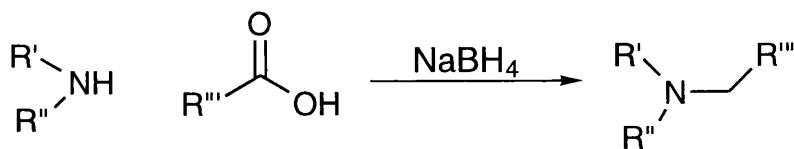
Scheme 12 Attempted preparation of (105)

3.7.3 Attempted Preparation of (104)

Due to the unsuccessful attempted preparation of (105) we decided to prepare bisquinoline (106) and then alkylate the nitrogen atoms. (106) was prepared according to the procedure of Vennerstrom in reasonable yield (55%) with spectroscopic data comparable with the literature values (**Scheme 13**).¹⁰² Alkylation of amines had been reported by two groups using sodium borohydride-carboxylic acid systems.^{105, 106, 107} Using acetic acid various aromatic amines had been alkylated in good yield (**Scheme 14**).



Scheme 13 Attempted preparation of bisquinoline (104)



Scheme 14 Alkylation of amines with carboxylic acids

Using route A we attempted to alkylate (**106**) using the chemistry reported by Marchini *et al.* who used sodium borohydride-carboxylic acid systems to alkylate a wide range of aliphatic and aromatic amines.¹⁰⁵ They assigned $\text{Na}[(\text{RCOO})_3\text{BH}]$ as the *N*-alkylating species. Sodium borohydride was added portionwise to glacial acetic acid at 0 °C and (**106**) was then added. The suspension was heated at 80 °C for two hours until a clear yellow solution was formed. After cooling to room temperature the precipitate was collected ; however ^1H and ^{13}C NMR spectra confirmed the isolation of starting material.

For route B followed the procedure of Gribble *et al.* who had also reported the same reaction, only adding the reagents in a different order and carrying out the reaction under nitrogen.¹⁰⁷ Gribble and co-workers had looked at the alkylation of indoles and aromatic amines; however using their chemistry only starting material was isolated.

Both groups had successfully alkylated diphenylamine with acetic acid; however we saw no evidence of alkylation with our quinoline using either set of reaction conditions. This may be due to the lack of nucleophilicity of the nitrogen we want to alkylate and their chemistry, in our hands, was not extendable to alkylation of quinolines. If these types of compounds were studied in the future possible alkylation methods for aromatic amines have been discussed by Barton and Mohri.¹⁰⁸

3.8 Biological Evaluation - *In vivo* Studies

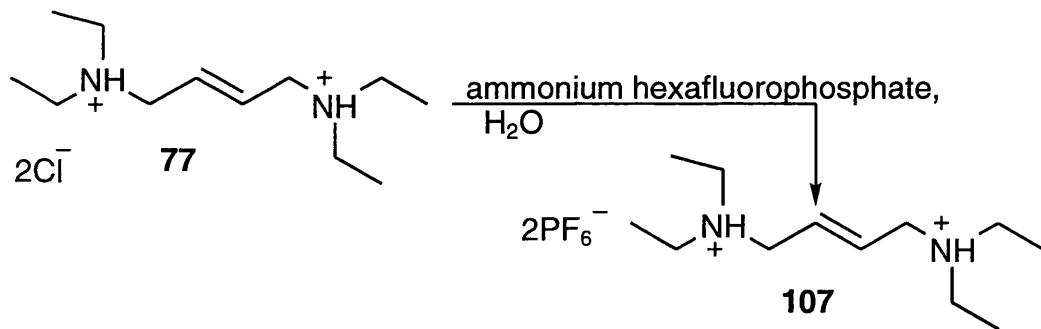
Five compounds were carried forward to *in vivo* testing, (**77**), (**92**), (**94**), (**96**) and (**100**), with chloroquine as a control. The compounds were tested in mice against a rodent malaria, *P. chabaudi*. Chloroquine at a dose of 50 mg kg⁻¹ was curative and no parasitaemia was detected in mice over 14 days. Our "lead" compound (**77**) delayed parasitaemia for a day, as did (**92**) when given at 100 mg kg⁻¹ or 300 mg kg⁻¹, but this did not show any significant antimalarial activity. Compound (**94**) was given at 150 mg kg⁻¹ in

three doses over 48 hours and had no activity but was toxic. (**96**) was given at 175 mg kg⁻¹ in two doses 24 hours apart and was inactive. The quinoline analogue (**100**) was given at 25 or 50 mg kg⁻¹ and was curative at the higher dose which compares well with chloroquine. This should be compared with the work of Singh¹⁰⁰ where (**99**) (free base of (**100**)) was curative at 160 mg kg⁻¹ on *P. berghei*.

These results were disappointing. None of our compounds had any activity *in vivo* and it was surprising that (**94**) was toxic. Edwards reported some toxicity with their spermine analogues but only when the central carbon chain had more than seven carbon atoms present. We have no explanation for the toxicity of (**94**). Again it was surprising that (**96**) had no activity *in vivo* after being so promising *in vitro*. The dibenzyl analogues prepared by Edwards are known to be debenzylated by polyamine oxidase and it has been speculated that this may be related to their activity as well as their ability to accumulate to high concentrations in the cell. If that was indeed important and our tetrasubstituted compounds could not be metabolised in such a way this may explain the lack of *in vivo* activity of (**96**).⁷⁷ This work has been submitted for publication.¹⁰⁹

3.9 Electron Density Studies on Compound (**107**)

As part of her physical chemistry PhD Dr Kirsty McCormack looked at the electron density over our "lead" compound (**77**).¹¹⁰ If the polyamine-biological anion interaction is important then it will depend on distribution of the 2+ charge on the cation. Hopefully results may allow us to design rationally future compounds that may be active. A recent paper by Cohen and co-workers described molecular modelling of the same sort of interactions on spermine phosphate hexahydrate.¹¹¹ To provide crystals suitable for the study was my responsibility. From the dihydrochloride salt growing crystals of the correct size proved difficult so we decided to try the bis(hexafluorophosphate) salt instead. This was known by a member of our group to produce a solid that was easily crystallized. The bis(hexafluorophosphate) salt precipitated out of water and crystals were grown from a solution of methanol/ether and obtained as colourless plates (**Scheme 15**).



Scheme 15 Ligand transfer to produce the bis(hexafluorophosphate) compound (107)

Unfortunately the hexafluorophosphate group was shown to be disordered at room temperature; but at the lower temperatures of the studies there was no significant dynamic disorder. A crystal structure was obtained (**Figure 16**) and this shows the bis(hexafluorophosphate) compound (107) to be centrosymmetric. The PF_6^- counter ion was disordered at room temperature with four of the F atoms spinning around the $\text{F}_2\text{-P-F}_4$ axis.

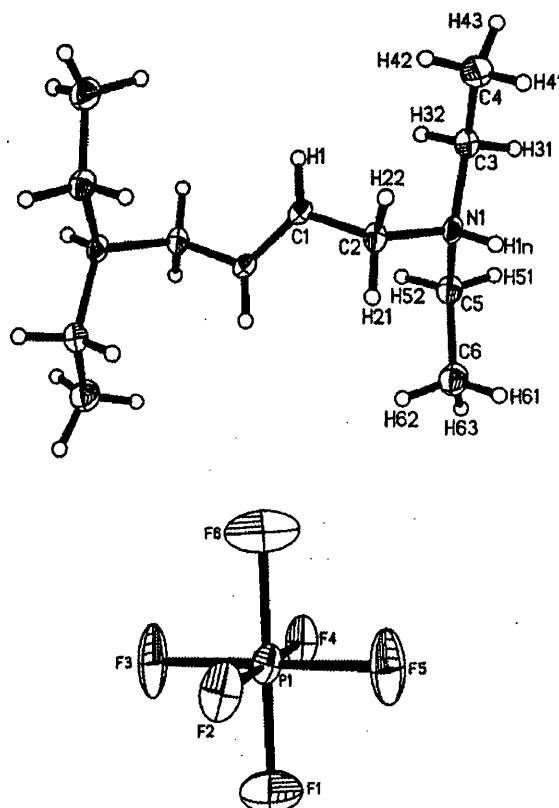


Figure 16 The crystallographic centrosymmetric structure of (107)

The work of Dr McCormack, both theoretical and experimental, showed that the carbon and nitrogen atoms carried partial positive charges whilst all the hydrogen atoms were slightly positive. This agrees with our NMR data when comparing the chemical shifts of the protons in free base and salt form i.e. (79) and (107) (**Figure 17**). In (107) each nitrogen carries a formal positive charge which acts as a powerful electron withdrawing group. The 2^+ charge of the dication is actually distributed over the hydrogen atoms which places them in a deshielded environment. This leads to a downfield shift in the NMR δ values, i.e. higher values of δ . The shift is largest for those hydrogen atoms on carbons α to the nitrogen and the effect diminishes for those β to the nitrogens. These results are consistently seen in NMR data for the amine salts (mostly hydrochloride) prepared throughout this thesis.

proton environment	79 (free base)	107 (ammonium salt)	Δ (ppm)
$\text{CH}_3\text{-CH}_2$	1.04	1.32	+0.28
$\text{CH}_3\text{-CH}_2\text{-N}$	1.54	3.22	+0.68
$\text{N-CH}_2\text{-CH}$	3.12	3.88	+0.76
CH	5.70	6.15	+0.45

Figure 17 NMR comparison for compounds (79) and (107)

On the basis of these results Dr McCormack showed that the negative charges on the carbons and nitrogens were diffuse and the overall electron density of (107) was positive shown by the solid lines (**Figure 18**). A model of the phosphate backbone of DNA was chosen (**Figure 19**) and data showed the electron density of the molecule to be negative (dotted lines). From this we may predict a strong interaction between the polyamine analogue (107) and the phosphate backbone of such molecules as DNA. This interaction is not site specific as all points on (107) have a large positive potential. Full details of this work have been published.¹¹⁰

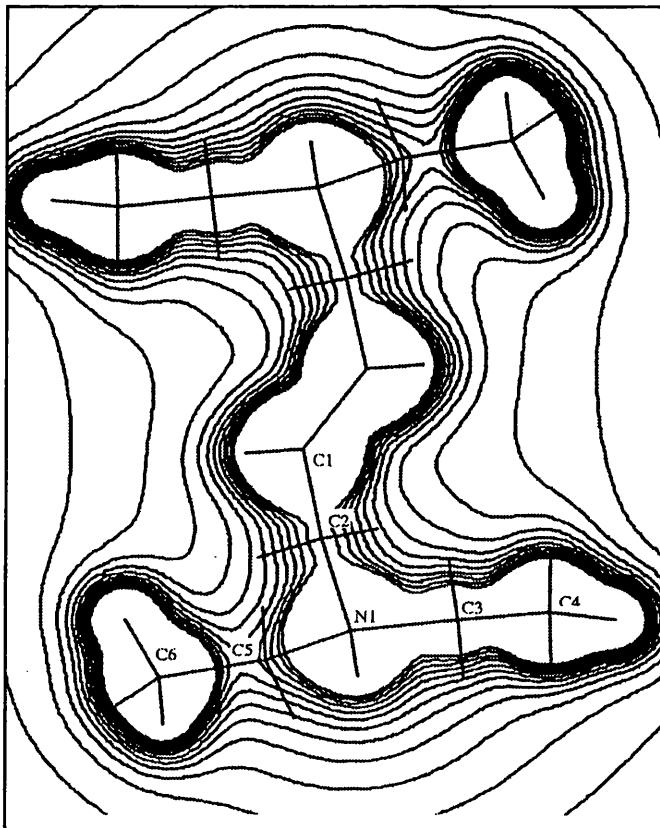


Figure 18 Map of the theoretical electrostatic potential of (107)

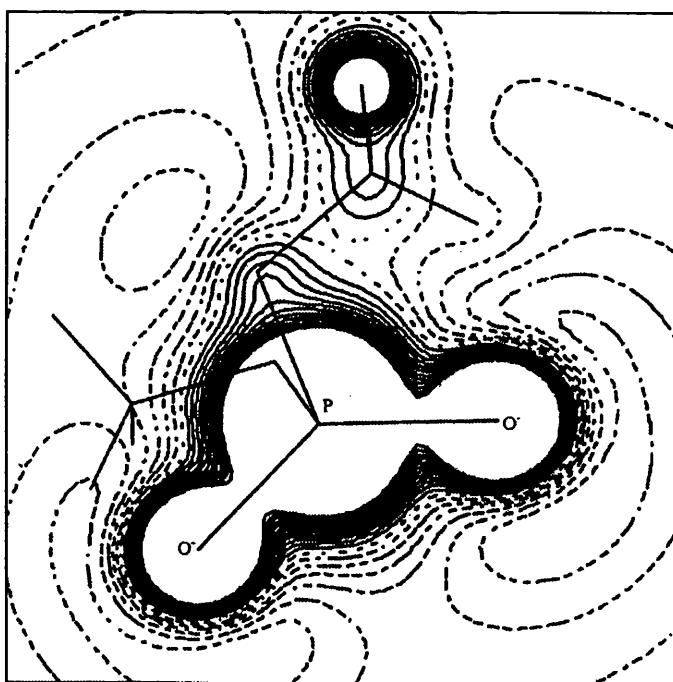


Figure 19 Map of the theoretical electrostatic potential of the model phosphate group

3.10 Summary

We started the project with our "lead" compound (**77**) and hopes of improving on its activity. We prepared a range of compounds varying the carbon chain and the *N*-alkylation patterns to give us an idea of the types of compounds that would be worth investigating further. It was not surprising that the dibenzyl analogues had the best *in vitro* activity; however the *in vivo* results were disappointing.

Looking again at previous work on therapeutic polyamines (spermidine or spermine), authors in the anti-cancer field had found di-alkylated analogues to be more active than the related putrescine compounds. We were looking to have strong interactions between our molecules and DNA and the spermidine analogues would have a 3⁺ charge whilst the spermine analogues would have a 4⁺ charge. This would give them a stronger interaction with DNA which might lead to improved activity. We decided to expand into synthesis of spermidine/spermine analogues to widen our biological test data and this chemistry is covered in the next chapter.

4

4 Investigation of Spermidine and Spermine Analogues

4.1 Introduction

In the last chapter we examined a range of putrescine analogues for antimalarial activity; however *in vivo* results were disappointing. We decided to extend our work to study some spermidine analogues. This idea was based on previous workers' observations in the cancer field that spermidine analogues were more active than corresponding putrescine analogues. The extra charge on the molecule is believed to enhance the activity due to improved binding properties.⁶⁴

Before the 1960s synthetic routes to polyamines were limited with few methods available for the selective modification of putrescine, spermidine, spermine and other polyamines. In the last few decades more literature was published, stimulated by the increased interest in this area. There are two major problems with functionalising polyamines: **1 attaching the group or groups to the correct nitrogen or nitrogens; and 2 having reactions that give reasonable yield.** For many years low yields were accepted as long as "some" of the right product was isolated.¹¹²

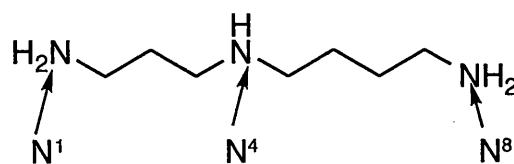


Figure 20 Numbering of spermidine and its derivatives

For ease of discussion the nitrogen atoms in spermidine are labelled as found in most older literature (**Figure 20**). Secondary amines are usually more basic than primary amines; however in spermidine they are not independent entities. Research has showed the primary nitrogens N¹ and N⁸

protonate in preference to N⁴, with N⁸ protonating first.¹¹³ N¹ and N⁴ are only three carbons apart and the electron withdrawing effects of each other cause a decrease in basicity and regioselectivity, of course, depends on these reactivity differences which are often difficult to exploit.³⁰ Over the years synthetic approaches have built up the desired polyamine using a combination of protecting group chemistry and selective reactions to construct the desired polyamines. In this chapter a wide range of chemistry used to produce polyamines will be highlighted.

4.2 Preparation of *N*-Alkyl Spermidine Analogues

Dr Carol McClintock in this group had prepared some *N*-alkyl spermidine analogues for anti-fungal testing.¹¹⁴ Previously, we had seen good antimalarial activity *in vitro* from compounds with good anti-fungal activity; therefore we decided to prepare a selection of these analogues for testing. Because these compounds are similar to spermidine they should be taken up by the cells efficiently and may have improved activity.

4.2.1 Synthesis of Spermidine Analogues (125), (126) and (127)

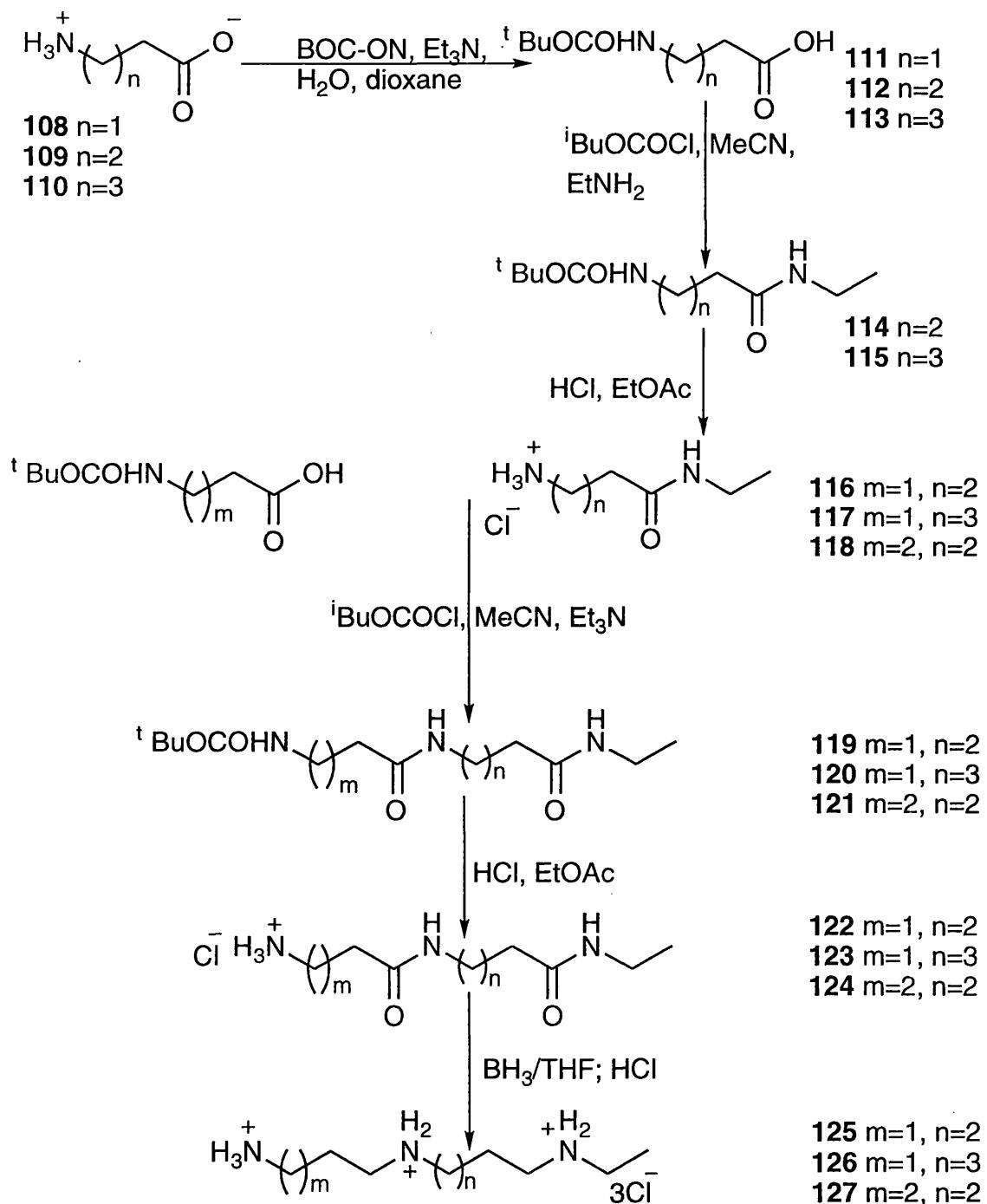
The synthetic route devised by Dr McClintock can be used to produce a wide range of compounds and is shown in **Scheme 16**. A number of key reactions are used:

- 1. Protection of amino acids as their *t*butylcarbamates (BOC) and subsequent deprotection**
- 2. Mixed anhydride formation of amides**
- 3. Borane reduction of amides.¹¹⁵**

We prepared three polyamines from this general reaction scheme which gave us (3,4) (**125**), (3,5) (**126**) and (4,4) (**127**) carbon skeletons. This would enable us to see the effect of altering the carbon backbone whilst holding the alkyl substituent constant. The ethyl group was chosen due to the success found with it in anticancer work and also our anti-fungal studies.

All steps went in yields comparable to those reported. One problematic stage was the BOC deprotections. The hydrochloride salts of the amides (**122**), (**123**) and (**124**) tended to be hygroscopic which led to problems with isolation. In many cases the salt was obtained as a sticky semi-solid which only on occasion would crystallise. The coupling reactions gave fairly low yields, although Dr McClintock had also reported problems

with this step. Sufficient quantities of (**125**), (**126**) and (**127**) were obtained for testing. Spectroscopic data were obtained and were consistent with literature values.

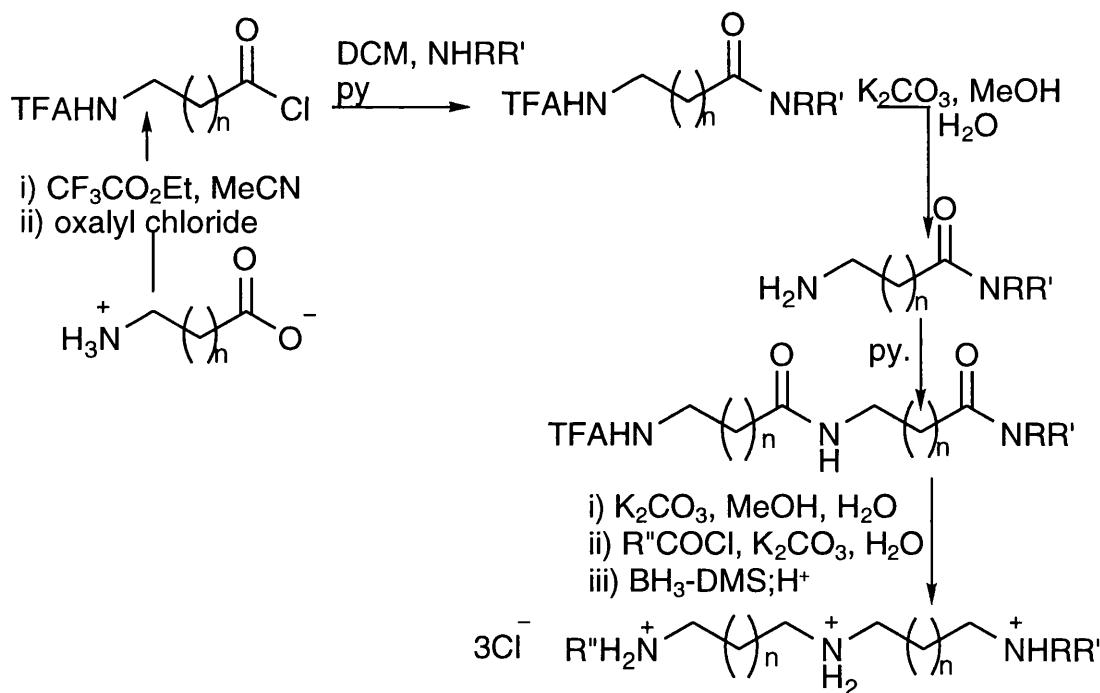


Scheme 16 Preparation of *N*-alkyl spermidine analogues (**125**), (**126**) and (**127**)

4.2.2 Other Reported Syntheses of *N*-alkyl Polyamines

A wide variety of chemistry is available to the synthetic chemist when building polyamines, especially an increasing number of protecting groups may be used.

Nordlander and co-workers studied the production of linear amines based on the formation of peptide bonds and subsequent reduction (**Scheme 17**).¹¹⁶ Rather than our approach of using carbamate protecting group chemistry the amino acids were converted into *N*-(trifluoroacetyl)amino acid chlorides by a combination of ethyl trifluoroacetate and then oxalyl chloride. The acid chlorides were coupled with amines to produce protected amides which were reduced using borane-dimethyl sulphide to give unsymmetrical putrescine analogues. The protecting group could also be removed and the free amine coupled with other *N*-(trifluoroacetyl)amino acid chlorides to give, after reduction, polyamides. This route leads to a potentially wide range of analogues.

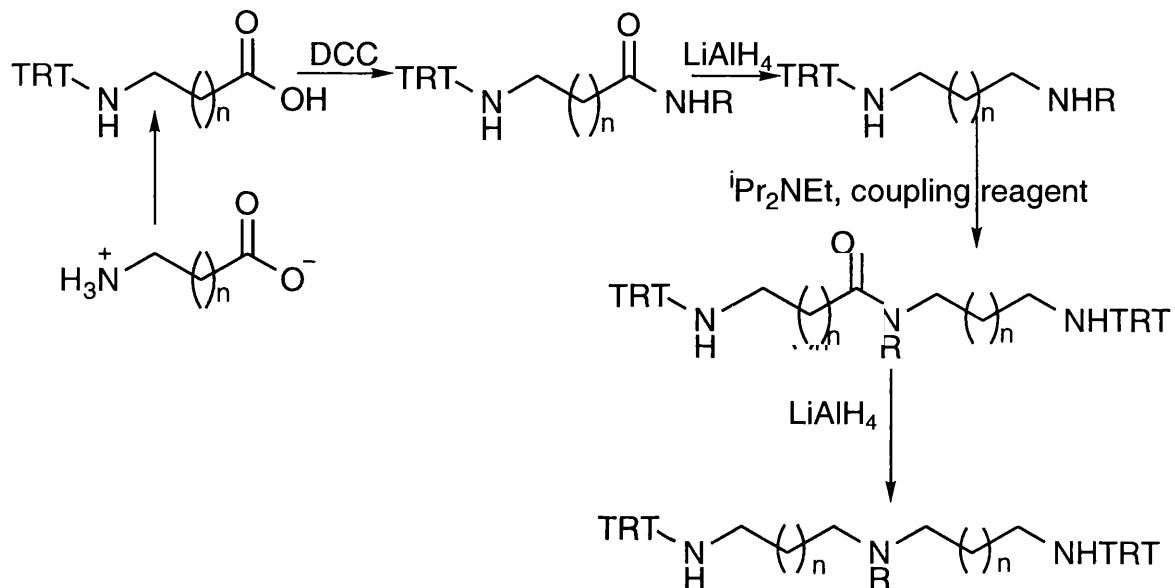


Scheme 17 Synthesis of polyamines

A similar approach was adopted by the group of Mamos who used the bulky triphenylmethyl (trityl, TRT) protecting group to protect their amino acids (**Scheme 18**).¹¹⁷ This protecting group was very useful as its hydrophobic and bulky nature facilitated work up and purification. The protected amino acid¹¹⁸ was coupled with various amines, using DCC, and

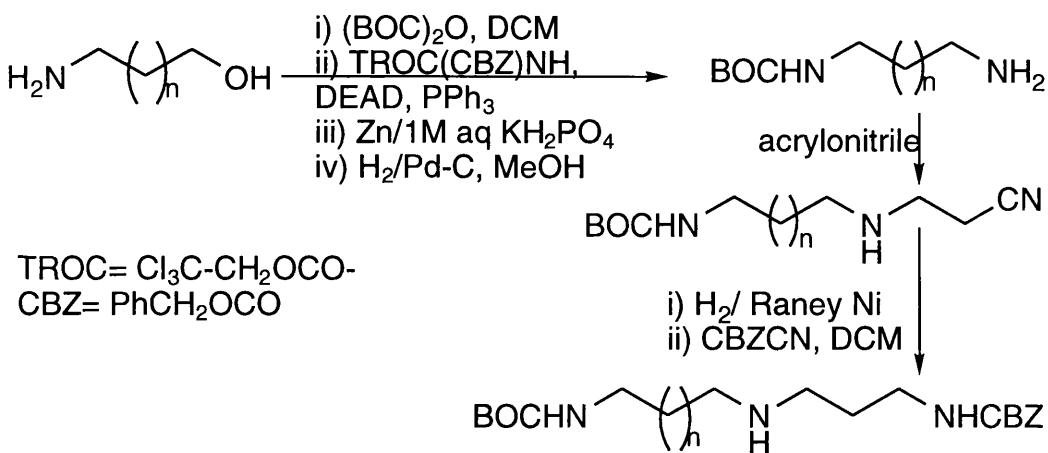
reduced by lithium aluminium hydride to form protected amines. These could be further coupled with protected amino acids to give amides which were again reduced to give a variety of spermidine analogues. Again this route may lead to a variety of products and is of a similar length to **Scheme 16**.

These approaches used similar ideas to our group as seen in **Scheme 16**, but with different choices of protecting group and a variety of reducing agents for the reduction of polyamides.



Scheme 18 Synthesis of polyamines

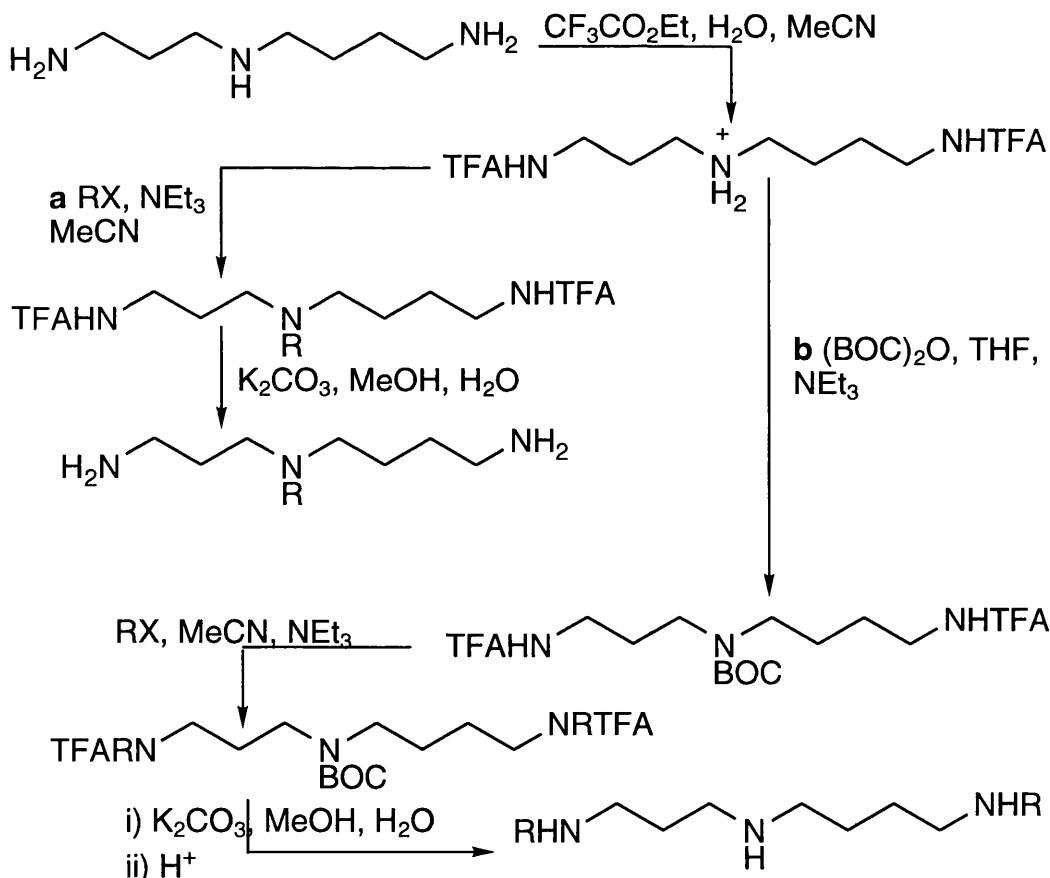
The group of Joao used carbamate protecting group chemistry similar to that shown in **Scheme 16** although functionalising amino alcohols, rather than acids (**Scheme 19**).¹¹⁹



Scheme 19 Preparation of polyamines

The aminoalcohol was BOC protected and the alcohol converted into a protected amine using the TROC (2,2,2-trichloroethyl carbamate) reagent under Mitsunobu conditions.¹²⁰ The protecting groups were removed to yield mono-protected diamines which were reacted with acrylonitrile. Protecting groups were added to produce compounds that could be further functionalised when desired.

The group of O'Sullivan had also been looking at alkylated spermidine derivatives for testing against parasitic diseases (**Scheme 20**).⁸¹ Spermidine was selectively protected on the primary nitrogens using ethyl trifluoroacetate, the secondary nitrogen was alkylated and the protecting groups were subsequently removed. This led to both primary (**b**) and secondary (**a**) functionalised derivatives in a low number of steps; however this route is only suitable where the carbon backbone is constant and the alkylating group changed. In order to produce analogues where the carbon chain varies the starting polyamine must be available.



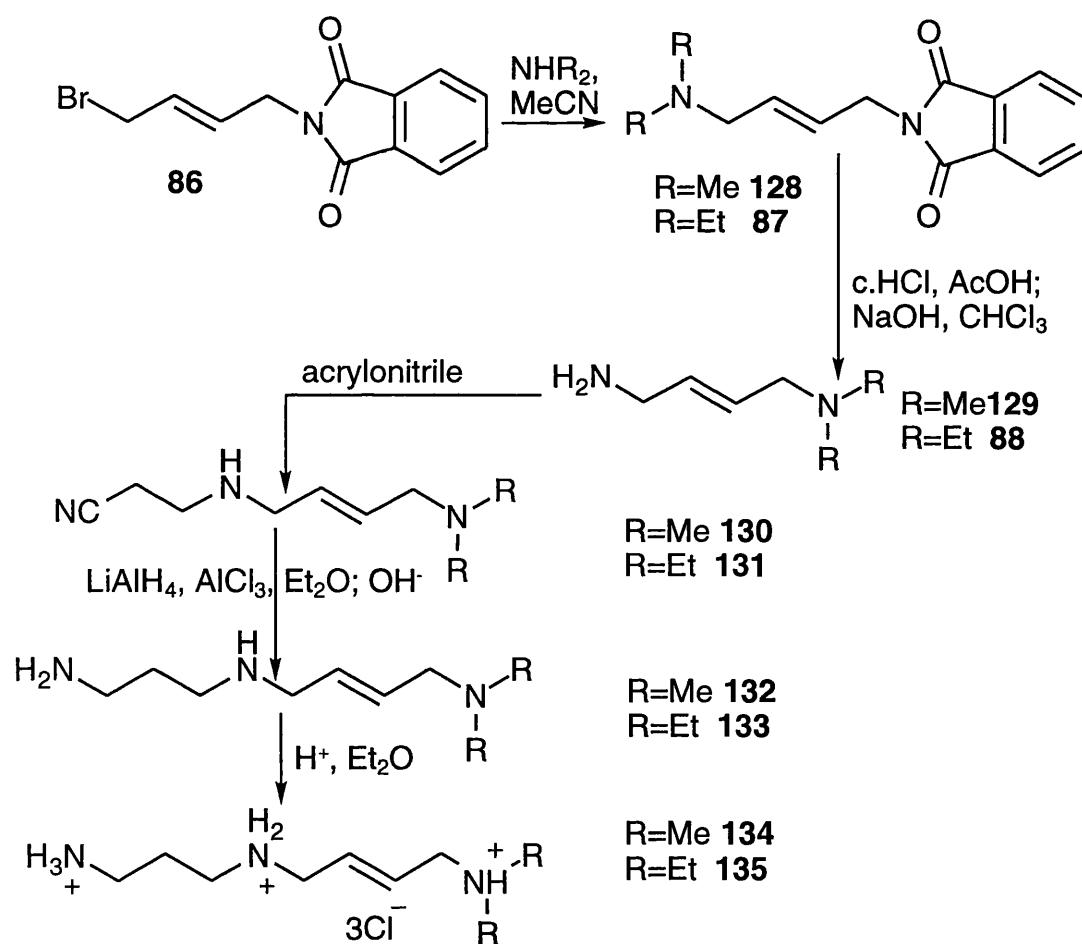
Scheme 20 Selective protection of spermidine in synthesis of its derivatives

4.3 Preparation of *N,N*-Dialkyl Polyamines

In tandem with the production of our *N*-alkyl spermidine analogues we decided to look at *N,N*-dialkyl polyamines. These would contain a free 3-aminopropyl unit and be disubstituted on N⁸.

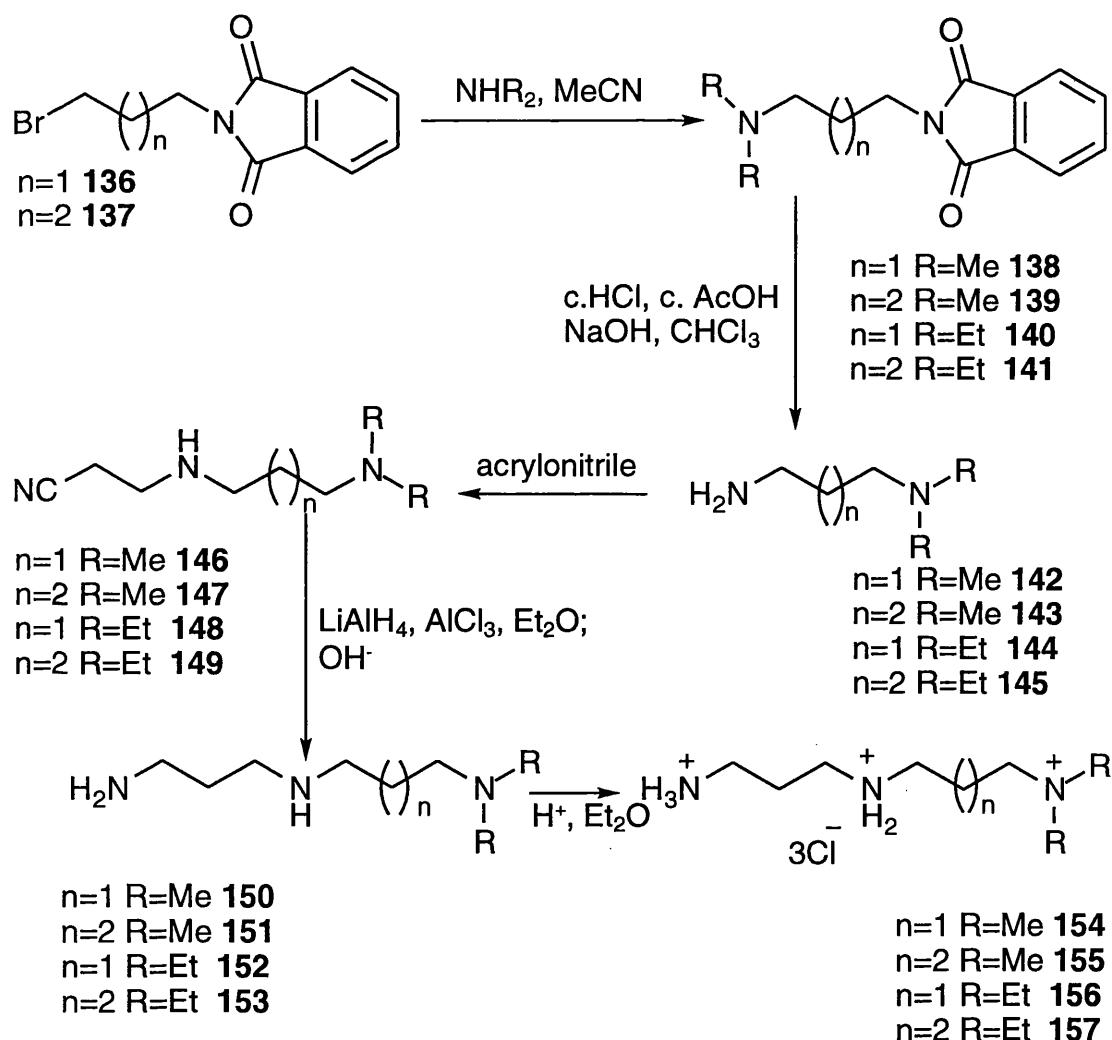
4.3.1 Synthesis of Saturated and Unsaturated Spermidine Analogues

The synthetic consideration was how to distinguish between the three available nitrogens of spermidine to functionalise where required. The approach of McClintock was fairly long with a number of low yielding steps and were designed for specific analogues. Our new approach used four key reactions: Gabriel synthesis; S_N2 displacement; Michael addition on acrylonitrile and lithium aluminium hydride reductions (**Scheme 21**, **Scheme 22**).



Scheme 21 Preparation of unsaturated *N,N*-dialkyl polyamines

The Gabriel synthesis has been well reviewed^{81, 121, 122, 123} and is widely used for the formation of primary amines, due to the excellent yields obtained and the variety of methods that may be used for removal of the phthalimide group.¹²⁴ In brief, it is the alkylation of a phthalimide anion with an alkyl halide and subsequent removal of the phthalimide group to leave a primary amine. We had access to unsaturated alkene (**86**) and the saturated phthalimides (**136**) and (**137**) shown in **Scheme 22** were commercially available. The use of the phthalimide group does limit the reaction to secondary amines as primary amines have a tendency to attack the protecting group; however we only wanted to use secondary amines so this was not a consideration.



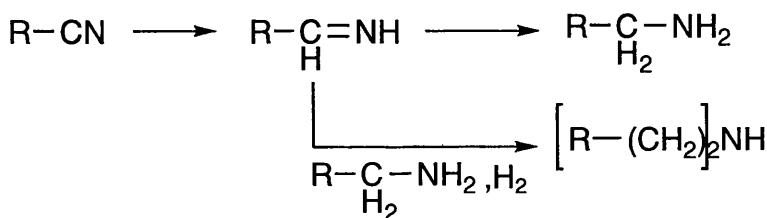
Scheme 22 Preparation of saturated *N,N*-dialkyl polyamines

The $\text{S}_{\text{N}}2$ reaction is widely reported in the literature, although there are problems associated with overalkylation of the halide when preparing primary and secondary amines. In general the order of reaction is $\text{I} > \text{Br} > \text{Cl}$

> F; however iodide ions can be added to improve the reactivity of other halogens.¹²⁵ We used the modification of potassium fluoride on Celite in our reactions.¹²⁶ The use of alkali metals in such reactions has been shown to promote *N*-alkylations in aprotic solvents by allowing the protic organic compound to form hydrogen bonds with fluoride ion. After acidic removal of the phthalimide group and basic work up the diamines, (129), (88), (142), (143), (144) and (145), were isolated in excellent yields.

The Michael addition involves the addition of amines to an activated carbon-carbon double bond using reagents such as acrylamide, vinylpyridines, acrylic acid and its esters and acrylonitrile.¹²⁵ The reaction on acrylonitrile is extremely useful in our work as after reduction it adds a 3-aminopropyl unit, one of the common substructures of polyamines.¹²⁷ The reaction can be controlled by using stoichiometric ratios of the reactants to give mono- and di-substituted 2-cyanoethylamines. Such a reaction was used by Israel *et al.* in the 1960s when preparing spermidine and spermine analogues.¹²⁸ Using the procedure of Israel *et al.* the diamine was stirred at 0 °C and acrylonitrile was added dropwise over 15 minutes. The reaction was gradually warmed until it was heated at 60-70 °C for 2 hours. This method was designed to prevent overalkylation of the nitrogen and provided excellent results with yields of (130), (131), (146), (147), (148) and (149) all over 60%. If necessary the amine could be purified by distillation; however temperatures lower than 120 °C were needed to prevent retro-Michael reactions and hence loss of product.

Reduction of the nitrile is not straightforward as hydrogenation leads to a mixture of products. The intermediate is an aldimine which can be reduced to give the primary amine or it can react further under reducing conditions to give secondary or tertiary amines.



In hydrogenation reactions the choice of catalyst is crucial with cobalt, nickel and ruthenium catalysts yielding primary amines; copper and ruthenium tend to give secondary amines; and platinum or palladium gives tertiary amine products. In the laboratory the most common reagent is lithium aluminium hydride or borane.¹²⁵ We chose lithium aluminium

hydride-aluminium chloride, for its ease of use and lack of reactivity with the carbon-carbon double bonds of (130) and (131). The use of aluminium chloride for aiding nitrile reductions is well documented and has been shown to increase the yield of such reductions.^{129, 130} This method has also been used by Bergeron and co-workers for the reduction of nitriles.¹³¹

The main problem with lithium aluminium hydride work up is the formation of a sticky precipitate which makes product extraction less efficient. This was overcome by the addition of strict quantities of water and 15% sodium hydroxide. For every n g of lithium aluminium hydride was added n ml of water, 3n ml 15% NaOH, and n ml water. This gave an excellent solid which was filtered and washed. In many cases the product of the reduction "stuck" to the aluminium and was hard to recover in good yields. A recent alternative that may be considered is the replacement of the aqueous work up with triethanolamine. This is reported to have improved yields.¹³² Widom and co-workers have also reported mild methods for extracting amines under neutral conditions.¹³³

The yields for the Michael addition and reduction are shown in **Figure 21**, with all but (133) formed in reasonable yield. The yield is related to the aqueous work up where, due to the hydrophilic nature of these compounds, some material is lost.

	nitrile yield (%)	product	reduction yield (%)
130	60	132	58
131	90	133	35
146	87	150	58
147	66	151	60
148	68	152	62
149	74	153	57

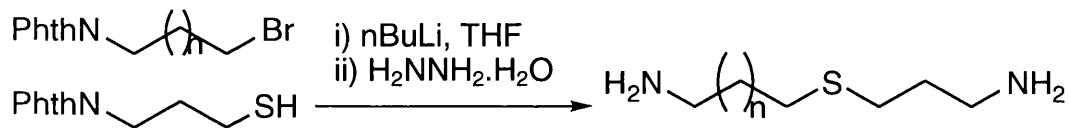
Figure 21 Comparison of yields for Michael addition and subsequent reduction

The increasing number of nitrogens in these compounds leads to problems in synthetic reactions. If the products are impure column chromatography is extremely difficult, due to amines "streaking" on silica and sometimes alumina, and distillation is sometimes problematic. Using the reactions shown in **Scheme 21** and **22** we obtained products in excellent yields and with high purity. The trihydrochloride salts were all formed by the

use of ethereal hydrochloric acid as discussed in chapter 3 and were stable enough to be handled for medium lengths of time in the air and were submitted for testing.

4.3.2 Other Reported Syntheses of Spermidine Analogues

Phthalimides had been utilised by Edwards and co-workers when preparing spermidine analogues for antitumour testing.⁶⁹ An appropriate bromophthalimide reacted with an aminosulphide, with the amine protected as a phthalimide. Removal of the protecting group with hydrazine resulted in spermidine analogues with the central nitrogen replaced by sulphur (**Scheme 23**).



Scheme 23 Sulphur-substituted spermidine analogues

The Michael addition was utilised by Israel and co-workers.¹²⁸ A variety of analogues was prepared using this chemistry (**Scheme 24**).



Scheme 24 The use of acrylonitrile for polyamine synthesis

4.3.3 Analytical Data on *N,N*-Polyamines

It was easy to confirm that a diamine, such as (**88**), had undergone Michael addition due to the nitrile absorption in the IR spectrum and its CN peak in the ¹³C NMR spectrum. This is exemplified by **Figure 22** below, showing the IR spectrum of (**131**). The nitrile resonance is observed as a sharp peak at 2250 cm⁻¹, which is common to all these adducts. In this case we also observed the C-H absorption characteristic of a *trans*-carbon-carbon double bond, seen at 976 cm⁻¹.

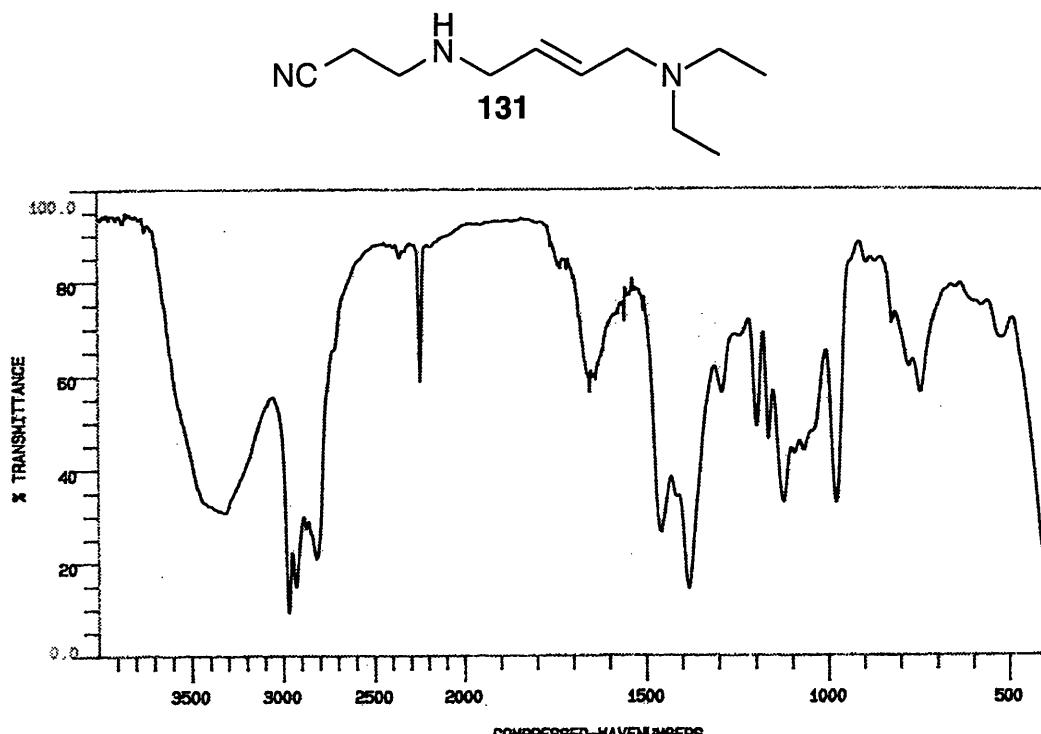


Figure 22 IR spectrum of compound (131), neat solution

The ^1H NMR data of polyamines are often inconclusive as many signals overlap whereas the ^{13}C NMR data are usually more useful as the types of carbons present are easily identified. The ^1H NMR (**Figure 23**) and ^{13}C NMR spectra (**Figure 24**) of (148) show the features common to this group of compounds. The ^1H NMR spectrum shows the expected quartet and triplet for the ethyl groups and various multiplets for the aminopropyl unit. The methylene protons α - and β - to the nitrile appear as triplets, coupled to each other, and this is characteristic of the 2-cyanoethyl subunit.

The ^{13}C NMR spectrum shows the aliphatic region where DEPT analysis identified all peaks as CH_2 apart from the highest field CH_3 . The quaternary nitrile peak is easily identifiable, occurring at δ 118.6. After the reduction the nitrile peak disappears from both IR and ^{13}C NMR spectra and the ^{13}C NMR spectrum shows an extra methylene peak.

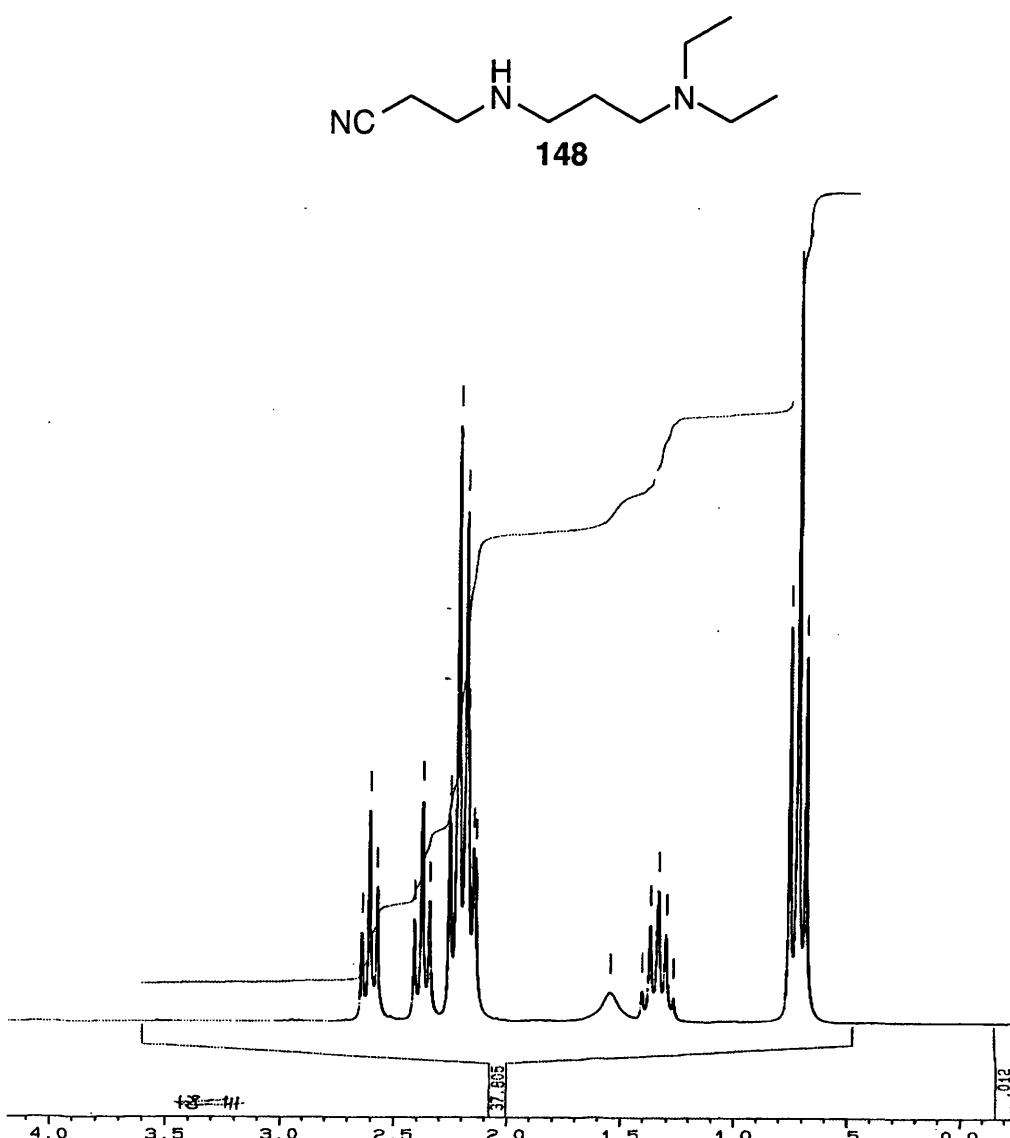


Figure 23 ^1H NMR spectrum of compound (**148**), 200 MHz, CDCl_3

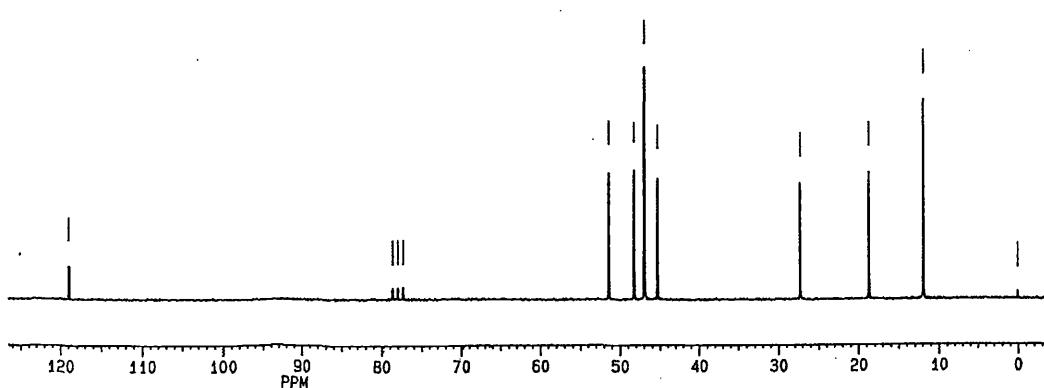


Figure 24 ^{13}C NMR spectrum of compound (**148**), 200 MHz, CDCl_3

A comparison of ^{13}C NMR nitrile resonances and IR absorptions can be seen in **Figure 25** with all values extremely similar.

	^{13}C NMR (ppm)	IR absorption (cm^{-1})
146	118.4	2250
147	117.7	2250
148	118.0	2250
149	118.6	2247
130	118.8	2250
131	118.6	2247

Figure 25 Comparison of nitrile peaks in IR and ^{13}C NMR spectra

The mass spectra of amines are not always particularly useful especially with the hydrochloride salts where FAB or Cl/NH₃ must often be used to identify molecular ions. Primary fragmentation of the molecular ion is favoured and amines fragment as shown in **Figure 26**. An example of a typical fragmentation pattern is shown below in **Figure 26** for compound (149). The lower molecular mass peaks seen in the spectrum are typical of most polyamines.¹³⁴

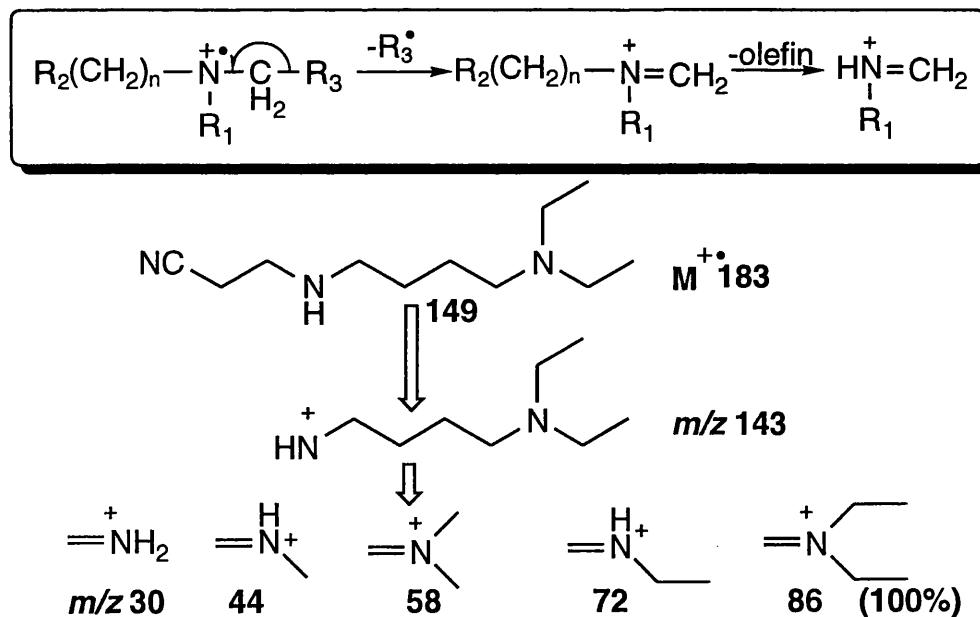


Figure 26 Typical mass spectrum fragmentation of a polyamine

Analytical data for polyamines are usually conclusive, although microanalysis is not often possible due to the hygroscopic nature of most of the salts prepared.

4.3.4 Biological Evaluation

Antimalarial Activity

The *N*-alkyl and *N,N*-dialkyl polyamines were tested *in vitro* against *P. falciparum* and the results are shown in **Figure 27**.

	activity ($\mu\text{g ml}^{-1}$)	LEC (μM)
3,4- <i>N</i> -ethyl 125	>1000	3540
3,5- <i>N</i> -ethyl 126	>1000	3370
4,4- <i>N</i> -ethyl 127	500	1690
3,4-unsat- <i>N,N</i> -dimethyl 134	500	1780
3,4-unsat- <i>N,N</i> -diethyl 135	500	1620
3,3-sat- <i>N,N</i> -dimethyl 154	100	370
3,3-sat- <i>N,N</i> -diethyl 155	>1000	3540
3,4-sat- <i>N,N</i> -dimethyl 156	1000	3370
3,4-sat- <i>N,N</i> -diethyl 157	10	30
chloroquine 2	<1	<1.9

Figure 27 *In vitro* activity of spermidine polyamines

The biological data were disappointing. Polyamines **(125)**, **(126)**, **(155)** and **(156)** were essentially inactive. Within the *N*-ethyl series the most active compound had a 4,4 carbon chain **(127)** although it showed no significant activity. The unsaturated analogues **(134)** and **(135)** had virtually no activity, which was in contrast to the putresine results where the (*E*)-1,4-diaminobut-2-ene moiety improved activity. Comparing the *N,N*-dialkyl saturated analogues there is no consistent structure-activity relationships: 3,3 dimethyl > 3,3 diethyl; 3,4 diethyl > 3,4 dimethyl. The most active compound of this group is **(157)**; diethyl substituted with a 3,4 carbon chain and with a LEC similar to that of our original lead compound **(77)** of 18-36 μM . We did not prepare any *N*-alkyl unsaturated analogues due to disappointing data for **(134)** and **(135)**.

