Synthesis of Novel Alkylating Agents for Biological Evaluation as Anti-Cancer Prodrugs

A Thesis presented in part fulfillment
of the requirement for the
Degree of Doctor of Philosophy

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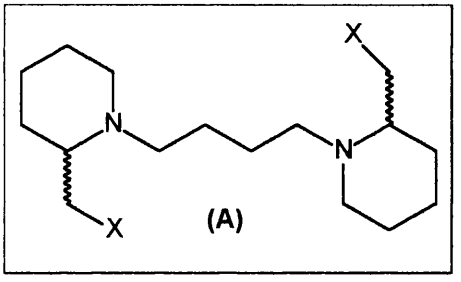
Finally I would like to dedicate this thesis to my parents for their support through my seven years at university.

Summary

Bioreducible antitumour agents are prodrugs which are intended to be inactive in normal cells, but are able to undergo metabolic reduction in cancer cells to produce toxic species which can damage biomolecules. This reduction can occur readily in solid tumours due to two factors which are unique to them: a) tumour hypoxia; and b) the expression of high levels of reductase enzymes. Each of these factors can promote specific cytotoxicity to the tumour and in principle could create a selective anti-tumour agent. The overall aim of this work was to synthesise powerful alkylating agents and then target them selectively to cancer cells as inactive prodrugs.

Following on from previous work on *N*-oxides of *cis*- and *trans*-2,6-disubstituted-*N*-methylpiperidine, compounds **(A)**, where **X** is a carbamate or halogen, were synthesised and subjected to biological evaluation against two human carcinoma cell lines, HT29 and BE cells, under oxic and hypoxic conditions. They showed promising cytotoxicity towards the cancer cells and good DNA crosslinking activity.

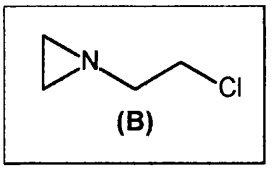


(A)

The DNA sequence specificity of these compounds for guanines warranted further study of structure/activity relationships. To optimise the selectivity and cytotoxicity of this type of compound a series of compounds was formed examining variables such as ring size; number of rings in the system; number of carbons between rings (2-8); stereochemistry; leaving group; number of atoms between nitrogen and the leaving group and the number of potential alkylating sites. These compounds underwent biological evaluation against the human leukaemic K562 cell line. Results from the biological testing were inconclusive in understanding structural/biological activity relationships.

An area of chemistry which is as yet unexplored is that of macrocyclic alkylating agents as potential anti-cancer agents. A selection of cyclic alkylating agents was synthesised from a ring size of three in the monoalkylating aziridine

chloride **(B)** to larger macrocyclic systems of up to 23 membered rings with the potential of six alkylating functions. Also macrocyclic systems incorporating other heteroatoms in the ring such as oxygen and sulfur were made. The incorporation of other functional groups such as amide or benzyl moieties in the macrocyclic systems was also examined as in the synthesis of **(C)**.



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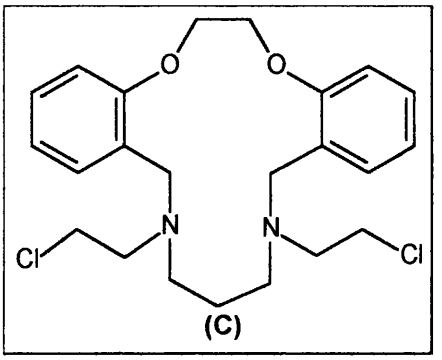

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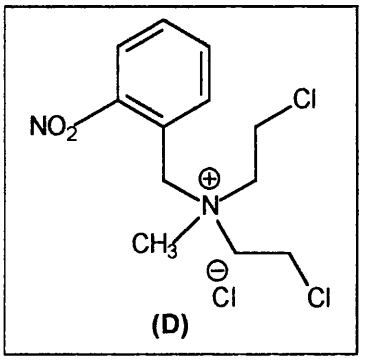

(D)

Variations on the size of the ring; the number of potential alkylating sites; the number of carbons between the nitrogens; and the number and type of heteroatoms in the macrocyclic system have been examined.

The methods used to form these compounds varied depending on available starting materials from high dilution techniques to condensation reactions between amines and esters or acid chlorides. The high dilution method employed the cyclisation between amines protected with tosyl groups, and alcohols, also protected as tosylates. This was followed by sulfonamide cleavage and alkylation using ethylene oxide to obtain the alcohols. The hydroxyl groups were substituted by chlorine atoms to form the alkylating *N*-chloroethyl moieties.

The results of the biological evaluation of the macrocyclic compounds indicated that **(C)** was an excellent DNA crosslinker and also very cytotoxic to the cancer cell line.

The potential hypoxia selectivity was investigated by the formation of nitro benzyl compounds such as **(D)**. Nitrobenzyl quaternary compounds have been shown to undergo fragmentation after one electron reduction to an alkylating system. A selection of the macrocyclic systems formed were developed into *o*- and *p*-subtituted nitrobenzyl quaternary compounds. The results of the biological evaluation of these compounds were very disappointing and showed that none of the compounds tested was reduced under hypoxic conditions to give an alkylating agent.

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Chapter 1

" Not people die but the worlds die within them"

-Yevgeny Yevitushenko [1]

1.1.1 Cancer

cancer 1. any type of malignant growth or tumour caused by abnormal and uncontrolled cell division: it may spread through the lymphatic system or blood stream to other parts of the body. **2.** the condition resulting from this. **3.** an evil influence that spreads dangerously. - The Concise Oxford English Dictionary, 1996.[2]

Cancer is one of the most feared diseases in the modern day world!

The fight against this dreaded enemy has been an ongoing battle since well before the beginning of the century and is currently the concern of many research groups and scientists the world over. In fact, cancer was reported as early as classical Greek literature. A study into literature on cancerous diseases by Jacob Wolff reported that Atossa, the wife of Darius the Great, had been cured of cancer of the breast by a captured Greek physician, Democedes.[3] However, this must be treated with caution as the difference between cancer and a lesion would not be known in the 5th Century BC. The difference between benign and malignant cancer was established more than 60 years after the first study of tumours in light of the tissue theory which alluded to the differences between lesions and cancer.

Many people today hear the word cancer and immediately think it is a death sentence and fear is instilled in them. This fear comes from the fact that the body has revolted against its owner, leading to destruction from within. Nowadays this is not always the case as there have been massive leaps made towards the achievement of cures for many cancers.

There has been a much greater insight into cancer as a whole and it is now recognised that it could also be a genetic disorder[4,5] and thus we have to understand the causes of the disease to find cures.

Cholera, the plague, yellow fever, tuberculosis, *etc.* which previously killed millions, have all but been eradicated. This is due to increased knowledge and improved hygiene during the past 150 years.[6] Cancer is now one of the most prevalent diseases of the modern day world.

One of the main problems in the control and erradication of cancer is that it is not a single disease but covers over 200 different types of disease[6] classed together as cancer. Not only are there many types of cancer (even within the same family of cancers) but they may be treated using different methods, thus making prognosis and treatment more difficult.

1.2.1 How Prominent is Cancer?

The answer to this question is that it is one of the most common diseases with very high mortality rates.

In the United States it is estimated that 1 in 3 will develop cancer at some stage in their life and that 22% of these will die from the disease.[7] This makes it the main cause of death in the USA followed by heart disease. Cancer of the skin, lung, colon and rectum are the four most common cancers in the USA and account for over 50% of cancer mortality. Lung cancer is the largest killer accounting alone for 28% of the deaths.[7] **Table 1** shows the distribution of cancer cases and deaths in the USA in 1990.[7]

Cancer Site	Cases per Year	Deaths per year
Lung	157,000 (15%)	142,000 (28%)
Colon/ Rectum	155,000 (15%)	61,000 (12%)
Breast	151,000 (14%)	44,000 (9%)
Prostate	106,000 (10%)	30,000 (6%)
Bladder	49,000 (5%)	10,000 (2%)
Uterus	47,000 (5%)	10,000 (2%)
Lymphomas	43,000 (4%)	20,000 (4%)
Oral Cavity	31,000 (3%)	8,000 (2%)
Pancreas	28,000 (3%)	25,000 (5%)
Leukaemias	28,000 (3%)	18,000 (4%)
Skin	28,000 (3%)	9,000 (2%)
	823,000 (79%)	377,000 (74%)
All Sites	1,040,000 (100%)	510,000 (100%)

Table 1 Data from 1990 (American Cancer Society, Cancer Facts and Figures, 1990)[7]

1.3.1 What Stage of Life are you Likely to Develop this Disease?

Cancer can affect humans of any age but is more prevalent in the elderly. This is due to the fact that many malignant tumours grow quite slowly over a long period of time. Cancer is also responsible for 15% of childhood deaths.[7]

Different types of cancers are also prevalent in certain age groups. Children tend to suffer from cancers of the blood such as leukemias and lymphomas which account for 50% of childhood deaths from cancer.[7] Other cancers which are frequently encountered in children are those of the brain, nervous system, bone and kidney, all of which are much rarer in adults.

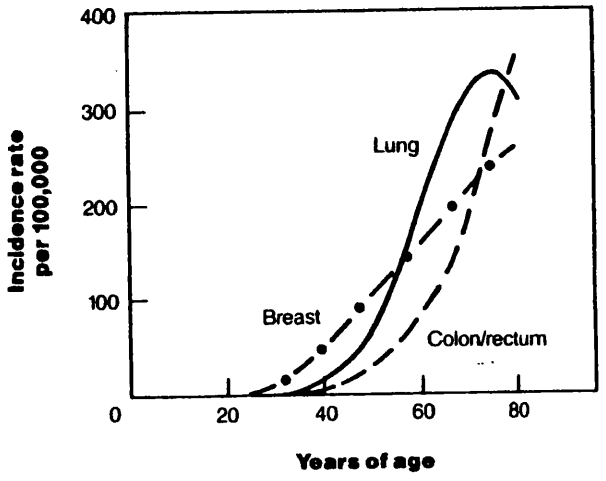


Fig 1

Fig 1 shows incidences of colorectal, breast and lung cancer. These cancers are most prevalent in adults over the age of 60 and practically negligible in people below 20 years of age.[7]

1.4.1 What Happens when Cancer Occurs?

Cancer is a disease which is partly characterised by uncontrollable cell proliferation. The human body is made up of over 10^{14} individual cells[8] which are subdivided into groups and classes that form various tissues and organs which are in themselves programmed to carry out specific allocated functions. Some cells within the body are apparently incapable of further division once they are formed during the development of the embryo or in infancy, e.g. nerve cells. Others such as the liver

are capable of rapid multiplication on demand, e.g. on partial destruction of the liver. Finally others are continuously replicating in order to replace cells that are lost or destroyed such as those in the blood, skin, hair etc.[7] From fertilisation of the egg to death at an old age the human being is the product of 10^{16} cell divisions.[9]

The problem of uncontrollable cell replication generally occurs after a number of factors affect a cell causing it to mutate. Thus when the mutated cell replicates it passes on its mutation(s) to the next line of cells and gives rise to an ever-increasing population of unrestrained progeny cells. A well-developed tumour is made up of 10^{13} cells which equates to one pound of tissue.

The word neoplasm (which literally means "new growth")[7] is generally applied to any abnormal growth of cells and this growth can be subdivided into benign and malignant.

A benign neoplasm is one which remains confined to the original location and is usually encapsulated in a fibrous surrounding and generally grows at a much slower rate than that of malignant neoplasms.[7]

Malignant neoplasms are capable of invading adjacent tissues and organs. This process of invasion of other cells and formation of secondary tumours which is known as metastasis and once it has occurred it is generally much harder to deal with the cancer by surgery or other localised treatments. The process of metastasis in itself poses barriers to the cancer cells including the journey through the blood system avoiding recognition by the immune system. If the tumour is in the brain it is necessary for the cell to cross the blood brain barrier and attach itself to the blood vessel at the distant site. Only 1 in every 10,000 cells successfully establishes a metastatic tumour.[7]

Malignant neoplasms are split into three classes.[9]

- Carcinomas account for ~90% of all human cancers. The tumour developd from epithelial cells which cover the surface of the body and line the internal organs which are affected.
- Sarcomas are relatively rare in humans. The cancer affects the connective tissues such as muscle and bone.
- Leukaemias and lymphomas account for ~ 8% of cancers and are malignancies of the blood and lymph systems.

or wound. A stimulant of these cells is the platelet derived growth factor which is stored in blood cells and is released during clotting. This stimulates the fibroblasts of the skin surrounding the clot leading to regrowth and healing of the wound.[2] Different growth factors stimulate different cells.

1.4.3 Programmed Cell Death

Programmed cell death, otherwise known as apoptosis (which is the Greek word for the falling away of petals from a flower or leaves from a tree)[8] is an active process in which cell death occurs as part of the normal developmental program of cells. It is critically important during embryonic development[7] and is an intrinsic part of the developmental program of some cell types. Regulation of cell death is just as critical as maintaining a constant cell population as it is basically the regulatory control of cell proliferation. Coincident with their failure to differentiate normally, cancer cells generally fail to undergo apoptosis and instead continue to proliferate. The defective differentiation and prolonged lifespan of cancer cells is closely related to the capacity for unregulated cell proliferation. In many cases the progressive growth of malignant cells in an organism is a combined effect of the continuous uncontrolled cell division and the failure of the cancer cells to undergo apoptosis. Thus we can see that the process of programmed cell death is important in the growth of many cancers.[8]

1.5.1 What Causes Cancer?

Cancer is not caused by a single factor but is in fact a complex disease requiring exposure to several carcinogenic factors to initiate the faulty genetic program. These so called "hits" can arise from exposure to a variety of factors such as chemical, radiation, viral, genetic and environmental. These will be discussed later.

By analysing the causes we can establish that, in general, cancer could theoretically be cured by identifying and eliminating these initiating factors.

For cancer to develop there must be an initiating agent which alters the critical cell regulating genes in the body which in turn leads to abnormal cell growth. Tumour promotion is the second stage, when the proliferating cells increase leading to tumour growth. There are a variety of tumour promoters present within the body, such as

hormones, e.g. oestrogen in endometrial carcinomas and dietary fat in colon carcinoma.[7]

1.5.2 Carcinogens

Carcinogens are the most frequently blamed agent for the initiation of cancer in today's society. The most commonly known intake of carcinogens is by smoking.

Since 1930 in the United States the incidence of lung cancer has increased 10 fold which correlates closely with the increased usage of tobacco in the early stages of the century.[7] Growth in cancer incidence is often slow as the gap between consumption of the carcinogen and detection of the cancer represents the multistep development of the cancer. Around the mid century throughout Europe it was actually seen to be fashionable to smoke and health warnings were ignored. This acts as evidence that drastic measures must be employed before a society will take any notice and subsequent action. After this period an increase was noted of cancer of the lung and larynx. An alarming statistic is that in the USA there are 27,000 deaths per year from cancer of the lung which could possibly be avoided.[7]

A second carcinogenic risk is that of excessive consumption of alcoholic beverages. This leads to increased risk of cancers of the oral cavity, pharynx, larynx and oesophagus as well as cirrhosis of the liver and in turn cancer of that organ.[7, 9] **Fig 3** shows a selection of chemicals which are considered to be carcinogenic.[7] However, it should also be remembered that very few people handle any large amounts of chemicals on a daily basis apart from scientists or chemical workers.

Environmental pollution may also be considered here as much of the general public is under the impression that large quantities of many carcinogens are released by industrial pollution.[7] This is actually a false belief. An example of one of the studies which has been performed is that of evaluation of lung cancer cases in Finland and Britain. Britain has a much higher degree of industrial pollution than Finland; however both countries have similar population and statistics for cigarette consumption. The findings were similar in each country verifying that industrial pollution in Britain was not having a detrimental effect on the number of lung cancer victims.[7, 10]

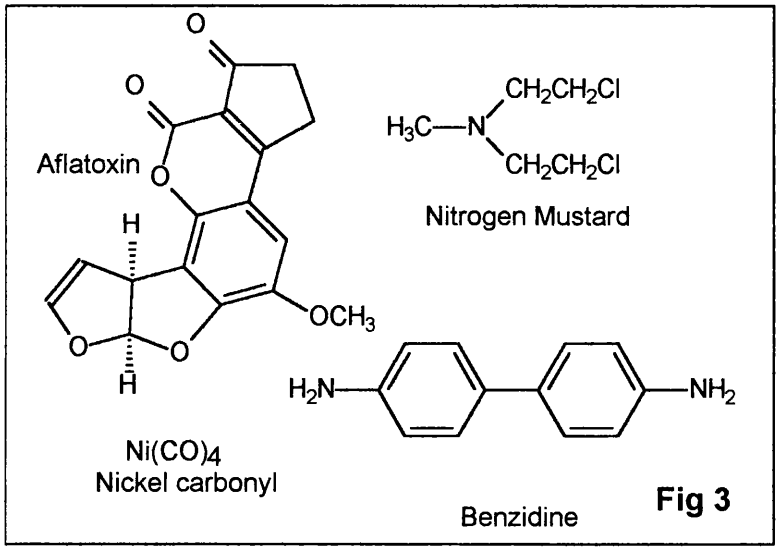


Fig 3

1.5.3 National Population

Examination of the epidemiology of cancers shows that there is an obvious factor which many people neglect to consider. The inhabitants of one country tend to be more prone to suffer from different types of malignancies than in another. **Table 2** shows a selection of high and low incidence rates in countries of specific cancers.

The differences in incidence rates could be due to genetic differences between populations or to environmental factors. The second explanation is more probable as studies have been completed into cancer incidence in immigrants.[7, 11] This indicated that immigrants within a short period of time were showing the same cancer statistics as the inhabitants who had lived in that country all their lives. On this basis it was estimated that up to 80% of cancers may be attributable to environmental factors.

Cancer	**High Incidence area** (per 100,000)	**Low Incidence area** (per 100,000)	**Ratio**
Melanoma	Australia (31)	Japan (0.2)	155
Prostate	U.S.A (91)	China (1.3)	70
Liver	China (34)	Canada (0.7)	49
Cervix	Brazil (83)	Israel (3.0)	28
Stomach	Japan (82)	Kuwait (3.7)	22
Lung	U.S.A (110)	India (5.8)	19
Colon	U.S.A (34)	India (1.8)	19
Brain	New Zealand (9.7)	India (1.1)	9
Breast	Hawaii (94)	Israel (14)	7
Leukaemia	Canada (11.6)	India (2.2)	5

Table 2[8]

1.5.4 Solar Radiation

Solar radiation in the form of ultraviolet light is the major cause of skin cancer. This particular type of cancer is very common but rarely is it lethal. In the USA there are around 600,000 cases of melanoma skin cancers (basal and squamous cell carcinomas) every year of which all are thought to be from exposure to sunlight. For comparison lung cancer has the highest cancer mortality rate in the USA. The incidence of lung cancer is around 160,000, which is less than 1/3 that of melanoma cases annually. Lung cancer accounts for a staggering 140,000 deaths per year whereas skin cancer accounts for 2,500 deaths per year. Thus skin cancer is responsible for perhaps only 1-2 % of the cancer mortality rates in the USA.[7]

1.5.5 Ionising Radiation

Ionisation is another form of radiation which can cause cancer. The first recorded case of radiation dermatitis was quoted by Haagensen in October 1902 at Hamburg Medical school. The patient was a worker from a factory which manufactured Roentgen tubes. He used his right hand as a test object in 1898. Two years later an ulcer developed on the irradiated area which turned out to be a carcinoma. The arm was amputated in 1902, but unfortunately reoccurrence of the cancer resulted in death in 1906.[12]

1.5.6 Medical Exposure

Around 250 million X-rays are performed each year in the USA, and this is a significant source of radiation. The average dose for diagnostic examinations is 0.5 rem, which would relate to induction of cancer of one in one million patients. This accounts for 250 cancers per year.[7]

1.5.7 Radon and Radium

A major source of radiation exposure is from radon in the home. Radon is formed as a product from the decay of uranium underground which seeps into houses through the floorboards. Many homes which have high levels of radon gas have higher lung cancer incidence rates than normal. Identification and modification of these types of houses would dramatically reduce this risk.[7]

Radon as a source of radiation has been known for centuries. Knowledge dates as far back as the 16th century, in the Erzgebirge (ore mountains) which separate Saxony from Bohemia, and in the uranium mines of Joachimsthal and Scheeberg.[6] Cases of lung cancer were attributed to working in an atmosphere high in radon.[13]

Radium induced cancers have also been known since 1896 with Becquerel's discovery. An example is that of women employed as watch dial painters who used luminous paint and habitually placed their brushes between their lips thus digesting small amounts of radium. Over a prolonged period of time this led to cases of aplastic anaemia, necrosis of the jaw and sarcomas.[6]

1.5.8 Diet

Variations in diet can increase the risk of developing some cancers as well as being capable of decreasing the risk of other cancers. There are many potential carcinogens which are found in food. But there are also many other dietary components which may prevent or decrease the risk of the development of certain cancers. This is also considered to be an environmental factor as eating habits differ in most countries.

Diets which are high in calories and fat have been linked to increased risk of cancer of the breast and colon.[14] Obesity is also related to increased cancer risks of the uterine endometrium (epithelial lining of the uterus) which reflects a high calorie diet. This may be attributable to the increased levels of oestrogens produced by the ovaries, which act to stimulate the proliferation of endometrial cells. Fat cells also produce oestrogen and contribute significantly to high levels of the hormone in post menopausal women.[7]

On the other hand diets which are high in certain vitamins, selenium[15] and compounds present in some vegetables, have been suggested to reduce cancer. Diets high in fresh fruits and vegetables are associated with decreased risk of many cancers. Diets plentiful in vitamin A and related compounds (retinoids) have been shown to block the development of epithelial cancers and high vitamin C diets have shown decreased risk of stomach cancer.[7]

1.6.1 Oncogenes

Oncogenes are genes which are capable of inducing one or more characteristics of cancer.[7] They are generally formed from proto-oncogenes during carcinogenesis.

Biologically active oncogenes are frequently found in carcinogenic cells and are absent from normal healthy cells. This would indicate that oncogenes are not genetically transmitted from parent to child. Instead, they are generated specifically in tumour cells as a consequence of genetic alterations on proto-oncogenes during the process of carcinogenesis. Once an active oncogene is formed it acts to drive abnormal cell proliferation, thus contributing to the development of the neoplasm.

The first known human oncogene was identified from a bladder tumour. The DNA of the bladder carcinoma was found to induce transformation of the recipient mouse cells efficiently, indicating that it contained a biologically active oncogene. Since the original discovery more than a dozen oncogene types have been identified in similar experiments.

The family of oncogenes which are most frequently involved in human carconogenesis is *ras*.[16, 17] Mutations which alter only a single nucleotide of the *ras* gene sequence suffice to convert normally functioning *ras* proto-oncogenes into potent oncogenes which efficiently induce neoplastic transformation. These mutations alter single amino acids at critical regulatory positions of the *ras* proteins, with the result that the *ras* oncogene proteins function in an uncontrolled manner. The *ras* proto-oncogenes are involved in the intracellular transmission of proliferative signals. Their activity within the cell is generally tightly controlled and they normally function as part of the response initiated by the growth factor binding to cell surface receptors. The proteins encoded by mutated *ras* oncogenes however are no longer properly controlled by cellular regulatory signals. Instead the oncogene proteins function in a continuous unregulated fashion leading to the abnormal stimulation of cell proliferation.

Gene amplification is a second method by which oncogenes are activated.[7] It occurs frequently in cancer cells resulting in increasing numbers of gene copying which leads to the overexpression of the amplified gene. A family of genes which is frequently amplified is that of the *myc* oncogene. These genes are frequently found

overexpressed in a variety of tumours such as cancer of the breast and lung and neuroblastomas.

1.6.2 Tumour Suppressor Genes

In contrast to oncogenes which initiate the cancer process there are tumour supressor genes which inhibit the development of cancer.[7] The function of tumour suppressor genes was first illustrated in experiments where normal cells and cancer cells were fused with each other. The resulting hybrid cells contain genes from both the parent cells and no longer have the full characteristics of the cancer cell. The tumourigenicity of the parent cancer cell is thus inhibited by the fusion of the cells which indicates that the genes derived from the normal parent cell act to suppress the tumour growth.[18]

An example of this type of suppressor gene is that of the *p53* gene.[7,8] This gene is found on the short arm of chromosome 17 and is frequently found as a mutated gene in human cancers such as brain, breast, colorectal, oesophageal, hepatocellular and lung carcinoma, osteosarcoma, rhabdomyosarcomas, leukaemias and lymphomas. The loss, mutation or inactivation of *p53* has been constantly observed in the above cancers.[19] Recently it has also been noted that inherited mutations of *p53* are responsible for the Li-Fraumeni cancer family syndrome, which is associated with the development of sarcomas, breast and brain tumours and leukaemias.[19] The inactivation of *p53* has been observed in ~ 50% of all types of lung cancer and the majority of breast and colorectal carcinomas.

Now that the causes of cancer have been discussed the next chapter will be concerned with the treatments of the disease and physical phenomena which may be exploited in the employment of treatment.

Chapter 2

2.1.1 Diagnosis

The early diagnosis of many forms of cancer will dramatically increase the possibility of a complete recovery without reoccurrence due to the nature of tumour development.

Prior to metastasis most cancers can be cured by localised treatment such as surgery or radiotherapy. After metastasis has occurred more dramatic treatments are employed in combination therapy such as chemotherapy, surgery and radiotherapy. Thus early diagnosis is imperative for positive prognosis.

One method for early diagnosis which is proving to be effective in certain cases is that of screening.

A fine example of the effectiveness of screening (if possible) is the Pap smear test. This was developed in the 1930s as a screening programme for cervical cancer. It is estimated that regular screening by Pap smear would prevent over 90% of deaths from cervical cancer. Such screening has in fact reduced the reported cases by 75% since 1940. Advantages of using the the Pap test, apart from early diagnosis, are that it is relatively inexpensive, safe, reliable and involves minimal discomfort for the patient.[20]

A second type of screening involving women is that for breast cancer. With an incidence of 150,000 cases per annum in the USA it accounts for about 30% of all female cancers. 1 in 10 women will suffer from this type of cancer even with effective mammograph screening. But it is very beneficial to catch this cancer in the early stages where it is likely that it could be controlled with minimal invasive treatments employed. The use of mammography is generally restricted to women over the age of 40. There are, however, a few disadvantages in the use of mammographs. These are the potential risk of exposure to X-rays, expense, the slight discomfort to the patient and the frequency of false results. The benefits outweigh the disadvantages immensely. Frequent physical examinations and self assessment of breasts are also encouraged in women of the age 40 and over to aid early diagnosis.[21] Younger women are also encouraged to carry out self assessment of their breasts.

Screening is employed in the diagnosis of colorectal cancer[20] and melanoma. The skin is easily accessible which means that pre-existing naevia can be studied for malignant change. There are problems here, however, due to the lack of definition of who should be screened. Even restricting the screening to identifiable risk factors such as large numbers of common naevi, freckling tendency, history of severe sunburn and atypical naevi would still mean that a large proportion of the population would require screening. Pancreatic cancer is also screened for in cases where there may be an inherited predisposition, with a certain amount of success. Tumour markers (discussed **2.1.2**) are used as a diagnostic tool in this case.

Generally annual physical examinations are recommended for people over the age of 40 including examination of the lymph nodes, oral cavity, prostate, testes, ovaries and thyroid as well as breast, cervix and colorectal cancers to aid early diagnosis. Unfortunately early detection may not be possible in many cases and symptoms may well be the first evidence of existing cancers.

The American Cancer Society has designed a method of calling attention to some of the early signs of many cancers which may or may not indicate the presence of cancers but will at least bring attention to the specific areas where symptoms exist and need treatment. Interestingly the symptoms spell out CAUTION![7]

- **C**hange of bowel or bladder habits.
- **A** sore that does not heal.
- **U**nusual bleeding or discharge.
- **T**hickening or lump in breast or elsewhere.
- **I**ndigestion or difficulty in swallowing.
- **O**bvious change in wart or mole.
- **N**agging cough or hoarseness.

2.1.2 Tumour Markers

Some cancers may be detected by substances known as tumour markers. These are substances which are found at higher than normal concentrations in the blood, urine or body tissues of some cancer patients.[22] A tumour marker may be produced by either the tumour itself or by the body in response to the presence of the cancerous growth. This type of phenomenon can at times be employed as a diagnostic tool in combination with other methods. It is not used singly due to the

fact that many markers are found in non-cancerous patients; not every cancer has tumour markers; and few markers are specific to any one tumour type.

There is, however, ongoing research into the possibilities of specific markers for tumour types and utilising this to detect tumours before symptoms appear and also to follow the progression of the disease.

Carcinembryonic antigen (CEA)[22] is a protein which is considered to be a tumour marker as more than half of the sufferers of colon, pancreas, stomach, lung and breast cancers have elevated CEA levels. Patients with ulcerative colitis, liver disease and lung infections have been shown to have elevated CEA levels but not as high as in the first examples.

Another tumour marker is prostate-specific antigen (PSA)[22] which is produced by both normal and abnormal prostate cells. The levels of the antigen are useful for monitoring the effectiveness of the treatment over time rather than purely as a diagnostic tool.

Other examples are prostatic acid phosphatase, CA 125, alpha-fetoprotein, human chorionic gonadotrophin, lactate dehydrogenase and neuron specific enolase which are all used as either diagnostic or monitoring tools.

A new method which is currently employed in detection of whether a cancer will spread is the use of a Neoprobe detector.[22] The physician uses the device to inject a radioactive dye into the original tumour and by placing the device around the tumour the sentinal lymph node, i.e. the lymph node to which a cancer is most likely to spread, can be detected. Thus scanning for radioactivity can identify the sentinal node as the one with most radioactivity. This detection system has been shown to be most effective in cases of melanoma and is now used in approximately 80% of such cases.[22]

2.1.3 Staging of Cancer

In order to treat the cancer it must first be established to what stage the cancer has developed. This is essential for determining the treatment to employ and likely prognosis. The concept of staging is applicable to most forms of cancer apart from leukaemias, since they involve the blood and are not anatomically localised.

The staging system is made up of two parts. These are grouped into four classes denoted by Roman numerals I-IV. Stage I cancers are usually small, localised and curable. Stage IV cancers are usually inoperable or have spread to be metastatic cancers. This definition varies from one cancer type to another and the prognosis will also differ, e.g. a stage II non small lung cancer will have a different prognosis than a stage II cervical cancer.[23]

The second method of staging is using the TNM system, where T stands for tumour, N for nodes and M for metastasis. T represents the tumour extent 0-4. T_0 is a tumour which has not started to invade local sites. N classifies the extent of regional lymph node involvement. This definition depends on the cancer type. N_0 means no lymph node involvement and N_4 indicates extensive involvement. M represents if there are metastases present, M_1, or not M_0. For example $T_1N_1M_0$ is defined as a tumour of type T_1, N_1 lymph node involvement and M_0 meaning no metastases.[7, 23]

A third method of cancer classification is that of the aggressiveness of the tumour growth. This refers to the appearance of the cells or to the percentage that appear to be dividing. This is graded I to IV from least to most aggressive. This is more important in some cancers than in others, e.g. it is very important in brain tumours and lymphomas.[23]

2.2.1 Treatments

After the cancer has been diagnosed and staged the first step on the ladder to recovery is to establish which is the best treatment to employ.

In general there is no one "cure" for cancer. This is because there are over 200 different types of cancer which are treated differently depending on the type and stage of the cancer. A blanket "cure" has not yet been found and is unlikely to be in the near future.

A cancer is classed as being cured if the neoplasm recedes (or is removed) and does not reappear within five years of treatment. In some cases the cancer may redevelop but has still been classed as cured if the reoccurence is not within the five year limit.

There are three main types of treatment for cancer. These are surgery, radiotherapy and chemotherapy.[7, 24] These are of varying value in different situations.

Frequently the cancer is treated by more than one, or even all three methods of therapy. Depending on the stage of the cancer the management may be as a curative or a palliative measure as many terminal patients only require treatment to ease pain and prolong life.

2.2.2 Surgery

Surgery is generally employed when the tumour growth is caught in the early stages of development. It is only successful if the neoplasm is localised and has not undergone metastasis and of course if it is accessible to the surgeon.

Surgery has proven to be effective in cases of malignant melanoma which is frequently identified early as the changes in colour of lesions etc. on the skin are quite noticeable. The prognosis for these patients is quite good if the growth is excised promptly. Unfortunately as many cancers start from inside the body it is often too late for this type of treatment to be successful in many patients as approximately 70% of cancers have already metastasised by the time of diagnosis.[24]

2.2.3 Uses of Surgery

Surgery is used principally as a curative treatment in cancers such as colorectal, breast, skin, stomach, female genital organs and prostate with a reasonable amount of success. It is also employed as palliative therapy in amputation of extremities, resection of infected, bleeding or obstructive cancers, severance of sensory nerve tracts and in cancers of the stomach, colorectal, lung, liver, pancreas and oesophagus.[24] This type of therapy is more often than not used in combination with radiotherapy and/or chemotherapy.

2.2.4 Radiotherapy

Radiation is a DNA mutation initiator but in many cases can be used as a method of treatment. This is a type of treatment employed during Stages I and II of the disease with most success. Generally, in developed countries, around 80% of patients survive five years after treatment. This falls to around 20% patient survival rate if the disease is in the later III-IV Stages. Similar to surgery this type of

treatment is primarily for localised cancers but can also be used for cells which have spread through normal tissue beyond the scope of surgery.

A drawback of this procedure is that it is not selective to cancer cells alone but kills normal healthy cells, thus it may be highly toxic to the patient. This limits the use of such treatment.

There are five types of radiotherapy which are employed. These use photons, electrons, neutrons, protons and heavy nuclei.

The primary action of radiation is to damage the DNA directly or by the generation of chemical species such as oxygen free radicals within the cell. Epithelial cells are very sensitive to radiation and cause unwanted side effects such as anaemia, nausea, skin damage, hair loss and sterility. The extent of these depends on the amount of radiation and to which area of the body it is delivered.

Radiotherapy is sometimes used instead of surgery in cases such as cancer of the cervix, oesophagus and oral cavity where the radiation can be directed specifically at the tumour leaving the normal healthy cells unharmed. Hodgkin's disease is a form of lymphoma and is often frequently treated by direct radiation of the lymph nodes.[24]

2.2.5 Uses of Radiotherapy

Radiotherapy is used as a principally curative treatment in cancers of the breast, female genital organs, skin, lymphomas, primary brain tumours, prostate and unresectable sarcomas. Alternatively it can be used as a palliative treatment for bone or intracranial metastasis, control of chronic bleeding, cancer of the lung or reduction of elevated intracranial pressure. A factor which must also be considered is that cancers have varying degrees of radiosensitivity. Lymphomas and leukaemias have a high radiosensitivity whereas melanoma, hepatoma and other sarcomas have low sensitivity and respond poorly.[24]

2.2.6 Chemotherapy

There are a variety of different chemotherapeutic agents which are active against cancer *via* different mechanisms. These can be split into several classes

which include alkylating agents, antimetabolites, natural products, anthracyclines and a miscellaneous group.

2.2.6.1 Alkylating agents

In 1962 Ross defined a biological alkylating agent as one which can replace a hydrogen atom by an alkyl group under physiological conditions (pH 7.0 - 7.4, 37 °C).[24, 25] They often have a paradoxical activity whereby they can inhibit tumour activity and also act as potent carcinogens.

Alkylating agents exert their anticancer action by reacting with amines, phosphates, sulfhydryl and hydroxy groups on crucial cellular molecules like nucleic acids, nucleotides and enzymes and thus inhibit their normal activity. The most active alkylating agents are those with two alkylating arms as they can form an intermolecular crosslink between different DNA strands which prevents the DNA from unwinding and replicating. The cell then becomes locked in the S phase and cannot progress through the cell cycle which eventually leads to death of the cell.

Fig 4[8] shows a β-DNA strand illustrating the major and minor grooves. The alkylation can take place in either of the grooves. Specific agents are selective to either major or minor groove which is generally dependent on size and preferred site of alkylation.

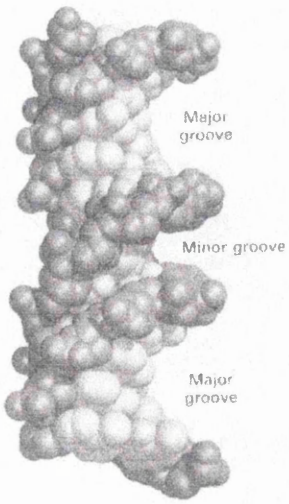


Fig 4

Single alkylation may lead to mispairing of bases or strand breakage which can also ultimately lead to cell death. The most common site of alkylation on the DNA is that of the N-7 position on the guanine base[26] (**Fig 5**).

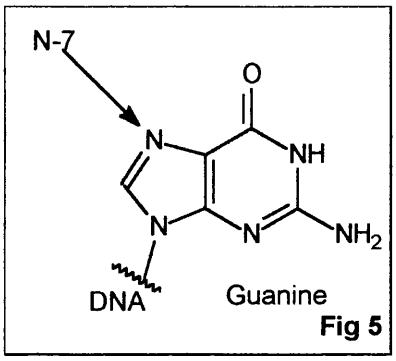


Fig 5

The agents form alkyl adducts either directly or after spontaneous or metabolic breakdown thus inhibiting DNA replication and transcription. Steric hindrance limits the access of electrophiles to some nucleophilic sites whereas phosphodiester groups and sugar hydroxyl groups remain largely exposed to electrophilic attack. Some nucleophilic centres of the purine and pyrimidine bases are partially or totally hindered by either:

- their involvement in hydrogen bonds; or
- their position in the interior of the DNA helix and the superposition of the electrostatic potential associated with the phosphate groups and bases themselves which greatly reduce their nucleophilic reactivity.

This is the largest group of anticancer agents and is split into sub groups which are nitrogen mustards, nitrosoureas, alkyl sulfonates, triazenes and aziridines.

Nitrogen mustards generally act via an intramolecular nucleophilic substitution process as shown in **Fig 6,** forming an aziridine ring and crosslinking the DNA if the alkylating agent is bifunctional.

Fig 7 shows the crosslinking that can occur, i.e either inter or intra strand again dependent on size and preferential alkylation site sequence.

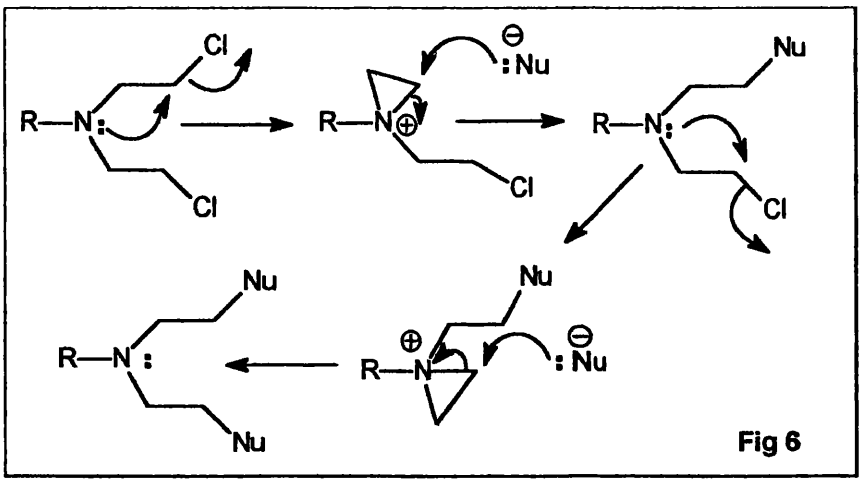
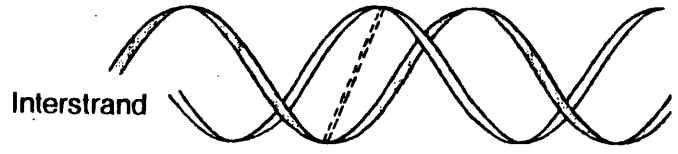


Fig 6



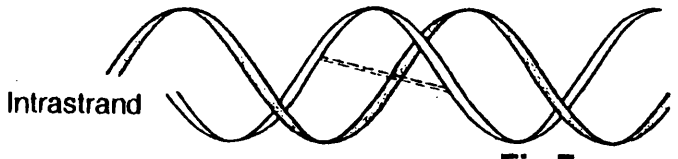


Fig 7

The war gas sulfur mustard $(ClCH_2CH_2)_2S$ was the first known alkylating agent[3] originally discovered during the second world war. Examples of *N*-mustards are mechlorethamine, chlorambucil, melphalan and lomustine. These are the most commonly employed group of anticancer agents.

Mechlorethamine was the first clinical chemotherapeutic agent used and is still in use today in Hodgkin's disease and is administered in stage IV of the progression of the disease.[3] Other cancers where this drug is administered are of mycosis fungoids and malignant, pleural and pericordial effusions.[3]

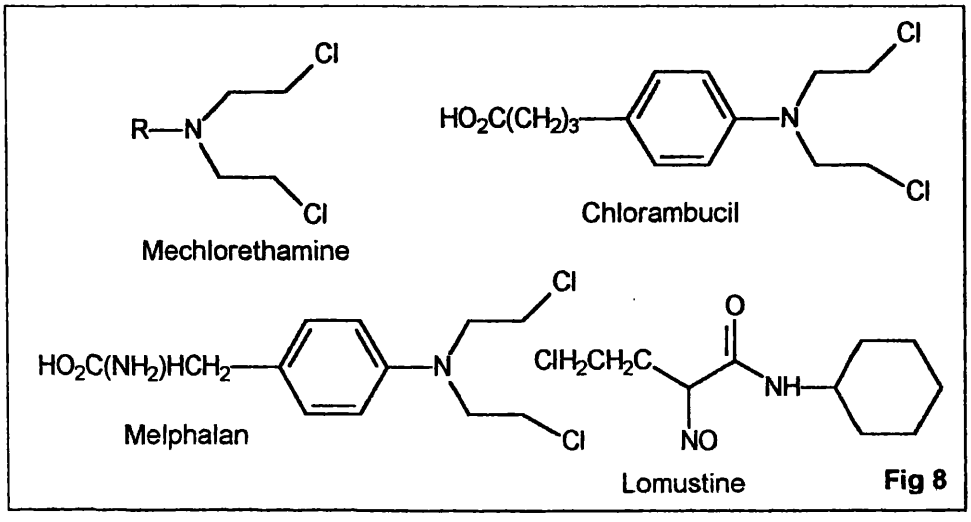


Fig 8

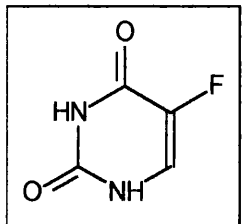
Chlorambucil is another alkylating agent in use today. This drug frequently encounters resistance which is attributable to cellular protective mechanisms. It is the phenylbutyric acid derivative of *N*-mustard and is used in the treatment of lymphoma, ovarian cancer and breast cancer. Combined with prednisone it is currently a standard treatment for chronic lymphocytic leukaemia.[3]

Melphalan is the phenylalanine derivative of *N*-mustard which was first synthesised in 1953 and is employed in the treatment of melanoma, breast cancer, ovarian cancer and multiple myeloma.[3] Finally lomustine is a rare example of an alkylating agent which has good activity against tumours of the brain. This is due to its high lipophilicity.[3]

2.2.6.2 Antimetabolites

Antimetabolites are compounds with structures which are very similar to those of essential metabolites. They act by inhibiting the metabolising enzymes. Antimetabolites are taken up by the rapidly replicating cells; therefore these cells are selectively inhibited and antimetabolites are S phase specific. The selective toxicity in this case derives from a difference in rate of growth rather than a qualitative difference in metabolism. Antimetabolites are primarily useful in rapidly growing lymphomas and leukaemias rather than slower growing solid tumours.

The main metabolite analogues are the purine and pyrimidine containing compounds such are 5-fluorouracil, methotrexate and 6- mercaptopurine involved in the biosynthesis of nucleic acids. They affect the biosynthesis of nucleic acids. This results in the cell being unable to produce purines or pyrimidines, thus ultimately DNA cannot be produced.



5-Fluorouracil

Fig 9

5-Fluorouracil[3] (**Fig 9**) is important as it is based on biochemical rationale and is extensively used in breast, gastrointestinal tract, ovary, bladder, prostate, pancreas and hepatoma cancers.

One mode of action of 5-fluorouracil is by the inhibition of thymidylate synthase (shown in **Fig 10**), which is essential for the biosynthesis of pyrimidines. It also acts by becoming fraudulently incorporated into RNA in the form of 5-fluorouracil nucleotides and so causes confusion in base pairing during the transcription process.