Previous work by Porter had seen success when spermidine analogues were tested as antitumour agents. N^1,N^8 - Spermidines had activity *in vitro* against L1210 cell lines.⁶³ The N^1,N^8 -derivatives decreased all polyamine pools; in contrast to DFMO which did not affect the spermine pool. We have no information on the ability of our *N,N*-dialkyl polyamine analogues to affect polyamine pools.

We can draw no logical conclusions from our results, other than the fact that the compounds were disappointing in activity and no correlation can be drawn between the variation of the carbon chain or the *N*-substitution patterns.

It was decided to take the most active analogue (**157**) and prepare some derivatives containing a benzyl group to see if this improved the activity.

Antifungal Activity

Spermidine is the predominant polyamine in fungi and perturbation of spermidine function may be effective at reducing fungal growth. Commercially available spermidine analogues have been shown to control powdery mildew on barley under experimental conditions. Two of our spermidine analogues (**125**) and (**126**) were tested for anti-fungal activity by Dr D. R. Walters, Scottish Agricultural College, Auchincruive, with the full results published.¹³⁵

Greatest control was achieved using 1.0 mM of (**125**) which reduced rust infection of broad bean plants by 77.6%. Analysis was done to determine the effects of (**125**) and (**126**) on fungal polyamine biosynthesis and to look at the incorporation of radiolabelled ornithine into polyamines in fungus grown in the presence of the analogues. Neither compound had any effect on the incorporation of radio-labelled ornithine into polyamines, which suggests that fungal biosynthesis was unaffected by (**125**) and (**126**). These results are in contrast to tests on norspermidine which was shown to perturb polyamine biosynthesis in potatoes. In view of the antifungal activity of these compounds more detailed studies on their effects on polyamine metabolism are needed.

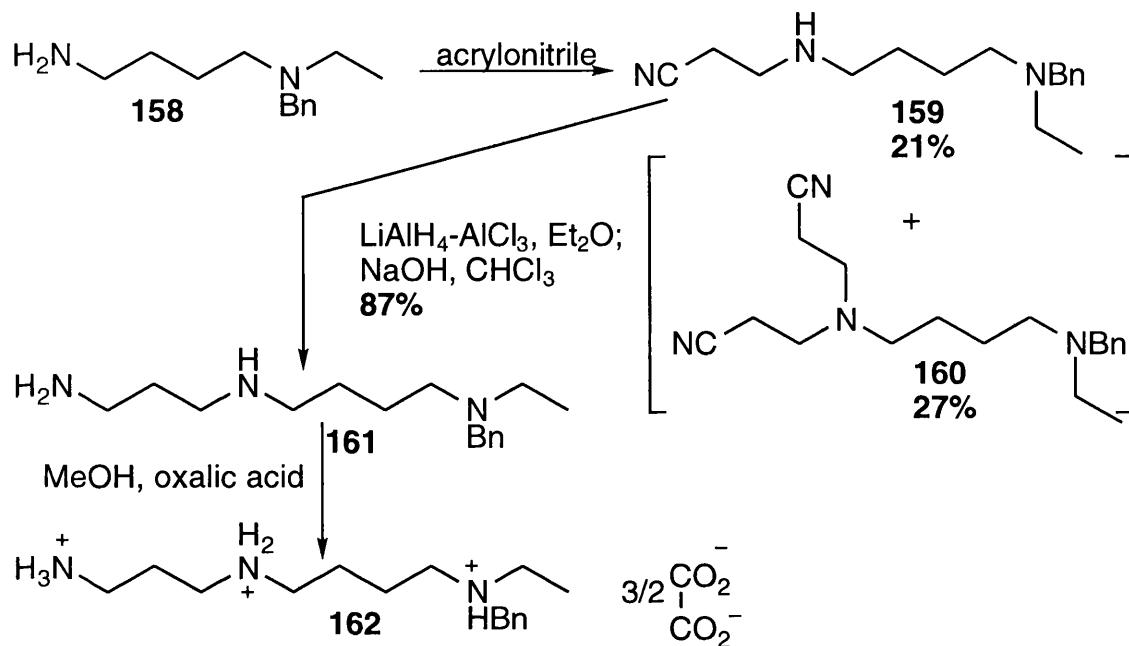
If these analogues are unable to perturb polyamine biosynthesis this may explain the lack of activity observed in our *in vitro* tests on *P. falciparum*. Certainly in other work the ability to disturb polyamine biosynthesis has been crucial for activity against antiproliferative diseases.^{1, 63}

4.4 Benzyl Substituted Spermidine Derivatives

There are three possible places that the benzyl group could be placed in (**157**) and therefore we have three target polyamines (**162**), (**165**) and (**167**).

4.4.1 Preparation of Compound (162)

Scheme 25 shows formation of (162) by the same reactions used to form the *N,N*-dialkyl spermidine analogues (Scheme 22).

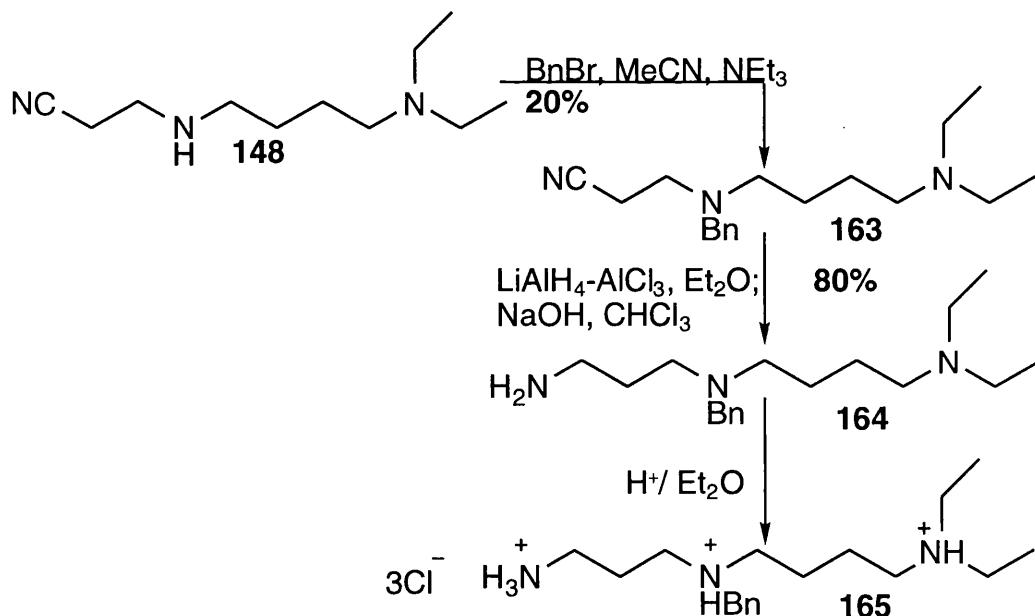


Scheme 25 Formation of spermidine derivative (162)

The diamine (**158**) was prepared from phthalimide (**137**) displacing with *N*-ethylbenzylamine. The Michael addition did not go cleanly as before with the desired nitrile (**159**) formed in only 21% yield and the dialkylated product (**160**) was isolated in 27% yield. This may be due to the viscous nature of (**158**) and stirring in a dilute solution of methanol or ethanol may improve this step. The mixture was separated by silica column chromatography with ethanol as eluent. Reduction went in good yield and the trihydrochloride salt of (**161**) was formed; however it was extremely hygroscopic and formed only as an orange thick oil. At this point we decided to examine an alternative salt for those hydrochlorides that were troublesome. Formation of the benzoate salt of (**161**) was attempted by stirring the free amine with benzoic acid in benzene; however no precipitation occurred. The same problem was observed when attempting to prepare the succinate salt. The use of oxalic acid is well known and has been previously used for formation of polyamine salts.¹¹⁹ The triamine was dissolved in methanol and a solution of oxalic acid in methanol was added. Precipitation occurred immediately and the solid could be recrystallised to yield (**162**).

4.4.2 Preparation of Compound (165)

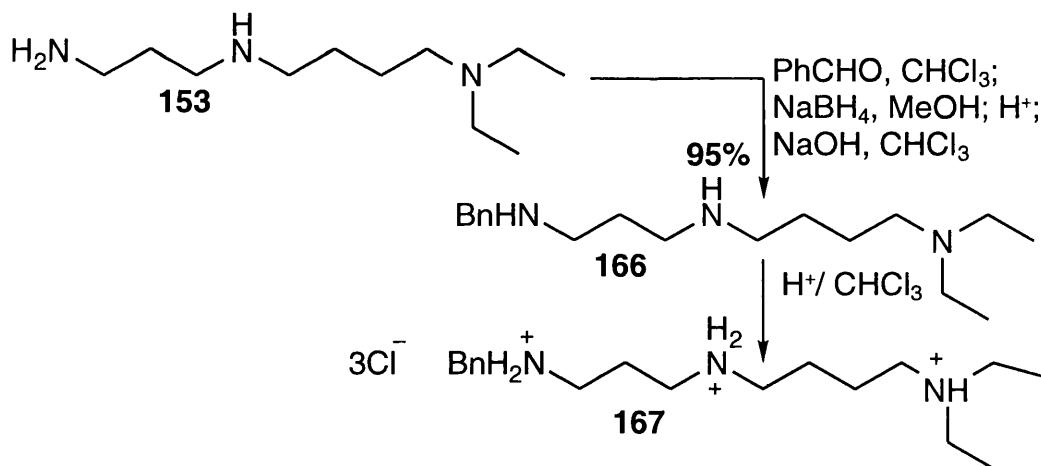
It is extremely difficult to functionalise a secondary nitrogen in the presence of the sterically more available primary nitrogen, so alkylation of N⁴ in the presence of N¹ was not attempted. The nitrile (148) contains the 3-aminopropyl unit effectively "hidden" as a 2-cyanoethyl moiety and was used to overcome this problem (**Scheme 26**). Alkylation of (148) was achieved with benzyl bromide in acetonitrile and triethylamine. The same problem of overalkylation occurred as in 3.4 and (163) was isolated in 20% yield. The reaction could be repeated in the presence of Hunig's base to try and prevent quaternary salts forming. Mohri *et al.* have also looked at this problem and have shown that tertiary amines can be prepared from secondary amines and the alkyl halide in the presence of potassium hydride and triethylamine in good yields.¹³⁵ Reduction of the nitrile in the usual manner afforded (164) and this was converted into the trihydrochloride salt. This was also hygroscopic; however the product was not obtained in sufficient yield to attempt further preparation of salts. It is interesting to note that the hydride reductions of (159) and (163) gave higher yields than those for the aliphatic triamines, the values being nearer 80% than 60%. This is probably due to the hydrophobic group present on these compounds which makes them more organic soluble and easier to extract during work up.



Scheme 26 Preparation of spermidine derivative (165)

4.4.3 Preparation of Compound (167)

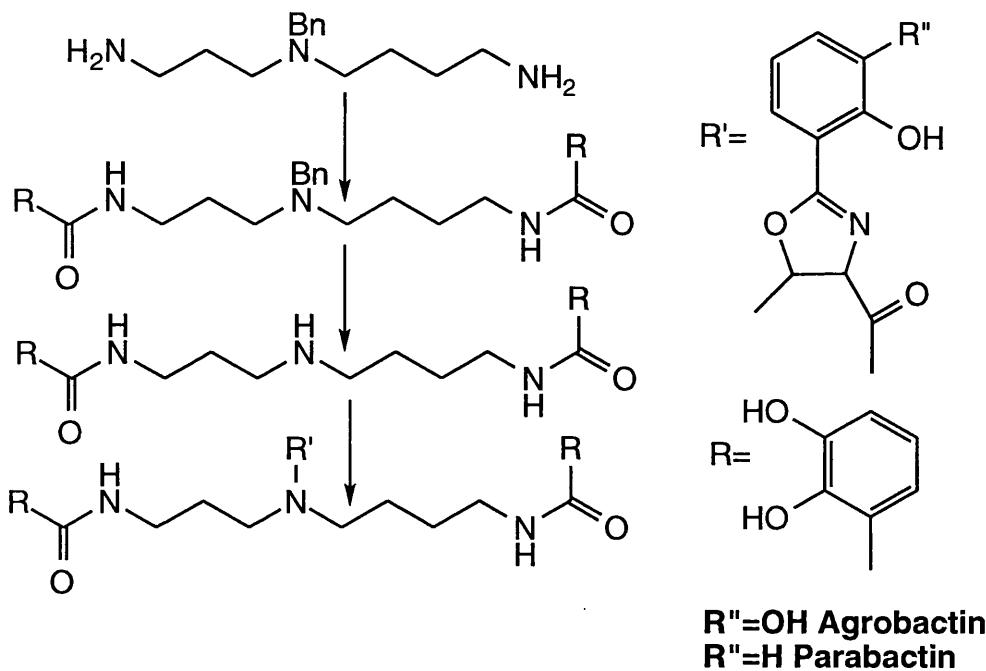
Reductive alkylation has been used widely in the alkylation of amines and has been widely reviewed.^{122, 136} It is a popular and longstanding route to amines, due to the high yields usually obtained and the ease of reaction. In this case alkylation must occur only at the primary nitrogen and work by Martell and co-workers had shown this was possible to achieve without protecting the internal nitrogens.¹³⁷ We used the chemistry of Sclafani *et al.* who had looked at selective alkylation of the primary nitrogens in polyamines with aromatic aldehydes.¹³⁸ Heating (**153**) at reflux in chloroform (dilute conditions) with 1 equivalent of benzaldehyde gave the imine in 2 hours which was not isolated but reduced with sodium borohydride in methanol using standard conditions. (**166**) was formed in excellent yield (95%) with no evidence of alkylation on the secondary amine (**Scheme 27**); presumably the primary amine of (**153**) is sterically more available than the secondary amine and reacts in preference with benzaldehyde. The trihydrochloride salt (**167**) was isolated as a cream solid.



Scheme 27 Formation of spermidine derivative (**167**)

4.4.4 Synthesis of *N*-Benzyl Spermidine (**173**) and Homologue (**175**)

So far we had only considered one compound with the benzyl on the secondary nitrogen (**165**). Bergeron and his group were interested in the formation of polyamine catecholamide iron chelators, including parabactin and agrobactin. They had been looking at the formation of *N*-benzylated triamines for use in the selective acylation of polyamines leading to the formation of these compounds (**Scheme 28**). This chemistry also led into other selectively *N*⁴-acylated compounds.¹¹²

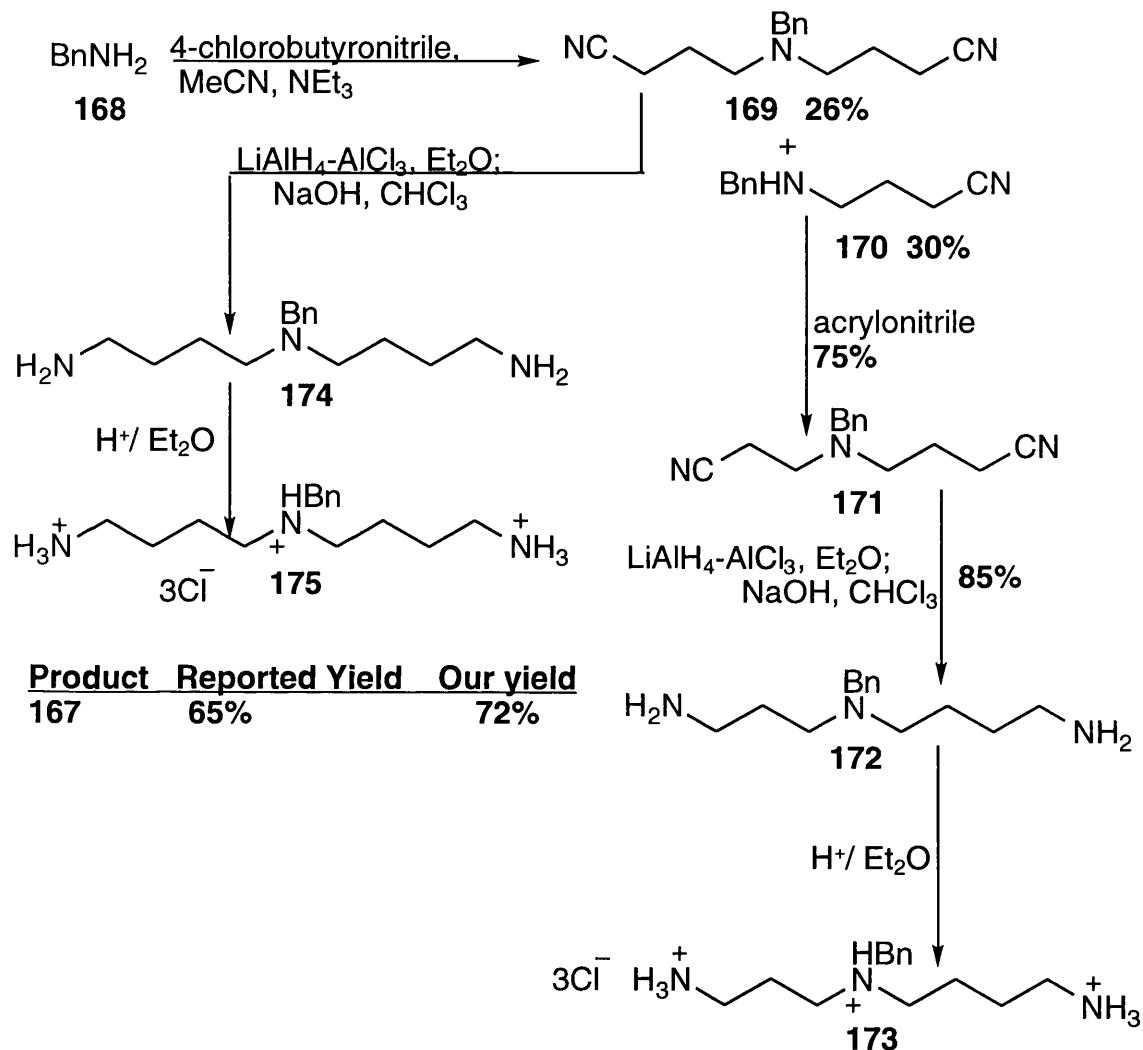


Scheme 28 Synthetic routes to parabactin and agrobactin

We were interested in the variety of *N*-benzyl compounds the group had prepared. We could then investigate the importance of the various nitrogens and carbon chains on antimalarial activity. The 3,4 (**173**) and 4,4 (**175**) analogues were prepared following the principles of Bergeron and co-workers (**Scheme 29**).^{112, 131, 139, 140}

Benzylamine was alkylated with 4-chlorobutyronitrile in acetonitrile and triethylamine. This differs from the approach of Bergeron *et al.* who alkylated in the presence of butanol and sodium carbonate. The alkylation is accompanied by formation of the mononitrile (**170**) as well as the desired dinitrile (**169**); however purification was achieved using silica gel chromatography with ethanol as eluent. This problem was also reported by Bergeron *et al.* who proposed that a number of equilibria were controlling the reaction. The mononitrile (**170**) however could be further alkylated with acrylonitrile and purified in the same manner to yield dinitrile (**171**). This gave us the two desired dinitriles which were reduced with lithium aluminium hydride to yield the 3,4 (**172**) and 4,4 (**174**) *N*-benzyl polyamines. Bergeron *et al.* chose to reduce the nitriles using hydrogen over Raney nickel, in ethanol, in the presence of sodium hydroxide. In a previous communication Bergeron had used lithium aluminium hydride for the same reduction and we followed this method, but using our basic work up.¹³¹ The mixed salt that precipitated from the reduction was, in our case, washed with methanol to ensure maximum recovery of the triamines. Occasionally, after

concentrating *in vacuo*, some salts still remained. The triamine (**172**) or (**174**) was then taken up in small amount of DCM and the unwanted salt removed by filtration. After production of the trihydrochloride salts (**173**) and (**175**) these compounds were submitted for testing.



Scheme 29 Preparation of *N*-benzylspermidines (**173**) and (**175**)

4.4.5 Biological Evaluation

The *in vitro* results for the benzyl polyamines are shown in **Figure 28**. No data are available on compound (**165**).

Compound (**167**) with the benzyl on the primary nitrogen did show slightly improved activity compared to (**157**). Neither of the compounds prepared following the work of Bergeron *et al.* had better activity than (**167**) and we found that the 3,4 carbon chain analogue was the most active. This may be related to the extra hydrophobic nature of the compound and this agrees with the work of Edwards *et al.* on spermine analogues where

terminal benzyl groups improved activity. It is speculated that the benzyl group is quickly removed by metabolism and that this action is related to the compounds activity.⁷⁷

	activity ($\mu\text{g ml}^{-1}$)	LEC (μM)
3,4-sat- <i>N,N</i> -diethyl 157	10	32
<i>N</i> -ethyl- <i>N</i> -benzyl 162	1	2.5
<i>N,N</i> -diethyl-2° <i>N</i> -benzyl 165	-	-
<i>N,N</i> -diethyl-1° <i>N</i> -benzyl 167	5-10	12-24
3,4- <i>N</i> -benzyl 173	10	60
4,4- <i>N</i> -benzyl 175	50	140
chloroquine 2	<0.1	<0.19

Figure 28 *In vitro* results for benzyl spermidine compounds

There is little in the literature on the biological activity of unsymmetrically substituted spermidine analogues; however the work of Porter⁶⁴ showed symmetrical *N*¹,*N*⁸-analogues did have better activity against L1210 cell lines than *N*⁴-alkylated derivatives of spermidine. The relatively lower activity of (**173**) and (**175**) may be related to the inability of these analogues to affect polyamine pools and (**173**) was evaluated by Porter against L1210 cell lines and had only negligible activity. The 3,4 carbon chain analogue (**173**) is more active than (**175**) maybe due to the similarity to spermidine and therefore more efficient cellular uptake. Work by O'Sullivan *et al.* on spermidine analogues as possible antiparasitic agents had shown that (bis)naphthylspermidine had excellent activity.⁸¹

In contrast to the cancer work where diethyl substituents seem sufficient for good activity, in the parasitic diseases some aromatic moiety seems necessary.

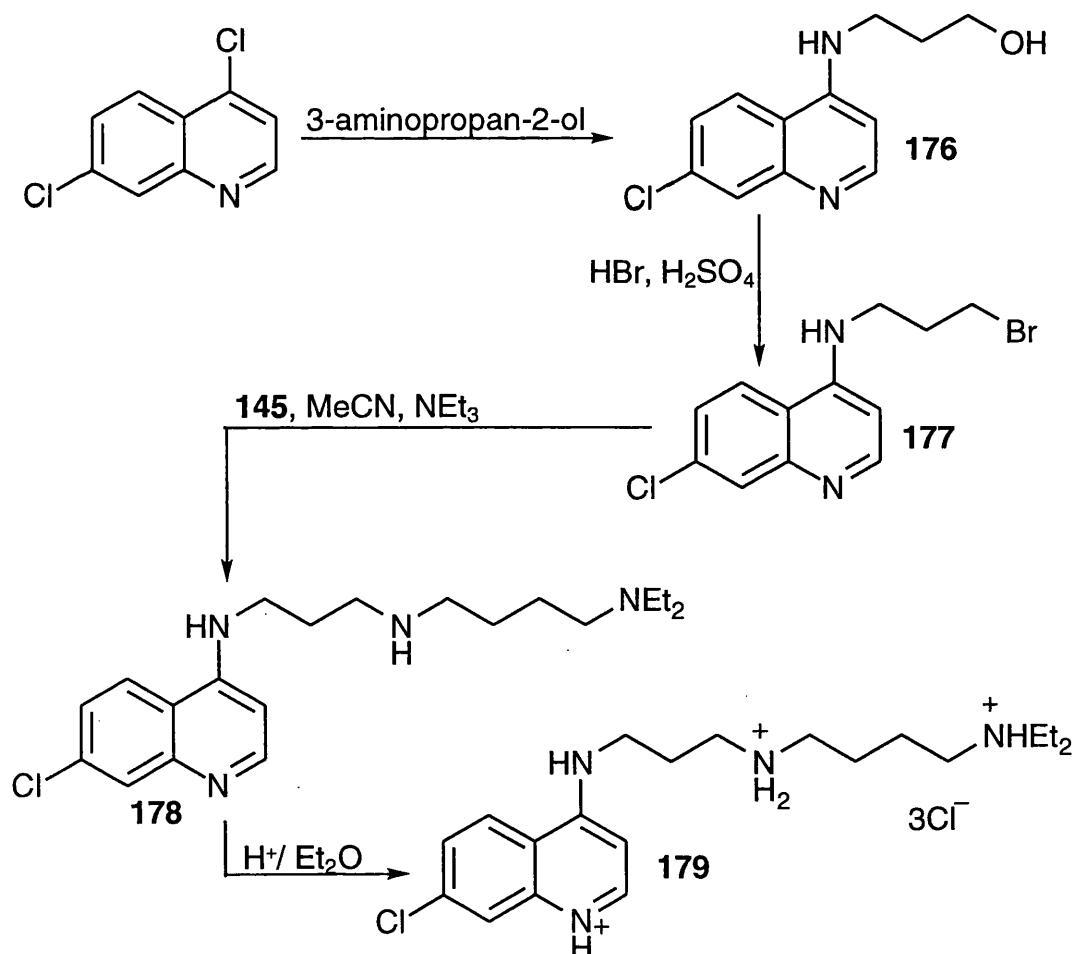
4.5 Preparation of Spermidine-Quinoline Derivatives

As in the previous chapter it was decided to prepare some quinoline-based compounds. Prior to 1942 little attention had focused on quinolines with alkylamino side chains in the four position, but since the discovery of chloroquine an explosion of literature was witnessed. Variations in the quinoline ring and the side chain were investigated.¹⁴¹ Work tended to concentrate on the formation of C-4 carbon chains attached to the quinoline

ring so we decided to investigate some spermidine derivatives; including attaching the quinoline ring to a primary or secondary nitrogen. Previous workers in this area had prepared a quinoline derivative with a 3,3 carbon backbone attached by condensation of triamine (**152**) with 4,7-dichloroquinoline.¹⁴² Marquez and co-workers had looked at a number of quinolines with polyamine side chains varying from 3,3; 3,4 or 3,4,3 with one quinoline ring attached. They had also looked at some bisquinolines with the two quinoline rings joined by 3,3 or 3,4,3 carbon chains.¹⁴⁷

4.5.1 Synthesis of Compound (**179**)

The reaction of the triamine (**153**) with 4,7-dichloroquinoline was not attempted due to the small amount of triamine available. Instead the alkyl halide (**177**) was prepared via the corresponding alcohol (**176**).

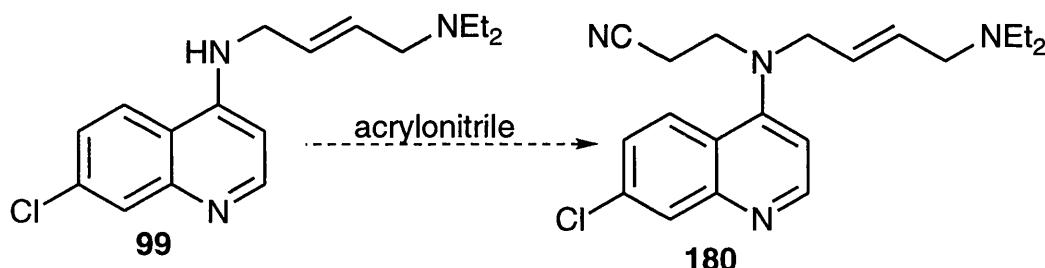


Scheme 30 Formation of a spermidine-quinoline conjugate (**179**)

The alcohol and bromide were previously prepared by Bolte¹⁴³ who heated 4,7-dichloroquinoline with 3-aminopropan-1-ol for 12 hours at 130 °C. We adapted this route by heating for a slightly longer 18 hours, and trituration in 1 mol dm⁻³ NaOH yielded a cream solid in 85% yield. The previous authors had precipitated the solid from ether and recrystallised it from ethanol-water to yield the product in 75% yield. The bromination was carried out, in our hands, by 48% hydrobromic acid and concentrated sulphuric acid compared to the previous authors who used only 48% hydrobromic acid. The quinoline derivative (**178**) was then prepared by the usual S_N2 reaction with diamine (**145**). The excess diamine was removed by Kugelrohr distillation and the trihydrochloride salt (**179**) was isolated in the usual manner (**Scheme 30**).

4.5.2 Attempted Synthesis of Compounds (**180**) and (**182**)

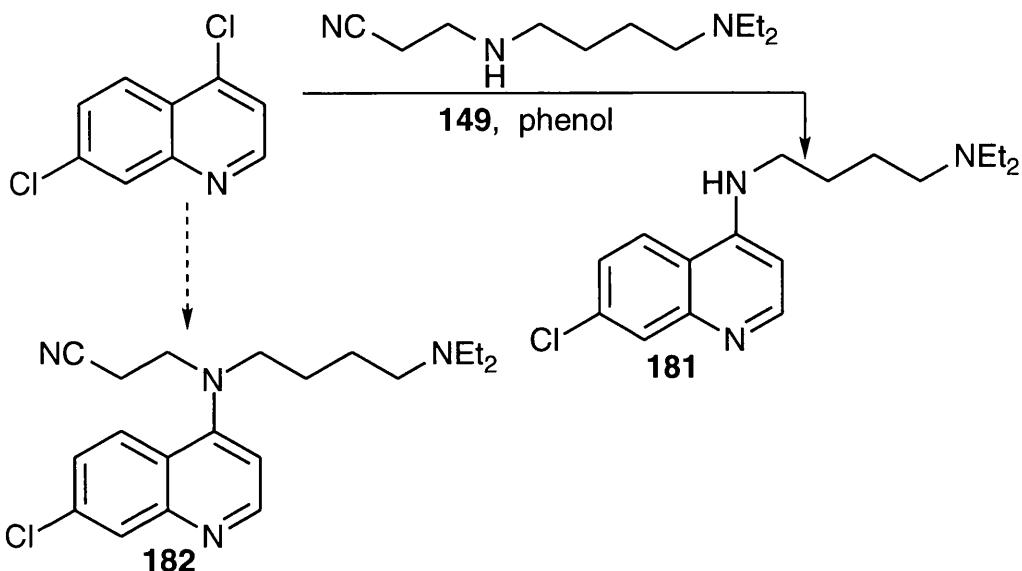
So far no attempts had been made to prepare spermidine analogues with a quinoline ring on N⁴. We had successfully prepared (**179**) with the quinoline on N¹ so it would be interesting to compare activities of these analogues. The first attempt is shown in **Scheme 31**. The diamine (**99**) was stirred in acrylonitrile; firstly with sodium hydroxide in methanol and then with ethanol at higher temperatures and for up to 1 week. In both cases no product (**180**) was formed.



Scheme 31 Attempted preparation of compound (**180**)

This is perhaps not a surprising result as the nitrogen we were trying to alkylate has reduced nucleophilic character due to delocalisation. Vitale and Chiocconi recently discussed alkylation of aromatic amines, although not quinolines, and used ⁿBuLi and an alkyl halide to achieve alkylation.¹⁴⁴

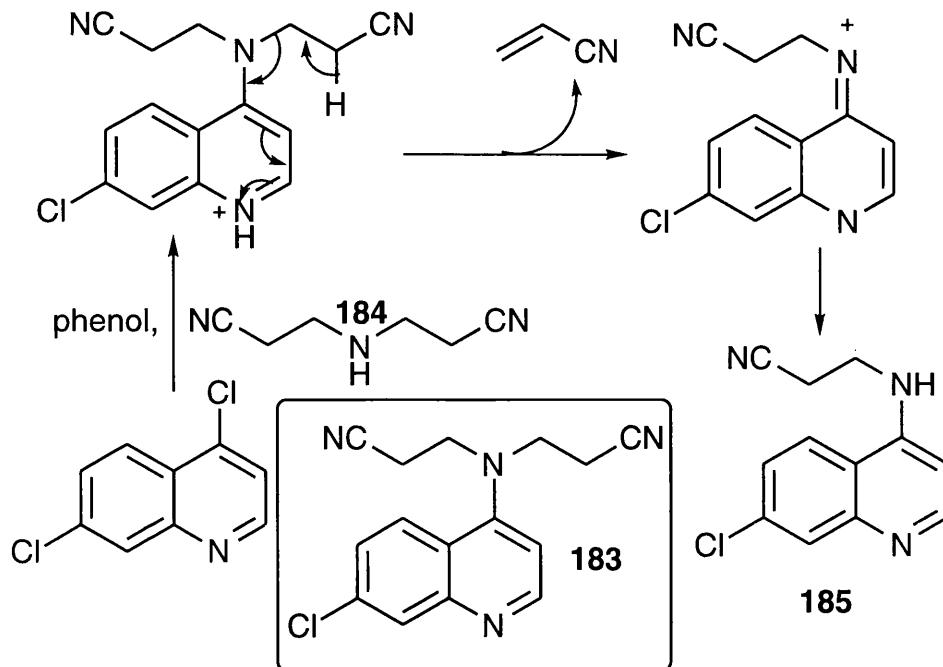
The second attempt involved diamine (**149**) with the nitrile group already attached (**Scheme 32**). Synthesis followed the usual method of heating in phenol for 6 hours at 160 °C. After work up a cream solid was isolated; however no nitrile absorbance was seen in the IR and no nitrile peak appeared in the ¹³C NMR spectrum.



Scheme 32 Attempted preparation of spermidine-quinoline conjugates

The resulting product was identified as (**181**) presumably due to a retro-Michael reaction occurring (**Scheme 33**). Previous authors had also shown that condensation with a secondary nitrogen was unlikely under these conditions¹⁴⁵ although Singh *et al.* have prepared bisquinolines using secondary amines under the same conditions.¹⁴⁶ Attempts to prepare these analogues were discontinued.

Marquez *et al.* had tried to prepare a quinoline derivative with the quinoline ring attached to the secondary nitrogen (**183**).¹⁴⁷



Scheme 33 Loss of acrylonitrile to give (**185**)

They had heated 4,7-dichloroquinoline with dinitrile (**184**) in phenol then intending to reduce the dinitrile to a diamine; however only the mononitrile (**185**) was recovered.

4.5.3 Analytical Data on Spermidine-Quinoline Derivatives

All singly charged ions in the mass spectrum which contain carbon atoms will give rise to another peak one mass unit higher due to the natural abundance of ^{13}C . Chlorine atoms consist of ^{35}Cl and ^{37}Cl present in an isotopic ratio 3:1, and bromine atoms consist of ^{79}Br and ^{81}Br in the ratio 1:1. Molecular ions or fragments that contain these atoms give rise to patterns in the spectrum due to this isotope effect (**Figure 29**).¹³⁵

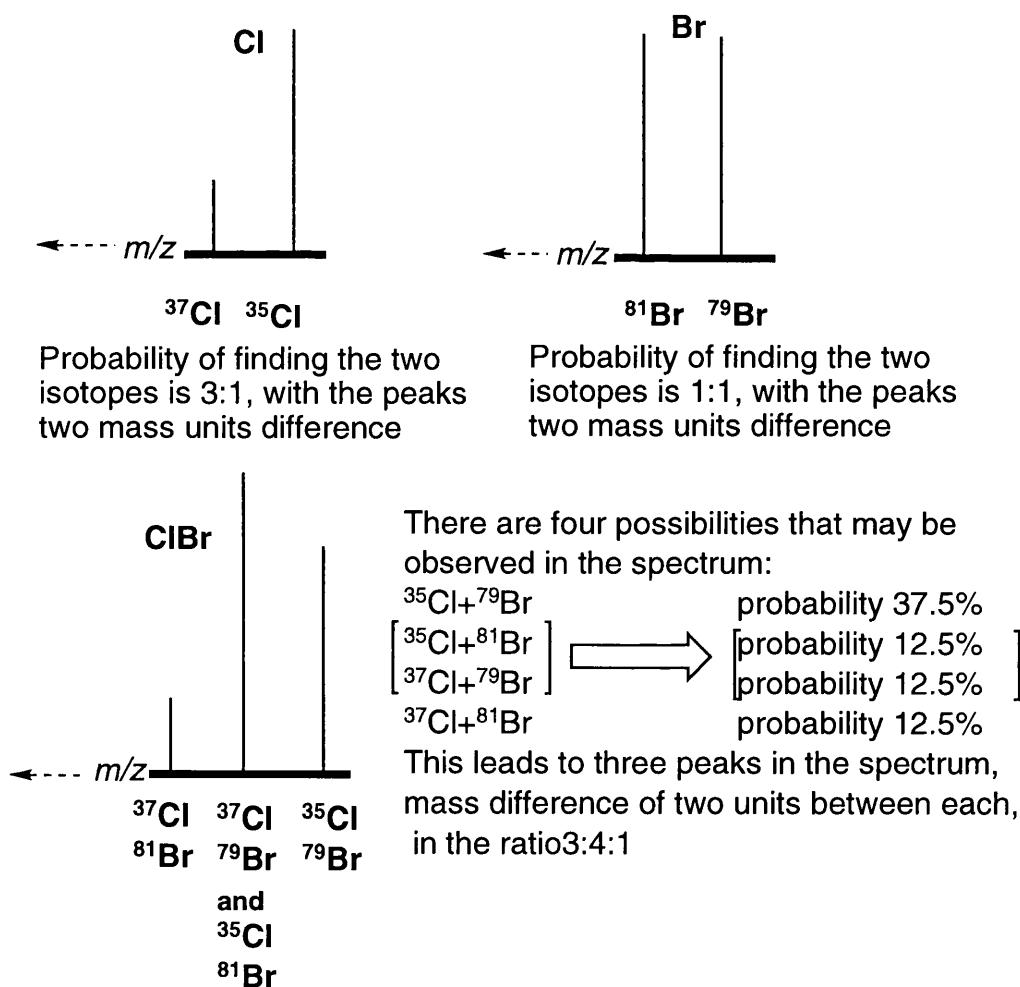


Figure 29 Isotope effects from chlorine and bromine in quinoline compounds

The quinoline derivatives prepared contain chlorine and sometimes bromine and show these expected effects. Compound (**177**) has three molecular ions at m/z 301, 299 and 297 in the ratio 1:4:3. The other

quinolines prepared in this work contain only chlorine and we observe the 1:3 ratio expected in those mass spectra.

The aromatic region of the ^1H NMR spectrum of quinoline compound (176) is shown in **Figure 30**. Many of the features of this spectrum are seen in all our analogues and are described below.

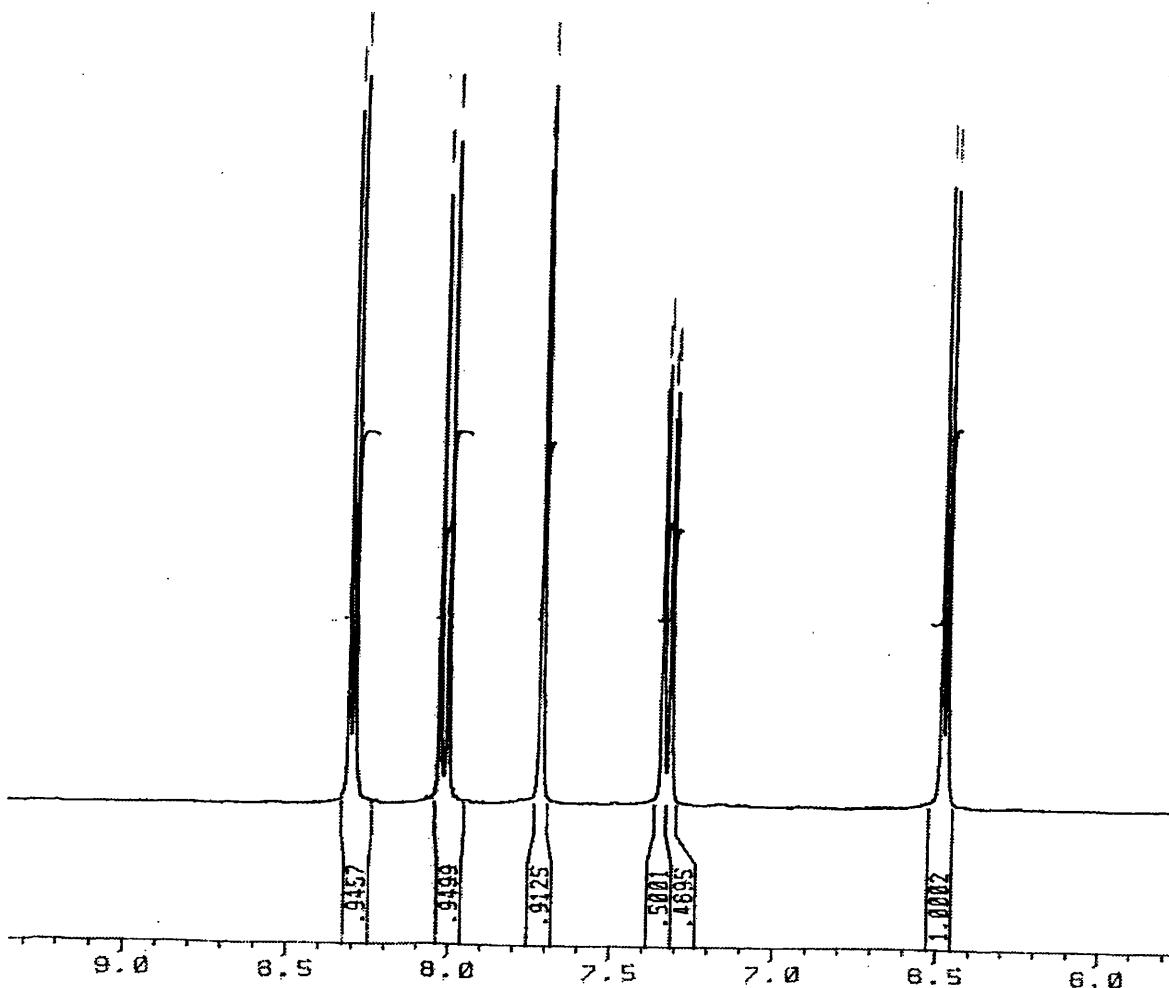
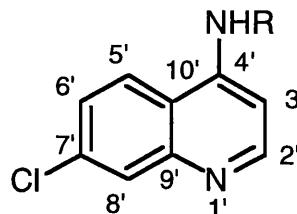


Figure 30 Typical aromatic region of a quinoline derivative, 360 MHz, CDCl_3

Protons 3' and 2' are coupled with a value of 5.7 Hz which is a little larger than the corresponding coupling in quinoline itself of 4.3 Hz. Proton 6' is easily identified as a doublet of doublets coupling to both H-5' (9.0 Hz) and H-8' (2.1 Hz). H-8' is seen only as a doublet coupling to 6' (2.1 Hz) with no longer range effects seen. H-5' is also a doublet with a coupling constant of 9.0 Hz again a little larger than that seen in quinoline (8.2 Hz). In the ^1H NMR spectra of quinoline derivatives protons at 3' and 6' are shielded by resonance effects, similar to those seen earlier (3.4.2) and this results in

them resonating at a higher field, i.e. lower δ . Proton 8' is shielded by resonance but deshielded by the nearby chlorine atom and appears at δ 7.65. The proton at 2' is deshielded by the electron withdrawing effects and resonance of the nearby nitrogen and occurs at the lowest field (**Figure 31**).



	176 (ppm)	176 J (Hz)	178 (ppm)	179 (ppm)
2'	8.22	5.7	7.75	7.81
3'	6.35	5.7	5.95	6.34
5'	7.91	9.0	7.66	7.25
6'	7.23	9.0, 2.1	6.60	6.82
8'	7.65	2.1	7.15	6.84

Figure 31 Comparison of ^1H NMR data for quinoline derivatives, 200 MHz, CDCl_3

A combination of coupling constants and chemical shift values make the protons in these compounds identifiable although the smaller coupling is not always observed. Similar effects are observed in the ^{13}C NMR spectra of related compounds (**Figure 32**).

	176 (ppm)	178 (ppm)	179 (ppm)
2'	151.9	151.3	142.8
3'	125.8	125.2	124.4
4'	149.1	148.3	139.9
5'	127.2	126.4	127.9
6'	99.4	98.9	99.3
7'	136.2	135.4	137.3
8'	124.1	124.1	118.7
9'	152.6	151.5	155.5
10'	118.5	117.7	114.6

Figure 32 Comparison of ^{13}C NMR data for quinoline derivatives

The carbons in C-2' and C-9' positions come at lowest field. C-4' is deshielded by its neighbouring nitrogen atom, whilst C-7' is deshielded by a

chlorine atom which produces a weaker effect. The chemical shift values for both ^1H and ^{13}C NMR spectra are extremely constant, with the only notable difference being evident after conversion of free base (**178**) into the trihydrochloride salt (**179**).

4.5.4 Biological Evaluation

The *in vitro* activity of (**179**) was, unsurprisingly, excellent and comparable with chloroquine. It would be interesting to compare (**100**) and (**179**) and see the effect of the side chain on activity (Figure 33).

	activity ($\mu\text{g ml}^{-1}$)	LEC (μM)
179	<0.1	<0.21
chloroquine 2	<0.1	<0.19

Figure 33 *In vitro* activity of a spermidine-quinoline conjugate

4.6 Investigations of Spermine Analogues

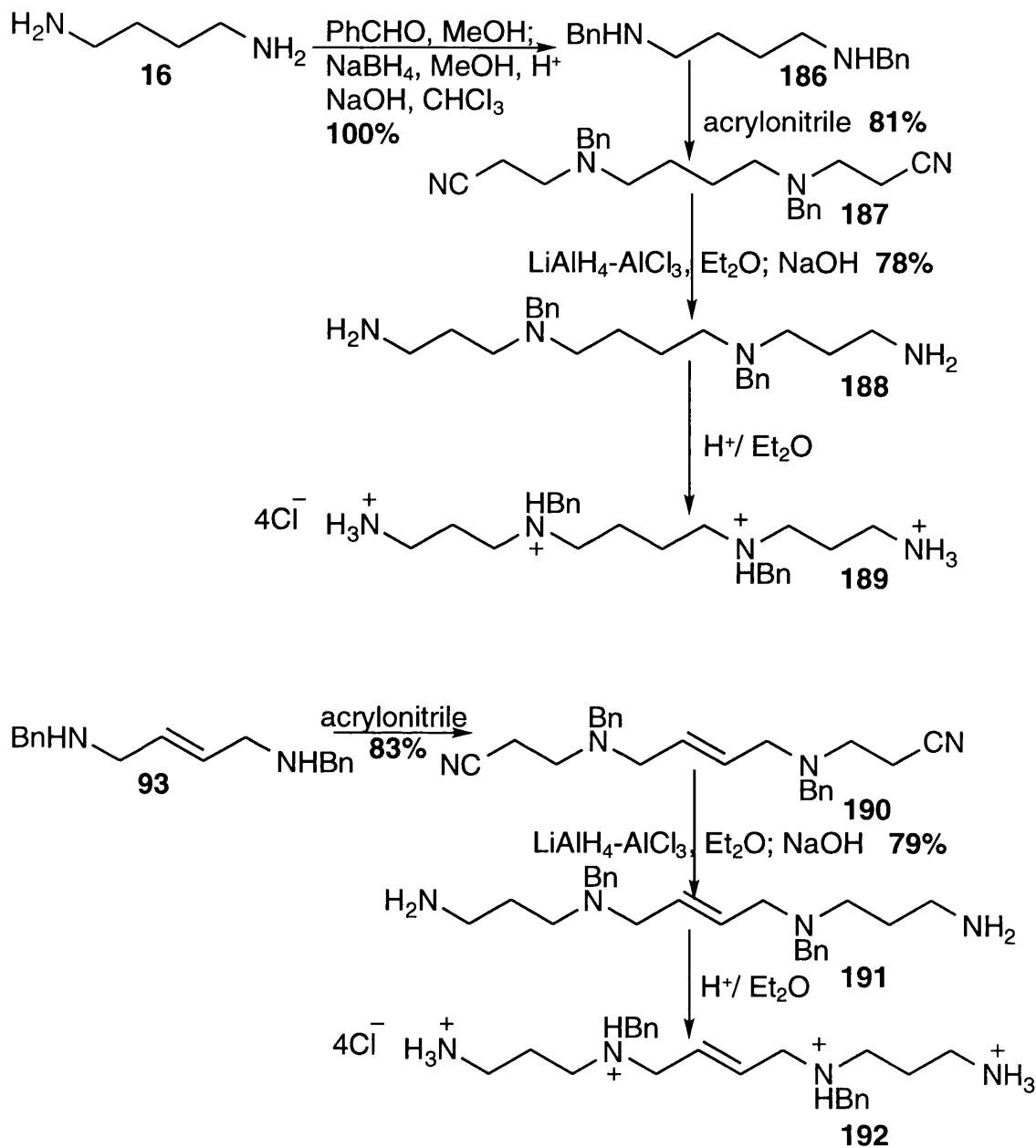
Previous work in the anticancer area had looked at the relative abilities of putrescine, spermidine and spermine analogues to inhibit cell growth.⁶⁴ Conclusions drawn stated that the relative order of activity was spermine > spermidine > putrescine. Edwards and co-workers working on polyamines as antimalarials had also looked at spermine analogues; varying the central carbon chain from 5-12 methylene units and they also found the compounds with benzyl groups on the terminal nitrogens to be active.⁷⁷

We decided to prepare the spermine analogues with benzyl groups on the internal nitrogens as these had not been investigated for antimalarial activity.

4.6.1 Preparation of Compounds (**189**) and (**192**)

The saturated analogue (**189**) was prepared in excellent yield starting from putrescine.^{148, 149, 60} Putrescine was reductively alkylated using benzaldehyde, (**186**) and was then stirred at 60-70 °C in an excess of acrylonitrile to yield (**188**) which was reduced in the normal manner to afford the tetraamine in good yield. 1,4-Diaminobut-2-ene is not readily available

and so diamine (**93**) was used, prepared as in **3.4**, and the same set of reactions were then followed. The two tetraamines were converted into the tetrahydrochloride salts (**189**) and (**192**) which were hygroscopic peach coloured solids (**Scheme 34**).



Scheme 34 Preparation of spermine analogues (**189**) and (**192**)

4.6.2 Biological Evaluation

In vitro activity of the two spermine analogues is shown in **Figure 34**. The spermine analogues had good activity, better than any putrescine or

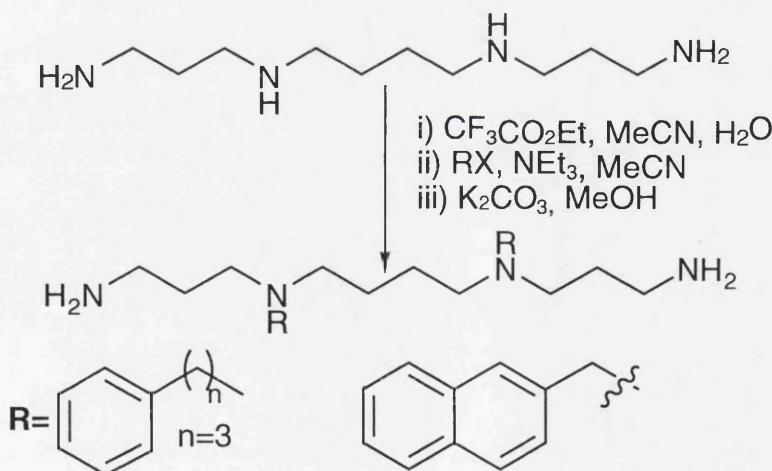
spermidine analogues prepared. Again (**189**) was more active than the unsaturated analogue (**192**) which was consistent with the spermidine analogue results.

	activity ($\mu\text{g ml}^{-1}$)	LEC (μM)
sat-dibenzyl 189	0.5-1	1-2
unsat-dibenzyl 192	5-10	10-20
chloroquine 2	<1	<1.9

Figure 34 *In vitro* activity of compounds (**189**) and (**192**)

Edwards *et al.* had isolated a polyamine analogues with a 3,7,3 backbone and benzyl substituents on the terminal nitrogens and it had an IC₅₀ value of 3.0 μM against *P. falciparum* and was curative when administered at 15 mg kg⁻¹ with DFMO.⁷⁷ Our saturated spermine analogue (**189**) compares favourably with this *in vitro* value.

In the search for active compounds against *T. cruzi* O'Sullivan and co-workers had extended the synthesis of spermidine derivatives to spermine derivatives.⁸¹ Using the same protecting group chemistry spermine analogues were prepared with internally alkylated nitrogen atoms. Their most active spermine derivatives had internal nitrogens alkylated with hydrophobic groups (**Scheme 35**).



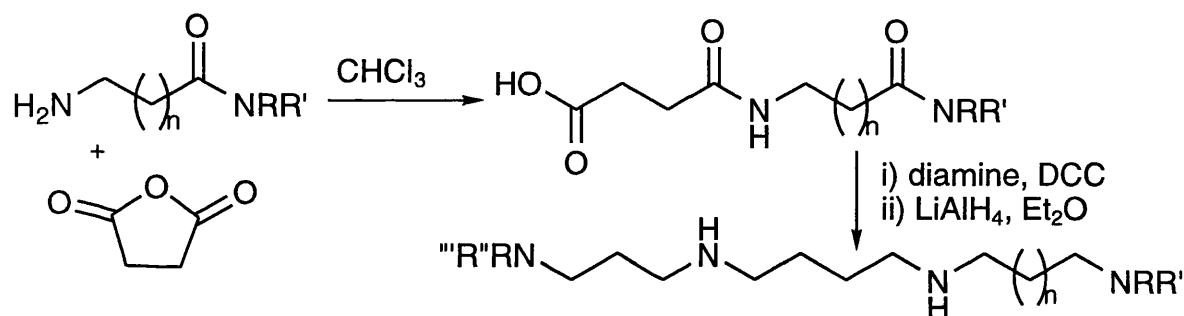
Scheme 35 Preparation of spermine derivatives

Synthetic routes to spermine analogues for study of antiparasitic activity were also investigated by Bellevue and co-workers⁸⁰ who found the most active compound had a 3,7,3 carbon chain with the terminal nitrogens alkylated with cycloheptylmethyl. The 3,7,3 analogues had little anticancer

activity so the carbon backbone seems to be of importance for parasitic diseases. Edwards in his work on spermine analogues had also found the 3,7,3 carbon skeleton to be active with benzyl substituted on the terminal nitrogens.⁷⁷

4.6.3 Other Reported Syntheses of Spermine Analogues

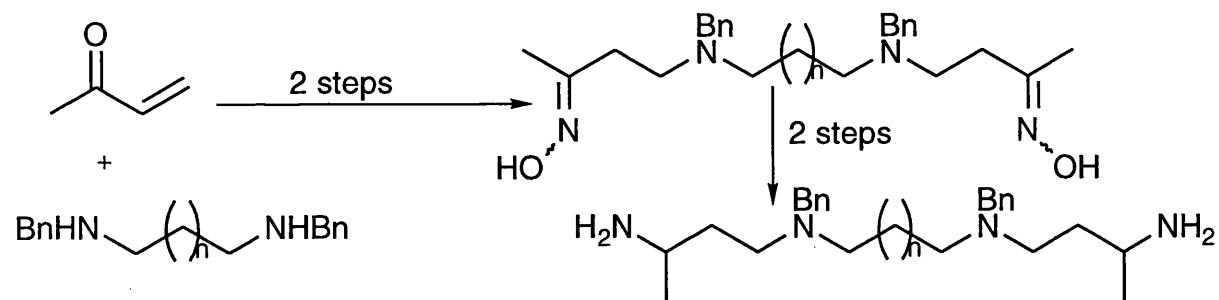
Nordlander *et al.*¹¹⁶ also looked at extending their methodology to higher chain polyamines. The amide with a free amino group could react with succinic anhydride and open the anhydride to add on a four carbon chain with a free acid group. This free acid group was converted into an amide and the amide reduced to yield the polyamine. This chemistry could be easily adapted to produce a wide range of analogues (**Scheme 36**).



Scheme 36 Preparation of higher polyamines

A similar approach was also used by Mamos *et al.* to give spermine analogues alkylated at either terminal or internal nitrogens.¹¹⁷ The choice of amines is easily altered to provide a number of closely related structures. Protection of terminal nitrogens as trifluoroacetates followed by reduction gives amines with reduced charge due to the electron withdrawing effects of the terminal groups.⁶⁰

The Michael addition with a variety of Michael acceptors has been used including methyl vinyl ketone, as shown in **Scheme 37**.⁶⁹



Scheme 37 Preparation of higher polyamines

4.7 Preparation of Guanidinylated Tetraamines

4.7.1 Introduction

The group of Kumar and co-workers were studying the formation of 1,3-diaminopropanes and their role in the activity of polyamines against leishmania.¹⁵⁰ Leishmania is a parasitic disease, as is malaria, so it was relevant to read about their studies. They prepared a number of compounds with the 1,3-diaminopropane pharmacophore where one nitrogen was present in a heterocyclic ring and the other in a guanidine group (**Figure 35**).

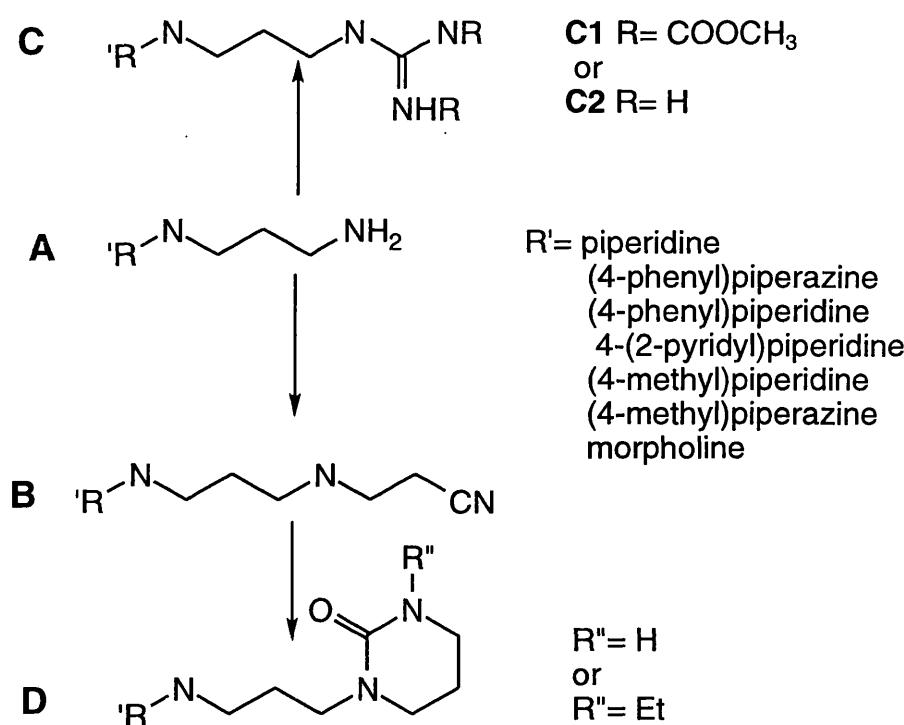


Figure 35 Structure-activity results on 1,3-diamines

They discovered that the 1,3-diaminopropane subunit was a good backbone for designing antileishmanial agents. If one nitrogen was incorporated in a cyclic system the compounds (**A**) had moderate activity. If the free amine was functionalised with a 2-cyanoethyl group (**B**) activity increased. If the free amine was converted into a *bis*carbomethoxy moiety (**C1**) activity reached a maximum compared to the free guanidine derivatives (**C2**) which had little activity. If both nitrogen atoms were in heterocyclic rings (**D**) the compounds had little activity (**Figure 35**).

Badry *et al.* had also looked at (bis)carbomethoxy guanidines as histamine-H₂ receptor antagonists¹⁵¹ and Rao *et al.* had studied the reaction

of a number of di- and polyamines with *N,N'*-biscarbomethoxy-S-methylisothiourea.¹⁵²

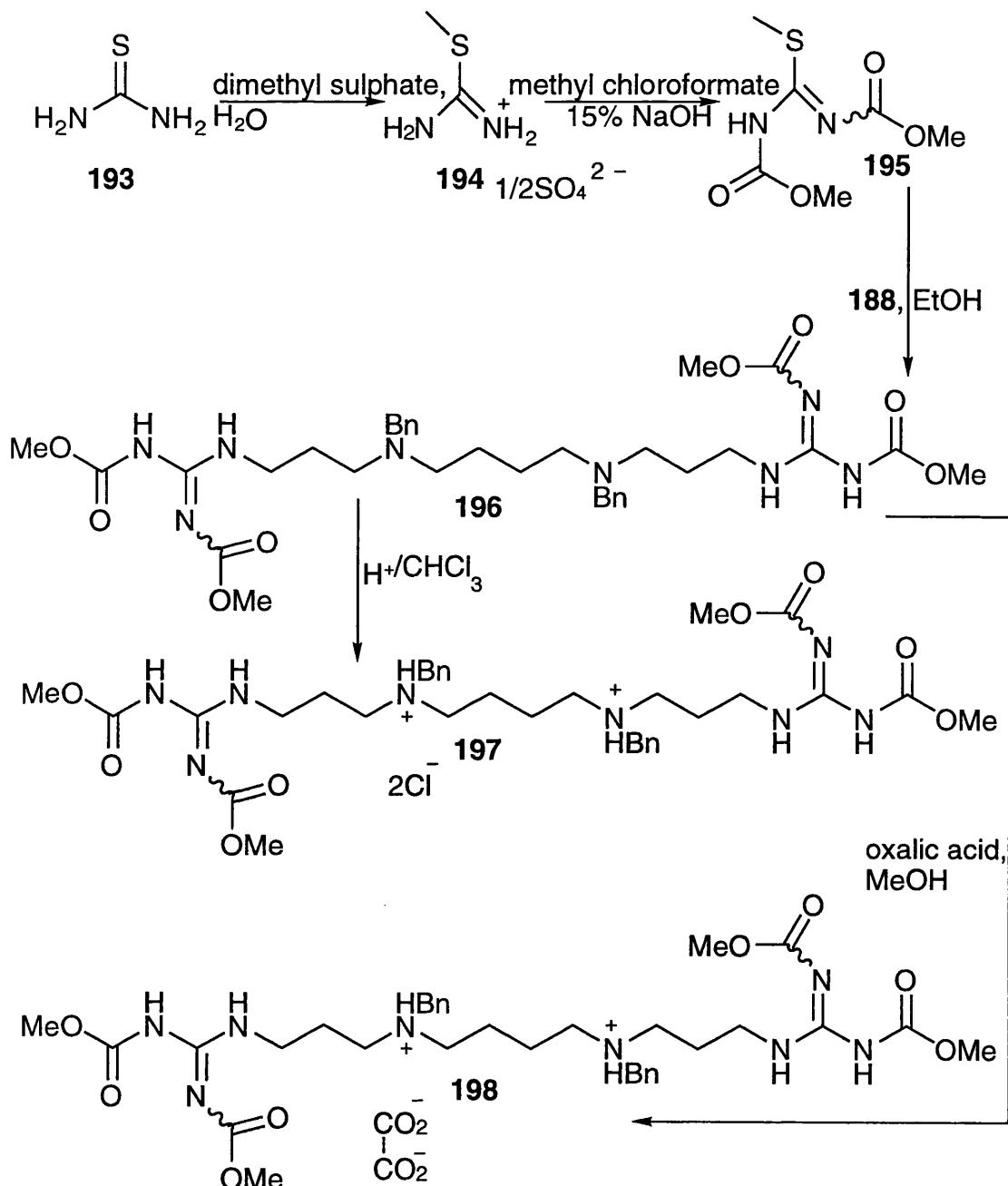
We decided to convert the primary amines of (188) into (bis)carbamethoxyguanidines to see the effect on activity.

4.7.2 Synthesis of Compound (196) and its Salts

To introduce the free guanidine into a compound and then functionalise is often difficult due to the basicity of the guanidine moiety. Thiourea (193) was to be converted into the *biscarbomethoxy* intermediate (195) which would react with our tetraamine to yield the desired product. First attempts to prepare (195) followed the route of Hazelton *et al.*¹⁵³ which did not isolate (194), but precipitated (195) from solution; however no product was isolated. The next attempt followed the procedure of Schildneck and thiourea was methylated with dimethyl sulphate in water to give (194) in good yield.¹⁵⁴ (194) was then dissolved in aqueous sodium hydroxide and reacted with methyl chloroformate to give (195) in reasonable yield.¹⁵⁵ The tetraamine was stirred with (195) in ethanol overnight and purification was achieved by silica chromatography.¹⁵⁰ The yield was low due to the large amount of aromatic impurities formed in the reaction and chromatography was repeated to isolate a pure product (196) (**Scheme 38**).

4.7.3 Analytical Data for Compound (196)

The ¹H NMR spectrum of (196) shows the expected multiplets for the methylene protons 2, 3 and 6 which are least affected by the β -nitrogen atoms. The protons α - to nitrogen atoms 1, 4 and 5 appear as a multiplet at δ 2.37 with protons at the 7 position appearing at slightly lower field due to the deshielding effects of the guanidine group (δ 3.35). The methylene protons at H-3 occur in the expected area for benzylic protons and are followed by two singlets for the carbamate methyl groups. We observe two different signals for these methyl groups (δ 3.62, δ 3.74), with an integral of 1:1, suggesting only one configuration of the (bis)carbamethoxy moiety although we have left stereochemistry undefined. The aromatic protons appear as a multiplet and we see a broad triplet for an NH group indicating coupling which must be CH₂NH (δ 8.29). The next NH peak is further deshielded and corresponds to NHCO₂Me (δ 11.59). Work by other groups on biscarbomethoxy guanidine compounds also report the NHCO₂Me at fairly high δ values.^{151, 152}



Scheme 38 Preparation of a biscarbomethoxy guanidine compound (**196**) and its salts (**197**) and (**198**)

Two methyl peaks also appear in the ¹³C NMR spectrum (δ 52.5, δ 53.1). The aromatic carbons appear as three CHs and one C in the usual order. We have three peaks left which are assigned to the two quaternary carbonyls and the guanidine quaternary carbon.

The guanidine moiety exists as tautomers. We also have the possibility of rotameric forms. Considering the wide variety of possible influences a fairly simple spectrum was obtained.

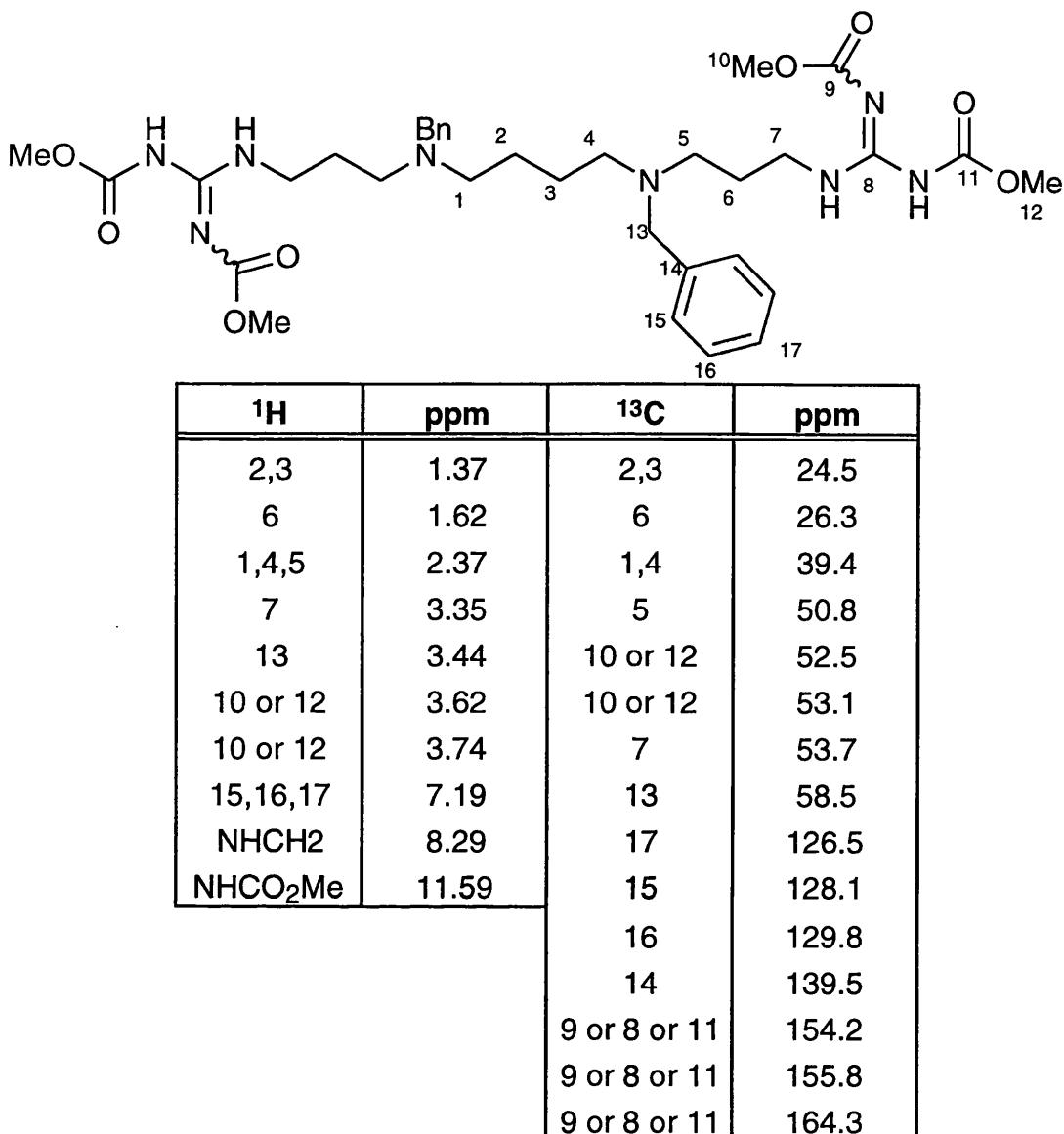


Figure 36 ¹H and ¹³C NMR data for compound (196)

Attempts were made to prepare the oxalate and hydrochloride salts of (196). The oxalate salt (198) was prepared by dissolving (196) in a small amount of methanol and adding one equivalent of oxalic acid, also in a small amount of methanol. Unfortunately (196) precipitated as a sticky gum, although ¹H and ¹³C NMR spectra were obtained. The ¹H and ¹³C NMR spectra were very similar to the free base with the two methyl signals appearing in a ratio of 1:1. Protonation of (196) will occur initially at the most basic position which are the nitrogens of the benzyl group in the centre of the molecule. Oxalic acid is only a weak acid and as one equivalent was used only these nitrogens appear to be protonated giving simple spectra for (198), as seen for (196).

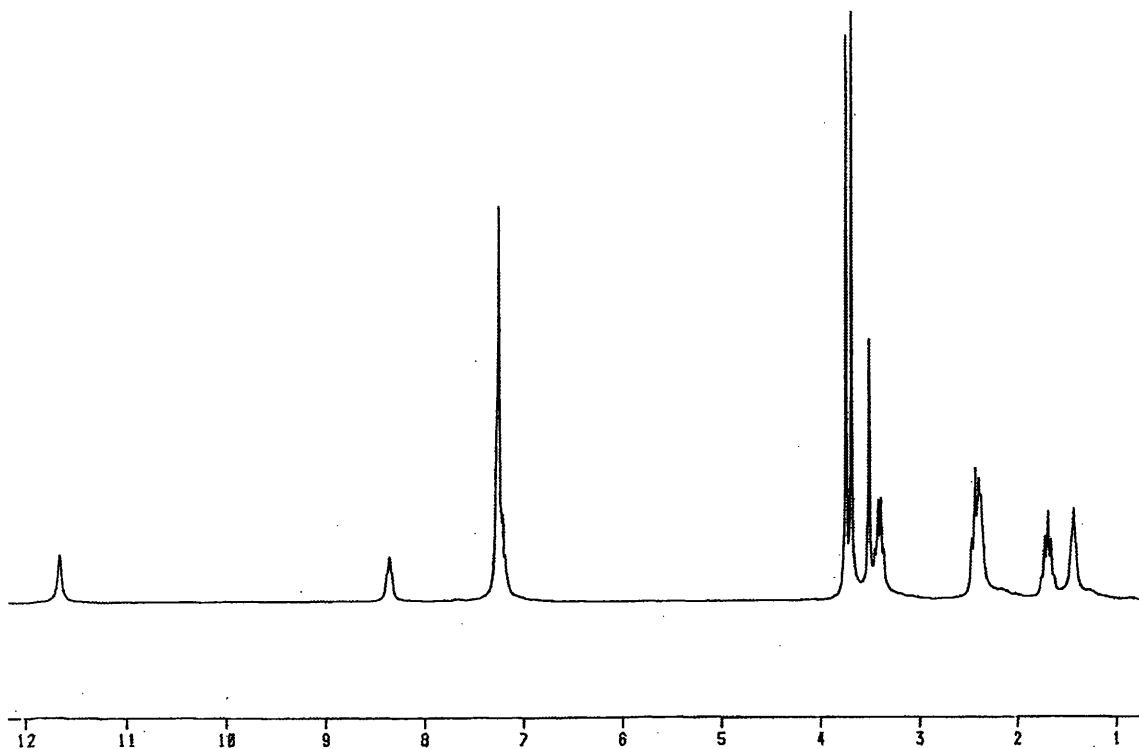


Figure 37 ^1H NMR spectrum of compound (196), 200 MHz, CDCl_3

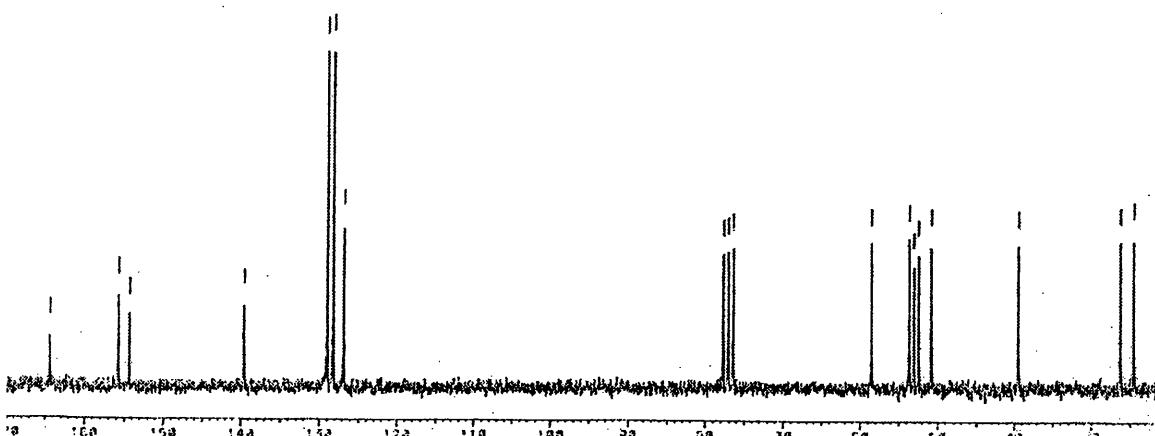


Figure 38 ^{13}C NMR spectrum of (bis)carbomethoxy guanidine derivative (196), 50 MHz, CDCl_3

The hydrochloride salt was prepared by precipitating the salt from chloroform with HCl/CHCl₃. Again only a sticky gum was obtained and the ^1H and ^{13}C NMR spectra of (197) were more complicated than those seen previously. The most noticeable difference was the appearance of three singlets in the ^1H NMR spectrum corresponding to three environments for the methyl groups and the ^{13}C NMR spectrum shows extra peaks in both the

aliphatic and aromatic regions. Using a stronger acid that was not added in a specific number of molar equivalents may have led to protonation of the (bis)carbomethoxyguanidine moiety (**Figure 39**).

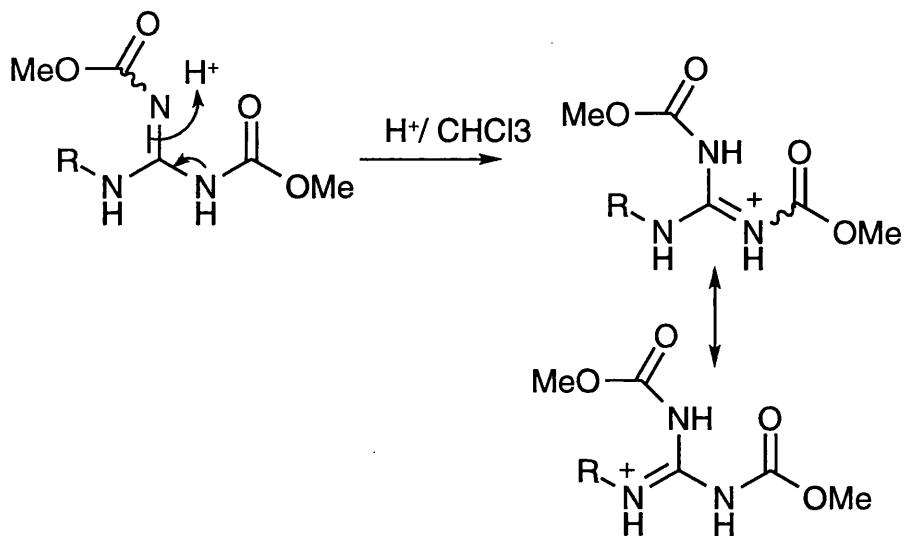


Figure 39 Protonation of the (bis)carbomethoxy moiety

If we assume complete protonation of the benzyl nitrogens we could have protonation of one (bis)carbomethoxyguanidine moiety or of both. For each of these possibilities we could also see rotamers, as discussed before. This leads to a very complicated spectrum, and no attempts were made to assign any peaks.

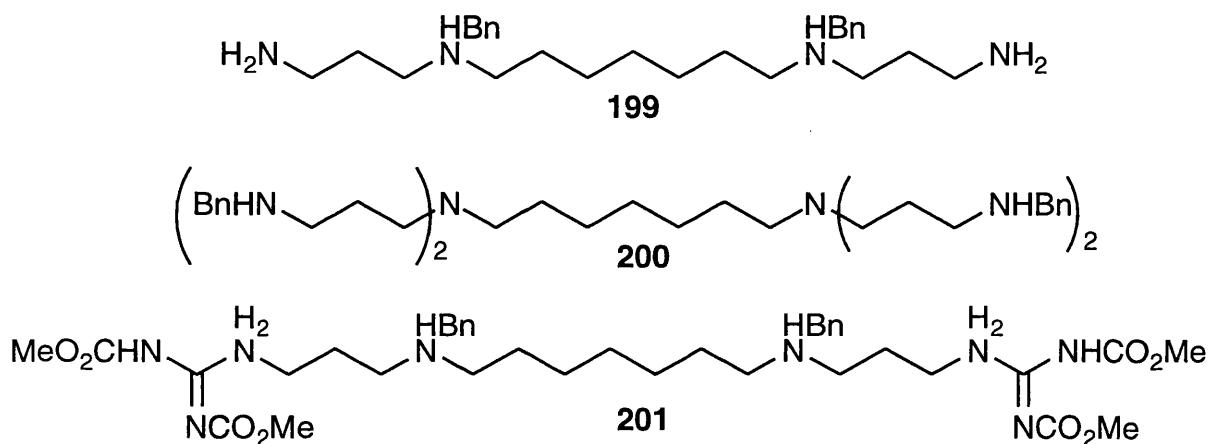
4.8 Future Work

The preparation of spermidine analogues was, at first, a promising lead; however after preparing a number of compounds we still did not see any great antimalarial activity. The antifungal results were more promising and. No future work is planned on the synthesis of such spermidine analogues.

Spermine analogues were more promising and there are a number of leads that would be worth following up in this area. The work of a number of research groups has shown that 3,7,3 carbon skeletons impart greater antimalarial activity on polyamine analogues and are possibly the best for parasitic disease chemotherapy. (**189**) was our most active spermine derivative to date and it may be worth preparing the 3,7,3 analogue of this (**199**) to see if the change in carbon skeleton improves activity. The 3,7,3-

bisbenzyl analogue (**200**) may also be worth preparing and is similar to (**67**) which was prepared by Edwards *et al.* and has the benzyl groups on the terminal nitrogens.

Strong guanidinium-carboxylate interactions have been observed in substrate-enzyme binding with MGBG, an anti-tumour agent, and its analogues are known to be taken up by polyamine transporters.^{156, 157, 158} We do not intend to prepare any free guanines but those functionalised as (bis)carbomethoxy are worth considering as Kumar and co-workers found that these were more active than the free guanidine. The preparation of (bis)carbomethoxyguanidine (**196**) and its salts should be pursued. Alternative salts, e.g. picrate, maleate and succinate should be prepared to produce crystalline salts that could be tested. Again, the 3,7,3 analogue (**201**) would also be worth preparing.



4.9 Summary

The preparation of spermidine analogues led to a number of compounds; however out of those tested we did not see any great antimalarial activity. The addition of a benzyl group did increase activity, especially in the spermine area where results have been more promising.

We were still interested in looking at DNA binding as it is often mentioned that this may be related to good activity of polyamine analogues as they displace the natural polyamines from DNA. In the next chapter a number of polyamines were prepared that may lead to greater activity against *P. falciparum*.

5

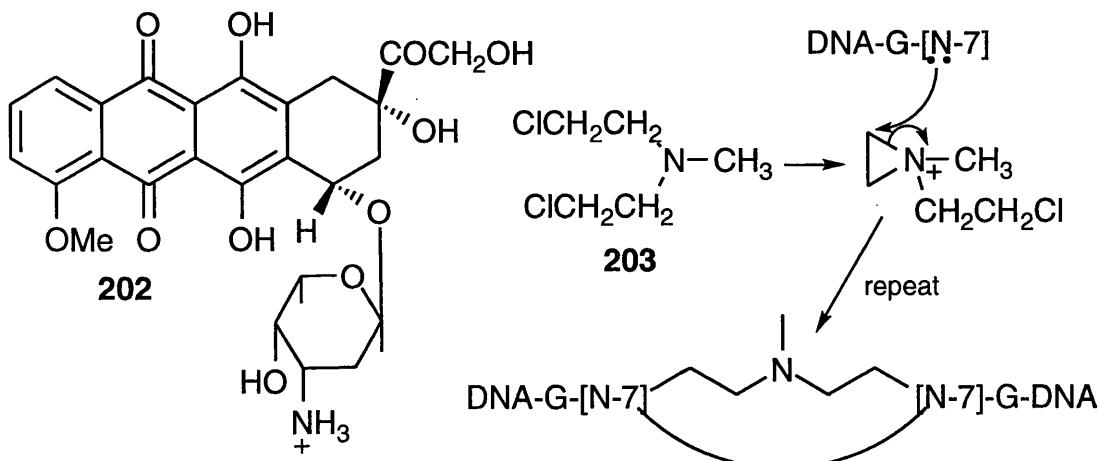
5 DNA Binding and Chemotherapy

5.1 Introduction and Rationale

The recognition of one molecule by another is crucial to many biological reactions such as DNA replication, molecular transport and also drug action. Within DNA the two antiparallel strands of adenine(A), thymine (T), guanine (G) and cytosine (C) are supported on the phosphate-sugar backbone and form different conformational types of DNA called A, B, D or Z, with B-DNA being the most common. The DNA structure that forms provides a variety of places for interaction with other molecules by hydrogen bonding, Van der Waals forces and electrostatic interactions.¹⁵⁹

A compound can bind in a number of ways to DNA and can also show selectivity for the major or minor groove, specific base sequences and sites of DNA base pairs. We can broadly categorise the modes of DNA binding as follows.

Intercalators are planar molecules, usually consisting of three or four fused rings that insert between stacked nucleic acid base pairs, e.g. adriamycin (**202**) which is important in cancer therapy.



Covalent groove binders contain electrophilic groups such as aziridines or cyclopropanes and are found in many compounds that are capable of covalent interaction with DNA. Nitrogen mustards are one of the most common classes acting by crosslinking DNA through covalent bonding to the guanine N-7 atoms in the major groove, e.g. mechlorethamine (**203**).

Non-covalent minor groove binders are held in position by hydrogen bonds, Van der Waals forces and electrostatic interactions, e.g. distamycin A (**204**) and netropsin (**205**). The interactions of netropsin with AT-rich DNA are shown in **Figure 40**.

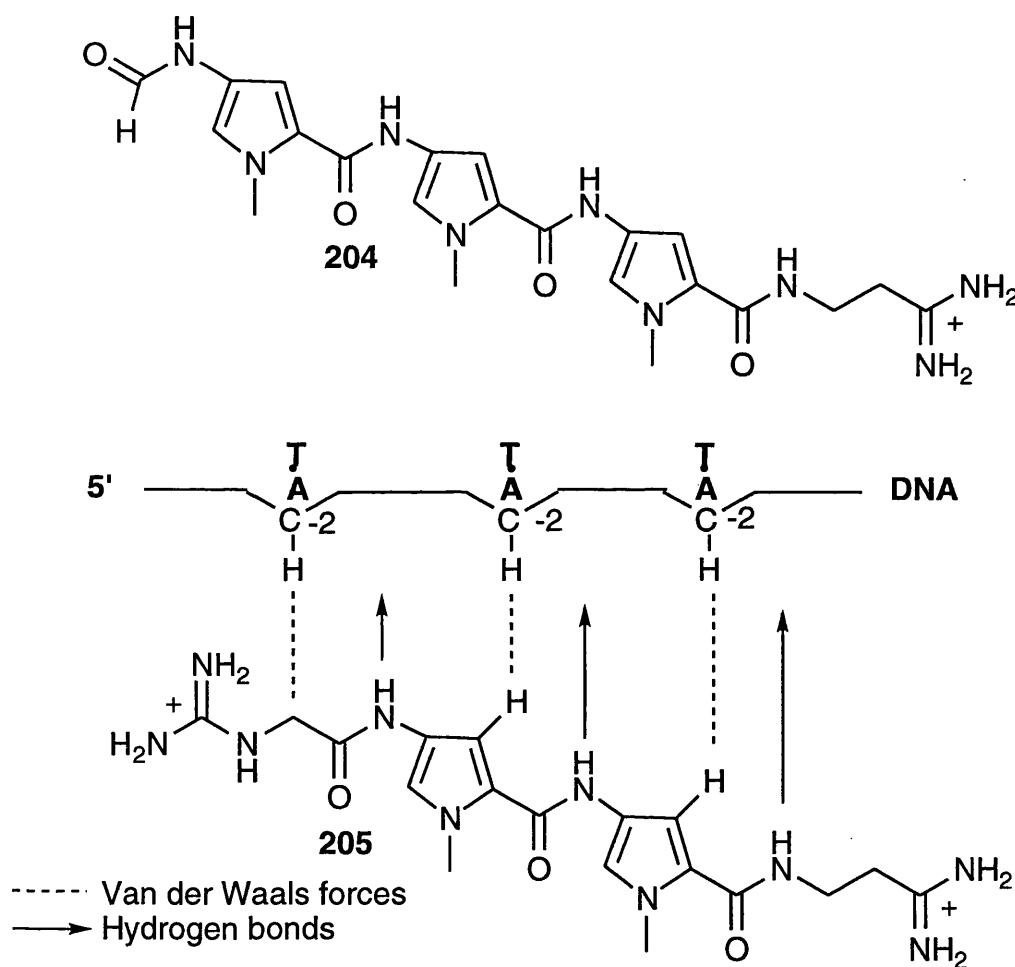


Figure 40 Interactions of netropsin with AT-rich DNA

Polyammonium salts bind to DNA through electrostatic interactions which are, in most cases, non-sequence specific. Whilst remaining close to the DNA the polycation retains a high degree of freedom within the polyamine-DNA complex. The exact position of the binding interaction is not known, e.g. minor groove, major groove or phosphate backbone, and this has caused some debate (**2.7**). Spermine has been shown by crystal

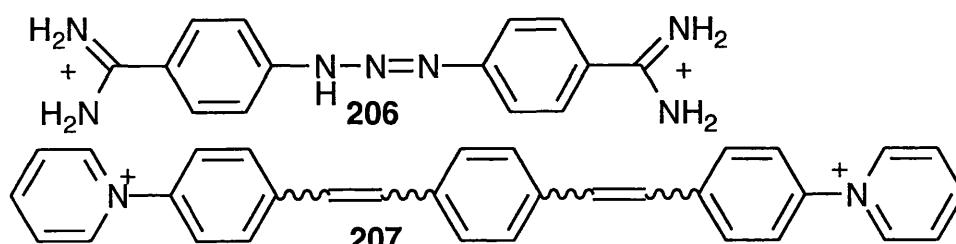
structure to bind to a dodecamer of DNA (CGCGAATTCGCG) and spans the major groove of a GC base pair with its terminal nitrogens making electrostatic interactions with phosphates on opposite strands of the DNA duplex. The central nitrogens are close to guanine O-6 and cytosine N-4 substituents.^{160, 161, 162} Larger molecular weight DNA binding compounds such as proteins make use of complementary side chain interactions with AT and GC base pairs to promote tight complexation. Lower molecular weight polyamines bind to DNA but lack the structural features necessary to give pronounced AT/GC selectivity.¹⁶³

The structure, dynamics and reactivity of DNA with biologically active molecules and non-intercalating ligands have been the subject of many studies recently.¹⁶⁴ Several ligands show DNA-binding specificity that leads to inhibition of DNA and RNA synthesis and a broad spectrum of antiviral, antibacterial, antitumour and antiprotozoal activity.¹⁶⁵

Distamycin A (204) has been shown to bind to the minor groove of DNA by Van der Waals forces between the CH protons of the pyrroles and C-2 hydrogens of adenine; and by electrostatic interactions between the terminal cationic groups and the phosphate DNA backbone. Distamycin A has a specific interaction with dA.dT rich areas of DNA, in contrast to other pyrrole-amidine antibiotics such as anthramycin which exert a preference for dG.dC base pairs.^{165, 166}

Berenil (206) is another AT selective DNA groove binder. It acts irreversibly on *T. brucei brucei* AdoMetDC and is used as an effective trypanocidal agent.¹⁶¹

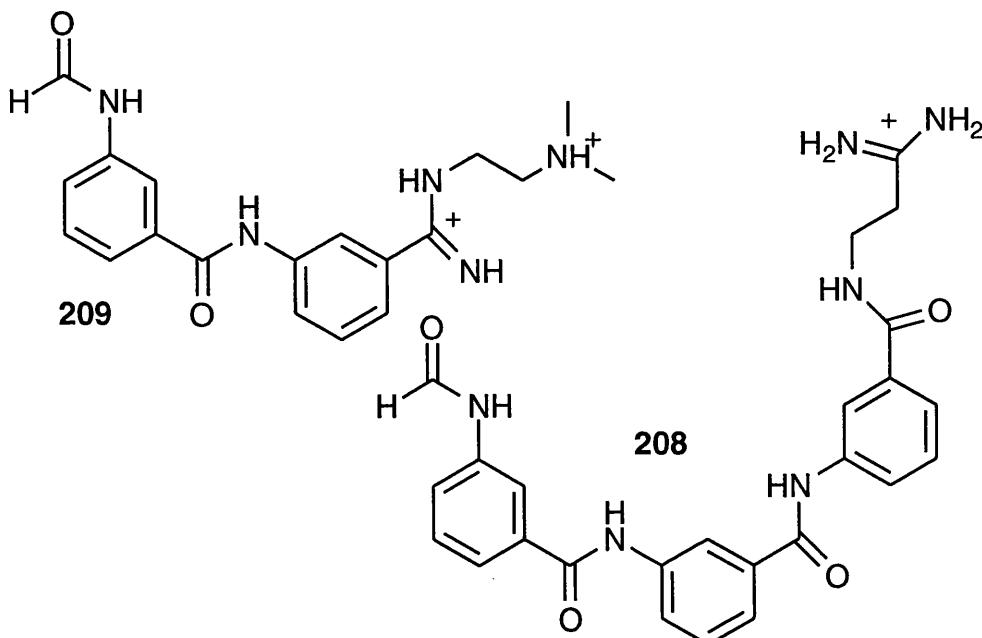
These AT-rich sequences may correspond to areas of maximum molecular electrostatic potential which may attract ligands with cationic functional groups. Hydrogen bonding is not essential for AT selectivity. This is shown by the bisquaternary ammonium heterocycle (**207**) which cannot hydrogen bond but still binds in the minor groove with AT selectivity. In general, good steric fit in the minor groove is a key requirement so that the adduct can be stabilised by electrostatic potentials; however hydrogen bonding, where possible, will also contribute significantly to stability.^{159, 167}



Our rationale for this area of work comes from work showing that the genome of *P. falciparum* is extremely rich in A+T (over 80% in most species) whilst the human host consists of only 59% A+T. Ligands specific for AT-rich DNA could be more inhibitory to the parasite than the host, and hence be effective antimalarials.^{165, 168}

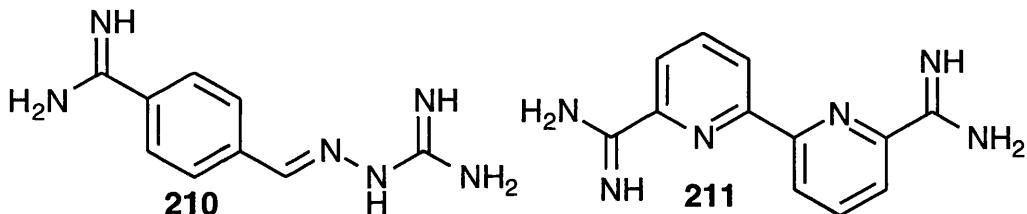
Those compounds with AT selectivity may be effective against parasitic diseases and indeed this is already the case with berenil. Distamycin A is also a potent inhibitor of parasite growth and investigations showed that this compound is more toxic to the ring stage of *P. falciparum* growth than trophozoite or schizont stages. This corresponds with the life cycle stage when most of the nucleic acid synthesis takes place. Distamycin A has been shown to be more toxic to parasites than mammalian cells. In contrast GC specific agents were more toxic to the mammalian cells than the parasite cells, as predicted by the rationale above. The use of distamycin A for anticancer work was abandoned, due to toxicity to the host. The toxicity against malaria parasites is 2000-3000 fold higher than against the host cells. There is hope that the extra permeability pathways found in the infected erythrocyte can be exploited to increase toxicity to the parasite and yet restrict the access to host cells, hence overcoming this problem.

Distamycin has suffered problems with low water solubility and there is concerted work to improve on this. A phenyl analogue of distamycin A (**208**) recognises AT-rich sequences of DNA through the minor groove as shown by Dasgupta and co-workers.¹⁶⁹



Lee *et al.* prepared (**209**) where the amide group was replaced by an amidine moiety, to improve water solubility.¹⁷⁰ The C-terminus in (**209**) contains the dimethylaminoethyl moiety which like the propioamidine group of (**208**) and distamycin A recognize an AT base pair. It is these functional groups we are interested in for our polyamine work.

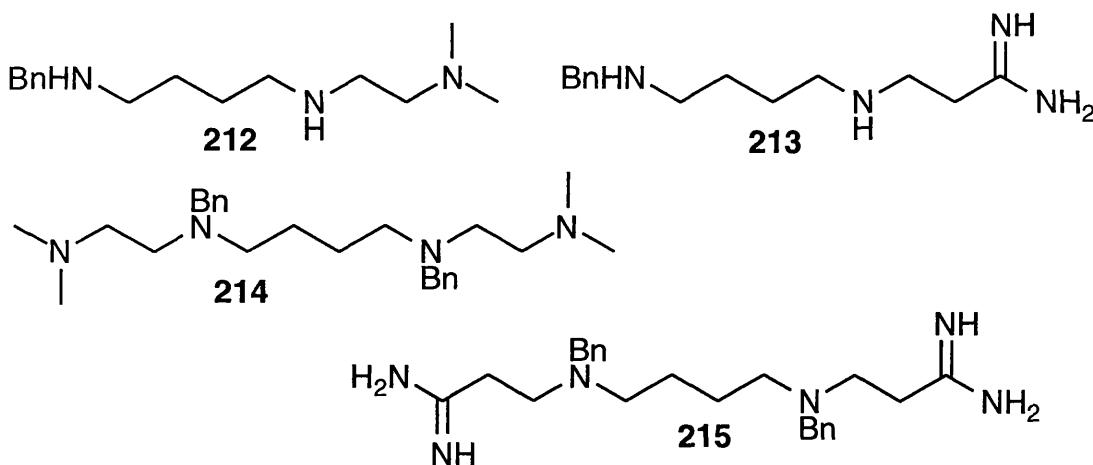
The use of diamidinic compounds on diseases caused by parasitic protozoa has greatly increased and Calonge and co-workers tested a number of diamidines, such as (**210**) and (**211**). The target of aromatic diamidines is DNA and binding takes place in the minor groove regions of DNA. Several other authors have found cationic diamidines to have strong inhibitory effects on AdoMetDC and putrescine transport in parasitic protozoa and host cells. This is shown by a depletion in polyamine pools and a reduction in cell proliferation.¹⁷¹



There is some dispute as to whether the antiproliferative effect of such compounds may be due to displacement of polyamines from their site of interaction with DNA thereby inhibiting replication/ transcription; however the formation of amidines is definitely worth pursuing.

The dimethylaminoethyl moiety and propioamidine group recognize an AT base pair and we wanted to incorporate these groups into some of our analogues to see if there was any antimalarial activity. We proposed two spermidine and two spermine analogues to synthesize and test for activity *in vitro* against *P. falciparum*.¹⁷²

The spermidine analogues (**212**) and (**213**) contain the 4 carbon backbone with an *N*-benzyl substituent on one end. On the other end is the functional group dimethylaminoethyl (**212**) or propioamidine (**213**). The spermine analogues also contain the four carbon backbone with dibenzyl substitution similar to our active spermine analogue (**189**) and the dimethylaminoethyl group on each end in (**214**) and propioamidine groups in (**215**).



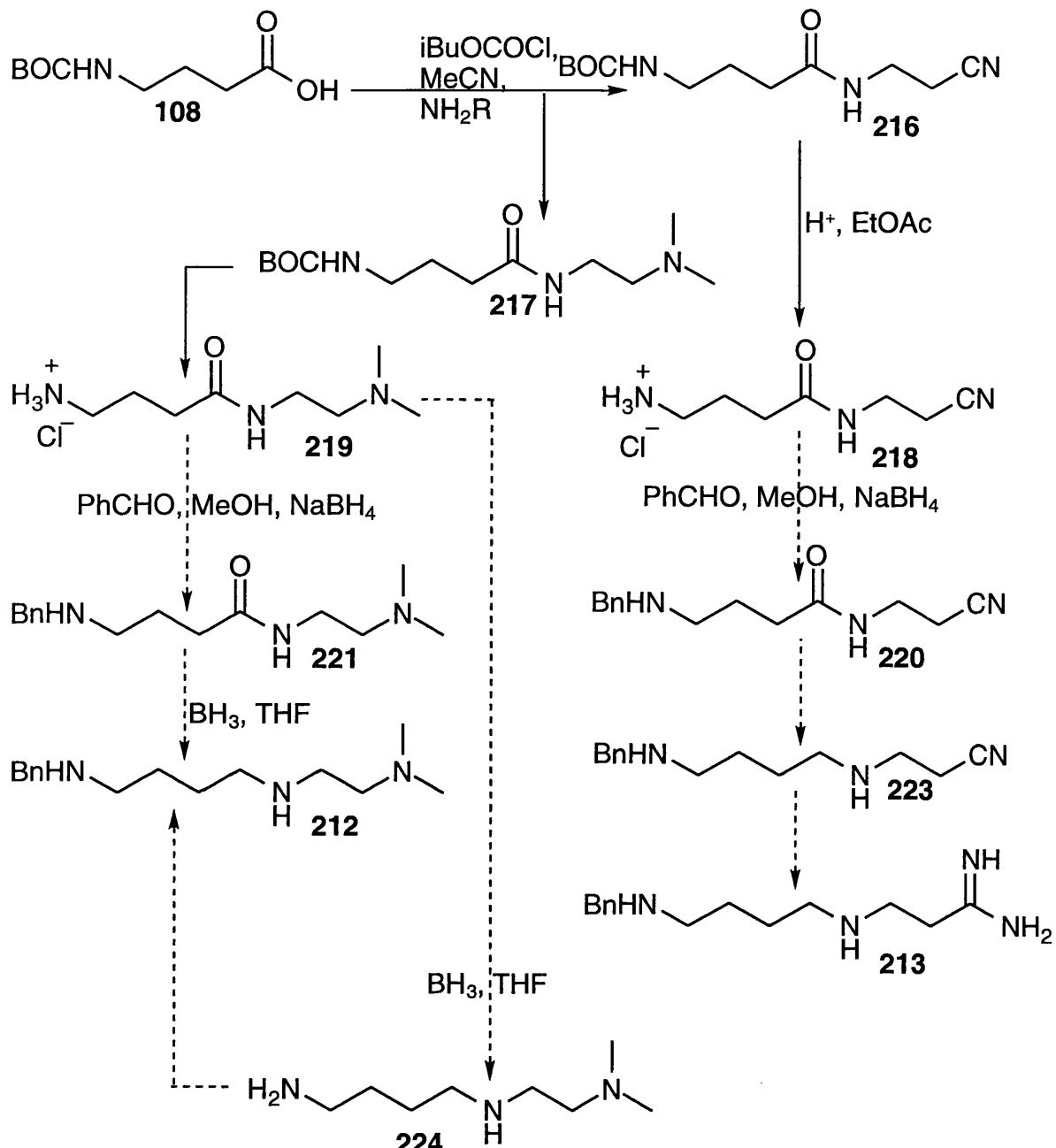
5.2 Approaches Towards Spermidine Analogues

5.2.1 Initial Approaches to Compounds (212) and (213)

Considering the structure of our two target compounds we decided that an approach similar to that of **Scheme 16** would provide a suitable starting point. This would use the BOC protected aminobutyric acid (**108**) to prepare amides (**216**) and (**217**) using mixed anhydride coupling conditions. BOC removal and reductive amination would provide intermediate amides which would be reduced to give the target products (including separate production of the amidine moiety) (**Scheme 39**).

Production of amide (**216**) went in reasonable yield (65%) and it was obtained as cream crystals. Removal of the BOC protecting group gave a hygroscopic semi-solid (**218**) which would not crystallise, so reductive amination to (**220**) was attempted on the crude material. Using 1 equivalent of benzaldehyde, 1 equivalent of triethylamine and 1.2 equivalents of sodium borohydride in methanol (**218**) were heated at reflux for 2 hours and then left at room temperature overnight. A complex mixture of products was obtained, shown by TLC, and purification was not attempted.

The functional groups that may be problematic are the amide and nitrile. Amides are not normally reduced by sodium borohydride, but require other reagents. This is also true about nitriles so the mixture of products was a surprise.¹⁷³ This route was discontinued as further stages in the sequence, e.g. selective reduction of amide functionality over nitrile in (**221**) would cause problems. An alternative was sought.



Scheme 39 Attempted synthesis of compounds (212) and (213)

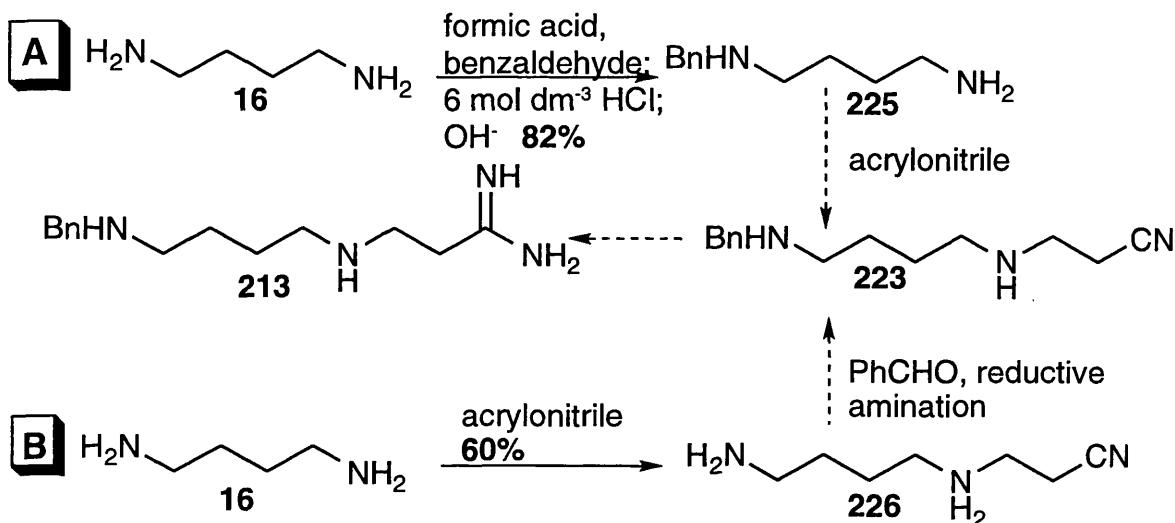
Purification of (217) caused problems when no crystallisation occurred. Alumina column chromatography was successful, eluting with hexane-ethyl acetate (4:1) enabling us to isolate (217), albeit in low yield (14%). Unfortunately the same problems with BOC deprotection and reductive amination occurred as with (220).

One alternative would be the reduction of (219) to the polyamine (224) and then selective reductive amination on the primary nitrogen as described before. This synthetic route was investigated briefly; however only intractable mixtures were obtained. The other consideration is use of the

BOC protecting group. It is acid labile and removed easily in 3 mol dm⁻³ acid; however these acid conditions might affect the nitrile group. If partial protonation was occurring in the deprotection step of (**216**) it could lead to the unwanted side reactions.

5.2.2 Other Approaches to (**213**)

We decided to prepare intermediate (**225**) which did not have the amide functionality and would allow direct formation of the amidine moiety from nitrile (**223**). In route A the benzyl group is added first and then the 2-cyanoethyl; whereas in route B the 2-cyanoethyl is added first followed by the benzyl group (**Scheme 40**).



Scheme 40 Attempted synthesis of compound (**213**)

Putrescine is particularly difficult to monofunctionalise due to the two identical amino groups and its tendency to give bifunctionalised compounds even if the diamine is used in excess. Bergeron and co-workers had looked at this problem and developed an alternative route for the preparation of (**225**).¹⁷⁴ Putrescine was dissolved in formic acid at 0 °C and 1 equiv. of benzaldehyde was added. The solution was warmed to room temperature and then refluxed until the benzaldehyde had disappeared by TLC. After cooling and the addition of 6 mol dm⁻³ hydrochloric acid the solution was heated at reflux for 18 hours to remove any formamide. Distillation afforded (**225**) in good yield. We wanted to alkylate the primary nitrogen with 1 equivalent of acrylonitrile whilst not affecting the benzylic nitrogen. The first attempt involved stirring diamine (**225**) at 0 °C and slowly adding 1 equiv. of acrylonitrile, following the procedure of Israel *et al.*¹²⁸ On work up, NMR

spectroscopy revealed a complex mixture of alkylated products, shown by the extra quaternary carbon peaks in the nitrile region. The second attempt used the same reaction conditions but on a solution of (225) in ethanol to provide a more dilute system. Overalkylation occurred here also.

Route B also started from putrescine, this time utilising the chemistry of Israel *et al.* to form (226).¹²⁸ Putrescine and 1 equiv. of acrylonitrile were stirred at 0 °C and then gradually warmed to 60-70 °C for 2 hours, and the product was purified by distillation. The benzyl group was then to be added by reductive amination. We chose to use the chemistry of Sclafani *et al.*¹³⁸ heating (226) at reflux in chloroform with 1 equivalent of benzaldehyde to form the imine selectively on the primary nitrogen. After concentration of the solution sodium borohydride in methanol was added to reduce the imine. Unfortunately this route resulted in a large number of products in the final mixture, shown by TLC. Purification was not attempted.

Reactant	Desired Product	Conditions	Result
225	213	1 equiv. acrylonitrile	overalkylation
225	213	1 equiv. acrylonitrile in ethanol	overalkylation
226	213	CHCl ₃ , PhCHO; NaBH ₄ , MeOH	mixture of products
226	213	CHCl ₃ , PhCHO; NaCNBH ₃ , MeOH	mixture of products

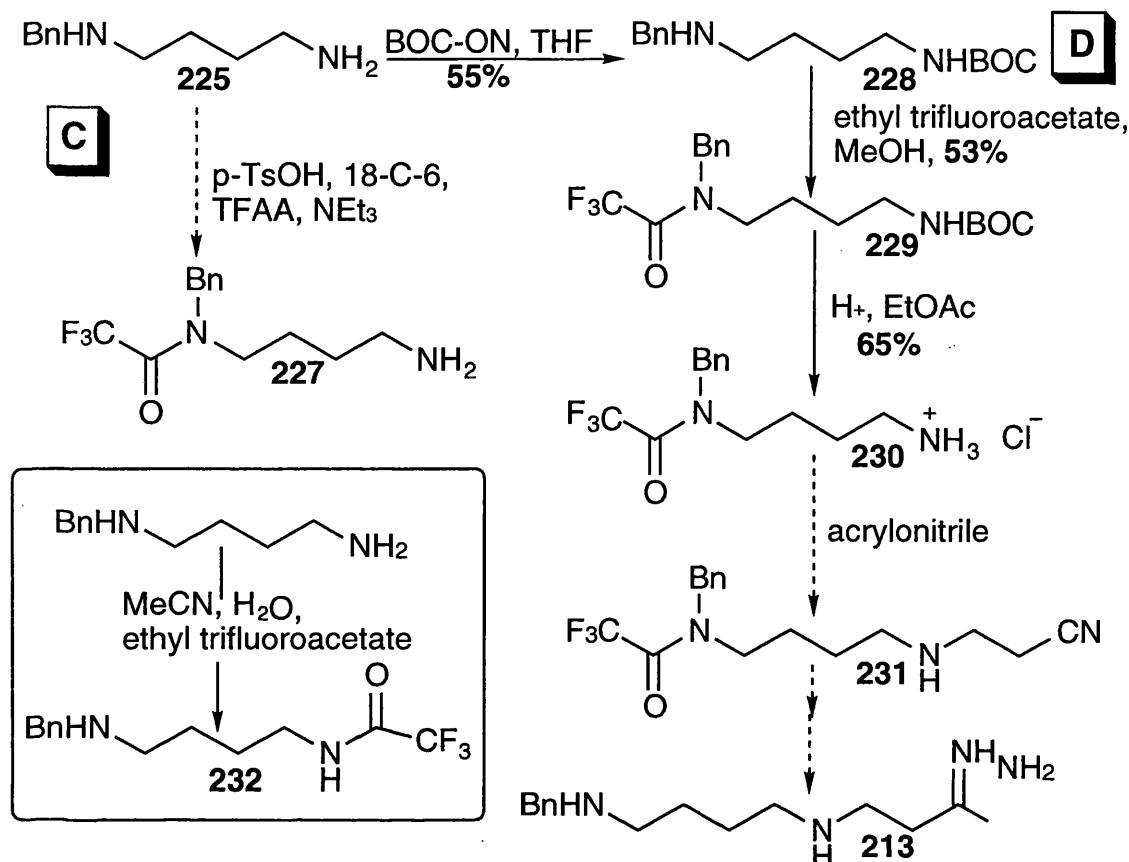
Figure 41 Attempts to prepare (213)

Examining the intermediate (226) the problem of side reactions may be related to the introduction of the benzyl group using reductive amination. The milder reducing agent sodium cyanoborohydride was used; however this also resulted in a number of products. This may be due to the slightly acidic nature of chloroform leading to side reactions of the nitrile group.

Looking at route A we have a problem of a mixture of alkylation products so if we can protect the secondary nitrogen we only have to concentrate on adding one 2-cyanoethyl unit to the primary nitrogen.

The quickest route to protect the secondary nitrogen would be directly (route C) rather than the more laborious route of protection of the primary

nitrogen then the secondary nitrogen and deprotection etc. shown in route D (Scheme 41).



Scheme 41 Attempted preparation of (213)

Few routes are available for the selective protection of a secondary amine in the presence of the more sterically favourable primary amine. Selective reaction of the secondary amine in the presence of a primary amine was investigated by Barrett and Lana using 18-crown-6.^{175, 176} 18-C-6 is able to complex primary ammonium salts by N-H···O hydrogen bonding and N⁺···O interactions. This is a form of dynamic protection with the primary nitrogen held in a complex whilst the secondary nitrogen is acylated (Figure 42).

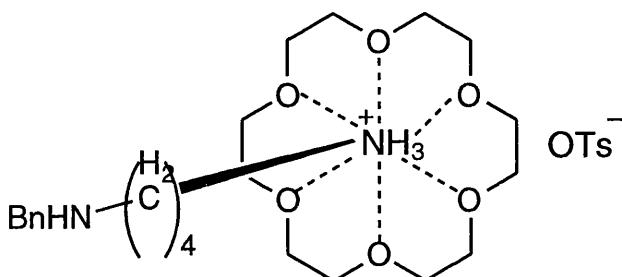


Figure 42 Complexation of NH₃⁺ by 18-C-6

Ganem *et al.* had also used this type of methodology in the selective acetylation of secondary amines.¹⁷⁷ Adapting this route of Barrett *et al.* diamine (**225**) was stirred in acetonitrile with *p*-toluenesulphonic acid. The solution was concentrated *in vacuo* to give a clear oil which was stirred in DCM at 0 °C. Triethylamine (1.1 equiv.) and then trifluoroacetic anhydride were added and the solution was stirred at room temperature overnight. Purification of the concentrated solution was attempted using a mild basic wash and also column chromatography; however the desired product (**227**) was not obtained as a pure sample.

The longer route D was then attempted. The diprotected putrescine (**228**) had been prepared by Bergeron and co-workers by stirring (**225**) in THF with BOC-ON.¹⁷⁴ Purification was carried out by column chromatography rather than their distillation techniques as the lower pressures needed for efficient distillation were not easily attained on our vacuum pumps. This may be the reason for the lower than reported yield. We then wanted to protect the secondary nitrogen. Obviously the protecting group should not be acid sensitive as we wanted to remove BOC and leave on this new group. Removing the protecting group at a later stage was also a consideration as we did not want any side reactions with the nitrile group i.e. no reducing conditions. The choice of group was trifluoroacetyl which is base labile under mild conditions. The protected putrescine (**228**) was stirred in methanol with ethyl trifluoroacetate for 48 hours before concentrating *in vacuo* to give a thick yellow oil. Purification was achieved by silica chromatography and (**229**) was isolated in 53% yield. The reaction had not gone to completion by TLC suggesting that the secondary nitrogen is not highly susceptible to the reaction with the ester. This is not surprising due to the steric hindrance around the nitrogen; however the use of a stronger acetylating agent was not investigated in case of lability of the BOC group under harsher conditions. Column chromatography resulted in isolation of both the desired product and starting material which could be recycled. The BOC group was removed in the usual way to yield (**230**) as a thick yellow oil; however upon trituration with ether a white powdery solid was obtained in good yield (65%). Attempted alkylation of (**230**) in ethanol with one equivalent of acrylonitrile was attempted; however initial indications were that overalkylation had again occurred. This route was only attempted once on a small scale and may be worth pursuing.

In brief, the selective protection of (**225**) on the primary nitrogen with the TFA group to give (**232**) goes in excellent (80%) yield; and could be an alternative strategy.

5.2.3 Analytical Data for Compounds (**228**), (**229**) and (**230**)

The variations in the NMR spectra as we introduce different protecting groups onto the polyamines are highly noticeable.

Formation of (**228**) led to the introduction of a BOC group, which is easily recognisable in the ^1H NMR (**Figure 43**) and ^{13}C NMR spectra because of the tert-butyl group.

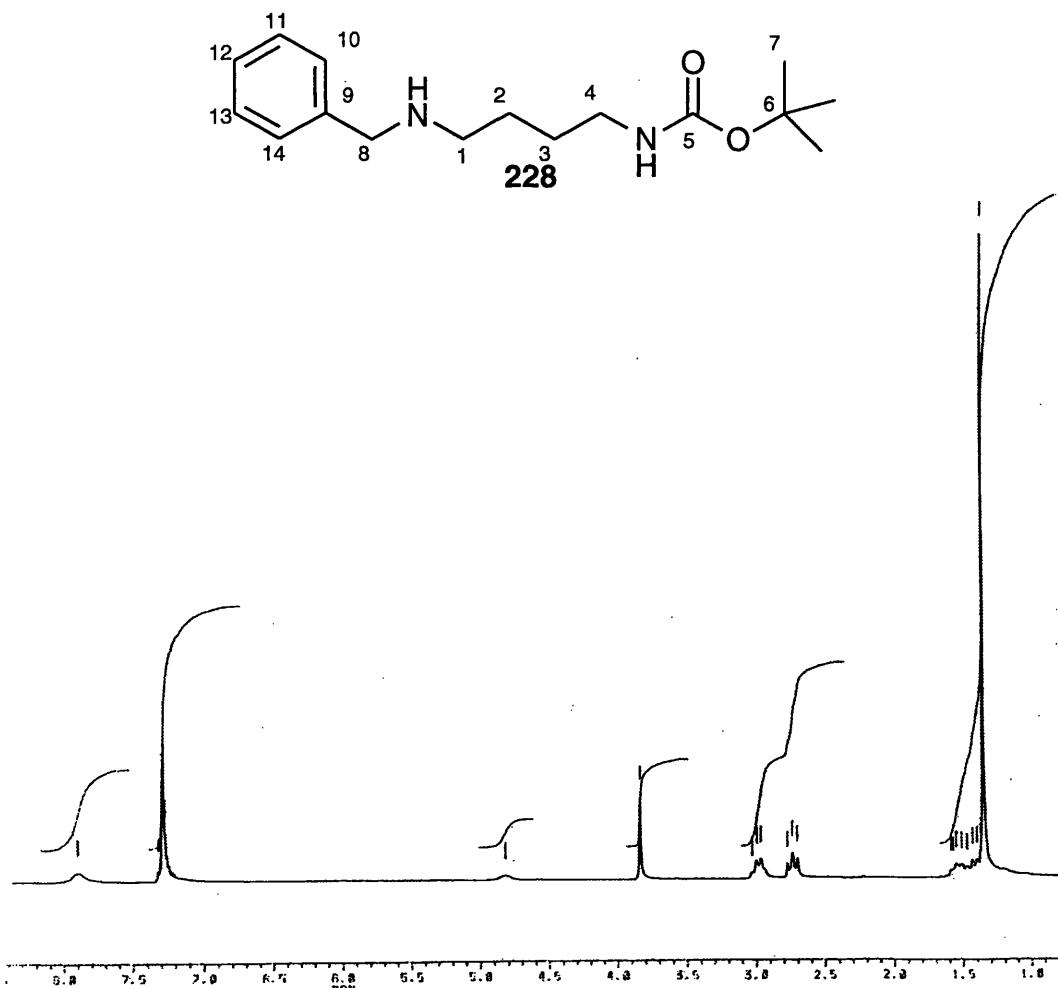


Figure 43 ^1H NMR spectrum of (**228**), 200 MHz, CDCl_3

Protons 2 and 3 form the expected multiplet followed by a multiplet for H-1 and then H-4. The benzylic protons occur as a singlet as does the aromatic region. In the ^{13}C NMR spectrum we have the usual four peaks for the aromatic ring with C10=C14 and C11=C13. The IR spectrum of (**228**)

shows a sharp stretch at 3335 cm^{-1} corresponding to the NH of the carbamate, and a variety of peaks around $1500\text{-}1700\text{ cm}^{-1}$ due to the C=O stretch of the carbamate (expected value ROCON $1740\text{-}1690\text{ cm}^{-1}$) and the C=C stretching of the aromatic ring. The C-H deformation of the aromatic ring gives two peaks at 754 and 700 cm^{-1} characteristic of a mono-substituted benzyl ring, and this is common to all these adducts.

Figure 44 shows the ^1H NMR spectrum of compound (**229**) containing the BOC and TFA protecting groups. The singlet for the BOC group is still present; however it appears very finely split, suggesting two environments for those protons. The usual multiplets appear for H-2 and H-3 with H-1 and H-4 appearing more complex than in the spectrum of (**228**). The benzyl protons no longer appear as a singlet, but as two singlets indicating magnetic non-equivalence. The aromatic region is also split into two distinct areas, rather than the usual singlet. The carbamate NH is slightly hidden underneath the benzylic protons signal. The IR spectrum is very similar to (**228**) with the C=O stretches of the amide and carbamate overlapping at 1688 cm^{-1} .

Figure 45 shows the ^{13}C NMR spectrum of compound (**229**). The tertiary butyl group is obvious as the large CH_3 identified by the use of DEPT. There is a large number of aliphatic CH_2 peaks in the spectrum and in the aromatic region of the spectrum there are 5 CH peaks corresponding to each separate carbon atom and two quaternary peaks appear for C-9. The carbamate quaternary carbon is found at δ 155.9 and the TFA quaternary carbon peak is not observed. This is due to the C signal being split into a quartet by the three neighbouring fluorine atoms, and hence the signal is too weak to be seen. All these factors suggest the presence of rotamers of (**229**) with the magnetic inequivalence this brings due to interconversion between rotamers being slow on the NMR time scale.