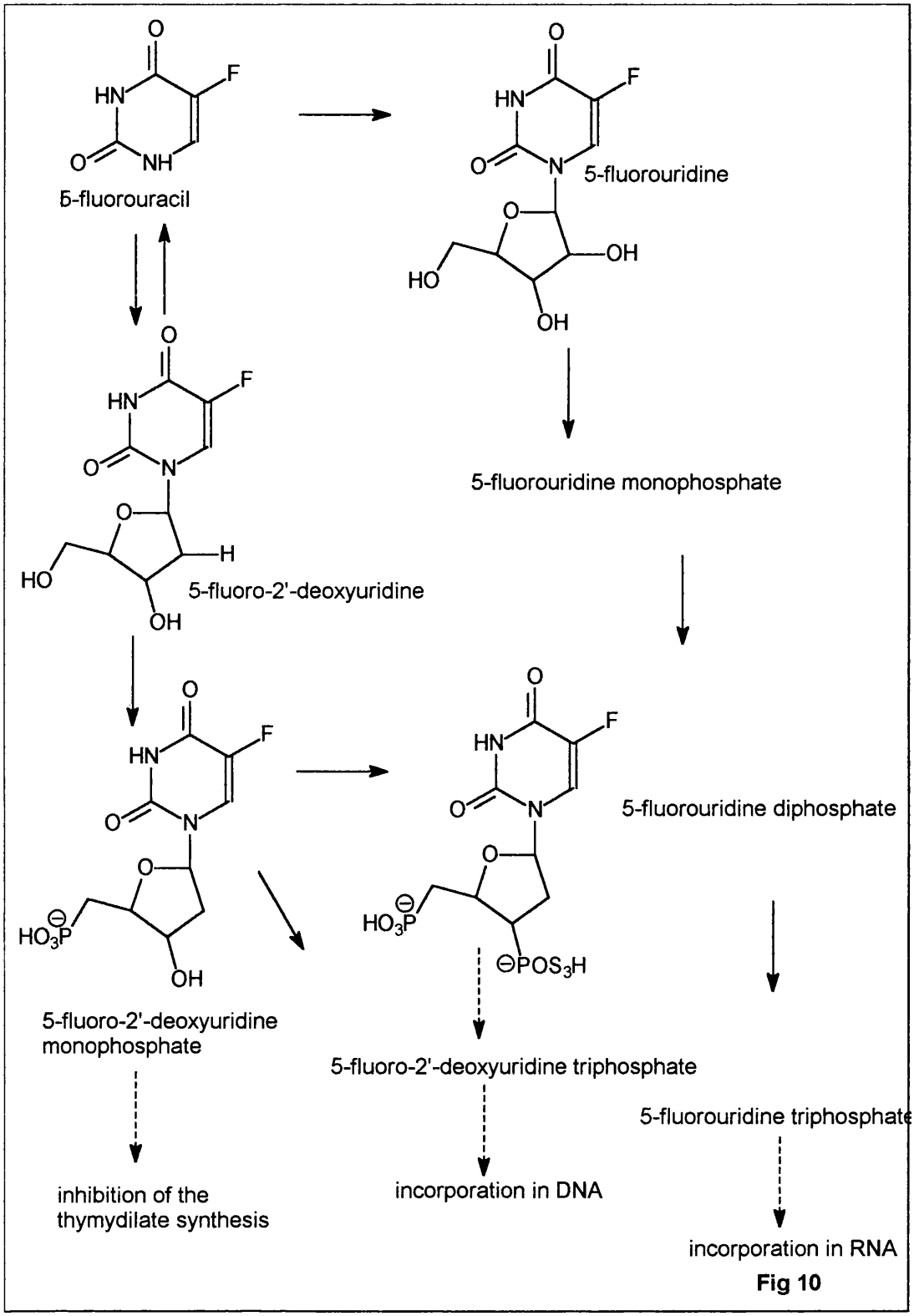


Fig 10

Methotrexate (**Fig 11**) is an analogue of folic acid and is effective against breast cancer and acute leukaemia. Its mode of action is by the inhibition of dihydrofolate reductase (DHFR), the enzyme which effects the reduction of folic acid. Without DHFR the cells are deprived of key metabolic intermediates which are needed to form nucleotides and ultimately nucleic acids.

Methotrexate inhibits the enzyme DHFR and since the affinity for methotrexate is much larger than that for dihydrofolic acid, the enzyme will preferentially bind to the antimetabolite. This results in the cell being unable to produce purines or pyrimidines, thus ultimately DNA cannot be produced.

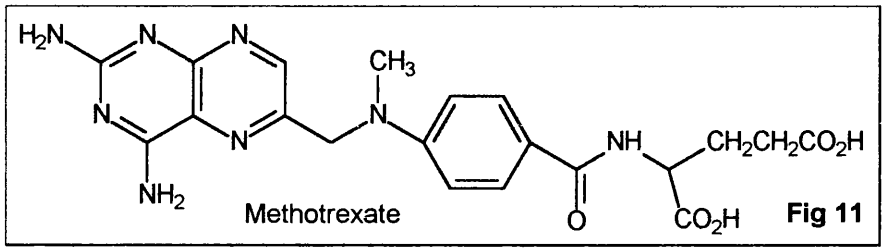


Fig 11

2.2.6.3 Natural Products

This is a group which is wide and varied in both structure and mode of action of inhibition of cancer. The group includes doxorubicin, bleomycin, podophyllotoxins, the Vinca alkaloids and taxol.

Taxol (**Fig 12**), otherwise known as Paclitaxel, was approved for sale by the US Food and Drug administration in 1992 due to its significant activity in the treatment of patients with refractory ovarian cancer. In 1994 it was then administered for breast cancer. It is highly likely that it will also be approved for other cancers such as that of the lung.[27, 28]

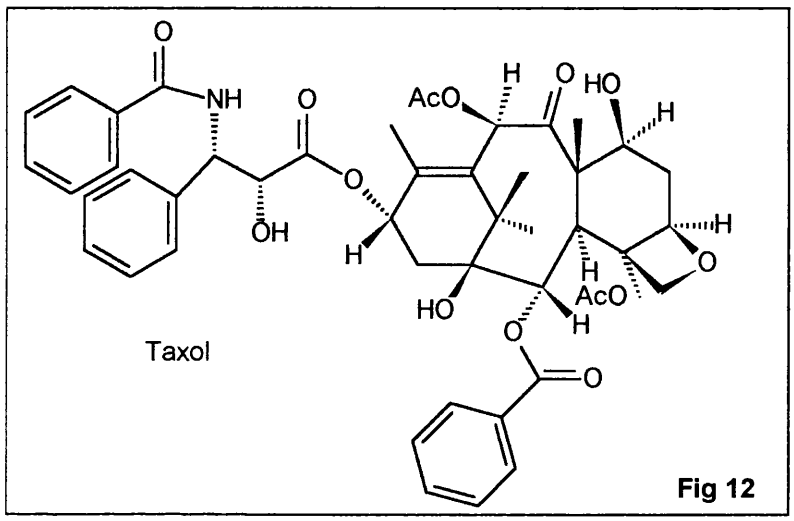


Fig 12

Taxol is an antitubulin agent which promotes the assembly of microtubules, resulting in a highly stable, non functional polymer.[29-31] There is however a problem with the supply of this drug as a suitable industrial synthetic procedure is still in development. Further development of this agent may result in an important treatment for cancers which are not presently well controlled.

2.2.6.4 Vinca alkaloids

Vincristine and vinblastine (**Fig 13**) are constituents of the Madagascan periwinkle plant *Vinca rosea* and act by binding to tubulin protein which inhibits polymerisation. They are generally administered in stage IV and are excreted by the biliary tract.[3]

Vinblastine is used in combination chemotherapy in testicular therapy, but has the drawback of being highly toxic to bone marrow, Vincristine is used in childhood leukaemia and is toxic to the CNS. When used in combination chemotherapy vincristine has revolutionised the treatment of childhood leukaemia by inducing complete remission in 90 - 100% of cases where it is employed.

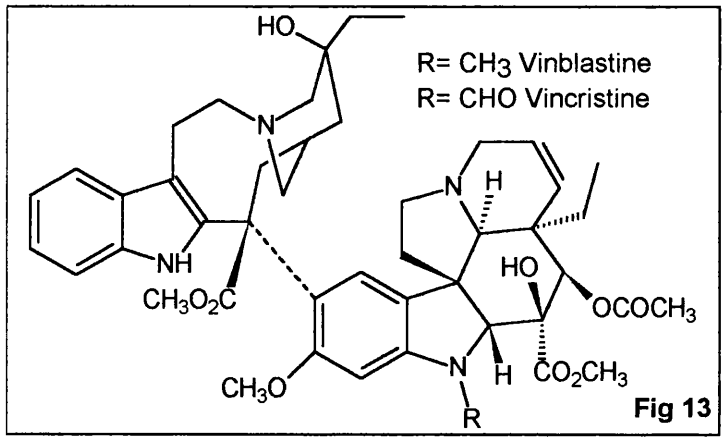


Fig 13

2.2.6.5 Anthracycline Antibiotics

This group of antitumour agents has an activity spectrum which is second only to that of alkylating agents. They are also the most important of the natural product anticancer agents.[3]

There are at least 5 potential mechanisms for action:

- intercalation between DNA strands;
- membrane binding which alters the permeability of the cell and may change the pattern of ion transport;
- radical formation under enzymatic transformations leading to indiscriminate alkylation;
- chelation of metal ions, thus impairing cell function and possibly forming toxic organometallic compounds;
- direct alkylation.

Examples of this class are doxorubicin (Adriamycin) and daunorubicin (Daunomycin) (**Fig 14**) which are isolated from different species of *Streptomyces*.

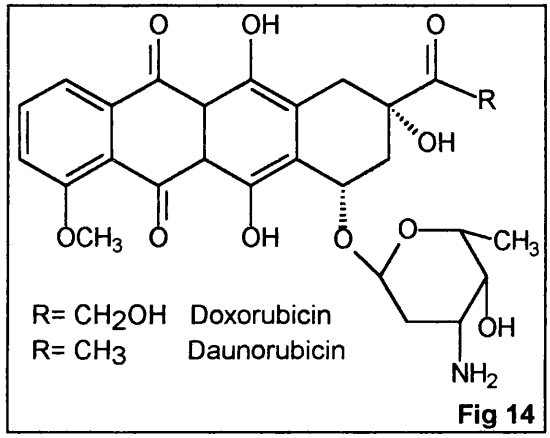


Fig 14

Despite the wide and varied spectrum of activity of these types of agents they have limited use due to their toxicity and unwanted side effects.

2.2.6.6 Miscellaneous

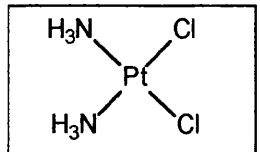
There are several compounds which do not fit into any of the above classifications. Hydroxyurea is one example which acts by inhibiting ribonucleotide reductases. This is the enzyme that generates the deoxyribose nucleotides necessary for DNA synthesis.

Another example is L-asparaginase which is the enzyme which destroys leukaemic cells by depriving them of the L-asparagine they require for survival.[3]

Another important example is that of cisplatin (**Fig 15**).

Cisplatin is an example of a platinum complex which again acts by crosslinkng DNA.[3] It acts in four ways:

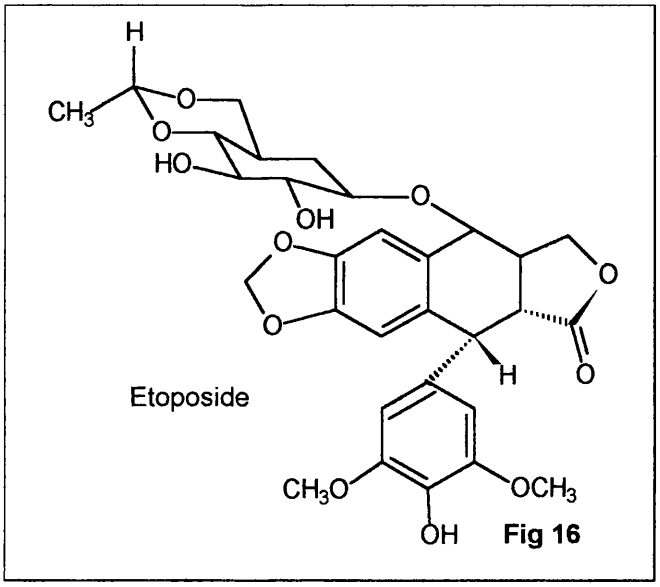
- it binds to DNA at specific sites;
- it selectively inhibits the DNA synthesis over RNA and protein synthesis;
- it is more toxic to cells which are deficient in DNA repair;
- for equal number of DNA lesions, cisplatin inhibits the DNA synthesis to a greater extent than the *trans* -isomer which has no antitumour activity.



Cisplatin

Fig 15

The podophyllotoxin derivatives, e.g. etoposide[26] (**Fig 16**), which are extracted from roots and rhizomes of *Podophyllum peltatum* and *P. emadi* and are formed into semi-synthetic derivatives. This drug is widely used in combination with drugs such as cisplatin in the treatment of testicular teratoma, lymphoma, small cell lung cancer, leukaemia and Kaposi's sarcoma. The method of action is by *O*-demethylation to form the *ortho*-dihydroxy (catechol) derivative which may be involved in covalent binding to cellular macromolecules. Further reaction to form the *ortho*-quinone probably involves the formation of a semiquinone free radical which may also cause DNA strand breakage.



Etoposide

Fig 16

2.2.7 Treatments by the Immune System

Biological therapies are known to use the body's immune system to fight cancer or to lessen the side effects which are often caused by some of the aforementioned treatments. The immune system itself is a complex network of organs and cells which work together to defend the body against any foreign invader. Research has proven that the immune system can recognise the difference between normal healthy cells and those of cancerous cells and can attempt to eliminate the latter. This system may, however, breakdown or become overwhelmed by these cells thus allowing the cancer cells to continue to proliferate.

Biological systems can be designed to repair, stimulate or enhance the natural anticancer function. They are often known as biological response modifiers (BRMs). In general they alter the interaction between the body's immune defences and the cancer. BRMs exist naturally in the body as cytokines and antibodies.

However, it is now possible to synthesise BRMs which can:[32]

- inhibit tumour cell growth;
- enhance the immune system against the growth;
- eliminate, regulate or suppress the body response that permits cancer cell growth by the immune system;
- make cancer cells more susceptible to destruction by the immune system;
- alter the cells growth patterns to promote behaviour similar to that of a healthy cell;
- block or reverse the process that changes healthy cells into cancerous ones;
- enhance the ability to repair normal cell damage by other forms of cancer treatment;
- prevent a cancer cell from spreading to other sites of the body.

These agents include a group called interferons which are a type of cytokine. Of the three types alpha is the most widely used in cancer treatments such as hairy cell leukaemia, Kaposi's sarcoma (a rare cancer of the cells lining blood vessels which often occurs in AIDS patients) and chronic myelogenous leukaemia.[32]

Another agent class is the group of interleukins. These are made in the body. IL-2 (5 classes) has been studied most widely. It stimulates the activities of the immune cells such as lymphocytes which can destroy the cancer cells. In studies

patients with advanced renal cell carcinoma or advanced melanoma have been shown to have the best response to IL-2 therapy.[32]

The tumour necreosis factor (TNF) is another type of cytokine, similar to the previous example in that it stimulates the immune system to fight the cancer. TNF, however, also directly affects the tumour cells damaging them and the blood vessels within the cancer. Mechanisms of the action which create the damage are unclear. Combined with the fact that TNF is extremely toxic it has been found to act best (and with least unwanted toxicity) when directed at a specific tumour site rather than being administered throughout the body.[32]

Colony stimulating factors (CSFs), sometimes called hematopoietic growth factors, on the other hand do not directly effect the tumour cells but encourage bone marrow cells to divide and develop into specialised platelets, white and red blood cells. Bone marrow is important to the body's immune system because it is the source of all blood cells. CSFs have been found to be particularly beneficial when employed in combination with high dose chemotherapy. This is because the drugs affect the ability to make the white blood cells which are responsible for fighting the cancer.[32]

Investigations into the use of agents such as these are ongoing.

In conclusion of this section BRMs show promising developments for the future of treatment for many cancers in a less harsh manner than classical methods employed.

2.3.1 Chemoprevention

As it has now been realised that cancer is a disease which is caused by many factors, a simple answer would be to eradicate the possible risk areas. Chemoprevention may be an alternative method of cancer control.

There are a variety of methods which can be employed here. Firstly there is the use of diet (previously mentioned in **Chapter 1**).

Dietary manipulation is a chemopreventative measure which can be employed as diets which are high in certain foodstuffs can promote the development of cancer, whereas diets high in other foodstuffs can help prevent certain cancers. An example of this is in head, neck and oral cavity cancers which are found predominantly in areas where smoking levels, tobacco chewing and alcohol intake levels are high.

The obvious answer would be to cut out the intake of carcinogenic compounds. However, to alter the general public's behaviour towards activities which have been enjoyed for long periods of time takes much more than the cases of cancers which are related to such pastimes.[33]

If you alter the diet this may have an effect. For example, retinoids, carotenoids and vitamin E have all been shown to prevent the progress of chemically induced carcinogenesis in animal studies.[33] The mechanism of the action is as yet unclear but is thought to be that the retinoids affect the cell differentiation by stimulating cells which are depressed during carcinogenesis and by inducing apoptosis. They are also thought to modulate the expression of a variety of genes involved in growth and differentiation. ß-Carotene acts as an antioxidant. It is a potent quencher of free radicals such as singlet oxygen as well as acting as an immunostimulant. Vitamin E also acts as a free radical scavenger and inhibits peroxidation at quite high oxygen pressure. This means that its mode of protection is by inhibiting lipid peroxidation and the damage caused by free radicals. Similarly to ß-carotene it acts as an immunostimulator.

As yet altering dietary components as a strategy to prevent head and neck cancers is a bit premature.

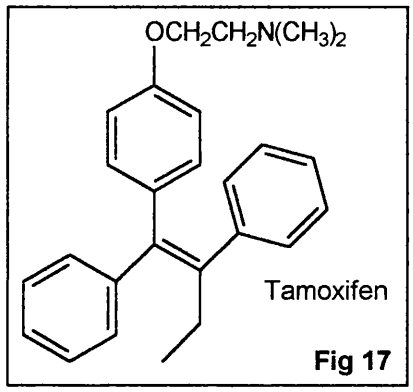
A second method where chemopreventative measures are being investigated is in the prevention of the development of liver cancer. Liver cancer manifests itself mainly as hepatocarcinomas (HCCs) and ranks as the 8th most common site for cancerous growths. It is a priority health problem in sub-Saharan Africa, East Asia, South East Asia and Melanesia. Over 90% of HCCs are related to environmental exposures. Chronic hepatitis, exposure to aflatoxins through food contamination and high alcohol consumption are though to be several of the main factors. In developing countries the exposure to hepatitis is thought to account for the majority of the HCC cases. Could this be prevented? The answer is yes, possibly through vaccination.

Oltipraz (5-2[pyrazinyl]-4-methyl-1,2-dithiole-3-thione) has been used as a single dose antischistosomal drug which effects the metabolism of aflatoxin B1 in rats. It acts by inducing the formation of glutatione S- transferase. The development of HCCs in rats has been shown to be inhibited with low doses of Oltripaz (0.075% Oltripaz with 25µg aflatoxin B1 *per oz* of body weight for two weeks gave a protection of 100%).[33] This drug is in phase I and II trials to establish the safe doses and

metabolic pathways during chronic administration. It is exhibiting some very promising properties as a chemopreventative agent in populations exposed to high doses of aflatoxins.

2.3.2 Tamoxifen

Tamoxifen, also known as Nolvadex, (**Fig 17**), is a drug which has been used in clinical practice for more than 20 years.[3] It is used to treat breast cancer in post-menopausal women in more than 100 countries. It acts by interfering with the activity of the female hormone oestrogen. Some breast cancer cells are oestrogen sensitive. The hormone binds to these cells and stimulates them to grow and divide. Tamoxifen prevents the binding of oestrogen. It may also benefit patients whose cells are not oestrogen sensitive by giving the effects of hormone replacement therapy. Other benefits of tamoxifen are decreased risk of osteoporosis and heart disease. However there are several disadvantages such as increased risk of cataracts, depression and cancer of the uterus.



Tamoxifen

Fig 17

Recently trials in the employment of this drug as a chemopreventative agent have been run. Promising results have been obtained. NCI have reported that from a 6 year study the use of tamoxifen has dramatically reduced the risk of developing breast cancer in women who are at risk by nearly half from 1 in 130 to 1 in 236.[23, 34]

Thus there appears to be great potential in the development in this area as a chemopreventative measure.

2.4.1 Selectivity & Multidrug Resistance

One of the major limiting factors in the development of chemotherapeutic treatment of cancer is that anticancer agents currently employed are not selective to cancer cells alone but kill normal healthy cells as well.

Selectivity can be encompassed on three levels, primary, secondary and tertiary.[38]

The primary (subcellular) level is the area in cell division which is controlled by small molecules that initiate the processes by binding to transmembrane receptors which in turn pass the signal onto the growth transduction pathway to the nucleus. There are two broad classes of potential target molecules in this area. These are oncogenes (previously discussed) and their nucleic acid proteins, e.g. mRNAs, and secondly the protein products. The first group includes antisense oligionucleotides[35, 36] and DNA minor groove binding drugs.[37, 38] The second group includes those aimed at selectively inhibiting protein function in the growth transduction pathway.[40, 41]

Secondary (cellular) level is where most of the clinically used chemotherapeutic agents act selectively. There is still, however, very poor selectivity, thus their action and cytotoxicity profiles need refining.[37]

On the tertiary (supracellular) level the tumours have a variety of microenvironments defined by the accessibility of oxygen to the cell due to limited vasculature networks. This leads to poor venous drainage, low pH due to increased glycolysis, a primitive vasculature and chronic hypoxia[38, 39] (discussed in **2.6.1**).

A second limiting factor in the fight against cancer successfully using chemotherapy is that of drug resistant tumours. Many tumours may be totally unresponsive to certain anticancer agents. Even in tumours which are initially responsive a small number of drug resistant cells may be left which can proliferate and form another tumour which will be resistant to the original form of treatment. This may be due to gene amplification which occurs in many cancer cells.

Drug resistant tumours may be resistant to more than one type of agent. This is called multidrug resistance (MDR). An explanation for some MDR is that it results from expression of the gene coding of a plasma membrane protein (P-glycoprotein) which acts by pumping a variety of anticancer agents out of the cell before they have time to act, thus leading to resistance to agents such as actinomycin D, daunomycin, doxorubicin, vincristine, vinblastine, mitomycin C, etoposide and teniposide.

A second area of MDR arises from alterations in topoisomerase II leading to resistance to daunomycin, doxorubicin, etoposide and teniposide by reacting with the enzyme and generating DNA breaks. Increased activity of cellular enzymes which repair damage to DNA can also lead to cross resistance of multiple alkylating agents.

A solution to this problem may be by combination chemotherapy, i.e. using a variety of different agents at once. This is useful as it is unlikely that any one cell will be resistant to all chemotherapeutic agents. The trick is discovering the correct combination of anti-tumour effects with limits on the toxicity which may be tolerated by the patient. A working example of this theory is the treatment of testicular cancer with cisplatin, bleomycin and etoposide.

2.5.1 Pharmacokinetics and Pharmacodynamics

The vast majority of conventional antineoplastic agents have a very small margin between the effective dose and the lethal dose, i.e. the therapeutic index is very low.[42]

The selectivity of the agents can be explained by pharmacodynamic and pharmacokinetic considerations.

The pharmacodynamics describe the therapeutic activity and toxicity of the treatment. MDR is a pharmacodynamic consideration which may be multifactorial and includes:[42]

- impaired uptake into cell or its rapid efflux by the P-glycoprotein pump;
- the utilisation of DNA repair;
- the elevation or reduction of target enzymes;
- the induction of protective mechanisms such as glutathione *S*- transferase.

Pharmacokinetics describes the absorption, distribution, metabolism and excretion of a drug. Examples of processes are:[42]

- the bioavailability from site of administration;
- extent of plasma protein binding;
- distribution to poorly vascularised areas or across the blood-brain barrier;
- extent and nature of biotransformation;
- rate and extent of clearance from the systemic circulation.

2.6.1 Hypoxia

As mentioned earlier tumour hypoxia is a phenomenon which is frequently found in solid tumours. If selective use of this feature can be incorporated into anti-tumour agents it would be a major breakthrough in the area.

2.6.2 What is Hypoxia?

Hypoxia is when cells exist in areas where the oxygen concentration is suboptimal for cell growth and metabolism but is high enough to maintain viability.

There are two types of hypoxia. The first of these is chronic or diffusion limited hypoxia. The distance at which molecular oxygen is found is limited to 150 - 200 microns from capilliaries, thus cells this far from capillaries will become hypoxic. This state of hypoxia can last from hours to days. If the cells are further away than this we find areas of necrosis which is when the cells die due to lack of oxygen. Many chronically hypoxic regions are starved of glucose and other nutrients as well as developing a surplus of metabolic waste products.[6, 7, 43]

Secondly we have acute hypoxia resulting from the intermittent opening and closing of tumour blood vessels, producing a more transient shortage of oxygen. This state can last from seconds to minutes. Tumour cell growth often outgrows that of the development of an adequate support system which may lead to the cells being grossly abnormal in appearance and performance.

There are several problems encountered in the existence of hypoxic regions in the treatment of cancer. These are the 2 - 3 fold increase in the resistance to radiation and also the resistance to some conventional anticancer agents, e.g. bleomycin and melphalan, due to the impaired vascular delivery system and the noncycling cell status.[6, 7, 43]

Hypoxia may lead to the overexpression of enzymes which may affect the drug response.

2.7.1 Bioreducibility

A second phenomenon which can be taken advantage of to induce selectivity is that of the hyperexpression of reductase enzymes in solid tumours.[43] Selectivity by

this route is called ' Enzyme Directed Bioreductive Drug Development'. The enzymes concerned are able to catalyse reduction of bioreducible agents.[43]

Thus if we design a prodrug which will undergo metabolic reduction in hypoxic cells to release a toxic species which can then damage biomolecules *in situ* the selectivity would be greatly increased. These are commonly referred to as bioreductive or bioreducible drugs.

As different enzymes are activated to differing extents by different agents we could tailor agents to be activated by the reductases which are active at high levels in tumours and eliminate features which will be activated by enzymes which are predominant in normal tissues.[3]

2.7.2 Bioreducible Agents

There are generally four types of compounds which are capable of undergoing oxygen sensitive biotransformations which are nitro, quinone, *N*- oxides and transition metal complexes.

Nitro (hetero) arenes: These are hypoxic selective cytotoxins because the reduction of the nitro group to more toxic products (**Fig 18**) is inhibited by oxygen. This is due to reoxidation of the initial $1e^-$ reduction product, the radical nitro anion by molecular oxygen.[44] This establishes a $1e^-$ futile cycle in aerobic cells.[45] Under hypoxic conditions, net reduction to the hydroxylamine ($4e^-$s) or amine ($6e^-$s) oxidation is observed. Hypoxia selective cytotoxicity requires the products of the nitro reduction to be more toxic than the superoxide (and other toxic species such as the hydroxyl radical) derived from the action of the $1e^-$ futile cycle.

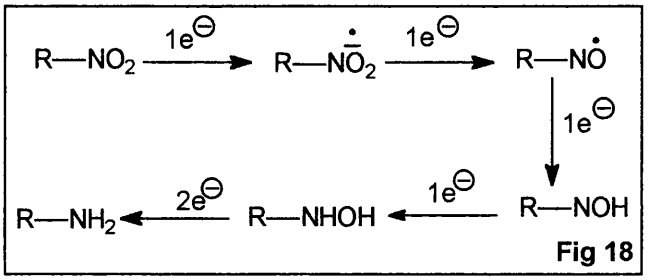


Fig 18

Quinones: The basis for oxygen selectivity is similar to that of the nitroreduction in that the 1 e^- reduction intermediate, in this case the semiquinone, can be reoxidised by oxygen to inhibit net reduction in aerobic cells (**Fig 19**). In some cases, autooxidation of the dihydroquinone can also be appreciable.[46] As for the

nitroarenes, this futile 2 e^- redox cycle generates potentially active oxygen species.[47] Since the dihydroquinones are (usually) less prone to redox cycling, they are substrates for conjugation. The alternative 2 e^- reduction route is generally considered a detoxification pathway.

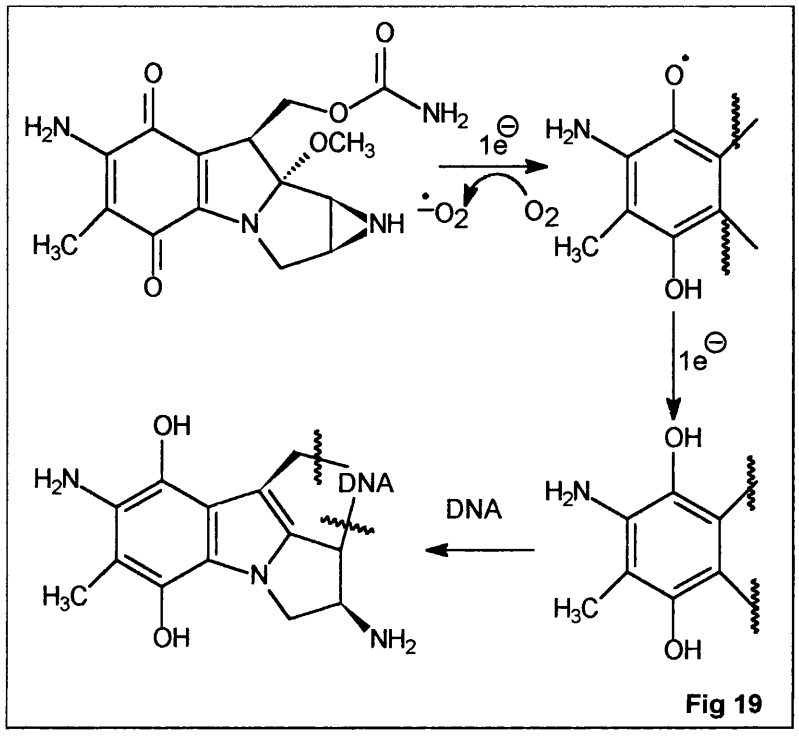


Fig 19

The reduced quinones themselves generally have low toxicity (apart from their propensity for reducing oxygen). Those in use usually contain potential alkylating functionality which is activated upon reduction. Wilson *et al*.[49] have pointed out that the high reactivity of many of these quinones with thiols may be the limiting factor of activity *in vivo*.

An example of this group of compounds is Mitomycin C shown in **Fig 19.**

***N*-Oxides: (Fig 21)**

Aromatic compound SR4233 has been found to be a highly selective hypoxic selective cytotoxin with 20-200 fold greater activity under hypoxic than aerobic conditions.[48] *In vivo* activity is excellent when combined with both single dose and fractionated radiotherapy, and the compound is now in clinical trials. It acts by bioreduction as shown in **Fig 20**[50] with oxygen inhibited reduction to the fluorescent triazine-1-oxide, as the major metabolite in hypoxic cells. It represents the paradigm for hypoxic selective cytotoxins based on the reduction of the *N*-oxide.

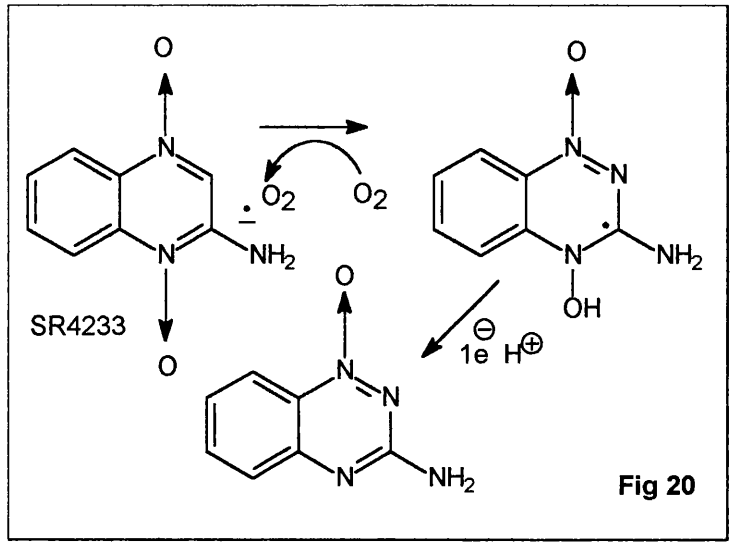


Fig 20

A variety of enzymes are known to catalyse the reduction of aliphatic *N*-oxides. The liver cytochrome P450 enzyme can reduce[51] imipramine *N*-oxide (**Fig 21**). Reduction of aliphatic *N*-oxides is easy and there is no evidence of toxic or reactive intermediates analogous to those generated during the reduction of nitro groups, quinones or aromatic *N*-oxides.

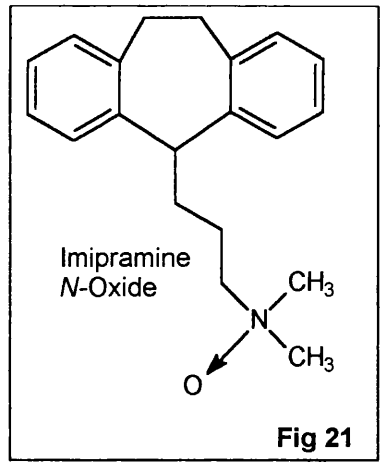


Fig 21

Transition Metals: Pt (II) complexes with nitroimadazoles have been shown to be HSCs.[52] The nitroaromatic ligand is the active redox centre rather than the metal. The suggestion is that the coordination of the alkylating agents to metal through the nitrogen lone pair would provide the complexes in which the alkylating moiety is deactivated, at which point it would be reduced and could release alkylating agents. An example is that of the Co(III) complex as shown in **Fig 22**.

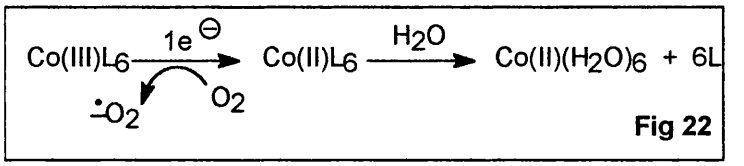


Fig 22

1 e^- reduction results in enormous labilisation of the ligands.[70] In hypoxic regions metabolism is possible by the displacement by water to form the stable hexa aqua Co(II) species. The free ligands are now more toxic than when bound to the metal.

Fig 23 shows SN24771 as an example of this type of agent which uses chelating alkylating agents as ligands. However, SN24771 shows limited hypoxia selectivity *in vitro*.[53]

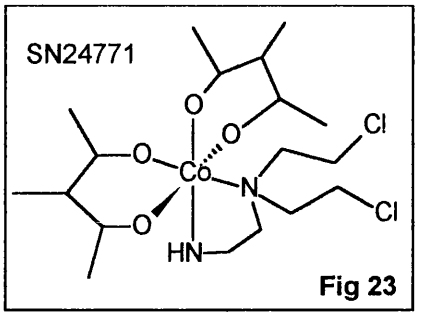


Fig 23

2.8.1 Gene Targeting

The presence of oncogenes and tumour suppressor genes provides a feature which may be exploitable. They provide a specific target for which agents may be developed to be specific. Is it possible to develop drugs which will interfere with the function of the oncogene proteins, or which will augment the activity of the tumour suppressor gene products? This is the main question to be asked.

A problem is posed by the fact that tumour suppressor genes and oncogenes are present in normal healthy cells as well as in the cancer cells. Since the products of the genes are critical regulators of normal cell proliferation they do not provide good chemotherapeutic targets. Thus the proposition is not straightforward but hopefully not an impossible one.

The first introduction of a recombinant gene into a human patient was on 22nd May 1989, at NIH in Bethesda,[54] with the purpose of marking tumour infiltrating lymphocytes to see where they are distributed in a patient with malignant melanoma. Since then there have been numerous gene marking and therapy protocols which have shown no serious undesirable side effects. Sustained improvement is however

a problem. Gene therapy requires a considerable technical expertise and specialised knowledge, therefore it will not become commonplace until vectors which can be used as drugs can be designed.

Techniques which have made gene therapy feasible include construction of "designer genes", gene cloning and the ability to insert functional genes into target cells. Diseases for which gene targeting may be feasible include various cancers, AIDS, haemophilia, cystic fibrosis, various anaemias and adenosine deaminase deficiency.[54]

There appears to be a wide opportunity for the development of this area and investigations are currently underway.

2.8.2 Angiogenesis

Angiogenesis, the process of new capillary blood vessels formation is a characteristic of a number of important physiological events. New blood vessels also provide exits for cancer cells to spread to other parts of the body. By cutting off the tumours blood supply the tumour could be starved of the nutrients it needs and the cells would die. A drug which can cut off the tumours blood supply is known as angiogenesis inhibitor. As of this year there and over 300 angiogenesis inhibitors discovered. Some of which are established cancer drugs which have been "re-discovered" to have antiangiogenic properties such as taxol, 5-fluorouracil and interferon alpha. Work in this area is currently ongoing in many research groups.

In the next chapter work which has previously been performed in the research group towards the development of hypoxic selective compounds will be discussed. This will be followed by the discussion of our work in this area.

Chapter 3

3.1.1 Previous Work

Previous work in the Robins research group by Dr Nicola Henderson centered around target compounds of 2,6-disubstituted piperidine derivatives based on the reported anticancer activity of 2,6-bis(halomethyl)piperidines.[54,55] Compounds of this type could form *N*-oxides, **Fig 24**, which should be non toxic to normal cells but under low oxygen conditions might be activated by reductive enzymes in hypoxic tumour cells to produce radicals and/or bifunctional alkylating agents.

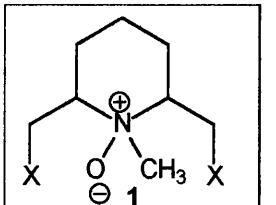
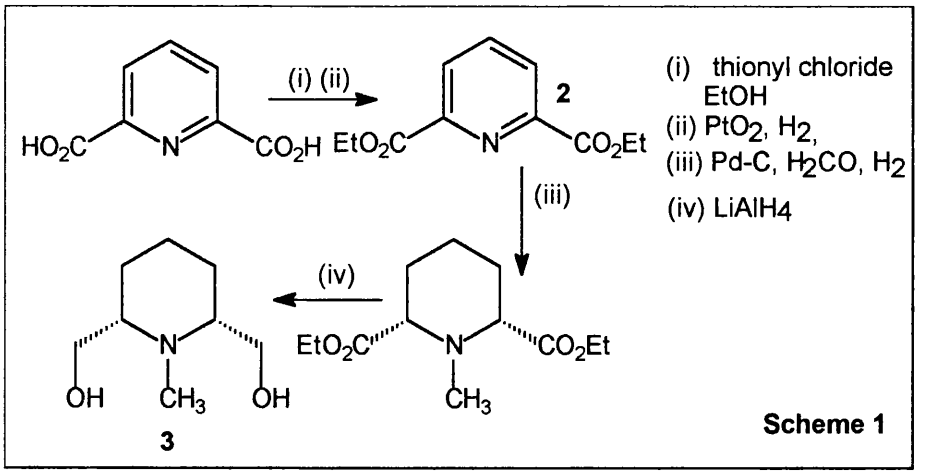


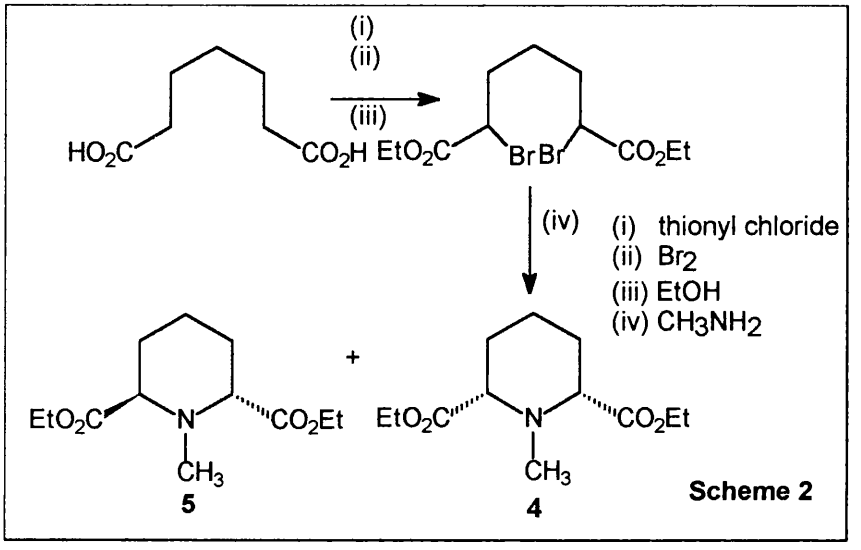
Fig 24

N-Oxides **1** of *cis* and *trans* 2,6-disubstituted *N*-methylpiperidine (**Fig 24**) with X as a carbamate or halogen were synthesised and evaluated for structure/ activity relationships as bioreducible prodrugs. The free bases were designed to be bifunctional alkylating agents active via aziridine ring formation. The *cis*-isomers were synthesised by hydrogenation of a diester, **2,** of dipicolinic acid followed by reductive methylation and further reduction to produce the diol, **3**, then the dihalides and the biscarbamates were formed as shown in **Scheme 1.**

The *trans*-isomers were formed by ring closure of a dibromopimelic acid derivative with methylamine as shown in **Scheme 2**. Equilibration of the *cis* and *trans*-diesters **4** and **5** in acid gave exclusively the *trans*-diacid, from which the diol was obtained and converted into the dihalides and biscarbamates.



Scheme 1

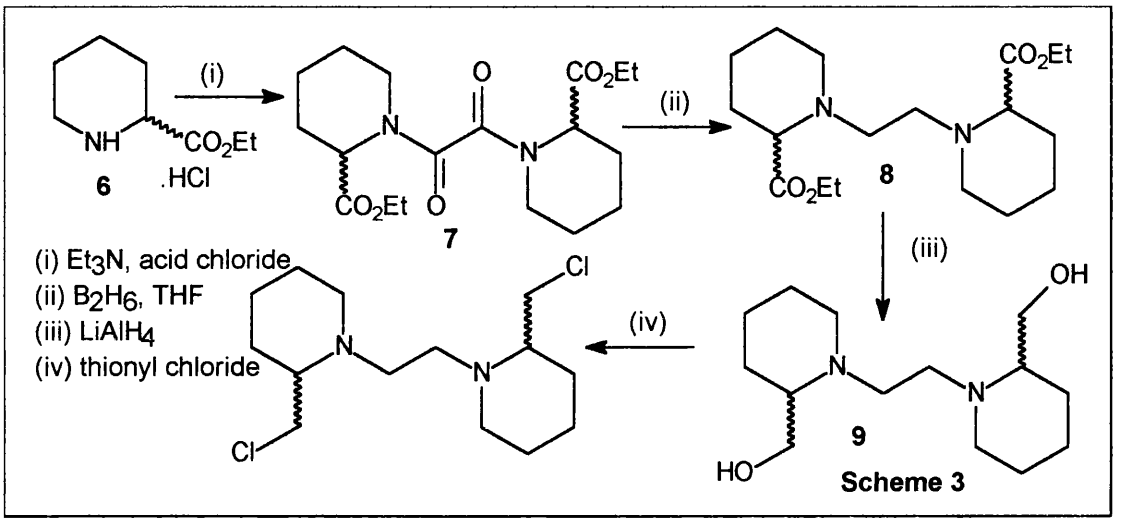


Scheme 2

A set of 22 novel compounds was tested against two human carcinoma cell lines, HT29 and BE cells, under oxic and hypoxic conditions. HT29 cells produce high levels of DT-diaphorase, a reductive enzyme which favors a two electron reduction pathway, whilst BE cells contain no DT-diaphorase and prefer a one electron reduction pathway. The IC_{50} values for this set of compounds were measured and results indicated that the free bases were more toxic than the *N*-oxides against both cell lines. Thus the compounds tested were unfortunately not selective to one type of reduction pathway rather than the other. The reduction potentials for the *N*-oxides were probably too high for the compounds to be reduced in biological systems.

This work was extended by the synthesis of a set of bispiperidine derivatives with different carbon chain lengths between the nitrogen atoms. These were prepared, as in **Scheme 3**, by esterification of DL-pipecolinic acid to give **6**, followed by the condensation of the free base with aliphatic diacid chlorides of varying chain lengths e.g. to give **7** if chain length is two carbons. This was followed by successive reduction of the diamide with borane-THF and the diester with lithium aluminium

hydride to give the diester **8** and diol **9** respectively. The resulting diol was chlorinated using thionyl chloride, and the carbamates were formed using *p*-nitrophenyl isocyanate. The final products were obtained as mixtures of a racemic and meso form, as in **Fig 25**.