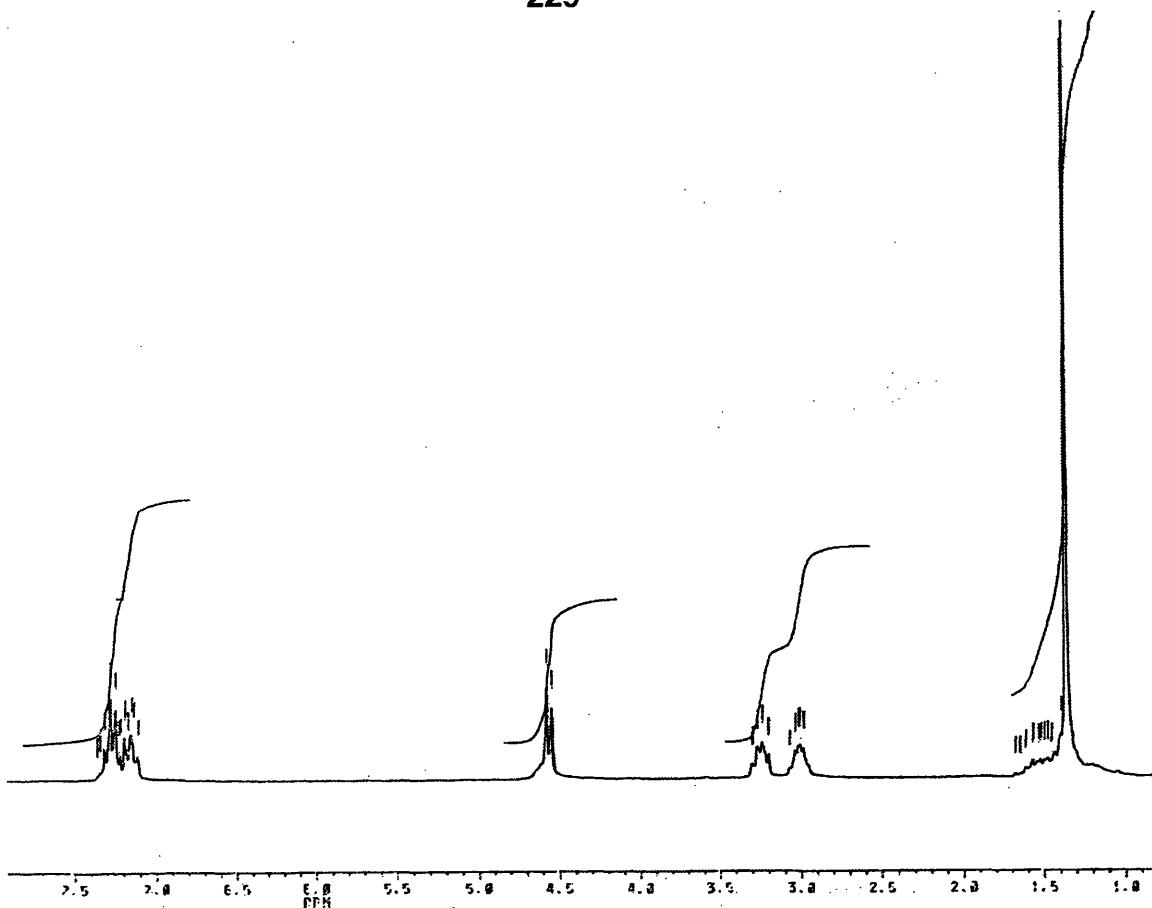
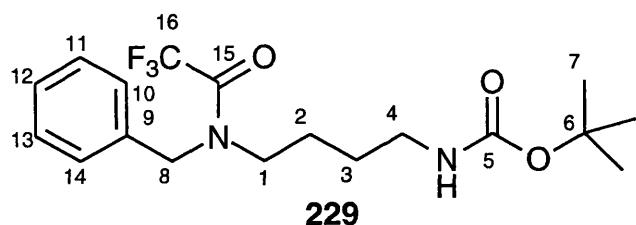


Figure 44 ^1H NMR spectrum of compound (229), 200 MHz, CDCl_3

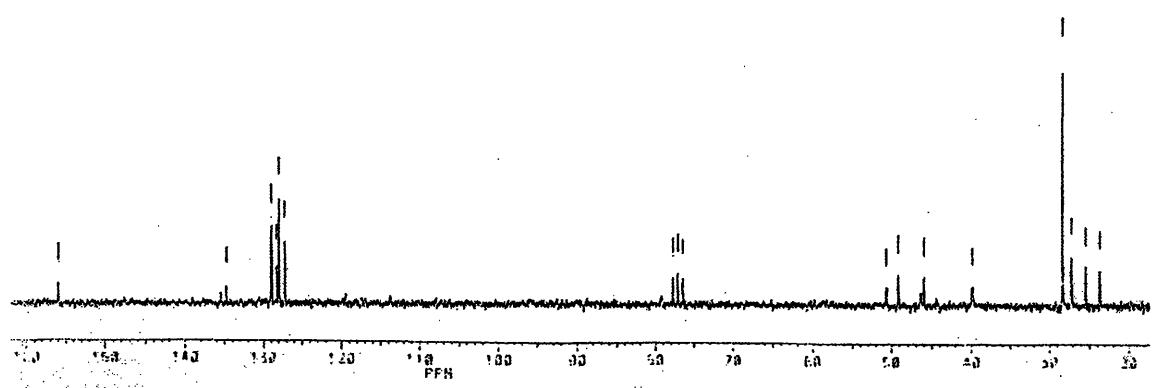


Figure 45 ^{13}C NMR spectrum of compound (229), 50 MHz, CDCl_3

The ^1H NMR spectrum of (230) contains broad multiplets corresponding to the methylene protons 2,3, and 4. H-1 appears as a triplet at lower field due to the electron withdrawing effects of the amide group. The benzylic protons H-8 are partially obscured by the DOH peak of the NMR solvent so we are unable to ascertain whether it appears as two singlets. The aromatic region is still split into two distinct areas. The ^{13}C NMR spectrum is more defining and is seen in **Figure 46**. The large CH_3 signal is no longer present confirming BOC deprotection. Protons 2 and 3 appear as four CH_2 peaks around δ 25. At lower field appears another CH_2 peak and then four more CH_2 carbons at approximately δ 50. This corresponds to 1-C and 8-C leaving the single peak at δ 40 due to C-4. This appearance of extra peaks in the ^{13}C NMR spectrum is characteristic of the presence of rotameric forms of (230). The two rotameric forms are magnetically equivalent in some regions of the spectrum as at δ 40 where only one peak is seen. Elsewhere multiple peaks are seen.

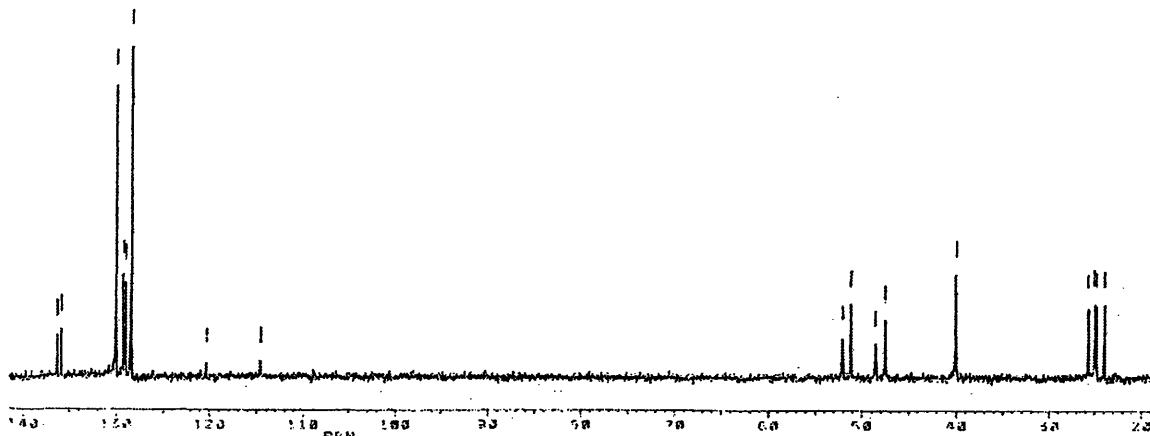
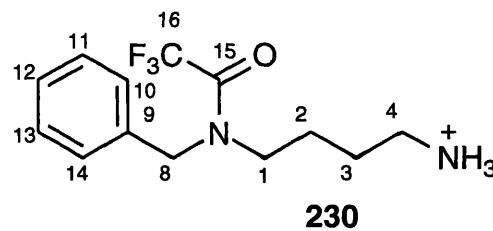
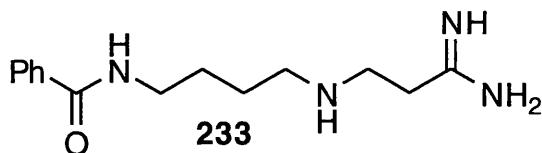


Figure 46 ^{13}C NMR spectrum of (230), 50 MHz, D_2O

5.2.4 Approaches Towards Compound (233)

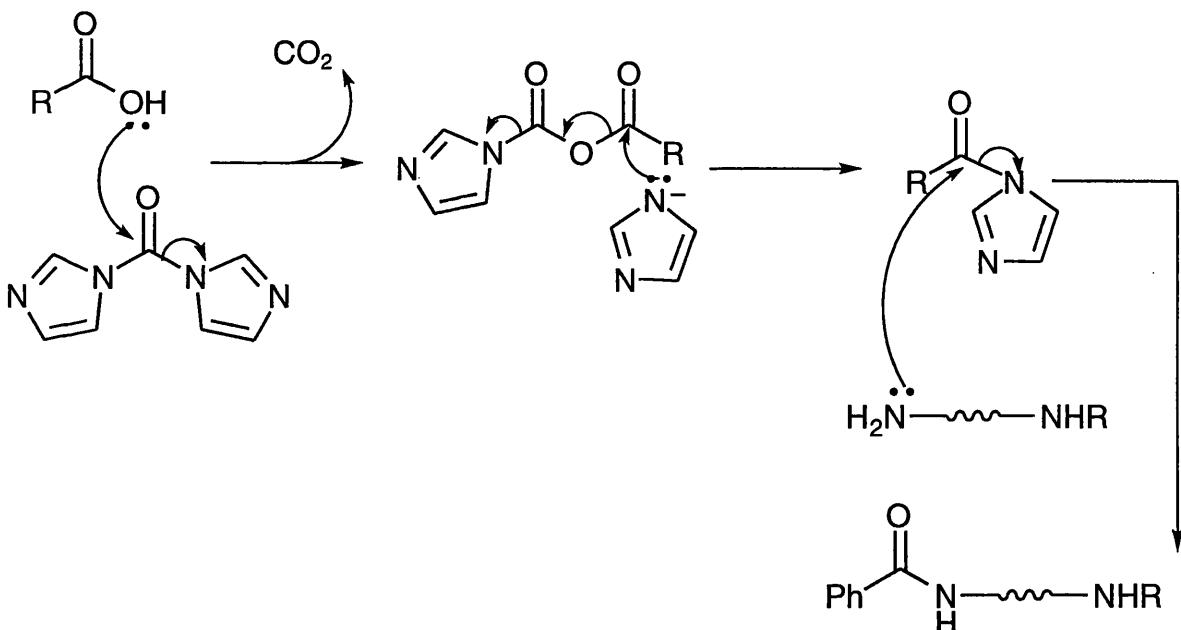
In order to produce a suitable compound for testing (233) was established as a new target, containing an amide linkage rather than the benzyl group. This compound was designed as the selective acylation of primary amines was reported in the literature and we would see if this type of

reaction occurred in reasonable yield compared to the failed attempts at selective reductive amination.



The nitrile (**226**) was available and we wanted to carry out selective acylation on the primary nitrogen. A number of synthetic routes exist for the selective acylation of primary nitrogens; however we wanted mild conditions so as not to effect the nitrile group. Acyl cyanides have been investigated by Murahashi in these types of reactions in solvents such as DCM and acetonitrile.¹⁷⁸ We decided to follow the procedure of Bergeron who had used *N,N*-carbonyldiimidazole (CDI) in the selective acylation of spermidine, spermine and other polyamines.¹⁷⁹

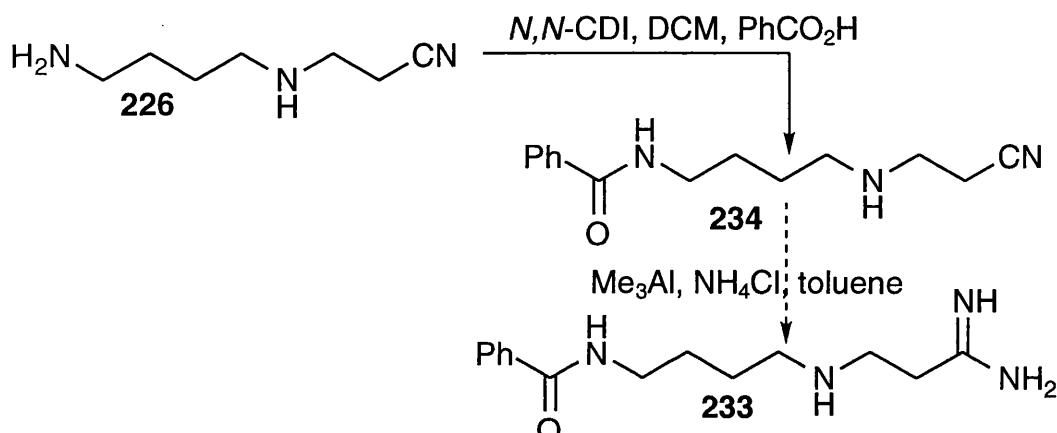
Acylimidazoles are generated easily from the carboxylic acid and CDI in DCM, at room temperature, in only one-two hours. The amine is then added and after stirring for 24-48 hours the acylated amine is formed. Imidazole as a side product is easily removed in an aqueous wash and any remaining carboxylic acid is removed by a slightly basic wash (**Scheme 42**).



Scheme 42 Mechanism of selective acylation of primary amines

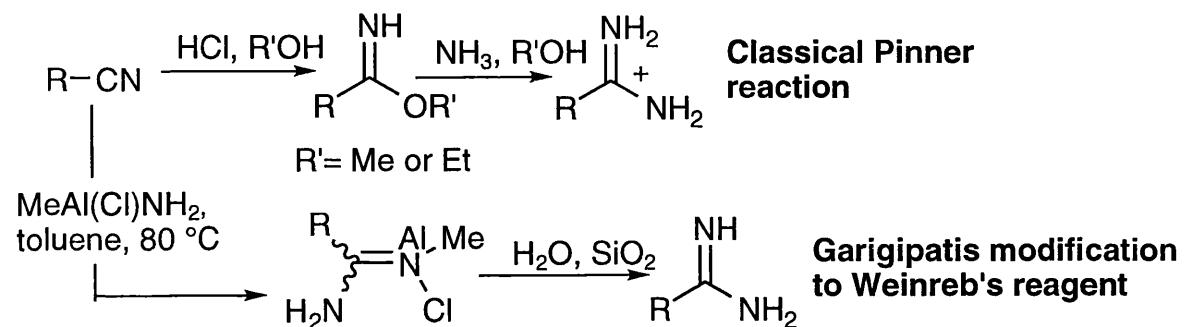
(**226**) was added to a pre-stirred solution of benzoic acid and CDI and the reaction was monitored by TLC. After 24 hours the reaction was worked

up as described above to yield a glassy solid. Purification by silica chromatography yielded (**234**) as a white solid. The yield (33%) was fairly low, maybe due to the polar nature of the product and therefore loss of product during the aqueous work up (**Scheme 43**). No side reactions were observed; maybe the reductive amination step would proceed in a non-acidic solvent such as DCM or tetrachloroethane although this was not pursued.



Scheme 43 Attempted synthesis of (**233**)

Conversion of a nitrile into an amidine is carried out by the Pinner reaction and has been used to form the amidine functionality in compounds such as Distamycin A (**204**). The reaction is a two step process: reaction with HCl/alcohol to give an intermediate imidate salt, followed by a reaction with NH₃/alcohol to yield the desired imidinium salt. An alternative to this traditional method is the use of the Garigipatis modification of Weinreb's reagent, which is particularly useful for sterically congested nitriles.^{180, 181} The aluminium reagent, originally developed by Weinreb, was prepared by the addition of ammonium chloride to commercially available trimethylaluminium and the amine complex was then hydrolysed by water adsorbed on silica gel (**Scheme 44**).



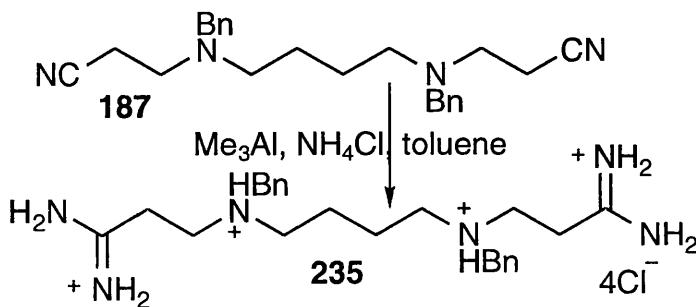
Scheme 44 Preparation of an amidine from a nitrile

One problem with the Pinner reaction is the need for extremely dry solvents to prevent hydrolysis of the intermediate. For ease of the reaction and availability of reagents we decided to utilise the Garigipatis method on compound (234) to prepare the amidine. After stirring the trimethyl aluminium solution with ammonium chloride the nitrile was added and stirred at 80 °C for 20 hours. The solution was poured onto a slurry of silica gel and water and after five minutes the silica was filtered and washed with methanol. The concentrated solution was redissolved in a small amount of methanol to remove any remaining ammonium chloride and HCl/Et₂O was added. A yellow hygroscopic solid was isolated which by NMR spectra showed a mixture of products (**Scheme 43**).

Lack of positive results from antimalarial testing of our other spermidine analogues prompted us to pursue the spermine analogues we had designed rather than the spermidine analogues which were proving troublesome.

5.3 Synthesis of Spermine Analogue (215) and its Tetrahydrochloride salt (235)

The amidine moiety is based around a three carbon unit which immediately suggests the dinitrile (187) used previously in the synthesis of benzylated spermine analogue (189) as a starting point (**Scheme 45**). The Garigipatis method for production of an amidine was utilised and, following the conditions described for (233) we were able to isolate (235) in 40% yield, as the hydrochloride salt. The lower than expected yield may be due to the polar nature of this compound. The work up involves hydrolysis on silica and the polar product will bind to very strongly to it. Although it was washed well with methanol some product may be retained on the silica. The tetrahydrochloride salt was prepared immediately from the free base.



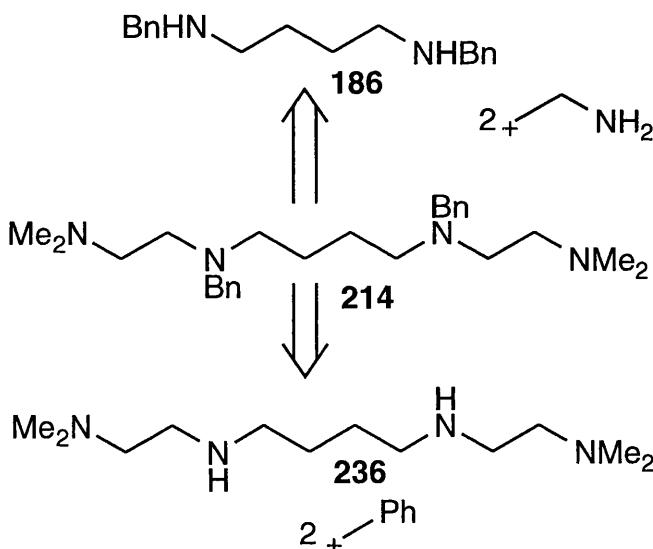
Scheme 45 Synthesis of compound (235)

Loss of the nitrile peaks from both IR and ^{13}C NMR spectra were indications that the reaction had occurred. An IR spectrum of the product showed a new absorption at 1689 cm^{-1} corresponding to the C=N stretch. The ^1H NMR spectrum was not useful due to the large number of multiplets for the methylene protons. The ^{13}C NMR spectrum also confirmed loss of the nitrile peak and the appearance of a new quaternary nitrogen at $\delta 167.0$, corresponding to the amidine group. We encountered problems trying to get a molecular ion for (**235**) due to extensive fragmentation, even when Cl or FAB were used.

5.4 Synthesis of Spermine Analogue (**214**) and its Tetrahydrochloride Salt (**243**)

5.4.1 Introduction

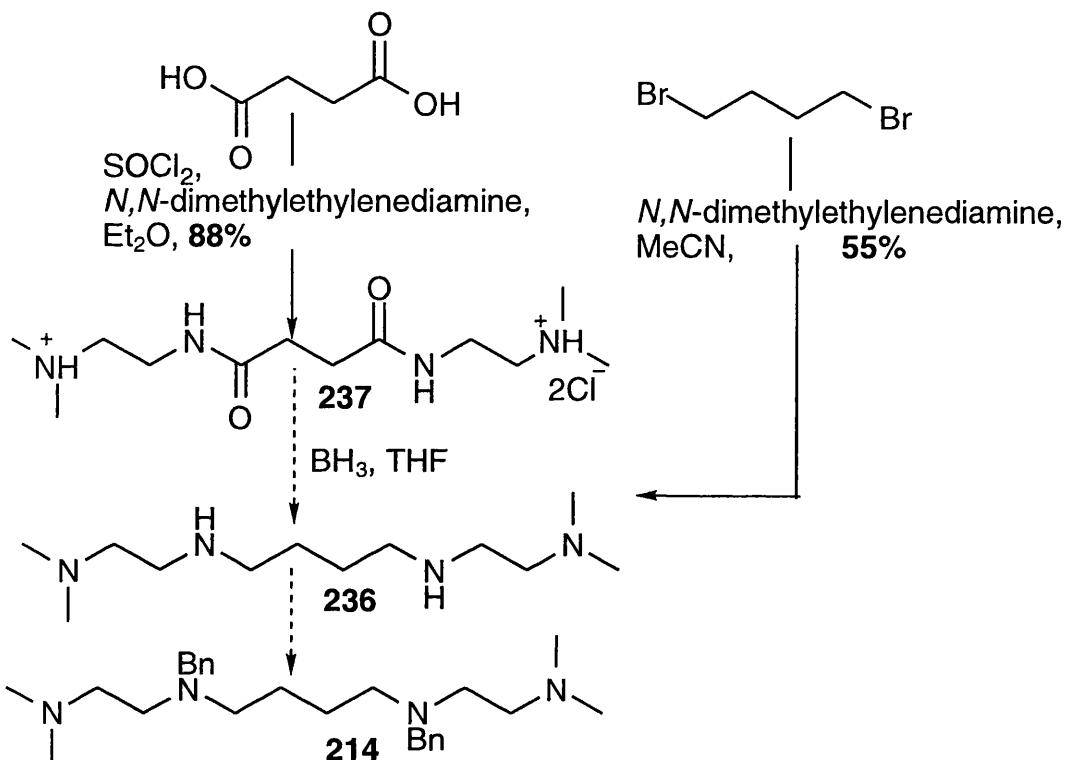
The synthesis of (**214**) can be considered in a number of ways and is shown in **Scheme 45**: in route 1 the carbon skeleton is prepared, with the *N,N*-dimethylaminoethyl moiety present, and the aromatic group added last; whereas in route 2 *N,N'*-dibenzylputrescine is utilised and a two carbon unit attached at each secondary nitrogen to form the *N,N*-dimethylaminoethyl moiety. We decided to pursue both routes.



Scheme 45 Analysis of routes to (**214**)

5.4.2 Attempted Synthesis of (214) via Route 1

To form a symmetrical polyamine with a central four carbon unit 1,4-dibromobutane or succinic acid are obvious starting materials and both routes are shown in **Scheme 46**.



Scheme 46 Attempted routes to compound (214)

Succinyl dichloride was stirred in dry ether at 0 °C and *N,N*-dimethylethylenediamine (2.2 equiv.) was added slowly. The resulting precipitate was filtered and recrystallised from MeOH-EtOH to yield (**237**). The amide reduction was attempted using borane in THF, as used previously for our polyamide reductions; however an impure and hygroscopic product was isolated which was difficult to purify.

The route from 1,4-dibromobutane was more direct using the usual S_N2 reaction to yield (**236**). Purification was slightly problematic due to co-distillation of the product with the starting materials; however on a small scale using Kugelrohr distillation the tetraamine was isolated.

We then looked at methods of alkylating the secondary nitrogens and a summary of our attempts can be found in **Figure 47**. The most obvious starting point was reductive amination using the usual conditions of benzaldehyde in methanol, followed by treatment with sodium borohydride.

After monitoring the reaction of the tetraamine with benzaldehyde by TLC no imine formation had occurred. Direct alkylation with benzyl bromide was attempted. Stirring (**236**) in acetonitrile with Hunig's base, benzyl bromide was added slowly; however a large number of UV active products were identified by TLC. We wanted alkylation only on the secondary nitrogen and had used Hunig's base to help prevent quaternisation; however a large number of side reactions had obviously occurred. Reactions with the tetraamine and benzoyl chloride in DCM failed to give any product.

Wright had looked at the alkylation of amines by esters and lithium aluminium hydride in a one step reaction.¹⁸² He had demonstrated that this reaction was unsuitable for preparation of secondary amines from primary amines, but was often suitable for the formation of tertiary amines. Low yields were recorded if an aromatic amine was alkylated; however the yields were generally good when dialkylamines were alkylated. Ethyl benzoate was useful as an alkylating agent for the introduction of a benzyl group and this method was thought to be good when the formation of quaternary salts was to be avoided.

Lithium aluminium hydride was stirred in THF for 30 minutes with tetraamine (**236**). Ethyl benzoate was then added dropwise and the solution was heated at reflux overnight. Work up was carried out using basic conditions and excess THF was removed by distillation. The resulting residue was isolated as a hydrochloride salt, however NMR spectroscopy showed only starting tetraamine to be present.

Reactant	Desired Product	Conditions	Outcome
236	214	PhCHO, MeOH, NaBH ₄	No imine formation
236	214	BnBr, MeCN, Hunig's base,	Complex mixture
236	214	PhCOCl, DCM	Starting material
236	214	PhCO ₂ Et, LiAlH ₄	Starting material

Figure 47 Summary of attempted routes to (**214**)

5.4.3 Synthesis of (**214**) via Route 2

The dibenzylputrescine derivative (**186**) was available in excellent yield from putrescine and we needed a way of adding a 2-aminoethyl unit. A review by Bradshaw *et al.* covers many synthetic routes to polyamines,

including extending the polyamines by the 2-aminoethyl unit. A large number of routes are available for this process and a few are summarised in **Figure 48**.^{183, 184}

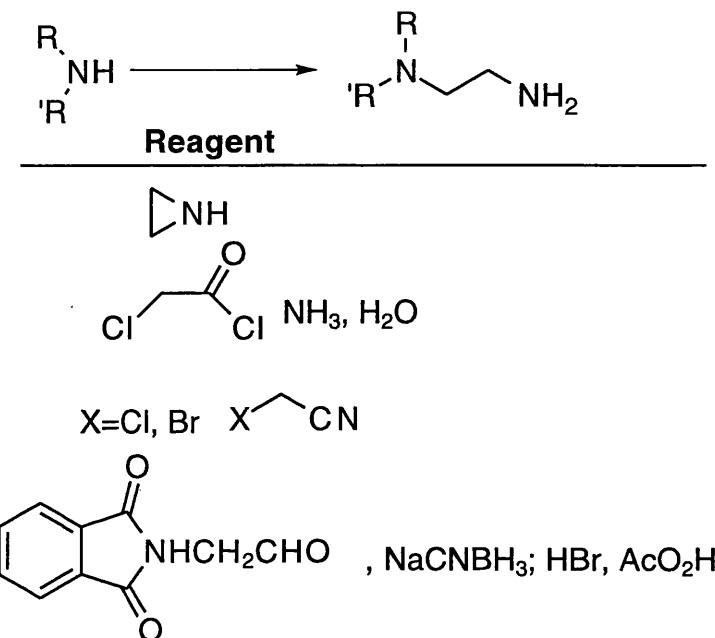


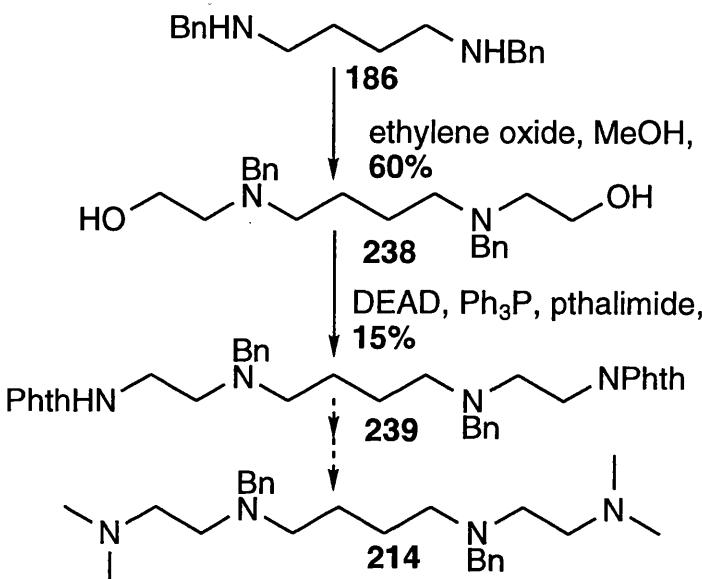
Figure 48 Synthetic routes to the 2-aminoethyl unit

Work started on the opening of an epoxide to form a 2-hydroxyethyl unit as prepared by Lee.¹⁴⁸ (**186**) was stirred in methanol and cooled with a dry ice bath as ethylene oxide was added. The flask was stoppered and stirred at room temperature overnight. After concentrating *in vacuo* the aminoalcohol (**238**) was isolated in 60% yield, comparable with the literature value. Transformation of the primary alcohol into a leaving group through, for example, bromination¹⁸³ or tosylation was not attempted due to possible side reactions from aziridine formation. Instead conversion into either a primary amine or the dimethylamino moiety was investigated.

Conversion of an alcohol into a primary amine may be achieved in a wide variety of ways.^{184, 185, 186, 187} Direct conversion from the primary alcohol into the dimethylamino group was considered however a suitable method was not found.^{188, 189} The chemistry of Mitsunobu was used allowing the transformation of a primary alcohol to a phthalimide followed by conversion into the NH₂ group and finally the tertiary amine (**Scheme 47**).

Alcohol (**238**) was stirred in THF with triphenylphosphine and phthalimide. Diethyl azodicarboxylate (DEAD) was added to produce a yellow solution which was stirred at room temperature for 12 hours. After concentrating *in vacuo* and triturating in ether-hexane to remove any

triphenylphosphineoxide the remaining solution was purified by column chromatography. Silica was used, eluting with hexane-ethyl acetate; however the product was still impure and chromatography was repeated to get a pure product. This resulted in a low yield of (**239**) of only 15%. Due to the troublesome nature of this reaction another route was investigated.



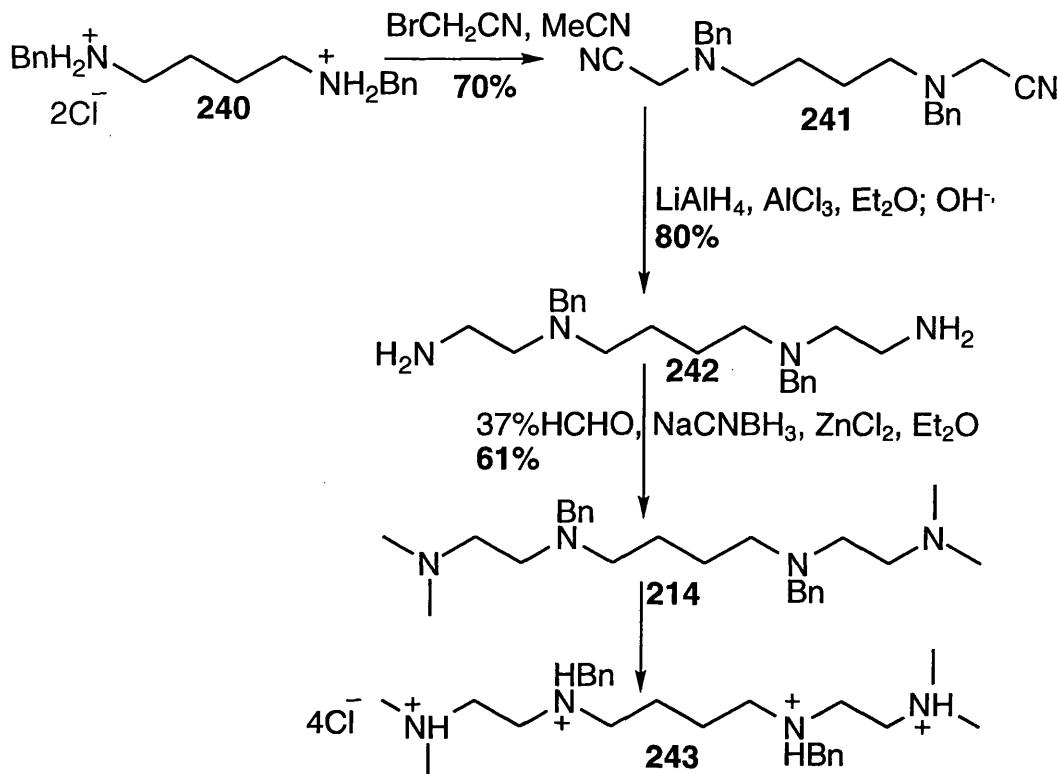
Scheme 47 Attempted preparation of (**214**) via Mitsunobu chemistry

Another reagent for the addition of a two carbon unit is bromoacetonitrile or chloroacetonitrile (**Scheme 48**).

(**240**) was stirred in chloroform with Hunig's base and bromoacetonitrile was added dropwise and the solution was stirred at room temperature for 18 hours. The dihydrochloride salt was easily prepared by the procedure of Lee *et al.*¹⁴⁸ After an aqueous work up the resulting solid was recrystallised from ethyl acetate-hexane to yield (**241**) as fine white needles. Reduction of the nitrile used the usual method of lithium aluminium hydride and aluminium chloride to yield (**242**) in good yield (80%).

The remaining consideration was how to form the *N,N*-dimethyl group. Conversion of primary amines into tertiary dimethylated amines may be achieved using formaldehyde and formic acid (Eschweiler-Clarke reaction) which is discussed in a review.¹⁹⁰ Reductive amination with formaldehyde can also be used¹⁸⁴ with a variety of reducing agents, the most efficient being sodium cyanoborohydride.¹⁹¹ Problems still existed with the reaction as a mixture of starting material and only partially methylated products were produced. Kim had used zinc-modified sodium cyanoborohydride to complete this type of reaction in excellent yields. The zinc-modified reducing

agent was formed by mixing sodium cyanoborohydride and anhydrous zinc chloride in a 2:1 molar ratio, in ether.¹⁹²



Scheme 48 Preparation of (214) and its salt (243)

The tetraamine was stirred in 37% aqueous formaldehyde in methanol and sodium cyanoborohydride and anhydrous zinc chloride were added. After 4 hours at room temperature basic work up yielded (214) as the free base in 61% yield. The tetrahydrochloride salt (243) was isolated from HCl/CHCl_3 to yield a white solid which was slightly hygroscopic, but stable enough to be handled.

5.4.4 Analytical Data on (241), (242) and (214)

Formation of nitrile (241) was established by the characteristic CN absorption in the IR spectrum at 2230 cm^{-1} and in the ^{13}C NMR spectrum a quaternary carbon peak occurs at $\delta 114.8$. The EI mass spectrum gives a molecular ion at $m/z 346$; however the most abundant peak is at $m/z 91$ indicating the presence of a benzyl group. Microanalysis also confirmed the formation of a pure compound.

Reduction of (241) to give (242) was also easily analysed due to the loss of the CN stretch from the IR and the ^{13}C NMR spectra. An extra peak

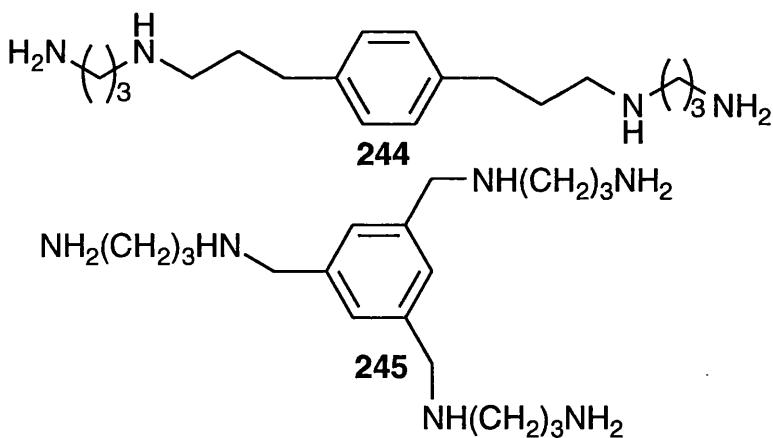
appears in the aliphatic region of the ^{13}C NMR spectrum due to the extra methylene formed in the reduction reaction. The ^1H NMR spectrum no longer shows a singlet for NCH_2CN but two triplets for $\text{NCH}_2\text{CH}_2\text{NH}_2$. CI was needed in order to gain accurate mass information; however the EI spectrum showed the most abundant peak to have m/z 91 again showing the benzyl group.

The formation of the tertiary N,N -dimethylaminoethyl group of (**214**) is accompanied by a large methyl peak in the ^{13}C NMR and ^1H NMR spectra. The methylene protons appear as groups of broad multiplets due to the similarity in chemical shifts and it is the ^{13}C NMR spectrum which is the most useful.

5.5 DNA Binding of Polyamines with a Central Aromatic Core

5.5.1 Introduction

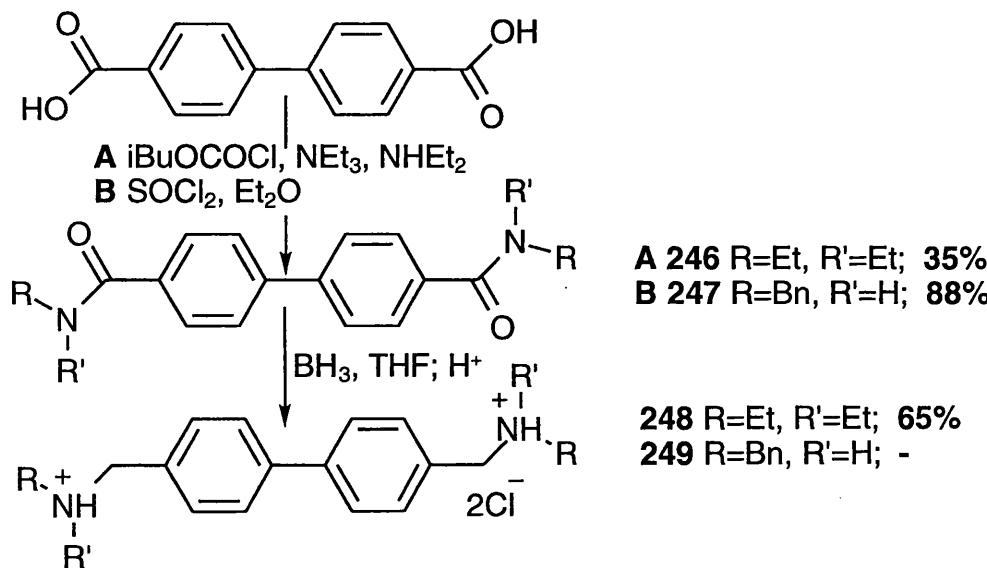
As discussed in **section 2.7** Edwards and co-workers had studied a number of polyamines with antitumour activity and looked for any correlations between activity and DNA binding. If the polyamine analogues act by displacing natural polyamines from their DNA binding sites then such an action may be relevant. Those polyamines, such as (**244**), with a central aromatic core were comparable or superior to spermine for DNA binding. This work also compared well with that of Stewart and Gray (**2.7**) who looked at a wide range of compounds, such as (**245**), studying their DNA interactions to establish structural parameters that would help in the design of DNA binding agents in the future.



Another colleague in our group was looking at aromatic compounds with a central phenyl core and we decided to expand this work into the biphenyl system.

5.5.2 Synthesis of Biphenyl Compound (248) and Attempted Synthesis of (249)

Preparation of amide (**246**) proceeded in fair yield from 4,4'-biphenyldicarboxylic acid using the mixed anhydride route. Alumina chromatography, eluting with ethyl acetate gave an orange oil which solidified on standing and recrystallisation produced (**246**) as cream crystals. Reduction of the amide went in good yield (65%) using borane in THF to produce the dihydrochloride salt (**248**). For the production of the dibenzyl analogue (**247**) the mixed anhydride route was not used due to the low yield obtained for (**246**). Instead the dicarboxylic acid was heated at reflux in thionyl chloride for one hour until it had all dissolved and then concentration *in vacuo* gave the diacid chloride. The acid chloride was stirred in dry ether at 0 °C and benzylamine was added dropwise. A vigorous reaction led to the formation of a precipitate which was filtered to yield (**247**) as a white powder. This material was extremely insoluble and a solvent system for recrystallisation was not found. Due to this lack of solubility borane reduction to produce (**249**) was not successful (**Scheme 49**).



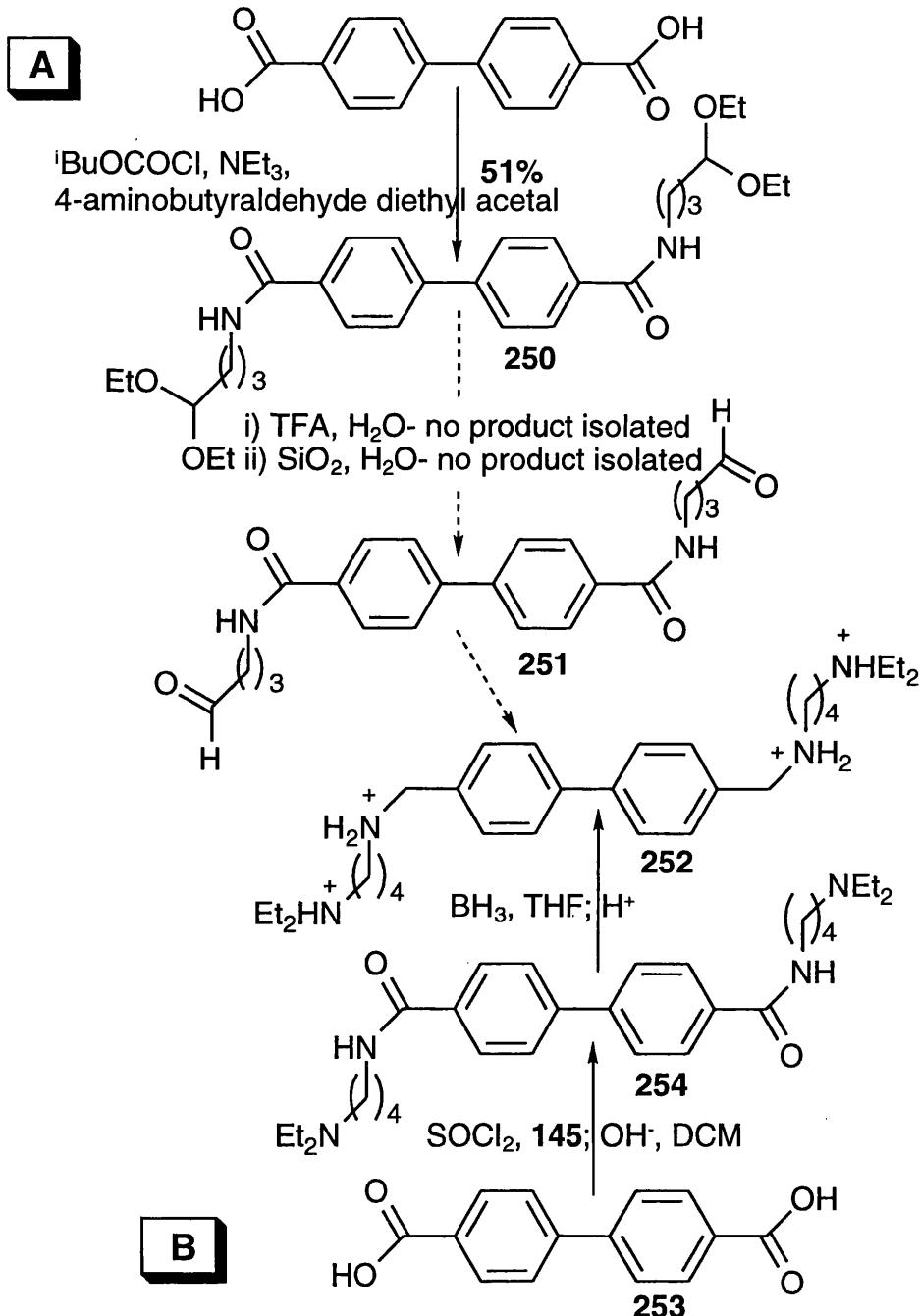
Scheme 49 Preparation of (**248**) and attempted preparation of (**249**)

Fisher and co-workers had investigated alternative reducing agents for tertiary amides including lithium diisopropylaminoborohydride and lithium

pyrrolidinoborohydride. They were reported to yield the amine or alcohol, depending on the steric requirements of the amide, in excellent yield. We did not investigate the use of such reagents.¹⁹³

5.5.3 Synthesis of Biphenyl Compound (252)

Initial investigations were concerned with adding the 4-carbon unit followed by the *N,N*-diethyl group (**Route A, Scheme 50**).



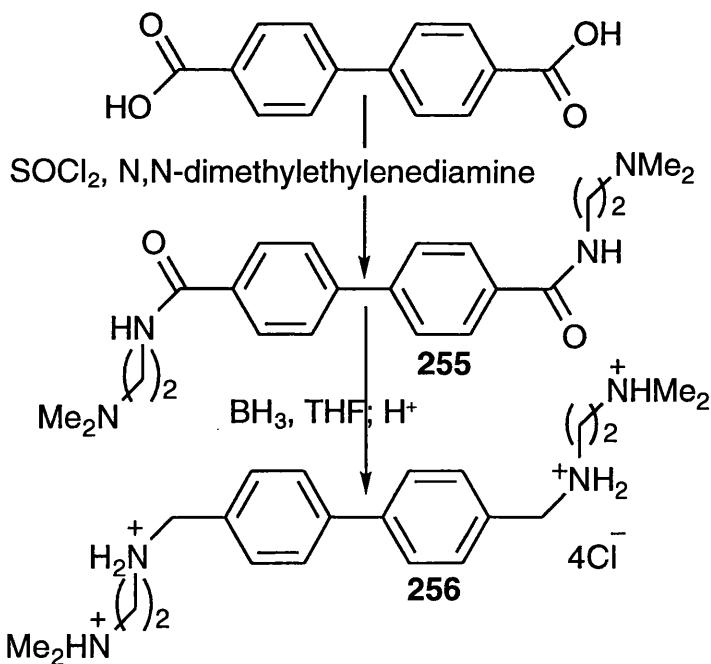
Scheme 50 Preparation of (252)

The dicarboxylic acid was reacted with 4-aminobutyraldehyde diethylacetal using mixed anhydride conditions, due to the instability of the diacetal in acid. This yielded (**250**) as a white solid in reasonable yield (51%). Attempts to hydrolyse the diacetal to the aldehyde using TFA or the milder water on silica gel both failed to give any product.

Instead the diamine (**145**) was coupled to the diacid to add the *N,N*-diethylaminobutyl moiety directly. The diacid was converted into the diacid chloride following the thionyl chloride route, and the diamine (**145**) was added to the acid chloride in dry ether. Due to possible protonation by any excess thionyl chloride, aqueous acid kept the salt of (**254**) in the aqueous solution and then basic work up allowed isolation of the free base (**254**) in 52% yield. Reduction in the usual manner went in 64% yield and the tetrahydrochloride salt (**252**) was isolated as a cream-green solid (**Route B, Scheme 50**).

5.5.4 Preparation of Compound (**256**)

Compound (**256**) was designed to have the central aromatic core and contain the dimethylaminoethyl group used in distamycin analogues. The amide (**255**) was prepared in reasonable yield (51%) following the thionyl chloride route and reduction went again in good yield (55%) to form the tetrahydrochloride salt (**Scheme 51**).



Scheme 51 Preparation of compound (**256**)

5.5.5 Analytical Data for Biphenyl Compounds

The aromatic region of the ^1H NMR spectra of these compounds appears as an AA'BB' system in all of the biphenyl systems prepared. Also common to most spectra is the coupling of the amide NH to the neighbouring CH_2 group, hence it appears as a triplet.

The aliphatic region of the ^1H NMR spectrum of (246) would be expected to consist of a triplet and quartet for the ethyl groups. Instead we observe a broad singlet and a broad doublet (**Figure 49**). The aliphatic region of the ^{13}C spectrum (**Figure 50**) consists of two CH_3 peaks and two CH_2 peaks. These results are due to the magnetic non-equivalence of the CH_2 and CH_3 peaks and is similar to that observed for *N,N*-dimethylformamide where the two methyl groups are also non-equivalent. The line broadening of the ^1H NMR spectrum is probably due to interconversion between rotameric forms.

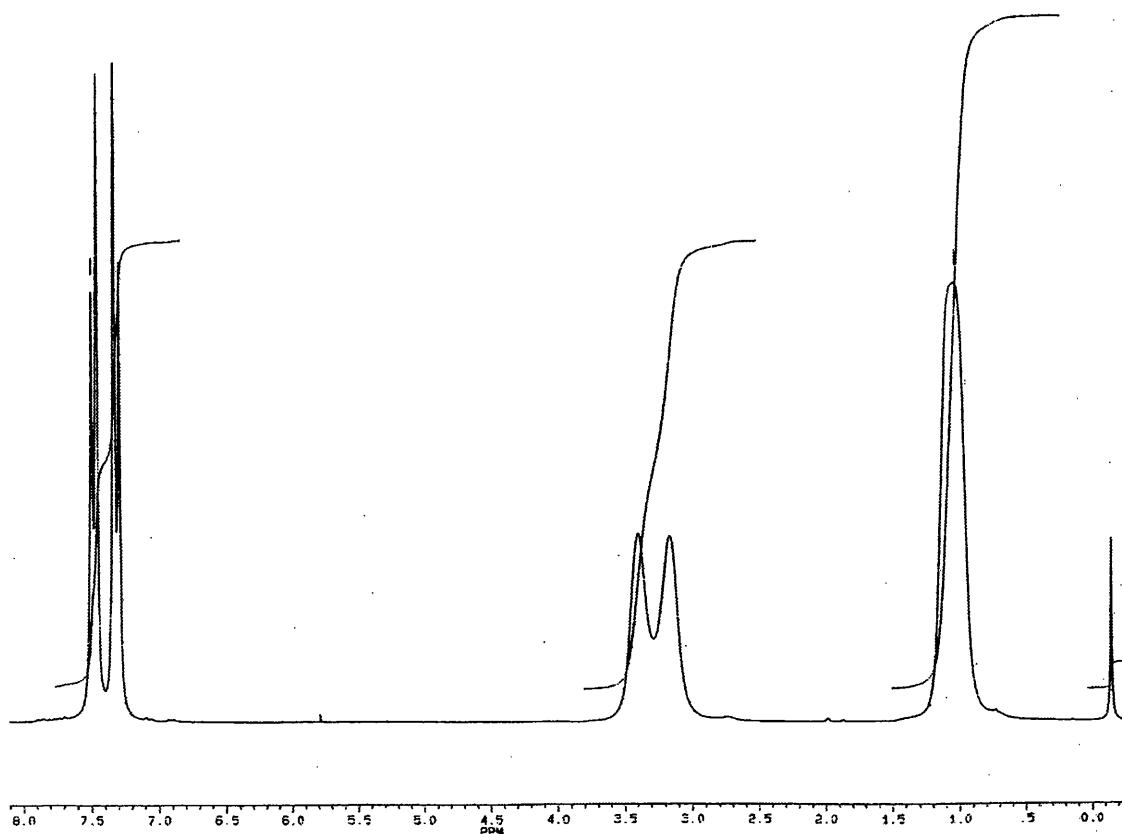


Figure 49 ^1H NMR spectrum of compound (246), CDCl_3 , 200 MHz

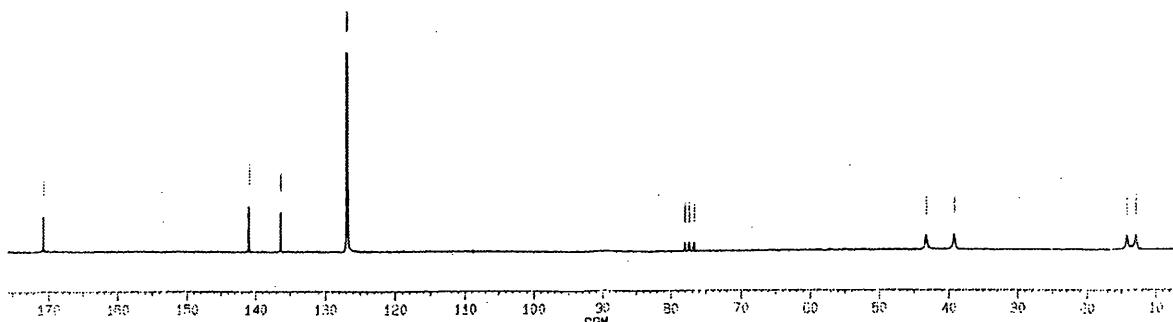


Figure 50 ^{13}C NMR spectrum of compound (246), CDCl_3 , 50 MHz

In general the mass spectra of these biphenyl compounds show peaks at m/z 91 and m/z 152 corresponding to fragmentation of the biphenyl system. Other peaks usually correspond to loss of fragments from either the amide or typical fragmentation patterns of an amine. The IR spectra consist of the expected amide stretch at about 1600 cm^{-1} and a peak between 860 and 800 cm^{-1} for the *paradisubstituted biphenyl* system.

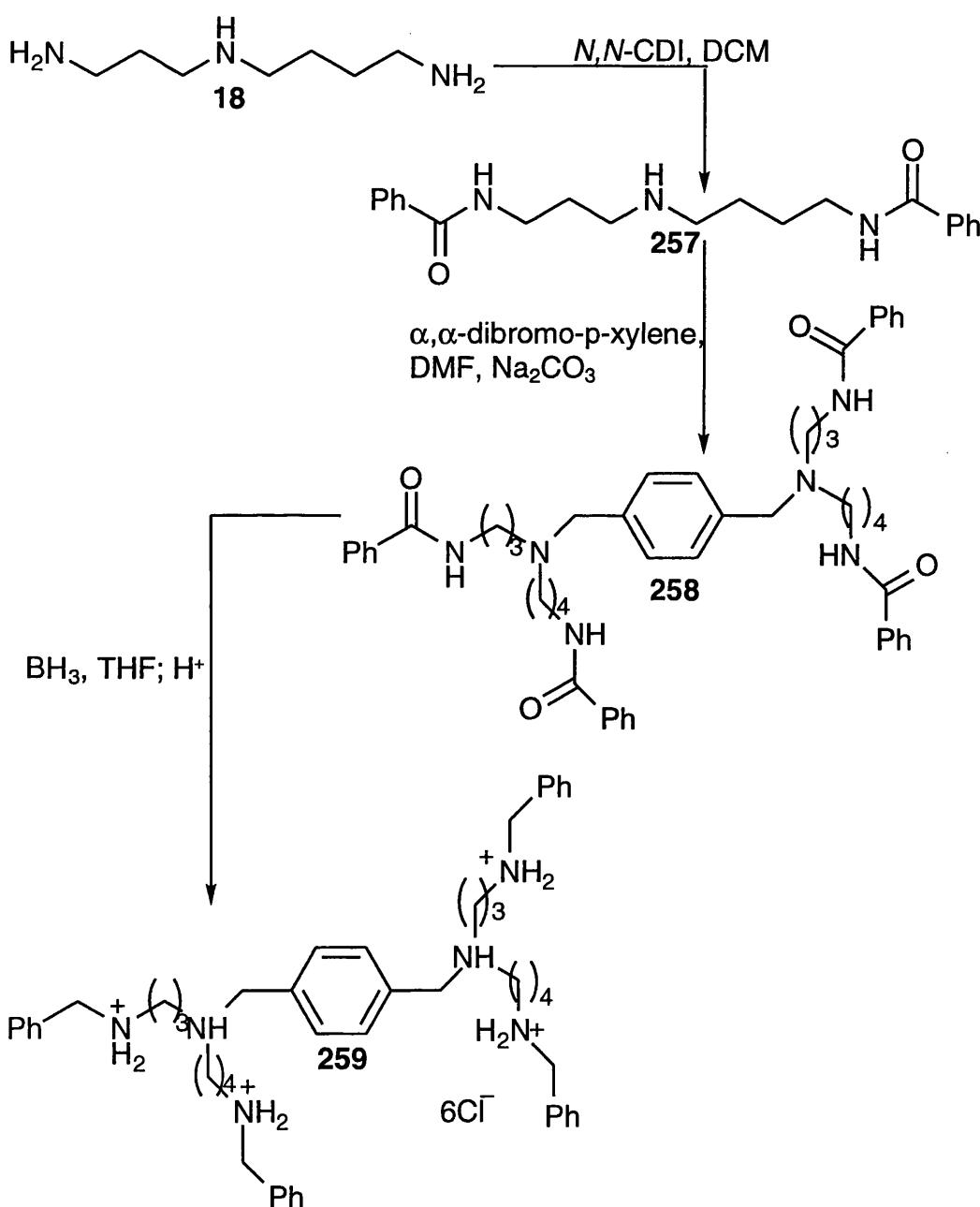
5.5.6 Preparation of Compound (259)

The work of Gray had established that the most active compounds had polyamine units attached to the central aromatic ring usually with free aminogroups in the terminal positions. Edwards speculated that the activity of the benzyl polyamines may be related to fast metabolism of the benzyl groups, so we designed compound (259). It was to be prepared as the hexahydrochloride salt which could lead to good DNA binding and the spermidine side chain with benzyl groups on the terminal positions which would be quickly metabolised to leave the free amino groups.

We wanted to carry out alkylation of the secondary nitrogen of spermidine so the two primary nitrogens were acylated. Following the procedure of Moss¹⁸⁰ spermidine was acylated with benzoic acid using CDI to provide selective reactions at the primary positions (see 5.2.4). (257) was isolated in 50% yield and was heated at reflux in DMF with α,α -dibromo-*p*-xylene and sodium carbonate. After work up (258) was isolated as a cream solid, in low yield (21%). Borane reduction of the tetraamide went in reasonable yield (57%) to yield the hexahydrochloride salt (259) (**Scheme 52**).

The ^1H NMR spectrum of (258) consists of a large number of multiplets due to the similarity in chemical shift of a number of the protons. Two amide NH peaks appear at δ 6.93 and 7.56 and the aromatic region

contains many multiplets for the protons of the three different phenyl rings. The protons for the central symmetrical ring appear as a singlet. Microanalysis of (**258**) confirms the purity of the sample. After the borane reduction to give (**259**) the ^1H NMR spectrum simplifies and the aromatic region consists of two singlets corresponding to the central phenyl ring and the terminal benzyl moieties. There are three benzylic proton environments shown by the three singlets at δ 4.03, 4.04 and 4.24. The rest of the spectrum consists of multiplets for the aliphatic protons. It was necessary to use FAB MS to obtain a molecular ion and therefore accurate mass details for (**259**).

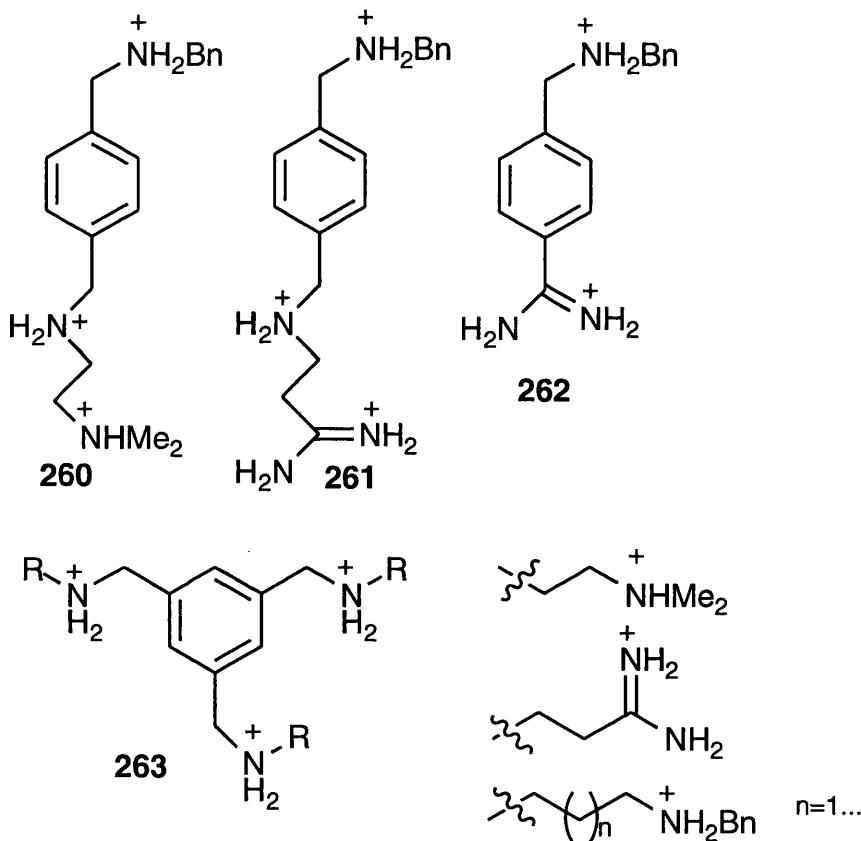


Scheme 52 Preparation of (**259**)

5.6 Future Work

Unfortunately a number of compounds are currently being tested so a lack of test data makes it hard to determine where this area will lead.

One promising area are those compounds with a central benzene ring. Possible future compounds include those shown below, (260), (261), (262) and variations of (263). The compounds with amidines are particularly interesting due to the high affinity such compounds have been shown to have for DNA. A comparison of DNA binding affinities for the polyamines we have prepared has not been made, but assays are available for such calculations if required.



5.7 Additional Biological Data

In the week leading up to the PhD viva for this thesis additional biological data for compounds prepared in chapter 5 were received and are shown in the table on p137.

	activity ($\mu\text{g ml}^{-1}$)	LEC (μmol)
sat-dibenzyl 189	1-0.5	2-1
amidine spermine 235	0.05-0.001	0.1-0.002
dimethylaminoethyl spermine 243	0.05-0.01	0.1-0.02
tetraethyl-biphenyl 248	10-5	25-12
dimethylaminoethyl-biphenyl 256	50	195
benzylspermidine-biphenyl 295	0.05-0.001	0.05-0.001
chloroquine	<0.01	<0.02

Results for compounds **235** and **243** were extremely good with activity close to that of chloroquine. It is not, however, correct to say whether this is due to increased DNA binding and/or greater selectivity of the compounds for AT base pairs although this could be studied in the future. The effect of these compounds on polyamine pools and/or polyamine biosynthetic enzymes would also be interesting.

The biphenyl compounds were generally not very active, apart from the benzyl spermidine analogue **295** which had excellent activity. This compound would also be worthy of further study.

5.8 Summary

Yet again the spermidine analogues have proven challenging to prepare. Synthetically the spermine analogues are easier due to the symmetry of the compounds and our two target compounds were prepared in good yield. The next chapter covers two areas where this project might lead in the future and gives a brief idea of the types of chemistry it may involve.

6

6 Iron Chelation and Macroyclic Polyamines

6.1 Introduction

This chapter does not seek to cover the wide area of iron chelation and macrocyclic polyamines as both are wide ranging and diverse topics. Instead a few ideas that were explored in the final stages of this PhD project will be discussed and also where it may lead in the future.

6.2 Iron Chelation

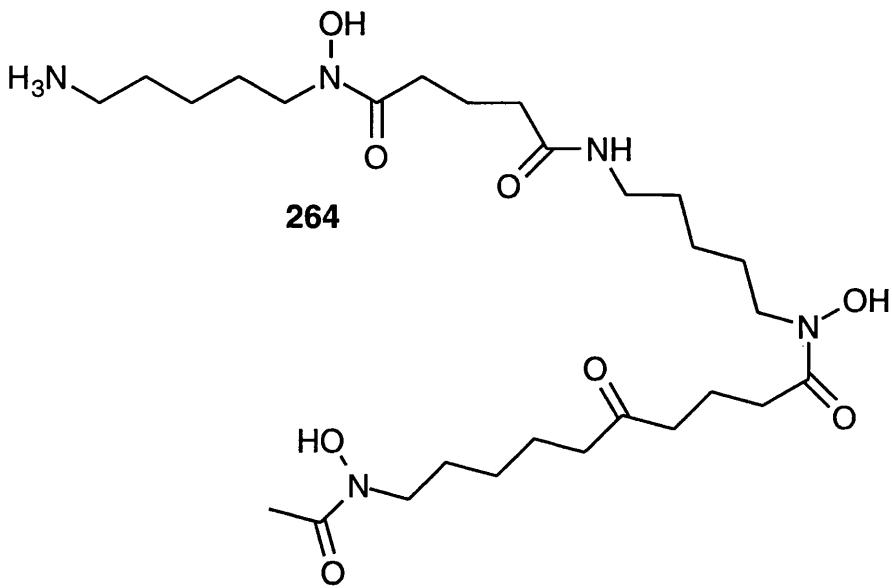
6.2.1 Introduction

A wide range of iron chelators, including some natural products, have been shown to possess antimalarial activity *in vitro* and *in vivo*. This includes desferrioxamine (264), although it is not orally active which is an essential requirement for treatment of the disease in the tropics.¹⁹⁴

Most clinical impetus for the development of iron chelators has been to find treatment for iron overload and this has met with some success. Desferrioxamine B has been used to treat iron overload and is one of a family of fungal siderophores. Coordination of Fe(III) occurs via the hydroxamate moieties; however hydroxamates are sensitive to the acidic environment in the stomach.¹⁹⁵

The malaria parasite spends an essential part of its life cycle in the human red blood cell. Many metabolic processes of the erythrocytic (human red blood cell) malarial parasite are dependent on iron and withholding iron from the parasite, by iron chelators, could conceivably disrupt the metabolism of the trophozoite. This may work by preventing

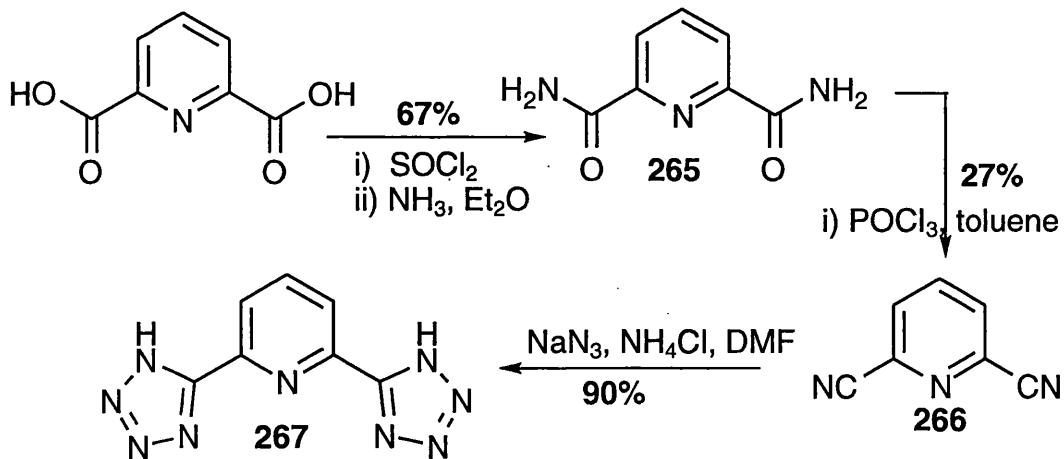
DNA synthesis, disrupting proteolysis of the host haemoglobin as well as other essential functions.^{194, 196, 197}



What is needed is an orally active compound with high affinity for Fe(III); which does not redistribute iron supplies in the body and is metabolically degraded.¹⁹⁸

6.2.2 Synthesis of Compound (267)

Following the procedure of McKendrick we prepared a tetrazole (267) to see if compounds of this type had any activity against the malaria parasite.¹⁹⁹



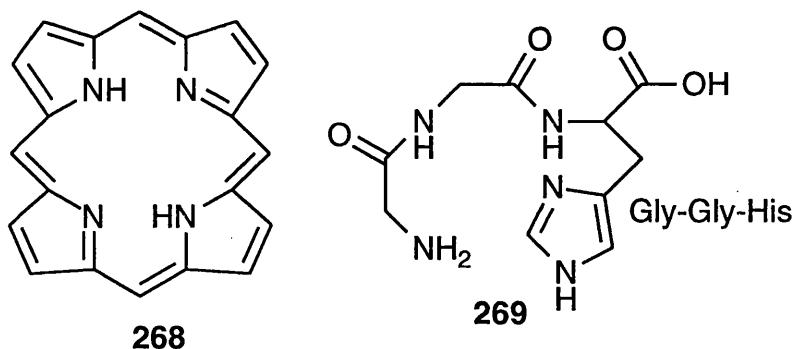
Scheme 53 Preparation of (267)

Starting from pyridine-2,6-dicarboxylic acid the diamide (**265**) was prepared and then dehydrated to give dinitrile (**266**). This was reacted with sodium azide to form the tetrazole (**267**) in excellent yield (**Scheme 53**). Unfortunately (**267**) was only active down to 50 µg ml⁻¹ (246 µmol) when tested in vitro against *P. falciparum*.

6.3 Macrocyclic Polyamines

6.3.1 Introduction

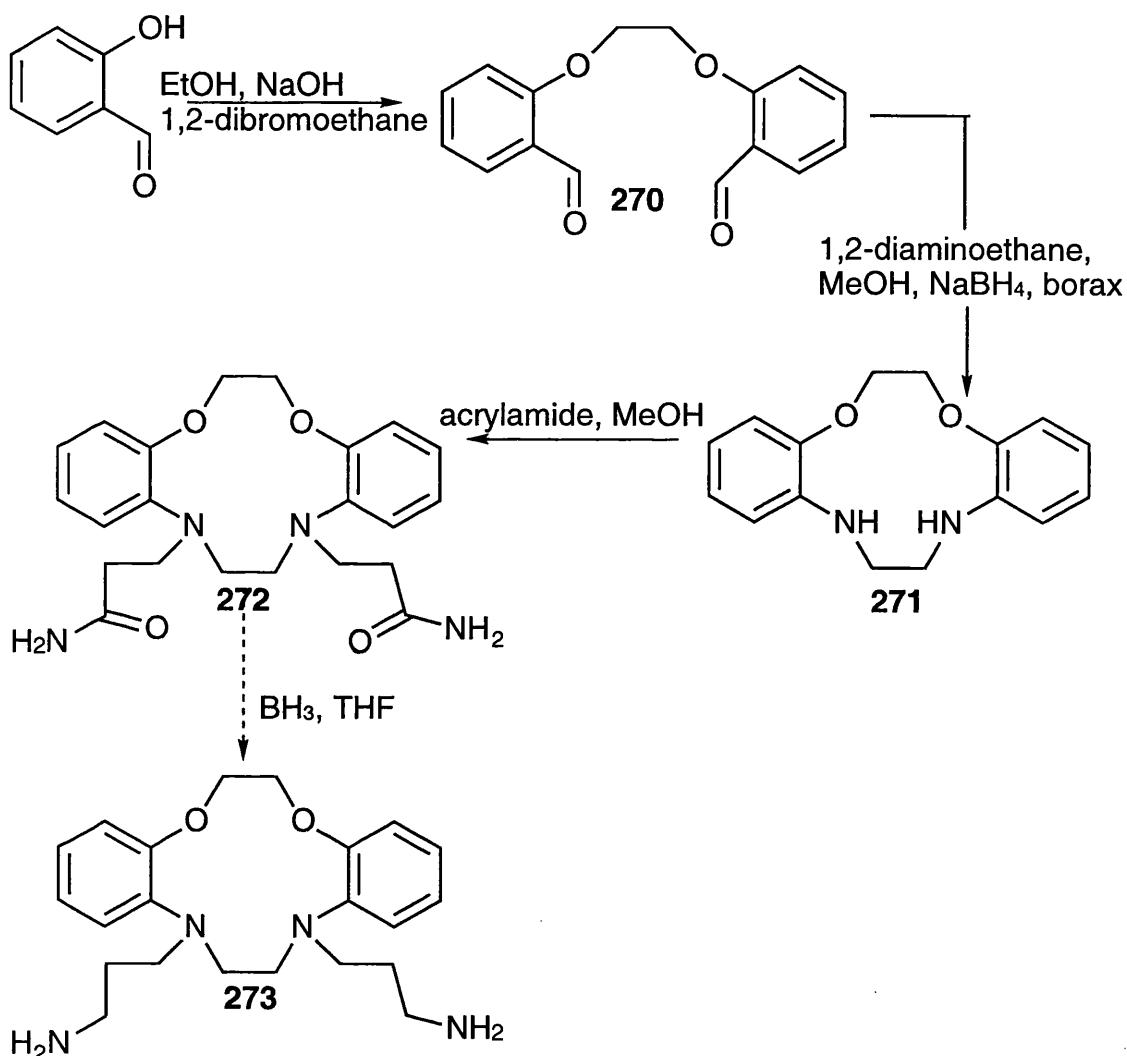
Saturated polyamine macrocycles have been known and studied for a long time and have been used mostly as chelating agents for transition metal ions. The literature covering this area is prolific and this brief introduction will not attempt to provide a comprehensive review of the area. Macrocytic polyamines were discovered to have properties similar to biomolecules such as porphyrins (**268**), peptides (**269**) and naturally occurring polyamines such as spermidine and spermine. One of the major properties of macrocytic polyamines is their strong basicity which leads to new properties not shown by the linear polyamines.²⁰⁰



Mixed donor macrocycles have been studied because they do not normally yield the extreme thermodynamic and kinetic stabilities of the tetraaza macrocycles. Recent work by Lindoy has looked into preparing "pendant arm" macrocycles with donor groups incorporated in the arms. It has been shown by Kaden that such compounds exhibit properties that often resemble their parent unsubstituted macrocycles as well as their open chain analogues, i.e. a substituted polyamine.²⁰¹ We decided to make initial investigations in this area to see if any antimalarial activity was observed.

6.3.2 Approaches Towards Compound (273)

Following the chemistry of Armstrong and Lindoy, Williamson condensation between 1,2-dibromoethane and salicylaldehyde gave (**270**) in good yield (**Scheme 54**).^{202, 203}



Scheme 54 Preparation of (**272**) and attempted preparation of (**273**)

The macrocycle (**271**) was prepared according to Grimsley *et al.* by warming the aldehyde and 1,2-ethanediamine in methanol with sodium borohydride and borax. Low yields (32%) of the macrocycle were obtained; however this was also reported in the literature. Formation of the bisamide by stirring (**271**) and acrylamide in methanol at reflux for two days was achieved following the procedure of Chia²⁰¹ isolating (**272**) in 37% yield. Reduction of the diamide to give (**273**) had previously not been reported and in our hands initial experiments led to only black intractable products.

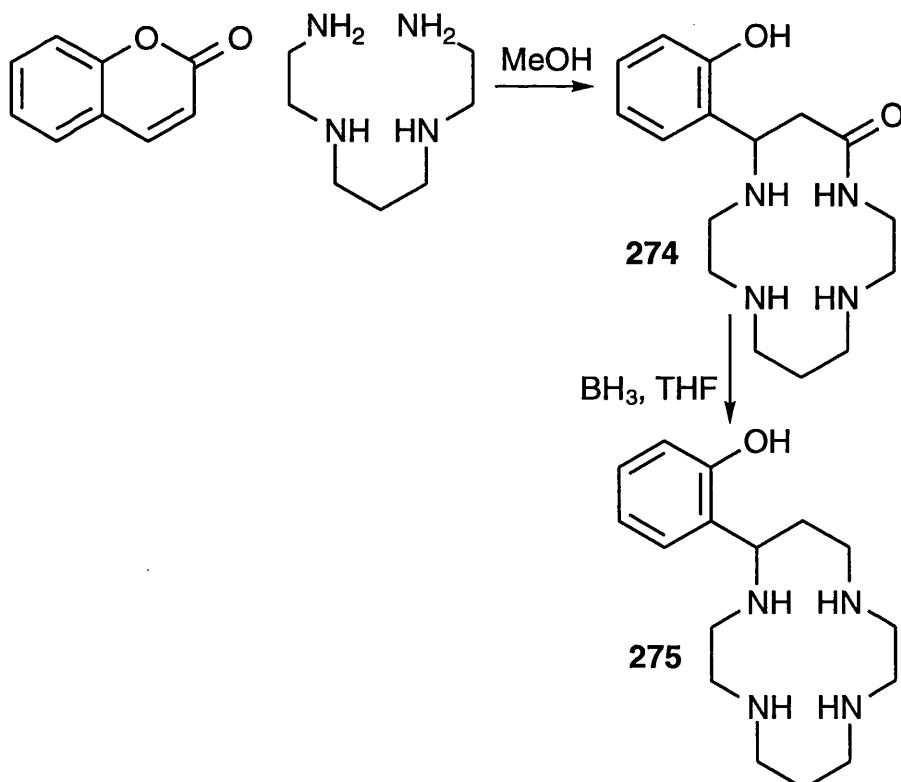
No biological data are available of these macrocycles; however the area is worth studying in further detail.

6.4 Future Work

6.4.1 Macrocycles That Chelate Iron (III)

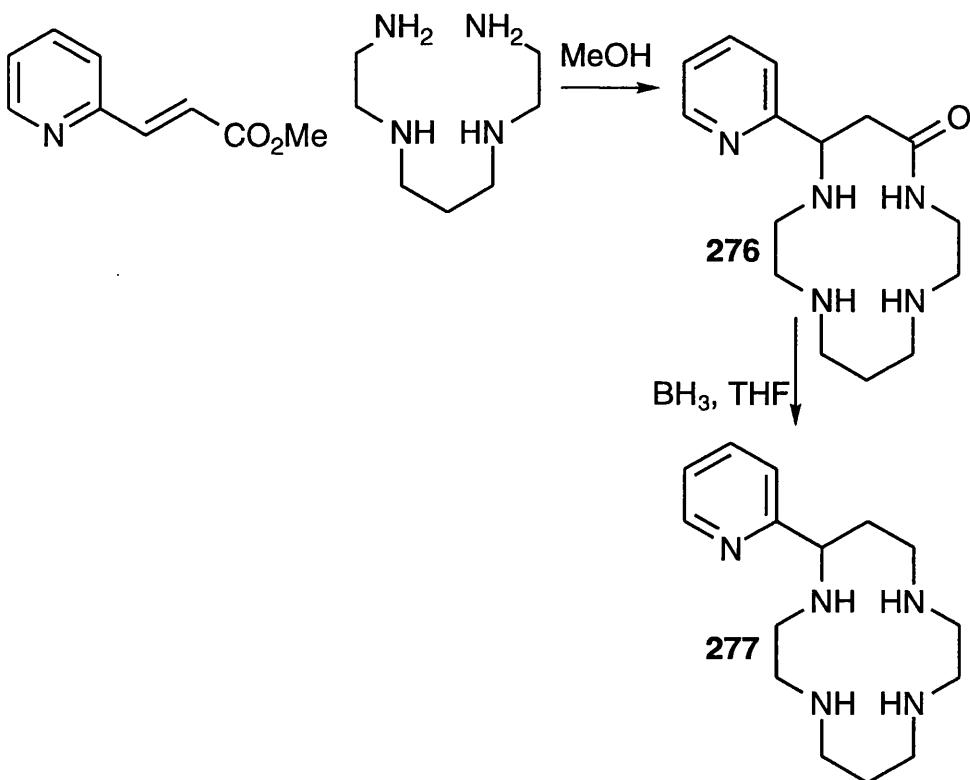
Macroyclic structure is extremely favourable for metal complexation so it might be possible to find macrocyclic polyamines that could chelate iron and hence disrupt the parasites' growth in the red blood cell and hence be used as an antimalarial.

Only limited information is available on macrocycles that will chelate iron (III); however Kimura had looked at macrocyclic polyamines with side arms on a ring carbon.^{204, 205} α,β -Unsaturated carboxylic acid esters and a linear polyamine react via a Michael addition and intramolecular lactam formation in a one step annulation reaction to yield (274). Borane reduction would then lead to the saturated macrocyclic polyamine with a side arm (275) (**Scheme 55**).



Scheme 55 Formation of a macrocycle with a pendant phenol moiety.

Starting from coumarin, polyamines with pendant phenols were prepared and Kimura speculated that such compounds would have interesting biological activities.²⁰⁶ Initial studies in this project prepared (**274**) successfully and this synthesis may be worth pursuing. Further work also led to the formation of macrocycles (**276**, **277**) with pendant pyridyl moieties (**Scheme 56**).²⁰⁷ Initial work in this area led to formation of (**276**), however the borane reduction gave a mixture of products.



Scheme 56 Macrocycles with pendant pyridines

These phenol and pyridyl pendant arms project into the cavity and are able to co-ordinate metal ions such as iron and nickel axially in contrast to a large number of the basic macrocycles that co-ordinate copper and zinc, among others (**Figure 51**).²⁰⁰

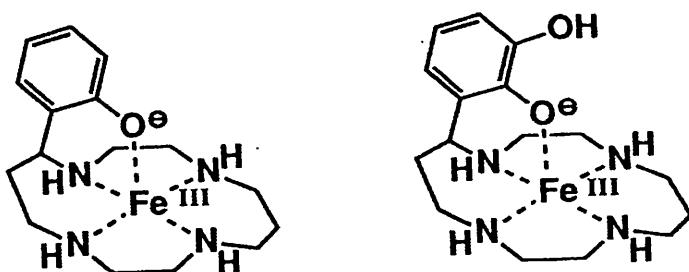


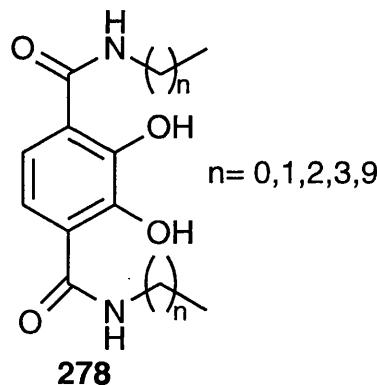
Figure 51 Chelation of Fe(III) by polyamine macrocycles.²⁰⁰

This area would be worth investigating by preparing some compounds for *in vitro* antimalarial testing varying the carbon chain to produce a variety of analogues. Both amide and amine are thought to have activity so a combination of both might lead to some useful structure-activity relationships.

g

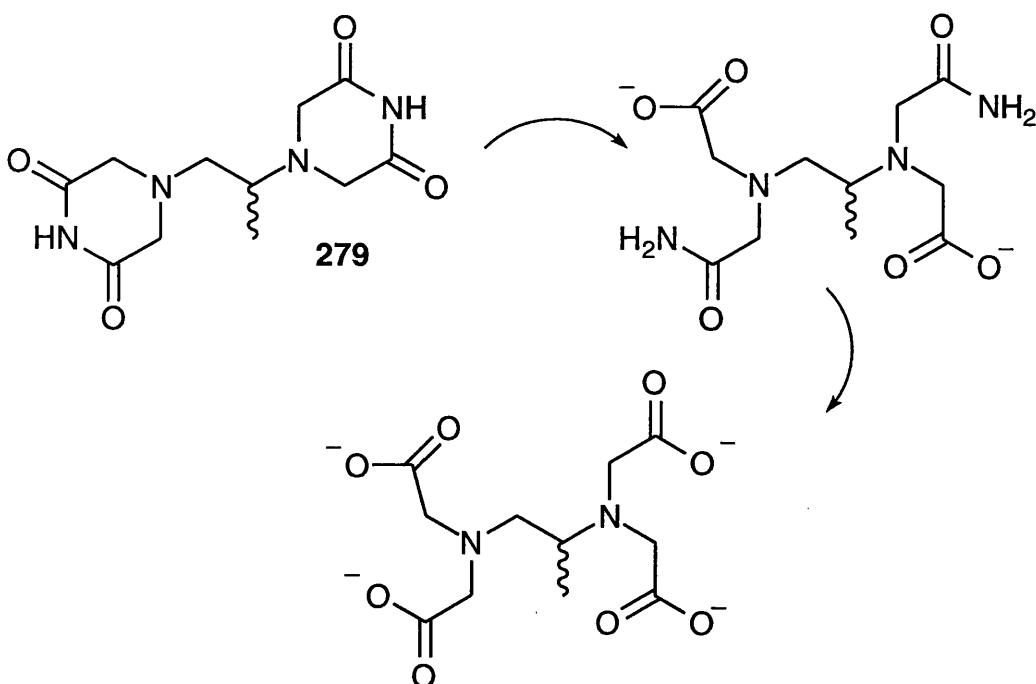
6.4.2 2,3-Dihydroxyterephthalimides

The naturally occurring siderophores are low molecular weight Fe(III) sequestering agents produced by microbes and they are able to accommodate the preferred octahedral geometry of Fe(III). Garrett *et al.* studied the coordination chemistry of the bidentate 2,3-dihydroxyterephthalimides (**278**) and found them to be excellent chelators of Fe(III) considering that they were such simple compounds.²⁰⁸ Our group had been looking at compounds similar to this, but as amines rather than amides; however it might be worth investigating a number of these compounds. We could envisage a variety of substituents on the amide from benzyl to extended polyamines.



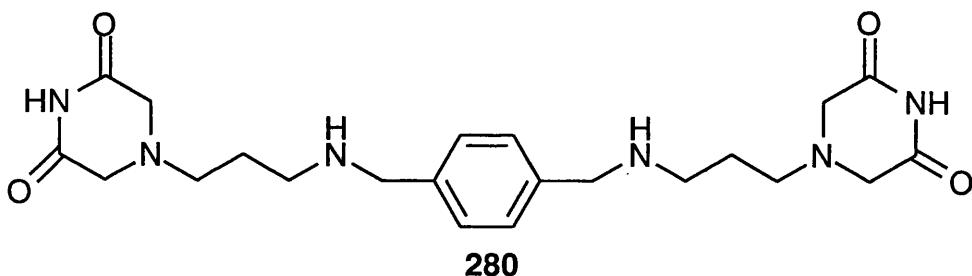
6.4.3 2,6-Dioxopiperazines

An excellent review by Hider¹⁹⁷ discusses a range of iron chelating agents with clinical potential. Herman *et al.* also cover the activity of such compounds.²⁰⁹ One compound discussed is "Zinecard" (**279**) one of a family of bisdioxopiperazines first synthesized by Ciba Geigy chemists in the late 1950s. It is uncharged and can therefore enter cells where the cyclic imide links are cleaved by intracellular enzymes to generate a chelator capable of binding Fe(III) (**Scheme 57**). In general this range of compounds were developed as potential anti-tumour agents.^{210, 211}



Scheme 57 Activation of (279) by enzymes to yield an Fe(III) chelator

This bisdioxopiperazine moiety may provide a group that we could add to our polyamines to produce a variety of compounds that could be tested for activity against *P. falciparum*, for example (280).



6.5 Summary

A few ideas for future areas our group may expand into have been briefly described. The chemistry of macrocyclic polyamines is new and interesting and little work has been done to ascertain if any of these compounds have antimalarial activity. Iron chelation has been studied in depth and there is evidence in the literature that it may prove a useful therapeutic tool against malaria. If we can combine both areas then we have a broad range of future work to cover!

7

7 Experimental

Reagents were purchased from Aldrich Chemical Company (Gillingham, UK) or Lancaster Synthesis (UK) and were used without further purification. Organic solvents were obtained from Rhone-Poulenc-Rorer and were dried, as necessary, using the procedures described by Perrin and Armarego. Melting points (m.p.) were determined in open capillaries using a Gallenkamp apparatus and are uncorrected. ¹H and ¹³C NMR spectra were obtained on a Bruker AM200-SY spectrometer operating at 200 MHz and 50 MHz respectively, or where stated, at 360 MHz and 90 MHz respectively on a Bruker AM 360 spectrometer. J Values are given in Hz. ¹³C NMR spectra were assigned with the aid of Distortionless Enhancement by Polarisation Transfer (DEPT)-edited spectra. The numbering schemes shown are used for ease of assigning the NMR spectra and do not refer to the system of nomenclature. Mass spectra (MS) were recorded on an updated AEI MS12 or MS902 spectrometers; percentile figures refer to relative intensity as a percentage of the base peak. MS were obtained using electron-impact ionisation (EI) mode or, if stated, chemical ionisation (CI) mode or fast atom bombardment (FAB). Infra-red (IR) spectra were obtained on a Perkin Elmer PU 9800 FT-IR spectrophotometer. Retention factors (R_f) were obtained by analytical Thin Layer Chromatography (TLC) on Merck aluminium-backed silica plates of 0.25 mm thickness or Merck aluminium-backed alumina plates thickness 0.2 mm; chromatograms were visualised using UV conditions at 154 nm or by staining with iodine. All column chromatography was carried out on silica gel (particle size 70-230 mesh) or alumina gel (particle size 150 mesh).

***In vitro* against *P. falciparum* asexual erythrocytic stages - S. R. Phillips and F. McMonagle, Division of Infection and Immunity.**

P. falciparum culture adapted strains FCR (Gambia) and JS (Zimbabwe) were used. Both these strains are chloroquine sensitive and were grown as stock cultures *in vitro* in petri dishes by a modification of the candle jar technique of Trager and Jensen.⁹² In culture the parasites were asynchronous in their growth and therefore all stages in the 48 hour asexual erythrocytic cycle would be represented at any one time. The RPMI 1640 culture medium was supplemented with 10% group AB (from the West of Scotland Blood Transfusion Service [WSBTS]) or occasionally A serum (from RSP) and the parasites grown in outdated group O (from WSTBS) or occasionally with fresh group A red blood cells (from RSP). The outdated red blood cells were obtained one month after donation into citrate dextrose and fresh erythrocytes were collected into heparin at 10 iu/ml final concentration. Comparisons were made between fresh and outdated red blood cells to demonstrate that compounds with antimalarial activity had comparable activity in both fresh and outdated red blood cells (data not shown) and that both sources of the serum were equally effective in supporting parasite growth. The AB and A serum was heated at 60 °C for 30 min before use (for reasons of safety). The assay system for screening compounds for activity against *P. falciparum* was that described by Desjardins *et al.*⁹³ In brief the parasites were grown in the presence or absence of the test compounds or control substances in 96 well flat-bottomed tissue culture plates, each well containing a total volume of 200 ml with erythrocytes at a 3% haematocrit and a starting parasitaemia of 0.5%. Each test concentration was carried out as a minimum in triplicate. After 24 h incubation in a candle jar 100 ml were removed from each well and replaced with 100 ml fresh medium containing 1 mCi ^{3}H -hypoxanthine.⁹⁴ The plate was incubated for a further 18 h before the parasites were harvested and parasite growth measured as the incorporation of the isotope, using conventional liquid scintillation counting. The mean and standard deviation of the counts per minute for each dilution of the test compounds or the control cultures were calculated and the results displayed using Cricket graph. The results were analysed using a standard t-test.

***In vivo* against *P. chabaudi* - S. R. Phillips and F. McMonagle,
Division of Infection and Immunity.**

P. chabaudi AS strain, in inbred NIH mice, was handled as described by McLean and Phillips.⁹⁵ In brief three months old male inbred NIH mice were infected intravenously with 1×10^6 *P. chabaudi* parasitized erythrocytes and groups of five mice immediately afterwards injected with either chloroquine at 50 mg/Kg body weight or the test compounds. as follows: **3** and **10** at 100 or 300mg/Kg body weight, **7** at 150 mg/Kg, **11** at 175 mg/Kg and **12** at 25 or 50 mg/Kg. Compound **7** was given as three equal doses over 48 hours and **11** as two equal doses 24 hours apart. The compounds were dissolved in physiological saline and injected intraperitoneally. A control group of mice received saline only. The parasitaemia was followed in the mice by daily thin blood smears which were stained with Giemsa's stain and recorded as the numbers of parasitized erythrocytes in 10^5 erythrocytes. The mean parasitaemia for each group was calculated and the time to reach 2% parasitaemia was determined and compared for each group using a student's t test. Differences were considered significant when $p < 0.05$.

7.1 Experimental to Chapter 3

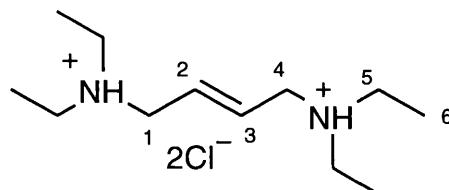
General Procedure A1-Formation of Hydrochloride Salts.

Ethereal hydrogen chloride was prepared by carefully bubbling dry HCl gas through a solution of dry ether until saturated. The free base was dissolved in an appropriate amount of ether and was then treated with an excess of dry HCl/Et₂O. The precipitate was removed by filtration and recrystallized using EtOH/Et₂O.

General Procedure A2-Formation of Hydrochloride Salts.

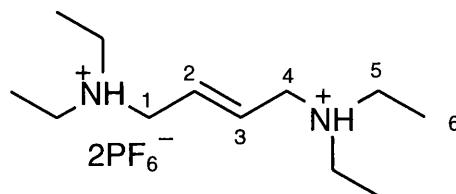
The free base was dissolved in an appropriate amount of CHCl₃ and then treated with an excess of dry HCl/CHCl₃ (prepared by bubbling dry HCl gas through chloroform until saturated). The solution was concentrated *in vacuo* and recrystallized using EtOH/Et₂O.

(E)-N,N,N',N'-Tetraethyl-1,4-diaminobut-2-ene Dihydrochloride 7756



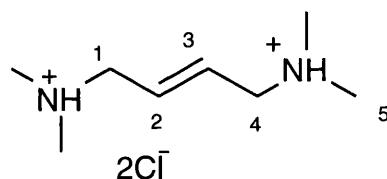
(E)-N,N,N',N'-Tetraethyl-1,4-diaminobut-2-ene **79** was prepared as reported and the dihydrochloride salt was prepared by general procedure A1 to yield **77** as white crystals (74%), mp 239.1-240.1 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2677 (-CH₂-), 1625 (C=C), 956 ((E)-C=C, C-H bend); δ_{H} (200MHz, D₂O) 1.07 (12H, t, *J* 7.0, 6-H₃), 2.86 (8H, q, *J* 7.0, 5-H₂), 3.50 (4H, m, 1,4-H₂), 5.86 (2H, m, 2,3-H); δ_{C} (50MHz, D₂O) δ 9.3 (CH₃-6), 48.2 (CH₂-5), 53.2 (CH₂-1 and -4), 129.6 (CH-2 and -3); *m/z* (EI) 198 (M⁺-2H, 1.2%), 126 (100), 86 (91), 56 (72), 42 (70), (Found: M⁺-2H, 198.2051. C₁₂H₂₆N₂ requires 198.2043).

(E)-N,N,N',N'-Tetraethyl-1,4-diaminobut-2-ene Bis(hexafluorophosphate) 107¹¹⁰



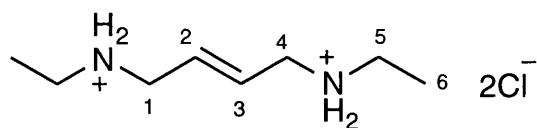
107 was prepared by this group as described by McCormack and co-workers and crystals grown from a solution of MeOH/Et₂O (100%). mp 189–189.5 °C.

(E)-N,N,N',N'-Tetramethyl-1,4-diaminobut-2-ene Dihydrochloride 83^{38, 57}



Synthesis of (E)-N,N,N',N'-tetramethyl-1,4-diaminobut-2-ene 80 was carried out as described by Cook.³⁸ The dihydrochloride salt was prepared by general procedure A1 to yield 83 as white crystals (30%), mp >250 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2662 (-CH₂-), 1620 (C=C), 961 ((E)-C=C, C-H bend); δ_{H} (200MHz, D₂O) 2.70 (12H, s, 5-H₃), 3.69 (4H, m, 1,4-H₂), 5.99 (2H, m, 2,3-H); δ_{C} (50MHz, D₂O) 43.2 (CH₃-5), 58.9 (CH₂-1 and -4), 130.2 (CH-2 and -3); *m/z* (EI) 142 (M⁺-2H, 1.1%), 97 (32), 82 (45), 58 (100), 42 (62), (Found: M⁺-2H, 142.1471; C, 44.42; H, 13.19; N, 9.16. C₈H₁₈N₂ requires 142.1467; C, 44.65; H, 13.02; N, 9.37.).

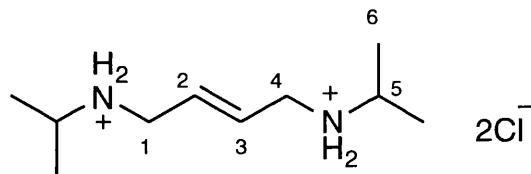
(E)-N,N'-Diethyl-1,4-diaminobut-2-ene Dihydrochloride 84^{38, 57}



The free base (E)-N,N'-diethyl-1,4-diaminobut-2-ene 81 was prepared as reported by Cook³⁸ and the dihydrochloride salt was prepared by general procedure A1 to yield 84 as white crystals (60%), mp >250 °C;

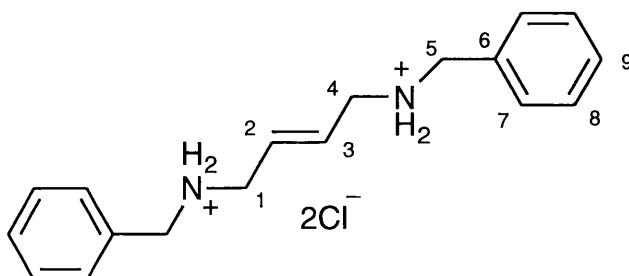
$\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2760 (N-H), 2548 (-CH₂-), 1591 (C=C), 976 ((E)-C=C, C-H bend); δ_{H} (200MHz, D₂O) 0.87 (6H, t, *J* 6.9, 6-H₃), 2.69 (4H, q, *J* 6.8, 5-H₂), 3.32 (4H, m, 1,4-H₂), 5.64 (2H, m, 2,3-H₂); δ_{C} (50MHz, D₂O) 13.0 (CH₃-6), 44.8 (CH₂-5), 49.9 (CH₂-1 and -4), 130.7 (CH-2 and -3); *m/z* (EI) 142 (M⁺ -2H, 1.1%), 97 (49), 82 (90), 68 (67), 56 (100), 41 (56), (Found: M⁺-2H, 142.1451; C, 44.66; H, 9.37; N, 13.02. C₈H₁₈N₂ requires 142.1465; C, 44.58; H, 9.33; N, 12.91).

(E)-N,N'-Diisopropyl-1,4-diaminobut-2-ene Dihydrochloride 85⁹⁶



The free base (*E*)-N,N'-diisopropyl-1,4-diaminobut-2-ene **82** was prepared as described. The dihydrochloride salt was prepared by general procedure A1 to yield **85** as white crystals (71%), mp 238.0-240.1 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2870 (N-H), 2531 (-CH₂-), 1585 (C=C), 970 ((E)-C=C, C-H bend); δ_{H} (200MHz, D₂O) 0.99 (12H, d, 6-H₃, *J* 6.8), 3.10 (2H, sept, 5-H, *J* 6.8), 3.41 (4H, m, 1,4-H₂), 5.71 (2H, m, 2,3-H); δ_{C} (50MHz, D₂O) 19.1 (CH₃-6), 46.2 (CH-5), 51.2 (CH₂-1 and -4), 129.1 (CH-2 and -3); *m/z* (EI) 170 (M⁺ -2H, 1.2%), 155 (12), 112 (64), 96 (50), 70 (100), 58 (22), (Found: M⁺-2H, 170.1794; C, 49.05; H, 9.58; N, 11.19. C₁₀H₂₂N₂ requires 170.1790; C, 49.38; H, 9.95; N, 11.52).

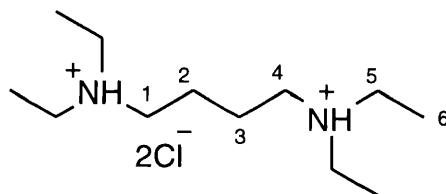
(E)-N,N'-Dibenzyl-1,4-diaminobut-2-ene Dihydrochloride 94



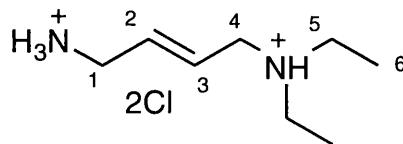
To a predistilled sample of benzylamine (4.99 g, 10 equiv.) was added (*E*)-1,4-diaminobut-2-ene (1.02 g, 4.67 mmol) in toluene (100 ml) over a period of one h at 40°C. A white precipitate appeared almost immediately and the

mixture was stirred at room temperature until no more starting material was present by TLC. CHCl_3 (100 ml) was added and the solution was washed well with water (3x50 ml). The organic extracts was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residual oil was distilled to remove any excess benzylamine (36-40 °C, 1 mm) and the residue, (*E*)-*N,N'*-dibenzyl-1,4-diaminobut-2-ene **93** was isolated as a clear oil. The dihydrochloride salt was prepared by general procedure A1 to yield **94** as white crystals (1.10 g, 3.27 mmol, 70%), mp >250 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3032 (Ar C-H), 2610 (- CH_2 -), 1585 (C=C), 1500 (Ar C=C), 978 ((*E*)-C=C, C-H bend), 745 (Ar C-H bend), 696 (Ar C-H bend); δ_{H} (200MHz, D_2O) 3.61 (4H, m, 1,4- H_2), 4.64 (4H, s, 5- H_2), 5.88 (2H, m, 2,3- H), 7.31 (10H, s, 7,8,9- H); δ_{C} (50MHz, D_2O) 48.4 (CH_2 -1 and -4), 51.4 (CH_2 -5), 129.4 (CH-2 and -3), 130.2 (CH-9), 130.6 (C-6), 130.7 (CH-7), 131.3 (CH-8); m/z (EI) 266 (M^+-2H), (Found: M^+-2H , 266.1762. $\text{C}_{18}\text{H}_{22}\text{N}_2$ requires 266.1758).

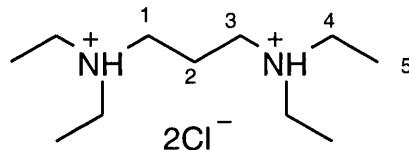
(*E*)-*N,N,N',N'*-Diethyl-1,4-diaminobutane Dihydrochloride **90**



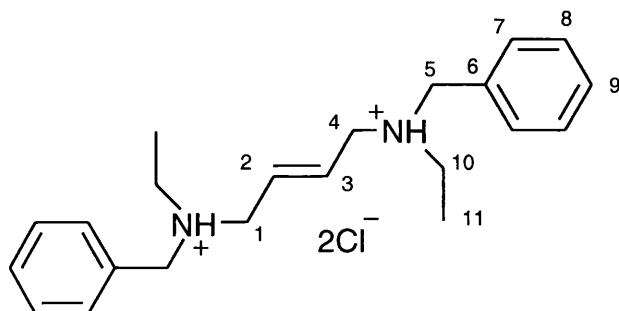
77 (0.765 g, 2.82 mmol), 10% Pd on charcoal (0.30 g) and MeOH were placed in a flask under hydrogen. The solvent was degassed 3 times and left under vacuum for a further 15 min. The hydrogen was released and the mixture was stirred for 72 h. The mixture was filtered through Celite which was washed with MeOH (3 x 20 ml) and the filtrate was concentrated *in vacuo*. The resulting solid was recrystallized from ethanol/diethyl ether to yield **90** as a fawn solid (50%), mp >250 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2970 (NH), 2667 (- CH_2 -), δ_{H} (200MHz, D_2O) 0.90 (12H, t, J 7.1, 6- H_2), 1.41 (4H, m, 2,3- H_2), 2.85 (8H, q,, J 7.1, 5- H_2), 2.80 (4H, m, 1,4- H_2); δ_{C} (50MHz, D_2O) 9.1 (CH_3 -6), 21.6 (CH_2 -5), 48.2 (CH_2 -2 and -3), 51.6 (CH_2 -1 and -4); m/z (EI) 200 (M^+-2H , 2.8%), 98 (39), 86 (100), 70 (12), 58 (46), 42 (40), (Found: M^+-2H , 200.2261. $\text{C}_{12}\text{H}_{28}\text{N}_2$ requires 200.2270). (Grateful thanks to RAC for preparation of this compound).

(E)-N,N-Diethyl-1,4-diaminobut-2-ene Dihydrochloride 89⁵⁷

This was prepared as described and gave ¹H, ¹³C NMR and IR spectra and yield were comparable to those already reported (65%). (Found: M⁺-2H, 142.1492. C₈H₁₈N₂ requires 142.1512)

N,N,N',N'-Tetraethyl-1,3-diaminopropane Dihydrochloride 92⁹⁷

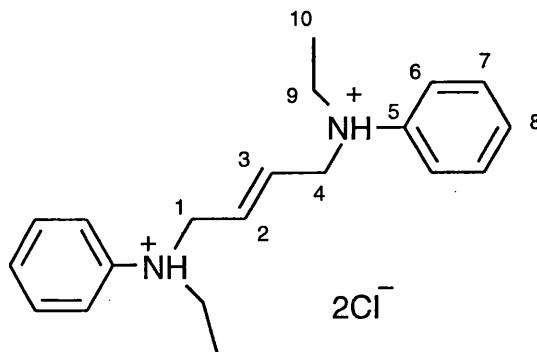
The free base *N,N,N',N'-tetraethyl-1,3-diaminopropane* **91** was prepared as reported. The dihydrochloride salt was prepared by general procedure A1 to give **92** as a white powdery solid (17%), mp >250 °C; ν_{\max} (KBr)/cm⁻¹ 3427 (N-H), 2926 (-CH₂-), 2663 (-CH₃); δ _H (200MHz, D₂O) 1.02 (12H, t, *J* 7.0, 5-H₃), 1.90 (2H, m, 2-H₂), 2.80 (4H, m, 1,3-H₂), 3.00 (8H, q, *J* 7.0, 4-H₂); δ _C (50MHz, D₂O) 9.3 (CH₃-5), 19.7 (CH₂-2), 48.5 (CH₂-1 and -3), 49.3 (CH₂-4); *m/z* (EI) 186 (M⁺-2H, 2.8%), 113 (34), 98 (68), 86 (100), 72 (17), 58 (30), (Found: M⁺-2H, 186.2100. C₁₁H₂₆N₂ requires 186.2096).

(E)-N,N'-Dibenzyl-N,N'-diethyl-1,4-diaminobut-2-ene Dihydrochloride 96

The free base **81** (0.5 g, 3.52 mmol) was diluted with MeCN at rt and NEt₃ (4 ml, 28.2 mmol) was added with stirring. Benzyl bromide (1.67 ml, 14.1

mmol) was added dropwise and the solution was stirred for 1 h. The solution was filtered and the filtrate was concentrated *in vacuo* to give a white residue. The residue was dissolved in water (10 ml), made basic with NaOH and extracted with EtOAc (2x10 ml). The organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give (*E*)-*N,N'*-dibenzyl-*N,N'*-diethyl-1,4-diaminobut-2-ene **95** as an oil, δ_{H} (200MHz, D_2O) 1.03 (6H, t, *J* 7.1, 11-H₃), 2.50 (4H, q, *J* 7.1, 10-H₂), 3.06 (4H, m, 1,4-H₂), 3.54 (4H, s, 5-H₂), 5.68 (2H, m, 2,3-H), 7.24 (10H, m, 7,8,9-H); δ_{C} (50MHz, D_2O) 11.7 (CH₃-11), 47.0 (CH₂-10), 55.0 (CH₂-1 and -4), 57.5 (CH₂-5), 126.7 (CH-2 and -3), 128.1 (CH-7), 128.9 (CH-9), 130.6 (CH-8), 139.6 (C-6). The dihydrochloride salt **96** was prepared by general procedure A1 as a creamy powder (0.608g, 1.54 mmol, 44%), mp 214.1-216 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3425 (N-H), 3084 (C-H), 2966 (-CH₂-), 2796 (N-CH₂), 2577 (NH), 1494 (C=C), 1452 (C=C), 974 ((*E*)-C=C, C-H bend), 739 (Ar C-H bend), 697 (Ar C-H bend), δ_{H} (200MHz, D_2O) 1.08 (6H, t, *J* 7.2, 11-H₃), 2.93 (4H, q, *J* 7.2, 10-H₂), 3.56 (4H, m, 1,4-H₂), 4.05 (4H, s, 5-H₂), 5.82 (2H, m, 2,3-H), 7.26 (10H, m, 7,8,9-H); δ_{C} (50MHz, D_2O) 9.1 (CH₃-11), 48.6 (CH₂-10), 53.3 (CH₂-1,4), 57.4 (CH₂-5), 129.6 (CH-2 and -3), 130.1 (CH-7), 130.3 (C-6), 130.8 (CH-9), 131.6 (CH-8); *m/z* (EI) 322 (M⁺-2H, 0.3%), 202 (0.2), 174 (8.3), 148 (19) and 91 (100), (Found: M⁺-2H, 322.2437. $\text{C}_{22}\text{H}_{30}\text{N}_2$ requires 322.2419).

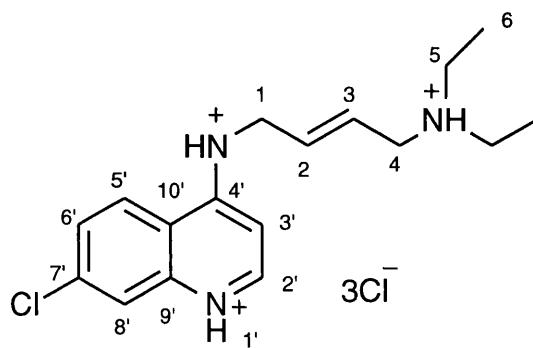
**(*E*)-*N,N'*-Diethyl-*N,N'*-diphenyl-1,4-diaminobut-2-ene
Dihydrochloride **98**⁹⁸**



The free base **97** was prepared by a slight modification of the procedure of Roberts and Ross. (*E*)-1,4-dibromobut-2-ene (1 g, 4.7 mmol) and *N*-ethylaniline (2.5 ml, 18.8 mmol) were heated at 100 °C for 4 h with stirring after which time the solution turned deep red. Water (10 ml) was added and the mixture was extracted with ether (2x10 ml) and the organic extracts

were dried (K_2CO_3), filtered and concentrated *in vacuo* to give a yellow residue. MeOH was added and a creamy precipitate appeared which was recrystallised from MeOH to give a white solid (0.93g, 3.14 mmol, 67%), $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2931 (C-H), 1598 (Ar C=C), 1506 (Ar C=C), 967 ((*E*)-C=C, C-H bend), 756 (Ar C-H bend), 694 (Ar C-H bend); δ_{H} (200MHz, D_2O) 1.12 (6H, t, *J* 7.05, 6-H₃), 3.33 (4H, q, *J* 7.05, 5-H₂), 3.85 (4H, m, 1,4-H₂), 5.62 (2H, m, 2,3-H), 6.65 (6H, m, 9,10-H), 7.20 (4H, m, 8-H); δ_{C} (50MHz, D_2O) 12.3 (CH₃-6), 44.6 (CH₂-5), 51.6 (CH₂-1 and -4), 112.2 (CH-2 and -3), 115.8 (CH-8), 127.8 (CH-10), 129.2 (CH-9), 148.1 (C-7); *m/z* (EI) 294 (M⁺, 15.9 %), 174 (84), 144 (9), 120 (24), 104 (43), 91 (83), 83 (100), 77 (57), (Found: M⁺, 294.2077. $\text{C}_{20}\text{H}_{26}\text{N}_2$ requires 266.2058). The dihydrochloride salt **98** was prepared following general procedure A2 in 80% yield, mp 189.1-190.2 °C decomp; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3424 (N-H), 3047 (Ar C-H), 2900 (-CH₂-), 1621 (C=C), 1596 (Ar C=C), 1480 (Ar C=C), 977 ((*E*)-C=C, C-H bend), 785 (Ar C-H bend), 701 (Ar C-H bend); δ_{H} (200MHz, D_2O) 0.84 (6H, t *J* 7.2, 6-H₃), 3.26 (4H, q, *J* 7.2, 5-H₂), 3.99 (4H, m, 1,4-H₂), 5.55 (2H, m, 2,3-H), 7.15 (4H, m, 8-H), 7.38 (6H, m, 9,10-H); δ_{C} (50MHz, D_2O) 10.3 (CH₃-6), 54.2 (CH₂-5), 59.2 (CH₂-1 and -4), 115.1 (CH-2 and -3), 122.8 (CH-8), 129.9 (CH-10), 131.5 (CH-9), 137.2 (C-7); *m/z* (EI) 294 (M^{+-2H}, 80%), 174 (100), 158 (58), 134 (52), 104 (38), 77 (35), (Found: M^{+-2H}, 294.2092. $\text{C}_{20}\text{H}_{26}\text{N}_2$ requires 294.2088).

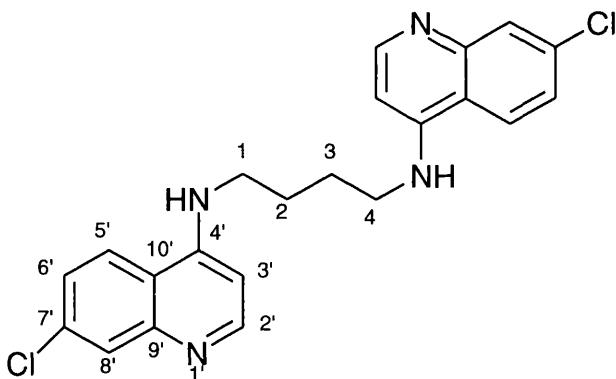
(*E*)-*N*-(7-Chloroquinolin-4-yl)-*N'*,*N'*-Diethyl-1,4-diaminobut-2-ene Dihydrochloride **100**



The free base was prepared as described and the solid was recrystallised from ether (25%), mp 113.0-115.1 °C [lit¹⁰⁰ 121-122 °C]; δ_{H} (200MHz, CDCl_3) 1.02 (6H, t, *J* 7.2, 6-H₃), 2.52 (4H, q, *J* 7.2, 5-H₂), 3.10 (2H, d, *J* 5.2, 4-H₂), 3.90 (2H, d, *J* 5.1, 1-H₂), 5.48 (1H, bs, NH), 5.80 (2H, m, 2,3-H), 6.40 (1H, d, *J* 5.4, 3'-H), 7.33 (1H, dd, *J* 8.9, 2.0, 6'-H), 7.72 (1H, d, *J* 8.9, 5'-H),

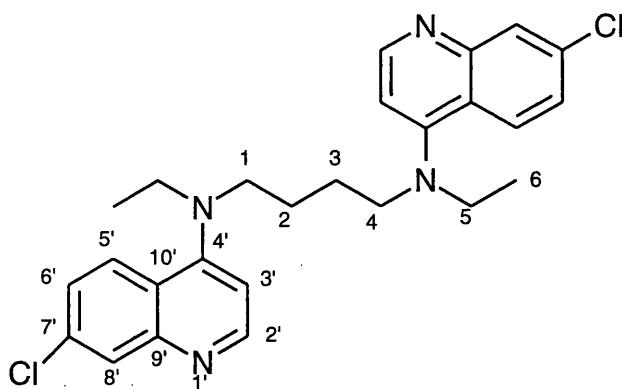
7.95 (1H, d, *J* 2.1, 8'-H), 8.50 (1H, d, *J* 5.3, 2'-H). The bulk of the free base was converted into the dihydrochloride salt following general procedure A1 to yield **100** as a hygroscopic brown solid (20%), $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3060 (Ar C-H), 2932 (C-H), 2866 (C-H), 1582 (Ar C=C), 1544 (Ar C=C), 978 ((E)-C=C C-H bend), 898 (Ar C-H bend), 804 (Ar C-H bend); δ_{H} (200MHz, D₂O) 0.97 (6H, t, *J* 7.2, 6-H₃), 2.86 (4H, q, *J* 7.2, 5-H₂), 3.51 (2H, d, *J* 5.2, 4-H₂), 4.04 (2H, d, *J* 5.1, 1-H₂), 5.52 (2H, m, 2 or 3-H), 5.90 (2H, m, 2 or 3-H), 6.43 (1H, d, *J* 5.3, 3'-H), 7.26 (1H, d, *J* 8.9, 2.0, 6'-H), 7.46 (1H, d, *J* 8.9, 5'-H), 7.76 (1H, d, *J* 2.1, 8'-H), 8.01 (1H, d, *J* 5.3, 2'-H); δ_{C} (50MHz, D₂O) 9.9 (CH₃-6), 43.1 (CH₂-5), 44.9 (CH₂-4), 53.1 (CH₂-1), 97.9 (CH-6'), 116.0 (C-10'), 119.5 (CH-2' or -3'), 123.7 (CH-2' or -3'), 125.9 (CH-8'), 127.1 (CH-3'), 129.6 (CH-5'), 133.5 (C-7'), 147.2 (C-4'), 148.2 (C-9'), 150.3 (CH-2'); *m/z* (EI) 305 (M⁺-2H, 0.3%), 303 (M⁺-2H, 1.0%), 288 (55), 232 (100), 178 (46), 162 (16), 144 (15), 110 (31) and 86 (57), (Found: M⁺-2H, 305.1496. C₁₇H₂₂N₃³⁷Cl requires 305.1473; Found: M⁺-2H, 303.1486. C₁₇H₂₂N₃³⁵Cl requires 303.1502).

***N,N'*-Bis(7-chloroquinolin-4-yl)-1,4-diaminobutane 106¹⁰²**



This was prepared according to the procedure of Vennerstrom on a 10 mmol scale and **106** was isolated in 55 % yield. mp 340.1-342 °C [lit. 339-341 °C]. IR, ¹H NMR and ¹³C NMR spectra were identical to published data.

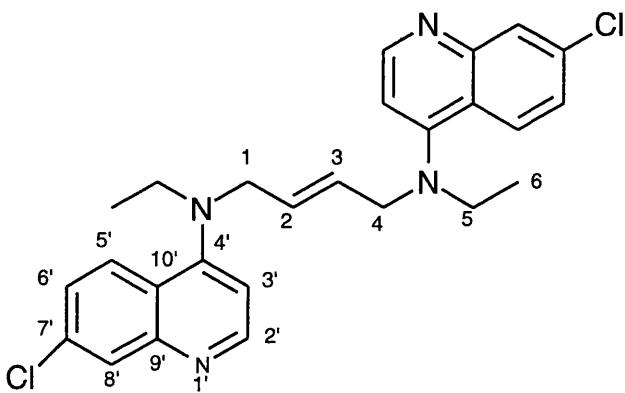
Attempted prepartion of *N,N'*-Bis(7-chloroquinolin-4-yl)-*N,N'*-diethyl-1,4-diaminobutane 104



Attempt 1: Following the procedure of Marchini¹⁰⁵ NaBH₄ (0.4630 g, 10 equiv) was added portionwise to glacial AcOH (11 ml) at 0 °C. The diamine **106** (0.5020 g, 1.22 mmol) was then added and the suspension heated at 80 °C for 2 h after which time the solution was clear yellow. After cooling to rt ppt occurred and the solid was collected by filtration. The cream solid was identical by ¹H and ¹³C NMR spectra to starting material.

Attempt 2: Following the procedure of Gribble¹⁰⁷ **106** (0.50 g, 1.27 mmol) was stirred in glacial AcOH (2 ml) at 50-55 °C under N₂ and NaBH₄ added portionwise over 30 min. The solution was heated for 20 h and the resulting solid triturated in 6 mol dm⁻³ NaOH. The cream solid was collected by filtration and was identical by ¹H and ¹³C NMR spectra to starting material.

Attempted preparation of (*E*)-*N,N'*-(7-Chloroquinolin-4-yl)-*N,N'*-diethyl-1,4-diaminobut-2-ene 105



81 (0.10 g, 0.71 mmol) was heated in phenol (0.3 g) with 4,7-dichloroquinoline (0.28 g, 2 equiv) and NaI (2 crystals) at 150-160 °C for 24 h. The residue was partitioned between 3 mol dm⁻³ HCl (10 ml) and EtOAc (10 ml) and the aqueous layer was washed with more EtOAc (3x10 ml). The aqueous was then made basic NaOH (s) and extracted with CHCl₃. The organic extracts were dried (K₂CO₃), filtered and concentrated *in vacuo*, however no product was observed.

7.2 Experimental to Chapter 4

General Procedure B1- Alkylation of Bromophthalimides with Diamines.

The phthalimide (12 mmol) was finely ground with 50% KF on Celite (12 mmol) and stirred in MeCN (60 ml). The diamine (10 equiv.) was added and the solution stirred at 40-60 °C for 24 h. The resulting solution was filtered and concentrated *in vacuo* to give the crude phthalimide which was taken up in c.HCl and c.AcOH (60 ml:60 ml) and heated at reflux for 72 h. Water (100 ml) and activated charcoal were added and the solution was heated for a further 1 h before filtering through a pad of Celite. The filtrate was concentrated *in vacuo* to 25 ml and the residue made basic (pH 12) with NaOH(s). The solution was extracted with CHCl₃ (3 x 50 ml) and the combined organic extracts were dried (K₂CO₃), filtered and concentrated *in vacuo* to yield the diamine as the free base.

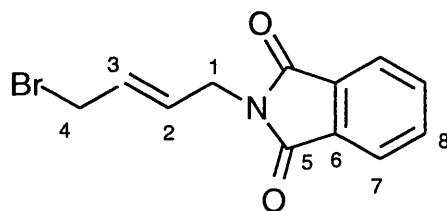
General Procedure B2- Michael Addition on Acrylonitrile.

Acrylonitrile (1 equiv.) was added dropwise over 15 min to the stirred amine (10 mmol) at 0 °C (ice-water bath). The solution was allowed to warm to rt then 45 °C for 30 min and heated at 60-70 °C for 2 h before being concentrated *in vacuo*, with care, to yield the desired nitrile. Purification, when necessary, was achieved by reduced pressure distillation (T<120 °C).

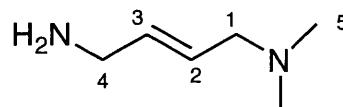
General Procedure B3- Reduction of Nitrile.