Scheme 3

Biological testing as before revealed that the cytotoxicity was dependent upon the length of the carbon bridge between the two nitrogens. The compounds with four carbons between the nitrogens were the most cytotoxic towards both of the human colon cell lines, e.g. IC_{50} 20 μm for n = 3 and X = Cl. The sequence selectivity of compounds with n = 1 to 5 and X = Cl as the free bases was studied using SV40 early promoter DNA by Professor Wakelin in Dublin and Professor John Hartley in London. The compounds which crosslink DNA were shown to alkylate guanines at the N-7 position in the major groove of DNA more selectively than melphalan. The bispiperidine system with two carbons between the nitrogens was the most reactive but it was less cytotoxic than melphalan in a human colon carcinoma cell line (IC_{50} value ca. 30 μM) and in a human chronic myeloid leukaemia cell line (IC_{50} value ca. 12 μM). The most cytotoxic compound was the *p*-nitrophenyl carbamate with an IC_{50} value of 0.3 μM against the chronic myeloid leukaemia cell line. In general the carbamates which were tested were less efficient crosslinkers of DNA. The biscarbamates with n = 1,3 and 4 as free bases were tested against a panel of ovarian carcinoma cell lines *in vitro* by Dr Kelland at the International Cancer Research laboratory in Surrey and showed IC_{50} values comparable to that of

cisplatin. Moreover none of cell lines showed cross resistance to the compounds tested.[56]

The DNA sequence specificity of bispiperidines and their potency warranted further study of structure/activity relationships. This is the concern of the remainder of this chapter.

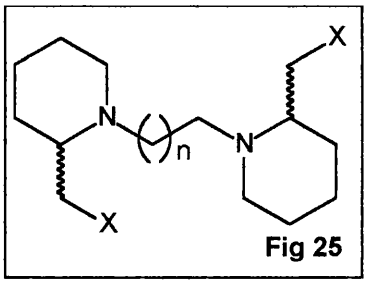


Fig 25

3.2.1 Variations on the Lead Compound

In examination of **Fig 26**, there is a sizable set of features within the system which can be altered to establish structure/activity relationships and to optimise the selectivity and the cytotoxicity towards cancer cells.

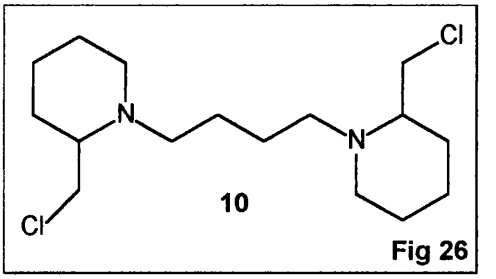


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Fig 26

The variables which were considered are:

- ring size;
- number of rings;
- number of carbons between the nitrogens;
- stereochemistry;
- leaving group;
- number of atoms between nitrogen and leaving group; and
- number of potential alkylating sites.

3.2.2 Ring Size

Previous compounds which have been tested have been six membered rings with one nitrogen present. What effect on the biological activity would reducing the

number of atoms in the ring to five with one nitrogen have? Using the five membered amino acid proline as the starting material would allow us to investigate this variable.

3.2.3 Number of Rings

The previously tested compounds contained two ring systems. What effect would having one ring or no ring systems present have on the biological activity if the alkylating potential was retained in a N-C-C-Cl system?

3.2.4 Number of Carbons between Nitrogens

The number of carbons between the nitrogens of the ring systems can be varied to find the optimum bridge length for crosslinking to occur. This also presents the possibility of alkylating at different sites within the major groove if the molecule is long and unconstrained, thus different sequence specificity may be observed.

3.2.5 Stereochemistry

Most of the previously tested compounds were mixtures of stereoisomers. If the compounds were enantiomerically pure would the biological activity increase or decrease? L-Proline is readily available and reasonably priced, which makes it a good starting material for the synthesis of the bispyrrolidine ring systems.

3.2.6 Leaving Group

The compounds previously tested were either chlorides or *p*-nitrophenyl carbamates. The systems synthesised in this work will have either of these two leaving groups or a 2,4-difluorophenylcarbamate as the leaving group. This derivative was chosen to avoid the possibility of radical formation from nitro groups in *in vitro* studies.

3.2.7 Number of Atoms between Nitrogen and Leaving Group

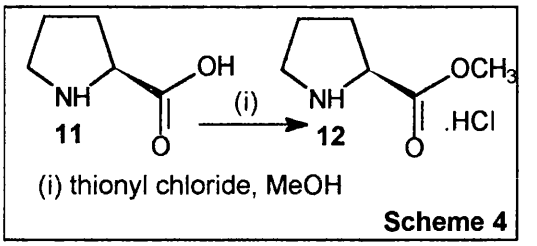
The mechanism of action of the previously synthesised and tested compounds is believed to be aziridinium intermediate formation. To provide further evidence for

this theory compounds with three rather than two atoms between the nitrogen and the leaving group will be synthesised.

3.3.1 Synthesis of Analogues

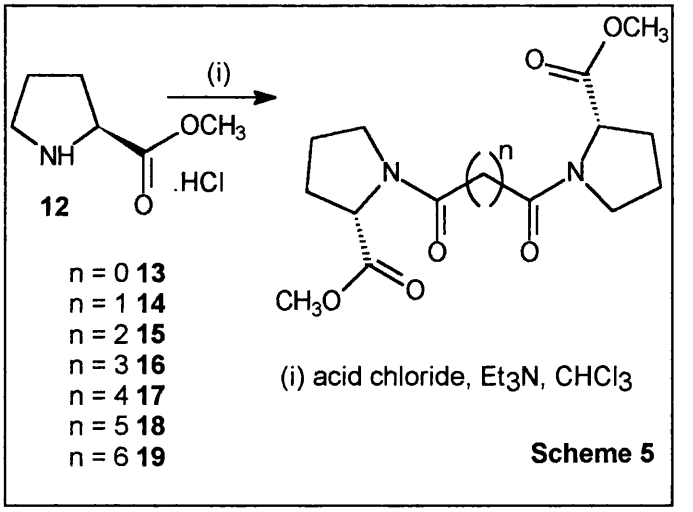
3.3.2 Bispyrrolidine Derivatives

The first set of compounds synthesised in this project contained two pyrrolidine rings with between two and eight carbons between the two nitrogens of the rings. The leaving group was consistently chlorine. This changes the ring size, varies the bridge length and should lead to enantiomerically pure products.



Scheme 4

The first step in the synthesis was the formation of the methyl ester as the hydrochloride salt, shown in **Scheme 4**. This was obtained by treating L-proline in excess MeOH with thionyl chloride and heating at reflux.[57] The product **12** was obtained as a white solid in high yield with spectroscopic data in agreement with literature values.

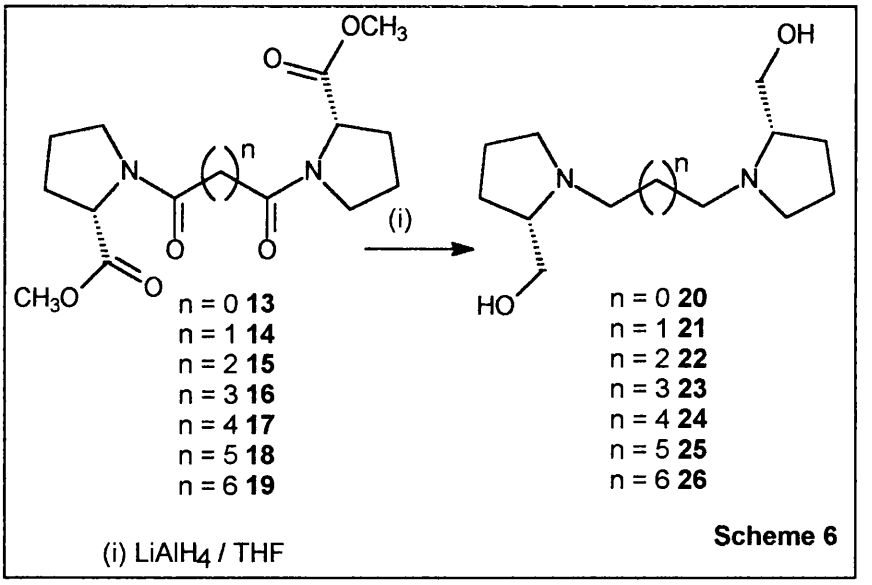


Scheme 5

The key step was to couple two of these molecules, as shown in **Scheme 5**, using the appropriate acid chloride e.g. oxalyl, malonyl, succinyl, glutaryl, adipoyl, pimeloyl and suberoyl dichloride to produce two through to eight carbons between the

nitrogens of the two proline systems. The reaction was performed in chloroform with two equivalents of triethylamine as a base to form the proline free base *in situ* and to mop up the HCl acid formed in the reaction and hence minimise ester hydrolysis. The bispyrrolidine systems were obtained generally as viscous clear oils after purification by column chromatography. The yields were reasonably high after optimisation for each of the coupling experiments.

The common trends throughout these seven diester diamide compounds were the presence of the two carbonyl carbon peaks in the ^{13}C NMR spectra at approximately δ 169 and δ 172 corresponding to the amide and ester carbonyl carbons. The IR spectra also showed the ester and amide carbonyl stretches at approximately 1737 and 1635 cm^{-1} respectively. All compounds gave satisfactory accurate mass data.

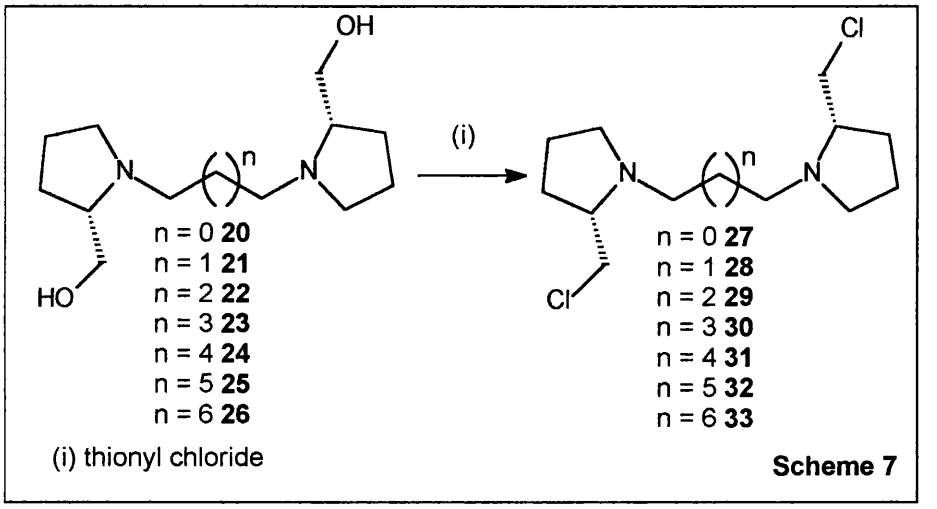


Scheme 6

The next step was the reduction of both the diamide and the diester to form the diol, shown in **Scheme 6**. In the bispiperidine systems previously formed[54] the amide was reduced using borane.THF followed by ester reduction with $LiAlH_4$ in THF as the solvent. Here the diester and the diamide were reduced simultaneously with $LiAlH_4$ in dry THF to afford the free base diols in good yields as clear viscous oils. In the ^{13}C NMR spectra of the diols there was the common feature of absence of any carbonyl carbon peaks. The carbonyl stretches were also absent from the IR spectra but with the addition of new bands at approximately 3400 and 1415 cm^{-1} corresponding to the hydroxyl stretch and bend. The ^{1}H NMR spectrum showed the

hydroxymethylene as a broad singlet at ~ δ 3.5. The accurate mass data were satisfactory.

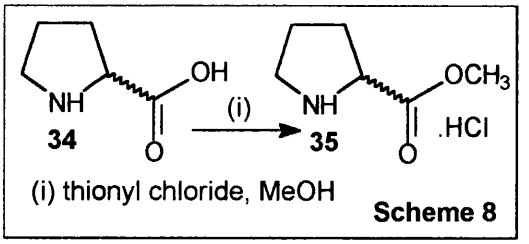
The final step in this sequence was the conversion of the diol into the dichloride. This was done simply using either the diol neat or the diol in chloroform and neat thionyl chloride, as shown in **Scheme 7**. The products were obtained as white to light yellow solids which were recrystallised from EtOH and diethyl ether. The IR spectra of the dichlorides showed an absence of the hydroxyl bands previously observed and additional bands at ~765 cm^{-1} for the CH_2-Cl stretch. The ^{13}C NMR spectra also indicated the presence of the CH_2-Cl carbon by the CH_2 signal at δ 55 and absence of the CH_2-OH carbon signal. The accurate mass data were consistent with the structures.



Scheme 7

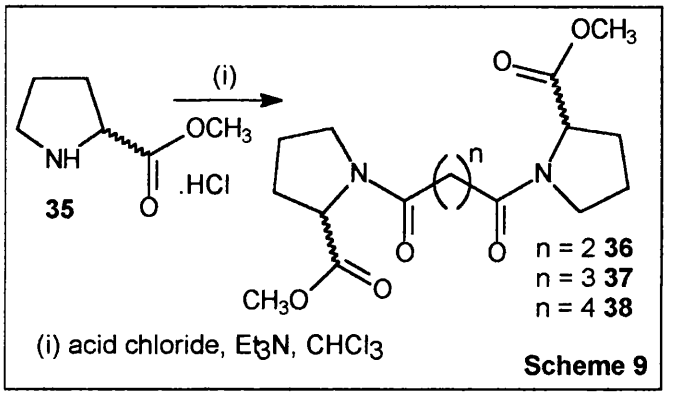
Analogous compounds were prepared from DL-proline. The methyl ester hydrochloride salt was formed from excess MeOH and thionyl chloride as a white solid in acceptable yields as shown in **Scheme 8**. The ^{13}C NMR spectrum showed the presence of the five carbons from the DL-proline and also the presence of the methyl ester carbon at δ 53.9. The ^{1}H NMR spectrum confirmed the methyl ester synthesis by the singlet at δ 3.8 corresponding to 3 protons intensity.

The coupling of the methyl ester hydrochloride salt was again the most problematic step using the appropriate acid chlorides e.g. succinyl, glutaryl and adipoyl dichloride to obtain four, five and six carbons between the nitrogens of the ring systems as shown in **Scheme 9**.

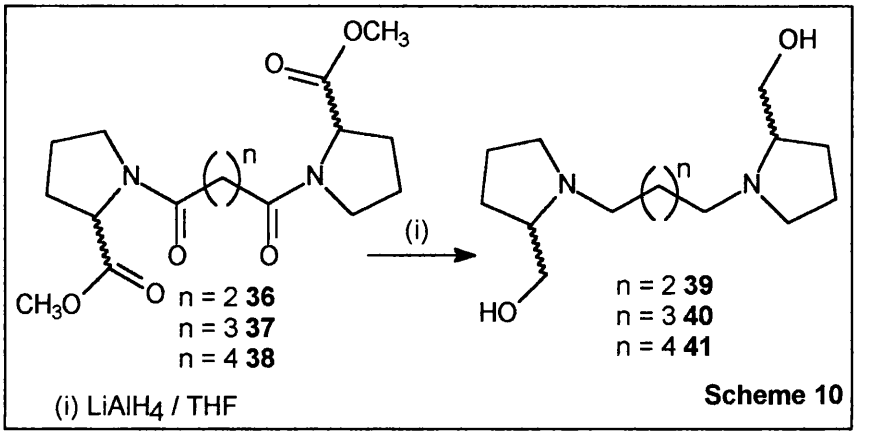


Scheme 8

The yields were acceptable and the products were clear viscous oils. The ^{13}C NMR spectrum showed the presence of the amide and ester carbons at δ 173.3, 173.2, 171.3 and 171.2 for n = 2. There are two signals for each carbon as there will be a mixture of stereoisomers arising from chiral centres being RR, SS (racemic) and RS (meso). The IR spectra also confirmed the presence of two carbonyl features by the presence of stretches at 1735 and 1635 cm^{-1} which again corresponds to the ester and amide carbonyl stretches. The accurate mass data was satisfactory.



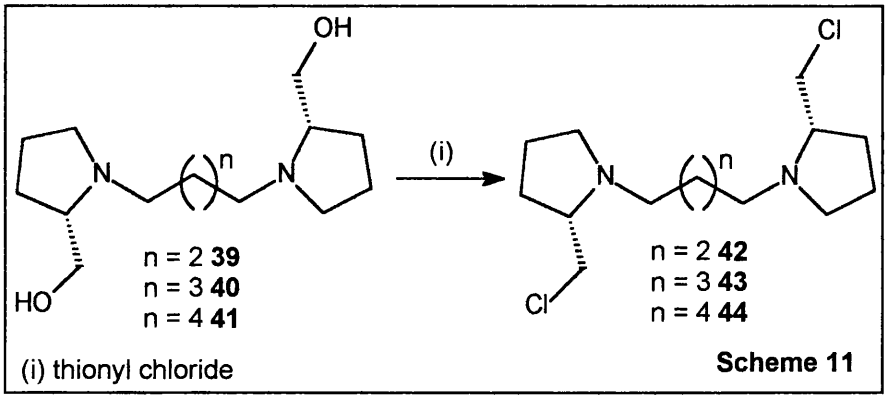
Scheme 9



Scheme 10

Reduction of the diester and diamides was performed simultaneously using $LiAlH_4$ to afford the appropriate diol systems as oils in reasonable yields as shown in **Scheme 10**. The IR and ^{13}C NMR spectra confirmed the structures with the absence of the carbonyl stretches and the carbonyl carbon respectively. The presence of hydroxyl stretches in the IR at ~1402 and 1081 cm^{-1} was observed. The final step

was the formation of the dichloride leaving group by substitution of the alcohol by the chloride. This reaction was performed by stirring the diol in neat thionyl chloride at room temperature as shown in **Scheme 11**. The products were obtained as off white solids after recrystallisation from EtOH and diethyl ether. The IR spectra indicated the absence of the hydroxyl group and presence of the chloride at ~ 745 cm^{-1}.



Scheme 11

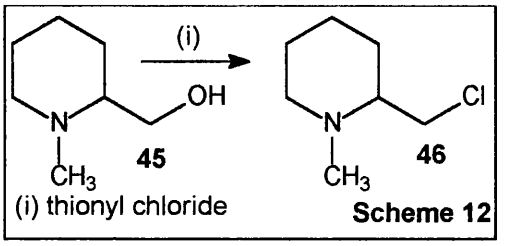
3.3.3 Single Ring Systems

The set of single piperidine ring systems with two and three carbons between the nitrogen and the leaving group were formed starting with *N*-methylpiperidine-2-methanol and piperidine-2-ethanol. This was done to investigate the biological mechanism of action in single rather than two ring systems with different leaving groups. These will be chloride, as before, and two carbamate systems: *p*-nitrophenyl and 2,4-difluorophenyl carbamates.

The carbamates were formed because:

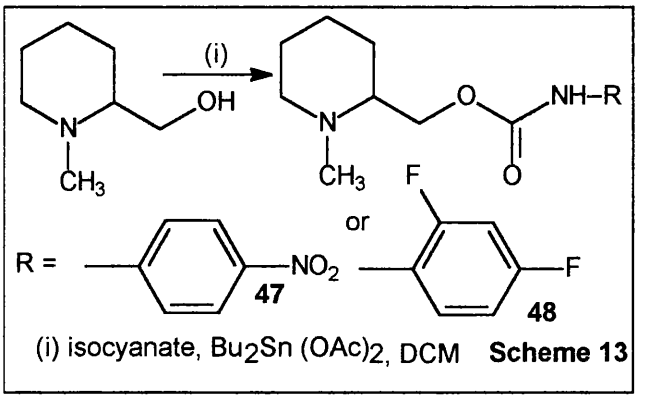
- they are reasonably stable which allows ease of handling;
- their ease of formation;
- carbamates are generally crystalline therefore are easily purified and characterised;
- the *p*-nitrophenyl carbamate has the potential for added cytotoxicity by the formation of radicals.

Starting with *N*-methylpiperidine-2-methanol, which is readily available, the only step was the conversion of the alcohol into the chloride. This was done in the usual manner by stirring in neat thionyl chloride at room temperature and the product was recrystallised from EtOH and diethyl ether. This was achieved in reasonable yield to give the desired product as shown in **Scheme 12**.



(i) thionyl chloride **Scheme 12**

Carbamate formation was performed using the appropriate isocyanate e.g. *p*-nitrophenyl isocyanate or 2,4-difluorophenyl isocyanate, the diol and a catalytic amount of dibutyltindiacetate as shown in **Scheme 13**. The reaction goes reasonably quickly and cleanly at room temperature in dichloromethane to afford the desired products. The mechanism is as shown in **Fig 27.** The ^{1}H NMR spectrum of **47** showed the presence of the *p*-nitrophenylcarbamate group by an AA'BB' system corresponding to the aromatic protons at δ 8.2- 7.6 and a broad singlet at δ 5.0 corresponding to the secondary amine proton and the tertiary amine methyl singlet signal at δ 2.6. The IR spectrum showed the presence of a carbonyl by the stretches at 1739 cm^{-1}, amine stretch at 3426 cm^{-1} and aromatic stretches at 1598 and 1508 cm^{-1}. Similarly for the 2,4-difluorophenyl carbamate compound **48** ^{1}H NMR signals at δ 7.4- 6.7 were observed corresponding to the aromatic protons and the tertiary amine methyl signal at δ 2.5. The IR spectrum showed the presence of the carbamate carbonyl stretch at 1730 cm^{-1} and aromatic stretches at 1506 and 1612 cm^{-1}.



(i) isocyanate, $Bu_2Sn(OAc)_2$, DCM **Scheme 13**

Both the *p*-nitrophenyl and 2,4-difluorophenyl carbamates were obtained as described above. The yields however were quite poor as purification by column chromatography on alumina was required with a large loss of product. The carbamate products were obtained as a yellow solid and white crystals respectively as shown in **Scheme 13**.

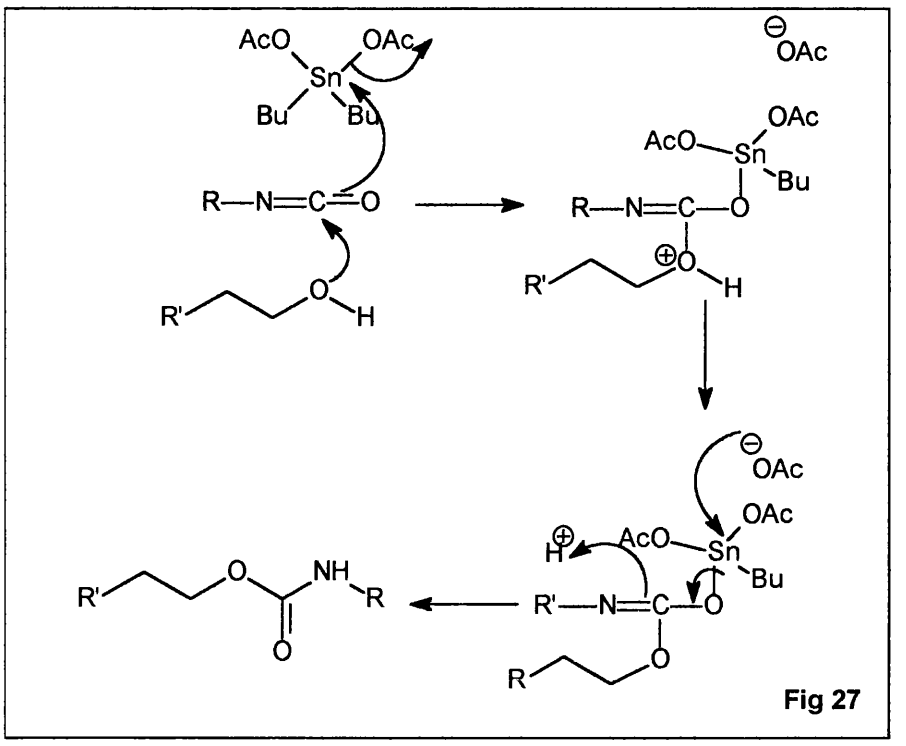
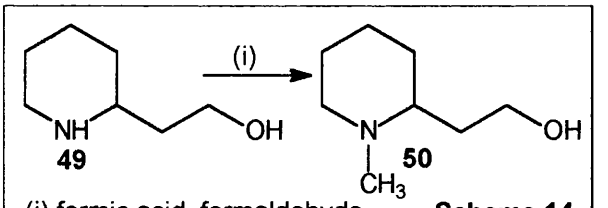


Fig 27

In order to make compounds with three carbons between the N and the leaving group piperidine-2-ethanol was protected by *N*-methylation using formaldehyde and formic acid as shown in **Scheme 14**. Here the use of formic acid is rather unusual as it is acting as a reducing agent. This is one of the few organic acids which can be used in such a manner. The reaction required heating to afford the desired product as a viscous slightly yellow oil.

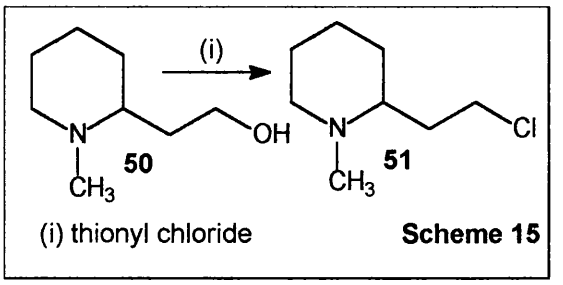


Scheme 14

The ^{1}H NMR spectrum for compound **50** showed the presence of the tertiary amine methyl by a singlet at δ 2.2 corresponding to 3 protons. The ^{13}C NMR spectrum also showed the presence of this carbon by a methyl signal at δ 41.5.

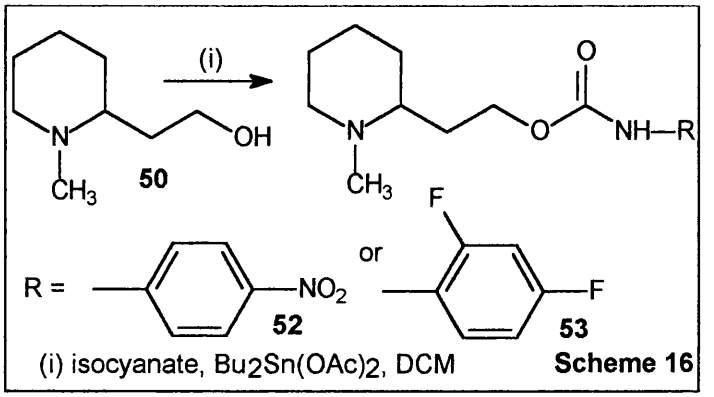
The chloride **51** was obtained by stirring the *N*-methylated piperidine-2-ethanol in thionyl chloride at room temperature as shown in **Scheme 15**. The resulting light yellow solid was then recrystallised using EtOH and diethyl ether in reasonable yield. The structure of **51** was confirmed by the absence of the hydroxyl stretch in the IR

spectrum and the presence of a CH_2 signal at δ 40.4 in the ^{13}C NMR spectrum representing the CH_2Cl carbon.



(i) thionyl chloride **Scheme 15**

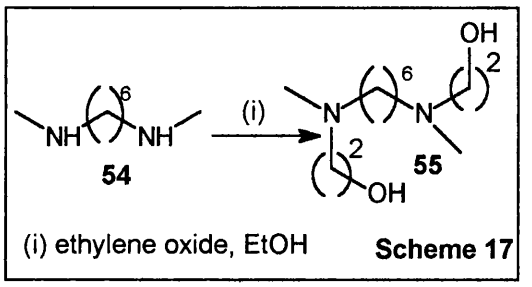
The carbamates **52** and **53** were obtained as previously mentioned using the appropriate isocyanates, dichloromethane and a catalytic amount of dibutyltin diacetate as shown in **Scheme 16**. They *p*-nitrophenyl carbamate was obtained as a canary yellow solid and the 2,4-difluorophenyl carbamate was obtained as white crystals. Again the yields were rather disappointing as alumina column chromatography purification was required. The structure of the *p*-nitrophenyl carbamate, **52**, was confirmed by its ^1H NMR and IR spectra showing carbamate carbonyl stretch at 1741 and aromatic stretch at 1505 and 1599 cm^{-1}. The 2,4-difluorophenyl carbamate **53** structure was also confirmed by the presence of aromatic signals at δ 7.3- 6.7 of intensity 3 protons as well as the signals of the original compound. The ^{13}C NMR spectrum showed the aromatic carbons at δ 111.2 to δ 124.1, the tertiary amine methyl at δ 39.8 and the carbonyl carbon at δ 154.2.



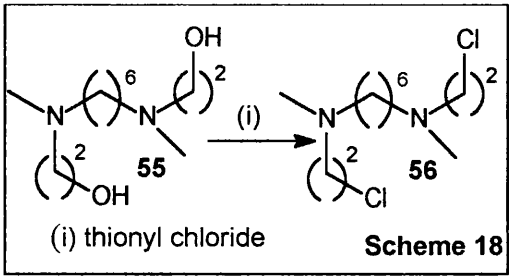
(i) isocyanate, $Bu_2Sn(OAc)_2$, DCM **Scheme 16**

3.3.4 Straight Chain Systems

To examine systems without a ring in the structure and with two or three carbons between the nitrogen and the leaving group and altering the leaving group the following compounds were synthesised .

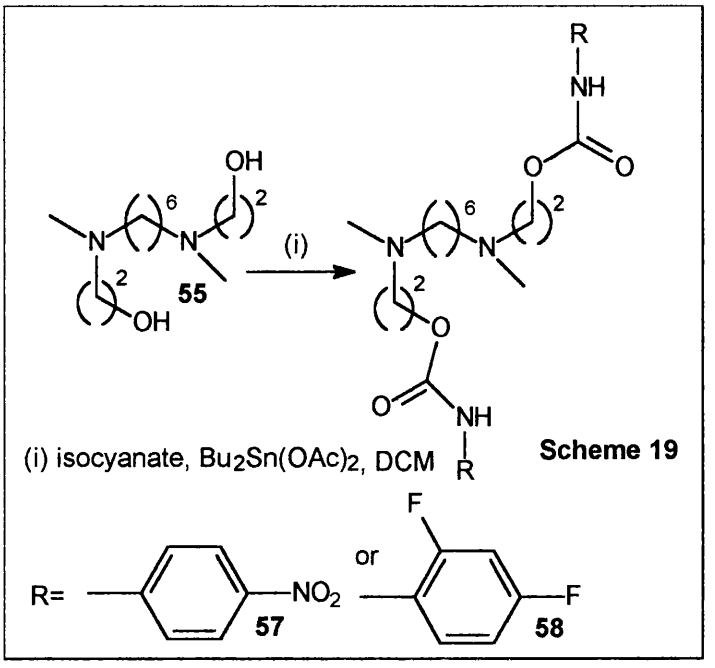


Starting from *N,N'*-dimethyl-1,6-hexanediamine, **54**, the hydroxyethyl arms of compound **55** were formed using an excess of ethylene oxide in EtOH by the method of Hancock *et al* [58], as shown in **Scheme 17.** The structure of compound **55** was confirmed by ^{1}H NMR spectroscopy showing methylene protons at δ 3.6-3.5 corresponding to the protons next to the hydroxyl and the ^{13}C NMR spectrum showed CH_2 signals at δ 61.7 and δ 56.9 corresponding to the carbons of the hydroxyethyl arms.



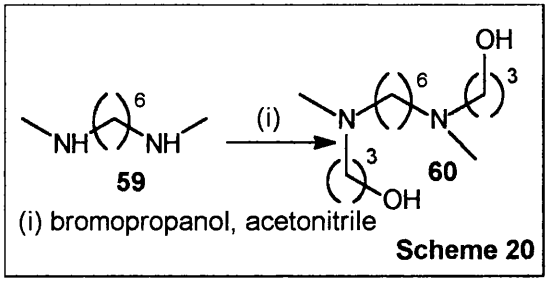
Again the dichloride, **56**, of the diol, **55**, was easily formed using excess neat thionyl chloride at room temperature as shown in **Scheme 18**. The structure of the product was confirmed by the absence of hydroxyl stretches and presence of CH_2Cl stretch at 619 cm^{-1} in the IR spectrum. The ^{13}C NMR spectrum showed the carbons of the chloroethyl arms at δ 57.2 and δ 56.6 which is much lower than those of the hydroxyethyl group.

The biscarbamates, **57** and **58**, were formed from the diol using the corresponding isocyanate, dibutyltindiacetate and dichloromethane as shown in **Scheme 19**.



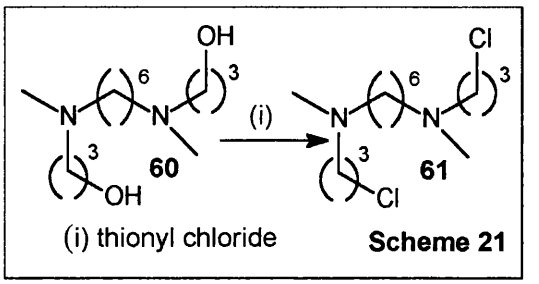
Scheme 19

The structure of the *p*-nitrophenyl compound was confirmed by the presence of aromatic stretches at 1498 and 1598 cm^{-1} and NH stretches at 3077 and 3365 cm^{-1} in the IR spectrum. The ^1H NMR spectrum showed a AA'BB' system for the aromatic protons of intensity 8 protons at δ 7.9-6.5 and the methyl on the tertiary amine as a singlet of intensity 6 at δ 2.9. The 2,4-difluorophenyl carbamate **58** was confirmed by ^{13}C NMR spectroscopy showing a signal at δ 154.2 corresponding to the carbonyl of the carbamate system and the methyl signal at δ 39.8 corresponding to the methyl on the amine. The ^1H NMR spectrum showed the presence of aromatic protons of intensity 3 at δ 7.3-6.7 and a singlet at δ 2.6 of intensity 6 protons, corresponding to the amine methyl protons. The IR spectrum showed the carbonyl stretch at 1734 cm^{-1}, the secondary amine stretch at 3441 cm^{-1} and the aromatic stretches at 1526 and 1626 cm^{-1}.



Scheme 20

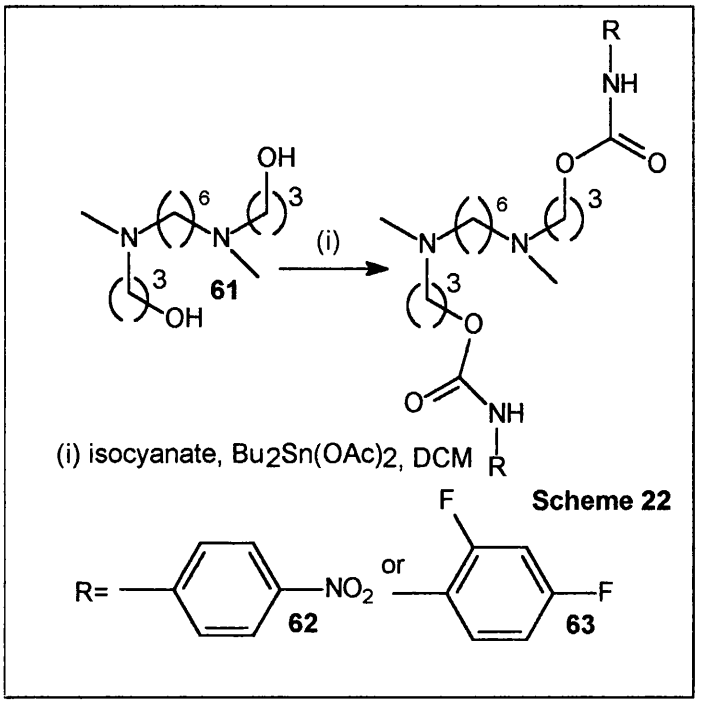
The hydroxypropyl groups were attached to form compound **60**, on treatment of *N,N'*-dimethyl-1,6-hexanediamine, **59**, with 3-bromopropan-1-ol in acetonitrile as shown in **Scheme 20**. The structure was confirmed by the presence of CH_2 signals at δ 60.9, 57.6 and 35.1 in the ^{13}C NMR spectrum corresponding to the hydroxypropyl group. The IR spectrum also shows the presence of the hydroxyl group with stretches at 3642 and 1092 cm^{-1}.



(i) thionyl chloride **Scheme 21**

The dichloride, **61**, was formed using excess thionyl chloride at room temperature as shown in **Scheme 21** and the structure was confirmed by the absence of hydroxyl stretches in the IR spectrum but the presence of the CH_2-Cl stretch at 750 cm^{-1}. The ^{13}C NMR spectrum showed the CH_2 next to the chloride at δ 56.7.

The biscarbamates, **62** and **63**, were formed from the diol using the corresponding isocyanate, dibutyltindiacetate and dichloromethane as shown in **Scheme 22**. The structure of the *p*-nitrophenyl carbamate **62** was confirmed by the ^1H NMR spectrum showing an AA'BB' system corresponding to the aromatic protons of intensity 8 at δ 8.1-6.6. A broad singlet at δ 4.3 indicated the secondary amine proton and the singlet of intensity 6 protons at δ3.05 corresponded to the methyl groups attached to the amine. The IR spectrum showed aromatic stretches at 1508 and 1628 cm^{-1} as well as the carbamate carbonyl stretch at 1736 cm^{-1} and the NH stretch at 3371 and 3481 cm^{-1}. The 2,4-difluorophenyl carbamate, **63**, showed similar signals in the IR spectrum of 1512 and 1612 cm^{-1} for the aromatic stretches, and for the NH stretches at 3083 and 3433 cm^{-1} and at 1728 $cm^{-1}$for the carbonyl stretch of the carbamate.



Scheme 22

3.4.1 Biological Evaluation

The compounds synthesised were tested by Professor John Hartley at University College London for cytotoxicity, DNA sequence specificity[59] and DNA crosslinking[60] ability against a human leukaemic K562 carcinoma cell line.

3.4.2 Sequence Specificity

A polymerase stop assay has been developed to determine the DNA nucleotide sequence specificity of covalent modification by antineoplastic agents using the thermostable DNA polymerase from *Thermus aquaticus* and synthetically labelled primers. The products of linear amplification are run on sequencing gels to reveal the sites of covalent drug binding. The method has previously been used to study a number of agents including nitrogen mustards, platinum analogues and mitomycin C which all agree with the sequence specificity obtained by other procedures. The assay is advantageous in that it is not limited to a single type of DNA lesion (as in the piperidine cleavage assay for guanine N-7 alkylation),[55] does not require a strand breakage step and it is more sensitive than other primer extension procedures which have only one polymerisation cycle. In particular this method has considerable potential for examining the sequence selectivity of damage and repair in single copy gene sequences in genomic DNA from cells.

The biological evaluation was carried out as reported in the literature.[59]

3.4.3 Agarose Gel method for the Determination of DNA Interstrand Crosslinking

Many agents used in the treatment of cancer are bifunctional and are able to crosslink biological macromolecules. The most important target is DNA. The formation of the interstrand crosslinks may be the most relevant cytotoxic lesion. Such lesions are formed by an initial covalent reaction of drug with an electrophilic site on the DNA to form a monoadduct which can be converted into a crosslink by a second reaction with the other DNA strand. Not all monoadducts are converted into crosslinks and the "second arm" reaction is generally assumed to be slow compared to the initial monoadduct formation.

There are several techniques for measuring DNA interstrand crosslinking. In many cases they are based on the fact that covalently crosslinked DNA strands are prevented from complete denaturation by heat or by alkali.[60] Many of these techniques are insensitive and time consuming. They may also require large amounts of DNA and drug and are generally not applicable to detailed time-course experiments. The method used by us is a simple highly sensitive agarose gel method for measuring interstrand DNA crosslinks and in particular the kinetics of formation of total crosslinks.

The densitometric quantitation of double and single stranded DNA within the same sample lane allows the extent of crosslinking to be calculated directly without having to take into account sample processing or loading variations. The use of ^{32}P end labeled DNA makes the technique sensitive enough to measure 10 ng DNA or less per lane (depending on required autograph exposure). This DNA is then denatured by either heat or alkali. The choice of buffer may be important, in particular phosphate buffers should be avoided as phosphate precipitates with ethanol which does not then dissolve in the strand separation buffer making gel loading difficult.

This method is particularly applicable to detailed time-course experiments such as "second arm" crosslinking and compliments the technique of alkaline elution experiments without the influence of complicating cellular factors such as DNA repair and the quenching of DNA monoadducts.

The biological evaluation was carried out as reported in the literature.[60]

3.4.4 MTT Based Cytoxicity Assay

Cytotoxicity tests are widely used to evaluate the effects of anticancer drugs on different cancer cell lines. Clonogenic assays use plating efficiency, either on plastic or on agar, as an end point such that the number of colonies relative to the control indicates the number of cells that survived the treatment. Also, the size of the colony can indicate whether the treatment was cytotoxic, cytostatic or mitogenic. These assays are slow and the development of microtitration assays has been of enormous benefit to those who screen new compounds for cytotoxic activity. The end point of a microtitration is an estimate of the number of cells. This can be found by either counts or by methods such as isotope incorporation. Use of MTT testing as a cell viability measure[61] can be chosen at an end point.[62] MTT is a yellow water soluble tetrazolium dye that is reduced by live, but not dead cells, to form a purple formazan product that is insoluble in aqueous solutions. It should be noted however that a number of factors can effect the reduction of MTT.[63]

Cells in exponential phase of growth were exposed to the cytotoxic drug of interest. The duration of the exposure was usually determined as the time required for maximal damage to occur but were also influenced by the stability of the drug. After removal of the drug the cells were allowed to proliferate for two to three doubling times in order to distinguish between cells which remain viable and are capable of proliferation and those which remain viable but cannot proliferate. Surviving cell numbers can then be determined indirectly by MTT dye reduction. The amount of MTT formazan produced can be determined spectrophotometrically once solubilised in a suitable solvent. It is important to note that the following conditions are met.

- MTT colour is shown to be in the linear range of dependence on the cell number;
- The colour produced is proportional to the time under MTT exposure.

3.5.1 Conclusions from Biological Testing

The four sets of compounds all underwent the above biological tests with results shown in **Tables 3, 4 and 5**.

The results overall were rather disappointing as the activity observed for compounds was nowhere near as promising as in the original set of compounds.[55] However, there were several interesting conclusions.

% Crosslinking

No.	IC50 μM	00.1 μM	0.1 μM	1.0 μM	10.0 μM
28	> 100	0	0	0	0
29	50	0	0	0	6.2
30	> 100	0	2	13	84
31	52	0	0	10.4	56.2
32	>100	0	0	4	41
33	> 100	0	2	2	52

Table 3

% Crosslinking

No.	IC50 μM	0.01 μM	0.1 μM	1.0 μM	10.0 μM
61	> 100	0	0	0	0
62	5	0	0	0	0
63	30	0	0	0	0
56	> 100	0	0	44	-
57	21	0	0	0	0
58	> 100	0	0	0	0

Table 4

% Crosslinking

No.	IC50 μM	0.01 μM	0.1 μM	1.0 μM	10.0 μM
46	> 100	0	0	0	0
47	30	0	0	0	0
48	> 100	0	0	0	0
51	> 100	0	0	0	0
52	> 100	0	0	0	0
53	> 100	0	0	0	0

Table 5

Chlorambucil IC50 45 μM

Melphalan IC50 8.5 μM

3.5.2 Leaving Groups

From the IC_{50} values of compounds **62** at 5 μM, **57** at 21 μM and **47** at 30 μM we observe that the most cytotoxic compounds were the ones containing the *p*-nitrophenyl carbamate functionality. The difluorophenyl carbamate is the second most cytotoxic leaving group and chlorides were the least in these compounds. This could be explained by the possibility of the lone pair on the nitrogen attacking the carbonyl group and expelling the amine ring with the nitro group still attached as shown in **Fig 28.** It could also be suggested that the nitro carbamates are more cytotoxic than the difluorocarbamates by the formation of radicals from the nitro groups.

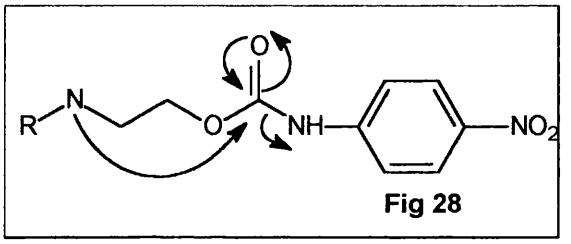


Fig 28

3.5.3 Ring Systems and Linker Length

It was also obvious that compounds with two ring systems were more active than the single piperidine or straight chain systems independent of leaving group featured. Compound **31** with the six carbon unit between the ring systems appears to be the best compound tested with IC_{50} value of 52 μM and crosslinking of 10.4 % at 1.0 μM and 56.2 % at 10.0 μM.

The single piperidine ring systems behaved as expected and there was no crosslinking activity observed as there is only one site for alkylation. There was also very little cytotoxicity shown in the piperidine ring system apart from **47** with IC_{50} value of 30 μM which was for the *p*-nitrophenyl carbamate system.

Reasonable crosslinking values were obtained from the bispyrrolidine systems with five and eight carbon linker units showing crosslinking at concentrations as low as 0.1 μM.

3.5.4 Ring Size and Stereochemistry

The bispiperidine systems were generally found to be better crosslinkers and more cytotoxic than the bispyrrolidine systems. Thus the conclusions are that the six membered ring systems were preferable to the five membered rings. It should be remembered, however, that the bispiperidine compounds were tested as a mixture of distereoisomers whereas the bispyrrolidine were tested as single LL enantiomers thus no conclusions can be drawn about the effect of stereochemistry on the biological activity observed. Results from testing of diastereoisomers of the bispyrrolidine systems were inconclusive due to solubility problems encountered by the tester.

Chapter 4

4.1.1 Macrocyclic Alkylating Agents

The main concern of this chapter is to discuss the progress from the synthesis of monofunctional and bifunctional alkylating agents to the design and synthesis of novel alkylating agents which contain a variable number of sites capable of alkylation.

The use of linear and branched polyamines in the treatment of cancer has previously been reported,[64,65,66] thus an alternative strategy would be the development of cyclic polyamines. An area which is, as yet, unexplored is that of multifunctional macrocyclic alkylating agents.

This chapter is concerned primarily with the formation of azamacrocycles as alkylating agents, i.e. cyclic systems with nitrogen as the only heteroatom present within the ring structure. These systems have been designed to incorporate a selection of structural variations in an attempt to establish structure/ biological activity relationships within the series.

4.2.1 History of Macrocycles

The basic defintion of a macrocycle is that it is a heterocyclic ring, comprising of nine or more atoms, of which at least three are non-carbon atoms such as nitrogen, oxygen, sulfur, phosphorus etc.[67]

Why should there be an interest in the synthesis of azamacrocycles? Macrocycles and their metal complexes have been of great chemical and biological interest for many years. Macrocyclic ligands which contain heteroatoms are important complexing agents for cations, anions and neutral molecules. The presence of similar compounds in many fundamental biological processes has stimulated their study. Such compounds include porphyrins and corrins which play an important part in the biological process of transporting oxygen in mammals and photosynthesis in plants, respectively.[67] [Other examples of biological processes involving macrocycles are reported by Hosseini and Lehn 1987,[68] Lehn 1985[69] and Yohannes *et al.* 1985[70].]

In particular azamacrocycles have undergone intensive investigation since their discovery over a hundred years ago when Bayer in 1886 prepared tetraazaquatren[71] **(Fig 29)**.

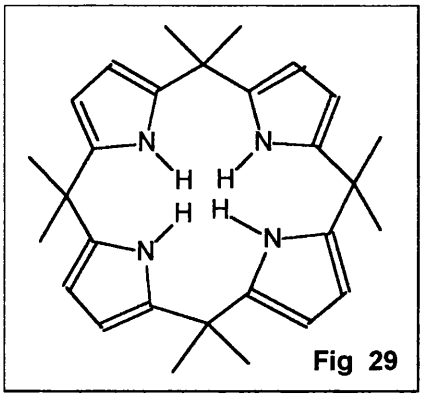


Fig 29

Azamacrocyles were established well before the discovery by Pedersen of the oxygen containing ligand 18-crown-6 in 1967.[72]

Properties of such compounds are determined by the fact that the nitrogen has a much stronger association with transition metal ions than oxygen. Oxygen is also more electronegative therefore the electron pair in nitrogen is more available for complexing purposes. This is an interesting phenomenon in this study as the potential for hypoxic selectivity could be exploited in the alkylating agent by the use of metal complexes which could be bioreduced in the body as mentioned previously. Azamacrocycles have properties which are intermediate between oxygen crown ethers, which strongly complex alkali and alkaline metal earth ions, and those of aza-oxa crowns which strongly complex heavy metal ions. Thus there is a wide scope for the development of azamacrocyclic alkylating agents.

4.3.1 Nomenclature

There are a variety of methods which are currently in use for naming macrocycles. In the method of Busch and co-workers[67] the size of the macrocycle is given in brackets followed by the number and types of heteroatoms present within the system. For example, cyclam (**Fig 30**), which is one of the more commonly known azamacrocycles, is written as [14]N_4. This, however, does not explain exactly where the nitrogens are positioned within the ring system. Therefore, in this thesis a variation on this theme will be employed. The structure will be written fully, by numbering the systems from the heteroatom with the highest atomic number as in Journal of Chemical Society papers listed in references. Cyclam will be written as

1,4,8,11-tetraazacyclobutadecane. Also shown in **Fig 30** are cyclen: 1,4,7,10-tetraazacyclododecane and tacn: 1,4,7-triazacyclononane which are both well known azamacrocycles. This means we are stating exactly what heteroatoms are positioned where within the macrocyclic systems.

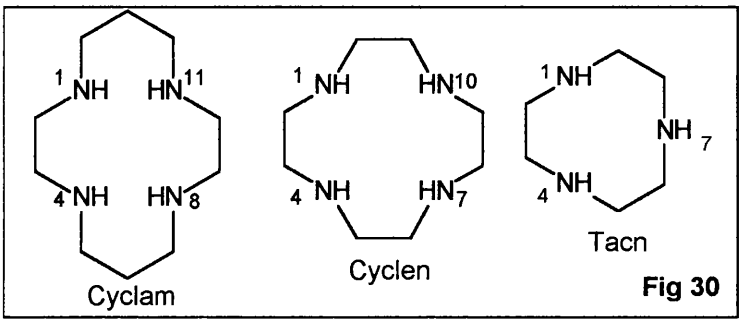


Fig 30

4.3.2 Methods of Synthesising Azamacrocycles

Generally there are two main commonly employed methods of synthesising macrocycles. These are by template synthesis and by high dilution techniques.

The first reported synthesis of a macrocycle was by Baeyer in 1886.[71] He used the condensation of pyrrole and acetone in the presence of mineral acid and achieved a 88% yield. Following this there were numerous attempted and successful syntheses of a wide variety of macrocycles. These are too numerous to mention and many are described in the review from a historical viewpoint by Newkome and coworkers.[67] The following workers have made considerable contributions in the area: E. Blasius (polymer-containing macrocyclic ligands); A. V. Bogatskii and I. G. Lukyanenko (prepared hundreds of aza-oxa macrocycles); D. H. Busch (template synthesis of aza-oxa macrocycles); J. M. Lehn (biological-like aza-oxa macrocycles); M. Okahara (ring closure of diols with tosyl chloride); P. Paoletti (complexing properties of aza-oxa macrocycles); J. E. Richman and T. J. Atkins (tosylamide ring closure reactions) and H. Stetter (first prepared macrocyclic diamides) all of which are discussed by Newkome.[67]

4.3.3 Synthesis Using Templates

There has been a wide and varied array of methods employed in the synthesis of macrocycles over the past 20 years. They have been in the main unsuccessful

and highly wasteful due to low yields of the desired product; use of large volumes of solvent; and competing side reactions which may occur.

The first reported synthesis of a macrocycle by employing template cyclisation techniques was in 1907 during the synthesis of phthalimide in a ferrous ion complex of phthalocyanine.[73] The metal ion acts as a template. Alternatively they may act in less obvious ways by masking or by activating individual functional groups or they may influence the reaction in other ways which are related to the more readily defined steric influences in the reaction sequence.

In general, the generation of the macrocyclic product is controlled by the presence of a metal template ion which interacts with the heteroatoms inducing the desired cyclic product. The main concern, to effect the cyclisation, is the orientation of the reactive sites to give the macrocyclic product rather than the linear polymer.

In the synthesis of medium to large rings (upwards of 13 members) there is an unfavourable entropy term to the overall free energy change. This leads to limitations in the temperatures which can be used in the reaction, as heating gives rise to an even more unfavourable $T\Delta S$ term and the competing reactions e.g. oligomerisation may be favoured at the expense of the desired cyclised product. These effects may be eradicated if an ion can be used as a template for the cyclisation step. Generally the metal can then be removed by either adding acid or by ligand exchange processes, e.g. by adding CN^-, sulfide or EDTA, or following reduction of the metal if it has a suitable redox couple.

The first reported template synthesis for polyazamacrocycles was by Curtis[74] in the reaction of $[Ni(1,2\text{-diaminoethane})]^{2+}$ with dry acetone to give the 14-membered system with four aza constituents as shown in **Fig 31**. The carbon nitrogen double bond can then be easily reduced with borohydride.

An alternative approach involving the cleavage of a common bond between two or more smaller rings, e.g. 5 or 6-membered, may also be employed. This is a well established method for medium to large ring synthesis. It is regarded as a template method wherein a tertiary or quaternary carbon serves as the template. An example is that of the synthesis of tricyclic orthoamides by the alkylation of bicycloguanidines.[75-78] The intermediates may be hydrolysed to give the free base triazamacrocycle or the opportunity may be taken to alkylate the nitrogens selectively (**Fig 32**).

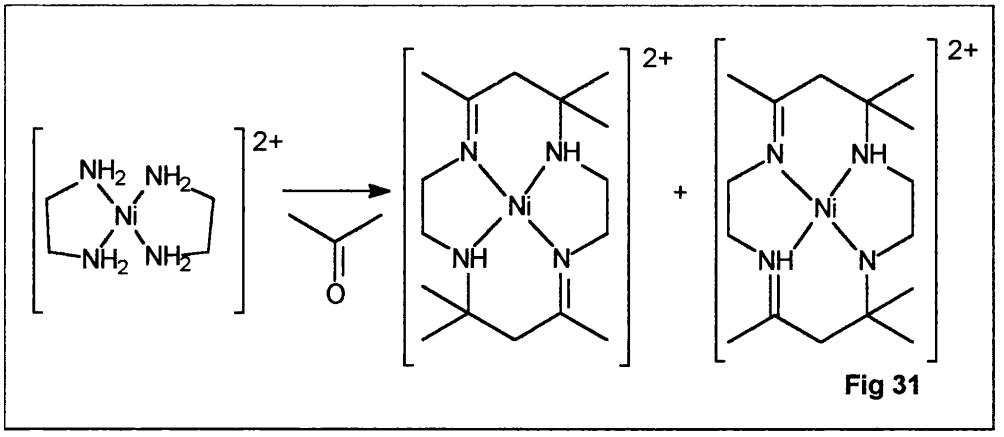


Fig 31

Today there are many similar methods for the synthesis of not only aza macrocycles but aza-oxa and aza-thia-macrocycles.

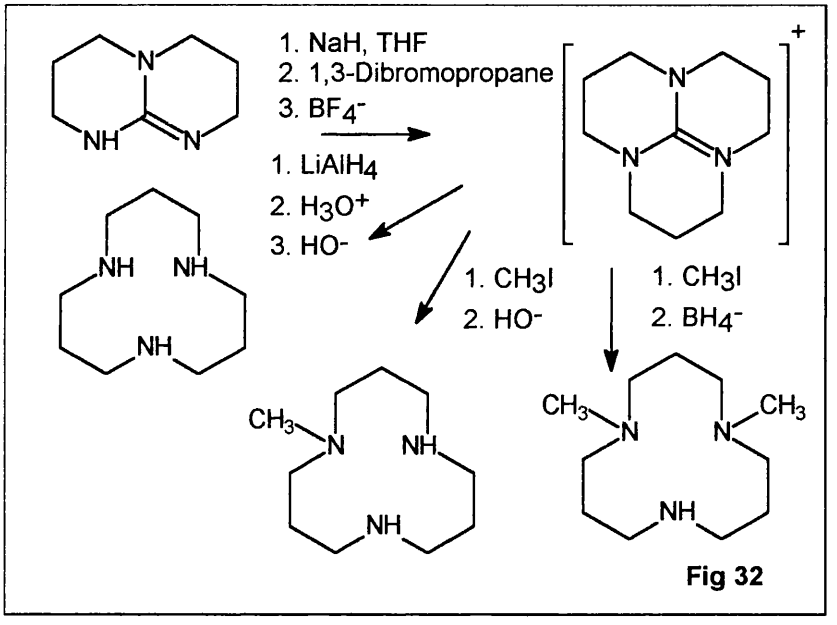


Fig 32

4.3.4 High Dilution Synthesis

The first non-template synthesis of a macrocycle was reported in 1954 by Stetter and Roos.[79] This involved the condensation reaction of terminal halides with bissulfonamide sodium salts under high dilution conditions to afford macrocyclic sulfonamides in moderate yields. Over a prolonged period this method has been honed and optimised for many macrocyclic compounds, each of which may require different conditions for the best results for the individual reactions to afford acceptable yields.

High dilution techniques take advantage of the fact that two groups can react forming the start of a linear chain and the remaining reactants are so dilute that the

two functional groups on each end can react together to form the cyclic product rather than encountering another open chain reactant. In large volumes of solvent it is statistically easier for the two ends of the short chain to find and react with each other than to find other short chain molecules. Also the functional groups at the ends of the short chains are reactive towards each other, while only half of the times the two separate chains meet will the groups be mutually reactive.

The reaction generally takes place in a dipolar aprotic solvent such as dimethyl formamide (DMF) and is now most commonly effected by the reaction of the dianion of a bistoluenesulfonamide with a ditosylate or dimesylate in dry DMF. The dianion of the toluenesulfonamide salt may be generated prior to the cyclisation step by employing sodium hydride in DMF or sodium in dry EtOH or alternatively the reaction can occur in DMF in the presence of caesium carbonate generating the anion *in situ*. **Fig 33** shows the general synthetic approach. Note that the tosylate group acts in two ways. Firstly it acts as a protecting group for the nitrogen and secondly as an activating group. It makes the secondary NH proton sufficiently acidic that the salt formation will occur under mild conditions. The sulfonamide group can then be removed by a variety of methods which will be discussed in section **4.4.1** to leave the azamacrocycle as the free base.

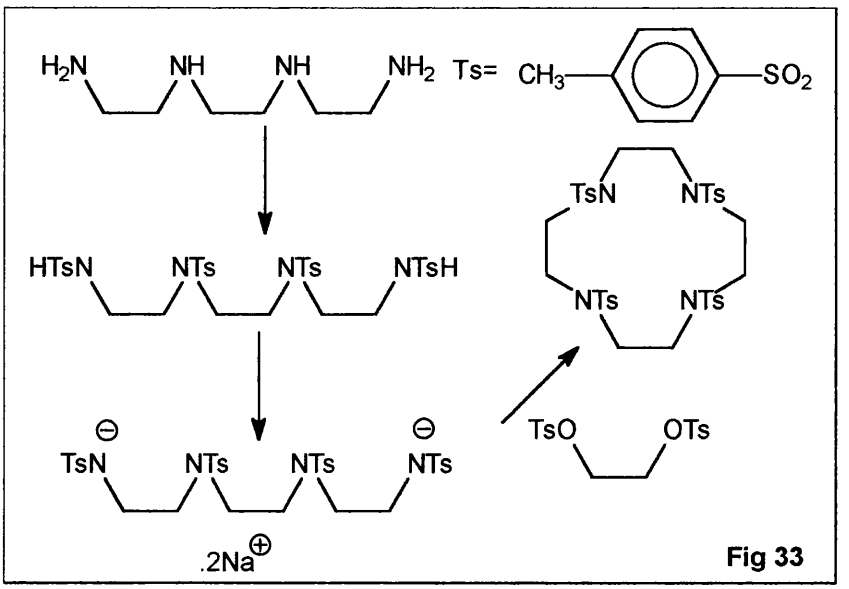


Fig 33

A second cyclisation method using a direct condensation may also be employed. The reaction of an ester or an acid chloride with linear polyamines will afford the cyclic amide. The amide can then be reduced, generally with lithium

aluminium hydride, to give the desired free base product as shown in **Fig 34**. This method employs more vigorous forcing reaction conditions than high dilution techniques.

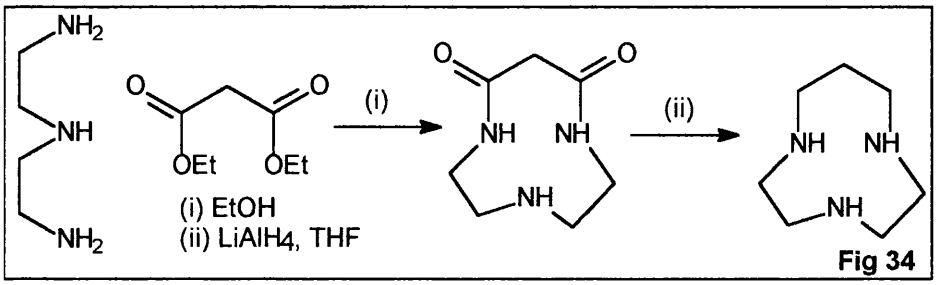


Fig 34

4.4.1 Sulfonamide Cleavage

The cleavage of sulfonamide groups from nitrogen is notorious for its difficulty, unpredictability, low yields and unreliability from one compound to the next.

There are several methods for sulfonamide removal reported in the literature. The most common ones are acid hydrolysis with concentrated sulfuric acid; reductive cleavage with hydrobromic acid and glacial acetic acid; reductive cleavage with lithium aluminium hydride; and electrochemical reduction.

- Acid hydrolysis using 90 - 97% concentrated sulfuric acid at high temperatures for a prolonged period is one of the most common sulfonamide cleavage methods. This is followed by treatment with base to give the free base product. This method may be unsatisfactory as some aza and oxa-aza compounds decompose under strongly acidic or basic conditions. The high temperatures and long reaction times along with difficulty in isolating the desired product also make this method unfavourable. It is, however, preferred for aza compounds giving acceptable yields, e.g. 98% sulfuric was used to give TACN in 90% yield by Searle and Geue in 1984,[80] McAuley *et al* in 1984[81] obtained 90% yield and Ciampolini *et al* in 1986[82] deprotected [14]N_4 -3Ts in 90% yield. A more recent publication by Lazaar in 1995[83] reports rapid detosylation of sulfonamide compounds on heating at 180 °C in concentrated sulfuric acid for 8 minutes.
- The use of hydrobromic acid and glacial acetic acid is also a common method employed in amine detosylations. Phenol is often used in the reaction as a bromine scavenger but it is not a necessity to the success of the reaction. This method allows the product to be obtained as the hydrobromide salt on addition of

large volumes of organic solvents such as diethyl ether. Thus isolation of the product is easier than in the use of concentrated sulfuric acid. Yields obtained using this method are in general acceptable e.g. 30% HBr/CH_3CO_2H gave [9]N_2O in 92% yield reported by Thom *et al in* 1986,[84] 40% HBr/CH_3CO_2H /phenol gave [5]N_5 in 94% yield by Osvath *et al* in 1987[85] and 33% HBr/CH_3CO_2H /phenol gave [32]N_6 in 92% yield by Hosseini and Lehn in 1986.[86] Thus this method is applicable for small to large sized macrocyclic rings.

- Reductive cleavage of the sulfonamide group with lithium aluminium hydride is a convenient reasonably clean route to isolate the desired product. However, if there are any carbonyl moieties within the structure they will also be reduced. Examples of employing this method in cleaving sulfonamide moieties are the formation of [18]N_4O_2 in 95% yield by Buhleier *et al.* in 1977[87] and [19]N_2O_4 in 85% yield by Pietraszkiewicz and Jurczak in 1984.[88]
- Electrochemical methods may be used to cleave tosylate groups. In general the yields are not as high as in other cleavage techniques. Examples of this technique being employed are yields of 92% of dibenzo-[15]N_2O_2 and 52% for cyclen both by Kossai *et al* in 1979.[89]

There are several other methods which have been used in sulfonamide cleavage such as the use of sodium with naphthalene.[90]

The most recent development in the area is that of reductive cleavage using Gilman's phenyldimethylsilyl lithium at 0 °C which selectively reduces secondary over primary amines.[91] This will be discussed later.

4.5.1 Results and Discussion

The first set of compounds which were synthesised consisted of alkylating agents containing only nitrogen.

This set of compounds was made to investigate the effect of:

- altering the number of alkylating arms by changing the number of nitrogens within the system;
- the size of the ring by changing the number of carbons between the nitrogens; and

- alkylation at different sites on the DNA strand.

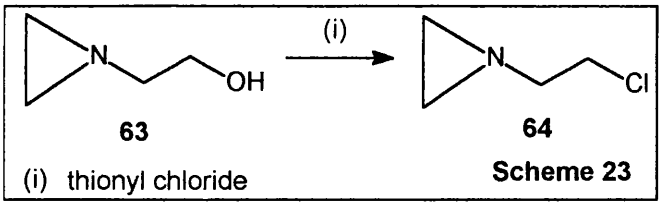
4.6.1 Synthesis of Macrocyclic Compounds

There were three major routes employed in the synthesis of the azamacrocyclic alkylating agents in this chapter:

- use of readily available macrocycles to form the alkylating agents;
- high dilution techniques; and
- cyclisation using condensation techniques.

4.6.2 Available Starting Materials

There are a very limited number of azamacrocycles which are available at a reasonable cost. The alkylating agents were formed by the reported method of Hancock *et al* [92] and Boeyens *et al* [93] using ethylene oxide in EtOH followed by chlorination with thionyl chloride as shown in **Scheme 23** and **Scheme 24**.



63 **64**

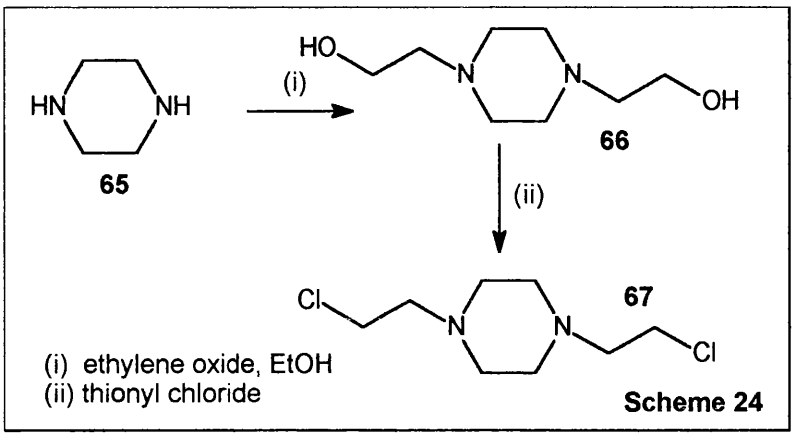
(i) thionyl chloride

Scheme 23

The first product synthesised was *N*-choloroethylaziridine. This was formed from *N*-hydroxyethylaziridine, **63**, which was available from Aldrich, and thionyl chloride. The chloride was recrystallised from EtOH and diethyl ether after continuous stirring in thionyl chloride for 48 h, as shown in **Scheme 23.** The IR spectrum of **64** showed a C-Cl stretch at 779 cm^{-1} and CH stretches at 2850 and 2981 cm^{-1}. The ^{13}C and ^{1}H NMR spectra showed the presence of three carbon signals at δ 55.1, 48.8 and 27.1 and the absence of the hydroxyl proton in the ^{1}H NMR spectrum.

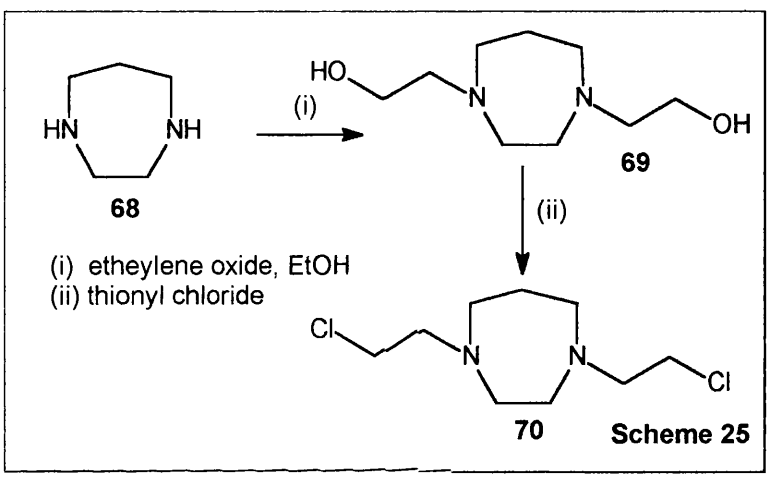
N,N'-Di(chloroethyl)piperazine, **67**, was formed from piperazine firstly forming the diol **66** and chlorination using thionyl chloride, as shown in **Scheme 24**. Product **67** was recrystallised from EtOH and diethyl ether in good yield as a white solid. Diol **66** showed stretches in the IR spectrum corresponding to the hydroxyl at 1282 and

3421 cm^{-1}. The ^{13}C NMR spectrum showed the three carbon signals at δ 59.6, 58.9 and 52.1 and the accurate mass spectrum in EI^+ mode showed the mass of **66** to be 175.1447 amu with the required mass being 175.1446 amu. The target compound showed the absence of hydroxyl stretches in the IR spectrum but the presence of a C-Cl stretch at 751 cm^{-1}.



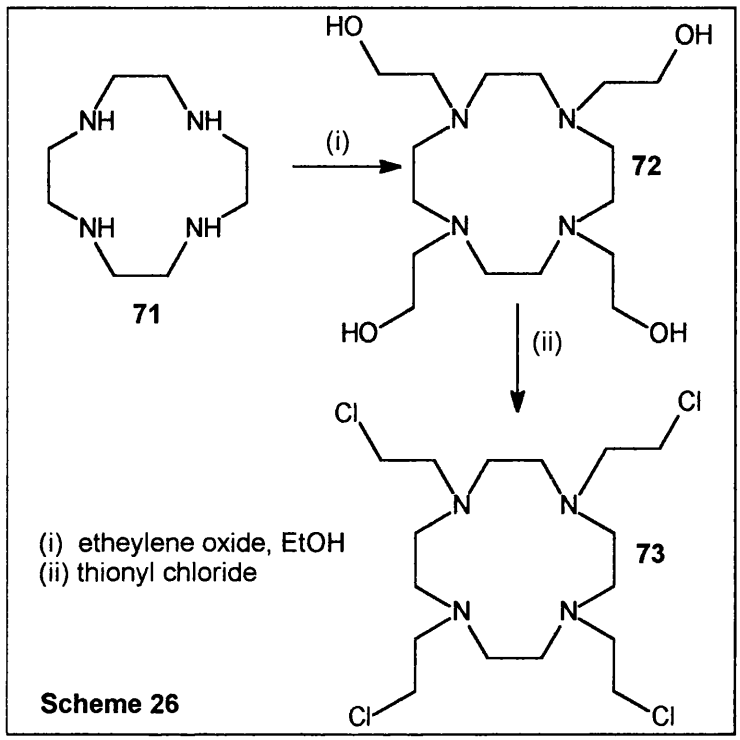
Scheme 24

The third compound, *N,N'*-di(chloroethyl)-1,4-diazacycloheptane, **70**, formed as shown in **Scheme 25**, increases the ring size by one carbon. Diol **69** was formed from 1,4-diazacycloheptane **68** and ethylene oxide in EtOH. The diol showed the presence of the hydroxyl proton in the ^1H NMR spectrum at δ 4.05. The ^{13}C NMR spectrum showed five methylene carbons and the IR spectrum showed hydroxyl stretches at 1251 and 1052 cm^{-1}. The target compound **70** was formed by continuous stirring of compound **69** in thionyl chloride for 24 h as a beige solid which was recrystallised from EtOH and diethyl ether in good yield as an off-white solid. The IR spectrum showed the presence of a C-Cl stretch at 751 cm^{-1} and the absence of the hydroxyl stretches. The ^1H NMR spectrum confirmed the absence of the hydroxyl proton.



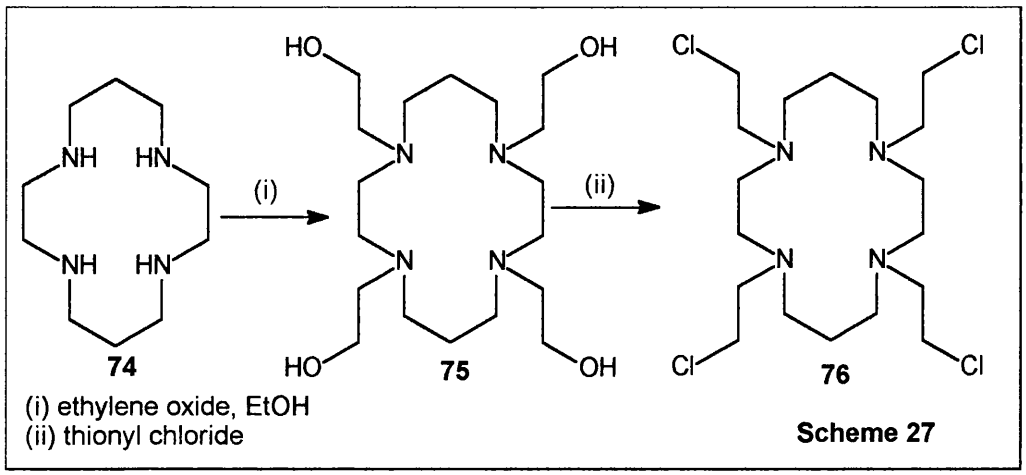
Scheme 25

The tetraol compound **72** was formed from cyclen (1,4,7,10-tetraazacyclododecane) **71** with ethylene oxide in the usual method. The target compound **73** was formed by stirring **72** in neat thionyl chloride as in **Scheme 26.**



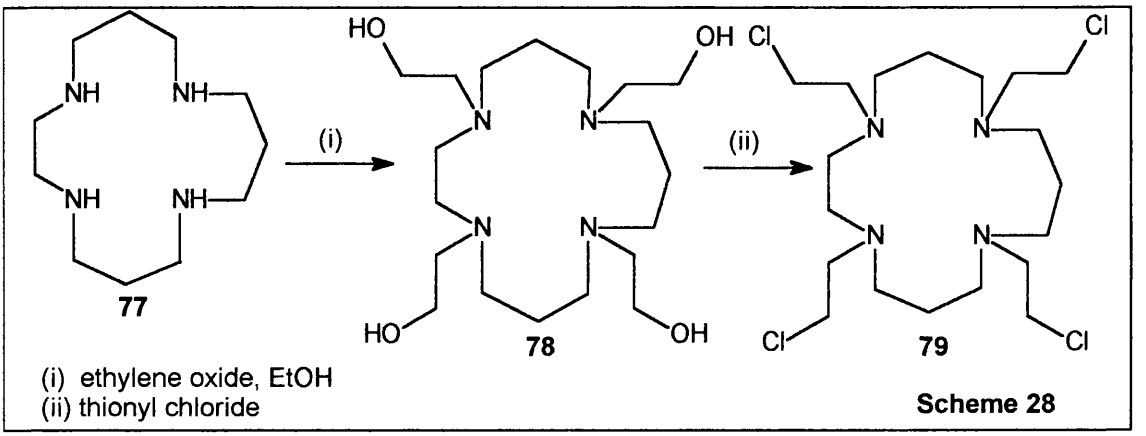
Scheme 26

The ^{1}H NMR spectrum for compound **72** showed signals at δ 2.16-2.23 with intensity 24 protons corresponding to the methylenes next to the nitrogens, δ 3.27-3.31 of intensity 8 protons representing the methylenes adjacent to the hydroxyl groups and finally the hydroxyl protons at δ 4.87. The corresponding tetrachloride compound **73** showed C-Cl stretches in the IR spectrum at 727 cm^{-1}. The microanalysis showed acceptable percentages for carbon, hydrogen and nitrogen.



Scheme 27

Tetrachloro compound **76** also has four potential alkylating sites contained within a macrocycle which has two carbon atoms more than compound **73** in the ring. Tetraol **75** was obtained in excellent yield as a viscous oil and gave spectroscopic data as in the literature.[93] The chlorinated compound **76** was obtained by stirring in thionyl chloride, as shown in **Scheme 27**, followed by recrystallisation from EtOH and diethyl ether in 74% yield. The IR spectrum showed a C-Cl stretch at 765 cm^{-1} and the absence of any hydroxyl stretches.

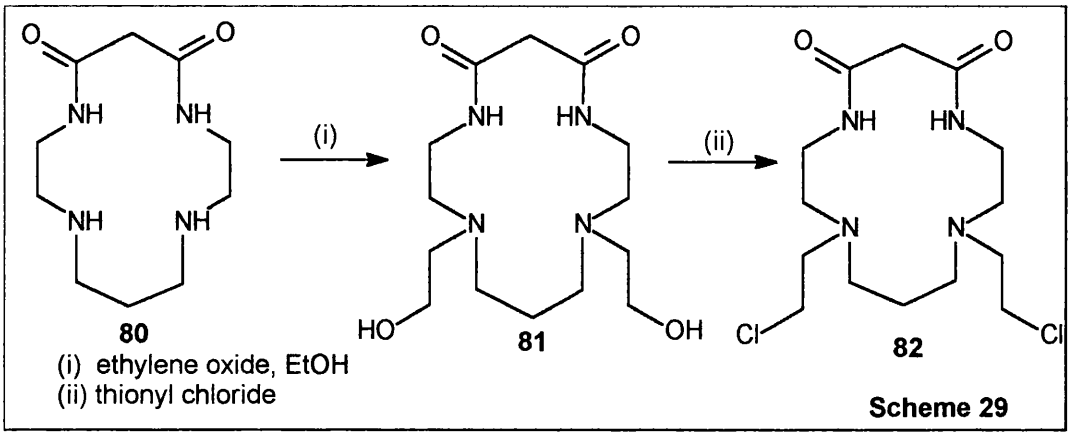


Scheme 28

By increasing the macrocyclic unit by one carbon another test compound **79** can be formed after alkylation of **77** using ethylene oxide followed by chlorination using thionyl chloride, as shown in **Scheme 28**. **79** was obtained in reasonable yield as an off-white solid. The tetraol **78** had an accurate mass spectrum found and required of 391.3284 amu in CI^+ mode. The ^1H NMR spectrum of **78** showed signals at δ 3.2-3.0 representing the eight methylene protons alpha to the hydroxyl group. The tetrachloride **79** had an accurate mass of 463.1929 amu found and required. The ^1H NMR spectrum also showed a shift in the position of the methylene protons alpha to the terminal chloride group of the alkylating arms.

By the inclusion of a diamide feature we can easily form a bifunctional alkylating agent **82** by alkylation of **80** using ethylene oxide in EtOH followed by chlorination using thionyl chloride as shown in **Scheme 29**. Starting with 1,4,8,11-tetrazacyclotetradecane-5,7-dione the diol **81** was formed which showed a ^{13}C NMR spectrum signal at δ 168 corresponding to the amide carbonyl carbon, and five signals between δ 63 and 53 corresponding to the carbons alpha to the amines and the carbons adjacent to the hydroxyl group. The target compound **82** showed

the absence of any hydroxyl stretches in the IR spectrum but the presence of a CH_2-Cl stretch at 752 cm^{-1}, along with the amide carbonyl stretch at 1686 cm^{-1}. The accurate mass spectrum showed the found mass to be acceptable.

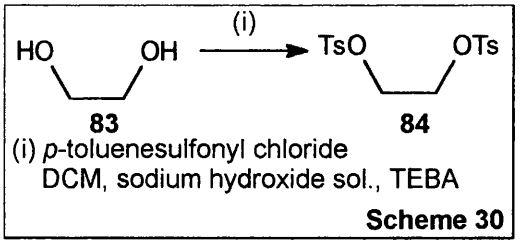


These are the only compounds which were formed from readily available starting materials.

4.6.3 High Dilution Synthesis

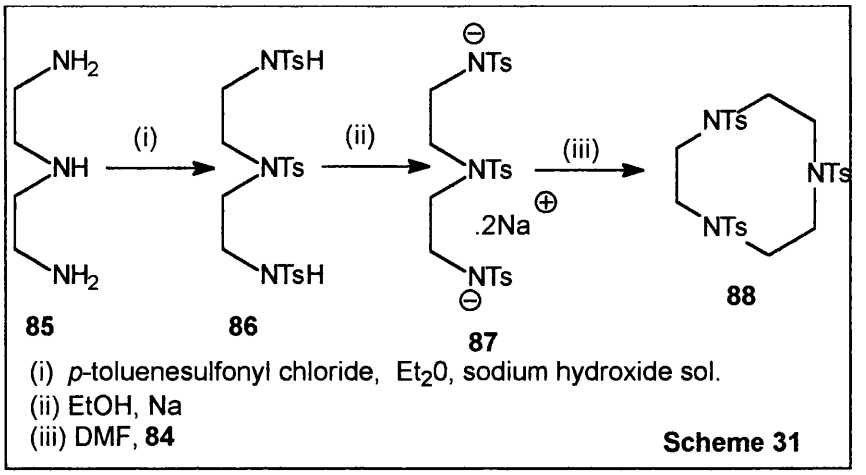
As discussed earlier high dilution techniques are an appropriate method to employ in the formation of macrocyclic compounds.

The general synthesis of this type of compound is by the protection and activation of the amine systems by a tosylate and activation of the alcohol to facilitate cyclisation.



To form the macrocyclic compound **88**, cyclisation with the bistosylated compound **84** was carried out as shown in **Scheme 31**. The ditosylated alcohol **84** was formed from 1,2-ethanediol, **83**, *p*-toluenesulfonyl chloride, DCM, sodium hydroxide solution and TEBA in a two phase reaction as shown in **Scheme 30**. This reaction went in excellent yield to afford compound **84** as a white solid (all tosylated compounds were recrystallised before use in cyclisation reactions). The disodium salt **87** was formed by dissolving the tosylate in EtOH and adding blocks of sodium. The

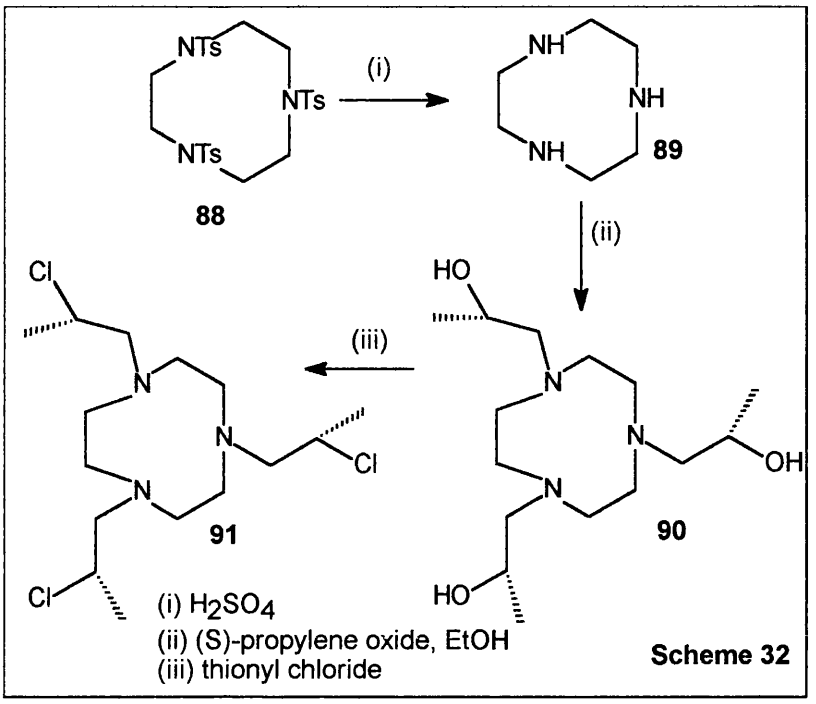
macrocyclic product **88** was obtained in good yield as a white fluffy solid. Spectroscopic data agreed with those reported and good analytical data were obtained.[80]



(i) *p*-toluenesulfonyl chloride, Et_20, sodium hydroxide sol.
(ii) EtOH, Na
(iii) DMF, **84**

Scheme 31

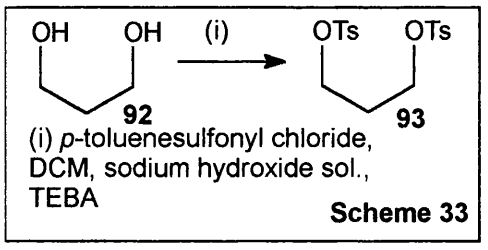
The trisulfonamide **88** was detosylated as discussed in section **4.4.1** to afford the free base 1,4,7-triazacyclononane **89** as a white solid. The ^1H NMR spectrum showed a singlet of intensity twelve protons at δ 2.47 corresponding to the NCH_2 protons and δ 1.75 of intensity 3 protons corresponded to the secondary amine protons. The ^{13}C NMR spectrum showed only one signal at δ 47.0. This material **89** was then alkylated to afford the triol **90**, which was chlorinated on stirring in neat thionyl chloride overnight as shown in **Scheme 32**. The ^1H NMR spectrum of the triol **90** showed signals at δ 1.14-1.16 intensity 9 protons, δ 2.41 intensity 18 protons and δ 3.71-3.90 with intensity 3 protons corresponding to the methyl, methylene adjacent to the nitrogen and the methine protons, respectively. The chloride **91** showed acceptable microanalysis results and IR stretches at 780 cm^{-1} representing the C-Cl stretch.



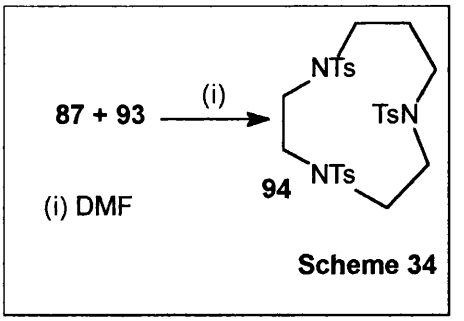
Scheme 32

The next two compounds were synthesised similarly to the last compound. *N,N',N"*-Tri(*p*-toluenesulfonyl)-1,4,7-triazacyclodecane **94** was formed using the trisulfonamide **87** and the ditosylated compound **93** as shown in **Scheme 34**.

Tosylated 1,3-propane diol compound **93** was obtained, as shown in **Scheme 33**, using *p*-toluenesulfonyl chloride, DCM, sodium hydroxide solution and TEBA as a white solid in good yield. Spectroscopic data were in agreement with the literature values.[80]

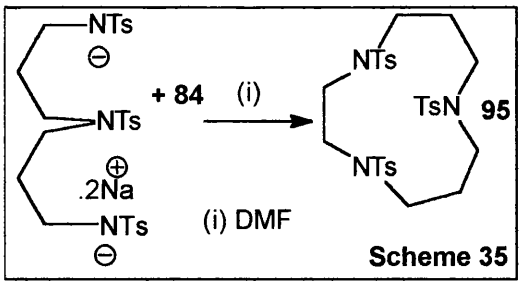


Scheme 33



Scheme 34

N,N',N"-Tri(*p*-toluenesulfonyl)-1,4,8-triazacyclounadecane **95** was prepared from *N,N',N"*-tri(*p*-toluenesulfonyl)-dipropylenetriamine and *O,O'*-di(*p*-toluenesulfonyl) -1,2-ethanediol as shown in **Scheme 35**. The accurate mass, ^{1}H and ^{13}C NMR spectra were all acceptable for these compounds.



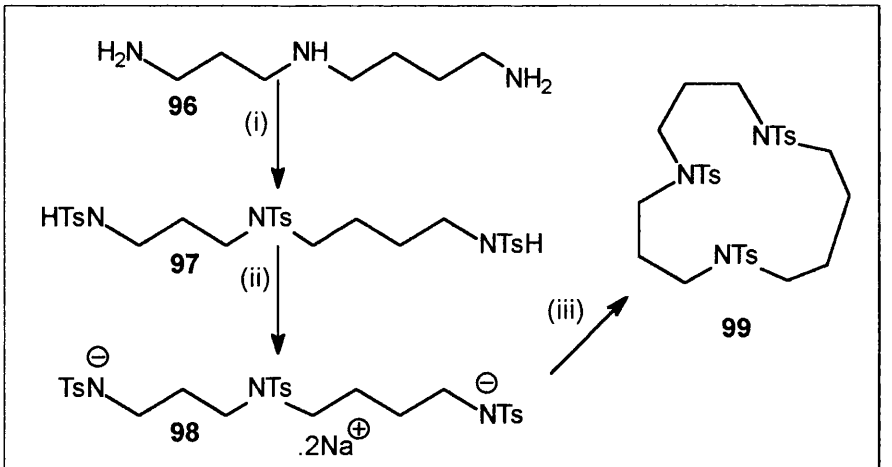
Scheme 35

4.6.4 Linear System Formation Before Cyclisation

An alternative method which can be employed in the formation of medium to large sized macrocylic compounds is that of forming linear amine systems before the cyclisation reaction.

Two linear polyamine systems which are readily available are those of spermine, **103**, and spermidine, **96**. Spermidine, **96**, has three nitrogens in the system with spacer lengths of three and four carbons. This allows us to form macrocycles where the spacer lengths between the carbons in the macrocycle are different from the previously mentioned compounds.

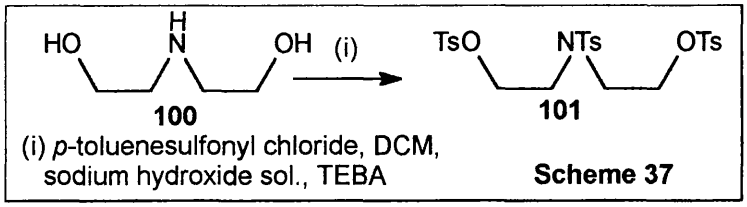
Spermidine was tritosylated using the usual amine tosylation method with *p*-toluenesulfonyl chloride, sodium hydroxide solution and diethyl ether at 0 °C as shown in **Scheme 36**.