Solid LiAlH₄ (2 equiv.) was stirred in dry ether (80 ml) under N₂. AlCl₃ (2 equiv.) was added carefully and the suspension was stirred for 10 min. The amine (8 mmol) in dry ether (10 ml) was added dropwise and the solution was stirred at rt for 18 h. The stirred solution was cooled with an ice bath and excess LiAlH₄ was decomposed via careful addition of water (1 ml/ g LiAlH₄), 15% NaOH (3 ml/ g LiAlH₄) and water (1 ml/ g LiAlH₄). The resulting solid was filtered off and washed well with ether and ethanol and the combined filtrates were concentrated *in vacuo*. The residue was partitioned between CHCl₃ (20 ml) and 15% NaOH (20 ml) and the aqueous layer was washed with further amounts of CHCl₃ (2x10 ml). The combined extracts were dried and concentrated *in vacuo* to give the triamine as the free base.

(E)-1-Phthalimido-4-bromobut-2-ene 86⁵⁷

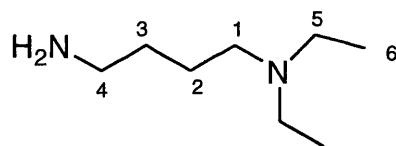


Following the procedure of Cook, **86** was prepared in 65% yield. ¹H, ¹³C NMR and IR spectra were identical to those of the literature compound. mp 99.5-100.7 °C (acetone) [lit. 95-96 °C (EtOH)].



86 (4.07 g, 14.3 mmol) and 50 % KF on Celite (8 g) were stirred in MeCN (60 ml) as in procedure B1 to yield the free base as a clear yellow oil (1.07 g, 9.4 mmol, 64%), $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$ 3384 (N-H), 1670 (C=C), 974 ((E)-C=C, C-H bend); δH (200MHz, CDCl₃) 0.55 (2H, s, NH₂), 1.17 (6H, s, 5-H₃), 1.84 (2H, d, *J* 5.8, 1-H₂), 2.24 (2H, d, *J* 5.1, 4-H₂), 4.63 (2H, m, *J* 5.0, 15.5, 2,3-H); δC (50MHz, CDCl₃) 42.8 (CH₂-1), 44.1 (CH₃-5), 60.6 (CH₂-4), 125.9 (CH-2 or -3), 133.9 (CH-2 or -3); *m/z* (EI) 114 (M⁺, 3.2%), 97 (38.7), 84 (21.8), 68 (13.2) and 58 (100), (Found: M⁺, 114.1159. C₆H₁₄N₂ requires 114.1157).

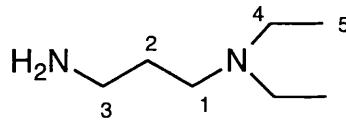
***N,N*-Diethyl-1,4-diaminobutane 145**



N-(4-Bromobutyl)phthalimide (4.03 g, 14.2 mmol) and 50% KF on Celite (8 g) were stirred in MeCN (70 ml) as in procedure B1 to yield the free base as a clear oil (1.72 g, 11.9 mmol, 84%), $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$ 3362 (N-H), 1466 (-CH₂-); δH (200MHz, CDCl₃) 1.07 (6H, t, *J* 7.1, 6-H₃), 1.53 (4H, m, 2,3-H₂), 2.44 (2H, t, *J* 6.9, 1-H₂), 2.55 (4H, q, *J* 7.2, 5-H₂), 2.75 (2H, t, *J* 6.7, 4-H₂), 3.04 (2H, s, NH₂); δC (50MHz, CDCl₃) 11.1 (CH₃-6), 23.9 (CH₂-2 or -3), 31.3 (CH₂-2 or -3), 41.6 (CH₂-1 or -5), 46.2 (CH₂-1 or -5), 52.2 (CH₂-4); *m/z* (EI) 144 (M⁺, 1.1%), 112 (12.6), 96 (71.2), 86 (51.5), 77 (92.3), 72 (51.8) and 58 (100), (Found: M⁺, 144.1636. C₈H₂₀N₂ requires 144.1645).

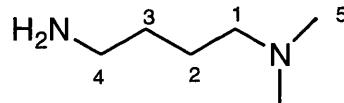
***N,N*-Diethyl-1,3-diaminopropane 144**

N,N-Diethyl-1,3-diaminopropane 144



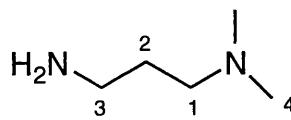
N-(3-Bromopropyl)phthalimide (4.06 g, 14.9 mmol) and 50% KF on Celite (8 g) were stirred in MeCN (70 ml) as in procedure B1 to yield the free base as a clear oil (2.04 g, 15.7 mmol, 94%), $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$ 3378(N-H), 1468 (-CH₂-); δ_{H} (200MHz, CDCl₃) 0.15 (6H, t, *J* 7.1, 5-H₃), 0.57 (2H, s, NH₂), 0.71 (2H, tt, 6.9Hz, *J* 7.1, 2-H₂), 1.61 (2H, t, 1-H₂), 1.61 (4H, q, *J* 7.1, 4-H₂), 1.86 (2H, t, *J* 6.7, 3-H₂); δ_{C} (50MHz, CDCl₃) 11.9 (CH₃-5), 31.1 (CH₂-2), 40.9 (CH₂-1 or -4), 46.9 (CH₂-1 or -4), 50.9 (CH₂-3); *m/z* (EI) 130 (M⁺, 4.4%), 113 (2.6), 100 (4.2), 96 (13.6), 86 (56.7), 74 (68.6) and 45 (100), (Found: M⁺, 130.1473. C₇H₁₈N₂ requires 130.1476).

N,N-Dimethyl-1,4-diaminobutane 143



N-(4-Bromobutyl)phthalimide (4.02 g, 14.2 mmol) and 50% KF on Celite (8 g) were stirred in MeCN (70 ml) as in procedure B1 to yield the free base as a clear oil (1.61 g, 13.9 mmol, 98%), $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$ 3380 (N-H), 1466 (-CH₂-); δ_{H} (200MHz, CDCl₃) 0.53 (2H, bs, NH₂), 0.53 (4H, m, 2,3-H₂), 1.26 (6H, s, 5-H₃), 1.30 (2H, t, *J* 6.8, 1-H₂), 1.74 (2H, t, *J* 6.6, 4-H₂); δ_{C} (50MHz, CDCl₃) 23.4 (CH₂-2 or -3), 29.9 (CH₂-2 or -3), 40.4 (CH₂-1), 43.7 (CH₃-5), 52.9 (CH₂-4); *m/z* (EI) 116 (M⁺, 5.7%), 96 (11.1), 85 (3.3), 77 (10.1), 74 (15.4), 58 (100), 56 (7.7) and 45 (18.1), (Found: M⁺, 116.1317. C₆H₁₆N₂ requires 116.1320).

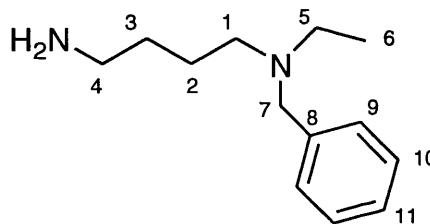
N,N-Dimethyl-1,3-diaminopropane 142



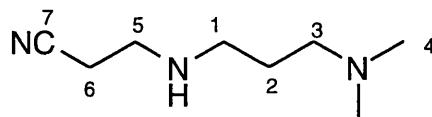
N-(3-Bromopropyl)phthalimide (4.10 g, 14.9 mmol) and 50% KF on Celite (8 g) were stirred in MeCN (70 ml) as in procedure B1 to yield the free base

as a clear oil (1.38 g, 13.3 mmol, 89%), $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3380 (N-H), 1464 (-CH₂-); δ_{H} (200MHz, CDCl₃) 0.87 (2H, tt, *J* 7.1, 7.1, 2-H₂), 0.99 (2H, s, NH₂), 1.47 (6H, s, 4-H₃), 1.58 (2H, t, *J* 7.1, 1-H₂), 2.01 (2H, t, *J* 6.8, 3-H₂); δ_{C} (50MHz, CDCl₃) 29.7 (CH₂-2), 38.9 (CH₂-1), 43.9 (CH₃-4), 55.9 (CH₂-3); *m/z* (EI) 102 (M⁺, 3.7%), 96 (1.6), 85 (18.4), 77 (5.0), 74 (2.3), 58 (100) and 44 (14.9), (Found: M⁺, 102.1147. C₅H₁₄N₂ requires 102.1137).

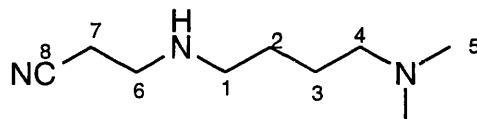
N-Benzyl-*N*-ethyl-1,4-diaminobutane 158



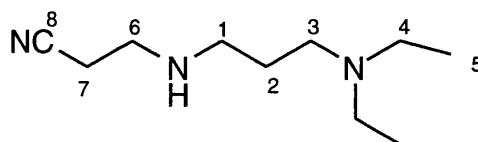
N-(4-Bromobutyl)phthalimide (5.09 g, 17.7 mmol) and 50% KF on Celite (8 g) were stirred in MeCN (40 ml) with *N*-ethylbenzylamine (26 ml, 10 equiv.) as in procedure B1. Excess *N*-ethylbenzylamine was removed by reduced pressure distillation and purification of the resulting oil achieved by alumina column chromatography, eluent Hex:EtOAc (1:1) and then flushed with MeOH to remove the product isolated as a red oil (2.2756 g, 11.1 mmol, 63%), $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3380 (NH), 2975 (-CH₂-), 2940 (-CH₂-), 2800 (N-CH₂), 1600 (Ar C=C), 1580 (Ar C=C), 1490 (Ar C=C), 730 (Ar C-H), 700 (Ar C-H); δ_{H} (200MHz, CDCl₃) 1.01 (3H, t, *J* 7.1, 6-H₃), 1.54 (4H, m, 2,3-H₂), 2.44 (4H, m, 1-H₂, NH), 2.50 (2H, q, *J* 7.1, 5-H₂), 2.77 (2H, t, *J* 6.3, 4-H₂), 3.56 (2H, s, 7-H₂), 7.27 (5H, s, 9,10,11-H); δ_{C} (50MHz, CDCl₃) 11.0 (CH₃-6), 24.4 (CH₂-2 or -3), 28.0 (CH₂-2 or -3), 40.4 (CH₂-1 or -5), 46.7 (CH₂-1 or -5), 52.6 (CH₂-4), 57.5 (CH₂-7), 127.0 (CH-11), 128.2 (CH-9), 129.2 (CH-10), 138.3 (C-8); *m/z* (EI) 206 (M⁺, 0.4%), 160 (4.5), 148 (27.6), 115 (16.7), 91 (100) and 70 (25.1), (Found: M⁺, 206.0059. C₁₃H₂₂N₂ requires 206.0062).

N-(2-Cyanoethyl)-N',N'-dimethyl-1,3-diaminopropane 146

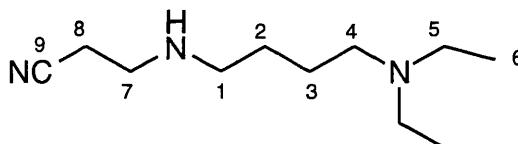
142 (0.90 g, 8.85 mmol) was treated with acrylonitrile (0.6 ml, 1 equiv.) according to general procedure B2 to yield the free base as a clear pale yellow oil (1.20 g, 7.74 mmol, 87%), $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3262 (N-H), 2250 (CN), 1464 (-CH₂-); δ_{H} (200MHz, CDCl₃) 1.64 (2H, m, *J* 7.0, 2-H₂), 1.87 (1H, bs, NH), 2.19 (6H, s, 4-H₃), 2.30 (2H, t, *J* 7.1, 3-H₂), 2.50 (2H, t, *J* 6.5, 1-H₂), 2.66 (2H, t, *J* 6.9, 6-H₂), 2.90 (2H, t, *J* 6.6, 5-H₂); δ_{C} (50MHz, CDCl₃) 18.5 (CH₂-2), 27.7 (CH₂-6), 45.1 (CH₂-3), 45.5 (CH₃-4), 47.5 (CH₂-1), 57.7 (CH₂-5), 118.4 (C-7); *m/z* (EI) 155 (M⁺, 2.6%), 141 (4.1), 115 (2.9), 96 (6.6), 85 (6.2), 77 (5.7), 72 (8.4), 70 (11.3), 58 (100) and 53 (28.7), (Found: M⁺, 155.1436. C₈H₁₇N₃ requires 155.1446).

N-(2-Cyanoethyl)-N',N'-dimethyl-1,4-diaminobutane 147

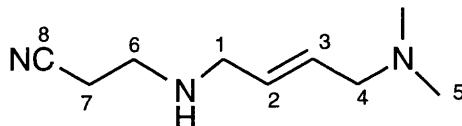
143 (1.05 g, 9.10 mmol) was treated with acrylonitrile (0.65 ml, 1 equiv.) according to general procedure B2 to yield the free base as a colourless oil (1.05 g, 6.19 mmol, 68%), bp 94-98 °C (0.1 mmHg); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3318 (N-H), 2250 (CN), 1466 (-CH₂-); δ_{H} (200MHz, CDCl₃) 1.11 (4H, m, 2,3-H₂), 1.47 (1H, bs, NH), 1.82 (6H, s, 5-H₃), 1.89 (2H, t, 4-H₂), 2.13 (2H, t, *J* 6.6, 1-H₂), 2.25 (2H, t, 7-H₂), 2.51 (2H, t, *J* 6.6, 6-H₂); δ_{C} (50MHz, CDCl₃) 17.7 (CH₂-2 or -3), 24.4 (CH₂-2 or -3), 26.9 (CH₂-7), 44.2 (CH₂-4), 44.5 (CH₃-5), 48.1 (CH₂-1), 58.6 (CH₂-6), 118.0 (C-8); *m/z* (EI) 169 (M⁺, 2.2%), 129 (2.4), 115 (2.3), 96 (4.4), 84 (6.1), 77 (3.0), 70 (3.9), 58 (100), 53 (5.6) and 45 (10.7), (Found: M⁺, 169.1586. C₉H₁₉N₃ requires 169.1594).

N-(2-Cyanoethyl)-N',N'-diethyl-1,3-diaminopropane 148

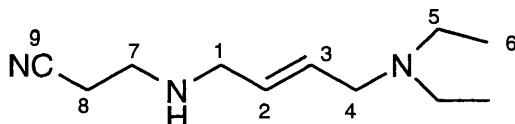
144 (1.48 g, 11.40 mmol) was treated with acrylonitrile (0.75 ml, 1 equiv.) according to general procedure B2 to yield the free base as a colourless oil (0.98 g, 7.54 mmol, 66%), bp 66-68 °C (0.03 mmHg); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3232 (N-H), 2250 (CN), 1468 (-CH₂-); δ_{H} (200MHz, CDCl₃) 0.75 (6H, t, *J* 7.1, 5-H₃), 1.37 (2H, m, 2-H₂), 1.58 (1H, bs, NH), 2.20 (4H, q, *J* 6.9, 4-H₂), 2.20 (4H, m, 1,3-H₂), 2.41 (2H, t, *J* 6.7, 7-H₂), 2.64 (2H, t, *J* 6.6, 6-H₂); δ_{C} (50MHz, CDCl₃) 10.6 (CH₃-5), 17.3 (CH₂-2), 26.0 (CH₂-7), 44.0 (CH₂-3 or -4), 45.6 (CH₂-3 or -4), 46.8 (CH₂-1), 50.1 (CH₂-6), 117.7 (C-8); *m/z* (EI) 183 (M⁺, 5.3%), 116 (15.3), 100 (4.0), 96 (7.2), 86 (100), 77 (25.4), 72 (39.8), 64 (2.8), 58 (31.2) and 53 (19.8), (Found: M⁺, 183.1748. C₁₀H₂₁N₃ requires 183.1758).

N-(2-Cyanoethyl)-N',N'-diethyl-1,4-diaminobutane 149

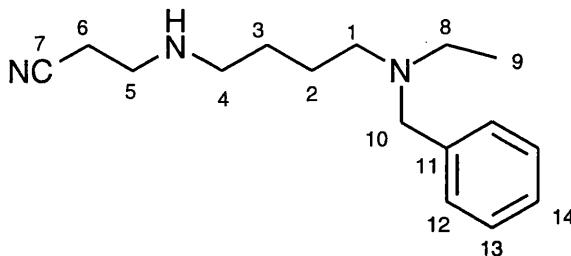
145 (1.81 g, 12.60 mmol) was treated with acrylonitrile (0.85 ml, 1 equiv.) according to general procedure B2 to yield the free base as a colourless oil (1.85 g, 9.37 mmol, 74%), bp 110-112 °C (0.7 mm); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3316 (N-H), 2247 (CN), 1469 (-CH₂-); δ_{H} (200MHz, CDCl₃) 0.71 (6H, t, *J* 6.8, 6-H₃), 1.18 (4H, m, 2,3-H₂), 1.44 (1H, bs, NH), 2.21 (4H, q, *J* 6.8, 5-H₂), 2.21 (4H, m, 1,4-H₂), 2.34 (2H, t, *J* 6.6, 8-H₂), 2.60 (2H, t, *J* 6.6, 7-H₂); δ_{C} (50MHz, CDCl₃) 11.4 (CH₃-6), 18.3 (CH₂-2 or -3), 24.4 (CH₂-2 or -3), 27.7 (CH₂-8), 44.7 (CH₂-4 or -5), 46.4 (CH₂-4 or -5), 48.7 (CH₂-1), 52.4 (CH₂-7), 18.6 (C-9); *m/z* (EI) 197 (3.6%), 143 (1.4), 123 (8.5), 112 (2.7), 98 (5.2), 86 (100), 72 (7.3) and 58 (18.6), (Found: M⁺, 197.1911. C₁₁H₂₃N₃ requires 197.1921).

(E)-N-(2-Cyanoethyl)-N',N'-dimethyl-1,4-diaminobut-2-ene 130

129 (0.92g, 8.10 mmol) was treated with acrylonitrile (0.55 ml, 1 equiv.) according to general procedure B2 to yield the free base as a colourless oil (0.79 g, 4.88 mmol, 60%), bp 76°C (0.07 mmHg); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3318 (N-H), 2250 (CN), 1458 (-CH₂-), 976 ((E) C=C, C-H bend); δ_{H} (200MHz, CDCl₃) 1.23 (1H, bs, NH), 1.87 (6H, s, 5-H₃), 2.18 (2H, t, *J* 6.6, 7-H₂), 2.54 (2H, d, 4-H₂), 2.54 (2H, t, *J* 6.6, 6-H₂), 2.94 (2H, d, 1-H₂), 5.31 (2H, m, 2,3-H); δ_{C} (50MHz, CDCl₃) 18.6 (CH₂-7), 44.2 (CH₃-5), 45.1 (CH₂-6), 50.4 (CH₂-4), 61.4 (CH₂-1), 118.8 (C-8), 129.6 (CH-2 or -3), 131.1 (CH-2 or -3); *m/z* (EI) 167 (M⁺, 8.5%), 122 (5.2), 97 (30.2), 84 (35.4), 68 (5.3), 58 (100) and 55 (8.5), (Found: M⁺, 167.1428. C₉H₁₇N₃ requires 167.1434).

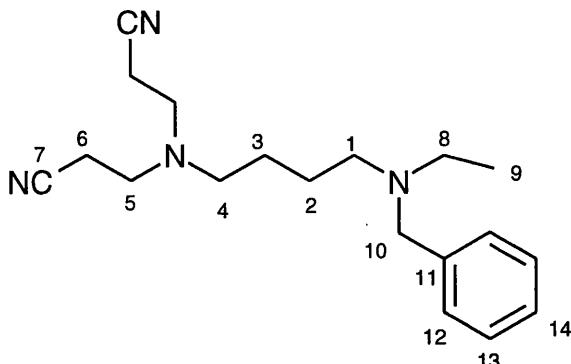
(E)-N-(2-Cyanoethyl)-N',N'-diethyl-1,4-diaminobut-2-ene 131

88 (0.99g, 6.95 mmol) was treated with acrylonitrile (0.46 ml, 1 equiv.) according to general procedure B2 to yield the free base as a pale yellow oil (1.22 g, 6.26 mmol, 90%), $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3321 (N-H), 2247 (CN), 1460 (-CH₂-), 978 ((E) C=C, C-H bend); δ_{H} (200MHz, CDCl₃) 0.95 (6H, t, *J* 7.1, 6-H₃), 1.52 (1H, bs, NH), 2.44 (4H, q, *J* 7.1, 5-H₂), 2.44 (2H, t, *J* 6.6, 8-H₂), 2.82 (2H, t, *J* 6.6, 7-H₂), 2.99 (2H, d, *J* 5.0, 4-H₂), 3.19 (2H, d, *J* 5.0, 1-H₂), 5.58 (2H, m, 2,3-H); δ_{C} (50MHz, CDCl₃) 11.4 (CH₃-6), 38.4 (CH₂-8), 44.0 (CH₂-5), 46.3 (CH₂-7), 50.3 (CH₂-4), 54.5 (CH₂-1), 118.6 (C-9), 129.6 (CH-2 or -3), 131.9 (CH-2 or -3); *m/z* (EI) 195 (M⁺, 12.3%), 180 (61.4), 123 (100), 112 (18), 86 (69.4), 70 (23.7), 58 (100) and 55 (46.8), (Found: M⁺, 195.1740. C₁₁H₂₁N₃ requires 195.1744).

***N*-Benzyl-*N*-ethyl-*N'*-(2-cyanoethyl)-1,4-diaminobutane 159**

158 (2.2756 g, 11.1 mmol) was treated with acrylonitrile (0.80 ml, 1 equiv.) according to general procedure B2. Purification was achieved using silica column chromatography, eluent EtOH to yield the free base as a colourless oil (0.5900 g, 2.33 mmol, 21%), $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3020 (Ar-H), 2940 (-CH₂), 2810 (N-CH₂), 2240 (CN), 1595 (Ar C=C), 1490 (Ar C=C), 745 (Ar C-H), 695 (Ar C-H); δ_{H} (200MHz, CDCl₃) 1.03 (3H, t, *J* 7.1, 9-H₃), 1.48 (4H, m, 2,3-H₂), 1.71 (1H, bs, NH), 2.43 (6H, m, 1,4,6-H₂), 2.54 (2H, q, *J* 7.3, 8-H₂), 2.80 (2H, t, *J* 6.7, 5-H₂), 3.56 (2H, s, 10-H₂), 7.32 (5H, m, 12,13,14-H); δ_{C} (50MHz, CDCl₃) 11.7 (CH₃-9), 16.5 (CH₂-2 or -3), 24.5 (CH₂-2 or -3), 27.6 (CH₂-6), 44.9 (CH₂-1 or -4 or -8), 47.1 (CH₂-1 or -4 or -8), 48.9 (CH₂-1 or -4 or -8), 52.6 (CH₂-5), 57.9 (CH₂-10), 116.7 (C-7), 126.7 (CH-14), 128.1 (CH-12), 128.8 (CH-13), 139.6 (C-11); *m/z* (EI) 168 (M⁺-Bn, 16.4%), 205 (M⁺-C₃H₄N, 0.3%), 190 (0.2), 148 (44.1), 134 (12.2), 120 (7.3), 106 (1.2) and 91 (100), (Found: M⁺, 168.1509. C₉H₁₈N₃ requires 168.1517; Found: M⁺, 205.1725. C₁₃H₂₁N₂ requires 205.1745).

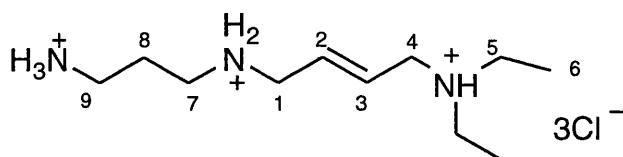
Also isolated from the column as a clear oil was ***N*-Benzyl-*N*-ethyl-*N'*-(2-cyanoethyl)-1,4-diaminobutane 160** (0.9254 g, 2.997 mmol, 27%),



$\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3020 (Ar-H), 2945 (-CH₂), 2820 (N-CH₂), 2245 (CN), 1600 (Ar C=C), 1480 (Ar C=C), 740 (Ar C-H), 695 (Ar C-H); δ_{H} (200MHz, CDCl₃) 1.04 (3H, t, *J* 7.1, 9-H₃), 1.44 (4H, m, 2,3-H₂), 2.38 (8H, m, 1,4,6-H₂), 2.51 (2H, q, *J* 7.1, 8-H₂), 2.77 (4H, t, *J* 6.8, 5-H₂), 3.54 (2H, s, 10-H₂),

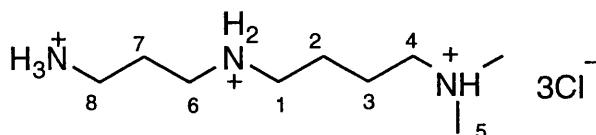
7.32 (5H, m, 12,13,14-H); δ C (50MHz, CDCl₃) 11.6 (CH₃-9), 16.7 (CH₂-2 or -3), 24.3 (CH₂-2 or -3), 24.8 (CH₂-6), 47.2 (CH₂-1 or -4 or -8), 49.4 (CH₂-1 or -4 or -8), 52.4 (CH₂-1 or -4 or -8), 52.9 (CH₂-5), 57.9 (CH₂-10), 116.7 (C-7), 126.7 (CH-14), 128.1 (CH-12), 128.9 (CH-13), 139.6 (C-11); *m/z* (EI) 312 (M⁺, 1.7%), 258 (8), 221 (4.1), 176 (4.8), 148 (72.7) and 91 (100), (Found: M⁺, 312.3128. C₁₉H₂₈N₄ requires 312.3132).

(E)-N-(3-Aminopropyl)-N',N'-diethyl-1,4-diaminobut-2-ene Trihydrochloride 135



131 (0.91 g, 4.64 mmol), LiAlH₄ (0.02 g, 2 equiv.) and AlCl₃ (0.17 g, 2 equiv.) in dry ether were treated according to general procedure B3 to yield the free base **133**. This was converted into the trihydrochloride salt **135** via procedure A1 and isolated as a pink powder (0.42 g, 1.37 mmol, 35%), mp 192.1-193.4 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3424 (N-H), 1462 (-CH₂-), 966 ((E)-C=C, C-H bend); δ H (200MHz, D₂O) 1.14 (6H, t, *J* 7.3, 6-H₂), 1.95 (2H, m, 8-H₂), 3.01 (4H, m, 7,9-H₂), 3.11 (4H, q, *J* 7.3, 5-H₂), 3.65 (2H, d, *J* 5.1, 1-H₂), 3.71 (2H, d, *J* 5.6, 4-H₂), 5.97 (2H, m, 2,3-H); δ C (50MHz, D₂O) 9.2 (CH₃-6), 24.6 (CH₂-8), 37.4 (CH₂-9), 44.9 (CH₂-5 or -7), 48.1 (CH₂-5 or -7), 49.1 (CH₂-1 or -4), 53.1 (CH₂-1 or -4), 127.8 (CH-2 or -3), 131.08 (CH-2 or -3); *m/z* (EI) 198 (M⁺-4H, 0.3%), 125 (70.6), 110 (66.8), 98 (34.5), 84 (100), 72 (32.3) and 58 (70), (Found: M⁺-4H, 198.1995. C₁₁H₂₄N₃ requires 198.2018).

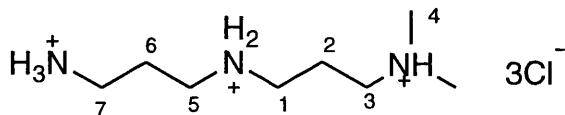
N-(3-Aminopropyl)-N',N'-dimethyl-1,4-diaminobutane Trihydrochloride 155



147 (0.51 g, 3 mmol), LiAlH₄ (0.23 g, 2 equiv.) and AlCl₃ (0.82 g, 2 equiv.) in dry ether were treated according to general procedure B3 to yield the free base **151**. This was converted into the trihydrochloride salt **155** via procedure B1 and isolated as a white powder (0.28 g, 1.80 mmol, 60%),

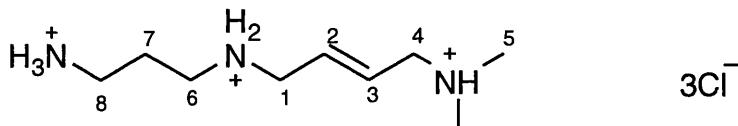
mp 204.5-206.5 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3424 (N-H), 1476 (-CH₂-); δ_{H} (200MHz, D₂O) 1.61 (4H, m, 2,3-H₂), 1.91 (2H, m, 7-H₂), 2.71 (6H, s, J 7.1, 5-H₃), 2.97 (8H, m, 1,4,6,8-H₂); δ_{C} (50MHz, D₂O) 22.1 (CH₂-2 or -3), 23.5 (CH₂-2 or -3), 24.6 (CH₂-7), 37.4 (CH₂-8), 43.5 (CH₃-5), 45.36 (CH₂-1 or -4 or -6), 47.8 (CH₂-1 or -4 or -6), 57.6 (CH₂-1 or -4 or -6); *m/z* (EI) 173 (M⁺-3H, 3.2%), 129 (6.8), 115 (4.2), 98 (8.5), 84 (22.3), 71 (10) and 58 (100), (Found: M⁺-3H, 173.1887. C₉H₂₃N₃ requires 173.1882).

N-(3-Aminopropyl)-*N,N*'-dimethyl-1,4-diaminopropane Trihydrochloride 154



146 (1.21 g, 7.77 mmol), LiAlH₄ (0.66 g, 2 equiv.) and AlCl₃ (2.40 g, 2 equiv.) in dry ether were treated to general procedure B3 to yield the free base **150**. This was converted into the trihydrochloride salt **154** via procedure B1 and isolated as a white hygroscopic solid (1.21 g, 4.51 mmol, 58%), $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3423 (N-H), 1466 (-CH₂-); δ_{H} (200MHz, D₂O) 1.97 (4H, m, 2,6-H₂), 2.75 (6H, s, 4-H₃), 3.01 (8H, m, 1,3,5,7-H₂); δ_{C} (50MHz, D₂O) 22.1 (CH₂-2), 24.6 (CH₂-6), 37.4 (CH₂-7), 43.7 (CH₃-4), 45.3 (CH₂-1 or -3 or -5), 45.6 (CH₂-1 or -3 or -5), 55.1 (CH₂-1 or -3 or -5); *m/z* (EI) 160 (M⁺-3H, 0.3%), 159 (M⁺-3H, 1.3%), 132 (3), 114 (5.2), 98 (4.5), 85 (50.3), 72 (14.5), 70 (18) and 58 (100), (Found: M⁺-3H, 159.1741. C₈H₂₁N₃ requires 159.1746).

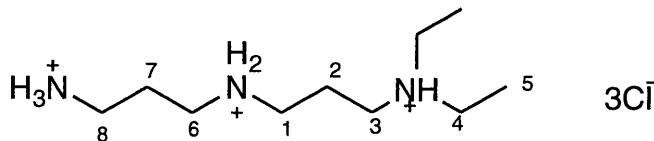
(E)-*N*-(3-Aminopropyl)-*N,N*'-dimethyl-1,4-diaminobut-2-ene Trihydrochloride 134



130 (0.68 g, 4.10 mmol), LiAlH₄ (0.31 g, 2 equiv.) and AlCl₃ (1.09 g, 2 equiv.) in dry ether were treated according to general procedure B3 to yield the free base **132**. This was converted into the trihydrochloride salt **134** via procedure B1 and isolated as a white powder (0.67 g, 2.40 mmol, 58%), mp 244.5-246.0 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3424 (N-H), 1470 (-CH₂-), 982 ((E)-C=C, C-H bend); δ_{H} (200MHz, D₂O) 1.98 (2H, m, 7-H₂), 2.75 (6H, s, 5-H₃),

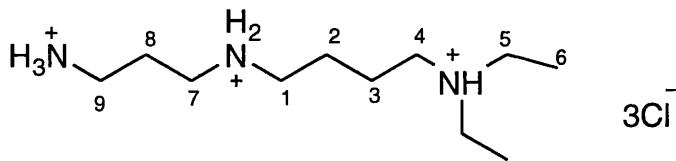
3.02 (4H, m, 6,8-H₂), 3.70 (4H, m, 1,4-H₂), 5.99 (2H, m, 2,3-H); δ C (50MHz, D₂O) 24.6 (CH₂-7), 37.5 (CH₂-8), 43.1 (CH₃-5), 44.9 (CH₂-6), 49.0 (CH₂-1 or -4), 58.9 (CH₂-1 or -4), 127.9 (CH-2 or -3), 131.6 (CH-2 or -3); *m/z* (Cl/NH₃) 173 (M⁺-1, 13%), 172 (M⁺-2, 100%), 141 (0.2), 129 (1.1), 115 (0.5) and 98 (0.3), (Found: M⁺-1, 173.1845. C₉H₂₃N₃ requires 173.1798; Found: M⁺-2, 172.1811. C₉H₂₂N₃ requires 172.1809).

***N*-(3-Aminopropyl)-N',N'-diethyl-1,3-diaminopropane Trihydrochloride 156**



148 (0.99 g, 5.40 mmol), LiAlH₄ (0.41 g, 2 equiv.) and AlCl₃ (1.44 g, 2 equiv.) in dry ether were treated according to general procedure B3 to yield the free base **152**. This was converted into the trihydrochloride salt **156** via procedure B1 and isolated as a cream powder (0.98 g, 3.35 mmol, 62%), mp 171.2-173.6 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3424 (N-H), 1470 (-CH₂-); δ H (200MHz, D₂O) 1.11 (6H, t, *J* 7.3, 5-H₂), 1.79 (4H, m, 2,7-H₂), 2.71 (4H, t, *J* 7.6, 6,8-H₂), 2.88 (2H, t, *J* 7.7, 1-H₂), 2.99 (2H, t, 3-H₂), 3.08 (4H, q, *J* 7.3, 4-H₂); δ C (50MHz, D₂O) 9.8 (CH₃-5), 23.1 (CH₂-2), 26.4 (CH₂-7), 38.2 (CH₂-8), 46.1 (CH₂-1, -3 or -4), 48.2 (CH₂-1, -3 or -4), 50.3 (CH₂-1, -3 or -4); *m/z* (EI) 187 (M⁺-3H, 1.3%), 113 (20.6), 98 (21.5), 86 (100), 72 (28.5) and 58 (41.1), (Found: M⁺-3H, 187.2039. C₁₀H₂₅N₃ requires 187.2030).

***N*-(3-Aminopropyl)-N',N'-diethyl-1,4-diaminobutane Trihydrochloride 157**

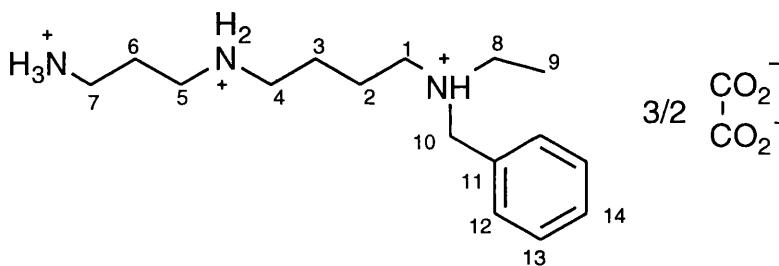


149 (0.63 g, 3.73 mmol), LiAlH₄ (0.28 g, 2 equiv.) and AlCl₃ (0.99 g, 2 equiv.) in dry ether were treated according to general procedure B3 to yield the free base **153**. This was converted into the hydrochloride salt **157** via procedure B1 and isolated as a cream hygroscopic powder (0.61 g, 2.15 mmol, 57%), $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3420 (N-H), 1466 (-CH₂-); δ H (200MHz, D₂O) 1.08 (6H, t, *J* 7.3, 6-H₃), 1.49 (4H, m, 2,3-H₂), 1.69 (2H, m, 8-H₂), 2.64 (2H, t,

J 6.9, 9-H₂), 2.68 (2H, t, *J* 7.0, 7-H₂), 2.77 (2H, t, *J* 6.9, 1-H₂), 2.95 (4H, q, *J* 7.3, 5-H₂), 3.01 (2H, t, *J* 7.0, 4-H₂); δ C (50MHz, D₂O) 9.2 (CH₃-6), 22.1 (CH₂-2 or -3,) 25.3 (CH₂-2 or -3), 27.4 (CH₂-8), 38.4 (CH₂-9), 46.2 (CH₂-1, -4, -5 or -7), 48.0 (CH₂-1, -4, -5 or -7), 48.3 (CH₂-1, -4, -5 or -7), 52.1 (CH₂-1, -4, -5 or -7); *m/z* (EI) 187 (M⁺-3H, 1.3%), 113 (20.6), 98 (21.5), 86 (100), 72 (28.5) and 58 (41.1), (Found: M⁺-3H, 201.2194. C₁₁H₂₇N₃ requires 201.2184).

***N*-Benzyl-*N*-ethyl-*N'*-(3-aminopropyl)-1,4-diaminobutane**

Oxalate 162

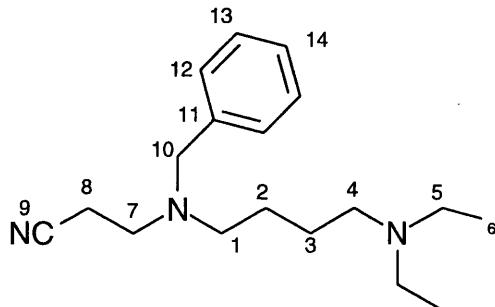


159 (0.50 g, 1.93 mmol) was stirred in Et₂O (30 ml) with LiAlH₄ (0.29 g, 4 equiv.) and AlCl₃ (1.03 g, 4 equiv.) according to general procedure B3 to yield *N*-Benzyl-*N*-ethyl-*N'*-(3-aminopropyl)-1,4-diaminobutane (0.4332 g, 1.65 mmol, 87%) as a clear oil, δ H (200MHz, CDCl₃) 0.92 (3H, t, *J* 7.1, 9-H₃), 1.38 (4H, m, 2,3-H₂), 1.51 (2H, m, 6-H₂), 2.29-2.65 (10H, m, 1,4,5,7,9-H₂), 3.45 (2H, s, 10-H₂), 7.16 (5H, m, 12,13,14-H); δ C (50MHz, CDCl₃) 11.7 (CH₃-9), 24.8 (CH₂-2 or -3), 27.9 (CH₂-2 or -3), 33.7 (CH₂-6), 40.3 (CH₂-1 or -4 or -8), 47.1 (CH₂-1 or -4 or -8), 47.7 (CH₂-1 or -4 or -8), 49.9 (CH₂-5), 52.9 (CH₂-7), 57.9 (CH₂-10), 116.7 (C-7), 126.7 (CH-14), 128.1 (CH-12), 128.8 (CH-13), 139.6 (C-11). The free base **161** (0.0621 g) was dissolved in MeOH (1 ml) and oxalic acid (0.032 g, 1.5 equiv.) in MeOH (1 ml) added. Precipitation appeared immediately and the product was recrystallised from H₂O-EtOH to yield a white solid in quantitative yield, $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3426 (NH₃⁺), 2953 (-CH₂-), 1608 (CO₂⁻), 1496 (Ar C=C), 1310 (CO₂⁻), 744 (Ar-H), 701 (Ar-H); δ H (200MHz, D₂O) 1.09 (3H, t, *J* 7.2, 9-H₃), 1.52 (4H, m, 2,3-H₂), 1.85 (2H, dt, 6-H₂), 2.83-3.04 (10H, m, 1,4,5,6,8-H₂), 4.11 (2H, s, 10-H₂), 7.28 (5H, m, 12,13,14-H); δ C (50MHz, CDCl₃) 8.79 (CH₃-9), 21.3 (CH₂-2 or -3), 23.6 (CH₂-2 or -3), 24.5 (CH₂-6), 37.3 (CH₂-1 or -4 or -8), 45.3 (CH₂-1 or -4 or -8), 47.7 (CH₂-1 or -4 or -8), 48.3 (CH₂-5), 51.5 (CH₂-7), 57.3 (CH₂-10), 129.8 (CH-11), 130.1 (CH-13), 130.9 (CH-14), 131.6 (C-12), 171.0 (C=O); *m/z* (Cl/NH₃) 264 (M⁺-1, 57%),

245 (5), 219 (4), 207 (10) and 136 (19), (Found: M⁺-1, 264.2445. C₁₈H₃₁N₃ requires 264.2450).

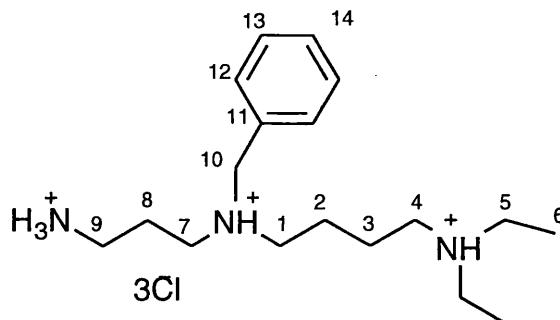
N-Benzyl-N-(2-cyanoethyl)-N',N'-diethyl-1,4-diaminobutane

163



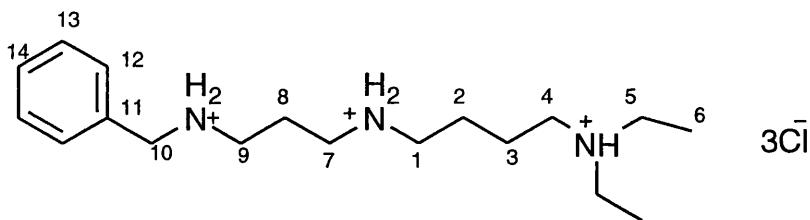
148 (0.2049 g, 1.04 mmol) was stirred in MeCN (5 ml) and NEt₃ (0.3 ml, 2 equiv.). BnBr (0.12 ml, 1 equiv.) was added dropwise and the solution was stirred for 2 h after which time it was concentrated *in vacuo*. The residue was partitioned between Et₂O (5 ml) and H₂O (5 ml) and the organic extracts washed with water (2x5 ml), dried (K₂CO₃), filtered and concentrated *in vacuo* to yield **163** as a clear oil (0.0558 g, 0.21 mmol, 20%) $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 2955 (C-H), 2254 (CN), 1524 (Ar C=C), 720 (Ar C-H bend) 724 (Ar C-H bend); δ_{H} (200MHz, CDCl₃) 0.97 (6H, t, *J* 7.1, 6-H₃), 1.45 (4H, m, 2,3-H₂), 2.39 (2H, t, *J* 6.9, 4-H₂), 2.48 (2H, t, *J* 7.0, 1-H₂), 2.50 (4H, q, *J* 7.1, 5-H₂), 2.52 (2H, t, *J* 7.0, 8-H₂), 2.78 (2H, t, *J* 7.0, 7-H₂), 3.61 (2H, s, 10-H₂), 7.28 (5H, m, 12,13,14-H); δ_{C} (50MHz, CDCl₃) 11.6 (CH₃-6), 16.3 (CH₂-2 or -3), 24.7 (CH₂-2 or -3), 25.2 (CH₂-8), 46.8 (CH₂-4 or -5), 49.2 (CH₂-4 or -5), 52.7 (CH₂-1), 53.7 (CH₂-7), 58.4 (CH₂-10), 118.9 (C-9), 127.2 (CH-14), 128.4 (CH-12), 128.6 (CH-13), 138.9 (C-11); *m/z* (EI) 287 (M⁺, 1.2%), 233 (1.6), 196 (23.4), 176 (2.7), 160 (10), 123 (20.7), 98 (7.5), 91 (47.3) and 86 (100), (Found: M⁺, 287.2378. C₁₈H₂₉N₃ requires 287.2361).

**N-(3-Aminopropyl)-N-benzyl-N',N'-diethyl-1,4-diaminobutane
Trihydrochloride 165**



163 (0.0440 g, 0.15 mmol) was reduced following general procedure B3 using LiAlH₄ (0.035 g, 6 equiv.) and AlCl₃ (0.1220 g, 6 equiv.). The free base was taken up in DCM, filtered and concentrated *in vacuo* to yield **164** as a clear oil (0.0348 g, 0.12 mmol, 80%), δ_H (200MHz, CDCl₃) 1.03 (6H, t, *J* 7.2, 6-H₃), 1.46 (4H, m, 2,3-H₂), 1.62 (2H, m, 8-H₂), 2.45 (6H, m, 1,4,7-H₂), 2.53 (4H, q, *J* 7.2, 5-H₂), 2.72 (2H, t, *J* 6.6, 9-H₂), 3.12 (2H, bs, NH₂), 3.53 (2H, s, 10-H₂), 7.29 (5H, m, 12,13,14-H); δ_C (50MHz, CDCl₃) 11.5 (CH₃-6), 24.6 (CH₂-2 or -3), 25.1 (CH₂-2 or -3), 30.7 (CH₂-8), 40.5 (CH₂-1, -4, -5 or -7), 46.8 (CH₂-1, -4, -5 or -7), 51.3 (CH₂-1, -4, -5 or -7), 52.8 (CH₂-1, -4, -5 or -7), 53.7 (CH₂-9), 58.7 (CH₂-10), 126.7 (CH-14), 128.1 (CH-12), 128.8 (CH-13), 140.7 (C-11). The trihydrochloride salt **165** was prepared by general procedure B1 and was isolated as a hygroscopic yellow semi-solid in essentially quantitative yield, ν_{max}(KBr)/cm⁻¹ 2940 (-CH₂-), 2791 (-NH⁺), 1610 (Ar C=C), 1580 (Ar C=C), 1499 (Ar C=C), 742 (Ar C-H bend), 695 (Ar C-H bend); δ_H (200MHz, D₂O) 1.20 (6H, t, *J* 7.3, 6-H₃), 1.70 (4H, m, 2,3-H₂), 2.10 (2H, m, 8-H₂), 3.11 (12H, m, 1,4,7,9-H₂), 4.37 (2H, s, 10-H₂), 7.47 (5H, m, 12,13,14-H); δ_C (50MHz, D₂O) 9.1 (CH₃-6), 21.4 (CH₂-2 or -3), 22.5 (CH₂-2 or -3), 37.3 (CH₂-8), 48.1 (CH₂-9), 48.2 (CH₂-1, -4, -5 or -7), 50.4 (CH₂-1, -4, -5 or -7), 51.5 (CH₂-1, -4, -5 or -7), 52.6 (CH₂-1, -4, -5 or -7), 58.3 (CH₂-10), 129.6 (C-11), 130.4 (CH), 131.2 (CH), 131.6 (CH); *m/z* (CI/NH₃) 292 (M⁺-2H 7.2%), 234 (0.09), 219 (0.03), 200 (0.3), 163 (0.06), 91 (0.36) and 86 (0.34), (Found: M⁺-2H 292.2757. C₁₈H₃₄N₃ requires 292.2762).

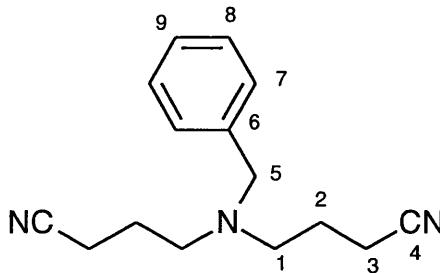
**N-(N-benzyl-3-aminopropyl)-N',N'-diethyl-1,4-diaminobutane
Trihydrochloride 167**



Following the procedure of Sclafani et al.¹³⁸ to a solution of **153** (0.1676 g, 0.84 mmol) in CHCl₃ was added benzaldehyde (0.08 ml, 1 equiv.). The solution was heated at reflux for 2 h and then concentrated *in vacuo* to yield the crude imine which was taken up in MeOH (15 ml). NaBH₄ (0.13 g, 4 equiv.) in MeOH (5 ml) was added and the solution was heated at reflux for 2 h before leaving at rt overnight. It was concentrated *in vacuo* and the residue was partitioned between CHCl₃ (15 ml) and 1 mol dm⁻³ NaOH (5 ml) and the organic layer was washed with 1 mol dm⁻³ NaOH (2 x 5 ml). 6 mol dm⁻³ HCl was added to the CHCl₃ extract and the aqueous layer was separated. The aqueous layer was rebasified to pH14 (NaOH), extracted with CHCl₃, dried (K₂CO₃), filtered and concentrated *in vacuo* to yield the free base **166** as a clear oil (0.2328 g, 0.80 mmol, 95 %), δ_H (200MHz, CDCl₃) 1.01 (6H, t, *J* 7.1, 6-H₃), 1.47 (4H, m, 2,3-H₂), 1.62 (2H, m, 8-H₂), 1.70 (2H, m, NH), 2.41 (2H, t, 4-H₂), 2.50 (4H, q, *J* 7.1, 5-H₂), 2.63 (2H, t, *J* 6.9, 1-H₂), 2.67 (2H, t, *J* 6.9, 7-H₂), 2.68 (2H, t, *J* 6.9, 9-H₂), 3.77 (2H, s, 10-H₂), 7.31 (5H, m, 12,13,14-H); δ_C (50MHz, CDCl₃) 11.6 (CH₃-6), 24.8 (CH₂-2 or -3), 28.1 (CH₂-2 or -3), 30.3 (CH₂-8), 46.7 (CH₂-1, -4, -5, -7 or -9), 47.9 (CH₂-1, -4, -5, -7 or -9), 48.5 (CH₂-1, -4, -5, -7 or -9), 50.0 (CH₂-1, -4, -5, -7 or -9), 52.8 (CH₂-1, -4, -5, -7 or -9), 54.1 (CH₂-10), 126.8 (CH-14), 128.0 (CH-12), 128.3 (CH-13), 140.5 (C-11); The trihydrochloride salt **167** was isolated following general procedure B1 as a cream powder in 82 % yield, mp 220-222.2 °C; ν_{max}(KBr)/cm⁻¹ 3423 (O-H), 2943 (-CH₂-), 2787 (-NH⁺), 1637 (Ar C=C), 1581 (Ar C=C), 1497 (Ar C=C), 748 (Ar C-H bend), 697 (Ar C-H bend); δ_H (200MHz, D₂O) 1.10 (6H, t, *J* 7.3, 6-H₃), 1.61 (4H, m, 2,3-H₂), 1.98 (2H, m, 8-H₂), 2.96 (8H, m, 1,4,7,9-H₂), 3.04 (4H, q, *J* 7.3, 5-H₂), 4.10 (2H, s, 10-H₂), 7.35 (5H, m, 12,13,14-ArH); δ_C (50MHz, D₂O) 9.01 (CH₃-6), 21.5 (CH₂-2 or -3), 23.5 (CH₂-2 or -3), 23.7 (CH₂-8), 44.7 (CH₂-1, -4, -5, -7 or -9), 45.3 (CH₂-1, -4, -5, -7 or -9), 47.9 (CH₂-1, -4, -5, -7 or -9), 48.2 (CH₂-1, -4, -5, -7 or -9), 51.7 (CH₂-1, -4, -5, -7 or -9), 52.1 (CH₂-10), 130.2 (CH-14), 130.6 (CH-12), 130.7 (CH-13), 131.2 (C-11); *m/z*

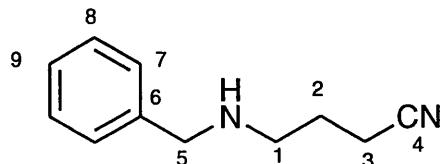
(Cl/NH₃) 292 (M⁺-2H, 100%), 263 (0.1), 217 (0.4), 202 (0.1), 145 (0.2) and 106 (0.2), (Found: M⁺-2H 292.2750, C₁₈H₃₄N₃ requires 292.2747).

N,N-Bis-(3-cyanopropyl)benzylamine 169^{112, 131, 139, 140}



Benzylamine (0.50 g, 4.66 mmol) and 4-bromobutyronitrile (1.02 ml, 2.2 equiv.) were stirred in MeCN (15 ml) and NEt₃ (0.65 ml, 2.2 equiv.) for 48 h at 40 °C. The solution was concentrated *in vacuo* and the residue was partitioned between 3 mol dm⁻³ HCl (15 ml) and CHCl₃ (15 ml). The aqueous layer was washed with CHCl₃ (2x10 ml) and then made basic to pH14 using NaOH(s). The aqueous was extracted with CHCl₃ (3x15 ml) and the organic extracts dried (K₂CO₃), filtered and concentrated *in vacuo* to give a clear yellow oil. Purification was achieved via silica gel column chromatography with EtOH as eluent to yield the desired product as a clear oil (0.2945 g, 1.22 mmol, 26 %), ¹H NMR data were identical with published data¹¹; Rf 0.76. (EtOH); ν_{max}(CHCl₃)/cm⁻¹ 3608 (N-H), 3022 (Ar-H), 2962 (-CH₂-), 2821 (N-CH₂), 2249 (CN), 1602 (Ar C=C), 1494 (Ar C=C), 727 (Ar C-H bend), 698 (Ar C-H bend); δ_C (50MHz, CDCl₃) 14.4 (CH₂-2), 22.8 (CH₂), 51.8 (CH₂), 58.0 (CH₂), 119.4 (C-4), 126.8 (CH-9), 128.0 (CH-7), 128.4 (CH-8), 138.2 (C-6); m/z (EI) 241 (M⁺, 1.5%), 187 (26.3), 164 (1.1), 118 (0.9), 104 (0.8), 91 (100), 77 (1.6) and 65 (11.3), (Found: M⁺, 241.1591. C₁₅H₁₉N₃ requires 241.1579).

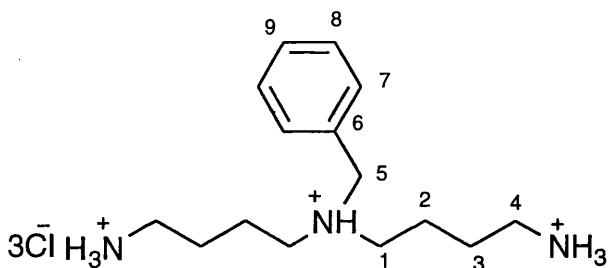
Isolated from the same column chromatography as a clear oil was **N-(3-cyanopropyl)benzylamine 170** (0.2536 g, 1.39 mmol, 30 %),



MS was identical with published data. Rf 0.41 (EtOH); ν_{max}(CHCl₃)/cm⁻¹ 3583 (N-H), 3022 (Ar-H), 3010 (-CH₂-), 2250 (CN), 1602 (Ar C=C), 1495 (Ar

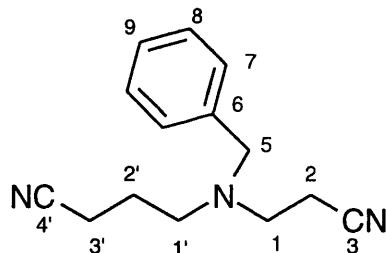
C=C), 768 (Ar C-H bend), 699 (Ar C-H bend); δ_{H} (200MHz, CDCl₃) 1.37 (1H, s, NH), 1.79 (2H, m, 2-H₂), 2.43 (2H, t, *J* 6.6, 3-H₂), 2.73 (2H, t, *J* 6.6, 1-H₂), 3.76 (2H, s, 5-H₂), 7.27 (5H, s, 7,8,9-ArH); δ_{C} (50MHz, CDCl₃) 14.9 (CH₂-2), 25.8 (CH₂), 47.4 (CH₂), 53.8 (CH₂), 119.8 (C-4), 127.1 (CH-9), 128.0 (CH-7), 128.4 (CH-8), 140.1 (C-6); *m/z* (EI) 174 (M⁺, 11%), (Found: M⁺, 174.1143. C₁₁H₁₄N₂ requires 174.1157).

N,N-Bis-(4-aminobutyl)benzylamine Trihydrochloride 175^{112, 131, 139, 140}



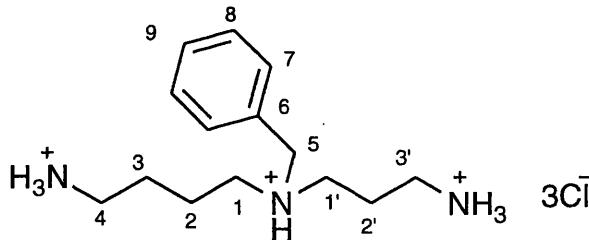
The dinitrile **169** (0.30 g, 1.20 mmol) was reduced using general procedure B3 and converted into the trihydrochloride salt via procedure A1 to give cream crystals (0.3081 g, 0.86 mmol, 72%), $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3423 (N-H), 2962 (C-H), 1998 (C-H), 1599 (Ar C=C), 1498 (Ar C=C), 746 (Ar C-H bend), 696 (Ar C-H bend); δ_{H} (200MHz, D₂O) 1.57 (4H, m, 3-H₂), 1.70 (4H, m, 2-H₂), 2.87 (4H, t, *J* 7.3, 4-H₂), 3.06 (4H, t, *J* 8.0, 1-H₂), 4.24 (2H, s, 5-H₂), 7.37 (5H, s, 7,8,9-H); δ_{C} (50MHz, D₂O) 21.5 (CH₂-2 or -3), 24.8 (CH₂-2 or -3), 39.8 (CH₂-4), 52.6 (CH₂-1), 58.1 (CH₂-5), 129.8 (C-6), 130.4 (CH-9), 131.2 (CH-7), 132.0 (CH-8);

N-(2-Cyanoethyl)-N-(3-cyanopropyl)benzylamine 171^{112, 131, 139, 140}



170 (0.2536 g, 1.46 mmol) was stirred in acrylonitrile (0.41 ml, 4 equiv.) at 70-80 °C for 24 h. The solution was concentrated *in vacuo* and purified by silica chromatography, with EtOH as eluent, to yield **171** as a clear oil (0.2481 g, 1.09 mmol, 75 %) ^1H NMR and IR were identical with published data^{z10}, Rf 0.81 (EtOH); δC (50MHz, CDCl_3) 14.4 (CH_2 -2'), 16.3 (CH_2 -3'), 23.1 (CH_2 -2), 48.9 (CH_2 -1') 51.5 (CH_2 -1), 58.1 (CH_2 -5), 118.9 (C-3 or -4'), 119.5 (C-3 or -4'), 127.2 (CH-9), 128.2 (CH-7), 128.4 (CH-8), 137.8 (C-6); m/z (EI) 227 (M^+ , 0.6%), 187 (20.4), 173 (4.7), 150 (0.6), 118 (0.9), 91 (100) and 65 (11.3), (Found: M^+ , 227.1433. $\text{C}_{14}\text{H}_{17}\text{N}_3$ requires 227.1422).

N-(4-Aminobutyl)-N-(3-aminopropyl)benzylamine Trihydrochloride 173^{112, 131, 139, 140}

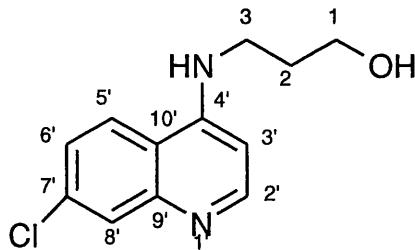


172 was prepared using general procedure B3 stirring dinitrile **171** (0.2481 g, 1.10 mmol), LiAlH₄ (0.38 g, 4 equiv.) and AlCl₃ (1.33 g, 4 equiv.) in dry ether (20 ml) to yield a clear oil (0.2026 g, 1.05 mmol, 95 %), ^1H NMR data were identical with published material.^{z10} New data: δC (50MHz, CDCl_3) 24.4 (CH_2 -2 or -3), 30.9 (CH_2 -2 or -3), 31.6 (CH_2 -2'), 40.5 (CH_2 -1 or -1'), 42.1 (CH_2 -1 or -1'), 51.3 (CH_2 -3' or -4), 53.6 (CH_2 -3' or -4), 58.7 (CH_2 -5), 126.7 (CH-9), 128.1 (CH-7), 128.7 (CH-8), 134.0 (C-7); The trihydrochloride salt **173** was isolated as cream crystals in 89% yield, $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3433 (N-H), 2966 (C-H), 1989 (C-H), 1601 (Ar C=C), 1499 (Ar C=C), 748 (Ar C-H bend), 699 (Ar C-H bend); δH (200MHz, D_2O) 1.49

(2H, m), 1.63 (2H, m), 1.96 (2H, m), 2.82 (4H, m), 3.04 (4H, m), 4.19 (2H, s, 5-H₂), 7.33 (5H, s, 7,8,9-H); δC (50MHz, D₂O) 21.4 (CH₂-2 or -3), 22.5 (CH₂-2 or -3), 24.7 (CH₂-2'), 37.4 (CH₂-3' or -4), 39.6 (CH₂-3' or -4), 50.2 (CH₂-1 or -1'), 52.7 (CH₂-1 or -1'), 58.2 (CH₂-5), 129.9 (C-6), 130.3 (CH-9), 131.0 (CH-7), 131.7 (CH-8); m/z (Cl/NH₃) 236 (M⁺-2H, 100%), 217 (0.1), 179 (0.45), 165 (0.4), 134 (0.3), 108 (0.5) and 91 (0.2), (Found: M⁺-2H, 236.2124. C₁₄H₂₆N₃ requires 236.2121).