(i) *p*-toluenesulfonyl chloride, Et_2O, sodium hydroxide sol.
(ii) EtOH, Na
(iii) DMF, **93**

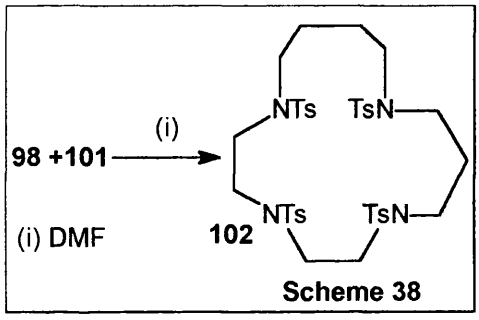
Scheme 36

The tritosylsulfonamide compound **99** was obtained on cyclisation of the disodium salt of **98**, with **93** and obtained as a white solid after dropwise addition of **93** in dry DMF to the tosylated amine at 100 °C, as shown in **Scheme 36**.



Scheme 37

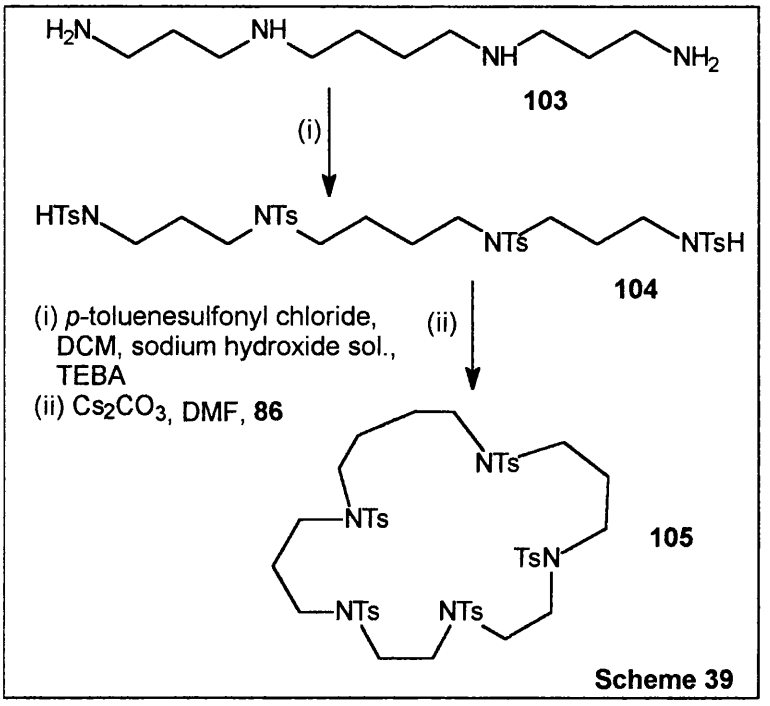
Tritosylation of diethanolamine **100** afforded **101** in good yield as a fluffy white solid as shown in **Scheme 37**. Spectroscopic data were in accord with the literature.[94] Compound **101** was reacted with the disodium salt of the tosylated spermidine compound **98**, as shown in **Scheme 38**, in dry DMF to afford the tetratosylsulfonamide 15-membered ring as a light yellow powder.



Scheme 38

The longer spermine chain allows us to have carbon spacer lengths between the nitrogens in the ring systems of three, four and three respectively. The final spacer length between the terminal nitrogens is dependent on the tosylated alcohol employed in the cyclisation step.

As previously the amine must be protected by tosylation. The tetratosylated compound **104** was formed in the usual manner with *p*-toluenesulfonyl chloride and sodium hydroxide solution in diethyl ether. The yield of the white solid was poor but acceptable. The nineteen membered tetratosylsulfonamide compound **105** was obtained on the cyclisation of the tosylated spermine **104**, with the tritosylated diethanolamine **86**, formed as shown in **Scheme 39**, by the usual manner, in DMF again utilising caesium carbonate to form the anion *in situ* as in **Scheme 39**.



Scheme 39

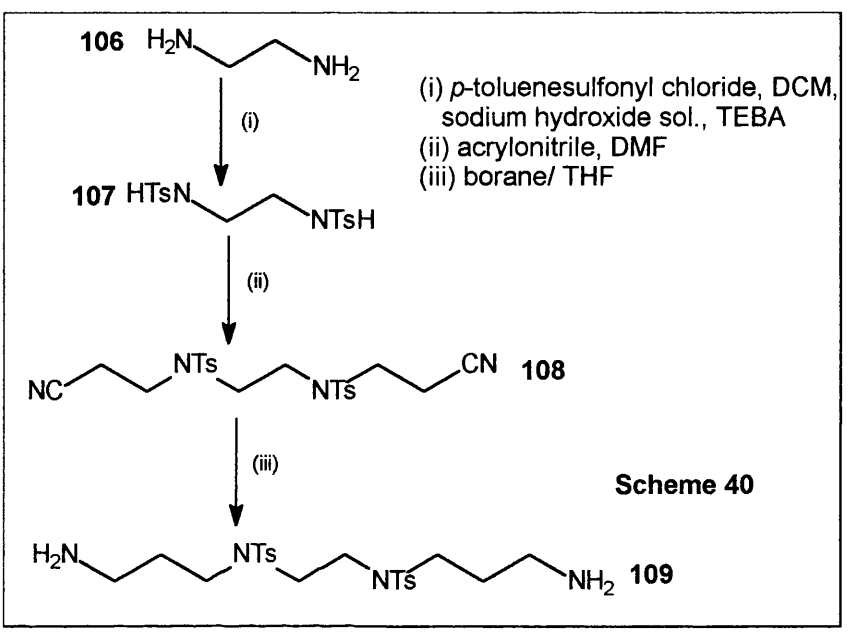
In a similar manner linear chains of varying lengths can be built up from readily available starting materials such as 1,2-diaminoethane and 1,3-diaminopropane as shown in **Scheme 40**. This allows larger macrocycles containing additional nitrogen atoms to be formed.

The synthesis of *N,N'*-4,8,11,15-hexa(*p*-toluenesulfonyl)-4,8,11,16-tetraaza octadecane-1,20-diamine from 1,2-diaminoethane was performed in a similar manner to reported, as shown in **Scheme 40**.[95] The linear hexaamine **109** was prepared as the acyclic form of the macrocyclic hexaamine product desired. Symmetrical elongation of diethylamine by tosylation, Michael condensation with acrylonitrile followed by reduction with borane gave rise to compound **108**. This was subjected to the same sequence of reactions to afford compound **110**.

Compound **107** was formed using the literature method[95] as shown in **Scheme 41** in good yield as white crystals. The ^{1}H NMR spectrum showed a doublet of doublets at δ 7.72-8.39 corresponding to the aromatic AA'BB' system, a singlet at δ 2.4 corresponding to the methyl group attached to the aromatic moiety, a broad singlet, of intensity 2 protons, at δ 5.53 representing the amine proton and a singlet of intensity 4 protons corresponding to the methylene groups alpha to the nitrogen atoms. The elongation of the compound proceeded by adding acrylonitrile in excess dropwise to a stirring mixture of compound **108** in DMF as reported.[95]

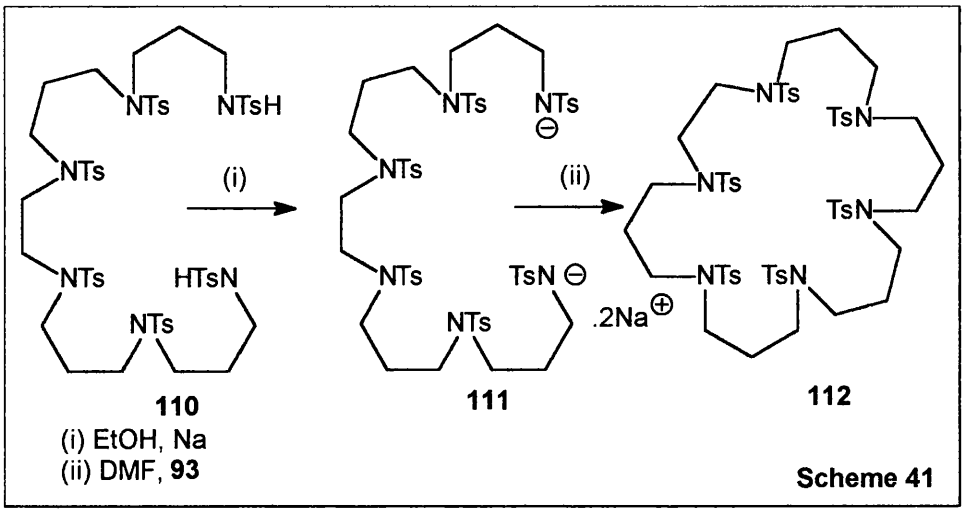
Characterisation of the white needles obtained in good yield, showed the presence of a nitrile stretch in the IR spectrum at 2250 cm^{-1} and the nitrile carbon in the ^{13}C NMR spectrum at δ 118.2. Compound **108** was reduced using borane to afford the primary terminal amine. The IR spectrum showed the absence of the nitrile stretch and the presence of broad N-H stretches at 2258 cm^{-1}. The terminal nitrogens were singly tosylated as before. This linear tetraaza compound was further elongated using the previous method. Again the nitrile carbon appeared in the ^{13}C NMR spectrum at δ 118.1 cm^{-1}. Compound **110** was obtained on reduction using borane and tosylation as before as a white solid in acceptable yield. The IR spectrum again showed the absence of nitrile stretches and the presence of primary amine N-H stretch at 2270 cm^{-1}. The ^1H NMR spectrum showed two singlets of intensity 12 and 6 protons at δ 2.36 and 2.33 corresponding to the internal and terminal methyl groups, with a doublet of triplets at δ 1.85-1.70 of intensity 8 protons and a broad singlet at δ 3.21 of intensity 2 protons corresponding to the secondary amine protons. The aromatic region was a complex multiplet of doublet of doublets corresponding to the AA'BB' aromatic systems of the sulfonamide groups.

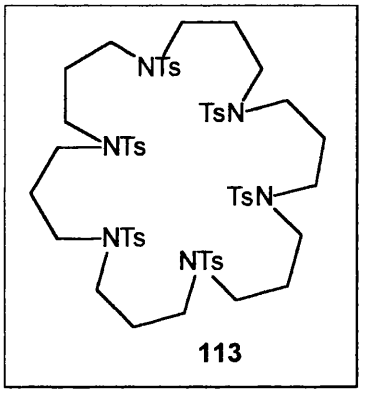
The disodium salt of the linear hexaamine **110** was formed in EtOH using sodium, to afford a white solid which was not characterised but cyclised using high dilution techniques, with *O,O'*-ditosylpropanediol **93**, to afford the tosylated analogue of the desired product as a biege solid in good yield. The tosylated 1,3-propanediol compound was synthesised as described.[95] The cyclisation of **93** and **111** afforded compound **112**.



Scheme 40

Compound **113** was synthesised in the same manner as compound **112** starting from 1,3-propanediamine. Characterisation of intermediates agreed with data reported.[95]



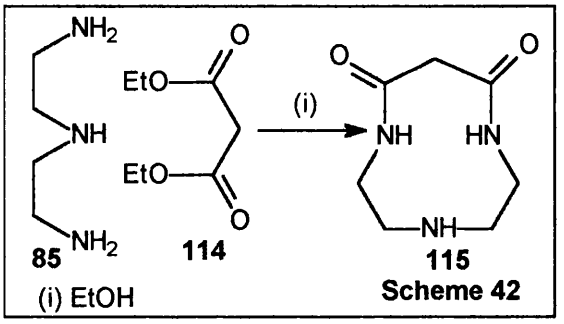


4.6.5 Condensation cyclisations

The use of condensation reactions between esters and amines is a convenient method for the synthesis of macrocyclic amines which may or may not contain other heteroatoms such as oxygen or sulfur. This procedure involves neither the use of high dilution techniques nor the protection and deprotection of amines to afford the free bases desired.

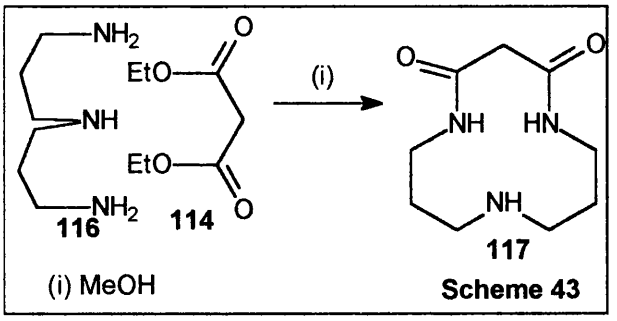
In compound **115** two of the nitrogens present in the ring system are protected as a direct consequence of the cyclisation technique employed as shown in **Scheme 42**. The condensation of esters with linear polyamines in EtOH generally gives the diamide cyclisation product. From this point selective alkylation of the unprotected nitrogens is possible or reduction of the amides to afford the free base. Two aza

macrocyclic compounds, **115** and **117**, were formed in this manner using literature procedures.[96,97]



Scheme 42

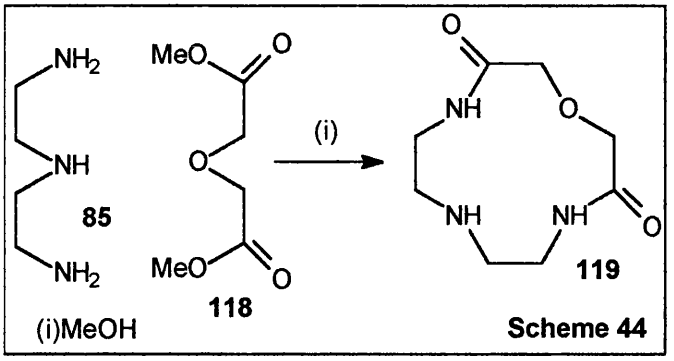
Compound **115** was formed by vigorously heating diethylenetriamine and diethyl malonate in EtOH refluxing for 5 days as reported.[97] **115** showed a doublet of triplets in the ^{1}H NMR spectrum at δ 3.24-2.64 and signals at δ 170 corresponding to the carbonyl carbon, and δ 47.2 and 39.2 corresponding to the other carbon types present. The diamide was reduced to afford the free base in very low yield. The ^{13}C NMR spectrum showed the absence of the carbonyl carbon and an additional signal at δ 52.7.



Scheme 43

The diamide **117** was formed from triaminodipropane and diethyl malonate in MeOH as in the literature and is shown in **Scheme 43.** ^{1}H and ^{13}C NMR signals corresponding to the literature. [97]

The final diamide prepared was 1-oxa-4,7,10-triazacyclododecane-3,11-dione **119** formed from diethylenetriamine **85** and the methyl ester **118** derived from diglycolic acid as shown in **Scheme 44**.[98] The ^{13}C NMR spectrum showed the presence of the amide carbonyl carbon at δ 172 and three other signals at δ 70.4, 47.4 and 38.3 representing the remaining carbons.



Scheme 44

Unfortunately the diamides were mainly resistant to reduction by borane or $LiAlH_4$. ^{13}C NMR spectra indicated starting material by the presence of a signal at ~ δ 170. The biological evaluation of the target compounds is discussed at the end of the next chapter.

Chapter 5

5.1.1 Heterocyclic Alkylating Agents

The previous chapter was concerned with the synthesis of alkylating agents containing only nitrogen as the heterocyclic atom. This chapter will, however, be concerned with the synthesis of alkylating agents containing atoms other than nitrogen (such as oxygen and sulfur) within the macrocycle.

Variations in the size of macrocycle, number and type of heteroatoms, and number of alkylating groups will be made.

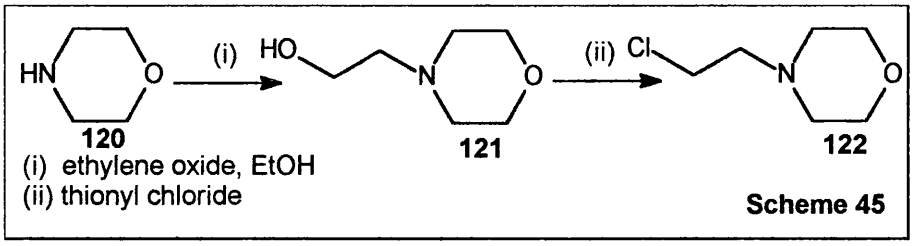
5.2.1 Synthesis of Oxa, Aza Macrocyclic Alkylating Agents

The first set of oxa, azamacrocyclic alkylating agents which were synthesised were formed similarly to some of the azamacrocycles in **Chapter 4**.

5.2.2 Available Starting Materials

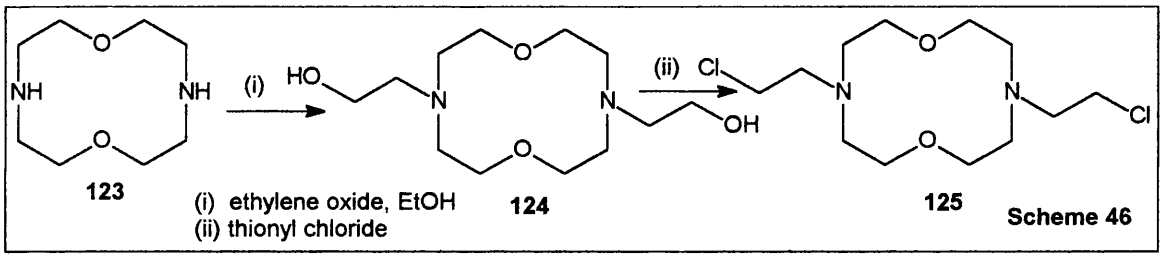
There are a very limited number of oxa, and azaoxamacrocycles which are readily available from chemical companies which are reasonably inexpensive. This limited availability reflects the difficulties which are generally encountered in the synthesis of macrocycles of this type. There are a few, however, which have been developed into alkylating agents for biological evaluation in this work.

The first compound formed was *N*-chloroethyl-1-oxa-4-azacyclohexane **122** as shown in **Scheme 45**. 1-Oxa-4-azacyclohexane **120** was successfully alkylated using ethylene oxide in EtOH to give the alcohol **121** as a light yellow oil in good yield. The accurate mass spectrum in EI^+ mode showed the found mass as 132.1024 amu and the required mass was 132.1025 amu. The alcohol **121** was stirred in thionyl chloride to afford the chloride, compound **122** as a cream coloured solid in high yield. The IR spectrum showed the presence of the C-Cl stretch at 659 cm^{-1}. The accurate mass spectrum in CI^+ mode was again acceptable. The found mass was 150.0685 amu with a required mass of 150.0686 amu.



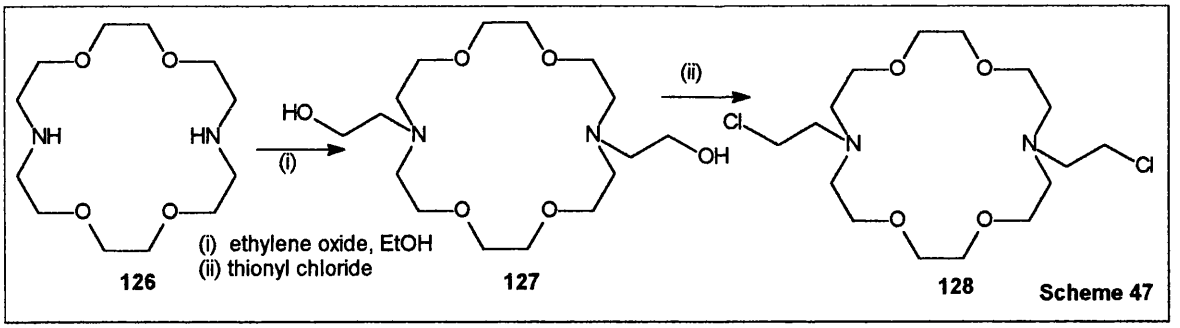
Scheme 45

Compound **125** was formed from 1,7-dioxa-4,10-diazacyclododecane. This was treated with ethylene oxide to afford the diol **124** which was treated with thionyl chloride to give the target compound as shown in **Scheme 46**. The diol **124** was obtained as an orange oil in good yield. Target compound **125** was obtained as a light beige solid in acceptable yield. The ^{13}C NMR spectrum of **125** showed the presence of four carbons at δ 67.4, 60.3 corresponding to the carbons next to an oxygen atom and signals at δ 57.7 and 53.4 corresponded to the methylene carbons adjacent to the nitrogen atom.



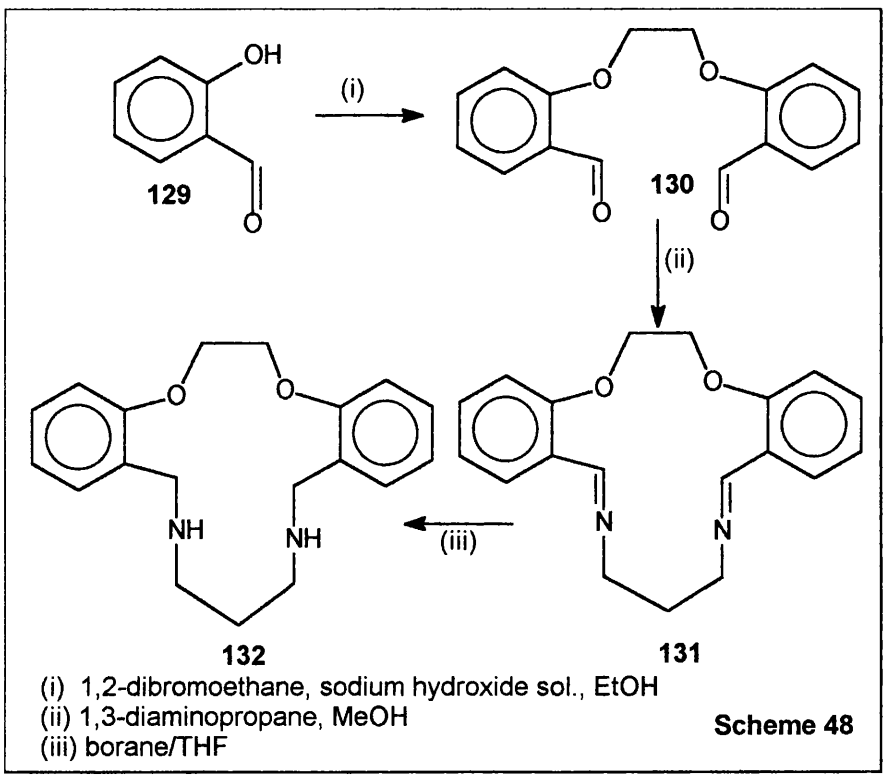
Scheme 46

The third target compound which was formed from readily available starting material was compound **128**. This was obtained when 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane was treated with ethylene oxide followed by thionyl chloride as shown in **Scheme 47**. The dichloride was obtained as a viscous orange oil. The diol **127** was obtained in high yield and its ^{1}H NMR spectrum showed signals at δ 2.58-2.63 as a multiplet corresponding to the methylene protons adjacent to the nitrogen atoms. Signals at δ 3.35-3.53 corresponded to the protons next to the oxygen atoms within the system. The hydroxyl protons were observed as a broad singlet at δ 4.78. In the ^{13}C NMR spectrum the shift of the signal of the methylene carbon next to the terminal atom from δ 59 in the diol to 39 supports the substitution of the hydroxyl for the chloride.



Scheme 47

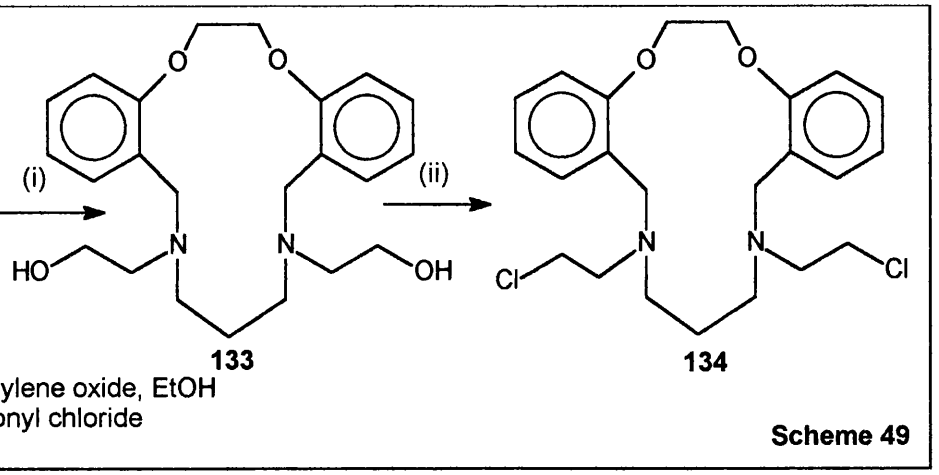
The dichloride compound *N,N'*-di(chloroethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane **128** was formed similarly to the previous target molecule, using the usual chlorination methods. The ^{13}C NMR spectrum of **127** showed two signals at δ 68.7 and 67.7 corresponding to the methylenes next to the oxygens and signals at δ 58.1, 58.0 and 53.5 corresponded to the carbons next to the nitrogen and hydroxyl atoms. The dichloride was obtained in acceptable yield and showed a shift of the methylene next to the terminal atoms to δ 42.7 supporting the substitution of the hydroxyls by the chlorides.



Scheme 48

Compounds containing a benzyl moiety have previously been shown to have significant biological activity against malaria parasities by Dr Lindsay Slater, who was previously a member of the research group.[99] The presence of benzyl groups increases the lipophilicity of the compounds. The synthesis of

N,*N*'-di(hydroxyethyl)-5,6:14,15-dibenzo-1,12-diaza-1,4-dioxacyclopentadecane, has been reported.[100]



(i) ethylene oxide, EtOH
(ii) thionyl chloride

Scheme 49

The aldehyde **130** was formed as in the literature by Williamson condensation from salicylaldehyde **129** and 1,2-dibromoethane in a 2 to 1 ratio with characterisation as in the literature.[101] Imine **131** was formed on treating the aldehyde **130** with 1,3-diaminopropane as a fluffy beige solid in low yield. The imine was reduced using an excess of borane in THF to afford the secondary amine **132** as a white solid in acceptable yield, as shown in **Scheme 48**. Characterisation of compounds was in accord with the literature data.[101] The free base **132** was alkylated in the usual manner with ethylene oxide and chlorinated using thionyl chloride as shown in **Scheme 49** to afford *N*,*N*'-di(chloroethyl)-5,6:14,15-dibenzo-1,12-diaza-1,4-dioxacyclopentadecane **134** as a white powder with spectroscopic data in agreement with those of Lindoy *et al.*[102]

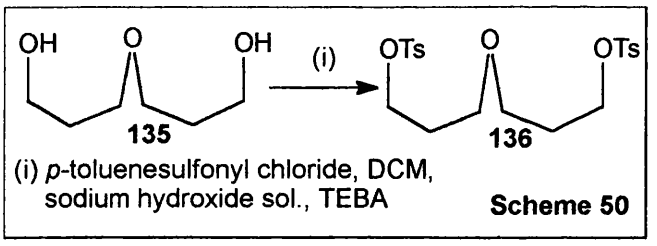
5.2.3 High Dilution Synthesis

There are a wide variety of macrocyclic alkylating agents which could be synthesised.

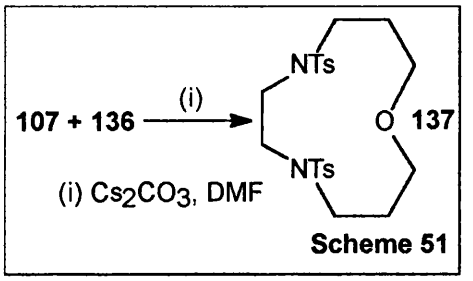
Using high dilution techniques it is relatively straightforward to form macrocyclic structures which contain sulfonamide moieties. In this work variations were made in the size of the macrocycle; the number and type of heteroatom; and their spacing within the macrocycle.

5.2.4 Diaza Macrocyclic *N*-Tosylated Compounds

We chose to start by forming a small aza-oxa macrocyle compound **137** using high dilution techniques. 1,3-Ethylenediamine was tosylated as previously to give compound **107**. Dipropylene glycol was tosylated as shown in **Scheme 50**, to afford compound **136**, [103] i.e. using *p*-toluenesulfonyl chloride, DCM, with sodium hydroxide solution acting as a base and TEBA as catalyst. The tosylated alcohol, **136**, was obtained as fluffy white needles in high yield. Spectroscopic data agreed with those of the literature.[104] The disodium salt of the tosylated amine was formed by adding sodium to a solution of the secondary amine compound **107** in EtOH. The cyclised product was obtained by dropwise addition of the tosylated alcohol **136** in dry DMF to a solution of the salt of the tosylated amine in dry DMF at 100 °C and the temperature was maintained over night. The ditosylated product was obtained as an off white solid in acceptable yield. Characterisation was in agreement with that of the literature.[105]

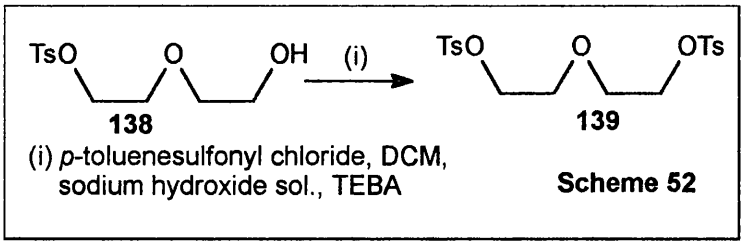


Scheme 50

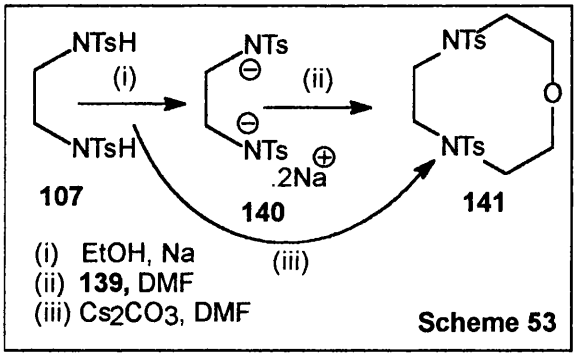


Scheme 51

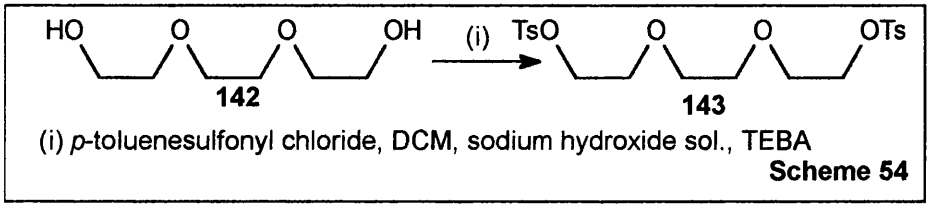
An alternative method would be to use two equivalents of caesium carbonate, in dry DMF along with the tosylated amine and tosylated alcohol compounds as shown in **Scheme 51**. The deprotonated tosylamide is formed *in situ*. The caesium ion does not play an active role in these types of reactions but acts as a means of solubilising the carbonate in the solvent. The tosylated alcohol was slowly added over a period of several hours and stirred overnight. This reaction involves a [1+1] Richman Atkins type co-condensation of the *N*-tosylated compound **107** with the linear ditosylate ester. The product was obtained on crystallisation from hot toluene. The yield from this method was very poor. Thus the former method was preferred.

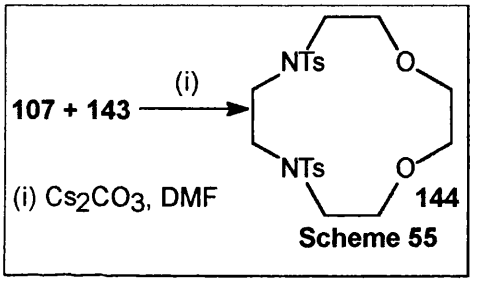


Compound **139** is very similar to the previous compound formed but contains two carbons less. Diethylene glycol was tosylated as shown in **Scheme 52**, using *p*-toluenesulfonyl chloride, DCM, sodium hydroxide solution and TEBA, as a white solid in good yield. The cyclic product was obtained by using caesium carbonate in dry DMF to facilitate the ring closure as shown in **Scheme 53**. However the yield of the white solid was very poor. Spectroscopic data were in agreement with those reported.[106]

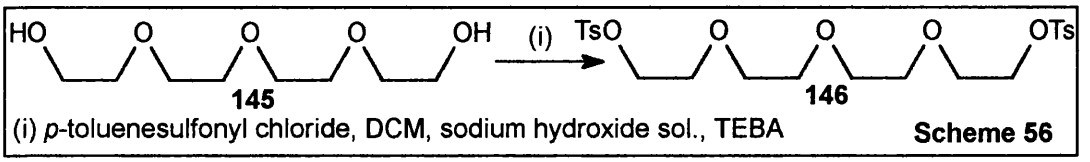


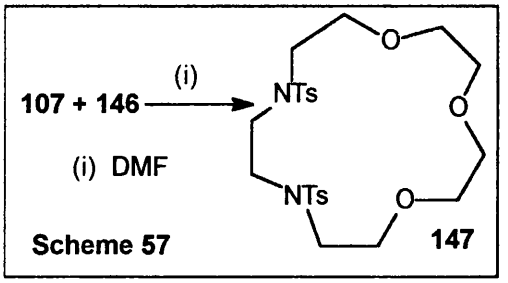
By extending the number of oxygens in the ring to two we can form medium sized macrocycles. The ditosylated alcohol **143** was formed from triethylene glycol, sodium hydroxide solution and TEBA in DCM as shown in **Scheme 54**.[103] The macrocycle contains two nitrogens, as shown in **Scheme 55**, using caesium carbonate to generate the salt *in situ*. Again this method leads to poor yields. Characterisation agreed with that in the literature.[107]





Finally the ditosylsulfonamide macrocyclic compound **147** was formed, as shown in **Scheme 57**, with three oxygen atoms in the ring. The disodium salt of **107**, was used along with ditosylated tetraethylene glycol compound **146** which was formed from tetraethyleneglycol, TEBA, sodium hydroxide and DCM in the two phase system shown in **Scheme 56**.[103] Spectroscopic data were in agreement with those in the literature.[108] The white solid macrocyclic product was obtained in good yield by this method and characterised data agreed with those reported.[109]

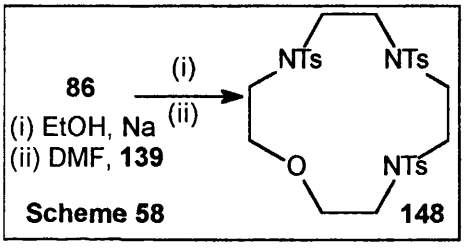




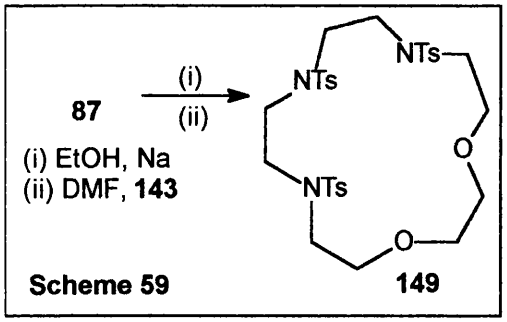
5.2.5 Triaza Macrocyclic *N*-Tosylated Compounds

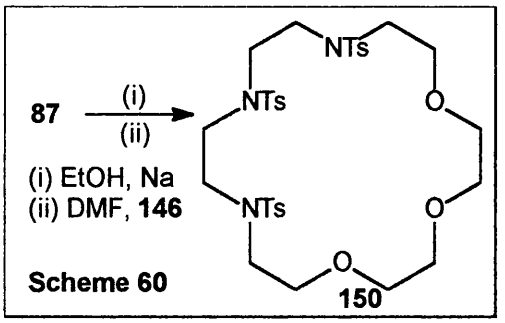
Incorporating three nitrogen atoms into the macrocycles allows us to extend the number of possible alkylation sites. It also increases the size of the macrocycle.

As in the previous section (**5.2.4**) the number of oxygens within the ring systems was increased stepwise. Compound **148** was formed from the tritosylsulfonamide **86**. The tosylation of diethylenetriamine was carried out as described in **Chapter 4** to afford compound **87** which formed the disodium salt also as in **Chapter 4**. The cyclisation occurred as before by the slow addition of the ditosylated alcohol **139** in dry DMF to the amine salt in dry DMF at 100 °C with vigorous stirring as shown in **Scheme 58**. Characterisation agreed with that in the literature.[110]



Compound **149** was synthesised from the tritosyl salt **87** and the ditosylated triethylene glycol **143**. The cyclisation occurred using the sodium salt, as shown in **Scheme 59**, to afford a white solid in acceptable yield.



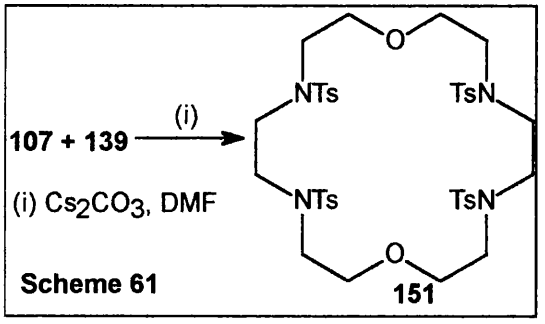


The trioxa, triaza compound **150** was synthesised from the tritosyl salt **87**, and the ditosylated tetraethylene glycol **146**, as shown in **Scheme 60**. The cyclisation was carried out as for the previous compound and afforded **150** as a white solid in good yield.

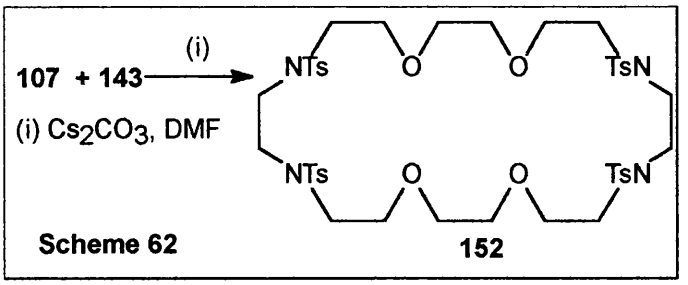
5.2.6 Tetraaza Macrocyclic *N*-Tosylated Compounds

The incorporation of a fourth nitrogen atom into the macrocycles allows us to extend the number of possible alkylation sites. In the compounds formed we are also altering the positioning of the nitrogen atoms relative to one another.

Compound **151** was previously formed in the synthesis of compound **141** as a [2+2] Richman Atkins type addition also takes place, i.e. two molecules of each of the tosylated amine and alcohol form the product as shown in **Scheme 61**. As with the smaller compound a very low yield was obtained.



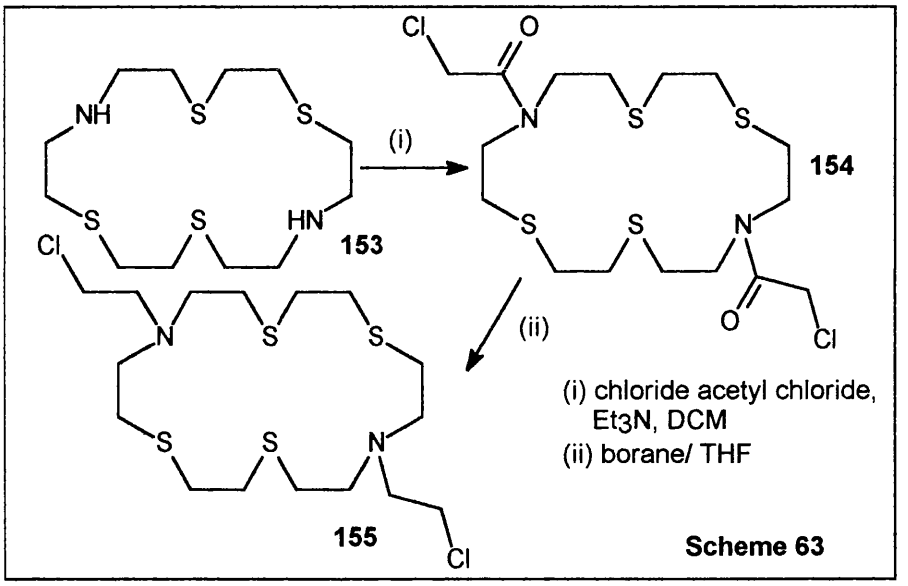
The final tetraaza macrocyle was synthesised in a similar way as shown in **Scheme 62**. As before low yields were obtained.



Scheme 62

5.3.1 Synthesis of Thia, Aza Macrocyclic Alkylating Agents

5.3.2 Available Starting Materials



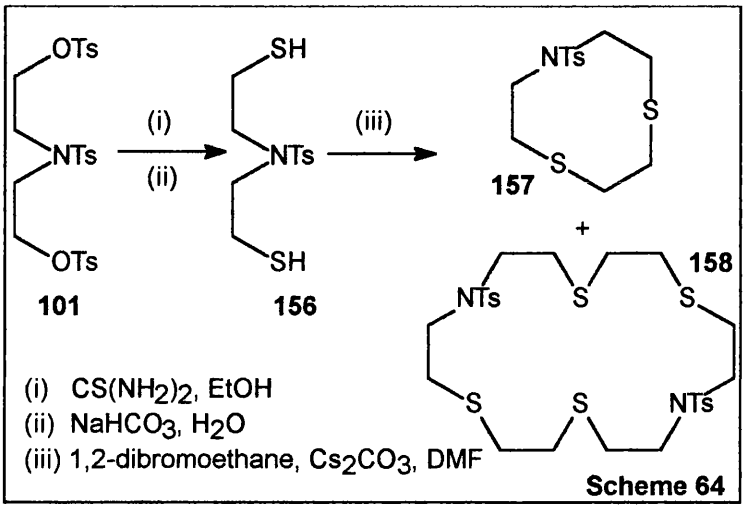
Scheme 63

As for the previous rings formed, there are very few cyclic starting materials from which we can work. 1,4,10,14-Tetrathia-7,17-diazacyclooctane **153**, is readily available. Using chloroacetyl chloride with triethylamine, the nitrogens were alkylated to afford compound **154**, by the method of Lehn, Parker *et al.*[111] The diamide was reduced using a solution of borane in THF, as shown in **Scheme 63**, to afford the target molecule **155** as an off white solid in good yield. The IR spectrum showed the absence of hydroxyl stretches and the presence of a CH_2-Cl stretch at 750 cm^{-1}.

5.3.3 High Dilution Synthesis

For comparison of biological activity a sulfur analogue of a previously prepared compound **125** was made.

Compounds **157** and **158** were formed as shown in **Scheme 64** from diethanolamine, which was tosylated in the usual manner to afford the tosyl compound **101**. The formation of two carbon to sulfur bonds proceeded using thiourea to afford compound **156**,[112] which was reacted with 1,2-dibromoethane in the usual manner with caesium carbonate to afford compounds **157** and **158**, with the [1+1] product as the major and the larger [2+2] product as the minor component.



Scheme 64

5.4.1 Detosylation Attempts on Sulfonamide Macrocycles

The process of removing tosylate groups from nitrogen atoms is one which is well documented as being very problematic. There are numerous methods as previously mentioned in **Chapter 4** which can be utilised for this process. However, none of these reactions are universally applicable. Thus each individual compounds formed as a sulfonamide macrocycle must be examined to decide an appropriate route for detosylation. There are numerous literature references to the difficulties encountered in successful performances of this reaction, relating to each of the different cleavage methods which can be employed.

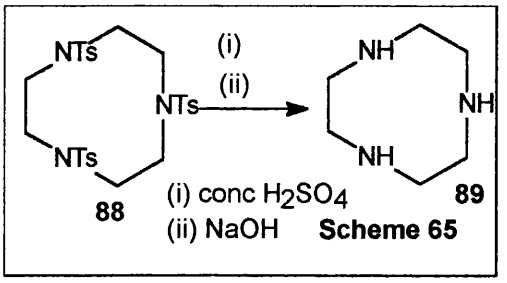
5.4.2 Concentrated Sulfuric Acid Method

One of the most commonly employed techniques in detosylation of amines is that of heating in concentrated sulfuric acid.

The reported temperature and times involved vary depending on the compound and author employing the method. The solution was then generally cooled and EtOH added cautiously. The aqueous solution was basified using sodium hydroxide pellets or solution and extracted using chloroform. This was the method reported by Lazaar[113], Hay and Norman[114] and Meunier *et al.*[115]

Variations of this method were employed for a selection of the sulfonamide macrocyclic compounds formed in **Chapter 4** and **Chapter 5**.

The only compound where complete detosylation occurred using this method was the tritosylated tacn **88** as shown in **Scheme 65**. Spectroscopic data of **89** agreed with those in the literature.[116]



Scheme 65

Compound **93** was heated at 160 °C for 8 h in concentrated sulfuric acid before cooling and addition to EtOH and diethyl ether which was stirring vigorously. A beige precipitate formed which was taken up in water and 47% hydrobromic acid was added. No precipitate occurred indicating that the hydrobromide salt of the triaza compound did not form.

The same technique, apart from heating at 180 °C was employed with compound **94**. ^{1}H NMR spectra indicated the precipitate was starting material obtained in low yield.

The penta aza compound **105** was treated with concentrated sulfuric acid and formation of the HBr salt was attempted. However, no precipate was observed on the addition of large volumes of diethyl ether.

Using the same method on oxa, aza compounds **151** and **152** heating the sulfuric acid solution at 190 °C for 1-2 h resulted in a small amount of precipitate of the detosylated salt. On analysis this was shown to be 75% detosylated. Thus this method looked promising for use on other oxa, aza compounds if the correct temperature and timing could be found. Further experiments using this method varying time and temperature of heating proved to be unfruitful; frequently cleavage

of the ether linkage was observed. Either starting material was recovered or an inseparable mixture of tosylated products and open ring compounds was obtained. This is not surprising due to the harsh conditions of the reaction. Compounds **144, 148** and **152** were all investigated by this technique, but to no successful conclusion.

The thia, aza compounds **157** and **158** were also subjected to treatment with concentrated sulfuric acid, but the compounds broke down.

5.4.3 Hydrobromic and Glacial Acetic Acid

This method of detosylation is also a frequently employed technique. Literature reports have shown a varied level of success using this technique from very low yield to virtually quantitative. The HBr salt was generally obtained from heating the sulfonamide compounds at reflux in 47% aqueous hydrobromic acid and glacial acetic acid. Alternatively prolonged stirring at room temperature sometimes facilitates the cleavage.[117] Phenol is frequently used as a bromine scavenger in this reaction, but is not necessary in all cases. The salt of the detosylated product was generally obtained on the addition of large volumes of organic solvents such as diethyl ether.

The *N,N',N"*-tri(*p*-toluenesulfonyl)-1,4,7-triazacyclononane **88**, was subjected to this method of reductive cleavage using literature methods.[117,118] with starting material being recovered.

The tosylate **99** formed from spermidine was stirred with a solution of phenol, HBr and glacial aetic acid. The major product obtained was phenol, without any recovery of the starting macrocyclic material.

The tetraaza, tetraoxa tosylate **152**, was subjected to this cleavage method. No salt was formed and no starting material were recovered.

5.4.4 Lithium Aluminium Hydride Reduction Method

This is one of the less vigorous methods of sulfonamide cleavage. It is also generally a clean and high yielding reductive technique. The products often prove difficult to isolate. At least 2.5 equivalents of lithium aluminium hydride are used per sulfonamide group, refluxing in THF for varied periods of time before basic work up.[119,120] This method was used for the detosylation of *N,N',N"*-tri

(*p*-toluenesulfonyl)-1,4,7-triazacyclononane **88**, using 2.5 equivalents of lithium aluminium hydride for each of the sulfonamide groups present. This method failed, affording a very small amount of white solid which was very impure starting material.

The treatment of the diaza, trioxa tosylate **147**, gave a similar result. However when the triaza, dioxa compound **142** was treated with 3.2 equivalents of the reducing agent a small amount of detosylation was observed, but the majority of the recovered material was starting material.

5.4.5 Na/ Liquid Ammonia Reduction Method

The use of liquid ammonia and lithium / sodium is a useful method for reductive cleavage of sulfonamides.[117] In literature reports this system has varied success and again has to be evaluated against each individual tosylate. It is carried out by adding sodium to a solution of liquid ammonia and the sulfonamide until a blue colour appears followed by quenching with ammonium chloride.

This method[117] was used in an attempt to detosylate *N,N',N"*-tri(*p*-toluenesulfonyl)1,4,7-triazacyclononane **88**. The product obtained was not that of the free base desired but an intractable mixture of starting material and impurities. There was no sign of detosylation. The reaction was repeated several times with no success.

5.4.6 Sodium/ Naphthalene Reduction Method

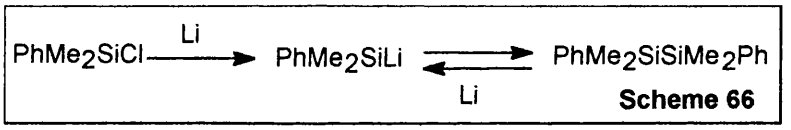
Reductive cleavage in this manner is useful in certain cases.[117,121,122] The tosylated compound was added to a sodium naphthalide solution and DME at low temperatures. The product was generally obtained with naphthalene still present and purified by column chromatography.

This technique was employed to cleave reductively the *N,N',N"*-tri(*p*-toluenesulfonyl)-1,4,7-triazacylononane **88** using the method of Heathcock and Blumenkopf.[123] This compound was chosen again as it was formed on a larger scale than that of the other macrocyclic compounds. Limited success was again observed with this technique as a viscous yellow oil was obtained which on analysis still showed the tosylate signals to be present in the ^{1}H NMR spectrum as well as signals for ring opened products.

5.4.7 Phenyldimethylsilyl Lithium Method

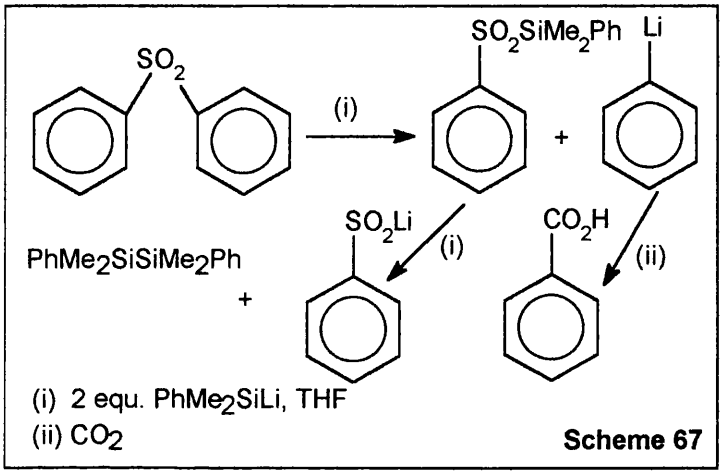
The use of Gilman's reagent (phenyldimethylsilyllithium) is one of the most recently employed techniques in sulfonamide cleavage, being pioneered by Dr Ian Fleming of Cambridge University. He admits that this, as with all of the other agents which can be employed in sulfonamide cleavage, has its limitations and incompatibilities since it is a powerful nucleophile which reacts with a wide range of electrophiles. This compound is suitable to afford secondary amines but not primary amines.

Gilman's reagent[124] is formed at 0 °C from lithium and phenyldimethylsilyl chloride by slow cleavage of the Si-Si bond of 1,1,2,2-tetramethyl-1,2-diphenyldisilane after the rapid formation of the disilane as shown in **Scheme 66**.[125] It was found that two or more molar equivalents were necessary for the cleavage as the by product from the cleavage, which was probably phenyldimethylsilyl sulfinate, reacts rapidly with the second equivalent of the silyllithium reagent. [91]



Scheme 66

The idea behind the use of this reagent as a sulfonamide cleaving agent is that on treating diphenyl sulphone with phenyldimethylsilyl lithium the organic layers from the work up were not organic products derived from the diphenyl sulfone but the hydrolysis products from the silicon reagent. This suggested that the lithium reagent had attacked the sulfur to give the silyl sulfinate ester, displacing phenyllithium as shown in **Scheme 67**. The ester will further react and form the lithium salt which will stay in the aqueous layer.



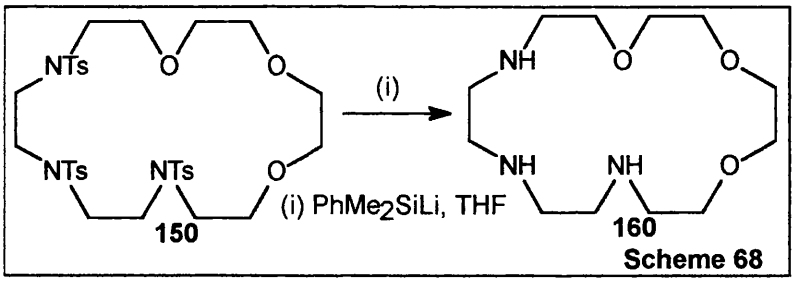
Scheme 67

This cleavage method was applied to several of the macrocyclic sulfonamide compounds synthesised, including both aza and aza, oxa systems of varying ring sizes and heteroatom content.

The tri(*p*-toluenesulfonamide) **88** was treated with phenyldimethylsilyl lithium.[91] This technique showed some potential as partial sulfonamide cleavage was observed. Possibly a larger excess of the reagent could improve the reaction.

Using the same sized ring but replacing a sulfonamide group with oxygen, compound **141**, was also treated with this reagent for 6 h at 0 °C. No success was achieved as starting material was recovered.

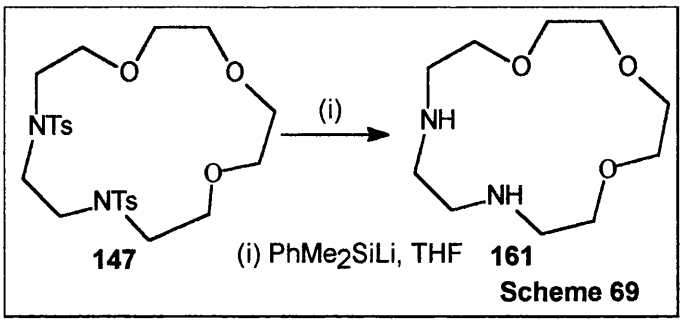
The triazatrioxa compound **150** with a larger ring size was partially detosylated (50-60 %). This was carried out using 3.5 equivalents of the reagent per sulfonamide group as shown in **Scheme 68**. The yield of the oil, however, was very poor and there were also a larger percentage of impurities and also the original starting material was present. The mixture could not be separated by column chromatography.



Scheme 68

A similar observation was found on the treatment of the diaza,trioxa compound **147** with the reagent. Detosylation appeared to increase to 70% to afford **160** in this instance **(Scheme 68)**.

Finally the triaza,dioxa compound **149**, was also treated with the phenyldimethylsilyl lithium reducing agent with 3.5 equivalents for each tosyl group. Surprisingly the ^{1}H NMR spectrum showed no detosylation had taken place. However the mixture which was obtained contained impurities as in previous cases which were inseparable from the starting material.



Scheme 69

This technique was the final one employed in the attempted detosylation of the sulfonamide macrocyclic systems. It was the one which showed the most potential for development.

5.4.8 Detosylation Conclusions

The sulfonamide cleavage methods attempted proved to be fruitless and in the majority of cases a waste of starting materials as the recovery of pure starting compounds by column chromatography yielded very small amounts.

The above attempts and different methods represent a long period of time and effort as the materials which were subjected to the detosylation techniques required to be resynthesised many times to allow further investigation into the different methods and conditions tried for the sulfonamide cleavage.

In hindsight it may have proved to be more beneficial if a different route for formation of the macrocyclic systems could have been attempted. Different nitrogen protecting groups such as mesyl groups may be used or further investigations carried on condensation reactions with acid chloride or ester moieties. These methods, however, afford different problems as the yields in the cyclisation step using mesyl groups are much lower than when using tosylate groups. Vigorous conditions and long periods are required for crab cyclisation techniques.

5.5.1 Biological Evaluation of Macrocyclic Compounds

As with the previous biological evaluation described in **Chapter 3**, it was performed against the P562 leukaemic cell line by Professor John Hartley at University College London. The cytotoxicity of the compounds was examined against the cell line. The DNA sequence specificity, crosslinking ability and single strand Taq stop assay were also studied.[59,60]

5.5.2 Results of Biological Evaluation of Aza Macrocyclic Alkylating Agents

Chlorambucil was run in parallel with the test compounds as a positive reference. It was also the control compound when examining sequence specificity.

The selection of compounds tested incorporates a variety of ring sizes from a three membered single alkylating agents through bis, tris and tetra aza compounds of up to fifteen atoms within the cyclic system. The results are shown in **Table 6**.

On examination of the cytotoxicity the lowest IC_{50} values were observed for the tri and tetra functional alkylating agents **76**, **73** and **91** with cytotoxicity values of 7.5, 10 and 13 μM respectively. These three compounds showed cytotoxicity values much lower than that of chlorambucil, which is a drug commonly used in chemotherapy, and about the same as melphalan (another drug used in chemotherapy). Although compound **79** is very similar structurally to compound **76** it appears to have a much lower cytotoxicity value which was rather unexpected.

The mono and bifunctional alkylating agents **67** and **64** showed very little cytotoxicity towards this cell line.

Compound No.	**IC_{50} μM**	**% Cross -linking**				**Ranking**
		0.01 μM	**0.1 μM**	**1.0 μM**	**10 μM**	
64	> 100	2	2	10	-	**6**
67	> 100	4	7	23	51	**5**
91	13	32	100	-	-	**3**
73	10	64	100	-	-	**2**
76	7.5	16	94	100	-	**1**
79	< 100	77	100	-	-	**4**

Chlorambucil has an IC_{50} value of 45 μM
Melphalan has an IC_{50} value of 8.5 μM
Table 6

The cytotoxicity results were mirrored by the activity levels in the DNA crosslinking experiments apart from compound **79** which had the best crosslinking value of 77% at 0.01 μM and 100% crosslinking at 0.1μM. The second best crosslinker was compound **73** with 64% crosslinking at 0.01 μM and 100% crosslinking at 0.1 μM. **91** was slightly less efficient at crosslinking with 32% at 0.01 μM and 100% crosslinking at 0.1 μM. The final tetra alkylating agent **76** showed 100% crosslinking at ten times the concentration as the other three tri and tetra functional compounds.

The three compounds **73**, **79** and **91** were described by Professor Hartley as "being amazing crosslinkers and amongst the most potent that he has passed through this specific screen". They are at least four logs better at crosslinking than chlorambucil.

Although the compounds **73**, **79** and **91** gave clear reactivity at 1 μM for crosslinking this was not reflected in the overall covalent binding. They produced much higher crosslinker to mono adduct ratio than the others tested such as **64, 67** and **76**. This was expected because of the multiple levels of chloroethyl groups present giving rise to increased chance of crosslinking.

76 revealed extensive covalent binding in the Taq stop assay. Stop sites are those of guanine bases with sequence specificity similar, but not identical to that of chlorambucil. The other compounds which were efficient crosslinkers gave good bonding patterns. However, they were not very sequence specific. Only at lower doses was there any evidence of sequence specificity similar to that of chlorambucil. There were also small differences in the intensity of some bands, but this was not very striking.

The pattern of alkylation of compounds **64**, **67** and **79** was again somewhat different from that of chlorambucil.

5.5.3 Results of Biological Evaluation of Aza, Oxa and Aza, Thia Macrocyclic Alkylating Agents

Compound No.	IC_{50} μM	% Cross -linking				
		0.01 μM	0.1 μM	1.0 μM	10 μM	
122	> 100	0	1	4	5	**4**
125	> 100	0	1	5	4	**3**
134	2.7	3	43	100	-	**1**
155	> 100	0	0	4	49	**2**

Table 7

In general the biological results, shown in **Table 7**, for the above compounds were very disappointing for all but one compound.

The IC_{50} values for compounds **122**, **125** and **155** were all above 100 μM which was rather disappointing. However, the most impressive compound tested overall was that of **134** which had a cytotoxicity IC_{50} value of 2.7 μM. This value is much lower than that of chlorambucil and melphalan. It also showed very good crosslinking values, but not quite as efficient as some of the aza alkylating agents in **Table 6**, but still showing evidence of good activity. Similarly to the aza compounds tested above, compound **134** gave stop sites in the Taq stop assay of guanine and was similar but not identical in sequence specificity to chlorambucil.

Compounds **67** and **79** showed no evidence of covalent bonding nor produced significant stops in the polymerase assay at the doses used.

The only other compound which showed any evidence of crosslinking was the thia, aza agent **155**. Neither of the oxa, aza compounds showed much evidence of crosslinking. A similar observation was noted in the Taq stop assay and sequence specificity.

5.6.1 Conclusions of Biological Evaluation

On consideration of the results obtained from the biological evaluation the agents with most potential for anti-cancer development were those of the aza tri and tetra chloroethyl compounds, **73**, **76** and **91** which showed excellent cytotoxicity and crosslinking values. The only compound containing heteroatoms other than nitrogen in the system which showed good potential was **134**. This was the compound containing benzyl moieties and shown in the other biological evaluation in **Chapter 6** and **Chapter 7** to have a range of biological activities.

Chapter 6

6.1.1 Hypoxia Selective Compounds

As the body of evidence for the presence of hypoxic regions[126] in many tumours grows so does the possibility of developing drugs which will be selective to cancer cells alone. The phenomenon of hypoxia could be extensively utilised in designing features within drug systems to be activated in regions of low oxygen levels.[127]

Many hypoxic regions have been implicated[128] in the emergence of drug resistance and metastatic phenotypes. Rice *et al* reported an amplification of the dihydrofolate reductase and P-170 glycoproteins genes with increased resistance to methotrexate and adrianamycin.[129] Young and Marshall reported that hypoxia resulted in the emergence of clones with markedly enhanced metastatic potential.[130] These data indicate that hypoxic cells can negatively impact the effect of tumour treatment making them important targets for novel compounds which may eradicate tumour cells in the hypoxic environment.

Previously this idea has been investigated in a variety of areas such as in the development of metal complexes of current active prodrugs which could be bioreduced in the body thus liberating the active moiety from the prodrug. Also quaternisation of *N*-mustards could be employed. The quaternisation of tertiary amines with suitable leaving groups which could be utilised in biological systems was thought of as far back as the 1950s[131] as these compounds would increase the water solubility of the drugs being employed.

6.2.1 Quaternary Nitrobenzyl Compounds

Hypoxic selective cytotoxins (HSCs) are generally capable of undergoing selective metabolism (usually reductive) under hypoxic conditions to generate a much more toxic species. Conceptually they consist of three domains:[132] a "trigger", which undergoes activation on hypoxic selective reduction, followed by the "effector" which is activated on the reduction of the "trigger"; and finally the "linker" which connects the other two domains and communicates the change.[133]

One set of compounds which have been extensively studied by Denny *et al* in New Zealand are nitrobenzyl halides.[132,133]

Nitrobenzyl quaternary compounds are known to undergo fragmentation after one electron reduction as shown in **Fig 35** using mechlorethamine **159** as an example.

The mechanism of the activity of compounds of this type is thought to be that of a one electron reduction to form the radical anion which in turn undergoes intramolecular transfer and decomposes to the radical anion and the halide ion. Evidence for the initial radical anion and the subsequently formed nitrobenzyl radical have been proven by the difference in intensity of absorption spectra between radical compounds and non radical starting materials. Thus the rates of transfer from starting material to radical anion can be measured; for example *p*-nitrobenzyl chloride, bromide and iodide are 4 x 10^3, 1.7 x 10^5 and 5.7 x 10^5 s^{-1} respectively.

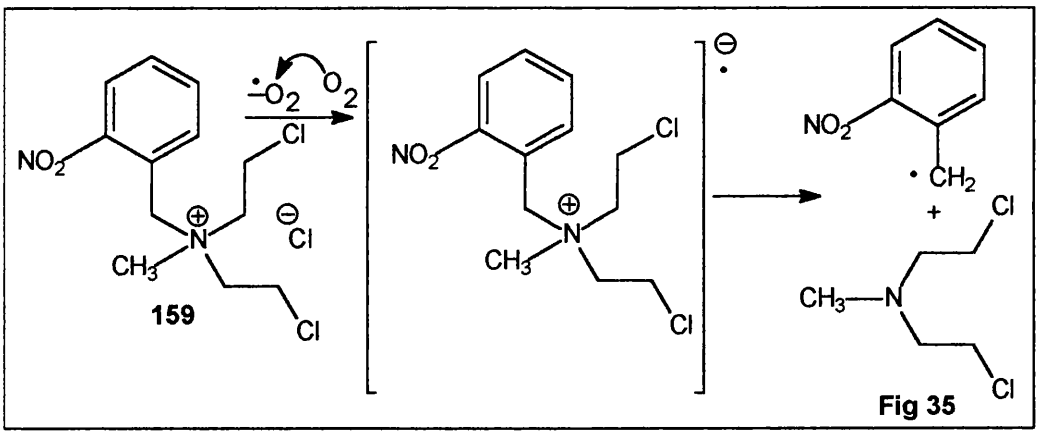


Fig 35

Fig 35 is shown as in the literature.[132, 133, 139]

Evidence also indicates that the *o*-derivative decomposes much more rapidly than the *meta*. The pKa of the *p*-nitrobenzyl bromide anion radical is 2.8 and the protonated form is found to undergo intramolecular transfer around 60 times more slowly than the anion radical. The reactivity of the various radical anions is rationalised in terms of spin density and charge distribution at the various positions on the ring and in terms of electrophilicities.[134] Thus *o*- or *p*- is preferred for a faster reaction.

Many of the compounds which have been synthesised contain a nitro aromatic moiety. This is because the compound must be highly activated to release the alkylating agents under hypoxic conditions in neoplastic cells. Nitrobenzyl halides are

regarded as prototypes in this area of nitroreductase catalysed bioreductive alkylating agents.

Under aerobic conditions *o*-, *p*- and *m*-nitrobenzyl chloride radical anions reduce oxygen to superoxide as is demonstrated by oxygen consumption and spin trapping of the superoxide with 5,5- dimethyl-1-pyrroline-*N*-oxide. At low oxygen conditions the *o*- and *p*-nitro radical anions undergo intramolecular electron transfer and decompose to nitrobenzyl radicals which can be spin trapped with *t*-nitrobutane. The radical adducts are characterised by a nitrogen hyperfine splitting of 16.5 G and two equivalent β hydrogen hyperfine splittings of 10.6 G.[135]

Some nitrobenzyl halides have been evaluated as HSCs and shown evidence of selective killing of hypoxic EMT6 cells. This evidence has been reviewed.[136,137]

It has been found that quaternary ammonium salts are more stable than those of the corresponding chlorides.[132,135,138,139] Analogues, such as *o*-nitrobenzylmethylamine have shown high selectivity for hypoxic cells *in vitro*[133, 136] and *in vivo*[133] along with greater cytotoxicity against intact rather than dissociated EMT spheroids. The *m*-nitro isomer was found to be less cytotoxic than either of the *o*-, or *p*-nitro isomers which is consistent with the previous literature[134] on the subject which indicates that the rates of fragmentation are lower for the *m*-isomer. The fragmentation can therefore be controlled by appropriate structural changes allowing the fragmentation to be competitive with the back oxidation process in particular oxygen concentrations. To some extent the toxicity is also dependent on the complete reductive activation of the nitro heterocyclic drugs by the enzymes within hypoxic systems.

A seperate factor which can be varied in the compounds is that of reduction potentials. Generally speaking the desired reduction potential for HSCs is between -300 and - 450 mV. The large positive Hammett substitutent parameter (σ *o,p*) of the quaternary function equates to -330 mV for the *p*-isomer which is within the desired range. Thus control of this factor can be maintained within the design of the compound. Mechlorethamine shows very high selectivity for hypoxic EMT6 cells in culture, rising to several thousand fold after 4 h. This is an unusual time dependance for which the reasons are unknown but may be due in part to the slow kinetics of release and subsequent build up of mechlorethamine within the hypoxic cultures. [139]

There are several advantages of these systems such as the cationic charge on the quaternary salts which ensures water solubility; control of the lifetimes of radicals; control of the reduction potential and rates of fragmentation of the nitro benzyl moeity. A disadvantage is that the cellular uptake of the compounds within the biological systems is slow.

For these reasons this type of anticancer agent merits further investigation.

6.3.1 Synthesis of Quaternary Nitrobenzyl Compounds

In an attempt to develop prodrugs which are hypoxic selective a selection of twelve novel quaternary nitrobenzyl nitrogen mustards were synthesised. The test compounds were synthesised from alkylating agents which had previously been synthesised and had undergone biological testing against either the human HT29 and BE cell lines or the human P562 leukaemic cell line and had shown promising results.

The compounds synthesised were either *o*- or *p*-nitro substituted derivatives, and not *m*- as the previously reported compounds which were *m*-nitrobenzyl alkylating agents were either inactive or much less active than the corresponding *o*- or *p*- derivatives. The influence of having different potential for alkylation has also been investigated by the incorporation of from one to four different alkylating sites in the molecule.

Compound **160** has previously been tested against the human colon carcinoma cell line HT29 and was found to be four times more toxic in Walker rat tumour cells under hypoxic conditions.[54] This, however is not as selective as nitromin, **Fig 36**, which operates by a one electron reduction intermediate. The nitrogen anion radical has been proposed to explain the DNA strand breakage observed in addition to DNA crosslinking in cells treated with this compound.[140]

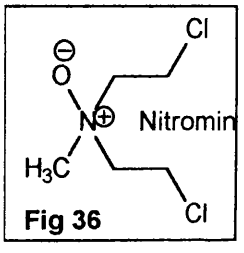
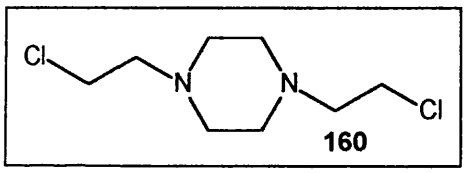
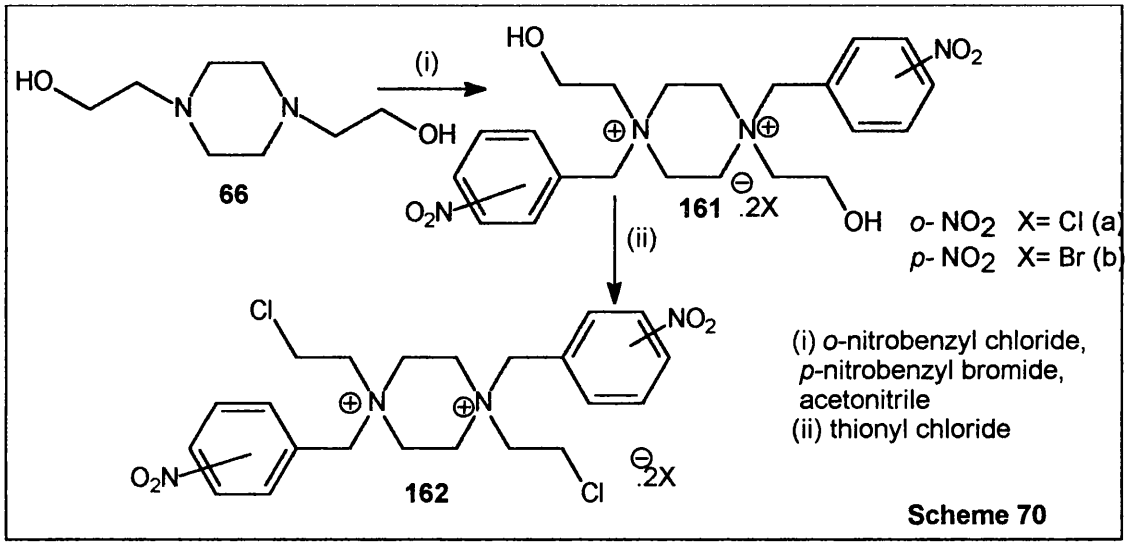


Fig 36



160

Compounds **162a** and **162b** were synthesised starting from the readily available piperazine (1,4-diazacyclohexane). 1,4-Bis(2-hydroxyethyl)piperazine **66** was obtained as a white solid on treatment of piperidine with ethylene oxide in EtOH according to literature,[58] in good yield. The *o*- or *p*-nitrobenzyl moiety was attached on treatment with *o*-nitrobenzyl chloride or *p*-nitrobenzyl bromide, respectively, in acetonitrile under reflux conditions as a white solid for the *o*-derivative and as a beige solid for the *p*-derivative. The target compounds **162a** and **162b** were formed as chlorides by substitution reaction in thionyl chloride and recrystallised from EtOH and diethyl ether as white solids.



Scheme 70

Synthesis of the diol has previously been discussed in **Chapter 4**. The IR spectrum for the *o*-nitrobenzyl derivative, compound **161a**, showed hydroxyl stretches at 3387, 2924 and 2853 cm^{-1} with hydroxyl bending at 1316 cm^{-1}. Also present were aromatic stretches at 1609 and 1526 cm^{-1}. The nitro group stretches were shown at 1577 and 1344 cm^{-1}. The ^{13}C NMR spectrum showed four CH carbon signals in the aromatic region as well as two quaternary carbon signals from δ 138.5-125.6. The benzyl CH_2 carbon was observed at δ 58.2 and the signals for the carbons of the CH_2 adjacent to the nitrogen were observed at δ 47.5 and δ 38.0.

The *p*-nitrobenzyl derivative, **161b**, showed stretches in the IR spectrum at 1525 and 1608 cm^{-1} for the aromatic vibrations. The hydroxyl stretches were observed at 3418, 2981, 2914 and 1350 cm^{-1}. The ^{13}C NMR spectrum showed only eight signals due to the symmetry within the compound. Four signals occurred in the aromatic region at δ 150.1, corresponding to the carbon with nitro group attached and

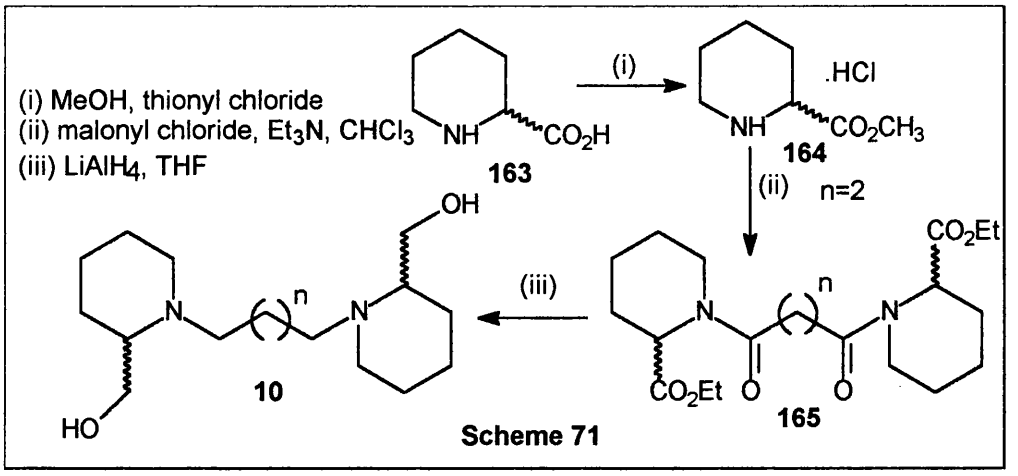
δ 135.4, 132.6 and 125.4 representing the remaining carbons in the aromatic moiety and the AA'BB' system. The benzyl CH_2 group carbon was observed at δ 58.4 and the CH_2 adjacent to the hydroxyl group occurred at δ 55.4. The ^1H NMR spectrum clearly showed the AA'BB' system at δ 8.13-8.08 and δ 7.51-7.42 as a complex signal. The methylene protons alpha to the nitrogen were shown as a broad singlet at δ 2.28 and the benzyl CH_2 protons at δ 3.70 as a singlet and finally the protons of the CH_2 group alpha to the hydroxyl group are represented as a singlet at δ 3.53.

The chlorides **162a** and **162b** were synthesised by general methods. The IR spectra showed the absence of the hydroxyl stretches and the addition of a stretch at approximately 750 cm^{-1}. In the ^1H NMR spectrum the signal for the carbon next to the chloride group occurred at δ 38.

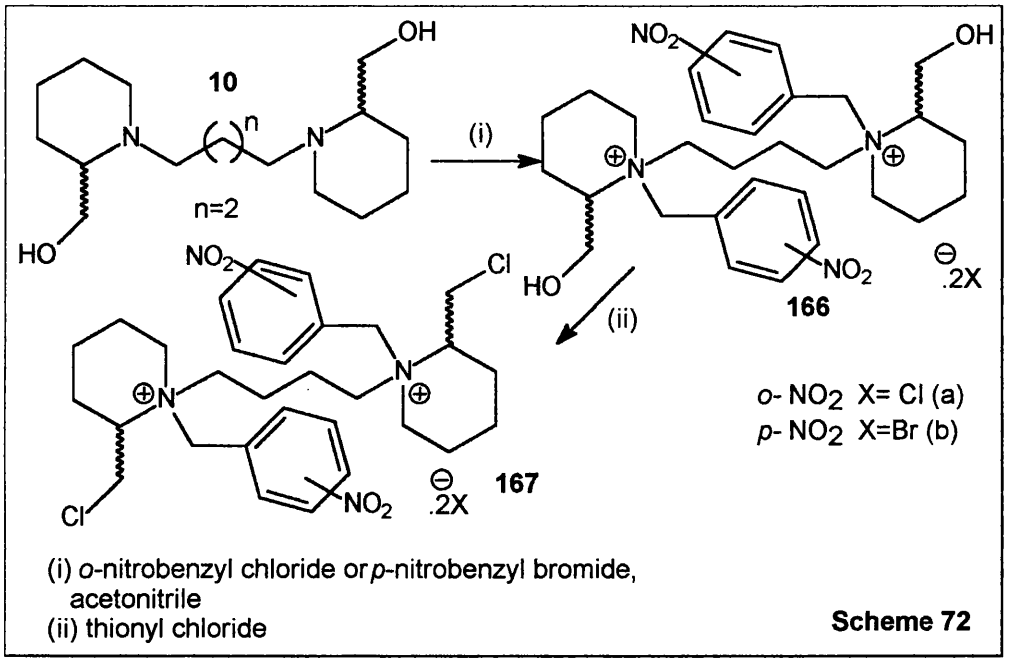
The previously synthesised compound **160** was chosen to develop into a quaternary nitrobenzyl alkylating agent due to the initial promising crosslinking activity and cytotoxicity values observed on biological evaluation of **160** as an anticancer agent.[54]

Compound **10 (Fig 26** in **Chapter 3)** was synthesised similarly to the route and methods employed by Dr Nicola Henderson starting from DL-pipecolinic acid forming the methyl DL-pipecolinate hydrochloride salt **164** using MeOH and thionyl chloride instead of 2,2-dimethoxypropane and concentrated hydrochloric acid previously used in the synthesis of this compound. Two of these molecules were coupled using succinyl dichloride in chloroform with two equivalents of triethylamine as a base to form the piperidine base *in situ* and to mop up the hydrochloric acid formed to minimise ester hydrolysis to give **165**. The amide and ester were reduced in one step using lithium aluminium hydride in good yield, as shown in **Scheme 66** to afford the desired compound **10**.

Compound **165** showed signals at δ 170.9 representing the ester carbonyl and at δ 57.5 representing the chiral centre carbon in the ^{13}C NMR spectrum. The rest of the signals in the ^{13}C and the ^1H NMR spectra were as reported by Henderson *et al.*[55]

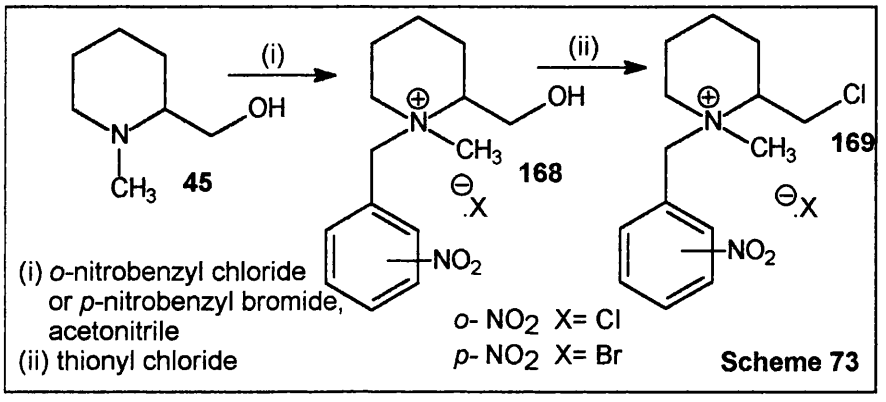


The chlorides **167a** and **167b** were obtained by substitution reaction on stirring in neat thionyl chloride and recrystallised from EtOH and diethyl ether as a white solid **167a** and an off white solid **167b,** respectively, as shown in **Scheme 72.** Diols **166a** and **166b** were isolated and immediately chlorinated without full characterisation other than ^1H NMR spectra. The final products **167a** and **167b** showed the absence of hydroxyl stretches and the addition of CH_2-Cl stretches at approximately 750 cm^{-1} in the IR spectra with the aromatic and nitro stretches as mentioned previously.



The third set of *o*- and *p*- substitued nitrobenzyl compounds were those shown in **Scheme 73**. These compounds are only capable of single alkylation. Compound **46** as previously mentioned in **Chapter 3** was tested against the P562 leukaemic cell line. Treatment of *N*-methyl-piperidine-2-methanol **45** with *o*-nitrobenzyl chloride or

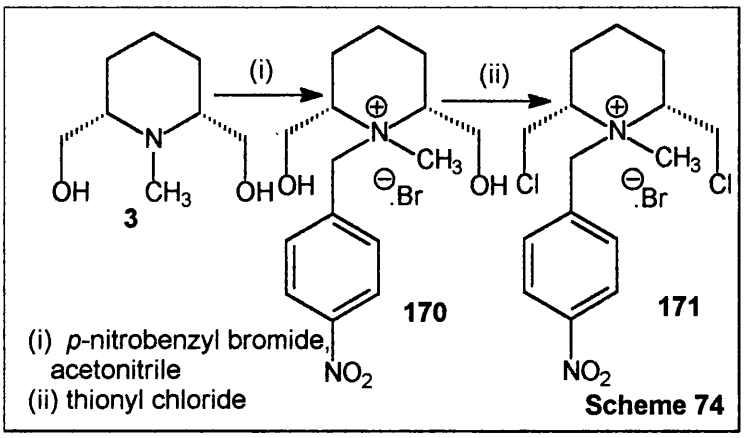
p-nitrobenzyl bromide in acetonitrile gave the quaternary salts, **168a** and **168b**, as white solids. Both the *o*- and *p*- substituted compounds showed hydroxyl stretches at 3200 to 3500 and ~1050 cm^{-1} in the IR spectra. Also observed were the aromatic stretches at 1518 and 1604 cm^{-1} and C-NO_2 stretches at 1330 cm^{-1}. On treatment with thionyl chloride the chlorides, **169a** and **169b**, were obtained and recrystallised from EtOH and diethyl ether as white crystals, in acceptable yields. The IR spectrum showed the absence of the hydroxyl stretches and the presence of a CH_2-Cl stretch at 750 cm^{-1}.



Quaternary nitrobenzyl chloride, **171**, was formed from the diol **3** which had been reported in **Chapter 3**.[141] Dipicolinic acid was refluxed with thionyl chloride for 48 h before the acid chloride which was formed was reacted with EtOH to give the diester **2**. Hydrogenation of the ester and methylation of the nitrogen was carried out using the method of Feldkamp *et al.*[142] The hydrogenation of the pyridine ring was carried out in aqueous acetic acid using Adams' catalyst. The isolation of the unmethylated product was unnecessary as *N*- methylation could proceed in the same solution using a reductive methylation procedure. This was completed by exchanging the platinum catalyst for palladium on charcoal and adding a slight excess of aqueous formaldehyde. The diester was then hydrogenated to afford the methylated product. This was the preferred method of methylation. The non aromatic cyclic compound was obtained as white crystals. This diester **2** was reduced using lithium aluminium hydride in THF to afford the diol product **3**, as shown in **Scheme 1** in **Chapter 3.**

The quaternisation of the nitrogen was performed in acetonitrile using the appropriate halogenated nitrobenzyl derivative to afford compound **170**. The test compounds were obtained by chlorination of the quaternised diol compound **171**

using thionyl chloride. These compounds were recrystallised from EtOH and diethyl ether, as shown in **Scheme 74.**



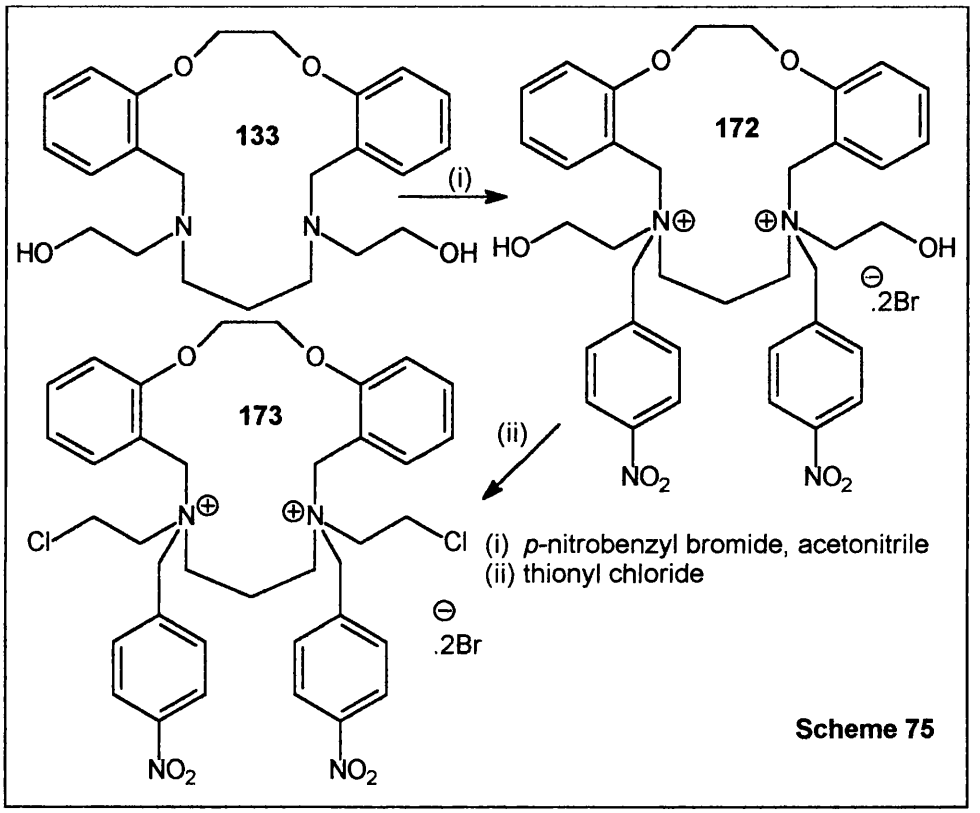
Scheme 74

The quaternised diol **170** was synthesised in reasonable yield. The IR spectrum showed vibrational stretches in the aromatic region and nitro stretches as in the previous *p*- nitro benzyl compounds. The final test compound **171** was also obtained in acceptable yield. The IR spectrum showed the absence of hydroxyl stretches. The presence of a CH_2-Cl stretch was observed at 704 cm^{-1}. The ^{13}C NMR spectrum showed four carbon signals for the aromatic moiety between δ 159.4 and δ 124.9. The rest of the signals observed were three CH_2 carbons, one methyl carbon and one quaternary carbon signal which corresponds to the remainder of the structure.

Compound **134** had previously been synthesised and had undergone biological evaluation against the P562 human leukaemic cell line by Professor John Hartley at University College London. The observed IC_{50} value of 2.5 μM was much lower, than that of chlorambucil or melphalan.

Compound **132** was formed following the method of Lindoy and Armstrong[101] using Williamson condensation between 1,2-dibromoethane and salicylaldehyde in good yields. This was followed by a condensation reaction or Schiff base cyclisation to afford the macrocyclic imine **131**. Reduction with borane THF afforded the free base **132** as shown in **Scheme 48** in **Chapter 5**. Structures of these compounds were confirmed by comparison of spectral data with those of Lindoy and Armstrong.[101]

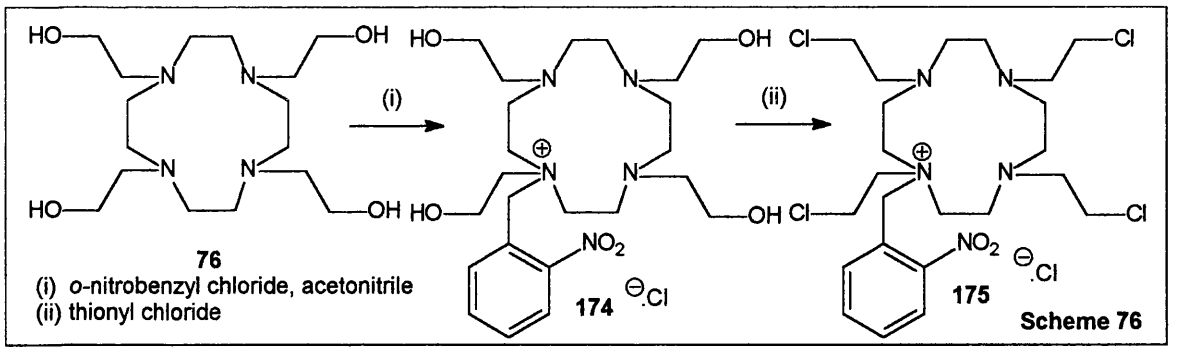
Diol **133** was formed on reaction of **132** with ethylene oxide in EtOH in a reasonable yield. As before the quaternary nitrogen was formed on reaction of diol **133** with *p*-nitrobenzyl bromide in acetonitrile and the product **172** was chlorinated by substitution reaction in neat thionyl chloride to afford **173** as a light green solid in good yield as shown in **Scheme 75**. The diol was not fully characterised before chlorination.