The following compounds were prepared following the procedure of McClintock.¹¹⁴ Their ¹H, ¹³C NMR and IR spectra were identical to those reported: 3-(t-butoxycarbonylamino)propanoic acid **111** (96%); 4-(t-butoxycarbonylamino)butanoic acid **112** (85%); 5-(t-butoxycarbonylamino)pentanoic acid **113** (90%); *N*-ethyl-4-(t-butoxycarbonylamino)butanamide **114** (43%); *N*-ethyl-5-(t-butoxycarbonylamino)pentanamide **115** (66%); *N*-ethyl-4-aminobutanamide hydrochloride **116** (95%); *N*-ethyl-5-aminopentanamide hydrochloride **117** (92%); *N*-ethyl-4-[3-(t-butoxycarbonylamino)propanamido]butanamide **119** (41%); *N*-ethyl-5-[3-(t-butoxycarbonylamino)propanamido]pentanamide **120** (50%); *N*-ethyl-4-[4-(t-butoxycarbonylamino)butanamido]butanamide **121** (32%); *N*-ethyl-4-(3-aminopropanamido)butanamide hydrochloride **122** (99%); *N*-ethyl-5-(3-aminopropanamido)pentanamide hydrochloride **123** (95%); *N*-ethyl-4-(4-aminobutanamido)butanamide hydrochloride **124** (90%); *N'*-(3-aminopropyl)-*N*-ethyl-1,4-diaminobutane trihydrochloride **125** (52%); *N'*-(3-aminopropyl)-*N*-ethyl-1,5-diaminopentane trihydrochloride **126** (62%); *N'*-(4-aminobutyl)-*N*-ethyl-1,4-diaminobutane trihydrochloride **127** (65%).

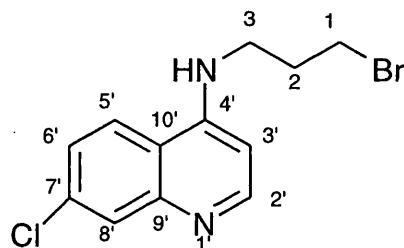
N-(7-Chloroquinolin-4-yl)-1-aminopropan-3-ol **176**¹⁴³



4,7-Dichloroquinoline (5 g, 25 mmol) was stirred in 1-aminopropan-3-ol (3.82 ml, 50 mmol) at 150-160 °C for 18 h. The solution was cooled to rt and then excess 1-aminopropan-3-ol removed by reduced pressure

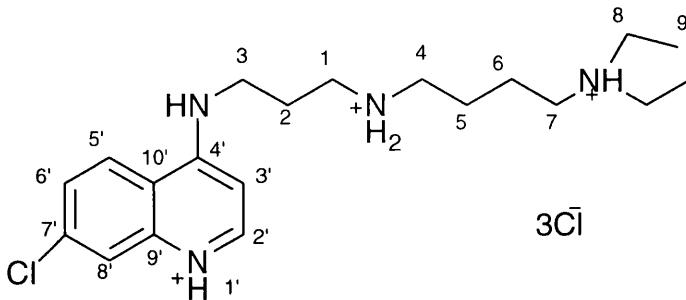
distillation (0.35 mmHg, 44 °C). After cooling to rt the amber residue was triturated in 1 mol dm⁻³ NaOH to give a cream solid which was collected by filtration to yield **176** (5.0240 g, 21.25 mmol, 85%), mp 134.9-137.1 °C (lit. 148-148.5 °C); IR, ¹H and ¹³C NMR spectra were consistent with published data. New data: *m/z* (EI) 238 (M⁺, 25.4%), 236 (M⁺, 76%), 205 (3), 191 (100), 178 (12), 164 (9.8), 156 (61.4), 128 (5.8), 99 (9.8), 75 (5.1) and 63 (3.5), (Found: M⁺, 238.0697. C₁₂H₁₃N₂O³⁷Cl requires 238.0686; Found: M⁺, 236.0719. C₁₂H₁₃N₂O³⁵Cl requires 236.0716).

N-(7-Chloroquinolin-4-yl)-1-aminopropan-3-bromide 177¹⁴³



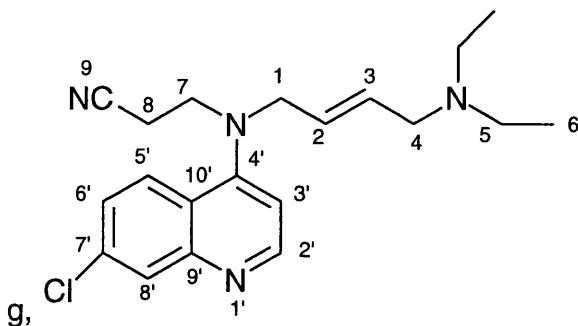
To a solution of 48 % HBr (4.6 ml, 10 equiv) was added c. H₂SO₄ (2.3 ml, 10 equiv) dropwise with T<0 °C (ice-bath). To this stirred orange solution was added the aminoalcohol (**176**) (1.01 g, 4.2 mmol), slowly, and with T<0 °C. The solution was heated at reflux until turbidity occurred (5 min) and then for a further 2 h. The aqueous layer was decanted and the residue was triturated with 2 mol dm⁻³ NaOH to give a white solid, collected by filtration, and recrystallized from EtOH-Et₂O to yield **177** as a cream solid (0.479 g, 1.59 mmol, 38 %), mp >250 °C (lit. 159-160 °C); IR, ¹H and ¹³C NMR spectra were consistent with published data. New data: *m/z* (EI) 301.9 (M⁺, 7.2%), 299.9 (M⁺, 27.9%), 297 (M⁺, 24.4%), 219 (23.1), 191 (100), 164 (7.3), 156 (77), 135 (3.6), 99 (15.3) and 78 (12.7), (Found: M⁺, 301.9804. C₁₂H₁₂N₂⁸¹Br³⁷Cl requires 301.9823; Found: M⁺, 299.9841. C₁₂H₁₃N₂⁷⁹Br³⁷Cl and C₁₂H₁₃N₂⁸¹Br³⁵Cl, requires 299.9852 and 299.9843. Found: M⁺, 297.9862. C₁₂H₁₂N₂⁷⁹Br³⁵Cl, requires 297.9873).

N-(7-Chloroquinolin-4-yl)-N'-(4-diethylaminobutyl)-1,3-propanediamine Trihydrochloride 179



177 (0.2293 g, 0.75 mmol) was stirred in acetonitrile (5 ml) with KF on Celite (0.8 g, 10 equiv) and diamine **145** (1.0590 g, 10 equiv) was added. Stirring was continued at 40 °C for 2 h before the solution was filtered and concentrated *in vacuo* to give a yellow oil. The oil was distilled using a Kugelrohr to give the free base **178** as a clear oil (0.2098 g, 0.58 mmol, 77 %), δH (200MHz, MeOD) 0.55 (6H, t, *J* 6.9, 9-H₃), 1.04 (4H, m, 5,6-H₂), 1.34 (2H, bt, *J* 6.1, 2-H₂), 2.35 (10H, m, 1,4,7,8-H₂), 2.90 (2H, t, *J* 6.0, 3-H₂), 5.95 (1H, d, *J* 5.5, 3'-H), 6.60 (1H, d, *J* 8.8, 6'-H), 7.15 (1H, s, 8'-H), 7.66 (1H, d, *J* 8.8, 5'-H), 7.75 (1H, d, *J* 5.1, 2'-H); δC (50MHz, MeOD) 10.1 (CH₃-9), 23.9 (CH₂-5 or -6), 26.5 (CH₂-5 or -6), 27.0 (CH₂-2), 41.1 (CH₂-7 or -8), 47.0 (CH₂-7 or -8), 47.3 (CH₂-4), 47.8 (CH₂-1), 52.7 (CH₂-3), 98.9 (CH-6'), 117.7 (C-10'), 124.1 (CH-8'), 125.2 (CH-3'), 126.4 (CH-5'), 135.4 (C-7'), 148.3 (C-4'), 151.3 (CH-2'), 151.5 (C-9'). The trihydrochloride salt was prepared by general procedure A2 to yield **179** as hygroscopic cream crystals (0.1796 g, 0.49 mmol, 85%), $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3050 (Ar C-H), 2942 (C-H), 2868 (C-H), 1586 (Ar C=C), 1545 (Ar C=C), 979 ((E)-C=C C-H bend), 890 (Ar C-H bend), 801 (Ar C-H bend); δH (200MHz, D₂O) 1.09 (6H, t, *J* 7.1, 9-H₃), 1.60 (4H, m, 5,6-H₂), 1.77 (2H, m, 2-H₂), 2.99 (10H, m, 1,4,7,8-H₂), 3.28 (2H, t, *J* 6.8, 3-H₂), 6.34 (1H, d, *J* 7.1, 3'-H), 6.82 (1H, d, *J* 9.4, 6'-H), 6.84 (1H, s, 8'-H), 7.25 (1H, d, *J* 9.5, 5'-H), 7.81 (1H, d, *J* 6.9, 2'-H); δC (50MHz, D₂O) 9.3 (CH₃-9), 21.7 (CH₂-5 or -6), 23.8 (CH₂-5 or -6), 25.4 (CH₂-2), 41.5 (CH₂), 46.0 (CH₂), 48.0 (CH₂), 48.2 (CH₂), 51.7 (CH₂), 99.3 (CH-6'), 114.6 (C-10'), 118.7 (CH-8'), 124.4 (CH-3'), 127.9 (CH-5'), 137.3 (C-7'), 139.9 (C-4'), 142.8 (CH-2'), 155.5 (C-9'); *m/z* (EI) 364 (M⁺-4H, 0.2%), 362 (M⁺-4H, 0.5%), 289 (15.1), 248 (9.4), 219 (13.1), 205 (46.8), 192 (100) and 112 (22.7), (Found: M⁺-4H, 364.2211. C₂₀H₃₁N₄³⁷Cl, requires 364.2208; Found: M⁺-4H, 362.2235. C₂₀H₃₁N₄³⁵Cl requires 362.2237).

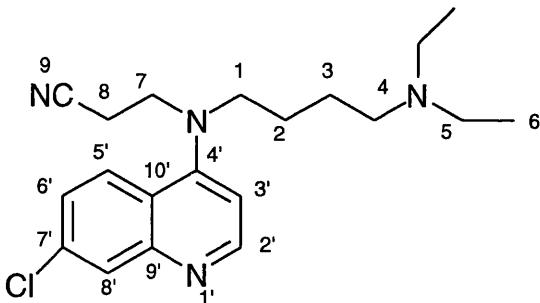
Attempted preparation of (*E*)-*N*-(7-Chloroquinolin-4-yl)-*N*-(2-cyanoethyl)-*N*',*N*'-diethyl-1,4-diaminobut-2-ene 180



Attempt 1: **99** (0.4279 g, 1.31 mmol) was stirred in MeOH (2 ml) and NaOH (0.05 g, 1 equiv) and acrylonitrile (0.10 ml, 1.1 equiv) added. The solution was stirred at rt for 48 h however no product was observed.

Attempt 2: **99** (0.1738 g, 0.57 mmol) was stirred in EtOH (2 ml) and NaOH (0.025 g, 1 equiv) and excess acrylonitrile (0.12 ml, 3 equiv) added. The solution was heated at 100 °C for 1 week, however no product was observed.

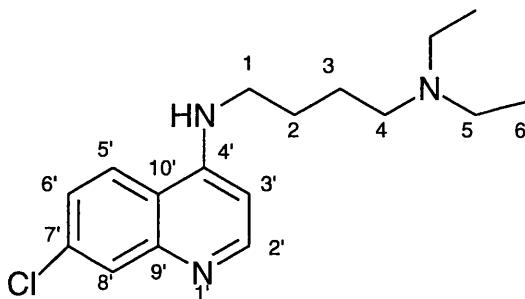
Attempted preparation of *N*-(7-Chloroquinolin-4-yl)-*N*-(2-cyanoethyl)-*N*',*N*'-diethyl-1,4-diaminobutane 182



149 (0.3044 g, 1.1 equiv) was heated in phenol (0.12 g) with 4,7-dichloroquinoline (0.2571 g, 1 equiv) at 160-165 °C for 6 h. On cooling the gummy solid was partitioned between CHCl₃ and 2 mol dm⁻³ NaOH and the aqueous layer was washed with CHCl₃. The organic extracts were concentrated *in vacuo* to give a brown tar. Purification was first attempted via silica flash chromatography hexaneane:EtOAc (1:1) with the product as baseline material eluting in EtOH. The concentrated residue was dissolved in iPrOH and precipitated with Et₂O asnd the filtrate concentrated *in vacuo*.

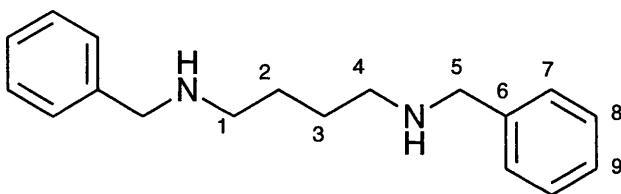
to give a bright yellow oil. No nitrile peak in NMR or IR spectra was observed.

The product was identified as ***N*-(7-Chloroquinolin-4-yl)-*N,N*'-diethyl-1,4-diaminobutane 181**

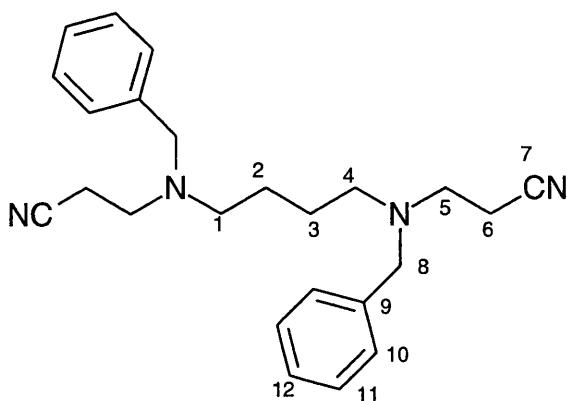


$\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3023 (Ar C-H), 2972 (-CH₂-), 1579 (Ar C=C), 1532 (Ar C=C), 802 (Ar C-H bend); δ_{H} (200MHz, MeOD) 0.90 (6H, t, *J* 7.2, 6-H₃), 1.50 (4H, m, 2,3-H₂), 2.36 (2H, t, 4-H₂), 2.41 (4H, q, *J* 7.2, 5-H₂), 3.20 (2H, t, 1-H₂), 6.33 (1H, d, *J* 5.7, 3'-H), 7.23 (1H, dd, *J* 9.0, 2.2, 6'-H), 7.62 (1H, d, *J* 2.1, 8'-H), 7.94 (1H, d, *J* 9.0, 5'-H), 8.20 (1H, d, *J* 5.6, 2'-H); δ_{C} (50MHz, D₂O) 11.2 (CH₃-6), 24.9 (CH₂-2 or -3), 28.1 (CH₂-2 or -3), 43.8 (CH₂-4), 47.6 (CH₂-4), 53.4 (CH₂-1), 99.6 (CH-6'), 118.7 (C-10'), 124.3 (CH-8'), 125.8 (CH-3'), 127.6 (CH-5'), 136.2 (C-7'), 149.6 (C-4'), 152.4 (CH-2'), 152.6 (C-9'); *m/z* (EI) 307 (M⁺-3H, 0.3%), 305 (M⁺-3H, 1.0%), 276 (5.5), 235 (1.4), 233 (5.6), 205 (2.4), 193 (1.6), 179 (1.5) and 86 (57), (Found: M⁺-3H, 307.1633. C₁₇H₂₄N₃³⁷Cl requires 307.1637; Found: M⁺-3H, 305.1659. C₁₇H₂₄N₃³⁵Cl requires 305.1659).

***N,N*'-Dibenzyl-1,4-diaminobutane 186**¹⁴⁸

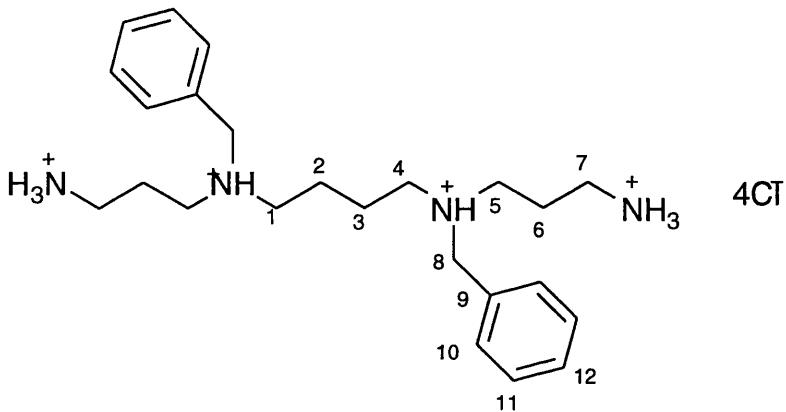


Following the procedure of Lee *et al.* the free base was isolated as a clear oil in 100% yield. ^1H , ^{13}C and IR were identical to reported data, (Found: M⁺, 268.1930. C₁₈H₂₄N₂ requires 268.1939).

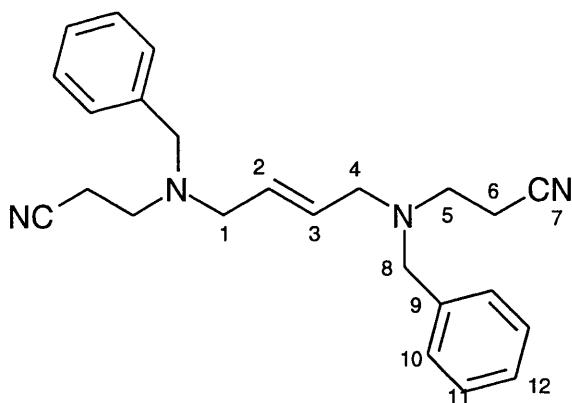
N,N'-Bis-(2-cyanoethyl)-N,N'-Dibenzyl-1,4-diaminobutane 187

186 (1.0116 g, 3.7 mmol) was stirred in neat acrylonitrile (1.5 ml, 6 equiv.) at 70-80 °C for 18 h before the solution was concentrated *in vacuo* to yield a yellow oil. Purification was achieved using silica chromatography with hexane:CHCl₃:EtOH (10:1:1) as eluent to isolate the free base as a clear oil (1.1383 g, 3.0 mmol, 81 %), R_f 0.35 (hexane:CHCl₃:EtOH, 10:1:1); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3065 (Ar C-H), 2942 (C-H), 2250 (CN), 1602 (Ar C=C), 1585 (Ar C=C), 1494 (Ar C=C), 763 (Ar C-H bend), 698 (Ar C-H bend); δ_{H} (200MHz, CDCl₃) 1.45 (4H, m, 2,3-H₂), 2.29 (4H, t, *J* 6.9, 6-H₂), 2.39 (4H, m, 1,4-H₂), 2.66 (4H, t, *J* 6.8, 5-H₂), 3.53 (4H, s, 8-H₂), 7.28 (10H, m, 10,11,12-H); δ_{C} (50MHz, CDCl₃) 16.2 (CH₂-2 and -3), 24.4 (CH₂-6), 48.9 (CH₂-1 and -4), 53.0 (CH₂-5), 58.3 (CH₂-8), 119.1 (C-7), 127.1 (CH-12), 128.3 (CH-10), 128.7 (CH-11), 138.9 (C-9); *m/z* (EI) 374 (M⁺, 3.2%), 283 (6.3), 213 (4.2), 173 (7.3), 123 (14.9) and 91 (100), (Found: M⁺, 374.2445. C₂₄H₃₀N₄ requires 374.2470).

N,N'-Bis-(3-aminopropyl)-N,N'-dibenzyl-1,4-diaminobutane Tetrahydrochloride 189⁶⁰

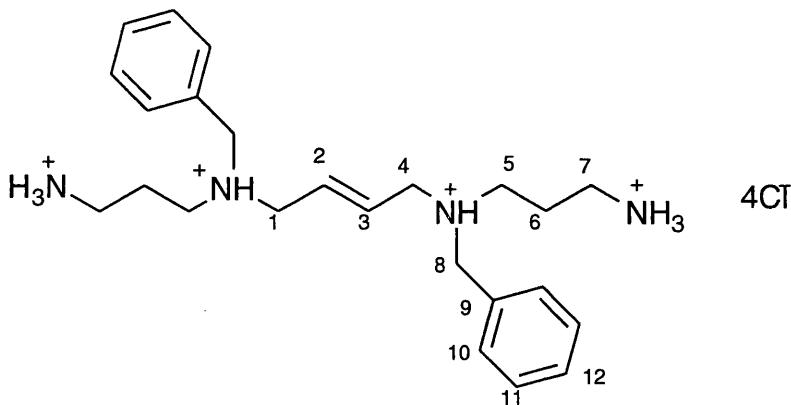


188 was prepared according to general procedure B3 reducing **187** (1.0720 g, 2.9 mmol) in ether (50 ml). The free base was isolated as a clear oil^{z14} (0.8569 g, 78%), δ_H (200MHz, CDCl₃) 1.44 (4H, m, 2,3-H₂), 1.53 (4H, m, 6-H₂), 2.35 (12H, m, 1,4,5,-H₂ and NH₂), 2.62 (4H, t, J 6.7, 7-H₂), 3.49 (4H, s, 8-H₂), 7.29 (10H, m, 10,11,12-H). The tetrahydrochloride salt **189** was isolated as a peach solid following general procedure A1 in 100% yield, $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3419 (N-H), 2930 (-CH₂-), 1602 (Ar C=C), 1585 (Ar C=C), 1497 (Ar C=C), 746 (Ar C-H bend), 701 (Ar C-H bend); δ_H (200MHz, D₂O) 1.60 (4H, m, 2,3-H₂), 2.03 (4H, m, 6-H₂), 2.88 (4H, t, J 7.6, 7-H₂), 3.09 (8H, m, 1,4,5-H₂), 4.21 (4H, s, 8-H₂), 7.36 (10H, m, 10,11,12-H); δ_C (50MHz, D₂O) 21.3 (CH₂-2 or -3), 22.5 (CH₂-7), 37.4 (CH₂-1 and -4 or -5), 50.5 (CH₂-1 and -4 or -5), 52.4 (CH₂-1 and -4 or -5), 58.2 (CH₂-8), 129.5 (C-9), 130.4 (CH-12), 131.3 (CH-10), 131.8 (CH-11); m/z (FAB) 386 (M⁺, 31.8%), 295 (3), 220 (0.5), 188 (1) and 91 (100), (Found: M⁺ xxx, C_xH_xN_x requires xxx).

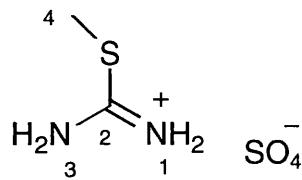
(E)-N,N'-Bis-(2-cyanoethyl)-N,N'-dibenzyl-1,4-diaminobut-2-ene**190**

190 was prepared following the general procedure B2 reacting **93** (0.4903 g, 1.8 mmol) with acrylonitrile (0.7 ml, 6 equiv.). Purification was via silica chromatography with hexane:CHCl₃:EtOH (10:1:1) as eluent, followed by hexane:CHCl₃:EtOH (3:1:1) to yield the free base as a clear oil (0.5441 g, 83%), R_f 0.47 (hexane:CHCl₃:EtOH, 6:1:1); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3022 (Ar C-H), 2931 (-CH₂-), 2812 (N-CH₂), 1602 (Ar C=C), 1494 (Ar C=C), 979 ((E)-C=C, C-H bend), 752 (Ar C-H bend), 722 (Ar C-H bend); δ_{H} (200MHz, CDCl₃) 2.36 (4H, t, J 6.7, 6-H₂), 2.73 (4H, t, J 6.7, 5-H₂), 3.10 (4H, m, 1,4-H₂), 3.58 (4H, s, 8-H₂), 5.67 (2H, m, 2,3-H), 7.28 (10H, m, 10,11,12-H); δ_{C} (50MHz, CDCl₃) 16.4 (CH₂-6), 48.5 (CH₂-5), 55.4 (CH₂-1 and -4), 58.0 (CH₂-8), 118.9 (C-7), 127.2 (CH-2 and -3), 128.3 (CH-12), 128.6 (CH-10), 130.5 (CH-11), 138.5 (C-9); m/z (EI) 372 (M⁺, 1.7%), 332 (13), 213 (5.5), 159 (4.2), 131 (3.1) and 91 (100). (Found: M⁺, 372.2334. C₂₄H₂₈N₄ requires 372.2314).

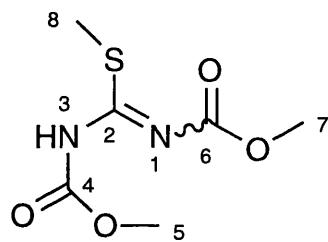
(E)-N,N'-Bis-(3-aminopropyl)-N,N'-Dibenzyl-1,4-diaminobut-2-ene Tetrahydrochloride 192



191 was prepared according to general procedure B3 reducing **190** (0.5423 g, 1.46 mmol) in ether (30 ml) with LiAlH₄ (6 equiv.) and AlCl₃ (6 equiv.). The free base was isolated as a clear oil (0.4332 g, 1.14 mmol, 78%), δ_H (200MHz, CDCl₃) 1.58 (4H, m, 6-H₂), 1.78 (4H, s, NH₂), 2.45 (4H, t, J 6.9, 5-H₂), 2.66 (4H, t, J 6.8, 7-H₂), 3.04 (4H, m, 1,4-H₂), 3.53 (4H, s, 8-H₂), 5.66 (2H, m, 2,3-H), 7.28 (10H, m, 10,11,12-ArH); δ_C (50MHz, CDCl₃) 30.7 (CH₂-6), 40.4 (CH₂-7), 50.8 (CH₂-5), 55.5 (CH₂-8), 58.2 (CH₂-1 and -4), 126.7 (CH-2 and -3), 128.1 (CH-12), 128.7 (CH-10), 130.6 (CH-11), 139.6 (C-9). The tetrahydrochloride salt **192** was isolated following general procedure A1 as white crystals in 99% yield, ν_{max}(KBr)/cm⁻¹ 3428 (N-H), 3065 (Ar C-H), 2934 (C-H), 1630 (C=C), 1458 (Ar C=C), 979 ((E)-C-H), 747 (Ar C-H), 702 (Ar C-H); δ_H (200MHz, D₂O) 2.20 (4H, m, 6-H₂), 3.03 (4H, t, J 7.4, 7-H₂), 3.27 (4H, t, J 8.0, 5-H₂), 3.93 (4H, m, 1,4-H₂), 4.39 (4H, s, 8-H₂), 6.16 (2H, m, 2,3-H), 7.52 (10H, s, 10,11,12-H); δ_C (50MHz, D₂O) 22.7 (CH₂-6), 37.3 (CH₂-7), 50.5 (CH₂-5), 54.5 (CH₂-1 and -4), 58.3 (CH₂-8), 129.5 (C-9), 129.8 (CH-2 and -3), 130.4 (CH-12), 131.2 (CH-10), 131.9 (CH-11); *m/z* (Cl/isobutane) 382 (M⁺-2H, 25.9%), 381 (M⁺-3H, 70.2%), 325 (0.35), 216 (0.28), 166 (0.43), (Found: M⁺-2H 382.3052, C₂₄H₃₈N₄ requires 382.3008; Found: M⁺-2H 3381.3020, C₂₄H₃₇N₄ requires 3381.3022).

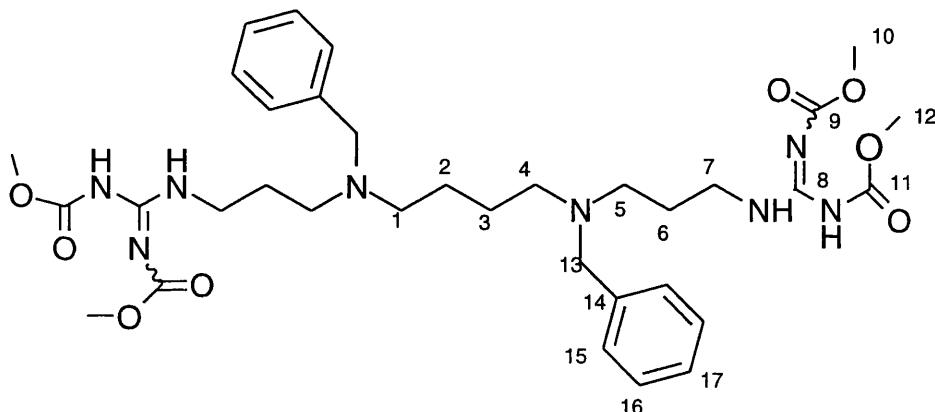
S-Methylisothiourea 194¹⁵⁴

Following the procedure of Schildneck thiourea **193** (5.9907 g, 79 mmol, 1 equiv.) was heated at reflux with dimethylsulphate (4.1 ml, 0.5 equiv.) in water (3.5 ml) to isolate **194** as white crystals (8.111 g, 29 mmol, 40%), mp 240.7-242.1 °C [lit. 235 °C]

1,3-Dimethoxy-S-methylisothiourea 195¹⁵⁵

Following the procedure of Weinhardt **194** (7.0071 g, 25 mmol, 1 equiv) was reacted with methylchloroformate (9.7 ml, 5 equiv.) to give **195** as white crystals (3.3025 g, 16 mmol, 64%), mp 102.1-104.8 °C [lit. 100-102 °C]. New data: δH (200MHz, CDCl₃) 2.34 (3H, s, 8-H₃), 3.72 (6H, s, 5,7-H₃), 11.75 (1H, bs, NH); δC (50MHz, CDCl₃) 14.3 (CH₃-8), 53.2 (CH₃-5 or -7), 151.9 (C-6), 161.5 (C-4), 172.5 (C-2).

N,N'-Bis-[bis(carbomethoxy)guanidinopropyl]-N,N'-dibenzyl-1,4-diaminobutane 196



Following the procedure of Kumar et al.¹⁵⁰ **195** (0.4878 g, 1.27 mmol) was stirred in EtOH (10 ml), pseudothioureacarbamate (0.48 g, 2.2 equiv.) added and the solution stirred at rt for 18 h. The solution was concentrated *in vacuo* to give a semi-solid. Purification was achieved using silica gel chromatography, EtOAc:hexane (1:1) increasing to EtOAc to yield the product **196** as a thick yellow semi-solid (0.1537 g, 0.24 mmol, 24%), $\nu_{\text{max}}(\text{CDCl}_3)/\text{cm}^{-1}$ 3342 (NH), 3020 (Ar-H), 2952 (-CH₂-), 1731 (C=O), 1711 (C=N), 1640 (C=C), 1576 (C=C), 1494 (C=C), 778 (Ar C-H), 669 (Ar C-H); δ_{H} (200MHz, CDCl₃) 1.37 (4H, m, 2,3-H₂), 1.62 (4H, m, 6-H₂), 2.37 (8H, m, 1,4,5,-H₂), 3.35 (4H, m, 7-H₂), 3.44 (4H, s, 13-H₂), 3.62 (6H, s, 10 or 12-H₃), 3.74 (6H, s, 10 or 12-H₃), 7.19 (10H, m, 15,16,17-H), 8.29 (2H, bs, NH), 11.59 (2H, bs, NH); δ_{C} (50MHz, CDCl₃) 24.5 (CH₂-2 and -3), 26.3 (CH₂-6), 39.4 (CH₂-1 and -4), 50.8 (CH₂-5), 52.5 (CH₃-10 or -12), 53.1 (CH₃-10 or -12), 53.7 (CH₂-7), 58.5 (CH₂-13), 126.7 (CH-17), 128.1 (CH-15), 129.8 (CH-16), 139.5 (C-14), 154.2 (C-9), 155.8 (C-11), 164.3 (C-8);

7.3 Experimental to Chapter 5

General Procedure C1

A solution of 2-t-butoxycarbonyloxyimino-2-phenylacetonitrile (BOC-ON) (1.2 equiv.) in 1,4-dioxane (1 ml/ mmol amino acid) and water (1 ml/ mmol amino acid) was added to a stirred solution of the amino acid (1 equiv.) in distilled triethylamine (3 equiv.). The solution became homogeneous and was stirred at rt for 2 h. Water (1.3 ml/ mmol amino acid) and EtOAc (1.6 ml/ mmol amino acid) were added and the aqueous layer was washed with EtOAc. The aqueous layer was acidified (pH 4) using 5% citric acid and extracted using EtOAc (3x50 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give the N-BOC aminoacid.

General Procedure C2

A solution of the *N*-BOC amino acid (1 equiv.) and NEt_3 (1.2 equiv.) in acetonitrile was cooled to -5 °C (ice bath). *i*Butylchloroformate (1.2 equiv.) was added and the resulting brightly coloured solution was stirred for 5 min. The appropriate amine (5 equiv.) was added and the solution stirred at 0 °C for 2 h. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (3x50 ml) and the combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to yield the crude product. Purification was achieved, where possible, through recrystallization from EtOAc.

General Procedure C3

The *N*-BOC amide was stirred in 3 mol dm^{-3} HCl/ EtOAc (1:1) for 30 min. The solvents were removed *in vacuo* to give a clear oil which solidified upon trituration in dry ether to give the amide as the hydrochloride salt.

General Procedure C4

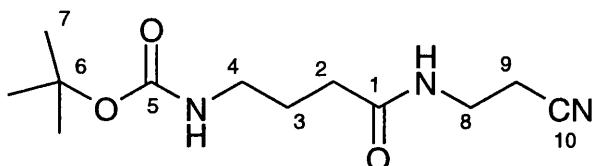
A solution of the *N*-BOC amino acid (1 equiv.) and NEt_3 (2.4 equiv.) in acetonitrile (8 ml/ mmol amino acid) was cooled to -5 °C (ice bath). *i*Butylchloroformate (1.2 equiv.) was added and the solution was stirred for 5 min. The amide hydrochloride salt (1.1 equiv.) was added portionwise and the resulting solution was stirred for 2h. Solvent was removed *in vacuo* and the resulting solid partitioned between EtOAc and water. The organic phase was separated and the aqueous layer was extracted with EtOAc (3x50 ml). The combined organic phases were dried (Na_2SO_4) and

concentrated *in vacuo* to yield the crude product which was recrystallized from EtOAc.

General Procedure C5

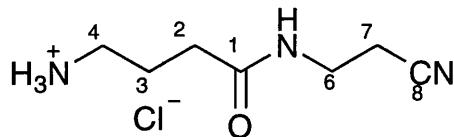
To a suspension of the amino amide hydrochloride salt (1 equiv.) in dry THF was added a solution of BH₃ in THF (1 mol dm⁻³, 8 equiv.). The resultant mixture was heated at reflux for 24 h and then cooled to rt. 6 mol dm⁻³ HCl was added slowly to the solution until no more H₂ was evolved and THF was removed by distillation at atmospheric pressure. MeOH and c.HCl were added to the residue and the solvents were removed *in vacuo* to yield the crude product. This was recrystallised from dry methanol to afford the desired polyamine hydrochloride salt.

N-(2-Cyanoethyl)-4-(t-butoxycarbonylamino)butanamide 216



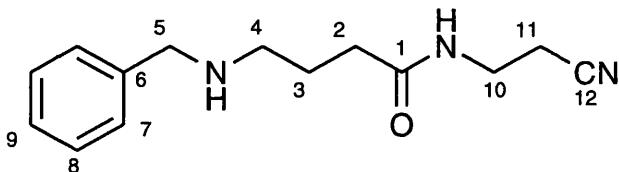
4-(t-Butoxycarbonylamino)butanoic acid **108** (3.04 g, 14.8 mmol) was stirred in MeCN (100 ml) and NEt₃ (4 ml, 2 equiv.). iBuOCOCl (2.3 ml, 1.2 equiv.) was added followed by 3-aminopropionitrile (4 ml, 4 equiv.) according to general procedure C1 and gave a cherry pink solution. After work up a thick yellow oil was isolated which crystallized overnight. Recrystallization from EtOAc-hexaneane yielded **216** as pale cream crystals (2.4540 g, 9.6 mmol, 65 %), mp96.4-98.5 °C; Rf 0.51 (EtOAc); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3343 (N-H), 3300 (N-H), 2873 (-CH₂-), 2247 (CN), 1699 (C=O), 1644 (C=O), 1549 (C=O); δ_{H} (200 MHz, CDCl₃) 1.45 (9H, s, 7-H₃), 1.61 (2H, m, 3-H₂), 2.27 (2H, t, J 7.0, 2-H₂), 2.65 (2H, t, J 6.4, 9-H₂), 3.18 (2H, q, J 6.3, 4-H₂), 3.50 (2H, m, 8-H₂), 4.97 (1H, bt, J 5.6, NH), 7.28 (1H, bs, NH); δ_{C} (50 MHz, CDCl₃) 19.6 (CH₂-3), 19.4 (CH₃-7), 26.4 (CH₂-2), 28.4 (CH₂-9), 33.3 (CH₂-4), 35.5 (C-6), 39.5 (CH₂-8), 116.4 (C-10), 157.6 (C-5), 173.0 (C-1); *m/z* (EI) 256 (M⁺, 2.2 %), 199 (60), 182 (33), 154 (33), 139 (21), 112 (100), 84 (23) and 57 (40), (Found: M⁺, 256.1649. C₁₂H₂₂N₃O₃ requires 256.1636).

N-(2-Cyanoethyl)-4-aminobutanamide Hydrochloride 218

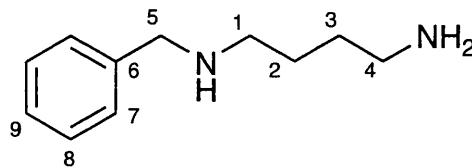


216 (2.002 g, 7.8 mmol) was stirred in 3 mol dm⁻³ HCl (15 ml) and EtOAc (15 ml) according to general procedure C3. The hydrochloride salt **218** was isolated as a hygroscopic solid and was used without purification, δ H (200 MHz, CDCl₃) 1.70 (2H, m, 3-H₂), 2.12 (2H, t, *J* 7.4, 2-H₃), 2.36 (2H, t, *J* 6.6, 7-H₂), 2.76 (2H, t, *J* 7.4, 4-H₂), 3.19 (2H, t, *J* 6.5, 6-H₂); δ C (50 MHz, CDCl₃) 23.8 (CH₂-3), 33.2 (CH₂-2), 35.3 (CH₂-7), 36.5 (CH₂-4), 39.6 (CH₂-6), 120.2 (C-8), 170.2 (C-1).

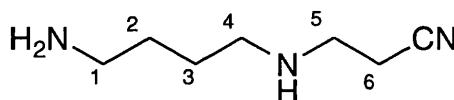
Attempted preparation of *N*-(2-Cyanoethyl)-4-benzylaminobutanamide 220



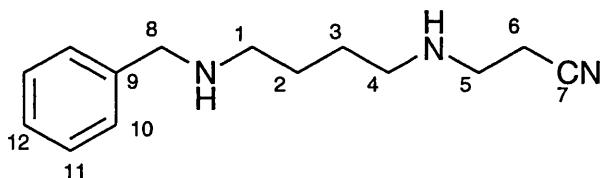
Crude nitrile **218** (1.49 g, 7.8 mmol) and benzaldehyde (0.8 ml, 1 equiv) were heated at reflux in MeOH (20 ml) for 2 h. NaBH₄ (0.36 g, 1.2 equiv) was added portionwise and the mixture was heated at reflux for 2 h before leaving at rt overnight. The solution was concentrated *in vacuo* to give a cream solid which was dissolved in H₂O (10 ml) and made basic pH 12 using NaOH (s). The aqueous layer was extracted with CHCl₃ (3x10 ml) and the combined organic extracts dried (Na₂SO₄), filtered and concentrated *in vacuo* to yield a yellow oil which contained a complex mixture of products.

N-Benzyl-1,4-diaminobutane 225¹⁷⁴

225 was prepared as a colourless oil according to the procedure of Bergeron et al. in 90 % yield. ^1H , ^{13}C and IR were identical with literature values. (Found: M $^+$, 178.1462. C₁₁H₁₆N₂ requires 178.1454)

N-(2-Cyanoethyl)-1,4-diaminobutane 226¹²⁸

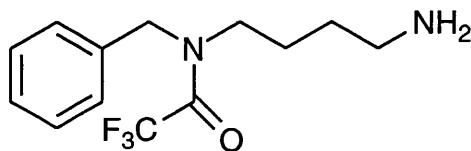
226 was prepared according to the procedure of Israel et al. to yield a clear oil in 60 % yield. IR and bp [lit 84-87 °C, 0.7 mmHg] were consistent with published data.

Attempted preparation of *N*-Benzyl-*N'*-(2-cyanoethyl)-1,4-diaminobutane 223

Attempt 1: **226** (0.7784 g, 5.52 mmol) was heated at reflux in CHCl₃ (100 ml) with benzaldehyde (0.56 ml, 1 equiv.). After 2 h the solution was concentrated *in vacuo* and dissolved in MeOH (100 ml). NaBH₄ (0.3 g, 1.2 equiv.) was added portionwise and the mixture left at rt overnight. Work up was as for **166** and gave a complex mixture of products.

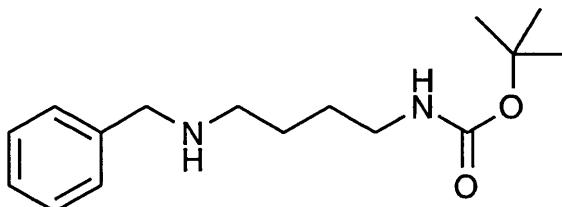
Attempt 2: To a stirred solution of **225** (0.51 g, 286 mmol) was added acrylonitrile (0.19 ml, 1 equiv.) dropwise at 0 °C. The solution was stirred at rt for 18 h and concentrated *in vacuo* to give a thick oil which by TLC contained inseparable products.

Attempted syntythesis of N-Benzyl-N-trifluoroacetyl-1,4-diaminobutane 227



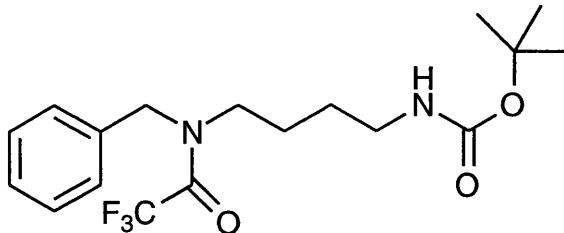
Following the procedure of Barrett et al.^{175, 176} **225** (0.5200 g, 2.92 mmol) was stirred in MeCN (15 ml) and p-toluenesulphonic acid (0.5557 g, 1 equiv.) was added. The solution was concentrated *in vacuo* to give a clear oil which was dissolved in DCM (15 ml) and 18-C-6 (0.78 g, 1 equiv.) added. The solution was again concentrated *in vacuo*, dissolved in DCM (25 ml) and cooled to 0 °C. NEt₃ (0.45 ml, 1.1 equiv.) was added followed rapidly by TFAA (0.44 ml, 1.06 equiv.) and the solution was stirred at rt overnight. The solution was concentrated *in vacuo* and purification attempted by silica gel chromatography, eluent DCM:MeOH:NH₄OH (17:2:1). Further purification was attempted however the desired product was difficult to obtain without contamination.

N-Benzyl-N'-(t-butoxycarbonylamino)-1,4-diaminobutane 228¹⁷⁴



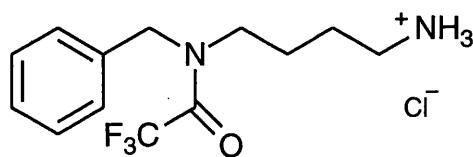
228 was prepared according to the procedure of Bergeron and co-workers on a 2.2 mmol scale in 55% yield. ¹H data was identical to published results. $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3426 (N-H), 3335 (N-H), 2930 (-CH₂-), 1707 (C=O), 1686 (C=O), 1542 (Ar C=C), 1470 (Ar C=C), 1390 (C(CH₃)₃), 754 (Ar C-H), 700 (Ar C-H); δ_{C} (50 MHz, CDCl₃) 23.4 (CH₂), 26.9 (CH₂), 28.2 (CH₃), 48.5 (CH₂), 51.4 (CH₂), 128.9 (CH), 129.0 (CH), 129.6 (CH), 131.7 (C); m/z (EI) 278 (M⁺⁺¹, 3%), 221 (28), 205 (14), 187 (12), 176 (1), 160 (10), 120 (46), 106 (31) and 91 (100), (Found: (Cl/NH₃) M⁺⁺¹, 279.2079 C₁₆H₂₇N₂O₂ requires 279.2086).

N-Benzyl-N-trifluoroacetyl-N'-(t-butoxycarbonylamino)-1,4-diaminobutane 229



228 (0.2556 g, 0.91 mmol, 1 equiv.) was stirred in MeOH (1 ml) and ethyltrifluoroacetate (0.14 ml, 1.1 equiv.) added and the resulting solution was stirred for 48 h at rt before concentrating in *vacuo* to give a thick yellow oil. Purification was achieved by silica chromatography, eluent EtOAc, to yield **229** as a thick yellow oil (0.1814 g, 0.48 mmol, 53%), R_f 0.8 (EtOAc); $\nu_{\text{max}}(\text{CDCl}_3)/\text{cm}^{-1}$ 3456 (N-H), 3035 (Ar-H), 2979 (-CH₂-), 1688 (C=O), 1573 (Ar C=C), 1508 (Ar C=C), 735 (Ar C-H), 678 (Ar C-H); δ_{H} (200 MHz, CDCl₃) 1.35 (9H, s, -H₃), 1.39-1.66 (4H, bm, -H₂), 2.29 (2H, m, -H₂), 3.24 (2H, m, -H₂), 4.54 (2H, d, J 6.5, -H₂), 4.60 (1H, bt, NH), 7.20 (5H, m, -H); δ_{C} (50 MHz, CDCl₃) 23.6 (CH₂-), 25.3 (CH₂-), 27.1 (CH₂-), 28.2 (CH₃-), 45.9 (CH₂-), 48.1 (CH₂-), 50.5 (CH₂-), 127.1 (CH), 127.9 (CH), 128.1 (CH), 128.8 (CH), 128.9 (CH), 134.7 (C), 155.9 (C); m/z (CINH₃) 392 (M⁺⁺NH₄, 3%), 336 (100), 320 (98), 301 (1), 276 (12), 246 (1), 221 (1), and 96 (6), (Found: M⁺⁺NH₄, 392.2175 C₁₈H₂₉N₃O₃F₃ requires 279.2086).

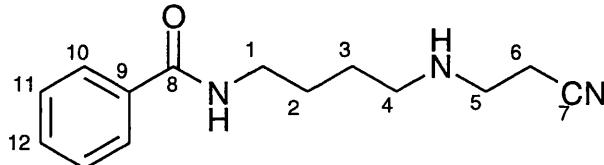
N-Benzyl-N-trifluoroacetyl-1,4-diaminobutane 230



229 (0.1776 g, 0.47 mmol) was stirred in EtOAc (1 ml) and 3 mol dm⁻³ HCl (1 ml) for 1 h. The solution was concentrated *in vacuo* to give a thick oil which was triturated with Et₂O to give a white powdery solid (0.0590 g, 65%), $\nu_{\text{max}}(\text{CDCl}_3)/\text{cm}^{-1}$ 3427 (NH₃⁺), 3032 (Ar-H), 2950 (-CH₂-), 1680 (C=O), 1605 (Ar C=C), 1560 (Ar C=C), 1497 (Ar C=C), 755 (Ar C-H), 701 (Ar C-H); δ_{H} (200 MHz, D₂O) 1.27 (4H, bm, -H₂), 2.62 (2H, m, -H₂), 3.06 (1H, bm, -H₂), 3.22 (1H, t, J 7.1, -H₂), 4.38 (2H, s, -H₂), 7.00 (2H, m, -H), 7.14 (3H, m, -H); δ_{C} (50 MHz, CDCl₃) 23.7 (CH₂-), 24.6 (CH₂-), 24.8 (CH₂-),

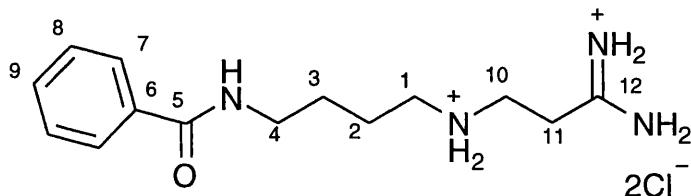
25.5 (CH₂-), 39.8 (CH₂-), 47.3 (CH₂-), 48.4 (CH₂-), 50.9 (CH₂-), 51.8 (CH₂-), 114.5 (C), 120.1 (C), 128.2 (CH), 128.8 (CH), 129.0 (CH), 129.6 (CH), 135.7 (CH), 136.1 (CH); *m/z* (EI) 274 (M⁺-1, 4.8%), 273 (M⁺-2, 0.9%), 257 (12), 244 (4), 231 (2), 218 (6), 205 (15), 202 (8), 177 (11), 166 (16) and 91 (100), (Found: M⁺-1, 274.1291. C₁₃H₁₇N₂OF₃ requires 274.1289; M⁺-2, 273.1222. C₁₃H₁₆N₂OF₃ requires 273.1229).

***N*-Benzoyl-*N'*-(2-cyanoethyl)-1,4-diaminobutane 234**



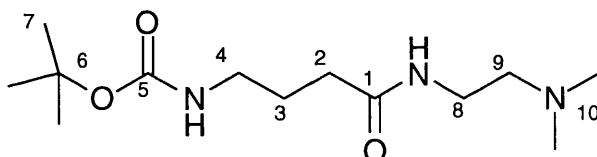
N,N'-Carbonyldiimidazole (0.57 g, 1 equiv) was stirred in dry DCM with benzoic acid (0.43 g, 1 equiv) for 1 h. **226** (0.51 g, 3.55 mmol) was added and the solution was stirred overnight. DCM (10 ml) was added and the solution washed with 1 mol dm⁻³ NaOH and H₂O before drying (Na₂CO₃), filtering and concentrating *in vacuo* to yield a glassy solid. Purification was achieved via silica chromatography, eluent EtOH, to yield a white solid (0.2908 g, 1.27 mmol, 33 %), $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3050 (Ar C-H), 2937 (-CH₂-), 2858 (N-CH₂), 1603 (Ar C=C), 1579 (Ar C=C), 1524 (Ar C=C), 766 (Ar C-H bend), 688 (Ar C-H bend); δ_{H} (200 MHz, CDCl₃) 1.49-1.62 (4H, m, 2,3-H₂), 2.45 (2H, t, *J* 6.6, 4-H₂), 2.61 (2H, t, *J* 6.6, 6-H₂), 2.84 (2H, t, *J* 6.6, 5-H₂), 2.77 (1H, s, NH), 3.39 (2H, q, *J* 6.4, 1-H₂), 7.31 (4H, m, 11,12-H), 7.78 (2H, m, 10-H); δ_{C} (50 MHz, D₂O) 18.3 (CH₂-3), 26.9 (CH₂-2), 27.1 (CH₂-4), 39.6 (CH₂-6), 44.7 (CH₂-5), 48.4 (CH₂-1), 118.8 (C-7), 126.9 (CH-10), 128.4 (CH-11), 131.2 (CH-12), 134.5 (C-9), 167.0 (C-8); *m/z* (EI) 245 (M⁺, 91%), 218 (10), 205 (35), 176 (8), 163 (28), 134 (37), 105 (100) and 77 (60), (Found: M⁺, 245.1526. C₁₄H₁₉N₃O requires 245.1523).

Attempted preparation of *N*-(2-Amidinoethyl)-*N'*-benzoyl-1,4-diaminobutane Dihydrochloride 233

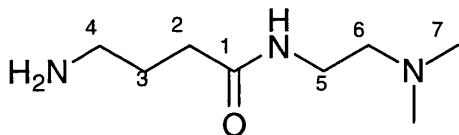


Following the procedure of Moss *et al.*¹⁸⁰ 2 mol dm⁻³ Me₃Al in toluene (1.01ml, 3.4 equiv) was added dropwise to a solution of NH₄Cl (0.12 g, 3.6 equiv) in toluene (2 ml), under N₂, and stirred for 2 h. The nitrile (0.3210 g, 1.86 mmol) in toluene (1 ml) was added dropwise and stirred at 70-80 °C overnight. The mixture was poured onto a slurry of silica gel (0.7 g) in CHCl₃ (2 ml) and stirred for 5 min before filtering. The silica was washed with MeOH and the filtrate concentrated *in vacuo*. The residue was redissolved in MeOH (1 ml) and filtered to remove NH₄Cl. To the MeOH solution was added c.HCl (5 drops) and the solution concentrated *in vacuo* to give a complex mixture of products.

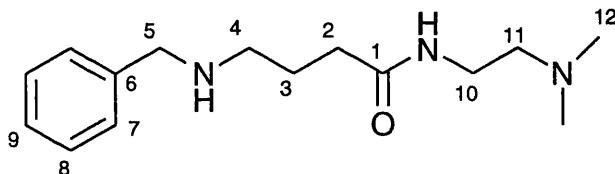
N-(2-Dimethylaminoethyl)-4-(t-butoxycarbonylamino)butanamide 217



108 (3.02 g, 14.7 mmol) was stirred in MeCN (100 ml) and NEt₃ (4 ml, 2 equiv). iBuOCOCl (2.3 ml, 1.2 equiv) was added followed by N,N-dimethylethylenediamine (6.4 ml, 5 equiv) according to general procedure C1 and gave a orange solution. After work up a thick yellow oil was isolated, however no crystallization occurred. Purification was achieved using alumina chromatography, eluent hexaneane:EtOAc (4:1) (0.5735 g, 2.1 mmol, 14 %), $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3452 (N-H), 2980 (-CH₂-), 2779 (N-CH₂), 1706 (C=O), 1659 (C=O), 1508 (C=O); δ_{H} (200 MHz, CDCl₃) 1.36 (9H, s, 7-H₃), 1.74 (2H, m, 3-H₂), 2.10 (2H, t, 2-H₂), 2.17 (6H, s, 10-H₃), 2.36 (2H, t, J 6.2, 9-H₂), 3.06 (2H, q, J 6.1, 4-H₂), 3.26 (2H, q, 8-H₂), 5.62 (1H, bs, NH), 7.28 (1H, bs, NH); δ_{C} (50 MHz, CDCl₃) 26.0 (CH₂-3), 28.4 (CH₃-7), 33.6 (CH₂-2), 36.6 (CH₂-9), 39.7 (CH₂-4), 39.9 (CH₂-8), 45.1 (CH₃-10), 156.3 (C-5), 172.8 (C-1); *m/z* (CI isobutane) 274 (M+H⁺, 20%), 218 (8), 200 (6), 173 (0.5), 147 (6), 130 (5.5), 71 (26) and 58 (70), (Found: M+H⁺, 274.2128. C₁₃H₂₈N₃O₃ requires 274.2125).

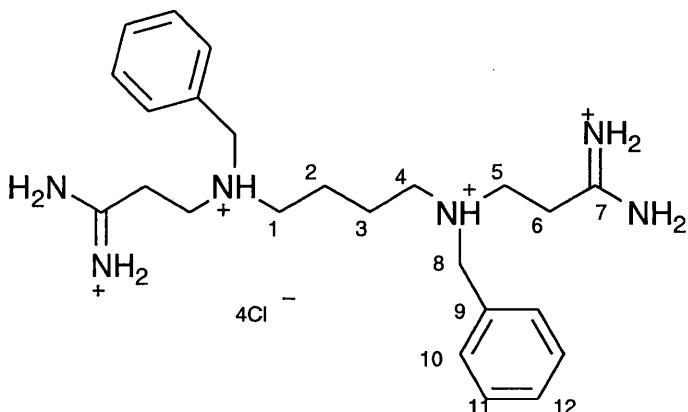
N-(2-Dimethylaminoethyl)-4-aminobutanamide 219

217 (0.5735 g, 2.1 mmol) was stirred in 3 mol dm⁻³ HCl (7 ml) and EtOAc (7 ml) according to general procedure C3. The hydrochloride salt was taken up in water (10 ml), made basic with NaOH (s) (pH14) and extracted with DCM (2x10 ml). The combined organic extracts were dried (K₂CO₃), filtered and concentrated *in vacuo* to yield **219** as a clear oil (0.2754 g, 1.75 mmol, 83 %), $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3444 (N-H), 2979 (-CH₂-), 2493 (NH₂), 1657 (C=O), 1511 (C=O); δ_{H} (200 MHz, CDCl₃) 1.40-1.60 (4H, m, 3-H₂, NH₂), 1.93 (6H, s, 7-H₃), 2.00 (2H, t, *J* 7.2, 2-H₂), 2.10 (2H, t, *J* 6.3, 4-H₂), 2.43 (2H, 4-H₂, *J* 6.8, 6-H₂), 2.99 (2H, m, 5-H₂), 6.67 (0.65 H, bs, NH), 7.33 (0.35H, bs, NH); δ_{C} (50 MHz, CDCl₃) 29.6 (CH₂-3), 33.7 (CH₂-2), 36.9 (CH₂-4), 41.5 (CH₂-6), 45.2 (CH₃-7), 58.0 (CH₂-5), 173.0 (C-1); *m/z* decomposes.

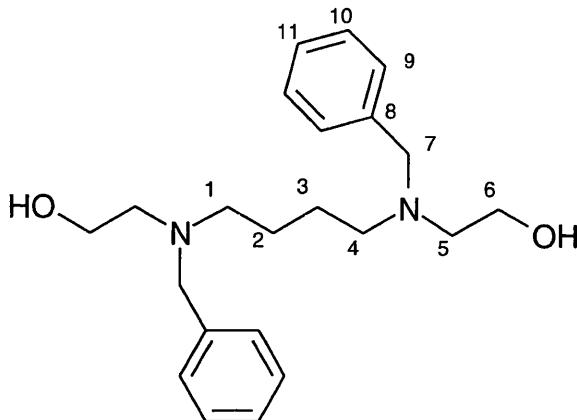
Attempted preparation of *N*-(2-dimethylaminoethyl)-4-benzylaminobutanamide 221

219 (0.27 g, 1.72 mmol) and benzaldehyde (0.17 ml, 1 equiv) were heated at reflux in MeOH (6 ml) for 2 h. NaBH₄ (0.066 g, 1.1 equiv) was added portionwise and the mixture was heated at reflux for 2 h before leaving overnight. Work up was according to the procedure for **220** to yield a complex mixture of products.

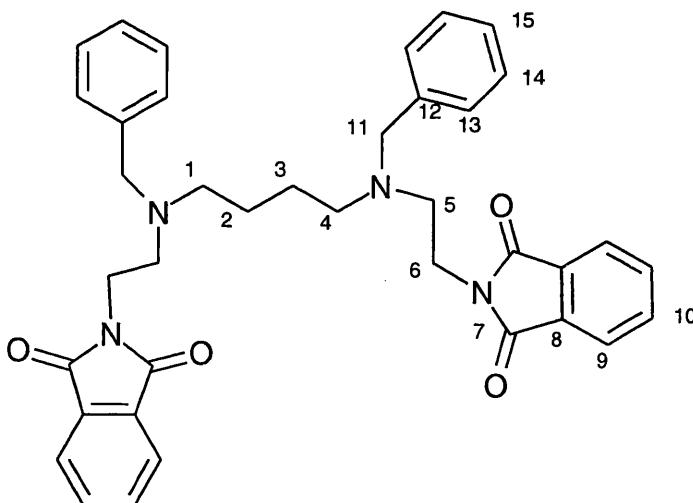
N,N'-Bis-(3-amidinopropyl)-N,N'-dibenzyl-1,4-diaminobutane Tetrahydrochloride 235



Following the general procedure of Moss *et al.*¹⁸⁰ 2 mol dm⁻³ Me₃Al in toluene (3.2 ml, 3.4 equiv) was added dropwise to a solution of NH₄Cl (0.36 g, 3.6 equiv) in toluene (3 ml), under N₂, and stirred for 2 h. The nitrile 187 (0.6937 g, 1.86 mmol) in toluene (1 ml) was added dropwise and stirred at 70-80 °C overnight. The mixture was poured onto a slurry of silica gel (1 g) in CHCl₃ (2 ml) and stirred for 5 min before filtering. The silica was washed with MeOH and the filtrate concentrated *in vacuo*. The residue was redissolved in MeOH (1 ml) and filtered to remove NH₄Cl. To the MeOH solution was added c.HCl (5 drops) and the solution was concentrated *in vacuo* to yield the hydrochloride salt as a yellow powder (0.2770 g, 0.67 mmol, 40 %), mp >200 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3060 (Ar C-H), 1689 (C=N), 1624 (Ar C=C), 1496 (Ar C=C), 746 (Ar C-H bend), 700 (Ar C-H bend); δ_{H} (200 MHz, D₂O) 1.58 (4H, bm, 2,3-H₂), 2.72-3.40 (12H, m, 1,4,5,6-H₂), 4.24 (4H, s, 8-H₂), 7.33 (10H, m, 10,11,12-H); δ_{C} (50 MHz, D₂O) 21.1 (CH₂-2 and -3), 28.0 (CH₂-), 50.0 (CH₂-), 52.3 (CH₂-), 58.4 (CH₂-), 129.1 (C-9), 130.3 (CH-), 131.2 (CH-), 131.7 (CH-), 167.0 (C-7).