Scheme 75

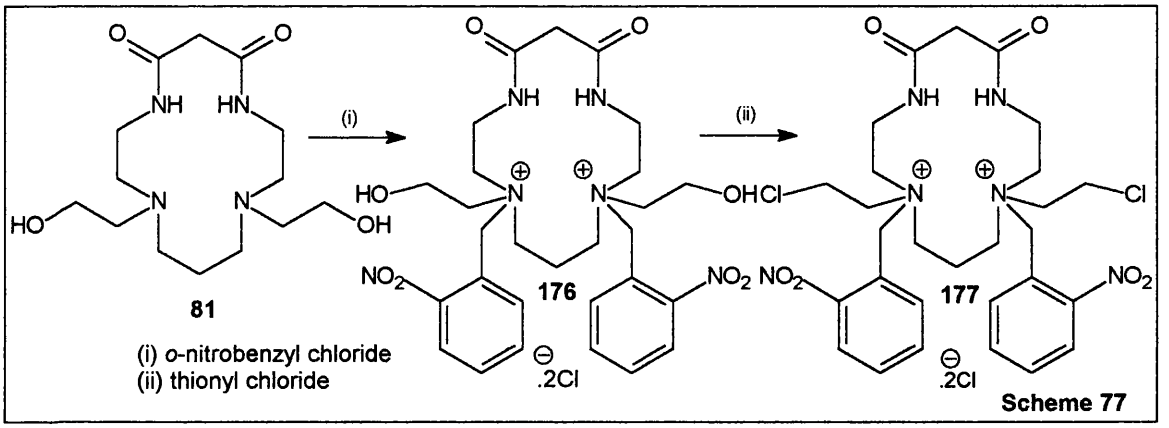
Cyclen is a readily available starting material and presents the potential for introducing four alkylating arms thus increasing the possibilities for crosslinking if this compound was developed into an multifunctional alkylating agent.

The tetraalcohol **76** was formed on reacting cyclam with an excess of ethylene oxide in EtOH as reported by Hancock and co-workers using general methods.[58] On attempting the formation of the quaternary amines only one quaternisation reaction occurred leading to **174**. The tetraol was then converted into the desired chloride product **175** as shown in **Scheme 76.**



Product **175** was obtained as an off white solid which was recrystallised from EtOH and diethyl ether. The IR spectrum showed CH_2-Cl stretching at 744 cm^{-1}. The nitro group showed stretching at 1546 and 1350 cm^{-1} and aromatic vibrations at 1526 and 1628 cm^{-1}.

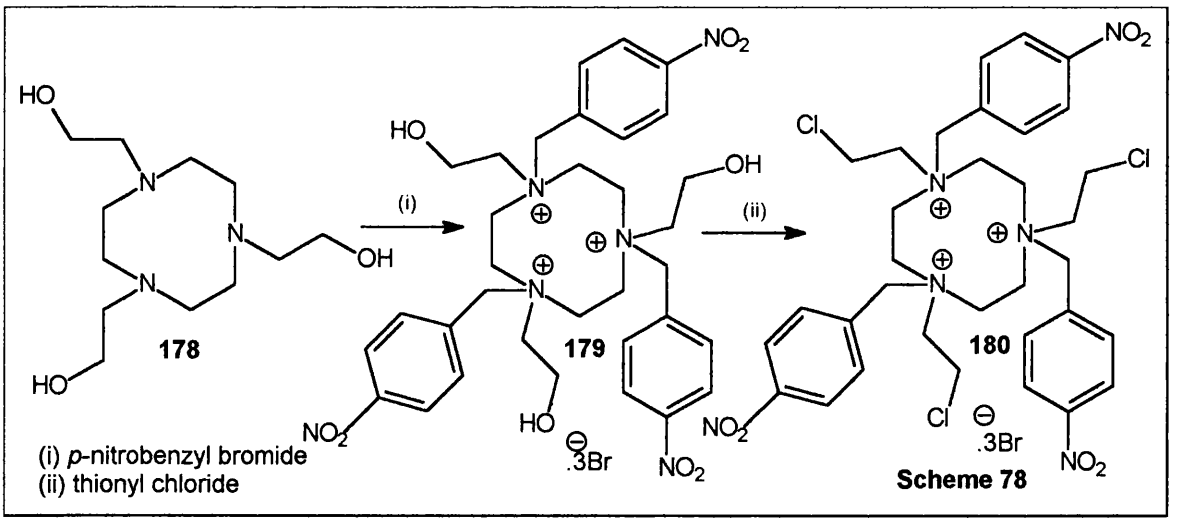
A variation from a cyclam type would be the incorporation of an amide group, as in compound **177**. This presents the possibility of developing bifunctional alkylating agents as the nitrogen lone pairs on the amides are delocalised and not available for alkylation. The free base nitrogens reacted with the ethylene oxide to form the diol **81**, which was in turn converted into the quaternary amine using *o*-nitrobenzyl chloride in acetonitrile to form compound **176**. The chlorination occurred as before using neat thionyl chloride to yield the desired product **177** as shown in **Scheme 77**. The IR spectrum of the target compound showed CH_2-Cl stretches at 749 cm^{-1}, the carbonyl stretch was present at 1685 cm^{-1} and aromatic stretches at 1529 and 1605 cm^{-1}.



Continuing the formation of multifunctional alkylating agents a system with three alkylating arms was synthesised from 1,4,9-triazacyclononane (tacn), as shown in **Schemes 31** and **32** in **Chapter 4**. The synthesis of 1,4,9-triazacyclononane is

well documented and proceeds under high dilution conditions as described in **Chapter 4**. The tosylated macrocycle was obtained as a white solid in reasonable yield. This underwent deprotection using concentrated sulfuric acid and sodium hydroxide to leave the free base as a white solid. The yield was very poor but was sufficient to carry on as in **Scheme 78**. As before the alcohol **178** was formed from ethylene oxide and quaternised in acetonitrile with the benzyl halide to give **179**. Finally the chloride **180** was obtained using neat thionyl chloride as an off white solid.

The ^{13}C NMR spectrum of the quaternised triol **179** showed signals at δ 144.8, 129.8, 127.6 and δ 123.51 representing the aromatic carbons. Signals were also observed at δ 58.6 58.0, 49.9 and 30.9 corresponding with the remaining carbons. The ^{1}H NMR spectrum of the chloride **180**, showed singlet signals at δ 3.37, 4.44 and 4.48 and doublet signals at δ 7.47-7.52 and 8.12-8.17 representing the methylene groups adjacent to the nitrogen, the methylene next to the aromatic moiety, the chloride and finally the AA'BB' aromatic system. The IR spectrum showed the presence of CH_2Cl stretches at 730 cm^{-1} along with the aromatic stretches at 1524, 1606 cm^{-1} and the nitro group stretch at 1349 cm^{-1}.



Scheme 78

6.4.1 Biological Evaluation

The compounds synthesised above were tested by Professor Ian Stratford from the School of Pharmacy and Pharmaceutical sciences at the University of Manchester.

6.4.2 Experimental Detail

Selective toxicity to hypoxic A549 cell lines was determined using the MTT assay as reported by Stratford and Stephens,[143] and also Jaffarr *et al* .[144]
The cells were treated with the drug for 3 hours at 37 °C under aerobic or hypoxic (N_2) conditions. The drug was then removed and the cells allowed to proliferate for three days prior to the MTT assay.

The results of the biological testing are presented in **Table 8**. IC_{50} (air) values, the concentrations required to kill 50 % of aerobic cells under the conditions of the assay, are divided by the IC_{50} (N_2) values, the concentrations required to kill 50 % of hypoxic cells, to give the hypoxic cytotoxicity ratio. These values enable quantitative comparisons of bioreductive cytotoxicities of the drugs. The aerobic and hypoxic toxicities of selected compounds against this cell line were described by Robertson *et al.*[145]

Compound Number	IC_{50}(air) mM	IC_{50} (N_2) mM	HCR
175	0.097 ± 0.026	0.075 ± 0.016	1.3
173	0.031 ± 0.027	0.027 ± 0.005	1.1
180	6.61 ± 1.73	5.77 ± 1.09	1.1
171	0.864 ± 0.172	0.80 ± 0.11	1.1
169b	1.50 ± 0.196	1.23 ± 0.151	1.2

Table 8

6.5.1 Conclusions

Only the five compounds listed in the table were actually tested. This was due to Professor Stratford having limited time and the initial tesing indicating that the remainder of the testing would be of limited value.

In all cases the compounds had marginally lower cytotoxicity values under nitrogen atmosphere than in the aerobic environment. The most active compound was compound **173** followed by **175** according to the lowest cytotoxicity values rather than differences between the two enviroments.

There can be no conclusions drawn about the activity being related preferentially towards either the *o*- or *p*-nitrosubstituted compounds as the compounds tested were all *p*-nitro substituted compounds.

Indications were that the best activity would be obtained by bifunctional agents rather than trifunctional agents as **180**, which was the trifunctional compound has the lowest activity. However, conclusions as to preferential activity by single or bifunctional agents is unclear as the second most active compound is a single functionality cyclam derivative. However, on comparison of compounds **171** and **169b** which are very similar compounds in structure, the bifunctional compound **171** is slightly more cytotoxic than the single functional compound **169b**. Thus indicating that bifunctional molecules have more potential than structurally similar single functionality agents.

The results found on biological evaluation of the above compounds were very disappointing and on examining the error margins on the values there is no significant differences in activity between the aerobic and hypoxic conditions. It was therefore decided that research in this area within the group would be brought to a close.

Chapter 7

7.1.1 Other Biological Testing

Occasionally compounds which are found to be active in one biological system can be active in a different biological environment. Two sets of compounds which have previously been synthesised and undergone anti-cancer biological evaluation have been selected to undergo a seperate biological evaluation process as potential anti-fungal *or* anti-parasitic agents (here we are going to examine action against *Leishmania* and *Trichomanosis* which are two parasitic tropical diseases).

7.2.1 Anti-fungal Investigations

Fungi are eukaryotic organisms which differ from plants in that they cannot photosynthesise food and therefore have to consume resources from dead matter as parasites on living organisms or in symbosis with another living being.

There are over 64,000 different species of fungi but due to difficulties in taxonomy there may be many more which have as yet not been discovered.[146] Of these species over 13,000 exist as lichens. This type of fungal species is widespread and varies from simple moulds, yeast and rusts to larger members of the family such as toadstools.

The family is split into five groups: zygomycotina, mastiyomycotina, ascomycotina, basidiomycotina and deuteromycotina. The classes are distinguished by distinctive sexual and developmental differences.

Plant pathogenic fungi adopt a parasitic lifestyle, i.e. they eat living plants, and form a parasitic symbiosis in which only the fungi benefit. Some fungi only grow in association with a living host, i.e. obligate parasites. Others can only exist away from the host, i.e. saprotrophs which live on dead matter. Parasitic fungi can also be described according to their modes of nutrition. Nectrophoic fungi kill cells in the host before eating them whereas biotrophs consume only living cells.

Fungicides act by killing the development of fungal species (seeds) or mycelium (feeding system). There are generally two types of fungicides which are classified

according to their uptake and mobility in the plants, protectant systems and eradication fungicides.

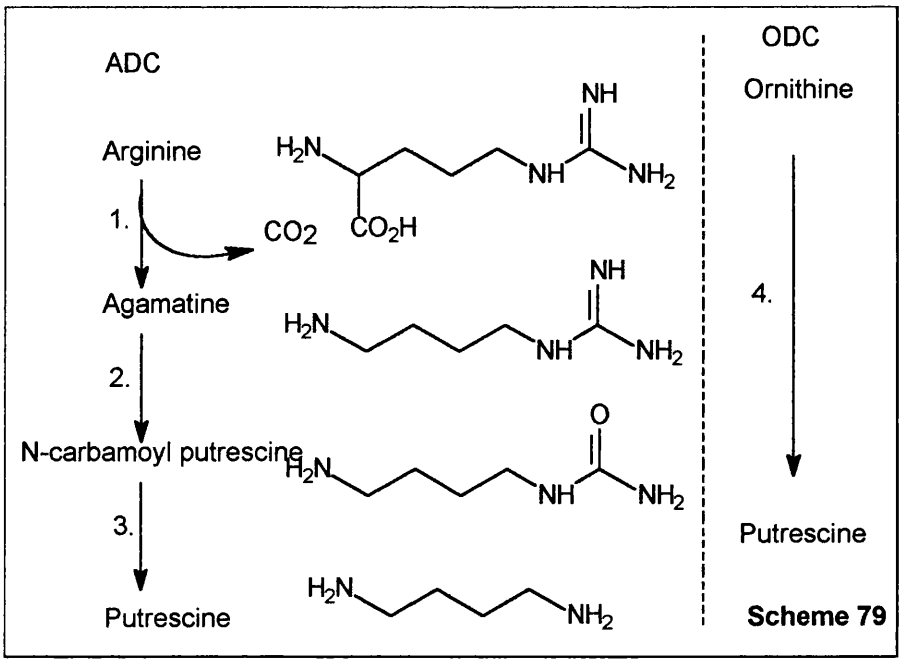
Protectant systems are a form of prophylaxis, i.e. treatment which prevents a disease. They are applied directly to an area of possible infection. They are incapable of penetrating plant tissues. Eradicant systems are applied after infection has occurred and have limited penetrative action. Both of these classes are non-systemic, i.e. not penetrative of living plants.

Chemical treatment is split into three forms: direct activity; toxic inactivation; or enhancement of resistance in the host plant.

Chemicals which act by killing or inactivating a fungus are directly acting. Those which prevent toxic secretions are inhibitory acting and those which enhance the resistance of the plant by altering its metabolism fall into the final category.

7.2.2 Polyamines

Polyamines are found in many forms throughout nature. They are found in every cell in the human body and play crucial but ill-defined roles in cell division and growth, e.g. putrescine, $NH_2(CH_2)_3NH_2$; cadaverine $NH_2(CH_2)_5NH_2$, spermidine $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$; spermine $NH_2(CH_2)_3NH(CH_2)_4NH_2$ and ornithine $NH_2CHCO_2H(CH_2)_3NH_2$. Spermine and spermidine are synthesised from putrescine which is synthesised from ornithine in fungi. Plants are also capable of synthesising putrescine from arginine as shown in **Scheme 79**.



1. Arginine decarboxylase, 2. Agmatine iminohydrolase, 3. *N*-caramoyl putrescine amido hydrolase, 4. Ornithine Decarboxylase.

Ornithine decarboxylase (ODC) is a 5'-pyridoxal phosphate (PLP) dependent enzyme and is a dimer of 80000- 82000 amu and a recent X-ray has shown ODC to exist as doughnut shaped dodecamers.[147]

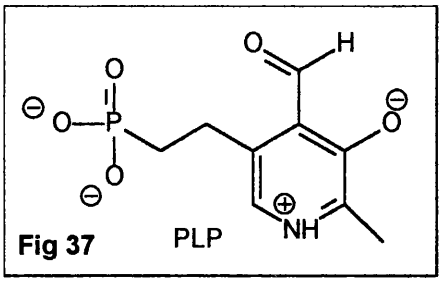


Fig 37 PLP

PLP, **Fig 37**, has a high specificity for ornithine and decarboxylates it to putrescine, but it can also catalyse the decarboxylation of lysine to cadaverine. As fungi can only use the ODC route to produce putrescine whereas plants can use both routes there is the possibility of blocking ODC formation which would effect the fungi, i.e. the fungal growth can be curtailed by inhibiting ODC leaving host plants to use the ADC route. There are three classes of ODC inhibitors, the most significant class being suicide inhibitors, including DMFO (**Fig 38**) which has previously been shown to have anti-fungal and anti-cancer activity.[148]

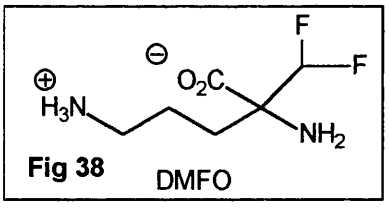


Fig 38 DMFO

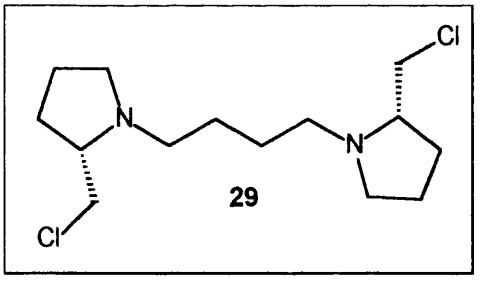
The second class is that of ornithine and putrescine analogues which are competitive inhibitors. This is the main area of interest of our research group in anti-fungal compounds. The final set of compounds are those which are capable of interacting or combining with PLP.

7.2.3 Selection and Synthesis of Compounds Tested for Anti-fungal Activity

After examining the biological evaluation results on all of the compounds synthesised and tested against the human leukaemic P562 cell line, the following small selection was submitted for further biological evaluation as potential anti-fungal agents to examine if they are active by inhibiting ODC.

The best anticancer activity was observed with the macrocyclic systems. Thus the majority of the compounds tested for anti-fungal activity are macrocyclic compounds with the exception of compound **30**. The leaving group throughout the selection was maintained as chloride, to allow for comparison. All compounds were tested as the hydrochloride salts.

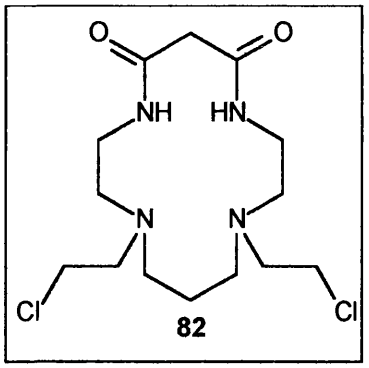
The first compound which was chosen was the bis alkylating agent, **29**, which has the pyrrolidine system, formed from two L-proline rings, joined with a four carbon spacer length. The compound was synthesised as discussed in **Chapter 3**. The reason for choosing this particular compound was that it was shown to have reasonable activity against the human leukaemic P562 cell line.

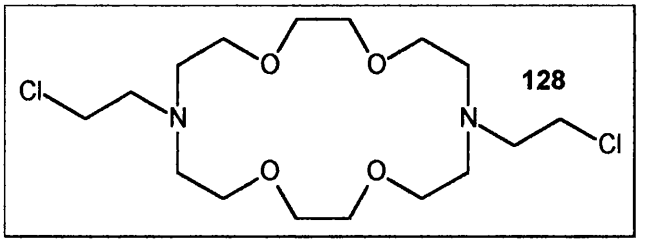


29

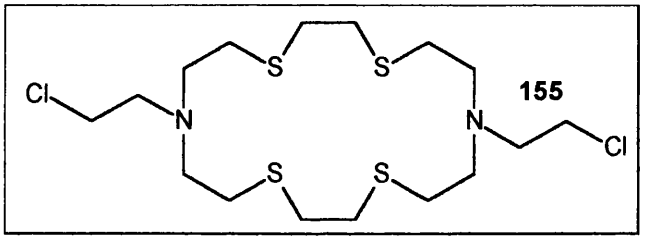
The second compound chosen for testing against fungi was **82**, which is the diamide bisalkylating system formed from cyclam. The potential of bisalkylation is maintained. The compound was synthesised as described in **Chapter 4**.

The third compound examined was the compound **128**. The alkylating potential is bifunctional. The incorporation of oxygen into the system may show a marked difference from the aza systems. The compound was formed as described in **Chapter 4**.

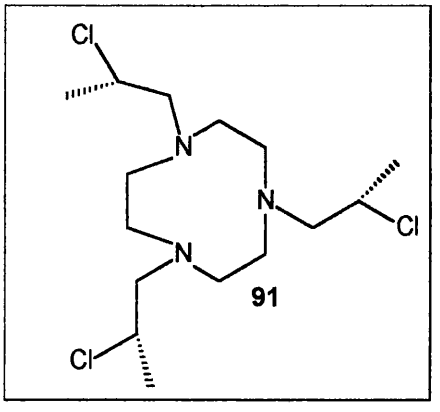




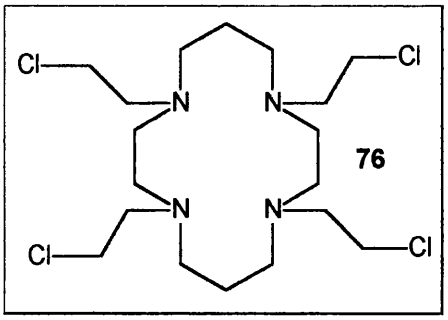
Compound **155** with bifunctional alkylating potential was also synthesised to investigate any effects the inclusion of sulfur into the systems would have on the activity. Thus the previous compound, **128**, and this one can be compared as they differ only with respect to the heteroatom included. Although sulfur and oxygen are both Group VIA (16) elements they show large differences in character. They are both $2e^-$ away from a noble gas configuration with essentially nonmetallic nature discussed in **Chapter 5**. **155** was synthesised as shown in **Chapter 5**.



The potentially tri alkylating compound **91** was formed as described in **Chapter 4**. **91** was chosen to examine the effect of increasing the number of nitrogens present within the system and also increasing the number of alkylating sites present.



The final alkylating compound contained the potential of tetra functionality. Compound **76** was formed from cyclam as in **Chapter 4**.



7.2.4 Biological Evaluation of Compounds

The fungicidal activity of the compounds was examined on infection of barley seedlings with the powdery mildew fungus *Erysiphe graminis* f.sp.*hordei* Marchal by Dr Dale Walters at the Scottish Agricultural College, Auchincruive. The effects were determined as in the literature.[149(preprint)]

The effects of compounds on growth and polyamine biosynthesis of *Pyrenophora avenae* were also examined. Growth of the *Pyrenophora avenae* was as in literature publication[150] as is the determination of the formation of polyamines from radiolabelled ornithine in the fungus.

Radial growth was measured after five days; the % leaf area covered with powdery mildew was assessed ten days after inoculation. Compounds were applied to seedlings two days after inoculation and all compounds were used at 25 μM.
All compounds were tested as the hydrochloride salts.

Compound	Radial Growth, mm	% leaf area infected
control	**3.7 ± 0.03**	**48.8 ± 4.48**
29	3.7 ± 0.03	39.1 ± 2.96
76	3.1 ± 0.02	33.2 ± 2.15
82	3.4 ± 0.03	26.0 ± 2.21
128	3.3 ± 0.02	21.3 ± 1.90
91	3.1 ± 0.03	38.8 ± 2.95
155	3.8 ± 0.03	33.8 ± 5.9

Table 9

Table 9 shows the results of the two tests performed on the compounds. The first was the *in vivo* test on *Pyrenophora avenae* and the second was the *in vitro* test with powdery mildew. As in the previous chapter the error margins are significant.

None of the tested compounds had any significant activity against the *Pyrenophora avenae* as the radial growth measured in mm was only slightly below that of the control. On the other hand significant activity was observed against the powdery mildew. All of the tested compounds had at least 9% less infected leaf area than the control. Two of these compounds were active, compounds **82** and **128**. On examination of the structures of these compounds there did not appear to be any obvious similar features which would be responsible for the activity. The only similarities between compounds **82** and **128** is that they are both bifunctional alkylating agents containing oxygen atoms within the system and that they contain medium sized macrocycles. **91** was nearly inactive. This was surprising as it contains three alkylating moieties which would be expected to confer activity. As compound **29** showed very little activity in either of the tests the indication was that pyrrolidine systems do not possess the potential of anti-fungal activity, whereas certain multifunctional macrocyclic alkylating agents posess some anti-fungal activity.

Compound Radioactivity in polyamine (dpm mg protein-1)

	Putrescine	**Spermidine**	**Spermine**
control	**29.2 ± 3.48**	**30.4 ±3.78**	**65.9 ± 3.14**
29	36.5 ± 1.91	53.2 ± 4.81	79.8 ± 6.55
76	16.6 ± 0.57	5.7 ± 0.73	44.3 ± 5.81
82	5.7 ± 0.63	4.9 ± 0.53	61.2 ± 7.28
128	5.3 ± 0.39	4.9 ± 0.56	26.2 ± 3.81
91	3.1 ± 0.38	22.6 ± 1.97	11.8 ± 2.91
155	10.0 ± 0.90	51.0 ± 4.80	22.0 ± 1.80

Table 10

Table 10 shows the effect on the flux of the label from ornithine into the polyamines. Compounds **82**, **128**, **91** and **76** reduce the levels of putrescine dramatically. This is a very positive result as it indicates that the ODC levels would also be reduced. The spermidine levels were comparable to that of putrescine apart from one anomally. **155** gave conflicting results where the levels increased by 10 fold to that of putrescine. This may be misleading as you require putrescine to form spermidine. The spermine levels then drop back to 22.0 dpm mg protein^{-1}. Experimental error may be to blame for this inconsistency. The spermine levels for the other compounds were comparable to the control apart from **128** and **91** which were much lower.

Thus overall **128** was the best compound *in vitro* against powdery mildew and also in *in vivo* tests againt *Pyrenophora avenae.*

7.3.1 Tropical Parasitic Disease Investigations

There are now over 30,000 named species of protozoa of which 10,000 are parasitic invertebrates and they exist in almost every species of vertebrate. Thus it is not surprising that man and most domesticated pets act as hosts to these parasites. The diseases which are caused by these parasites are out of proportion for the number of species involved. The protozoa which infect humans range from forms which are never fatal to those which cause diseases such as malaria, sleeping sickness, Chaga's disease and leishmaniasis, which are now regarded as being major diseases in tropical countries and together threaten over one quarter of the population of the world.

Parasitic protozoa lie between the prokaryotic and higher eukaryotic organisms and share some of the characteristics of each. They are usually small, have short generation times, high rates of reproduction and have a tendency to induce immunity to reinfection in those hosts that survive.

7.3.2 Leishmania[151,152,153]

One such tropical disease which is caused by parasitic protozoa is that of *leishmaniasis.* This is a very unpleasant disease which causes blistering and lesions on the skin. The typical infection is cutaneous but may invade subcutaneous or deeper tissues causing hideous and permanent disfiguration in man. *Leishmaniasis* in man is known to be caused by a complex of six species and fifteen or sixteen subspecies. Cutaneous leishmaniasis is caused by *Leishmania tropica* and visceral leishmaniasis is due to *Leishmania donovani.* Some are self healing dermal forms such as *L. tropica* and *L. mexicana* whereas others such as *L. donovani* are found effectively to be non-healing visceral forms. *L. donovani* infection frequently progresses to fatal conclusions over a number of years. Approximately only one quarter of patients with visceral leishmaniasis will recover.

Cutaneous leishmaniasis is an infection of the skin and includes three distinct clinical entities: tropical sores; mucocutaneous leishmaniasis and disseminated anergic cutaneous leishmaniasis.

After inoculation into the skin leishmaniasis parasites multiply by binary fission within histocytes which eventually rupture and release the parasites which enter other histocytes. The vector for this very unpleasant disease is that of the common bloodsucking sandfly. After ingestation by the insect vector the leishmania parasites change into flagellated leptomanads which multiply in the fly's gut, giving rise to huge numbers of organisms. The fly then bites and regurgitates the organisms into the next host. Tropical sores then develop where the sand fly bites, often on hands, arms, legs, face and ears. The incubation period can be anything from two to eight weeks to as long as three years. Biopsy specimens reveal that many organisms are found both in histocytes and free in tissue. As immunity develops the overlying epithelium becomes hyperplastic and necrosis of the dermis causes ulceration. Later there is a shift from a pure histocytic reaction to a granulamatus reaction, which is characterised by increasing numbers of lymphocytes and plasma cells. As this occurs the organisms become reduced in numbers and are finally impossible to find. Healing proceeds when granulation tissue and scar tissue fill the crater. Lesions of mucocutaneous leishmaniasis are characterised by a necrotizing granulomatus reaction with many inflammatory cells with very few organisms. These lesions of mucocutaneous leishmaniasis are resistant to treatment.

In anergic cutaneous leishmaniasis there are many large histocytes in the dermis which contain the cutaneous leishmaniasis. Necrosis does not occur nor do granulomas form, but the parasites continue to multiply with increasing numbers of histocytes which accommodate them.

Diagnosis is generally by smears or biopsy specimens. *Leishmania* parasites are recognised by their shape, size and staining characteristics. Treatment for tropical sores within the first year with anti-malarials remains the most effective way of dealing with the disease.

7.3.3 Trichomanosis[151,152,153]

Trichomanosis is another protozoan parasitic tropical disease. There are three species which are common in humans throughout the world. These are *Trichomanosis hominis* in the caecum and large intestine, Trichomanosis *tenax* in the mouth and Trichomanosis *vaginalis* in the vagina and urethra of women and seminal vesicles and prostate of men. *T. tenax* is harmless whereas *T.vaginalis* is often

harmless but may cause inflammation and discharge and is an increasingly important venereal disease infecting an estimated 180 million people worldwide. None of the *Trichomanosis* species form cysts but have rounded resistant stages. This means that the organism can survive in an external environment. *T.tenax* and *T. vaginalis* are both transmitted by direct contact. *T. vaginalis* infection in males rarely lasts more than four weeks as it is limited whereas in women it may persist for up to several years.

The genus *Trichomanosis* comprises flagellates that have three to five anterior flagella, an undulating membrane, an axostyle, and usually a cytosme.

These parasites are widely spread and effect nearly every mammal associated with man. T. *vaginalis* is the largest and most robust and is the only pathogen, although it had been suggested that heavy infections with *T. hominis* may cause diarrhoea.

T. vaginalis is a colourless pyriform flagellate. Its habitat is the vagina of the female and the urethra, epididymis, and prostate of the male. Hence it is frequently found in the urine. Reproduction takes place by binary longitudinal fission, with mitotic division of the nucleus. The trophozite is one of the most resistant parasitic protozoa. The incidence of infection is 10 to 25 % women, and higher in groups where hygiene is deficient. Only about 1/7 of the infected women get symptoms, although the vaginal secretions are invariably altered. There are several modes of transmission. Sexual intercourse, especially of asymptomatic infected males is probably the most important. However since young virgins are found infected, it appears that direct contact with infected females, contaminated toilet articles and toilet seats will transmit the infection. *T. vaginalis* is the causative agent of persistent vaginitis. The flagellate is responsible for low grade inflammation. The bacterial flora and physiological status of the vagina, including pH, are among factors which determine infection. Clinical diagnosis is based on symptoms of burning, a frothy creamy discharge, and punctuate lesions and hyperemia of the vagina. The microscopic examination of a drop of saline for motile trichomonads of a fresh vaginal discharge is the most practical method of diagnosis. Prostatic secretions following prostatic massage and urine of the male should be examined. The most effective drug for treatment for both sexes is metronidazole. Because the drug is carcinogenic and mutagenic under experimental conditions it has been recommended only if the

infection is severe and other measures have failed. Other drugs which have been applied with varying success to the vaginal mucosa by insufflation or by suppository are silver pictrate, furazolidone or iodochlorohydroxyquin. Attention to personal hygiene is the most important preventative measure and the detection of infected males could help in reducing infections.

This is an area where further development of the treatment could be exceedingly helpful to a large number of people.

7.4.1 Biological Evaluation

The biological evaluation was carried out by Professor Coombes at the University of Glasgow in the Parasitology department as follows.

7.4.2 Experimental Detail

The compound was dissolved to approximately 40 mg ml^{-1} in water (ideally) then dilutions of 1000, 500, 250, 100, 50, 25 and 10 μg ml^{-1} were made. 20 ml of the above dilutions were added to 180 μl culture at 105 μl^{-1} which means that the final drug concentrations were 100, 50, 25, 10, 5, 2.5 and 1 μg ml^{-1}. The cultures were initiated at 105 ml^{-1} and incubated for about 24 h anaerobically at 37 °C for *Trichomonads* and 120 h aerobically at 25 °C for *Leishmania* promastigates.
Controls, i.e. no drug, were run in tandem and the cultures and drugs were cultivated in 96 microlitre plates. 5 ml of the 40 mg ml^{-1} stock and 195 ml of water were combined to give 1 mg ml^{-1} in 2.5 % DMSO. The remaining dilutions were also made up in 2.5 % DMSO. The procedure was then followed as above to have final concentrations of 0.25 % DMSO which did not affect the growth of the organism.
At the end of the incubation period, the cultures are first assessed by eye (microscope) and assigned arbitrary indications of cell growth.

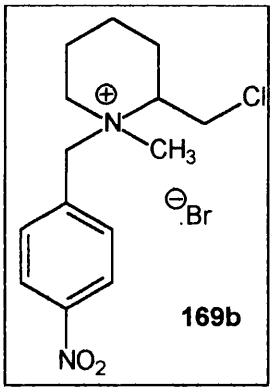
7.4.3 Selection and Synthesis of Compounds Tested for Anti-*Leishmania* and Anti-*Trichomanosis* Potential

The compounds which were synthesised for testing against the two diseases were chosen to cover a selection of variables such as:

- number of alkylating arms;
- number of atoms in heterocycles;
- number of nitrogens within the systems;
- other heteroatoms within the system;
- functional groups;
- number of quaternary nitrogens; and
- *ortho* and *para* substituted nitrobenzyl derivatives.

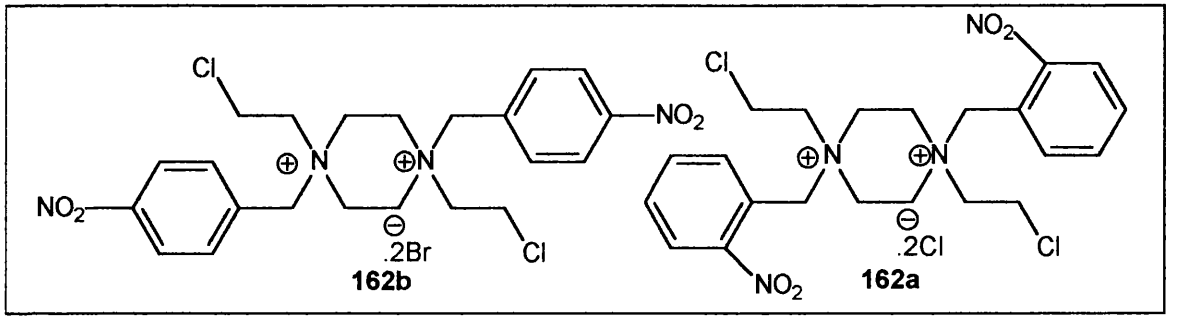
The compounds were all alkylating agents and tested as quaternary (either *o*- or *p*-substituted) nitrobenzyl salts. Most of the compounds tested were developed from compounds which had previously undergone biological evaluation against the leukaemic P562 cell line or BE29 or HT cell lines as described in **Chapter 6** and had shown some promising cell selectivity or/ and cytotoxicity.

The synthesis of the first compound, **169b**, was previously described in **Chapter 6**



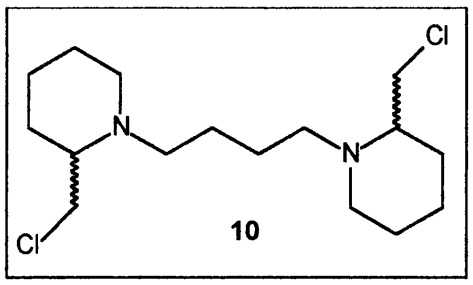
169b

The second and third compounds **162a** and **162b** were synthesised to study anticancer activity against HT29 and BE carcinoma cell lines as the free bases. They incorporate bifunctional alkylating potential within a small azamacrocycle. They also investigate if there are any differences in biological activity between *o*-substituted, **162a,** and *p*- substituted, **162b**, nitrobenzyl substituted compounds.
Their synthesis was discussed in **Chapter 6**.

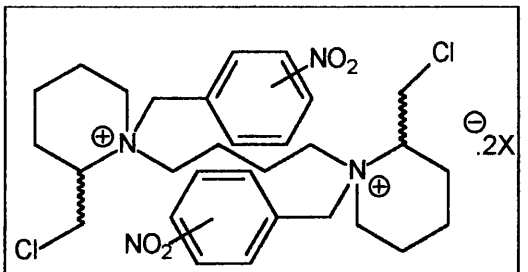


162b **162a**

Compound **10**, was previously synthesised as the free base by Dr Henderson and showed promising anticancer activity against HT29 and BE carcinoma cell lines. The compound was synthesised as reported.[54]

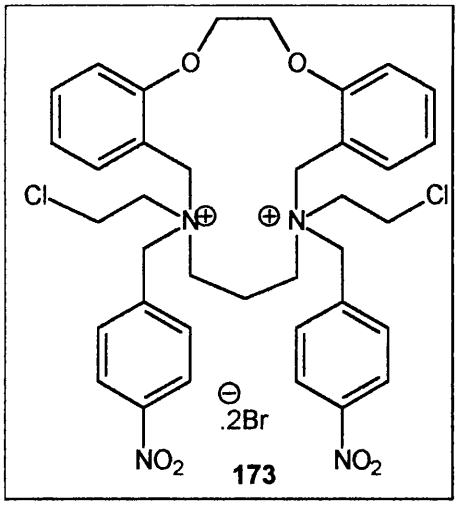


10

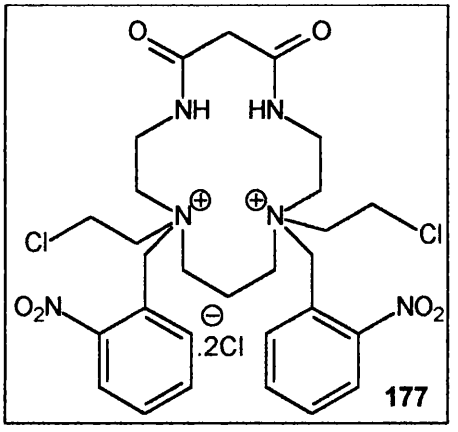


NO_2 *o* X= Cl **167a**
NO_2 *p* X= Br **167b**

Product **10** showed a level of anti-cancer activity thus meriting further biological evaluation. The synthesis of the free base was as described in **Chapter 5** followed by quaternisation, with both *o*- and *p*- derivatives, as discussed in **Chapter 6** to afford **167a** and **167b**.



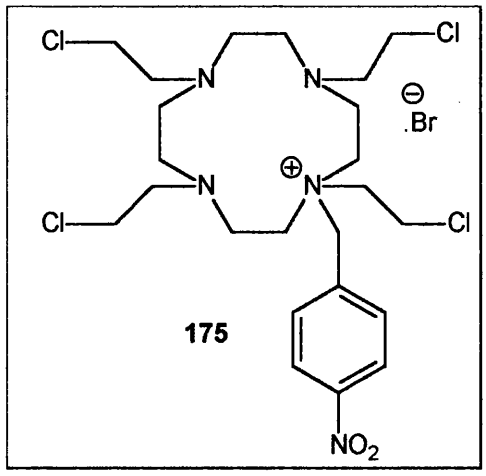
173



177

The penultimate bifunctional alkylating agent is compound **173**. This is a large macrocyclic compound with four nitogen atoms within the cycle with two alkylating arms and incorporation two aromatic rings functionality. The nitrobenzyl groups attached are *p*- substitiuted. Compound **173** was synthesised and characterised in **Chapter 6**.

The diamide cyclam derivative **177** offered a bifunctional alkylating potential and incorporated the amide functionality. The *o*-substituted quaternary nitrogen compound **177** was chosen for testing.



175

Potentially tetra alkylating agent **175** was synthesised as in **Chapter 6**. This compound is a large aza macrocycle quaternised with an *o*-substituted nitrobenzyl group. As stated in **Chapter 6** the compound only had one nitrobenzyl moiety attached.

7.5.1 Biological Evaluation: Conclusions

The compounds were tested against the *Leishmania* and *Trichomanosis* and **Table 11** shows the results of this testing.

	Leishmania		***Trichomanosis***	
	MLC	**LD50**	**MLC**	**LD50**
176	>25	>25	>25	10
162a	>25	>25	>25	10
167a	>25	>25	>25	>25
177	100	50	>100	25
173	50	10	>100	10
167b	>100	>100	>100	>100
175	>100	50	>100	25
162b	>100	>100	>100	>100
169b	>100	>100	>100	>100

Table 11

None of the results were particularly promising against either of the parasites.

There were, however, similarities in comparing the two sets of data.

The most active compounds against *Leishmania* were **162a, 167a, 173** and **175**. Thus indicates that bifunctional alkylating agents were preferential for activity. The *p*-nitrosubstituted compounds appeared to have limited biological activity. The most active compounds were of varying sizes thus no conclusions can be drawn about size preference. An interesting fact is that the most active compounds are those that contain nitrogen as the only heteroatom.

The most active compounds against *Trichomanosis* were again **162a**, **176** and **167a** followed by **177** and **173**. This indicates that the *o*-nitrosubstituted compounds had better activity than compounds which were *p*-nitrosubstituted. The bifunctional alkylating agents were also those preferred for biological activity. Again the size of the most active compounds varies thus no conclusions can be drawn for preferential activity. Finally the most active compounds against *Trichomanosis* were those containing nitrogen as the only heteroatom within the cyclic system

This information indicates that the macrocyclic aza compounds are consistently the ones which show the most promise for further development.

Chapter 8

8.1.1 Experimental Detail

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 of Perrin and Armarego. Melting points, m.p., were determined in open capillaries using Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM200-SY spectrometer operating at 200 MHz and 50 MHz respectively. ^{13}C NMR spectra were assigned with the aid of Distortionless Enhancement by Polarisation Transfer (DEPT) edited spectra. The numbering schemes do not refer to the system of nomenclature but to the assigned carbon numbers in the diagrams in the experimental. Mass spectra (MS) were recorded on an updated AE1 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 (Rf) were obtained by analytical Thin Layer Chromatography (TLC) on Merck aluminium backed silica plates of 0.25 mm thickness or Merck aluminium alumina backed plates of thickness 0.2 mm; chromatograms were visualised using UV conditions at 254 nm or by staining with iodine. All column chromatography was carried out on silica gel (particle size 70-230 mesh) or alumina (particle size 150 mesh). Micro analysis were performed by the department micro analyst.

8.2.1 General Methods

General method (a):

Methyl prolinate hydrochloride salt **12** in chloroform (30 ml) was added to a round bottomed flask along with triethylamine (two equivalents) under nitrogen. The solution was stirred for 10 min before being cooled to between -5 and -10 °C. The acid chloride (0.5 equivalents) was slowly added dropwise. The reaction mixture was

allowed to return to room temperature and stirred overnight. The solvent was removed under reduced pressure and the residue was taken up in water and extracted with ethyl acetate (4 x 50 ml). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. The products were purified by column chromatography on alumina.

General method (b):

The diester diamide was taken up in dry THF and slowly added to a solution of $LiAlH_4$ (4.5 equivalents) in dry THF under nitrogen at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The flask was then cooled to 0 °C and water: THF (1:1) was added dropwise followed by water to quench the reaction completely. The solvents were removed under reduced pressure and to the resulting white solid was added DCM. The solution was stirred for 30 min before filtering and concentration of the filtrate to leave the required diol.

General method (c):

The alcohol was slowly added to neat thionyl chloride (20-30 ml) at room temperature (if a vigorous reaction occurred and the alcohol was taken up in chloroform and the thionyl chloride was cooled to 0 °C before addition). The reaction stirred at room temperature before any solvent and excess thionyl chloride were removed under reduced pressure. The resulting solid was taken up in EtOH and recrystallised on the addition of diethyl ether.

General method (d):

A mixture of the diol, isocyanate (quantities shown for individual compounds) and 3 drops of dibutyltindiacetate were stirred together in DCM at room temperature and the reaction was monitored by TLC until complete. The solution was then concentrated to leave a solution which was purified by column chromatography.

General method (e):

As in literature method[58] with further purification by taking the residue up in water and extracting with chloroform. The desired alcohol was obtained on concentration of the extracts *in vacuo*.

General method (f):

For *N,N'*-di(*p*-toluenesulfonyl) diethylenetriamine, diethylenetriamine (41.3 g, 0.4 mol) in distilled water (250 ml) was added to a three necked flask. NaOH pellets (48.0 g, 1.2 mol) were slowly added keeping the temperature below 40 °C. The solution was cooled to 15 °C and diethyl ether was added (250 ml) with vigorous mixing. *p*-Toluenesulfonyl chloride (229.0 g, 1.2 mol) was added keeping the temperature below 20 °C. The solution was cooled to 0 °C and stirring was maintained for 1 h. The white solid was filtered off and washed with diethyl ether (250 ml). The product was recrystallised from chloroform.

General method (g):

N,N',N"-Tri(*p*-toluenesulfonyl)diethylenetriamine (31.0 g, 55.0 mmol) was suspended in dry EtOH (250 ml). Sodium metal (4.5 g, 0.2 mol) was added, whilst keeping the temperature between 60 and 70 °C. After the last of the sodium was added the solution was clear and on cooling the disodium salt precipitated. After refrigeration overnight the product was filtered off and oven dried at 120 °C for several hours.