N,N'-Bis-(2-hydroxyethyl)-N,N'-dibenzyl-1,4-diaminobutane**238**¹⁴⁸

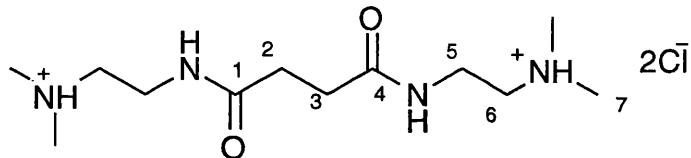
238 was prepared according to the procedure of Lee et al. to yield the desired alcohol in 60% yield. ¹H and ¹³C NMR spectra were identical with literature values and **238** was used without further purification.

N,N'-Bis-(2-phthalimidoethyl)-N,N'-dibenzyl-1,4-diaminobutane**239**

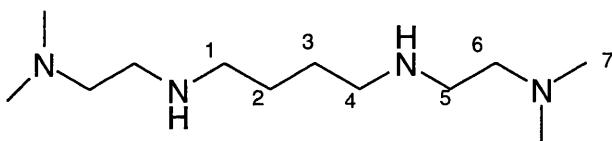
To a stirred solution of **238** (0.7487 g, 2.1 mmol) in THF (15 ml) was added triphenylphosphine (1.10 g, 2.2 equiv) and phthalimide (0.62 g, 2.2 equiv). DEAD (0.79 ml, 2.2 equiv) was added dropwise to produce a yellow solution which was stirred at rt for 12 h. The solution was concentrated *in vacuo* and the resulting oil triturated in Et₂O:hexaneane (1:1). After filtration the filtrate was concentrated *in vacuo* and the oil purified by silica column chromatography, eluent hexaneane:EtOAc (3:1) (0.1513 g, 0.25 mmol, 15

%), R_f 0.63 (hexaneane:EtOAc); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2982 (-CH₂-), 2872 (N-CH₂), 1773 (C=O), 1707 (C=O), 1613 (Ar C=C), 1494 (ArC=C), 1466 (-CH₂-), 739 (Ar C-H bend), 697 (Ar C-H bend); δ_{H} (200 MHz, CDCl₃) 1.27 (4H, m, 2,3-H₂), 2.32 (4H, m, 1,4-H₂), 2.55 (4H, t, J 6.2, 5-H₂), 3.41 (4H, s, 11-H₂), 3.62 (4H, t, J 6.2, 6-H₂), 7.04 (10H, m, 13,14,15-H), 7.58 (4H, m), 7.67 (4H, m); δ_{C} (50 MHz, CDCl₃) 24.6 (CH₂-2 and -3), 36.0 (CH₂-1 and -4), 51.1 (CH₂-6), 53.7 (CH₂-5), 58.2 (CH₂-11), 123.0 (CH-15), 126.6 (CH-13), 127.9 (CH-9), 128.8 (CH-14), 132.2 (C-8), 133.6 (CH-10), 139.5 (C-12), 168.1 (C-7); *m/z* (EI) 614 (M⁺, 11.8 %), 523 (36), 454 (42), 333 (46), 319 (8), 174 (55), 160 (19) and 91 (100), (Found: M⁺, 614.2868. C₃₈H₃₈N₄O₄ requires 614.2843).

N,N'-Bis-(2-dimethylaminoethyl)-succinyl diamide Dihydrochloride 237

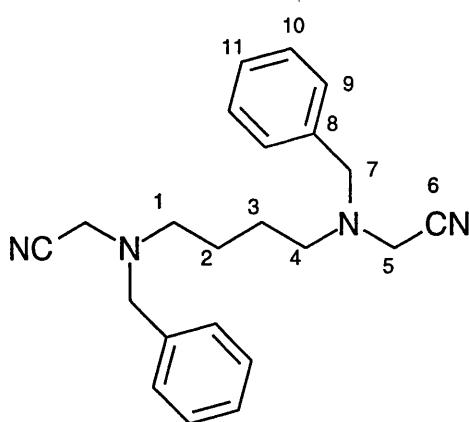


Succinyl dichloride (1 g, 6.45 mmol) was stirred in dry ether (20 ml) at 0 °C and *N,N*-dimethylethylenediamine (1.6 ml, 2.2 equiv) was added slowly. The resulting precipitate was filtered and recrystallised from EtOH:MeOH (4:1) to yield **237** as white crystals (2.42 g, 5.67 mmol, 88 %), mp >200 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3433 (N-H), 3263 (N-H), 2944 (-CH₂-), 1657 (C=O), 1535 (C=O), 961 ((E)-C=C, C-H bend); δ_{H} (200 MHz, D₂O) 2.39 (4H, s, 2,3-H₂), 2.74 (12H, s, 7-H₃), 3.12 (4H, t, J 5.8, 6-H₂), 3.41 (4H, t, J 5.8, 5-H₂); δ_{C} (50 MHz, D₂O) 31.1 (CH₂-2 and -3), 35.3 (CH₂-6), 43.0 (CH₃-7), 57.4 (CH₂-5); *m/z* (EI) 258 (M⁺ -2H, 2.6 %), 215 (1), 200 (2), 188 (37), 171 (1.3), 144 (0.6), 117 (2.3), 98 (1.4), 71 (15) and 58 (100), (Found: M⁺-2H, 258.2042. C₁₂H₂₆N₄O₂ requires 258.2028).

N,N'-Bis-(2-dimethylaminoethyl)-1,4-diaminobutane 236

Attempt 1: **237** was heated at reflux in THF with 1 mol dm⁻³ BH₃ in THF according to general procedure C5. The product isolated was extremely impure and difficult to purify.

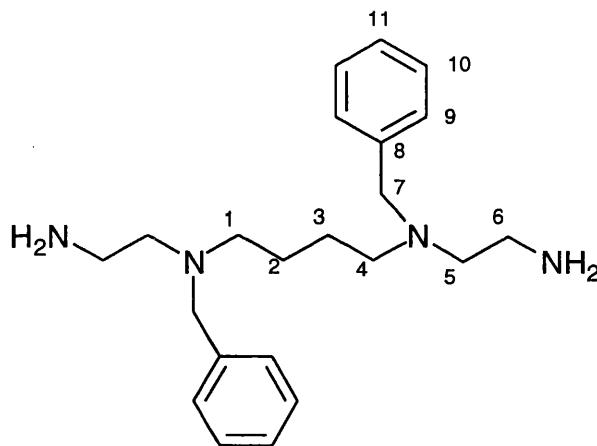
Attempt 2: 1,4-Dibromobutane (5.0 g, 23.16 mmol) was stirred in MeCN (20 ml) and NEt₃ (15 ml, 6 equiv.) followed by N,N-dimethylethylenediamine (5.3 ml, 2.2 equiv.) were added. The solution was stirred at rt for 18 h and then filtered and the filtrate concentrated *in vacuo*. A number of methods of purification were attempted however Kugelrohr distillation was only successful on a small scale to yield **236** as a clear oil (3.33 g, 12.743 mmol, 55%), bp 60–65 °C (1mm); $\nu_{\text{max}}(\text{CDCl}_3)/\text{cm}^{-1}$ 3263 (N-H), 2955 (-CH₂-); δ_{H} (200 MHz, CDCl₃) 1.37 (4H, m, 2,3-H₂), 1.84 (12H, s, 7-H₃), 2.12 (12H, m, 1,4,5,6-H₂); δ_{C} (50 MHz, CDCl₃) 23.0 (CH₂-2 and -3), 45.5 (CH₃-7), 54.0 (CH₂-1 and -4), 54.1 (CH₂-6), 58.3 (CH₂-5); *m/z* (EI) 262 (M⁺, 5.0 %), 218 (1), 174 (6.2), 146 (0.8), 118 (4.3), 84 (20) and 58 (100), (Found: M⁺, 262.4868. C₁₂H₃₀N₄O₂ requires 262.4870).

N,N'-Bis-(cyanomethyl)-N,N'-dibenzyl-1,4-diaminobutane 241

240 (0.5446 g, 1.60 mmol) was stirred in CHCl₃ (15 ml) and Hünigs base (1.22 ml, 4.4 equiv.) was added. Bromoacetonitrile (0.49 ml, 2.2 equiv.) was added dropwise and the solution stirred at rt for 18 h. The solution was washed with water (2x40 ml) and the organic extracts were dried (K₂CO₃),

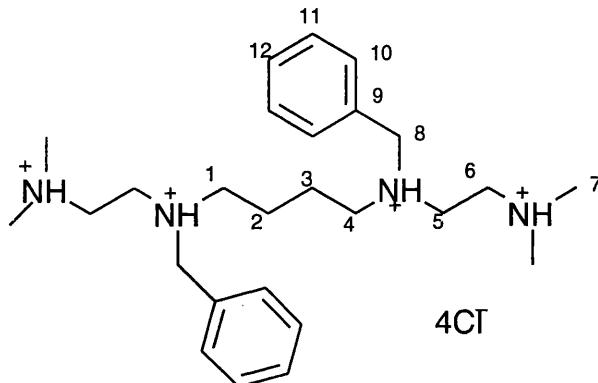
filtered and concentrated *in vacuo* to give a cream solid which was recrystallized from EtOAc-hexaneane to yield **241** as fine cream needles (0.3887 g, 1.12 mmol, 70 %), mp 81.9-83.6 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3030 (Ar-H), 2948 (-CH₂-), 2780 (N-CH₂), 2230 (CN), 1601 (Ar C=C), 1492 (Ar C=C), 748 (Ar-H bend), 697 (Ar C-H bend); δ_{H} (200 MHz, CDCl₃) 1.50 (4H, m, 2,3-H₂), 2.51 (4H, m, 1,4-H₂), 3.31 (4H, s, 5-H₂), 3.41 (4H, s, 7-H₂), 3.62 (4H, t, *J* 6.2, 6-H₂), 7.14 (4H, m, 9-H), 7.23 (6H, m, 10,11-H); δ_{C} (50 MHz, CDCl₃) 24.5 (CH₂-2 and -3), 41.2 (CH₂-1 and -4), 53.4 (CH₂-5), 58.4 (CH₂-6), 114.8 (C-6), 127.7 (CH-11), 128.6 (CH-9), 128.9 (CH-10), 137.2 (CH-8); *m/z* (EI) 346 (M⁺, 70.9%), 319 (5), 306 (4), 280 (2), 255 (3), 228 (7), 160 (18), 138 (7) and 91 (100), (Found: M⁺ 346.2161; C, 76.35; H, 7.37; N, 16.21. C₂₂H₂₆N₄ requires 346.2165; C, 76.25; H, 7.57; N, 16.18).

N,N'-Bis-(2-aminoethyl)-N,N'-dibenzyl-1,4-diaminobutane 242



Following general procedure B3 **241** (0.4487 g, 1.29 mmol) was reduced using LiAlH₄ (0.30 g, 6 equiv.) and AlCl₃ (1.03 g, 6 equiv.) to yield the tetraamine as a clear oil (0.3629 g, 1.03 mmol, 80 %), $\nu_{\text{max}}(\text{CDCl}_3)/\text{cm}^{-1}$ 3019 (Ar-H), 2400 (N-H), 1522 (Ar C=C), 744 (Ar C-H), 670 (Ar C-H); δ_{H} (200 MHz, CDCl₃) 1.20 (4H, m, 2,3-H₂), 1.35 (4H, m, 1,4-H₂), 2.32 (8H, m, 5-H₂ and NH₂), 2.57 (4H, t, *J* 5.8, 6-H₂), 3.42 (4H, s, 7-H₂), 7.19 (10H, m, 9,10,11-H); δ_{C} (50 MHz, CDCl₃) 24.8 (CH₂-2 and -3), 39.7 (CH₂-1 and -4), 53.7 (CH₂-5), 57.0 (CH₂-6), 58.8 (CH₂-7), 126.7 (CH-11), 128.1 (CH-9), 128.7 (CH-10), 139.7 (CH-8); *m/z* (Cl/NH₃) 355 (MH⁺, 100%), 325 (0.1), 313 (0.5), 267 (0.4), 222 (0.2), 203 (0.4), (Found: MH⁺ 355.2854, C₂₂H₃₅N₄ requires 355.2847).

N,N'-Dibenzyl-N,N'-bis-(2-dimethylaminoethyl)-1,4-diaminobutane Tetrahydrochloride 243



Attempt 1: Following the procedure of Wright¹⁸² **236** (0.2677 g, 1.16 mmol) in THF (6 ml) was added to a stirred solution of LiAlH₄ (0.22 g, 5 equiv.) in THF (6 ml) and stirring continued for 30 min. Ethyl benzoate (1 ml, 6 equiv.) was added dropwise and the solution heated at reflux for 18 h. H₂O (0.2 ml), 6 mol dm⁻³ (0.6 ml) and H₂O (0.6 ml) were added and the residue filtered and washed with THF. THF was removed by distillation at atmospheric pressure and the residue converted into the hydrochloride salt by general procedure A2. NMR analysis showed starting material only.

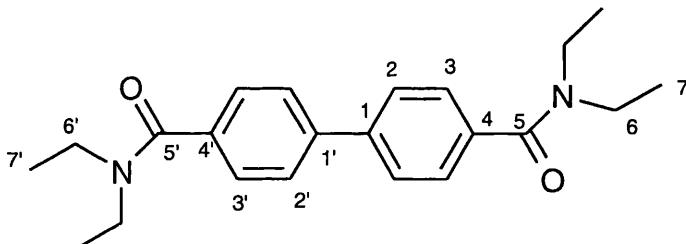
Attempt 2: To a stirred solution of **236** (0.1599 g, 0.69 mmol) in methanol was added PhCHO (0.16 ml, 2.2 equiv.) with stirring. The reaction was monitored by TLC and no imine formation was observed.

Attempt 3: To a stirred solution of **236** (0.3426 g, 1.5 mmol) in MeCN (10 ml) was added Hünig's base (0.55 ml, 2.2 equiv.) and benzyl bromide (0.37 ml, 2.2 equiv.). A number of inseparable products were observed by TLC.

Attempt 4: Following the procedure of Kim¹⁹² et al. **242** (0.3547 g, 1.00 mmol) was stirred in MeOH and 37 % aqueous formaldehyde (5.0 ml, 6.4 equiv.) added. A solution of NaCNBH₃ (0.1260 g, 2 equiv.) and ZnCl₂ (0.136 g, 1 equiv.) in MeOH (6 ml) was added and a white emulsion appeared after addition was complete. The solution was stirred at rt for 4 h before addition of 0.1 mol dm⁻³ NaOH (15 ml) and concentrating *in vacuo*. The residue was partitioned between H₂O (10 ml) and EtOAc (10 ml) and extracted with EtOAc (3x20 ml). The organic extracts were washed with H₂O (10 ml) and brine (10 ml) then dried (K₂CO₃), filtered and concentrated

in vacuo to yield a yellow oil **214** (0.2511 g, 0.61 mmol, 61 %), δ_{H} (200 MHz, CDCl₃) 1.37 (4H, m, 2,3-H₂), 2.01 (12H, s, 7-H₃), 2.14-2.28 (8H, m, 1,4,6-H₂), 2.45 (4H, m, 5-H₂), 3.47 (4H, s, 7-H₂), 7.20 (10H, m, 9,10,11-H); δ_{C} (50 MHz, CDCl₃) 24.7 (CH₂-2 and -3), 45.9 (CH₃-7), 51.9 (CH₂-1 and -4), 54.1 (CH₂-5), 57.6 (CH₂-6), 59.0 (CH₂-7), 126.7 (CH-12), 128.0 (CH-9), 128.7 (CH-10), 139.8 (CH-8). The tetrahydrochloride salt was prepared according to general procedure A2 to yield **243** as a white powder, $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3423 (N-H), 2955 (-CH₂-), 1618 (Ar C=C), 1496 (Ar C=C), 748 (Ar C-H deform.), 702 (Ar C-H deform); δ_{H} (200 MHz, D₂O) 1.75 (4H, m, 2,3-H₂), 2.87 (12H, s, 7-H₃), 3.23 (4H, m, 5-H₂), 3.58 (8H, m, 1,4,6-H₂), 4.41 (4H, s, 7-H₂), 7.48 (10H, m, 9,10,11-H); δ_{C} (50 MHz, D₂O) 21.0 (CH₂-2 and -3), 43.9 (CH₃-7), 47.5 (CH₂-1 and -4), 51.5 (CH₂-5), 53.0 (CH₂-6), 58.7 (CH₂-7), 126.7 (CH-12), 128.0 (CH-9), 128.7 (CH-10), 139.8 (CH-8); *m/z* (CI/ isobutane) 412 (M⁺-2H, 30.4%), 411 (M⁺-3, 100%), 352 (2.5), 307 (0.5), 262 (0.2), 233 (0.4) and 179 (1.8), (Found: M⁺-2H 412.3522, C₂₆H₄₄N₄ requires 412.3478; Found: M⁺-3H 411.3491, C₂₆H₄₃N₄ requires 411.3488).

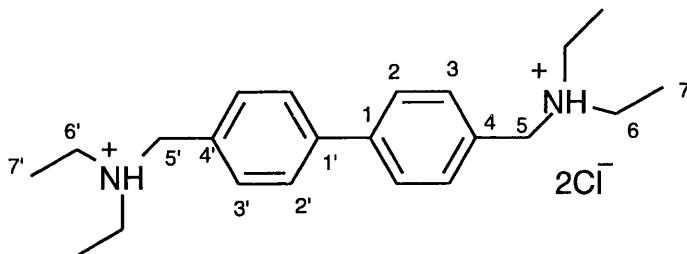
N,N,N',N'-Tetraethyl-4,4'-biphenyldicarboxamide 246



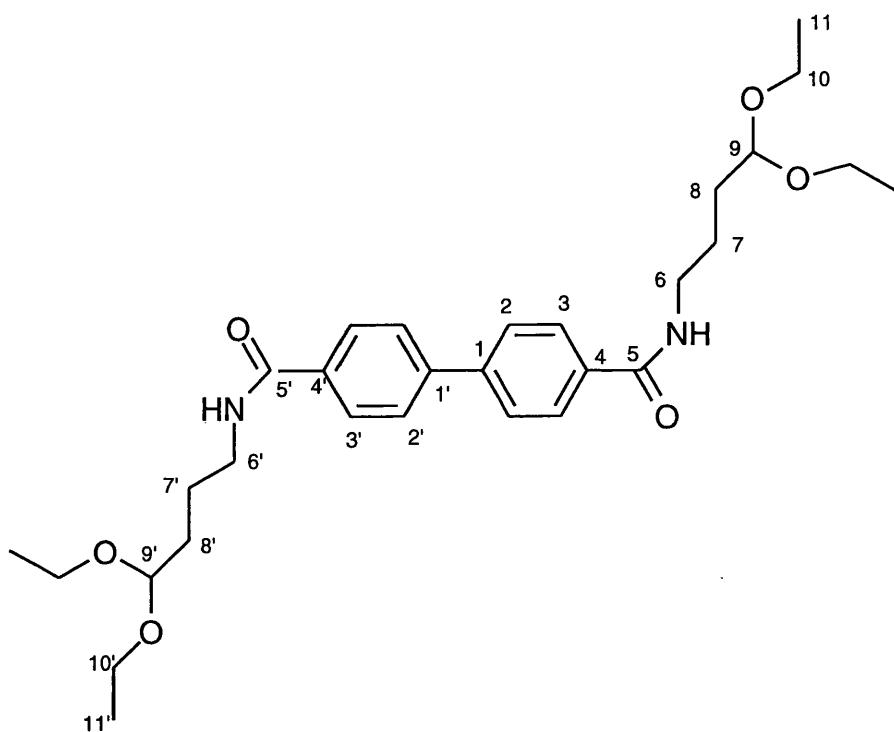
4,4'-Biphenyldicarboxylic acid (2g, 8.26 mmol) was stirred in MeCN (35 ml) with diethylamine (10 ml, 10 equiv) according to general procedure C2 to give an orange oil which solidified on standing. Purification was achieved using alumina chromatography with EtOAc as eluent to give **246** as fine white crystals (1.00 g, 2.89 mmol, 35 %), Rf 0.66 (EtOAc); mp 137.4-138.7 °C; $\nu_{\text{max}}(\text{CDCl}_3)/\text{cm}^{-1}$ 3054 (C-H), 2976 (C-H), 2935 (-CH₂-), 1622 (C=O amide), 1503 (Ar C=C), 845 (Ar C-H bend); δ_{H} (200MHz, CDCl₃) 1.18 (12H, bs, *J* 7.3, 7,7'-H₃), 3.55 (8H, bd, 6,6'-H₂), 7.31 (4H, d, *J* 8.0, 2,2'-H), 7.47 (4H, d, *J* 8.0, 3,3'-H); δ_{C} (50MHz, CDCl₃) 12.8 (CH₃-7), 14.1 (CH₃-7'), 39.1 (CH₂-6), 43.2 (CH₂-6'), 126.8 (CH-2 and 2'), 126.9 (CH-3 and 3'), 136.3 (C-4 and 4'), 140.9 (C-1 and 1'), 170.8 (C-5 and 5'); *m/z* (EI) 352 (M⁺, 40%), 280 (100), 251 (9.4), 180 (55), 152 (41) and 91 (32), (Found:

M^+ , 352.2168; C 74.84; H 8.02; N 7.87. $C_{22}H_{28}N_2O_2$ requires 352.2151; C 74.95; H 8.01; N 7.95; O 9.08).

Bis-(diethylaminomethyl)-4,4'-biphenyl Dihydrochloride 248

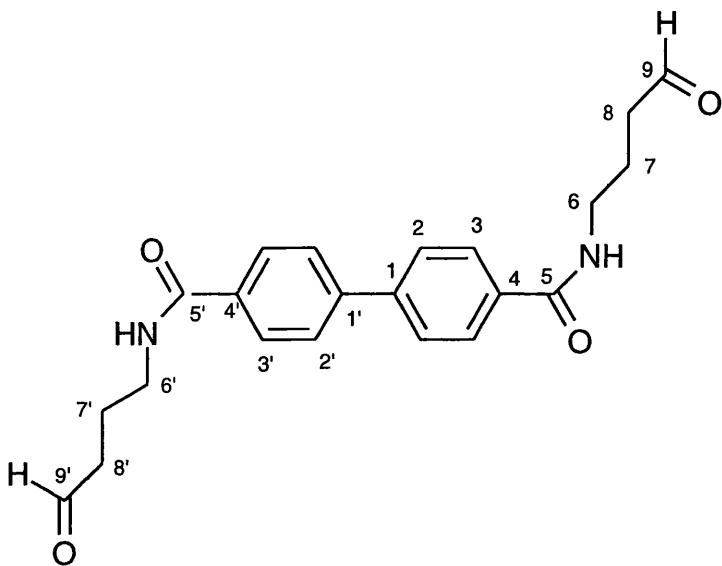


1 Mol dm⁻³ BH_3 in THF (5.6 ml, 8 equiv) was added dropwise to a solution of **246** (0.2452 g, 0.69 mmol) in THF (3 ml) under N_2 and heated at reflux for 24 h following general procedure C5. The resulting solid was recrystallized from ethanol-ether to give the dihydrochloride as cream crystals (0.1789 g, 0.45 mmol, 65 %), mp 244.0-244.9 °C; $\nu_{max}(KBr)/cm^{-1}$ 3487 (O-H), 3027 (Ar-H), 2934 (-CH₂-), 2756 (N-CH₂), 1614 (Ar C=C), 1503 (Ar C=C), 764 (Ar C-H bend), 695 (Ar C-H bend); δ_H (200MHz, MeOD) 0.96 (12H, t, *J* 7.3, 7,7'-H₃), 2.81 (8H, q, *J* 7.2, 6,6'-H₂), 3.99 (4H, s, *J* 8.3, 5,5'-H₂), 7.28 (4H, d, *J* 8.3, 3,3'-H), 7.35 (4H, d, *J* 8.4, 2,2'-H); δ_C (50MHz, MeOD) 7.1 (CH₃-7 and 7'), 45.9 (CH₂-6 and 6'), 54.7 (CH₂-5 and 5'), 126.8 (CH-2 and 2'), 128.6 (C-1 and 1'), 130.9 (CH-3 and 3'), 140.7 (C-4 and 4'); *m/z* (EI) 324 (M^- -2H, 19.5%), 252 (100), 180 (42.6), 118 (20) and 86 (14), (Found: M^- -2H, 324.2562. $C_{22}H_{32}N_2O_2$ requires 324.2565).

N,N'-Bis-(4,4-diethoxybutyl)-4,4'-biphenyldicarboxamide 250

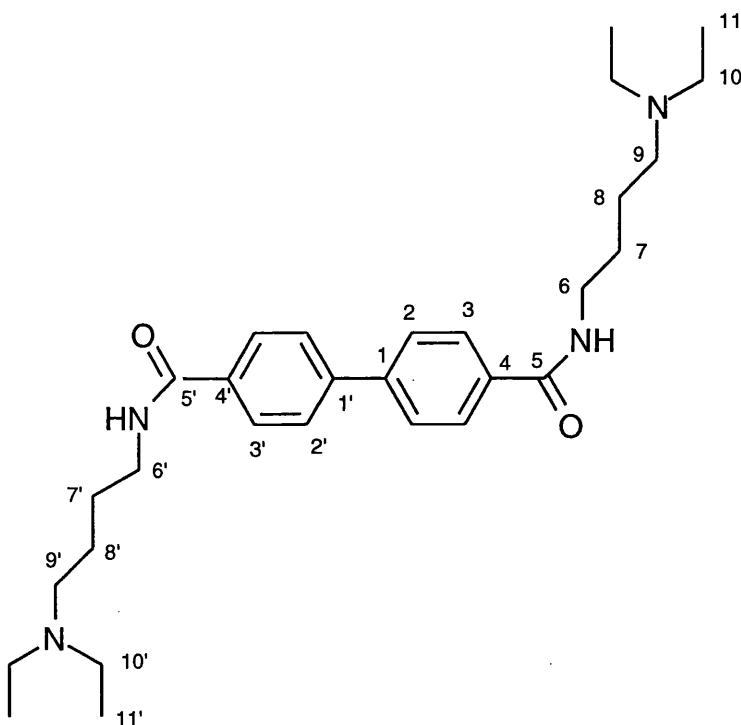
Following general procedure C3 4,4'-biphenyldicarboxylic acid (0.9632 g, 4.13 mmol) was stirred with 4-aminobutyraldehyde diethylacetal (3.6 ml, 5 equiv.). The white solid was recrystallised from EtOH-EtOAc to yield **250** as a spongy white solid (0.85 g, 1.66 mmol, 40 %), mp 158.1–159.4 °C; $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3457 (N-H), 2978 (Ar C-H), 2957 (-CH₂-), 1627 (Ar C=C), 1560 (C=O), 1420 (Ar C=C), 863 (C-H Ar deform.); δ_{H} (200MHz, CHCl₃) 1.20 (12H, t, *J* 7.0, 12,12'-H₃), 1.74 (8H, m, 8,8',9,9'-H₂), 3.51 (8H, m, 11,11'-H₂), 3.70 (4H, m, 7,7'-H₂), 7.36 (4H, d, *J* 8.2, 2,2'-H), 7.55 (4H, d, *J* 8.2, 3,3'-H); δ_{C} (50MHz, CHCl₃) 15.4 (CH₃-12 and 12'), 24.5 (CH₂-8 and -8' or -9 and -9'), 31.1 (CH₂-8 and -8' or -9 and -9'), 39.8 (CH₂-11 and -11'), 61.5 (CH₂-7 and -7'), 102.7 (CH-10 and -10'), 127.1 (CH-2 and -2'), 127.6 (CH-3 and -3'), 128.2 (C-4 and -4'), 142.7 (-1 and 1'C), 167.1 (C-5 and -5'); *m/z* (EI) 348 (M⁺-4OEt, 3%), 322 (44), 276 (100), 180 (87), 152 (44) 104 (37), 90 (17) and 76 (21) (Found: M⁺-4OEt, 348.1798. C₂₂H₂₄N₂O₂ requires 348.1803).

Attempted preparation of *N,N'*-Bis-(4-oxobutyl)-4,4'-biphenyldicarboxamide 251



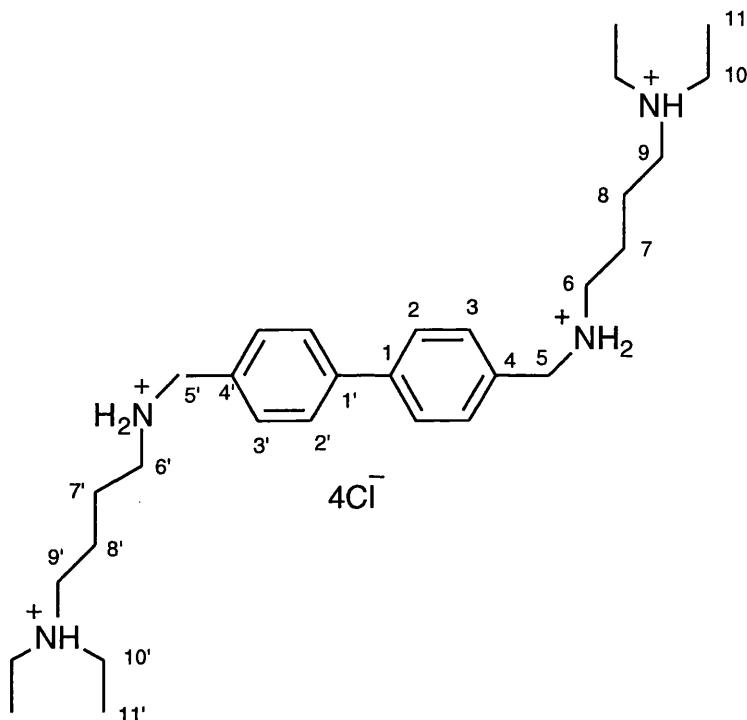
Attempt 1: **250** (0.20 g, 0.38 mmol) was stirred in CHCl₃ at 0 °C and 99 % TFA (0.15 ml) in H₂O (0.15 ml) added dropwise. Stirring continued for 1 h and the solution was then made basic, NaOH(s), and extracted with DCM. No product was observed by NMR spectra.

Attempt 2: Water (11 drops) was added to a suspension of silica gel (3 g) in DCM (8 ml). After 2 min **250** (0.3008 g, 0.57 mmol) was added with stirring and left for 1 h. Silica was filtered and washed well with DCM and the organic layer was concentrated *in vacuo*. No product was observed by NMR spectra.

N,N'-Bis-(4-diethylaminobutyl)-4,4'-biphenyldicarboxamide 254

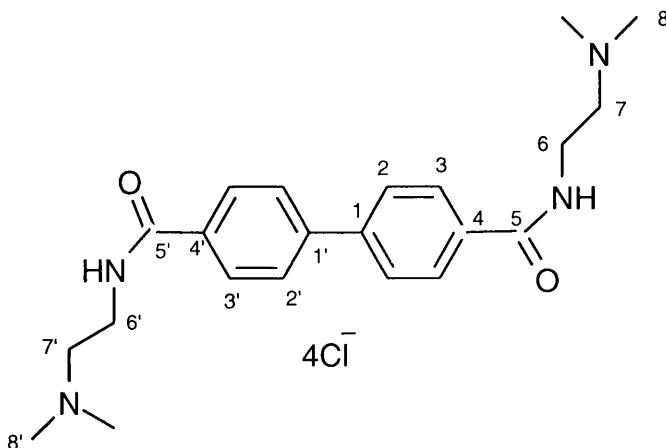
4,4'-Biphenyldicarboxylic acid (0.5g, 2.06 mmol) was stirred at reflux in thionyl chloride (8 ml) for 1 h and the solution was concentrated *in vacuo*. The residue was taken up in dry ether (10 ml) and stirred at 0 °C before the careful addition of **145** (1.2 g, 4 equiv) in dry ether (5 ml). 3 Mol dm⁻³ HCl (10 ml) was added and the aqueous layer washed with ether. The aqueous was made basic with NaOH (s) (pH14) and extracted with CHCl₃ (3x10 ml). The organic extracts were combined, dried (Na₂SO₄), filtered and concentrated *in vacuo* to give a grey/cream solid which was recrystallized from CHCl₃/hexaneane to yield **254** (0.4887 g, 1.06 mmol, 51 %), δ_H (200MHz, CDCl₃) 0.98 (12H, t, *J* 7.1, 11,11'-H₃), 1.64 (8H, m, 7,7',8,8'-H₂), 2.49 (12H, m, 9,9',10,10'-H₂), 3.45 (4H, m, 6,6'-H₂), 7.50 (2H, d, *J* 8.2, 2,2'-H), 7.81 (4H, d, *J* 8.2, 3,3'-H), 7.84 (2H, t, *J* 5.3, NH); δ_C (50MHz, CDCl₃) 11.2 (CH₃-11 and 11'), 24.9 (CH₂-7 and 7' or 8 and 8'), 27.6 (CH₂-7 and 7' or 8 and 8'), 40.1 (CH₂-9 and 9'), 46.6 (CH₂-10 and 10'), 52.3 (CH₂-7 and 7'), 126.8 (CH- 2 and 2'), 127.7 (CH-3 and 3'), 134.2 (C-4 and 4'), 142.4 (C-1 and 1'), 167.4 (C-5 and 5'); *m/z* (EI) 494 (M⁺, 1.4%), 351 (5), 276 (3.7), 180 (9.7), 152 (4.9) and 86 (100), (Found: M⁺, 494.3628. C₃₀H₄₆N₄O₂ requires 494.3636).

***N,N'*-Bis-(4-diethylaminobutylaminomethyl)-4,4'-biphenyl
Tetrahydrochloride 252**



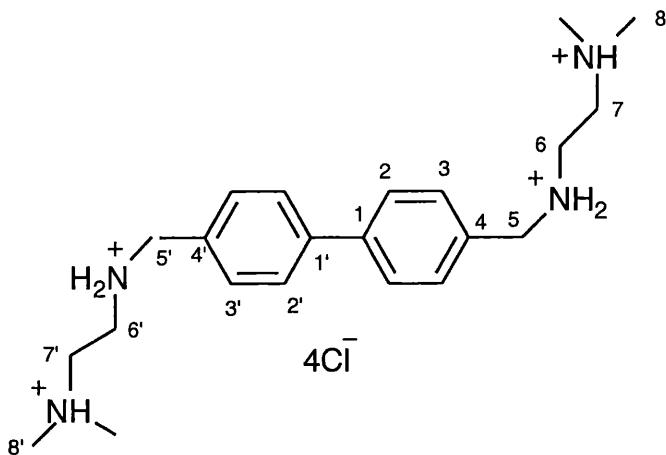
Following general procedure C5 **254** (0.4887 g, 1 mmol) in THF (5 ml) and 1 mol dm⁻³ BH₃ in THF (8 ml, 8 equiv) yielded **252** (0.34 g, 0.64 mmol, 64 %), mp >247.6-249.1 °C decomp.; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3420 (N-H), 3030 (Ar C-H), 2940 (-CH₂-), 1502 (Ar C=C), 840 (C-H Ar bend); δ_{H} (200MHz, D₂O) 1.06 (12H, t, *J* 7.3, 11,11'-H₃), 1.58 (8H, m, 7,7',8,8'-H₂), 2.98 (16H, m, 6,6',9,9',10,10'-H₂), 4.08 (4H, s, 5,5'-H₂), 7.36 (4H, d, *J* 8.2, 3,3'-H), 7.55 (4H, d, *J* 8.2, 2,2'-H); δ_{C} (50MHz, D₂O) 9.0 (CH₃-11 and 11'), 21.5 (CH₂-7 and 7' or 8 and 8'), 23.6 (CH₂-7 and 7' or 8 and 8'), 47.1 (CH₂-9 and 9'), 48.1 (CH₂-10 and 10'), 51.5 (CH₂-6 and 6'), 51.6 (CH₂-5 and 5'), 128.5 (CH-2 and 2'), 131.0 (C-1 and 1'), 131.3 (CH-3 and 3'), 141.6 (C-4 and 4'); *m/z* (EI) 466 (M⁺-4H, 0.4%), 322 (12), 180 (63), 144 (100) 112 (54), and 99 (26), (Found: M⁺-4H, 466.4031. C₃₀H₅₀N₄ requires 466.4027).

N,N'-Bis-(2-dimethylaminoethyl)-4,4'-biphenyldicarboxamide
255

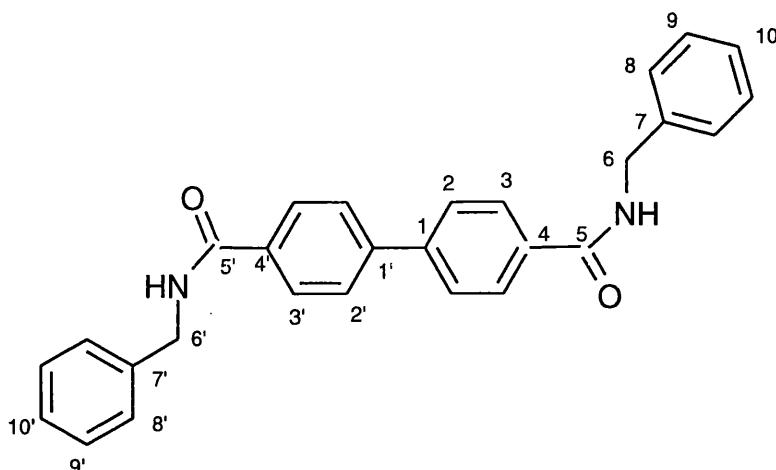


This was prepared as for **254** using 4,4'-biphenyldicarboxylic acid (1.0g, 4.13 mmol) and heated at reflux in thionyl chloride (15 ml) for 1h and the solution was concentrated *in vacuo*. The residue was taken up in dry ether (10 ml) and stirred at 0 °C before the careful addition of *N,N*-dimethylethylenediamine (1.8 ml, 4 equiv) in dry ether (5 ml). Work up was as for **254** to yield **255** as peach crystals (0.7936 g, 2.10 mmol, 51 %), mp 221.2-222.5 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3315 (N-H), 3067 (N-H), 3018 (Ar-H), 2941 (-CH₂-), 2795 (N-CH₂), 1635 (C=O), 1610 (Ar C=C), 1556 (C=O), 1490 (Ar C=C), 841 (Ar C-H bend); δ_{H} (200MHz, CDCl₃) 2.29 (12H, s, 8,8'-H₃), 2.55 (4H, t, *J* 7.2, 7,7'-H₂), 3.54 (4H, dt, *J* 7.1, 5.3, 6,6'-H₂), 7.00 (2H, t *J* 5.4, NH), 7.65 (4H, d, *J* 8.05, 2,2'-H), 7.89 (4H, d, *J* 8.05, 3,3'-H); δ_{C} (50MHz, CDCl₃) 37.2 (CH₂-7), 45.2 (CH₃-8), 57.7 (CH₂-6), 127.2 (CH-2), 127.7 (CH-3), 133.7 (C-4), 142.9 (C-1), 167.0 (C-5); *m/z* (CI) 383 (MH⁺, 100%), 377 (0.05), 300 (0.4), 279 (0.15), 223 (0.1), 177 (0.15) and 157 (0.32), (Found: MH⁺, 383.2442. C₂₂H₃₁N₄O₂ requires 383.2437).

***N,N'*-Bis-[2-(dimethylaminoethylaminomethyl)]-4,4'-biphenyl
Tetrahydrochloride 256**

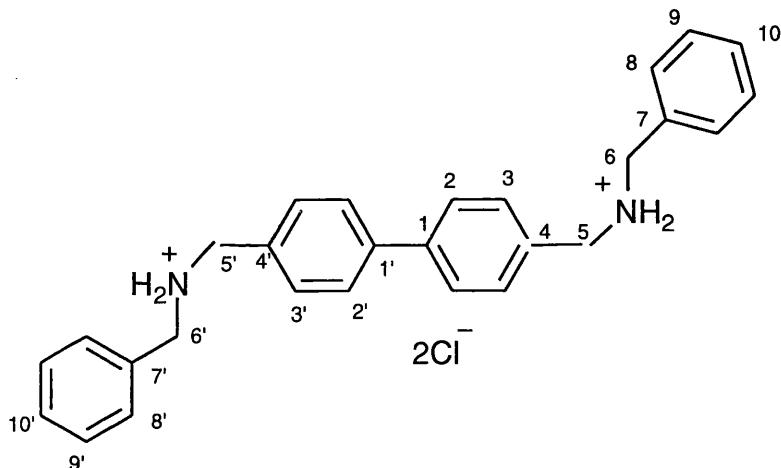


Following general procedure C5 **255** (0.5311 g, 1.40 mmol) in THF (10 ml) and 1 mol dm⁻³ BH₃ in THF (12 ml, 8 equiv) yielded **256** (0.3857 g, mmol, %), mp 250.5–252.1 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3337 (O-H), 2953 (-CH₂-), 2671 (-NH⁺), 1605 (Ar C=C), 1563 (Ar C=C), 1498 (Ar C=C), 831 (Ar C-H bend); δ_{H} (200MHz, D₂O) 2.90 (12H, s, 8,8'-H₃), 3.41 (8H, s, 6,6',7,7'-H₂), 4.20 (4H, s, 5,5'-H₂), 7.41 (4H, d, J 7.6, 2,2'-H), 7.89 (4H, d, J 7.5, 3,3'-H); δ_{C} (50MHz, D₂O) 42.0 (CH₃-8 and -8'), 44.2 (CH₂-6 and -6'), 52.2 (CH₂-8 and -8'), 53.3 (CH₂-7 and -7'), 128.7 (CH-3 and -3'), 130.5 (C-1 and -1'), 131.5 (CH-2 and -2'), 141.7 (C-4 and -4'); *m/z* (Cl/NH₃) 356 (M⁺-2H), 355 (M⁺-3H), 309 (0.5), 280 (0.8), 267 (0.3) and 208 (0.1), (Found: M⁺-2H, 356.2891. C₂₂H₃₆N₄ requires 356.2842; Found: M⁺-3H, 353.2704. C₂₂H₃₃N₄ requires 353.2703).

***N,N'*-Dibenzyl-4,4'-biphenyldicarboxamide 247**

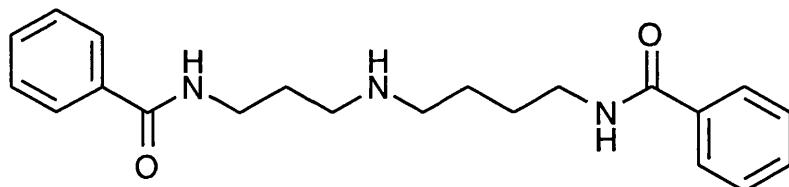
4,4-Biphenyldicarboxylic acid (1.06 g, 4.36 mmol) was stirred at reflux in thionyl chloride (15 ml) for 1h as for **254** and benzylamine (2.7 ml, 6 equiv) was added to the residue in dry ether. The precipitate was filtered and washed with ether to yield **247** as a white solid (1.6152 g, 88 %), however crystallisation failed due to the insoluble nature of this compound. mp >250 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3305 (N-H), 3060 (Ar C-H), 3020 (Ar C-H), 2920 (-CH₂), 1635 (Ar C=C), 1635 (C=O), 1605 (Ar C=C), 1560 (C=O), 1490 (Ar C=C), 840 (Ar C-H bend); δ_{H} (200MHz, D₆-DMSO) 4.56 (4H, d, *J* 5.3, 6,6'-H₂), 7.38 (10H, m, 8,8',9,9',10,10'-H), 7.90 (4H, d, *J* 7.7, 2,2'-H₂), 8.09 (4H, d, *J* 7.7, 3,3'-H₂), 9.26 (2H, t, *J* 5.4, NH); δ_{C} (50MHz, D₆-DMSO) 42.7 (CH₂-6 and 6'), 126.7 (CH-10 and 10'), 127.3 (CH-8 and 8'), 128.1 (CH-2 and 2'), 128.4 (CH-3 and 3'), 128.7 (CH-9 and 9'), 133.7 (C-4 and 4'), 139.8 (C-7 and 7'), 141.8 (C-1 and 1'), 165.8 (C-5 and 5'); m/z (EI) 421 (M⁺, 33%), 420 (M⁺, 100%), 419 (M⁺-1, 20%), 316 (85), 210 (4), 180 (27), 152 (14) and 91 (22), (Found: M⁺ 421.1892, C₂₈H₂₅N₂O₂ requires 421.1868; Found: M⁺ 420.1830; C, 79.79; H, 5.82; N, 6.76. C₂₈H₂₄N₂O₂ requires 420.1822; C, 79.96; H, 5.75; N, 6.66. Found: M⁺-1 419.1757, C₂₈H₂₃N₂O₂ requires 419.1754).

Attempted preparation of *N,N'*-Dibenzylaminomethyl-4,4'-biphenyl Dihydrochloride 249



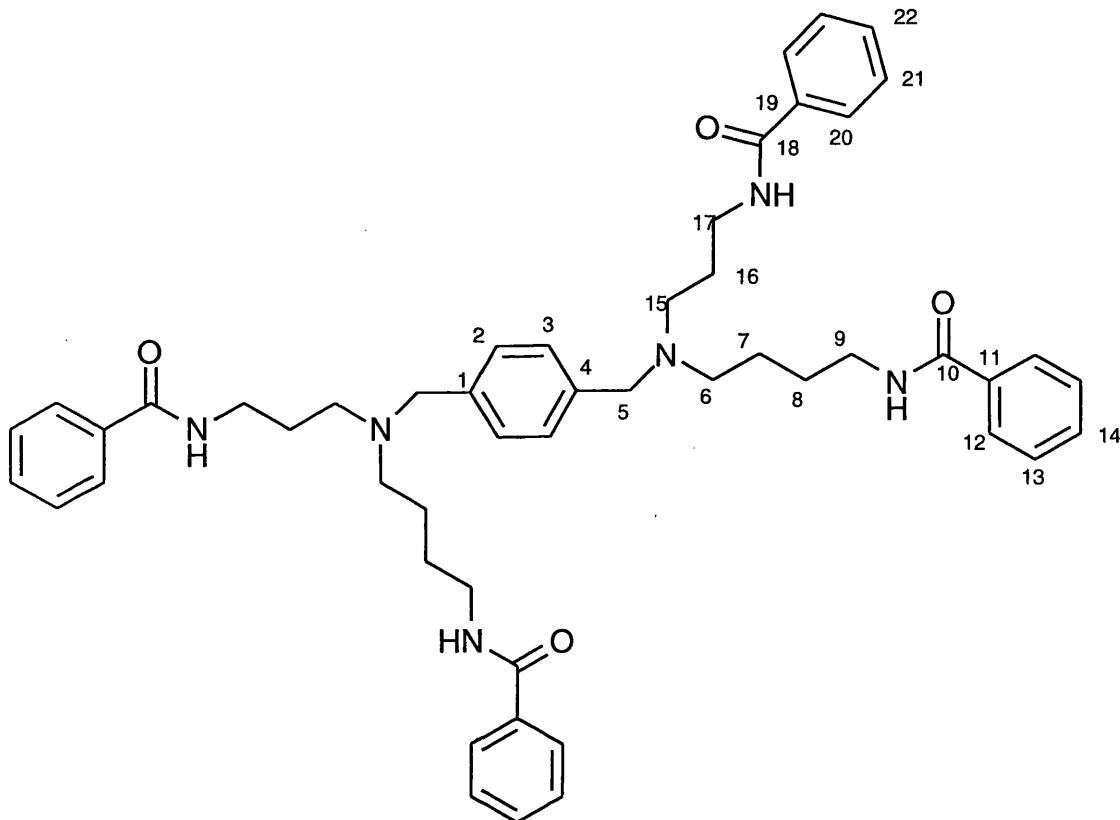
Due to the extremely insoluble nature of the diamide **247** attempts to reduce it using borane/THF as in general procedure C5 failed.

***N*-Benzoyl-*N'*-(3-benzoylaminopropyl)-1,4-diaminobutane
257¹⁸⁰**



This was prepared according to the procedure of Moss *et al.* in 50 % yield. ^1H , ^{13}C and IR spectra were identical with published data. mp 129.1-131.0 °C [lit. 129.5-130.5 °C]; (Found: M $^+$, 353.2108; C, 65.82; H, 4.68; N, 11.25. $\text{C}_{21}\text{H}_{27}\text{N}_3\text{O}_3$ requires 353.2113; C, 65.98; H, 4.70; N, 11.42).

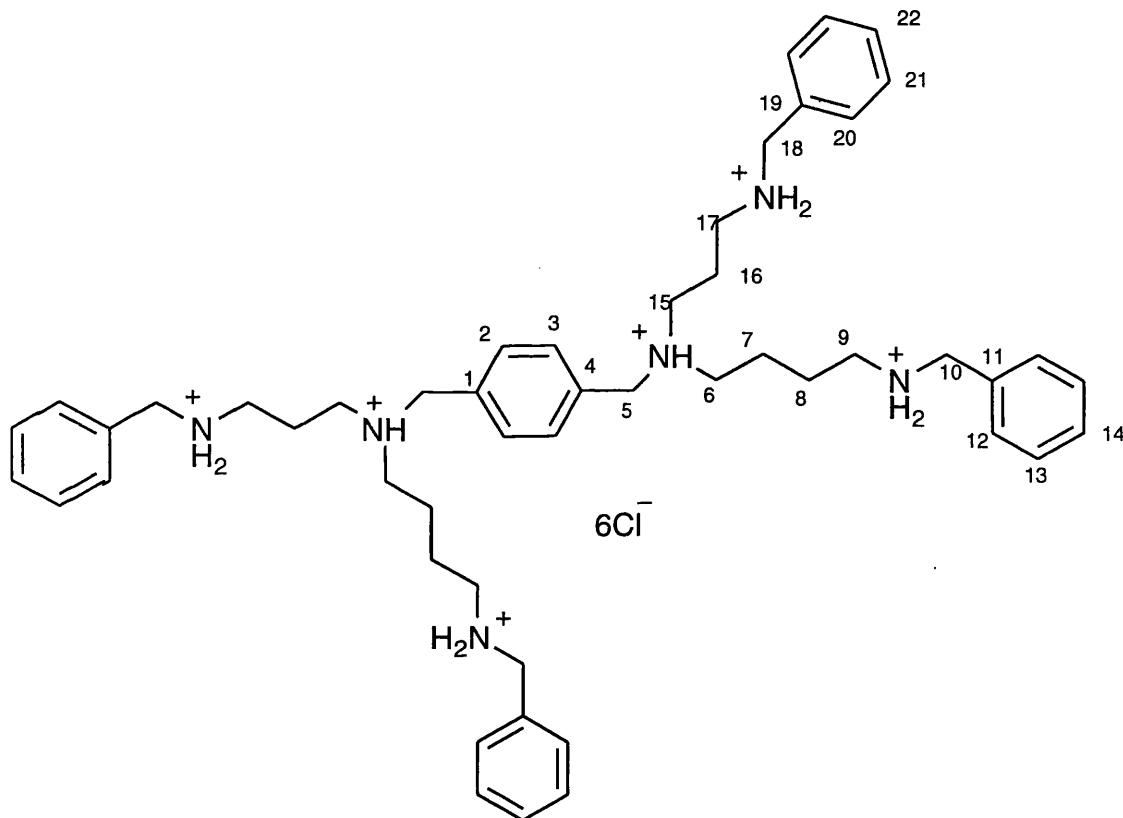
N,N'-Bis-(4-benzoylaminobutyl)-Bis-(3-benzoylaminopropyl)-1,4-xylylenediamine 258



α,α' -Dibromo-*p*-xylene (0.2065 g, 0.78 mmol) was heated at reflux in DMF (10 ml) with **257** (0.6448 g, 2.2 equiv) and Na₂CO₃ (0.2 g, 2.2 equiv) for 24 h. The solution was concentrated *in vacuo*, 2 mol dm⁻³ NaOH (10 ml) added and the solution extracted into EtOAc (3x10 ml). The combined organic extracts were dried (K₂CO₃), filtered and concentrated *in vacuo* and the product was recrystallised from EtOAc to yield **258** as a cream solid (0.2950 g, 0.37 mmol, 21 %), mp 126.5-128.5 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3315 (N-H), 3060 (N-H), 2940 (-CH₂), 2858 (-CH₂-), 1637 (C=O), 1602 (Ar C=C), 1578 (Ar C=C), 1541 (C=O), 1489 (Ar C=C), 849 (Ar C-H bend), 766 (Ar C-H bend), 695 (Ar C-H bend); δ_{H} (200MHz, CDCl₃) 1.57 (12H, bm, 7,8,16-H₂), 2.45 (8H, bt, 6,15-H₂), 3.38 (4H, bm, 9,17-H₂), 3.44 (4H, s, 5-H₂), 6.93 (2H, t, *J* 5.2, NH), 7.12 (4H, s, 2,3-H), 7.38 (12H, m, 13,14,21,22-H), 7.56 (2H, t, *J* 5.3, NH), 7.74 (8H, m, 12,20-H); δ_{C} (50MHz, CDCl₃) 24.5 (CH₂-7 or -8 or -16), 26.6 (CH₂-7 or -8 or -16), 27.3 (CH₂-7 or -8 or -16), 39.5 (CH₂-6 or -15), 39.8 (CH₂-6 or -15), 52.4 (CH₂-9 or -17), 53.4 (CH₂-9 or -17), 58.9 (CH₂-5), 127.0 (CH-2 and -3), 128.3 (CH-12 and -20), 128.4 (CH-13 and -21), 129.2 (CH-14 and -22), 134.8 (C-1 and -4), 138.2 (C-11)

and -19), 167.6 (C-10 and -18); m/z (FAB) 810 (MH^+ , 10%), 646 (1.7), 499 (0.7), 455 (47), 352 (5), 169 (22), 121 (8), 105 (100) and 77 (29), (Found: MH^+ , 809.4717. $C_{50}H_{61}N_6O_4$ requires 809.4680).

N,N'-Bis-(4-benzylaminobutyl)-Bis-(3-benzylaminopropyl)-1,4-xylylenediamine Hexahydrochloride 259

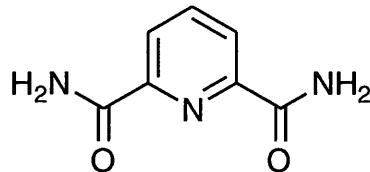


This was prepared following general procedure C5 heating at reflux **258** (0.3004 g, 0.37 mmol) and 1 mol dm⁻³ BH_3 in THF (6 ml, 16 equiv) in THF (4 ml) to yield **259** hexaneahydrochloride as a white powder (0.1970 g, 0.22 mmol, 57%), mp>200 °C; ν_{max} (KBr)/cm⁻¹ 3423 (N-H), 2948 (-CH₂), 2746 (-CH₂-), 1629 (Ar C=C), 1584 (Ar C=C), 1498 (Ar C=C), 766 (Ar C-H bend), 695 (Ar C-H bend); δ H (200MHz, CDCl₃) 1.58 (8H, bm, 7,8-H₂), 1.99 (4H, bm, 16-H₂), 2.91-3.02 (16H, bm, 6,9,15,17-H₂), 4.04 (8H, ds, 10,18-H₂), 4.24 (4H, s, 5-H₂), 7.28 (20H, s, 12,13,14,20,21,22-H), 7.41 (4H, s, 2,3-H); δ C (50MHz, CDCl₃) 21.4 (CH₂-7 or -8 or -16), 23.5 (CH₂-7 or -8 or -16), 44.6 (CH₂-7 or -8 or -16), 47.0 (CH₂-9 or -17), 50.1 (CH₂-9 or -17), 51.9 (CH₂-6 or -15), 52.1 (CH₂-6 or -15), 52.7 (CH₂-10 and -18), 57.4 (CH₂-5), 130.2 (CH), 130.6 (CH), 131.2 (CH), 131.5 (C), 131.6 (C), 132.9 (CH); m/z

(FAB) 753 ($M^+ - 5H$, 66%), 663 (15), 389 (0.5), and 326 (34), (Found: $M^+ - 5H$, 753.5636. $C_{50}H_{68}N_6$ requires 753.5686).

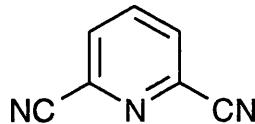
7.4 Experimental for Chapter 6

Pyridine-2,6-dicarboxamide 265¹⁹⁹



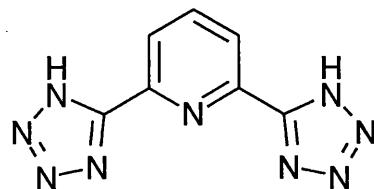
265 was prepared on a 1.0034 g, 6.0 mmol scale following the procedure of McKendrick to yield the product in 0.666 g, 67 % yield. 1H , ^{13}C and IR spectra were identical with those reported, (Found M^+ , 165.0539. $C_7H_7N_3O_2$ requires 165.0541).

Pyridine-2,6-dinitrile 266¹⁹⁹



266 was prepared on a 0.6654 g, 4.04 mmol scale following the procedure of McKendrick to yield the product in 0.1377 g, 27 % yield. 1H , ^{13}C and IR spectra were identical with those reported, mp 129.5-131 °C [lit 128-131 °C]; (Found M^+ , 129.0327. $C_7H_3N_3$ requires 129.0330).

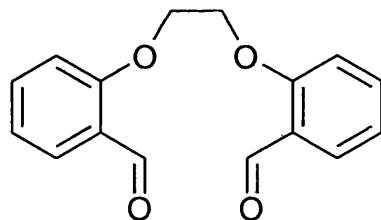
Pyridine-2,6-ditetrazole 267¹⁹⁹



267 was prepared on a 0.5100 g, 3.88 mmol scale following the procedure of McKendrick to yield the product in 0.7551 g, 90 % yield. 1H , ^{13}C and IR

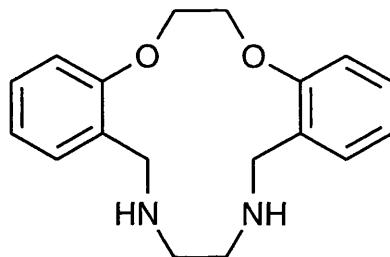
spectra were identical with those reported, mp >300 °C [lit. >300 °C]; (Found M⁺, 215.0670. C₇H₅N₉ requires 215.0669).

1,4-Bis(2'formylphenyl)-1,4-dioxabutane 270²⁰³



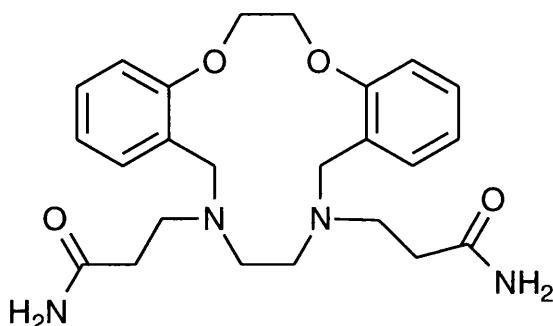
270 was prepared according to the procedure of Armstrong and Lindoy on a 200 mmol scale in 57% yield. ¹H, ¹³C and IR spectra were consistent with published data. mp 126.1-127.7 °C [lit. 129 °C], (Found M⁺, 270.0889. C₁₆H₁₄O₄ requires 270.0886).

5,6,7,8,9,10,16,17Octahydrodibenzo[e,m][1,4]dioxa[8,11]diazacyclotetradecine 271²⁰¹



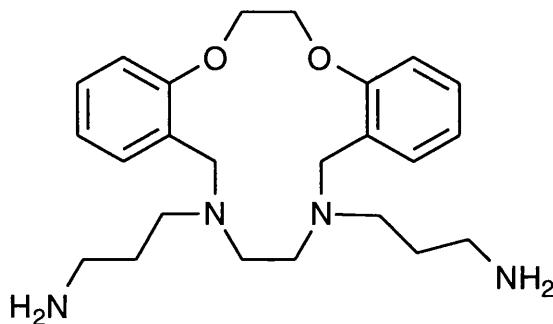
271 was prepared according to the procedure of Grimsley and co-workers on a 3.7 mmol scale in 32% yield. ¹H, ¹³C and IR spectra were consistent with published data. mp 151.3-152.8 °C [lit. 169 °C], (Found M⁺, 298.1680. C₁₈H₂₂N₂O₂ requires 298.1679).

7,8,16,17-Tetrahydrodibenzo[e,m][1,4,8,11]dioxadiazacyclotetradecine-6,9[5H,10H]dipropanamide 272²⁰²



272 was prepared according to the procedure of Chia and co-workers on a 1.34 mmol scale in 37% yield. mp 149-151 °C [lit. 151-153 °C]. ¹H, ¹³C and IR spectra were consistent with published data.

Attempted preparation of 7,8,16,17-Tetrahydrodibenzo[e,m][1,4,8,11]dioxadiazacyclotetradecine-6,9[5H,10H]bis(3-aminopropylamine)273



272 (0.22 g, 0.5 mmol) was refluxed in 1 mol dm⁻³ BH₃/THF (4 ml, 8 equiv.) according to general procedure C5 however only black residue was recovered with a large amount of aliphatic residues present by NMR. Purification was unsuccessful.

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