General method (h):

To a solution of *p*-toluenesulfonyl chloride (42.0 g, 0.2 mol) in DCM (50 ml) was slowly added 1,3-propanediol (7.61 g, 0.1 mol) in DCM (75 ml) at 0 °C followed by benzyltriethylammonium chloride (TEBA) (0.92 g, 4.0 mmol) then sodium hydroxide (8.0 g, 0.2 mol) in water (50 ml) at 0 °C with vigorous stirring. The solution was allowed to return to room temperature and stirring was maintained for 1 h. The solution was then poured into water (100 ml). The organic layer was removed and washed with water (3 x 150 ml). The solvent layer was dried over $MgSO_4$, filtered and concentrated *in-vacuo* followed by several hours on a vacuum line to remove any trace of solvent to leave the product as a fluffy white solid.

General method (i):

N,O,O'-Tri(*p*-toluenesulfonyl)diethanolamine (0.70 g, 1.2 mmol) in dry DMF (50 ml) was added dropwise via a pressure equalised dropping funnel over 4 h to a solution of caesium carbonate (0.2 g, 0.6 mmol) and

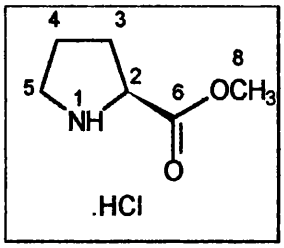
N,N',N'',N'''-tetra(*p*-toluenesulfonyl)spermine (1.0 g, 1.2 mmol) in dry DMF (50 ml) heated to 100 °C and stirring was maintained for 12 h. The solvent was distilled off to leave a white solid which was taken up in water (20 ml) and DCM (20 ml). The organic layer was removed and washed with water (3 x 20 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo.* The residue was taken up in minimal hot toluene and the desired product crystallised. The larger [2+2] Richman Atkins product precipitated at room temperature and the smaller product on refrigeration. On further cooling the Richman Atkins [1+1] product was obtained.

General method (j):

The disodium salt of *N,N',N''*-tri(*p*-toluenesulfonyl)diethylenetriamine (15.95 g, 26 mmol) was dissolved in dry DMF (100 ml). The mixture was heated to 100 °C and *O,O'*-bis(*p*-toluenesulfonyl)-1,2-ethanediol (9.68 g, 26 mmol) in dry DMF (100ml) was added dropwise with vigorous stirring at 100 °C and this was maintained for 12 h. The solution was cooled to room temperature and slowly added to ice water (750 ml) with vigorous stirring. The precipitate was filtered off and washed with water (5 x 200 ml). The product was oven dried at 120 °C overnight before recrystallisation from minimum hot chloroform and the addition of three times the volume of EtOH. The crystallisation occurred after cooling to 0 °C. The precipitate was filtered and washed with EtOH.

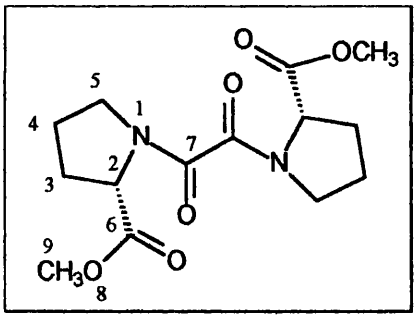
8.3.1 Experimental to Chapter 3

Methyl L-Prolinate hydrochloride 12



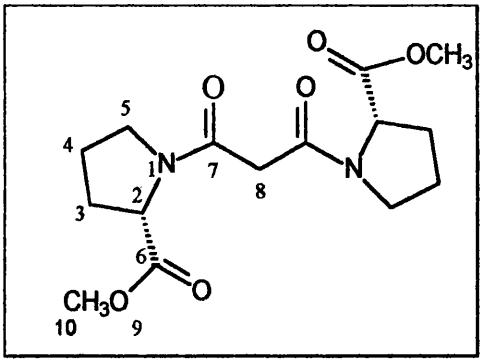
Ester **12** was prepared as in the literature[56] with L-proline (15.00 g, 0.13 mol) in methanol (125 ml) and thionyl chloride (18 ml) as a white solid, (19.0 g, 88 %); υ_{max} (KBr disc) 1747 and 2738 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.82-2.36 and 3.1 (6H, m, ring CH_2), 3.31 (3H, s, CH_3O), 3.35-3.41 (1H, m, CHN); δ_C (50 MHz, $CDCl_3$) 22.1 (C-4), 26.9 (C-3), 44.3 (C-5), 51.9 (C-8), 57.8 (C-2), 167.6 (C-6).

***N,N'*-(1,2-Dioxo-1,2-ethandiyl)bis(2S-2-methoxycarbonylpyrrolidine) 13**



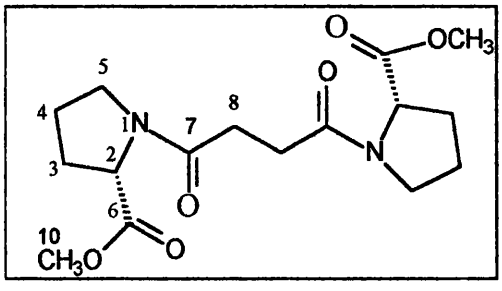
Bispyrrolidine **13** was prepared using general method (a) with L-prolinate hydrochloride **12** (2.00 g, 12 mmol) in chloroform (30 ml), dry triethylamine (3.04 g, 30 mmol) and oxalyl chloride (0.76 g, 6 mmol) which had been freshly distilled. The product was obtained as a light yellow coloured solid (1.42 g, 76 %); υ_{max} (KBr disc) 1192, 1429, 1632, 1737, 2882, 2988 and 3019 cm^{-1}; δ_H (200 MHz, CDCl3) 1.90-1.97 (8H, m, ring CH_2), 2.14-2.25 (4H, m, ring CH_2), 3.69 (6H, s, CH_3), 4.44-4.48 (2H, dd, CHN); δ_C (50 MHz, $CDCl_3$) 24.4 (C-4), 29.2 (C-3), 47.2 (C-5), 52.4 (C-9), 57.7 (C-2), 169.1 (C-6), 171.8 (C-7); *m/z* (CI^+) 313.15 (MH^+, 100%), 330.17 ($M+NH_4^+$, 65 %) (found: M^+ 312.1319; C, 53.89; H, 6.41; N, 8.83 %. $C_{14}H_{20}N_2O_6$ requires 312.1321; C, 53.84; H, 6.45; N, 8.97%).

N,N'-(1,3-Dioxo-1,3-propanediyl)bis(2S-2-methoxycarbonylpyrrolidine) 14



Bispyrrolidine **14** was prepared by method (a) using **12** (2.00 g, 12 mmol) in chloroform (30 ml), dry triethylamine (3.04 g, 30 mmol) and malonyl chloride (0.76 g, 6.0 mmol) which had been freshly distilled. The product was obtained as a light yellow oil (1.42 g, 76 %); υ_{max} (thin film) 1162, 1435, 1750, 2880, 2948, 2958 and 3017 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.68-2.17 (8H, m, $C\underline{H}_2CHN$, $C\underline{H}_2CH_2N$), 2.60-2.95 (4H, m, NCH_2), 3.59-3.76 (2H, m, $NCOCH_2$), 3.60 (6H, s, CH_3) and 4.36-4.45 (2H, m, CHN); δ_C (50 MHz, $CDCl_3$) 24.6 (C-4), 29.3 (C-3), 41.8 (C-8), 47.6 (C-5), 52.2 (C-10), 58.9 (C-2), 168.3 (C-6), 172.4 (C-7); *m/z* (CI/ Isobutane) 327.2 (MH^+, 100 %), 344.2 ($M+NH_4^+$, 5%) (found: MH^+ 327.1551. $C_{15}H_{23}N_2O_6$ requires 327.1556).

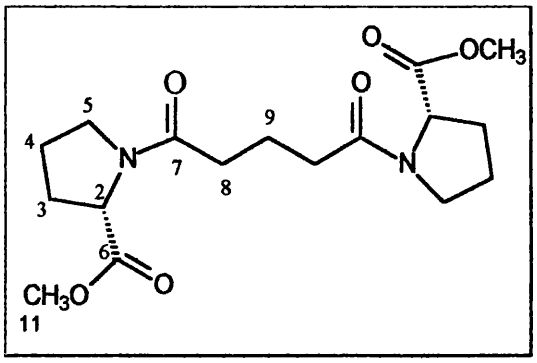
N,N'-(1,4-Dioxo-1,4-butanediyl)bis(2S-2-methoxycarbonylpyrrolidine) 15



Bispyrrolidine **15** was prepared using method (a) using **12** (2.00 g, 12 mmol) in chloroform (30 ml), dry triethylamine (3.04 g, 30 mmol) and freshly distilled succinyl chloride (0.93 g, 6.0 mmol). The product was obtained as a light pink oil (1.57 g, 78 %); υ_{max} (thin film) 1169, 1636, 1738 and 2882 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.7-2.09 (8H, m, CH_2CHN, CH_2CH_2N), 2.36-2.85 (4H, m, CH_2), 3.61 (6H, s, CH_3), 3.56-3.61 (4H, m, $NCOCH_2$), 4.36-4.58 (2H, m,CHN); δ_C (50 MHz, $CDCl_3$) 26.4, 29.1 (C-3),

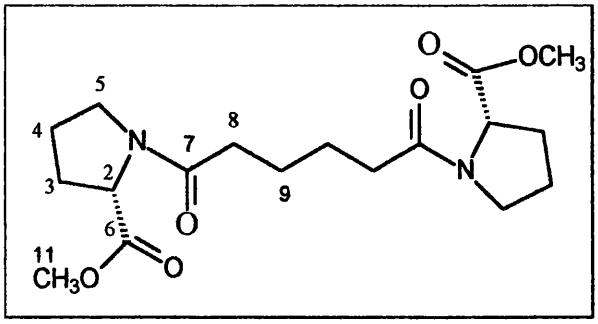
(C-4), 31.2 (C-8), 46.8 (C-5), 52.1 (C-10), 58.6 (C-2), 170.8 (C-6), 172.7 (C-7); *m/z* (Cl^+/ NH_3) 341.2 (*MH*$^+$, 100 %) (found: *MH*$^+$ 341.1706 $C_{16}H_{25}N_2O_6$ requires 341.1713).

N,N'-(1,5-Pentanediyl)bis(2S-2-methoxycarbonylpyrrolidine) 16



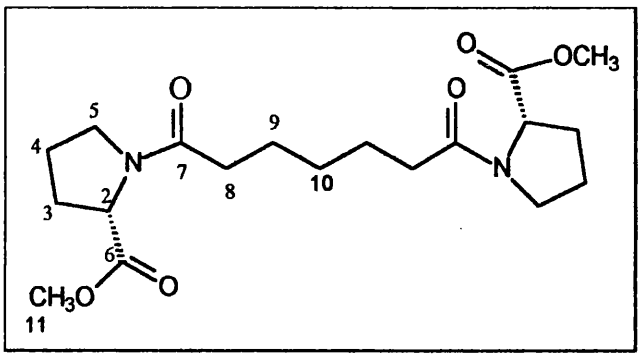
Bispyrrolidine **16** was prepared by method (a) using **12** (2.00 g, 12 mmol) in chloroform (30 ml), dry triethylamine (3.04 g, 30 mmol) and glutaryl dichloride (1.01 g, 6.0 mmol) which had been freshly distilled. The product was afforded as an off white solid (1.44 g, 68 %); υ_{max} (KBr disc) 1199, 1636, 1747, 2935, 2955 and 2988 cm^{-1}; δ_H (200 MHz, $CDCl_3$)1.90-2.15 (10H, m, $C\underline{H_2}CHN$, $C\underline{H_2}CH_2N$, $COCH_2C\underline{H_2}$), 2.33-2.45 (4H, m, CH_2N), 3.40-3.69 (4H, m, $COCH_2$), 3.65 (6H, s, CH_3) and 4.38-4.43 (2H, m, CHN); δ_C (50 MHz, $CDCl_3$) 19.7, 24.7 29.2 (C-3), (C-4), (C-9), 33.0 (C-8), 47.0 (C-5), 52.2 (C-11), 58.6 (C-2), 171.7 (C-6), 172.5 (C-7); *m/z* (EI^+) 355.2 (*MH*$^+$, 100 %) (found: *M*$^+$ 354.1790; C,57.46; H, 7.52; N, 7.82 %. $C_{17}H_{26}N_2O_6$ requires 354.1791; C, 57.61; H, 7.40; N, 7.92 %).

N,N'-(1,6-Dioxo-1,6-hexanediyl)bis(2S-2-methoxycarbonylpyrrolidine) 17



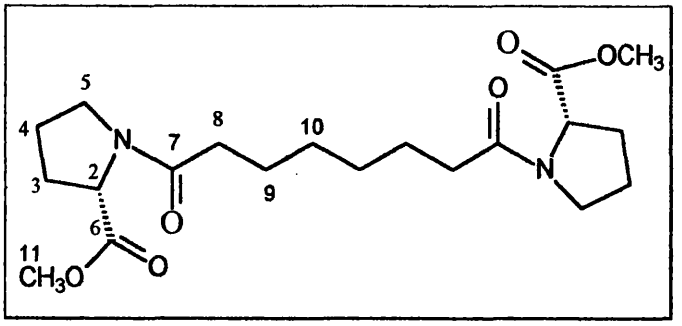
Bispyrrolidine **17** was prepared using method (a) with **12** (2.00 g, 12 mmol) in chloroform (30 ml), dry triethylamine (3.04 g, 30 mmol) and freshly distilled adipoyl dichloride (1.09 g, 6 mmol). The product was obtained as a white oil (1.92 g, 87 %);υ_{max} (thin film) 1173, 1431, 1632, 1730 and 2882 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.50-1.65 (4H, m, $COCH_2C\underline{H_2}$), 1.79-2.02 (8H, m, $C\underline{H_2}CHN$, $C\underline{H_2}CH_2N$), 2.06-2.19 (4H, m, CH_2N), 3.38-3.59 (4H, m, $COCH_2$), 3.54 (6H, s, CH_3) and 4.27-4.32 (2H, m, CHN); δ_C (50 MHz, $CDCl_3$) 22.4, 24.6, 29.0 (C-3), (C-4), (C-9), 34.0 (C-8), 46.9 (C-5), 52.0 (C-11), 59.2 (C-2), 171.7 (C-6), 172.7 (C-7); *m/z* (CI^+) 369.2 (*MH*$^+$, 100 %) (found: *MH*$^+$ 369.2026. $C_{18}H_{29}N_2O_6$ requires 369.2026).

N,N'-(1,7-Dioxo-1,7-heptanediyl)bis(2S-2-methoxycarbonylpyrrolidine) 18



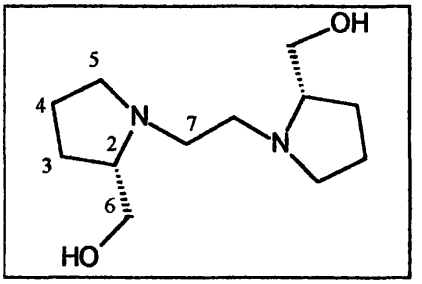
Bispyrrolidine **18** was prepared by method (a) using **12** (2.00 g, 12 mmol) in chloroform (30 ml), dry triethylamine (3.04 g, 30 mmol) and freshly distilled pimeloyl dichloride (1.18 g, 6.0 mmol). The product was obtained as a yellow oil (2.24 g, 98 %); υ_{max} (thin film) 1173, 1438, 1639, 2882, 2951, 2981 and 3020 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.18-1.36 (2H, m, $COCH_2CH_2C\underline{H_2}$), 1.54-1.63 (6H, m, $C\underline{H_2}CHN$, $COCH_2C\underline{H_2}$), 1.83-2.10 (6H, m, $C\underline{H_2}CHN$, CH_2CH_2N), 2.18-2.26 (4H, m, CH_2N), 3.50-3.64 (4H, m $COCH_2$), 3.68 (6H, s, CH_3) and 4.38-4.43 (2H, m, CHN); δ_C (50 MHz, $CDCl_3$) 22.4, 24.1,24.6, 29.1 (C-3), (C-4), (C-9), (C-10) 34.0 (C-8), 47.0 (C-5), 52.1 (C-11), 58.2 (C-2), 172.1 (C-6), 172.8 (C-7).

_N,N'_-(1,8-Dioxo-1,8-octanediyl)bis(2S-2-methoxycarbonylpyrrolidine) 19



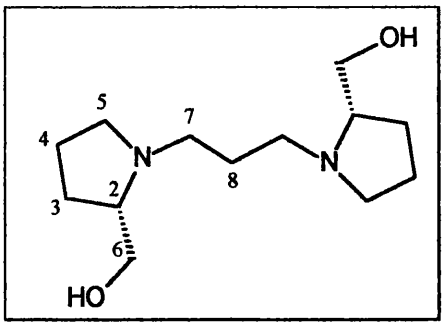
Bispyrrolidine **19** was prepared by method (a) using **12** (2.00 g, 12 mmol) in chloroform (30 ml), dry triethylamine (3.04 g, 30 mmol) and freshly distilled suberoyl dichloride (1.27 g, 6 mmol). The product was afforded as a clear oil (2.16 g, 91 %); υ_{max} (thin film) 1177, 1438, 1639, 1738, 2882, 2950, 2980 and 3026 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.25-1.32 (4H, m, CH_2 chain), 1.53-1.57 (4H, m, CH2 chain), 1.89-2.16 (8H, m, CH2 ring), 2.17-2.29 (4H, m, CH_2 ring), 3.40-3.60 (4H, m, $NCOCH_2$), 3.64 (6H, s, CH_3) and 4.40-4.58 (2H, m, CHN); δ_C (50 MHz, $CDCl_3$) 24.3, 24.6, 28.6, 28.9 (C-3), (C-4), (C-9), (C-10), 34.1 (C-8), 46.9 (C-5), 52.0 (C-11), 59.0 (C-2), 171.9 (C-6), 172.8 (C7); *m/z* (Cl^+) 396.2 (*M*$^+$, 100 %).

_N,N'_-(1,2-Ethanediyl)bis(2S-2-hydroxymethylpyrrolidine) 20



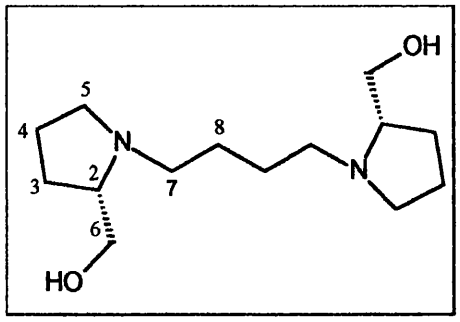
Diol **20** was synthesised using method (b) with **13** (1.40 g, 4.5 mmol) in dry THF (25 ml) and lithium aluminium hydride (0.94 g, 27 mmol) in dry THF (20 ml) as a clear oil (0.37 g, 36 %); υ_{max} (nujol) 1044, 1419 and 3000 (broad) cm^{-1}; δ_H (200 MHz, $CDCl_3$)1.16-1.46 (4H, m, $C\underline{H}_2CH_2N$), 1.63-1.87 (4H, m, $C\underline{H}_2CHN$), 2.24-2.32 (8H, m, NCH_2), 3.28-3.51 (6H, m, $C\underline{H}_2OH$, CHN) and 4.81 (2H, bs, OH); δ_C (50 MHz, $CDCl_3$) 25.3 (C-4), 27.2 (C-3), 46.0 (C-5), 55.9 (C-7), 64.5 (C-2), 65.4 (C-6); *m/z* (Cl^+) 229.21 (*MH*$^+$, 100%) (found: *MH*$^+$ 229.1913. $C_{12}H_{25}N_2O_2$ requires 229.1914).

N,N'-(1,3-Propanediyl)bis(2S-2-hydroxymethylproline) 21



Diol **21** was obtained using method (b) with lithium aluminium hydride (0.50 g, 13 mmol) in dry THF (25 ml) and **14** (1.0 g, 3.0 mmol) in dry THF (20 ml) as a light yellow oil (0.27 g, 37 %); υ_{max} (thin film) 1063, 1431, 3376 and 3627 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.45-2.51 (10H, m, C<u>H</u>$_2$CH$_2$N ring, C<u>H</u>$_2$CHN, C<u>H</u>$_2$CH$_2$CH$_2$N chain), 2.64-3.35 (4H, m, NCH$_2$ chain), 3.38-3.44 (2H, m, CHN), 3.46-3.78 (8H, m, CH$_2$O, CH$_2$N ring) and (2H, bs, OH); δ_C (50 MHz, $CDCl_3$) 24.0, 27.0, 27.2 (C-3), (C-4), (C-8), 54.5 (C-5), 55.3 (C-7), 64.7 (C-2), 65.8 (C-6); *m/z* (Cl^+) 243.0 (*MH*$^+$, 100 %) (found: *MH*$^+$ 243.2071. $C_{13}H_{27}N_2O_2$ requires 243.2073).

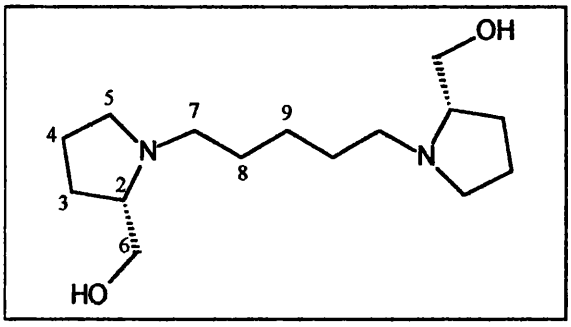
N,N'-(1,4-Butanediyl)bis(2S-2-hydroxymethylpyrrolidine) 22



Diol **22** was synthesised using general method (b) with **15** (1.50 g, 4.4 mmol) in dry THF (20 ml) and lithium aluminium hydride (0.75 g, 20 mmol) in dry THF (25 ml) as a clear oil (0.73 g, 82 %); υ_{max} (thin film) 1074, 1397, 3422 and 3612 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.46-1.64 (10H, m, C<u>H</u>$_2$CH$_2$ chain, C<u>H</u>$_2$CHN ring, <u>C</u>H$_2$CH$_2$N ring), 2.06 (4H, m, CH$_2$N chain, C<u>H</u>$_2$CH$_2$N ring), 2.46 (4H, m, CH$_2$N ring and chain), 2.71-2,80 (2H, m, CH$_2$N ring), 3.07-3.13 (2H, m, CHN), 3.33-3.67 (4H, dd and dd, C<u>H</u>$_2$OH) and 4.27 (2H, bs, OH); δ_C (50 MHz, $CDCl_3$) 23.1, 26.6, 27.2 (C-3), (C-4),

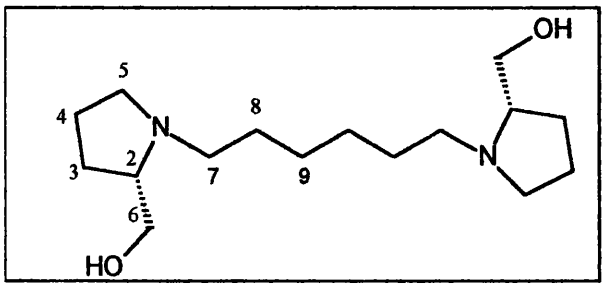
(C-8), 54.2 (C-5), 54.9 (C-7), 63.0 (C-2), 65.5 (C-6); *m/z* (Cl^+) 257.0 (*MH*$^+$, 100%) (found: *MH*$^+$ 257.2226. $C_{14}H_{29}N_2O_2$ requires 257.2229).

N,N'-(1,5-Pentanediyl)bis(2S-2-hydroxymethylpyrrolidine) 23



Diol **23** was synthesised using general method (b) with **16** (1.40 g, 4.0 mmol) in dry THF (25 ml) and lithium aluminium hydride (0.90 g, 26 mmol) in dry THF (20 ml) as a yellow oil (0.83 g, 75 %); υ_{max} (thin film) 1071, 1404, 3429 and 3627 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.25-1.41 (4H, m, C<u>H</u>$_2$CH$_2$CH$_2$N chain), 1.61-1.86 (8H, m, C<u>H</u>$_2$CH$_2$N ring and chain), 2.07-2.23 (2H, m, C<u>H</u>$_2$CHN), 2.33-2.46 and 2.58-2.72 (4H, m, CH$_2$N chain), 3.06-3.12 and 3.30-3.36 (4H, m, CH$_2$N ring) and 3.37-4.40 (8H, m, CH$_2$OH, OH, CHN); δ_C (50 MHz, $CDCl_3$) 23.6, 25.5 (C-4), 27.5, 28.5 (C-3), (C-8), (C-9), 54.1, 54.6 (C-5), (C-7), 62.2 (C-6), 64.9 (C-2); *m/z* (Cl^+) 271.26 (*MH*$^+$, 100%) (found: *MH*$^+$ 271.2382. $C_{15}H_{31}N_2O_2$ requires 271.2386).

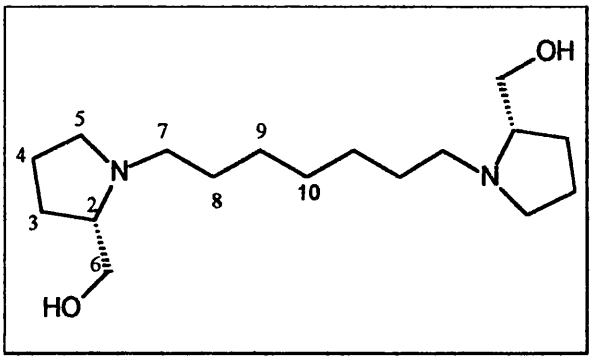
N,N'-(1,6-Hexanediyl)bis(2S-2-methoxycarbonylpyrrolidine) 24



Diol **24** was formed using general method (b) with **17** (1.85 g, 5.0 mmol) in dry THF (25 ml) and lithium aluminium hydride (0.84 g, 23 mmol) in dry THF (25 ml) as a yellowish oil (1.07 g, 75 %); υ_{max} (nujol) 1074, 1408, 3422 and 3627 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.25-1.63 (8H, m, C<u>H</u>$_2$CH$_2$N, C<u>H</u>$_2$CH$_2$CH$_2$CH$_2$N chain), 1.64-1.89 (8H, m,

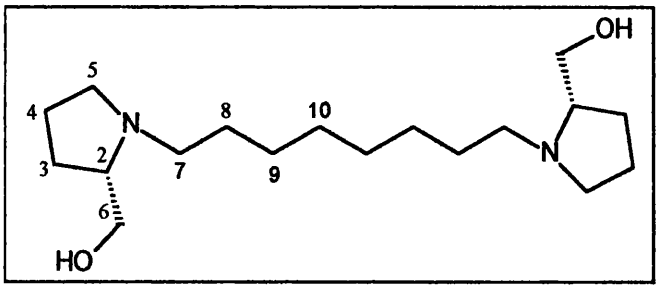
C$\underline{H}_2$CHN, C$\underline{H}_2$CH$_2$N ring), 2.06-2.46 (4H, m, CH$_2$N chain), 2.58-2.68 and 3.05-3.12 (4H, m, CH$_2$N ring), 3.30-3.36 (2H, m, CHN) and 3.49-3.55 (4H, m, C$\underline{H}_2$OH), 3.74 (2H, bs, OH); δ_C (50 MHz, $CDCl_3$) 27.1, 27.5, 28.7, 32.6 (C-3), (C-4), (C-8), (C-9), 54.1 (C-5), 54.7 (C-7), 62.1 (C-6), 64.9 (C-2); *m/z* (Cl^+) 285.23 (*MH*$^+$, 100 %) (found: *MH*$^+$ 285.2539. $C_{16}H_{33}N_2O_2$ requires 285.2542).

_N,N'_-(1,7-Heptanediyl)bis(2S-2-hydroxymethylpyrrolidine) 25



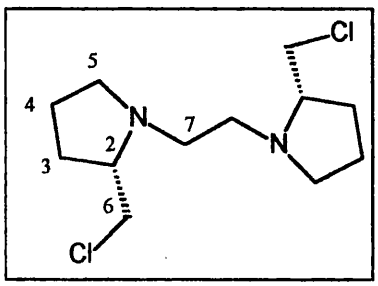
Diol **25** was synthesised via general method (b) using **18** (2.24 g, 5.9 mmol) in dry THF (20 ml) and lithium aluminium hydride (1.40 g, 37 mmol) in dry THF (20 ml) as a clear oil (1.47 g, 84 %);δ_H (200 MHz, $CDCl_3$) 1.09-1.45 (6H, m, C$\underline{H}_2$CH$_2$CH$_2$N chain), 1.48-1.73 (6H, m, C$\underline{H}_2$CH$_2$N chain), 1.95-2.06 (6H, m, C$\underline{H}_2$CHN, CH$_2$ ring), 2.15-2.29 (4H, m, CH$_2$N chain), 2.44-2.48 and 2.48-2.58 (4H, CH$_2$N ring), 2.89-2.93 (2H, m, CHN), 3.17-3.36 (4H, m, CH$_2$OH) and 3.92 (2H, bs, OH); δ_C (50 MHz, $CDCl_3$) 27.3, 27.4, 29.1, 32.5 (C-3), (C-8), (C-9), (C-10), 54.1 (C-5), 54.9 (C-7), 62.5 (C-6), 65.1 (C-2); *m/z* (Cl^+) 299.3 (*MH*$^+$, 100 %), 341.3 (*M*$^+$ C_3H_7, 65 %) (found: *MH*$^+$ 299.2698. $C_{17}H_{35}N_2O_2$ requires 299.2697).

N,N'-(1,8-Octanediyl)bis(2S-2-hydroxymethylpyrrolidine) 26



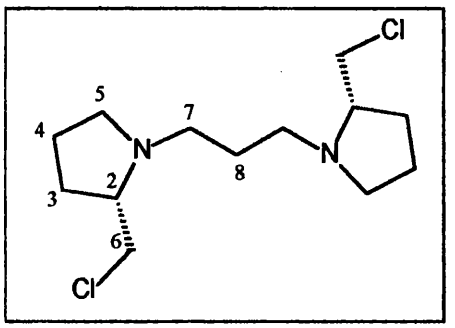
Diol **26** was produced using general method (b) with **19** (2.16 g, 5.4 mmol) in dry THF (20 ml) and lithium aluminium hydride (0.91 g, 25 mmol) in dry THF (20 ml) as a clear oil (1.39 g, 82 %); υ_{max} (thin film) 1116, 1385, 3440 and 3627 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.92-1.22 (6H, m, CH_2 chain), 1.22-1.44 (4H, m, CH_2 ring), 1.55-1.89 (8H, m, $C\underline{H_2}CHN$, CH_2 ring), 1.66-2.28 and 2.62-2.82 (8H, m, CH_2N ring and chain) and 3.35-3.70 (8H, m, $C\underline{H_2}OH$, CHN); δ_C (50 MHz, $CDCl_3$) 27.3, 27.5, 29.3, 29.2 (C-3), (C-4), (C-9), (C-10), 32.6 (C-8), 54.1 (C-5), 54.7 (C-7), 62.0 (C-6), 64.9 (C-2); *m/z* (CI^+) 313.26 (*MH*$^+$, 100 %) (found: *MH*$^+$ 313.2852. $C_{18}H_{37}N_2{}^{35}O_2$ requires 313.2855).

N,N'-(1,2-Ethanediyl)bis(2S-2-chloromethylpyrrolidine) 27



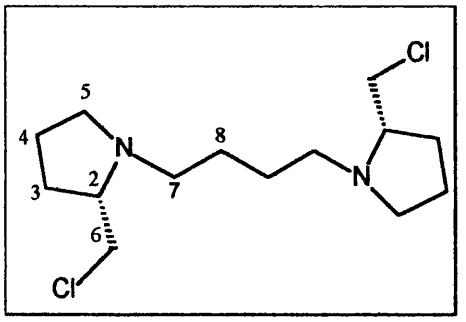
Chloride **27** as the free base was prepared using general method (c) with **20** (0.30 g, 1.31 mmol) and thionyl chloride (30 ml). The product was obtained as an off white solid and recrystallised from EtOH and diethyl ether (0.20 g, 57 %); υ_{max} (KBr disc) 764, 2873, 2947 and 2983 cm^{-1}; δ_H (200 MHz, D_2O) 1.67-1.85 (2H, m, CH_2 chain), 1.86-1.99 (4H, m, $C\underline{H_2}CHN$, CH_2 ring), 2.00-2.16 (2H, m, $C\underline{H_2}CHN$), 3.29-4.06 (4H, m, CH_2N), 3.51-3.54 (2H, m, CH_2N ring), 3.80-4.06 (6H, CH_2Cl, CHN); δ_C (50 MHz, D_2O) 24.2 (C-4), 29.3 (C-3), 55.8 (C-5), 55.9 (C-7), 61.8 (C-6) and 66.2 (C-2); *m/z* (CI^+) 265.1237 (*MH*$^+$, 100%) (found: *MH*$^+$ 265.1238. $C_{12}H_{23}N_2{}^{35}Cl_2$ requires 265.1238).

_N,N'_-(1,3-Propanediyl)bis(2S-2-chloromethylpyrrolidine) 28



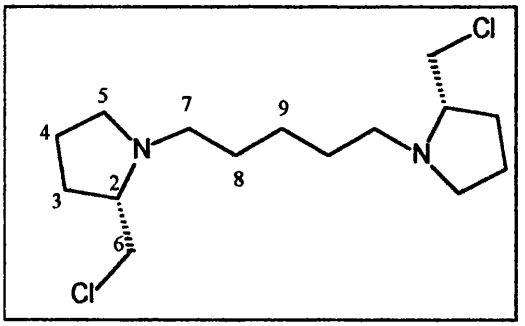
Chloride **28** as the free base was prepared using general method (c) with **21**, (0.20 g, 0.80 mmol) and thionyl chloride (15 ml). The white solid obtained was recrystallised from EtOH and diethyl ether (0.15 g, 68 %); υ_{max} (KBr disc) 752, 2871 and 2939 cm^{-1}; δ_H (200 MHz, D_2O) 1.66-1.84 (2H, m, CH_2 chain), 1.86-1.96 (6H, m, $C\underline{H}_2CHN$, CH_2 ring), 1.98-2.19 (2H, m, $C\underline{H}_2CHN$), 3.01-3.11 (4H, m, CH_2N chain), 3.28-3.34 (2H, m, CH_2N ring), 3.47-3.56 (2H, m, CH_2N), 3.69-3.85 (6H, m, CH_2Cl, CHN); δ_C (50 MHz, D_2O) 22.8 (C-4), 23.4 (C-8), 27.2 (C-3), 43.9 (C-5), 54.0 (C-6), 57.0 (C-7) and 68.4 (C-2).

_N,N'_-(1,4-Butanediyl)bis(2S-2-chloromethylproline) 29



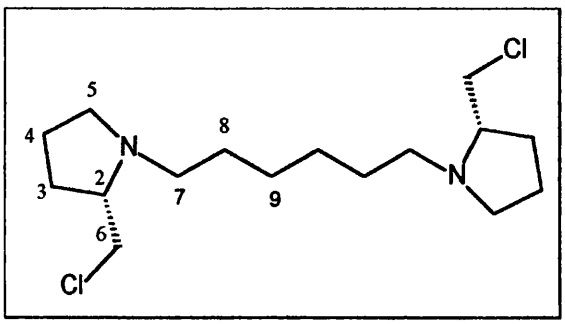
Chloride **29** as the free base was prepared by general method (c) using **22** (0.70 g, 2.8 mmol) and thionyl chloride (15 ml). The white solid was recrystallised from EtOH and diethyl ether (0.27 g, 33 %); υ_{max} (KBr disc) 752, 2947 and 2994 cm^{-1}; δ_H (200 MHz, D_2O) 1.46-1.63 (8H, m, $NCH_2C\underline{H}_2CH_2CH_2N$, $C\underline{H}_2CHN$), 2.06-2.21 (4H, m, $C\underline{H}_2CH_2N$), 2.46-2.49 (4H, m, NCH_2 ring and chain), 2.71-2.80 (4H, m, CH_2N ring), 3.07-3.13 (2H, m, CHN) and 3.35-3.56 (4H, m, CH_2Cl); δ_C (50 MHz, D_2O) 22.7, 23.03, 28.0 (C-3), (C-4), (C-8), 42.9 (C-5), 54.9 (C-7), 55.6 (C-6), 69.34 (C-2); *m/z* (Cl^+) 293.2 (*MH*$^+$, 100 %) (found: *MH*$^+$ 293.1551. $C_{14}H_{27}N_2{}^{35}Cl_2$ requires 293.1551).

N,N'-(1,5-Pentanediyl)bis(2S-2-chloromethylpyrrolidine) 30



Chloride **30** as the free base was prepared by general method (c) using **23**, (0.80 g, 2.96 mmol) and thionyl chloride (30 ml). The product was obtained as an off white/ yellow solid and recrystallised from EtOH and diethyl ether (0.37 g, 40 %); υ_{max} (KBr disc 750, 2874 and 2940 cm^{-1}; δ_H (200 MHz, D_2O) 1.27-1.30 (2H, m, C<u>H</u>$_2$ chain), 1.59-1.62 (4H, m, C<u>H</u>$_2$ chain), 1.84-1.98 (6H, m, C<u>H</u>$_2$CHN, C<u>H</u>$_2$ ring), 2.06-2.19 (2H, m, C<u>H</u>$_2$CHN), 2.94-3.07 (4H, m, CH_2N ring), 3.19-3.33 and 3.49-3.54 (4H, m, CH_2N ring), 3.69-3.85 (6H, m, CH_2Cl, CHN); δ_C (50 MHz, D_2O) 22.7, 23.8, 25.3, 28.0 (C-3), (C-4), (C-8), (C-9), 43.0 (C-5), 55.3 (C-7), 55.5 (C-6) and 69.2 (C-2); *m/z* (Cl^+) 307.19 (*MH*$^+$, 100 %) (found: *MH*$^+$ 307.1700. $C_{15}H_{29}N_2{}^{35}Cl_2$ requires *MH*$^+$ 307.1708).

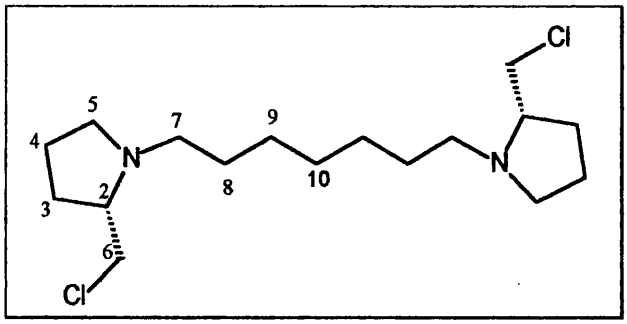
N,N'-(1,6-Hexanediyl)bis(2S-2-chloromethylpyrrolidine) 31



Chloride **31** as the free base was prepared using general method (c) from **24** (0.95 g, 3.34 mmol) and thionyl chloride (20 ml). The white solid obtained was recrystallised from EtOH and diethyl ether (1.07 g, 100 %); υ_{max} (KBr disc) 741, 2871 and 2940 cm^{-1}; δ_H (200 MHz, D_2O) 1.16-1.24 (4H, m, C<u>H</u>$_2$ chain), 1.44-1.58 (4H, m, C<u>H</u>$_2$ chain), 1.69-1.99 (6H, m, C<u>H</u>$_2$CHN, C<u>H</u>$_2$ ring), 2.03-2.20 (2H, m, C<u>H</u>$_2$CHN), 2.86-3.07 (4H, m, CH_2N chain), 3.11-3.29 and 3.45-3.57 (4H, m, CH_2N) and 3.60-3.85 (6H, m, CH_2Cl, CHN); δ_C (50 MHz, D_2O) 22.6, 25.5 , 26.1, 27.9 (C-3), (C-4),

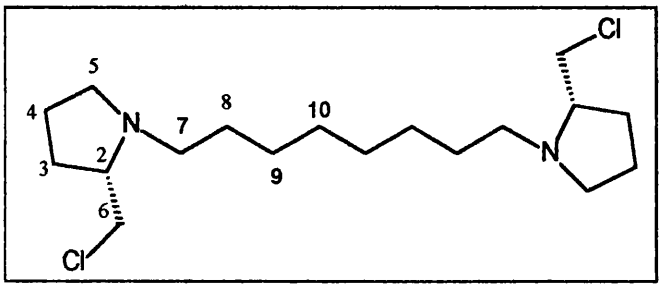
(C-8) (C-9), 43.0 (C-5), 55.5 (C-7), 55.8 (C-6) and 69.1 (C-2); *m/z* (Cl^+) 321.23 (MH^+, 50%) (found: MH^+ 321.1864. $C_{16}H_{31}N_2{}^{35}Cl_2$ requires 321.1864).

N,N'-(1,7-Heptanediyl)bis(2S-2-chloromethylpyrrolidine) 32



Chloride **32** as the free base was prepared using general method (c) with **25** (1.40 g, 4.7 mmol) and thionyl chloride (30 ml). The product was obtained as a brown solid and recrystallised from EtOH and diethyl ether (0.40 g, 26 %); υ_{max} (KBr disc) 749, 2860 and 2936 cm^{-1}; δ_H (200 MHz, D_2O) 1.00-1.15 (6H, m, CH_2 chain), 1.51-1.70 (4H, m, CH_2 chain), 1.72-1.94 (6H, m, $C\underline{H}_2CHN$, CH_2 ring), 1.98-2.15 (2H, m, $C\underline{H}_2CHN$), 2.81-3.02 (4H, m, CH_2N chain), 3.06-3.23 and 3.29-3.50 (4H, m, CH_2N ring) and 3.65-3.81 (6H, m, CH_2Cl, CHN); δ_C (50 MHz, D_2O) 22.6, 25.5, 26.3, 28.0, 28.4 (C-3), (C-4), (C-8), (C-9), (C-10), 43.1 (C-5), 54.5 (C-7), 55.9 (C-2) and 69.0 (C-6); *m/z* (Cl^+) 335.2 (MH^+, 85 %) (found: MH^+ 335.2021. $C_{17}H_{33}N_2{}^{35}Cl_2$ requires 335.2021).

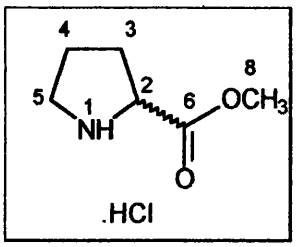
N,N'-(1,8-Octanediyl)bis(2S-2-chloromethylpyrrolidine) 33



Chloride **33** as the free base was prepared using general method (c) with **26** (1.19 g, 3.8 mmol) and thionyl chloride (20 ml). The product was obtained as a beige solid and recrystallised from EtOH and diethyl ether (1.17 g, 88 %); υ_{max} (KBr disc)

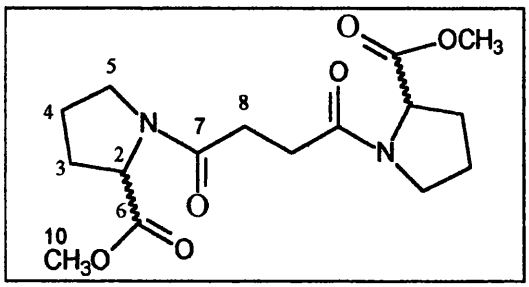
750 and 2932 cm^{-1}; δ_H (200 MHz, D_2O) 1.02-1.17 (8H, m, CH_2 chain), 1.56-1.71 (4H, m, CH_2 chain), 1.76-2.20 (8H, m, CH_2CHN, CH_2 ring), 2.86-3.21 (4H, m, CH_2N chain), 3.23-3.32 and 3.49-3.61 (4H, m, CH_2N ring) and 3.70-3.86 (6H, m, CH_2Cl, CHN); δ_C (50 MHz, D_2O) 22.6 (C-4), 25.6 (C-10), 26.4 (C-9), 28.0 (C-3), 28.6 (C-8), 43.0 (C-5), 55.5 (C-7), 56.0 (C-6) and 69.0 (C-2); *m/z* (CI^+) 349.2 (*MH*$^+$, 100 %) (found: *MH*$^+$ 349.2175. $C_{18}H_{35}N_2{}^{35}Cl_2$ requires 349.2177).

Methyl DL-prolinate hydrochloride 35



Ester **35** was prepared as in literature preparation[56] using DL-proline (5.00 g, 43.4 mmol) in MeOH (45 ml) and thionyl chloride (6 ml) as a white solid (19.0 g, 88 %); υ_{max} (KBr disc) 1747 and 2738,cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.04- 2.43 (4H, m, ring CH_2), 3.49-3.69 (2H, m, NCH_2), 3.84 (3H, s, CH_3), 4.46-4.52 (1H, m, CHN); δ_C (50 MHz, $CDCl_3$) 24.0 (C-4), 29.07 (C-3), 46.4 (C-5), 53.9 (C-8), 59.6 (C-2), 169.6 (C-6).

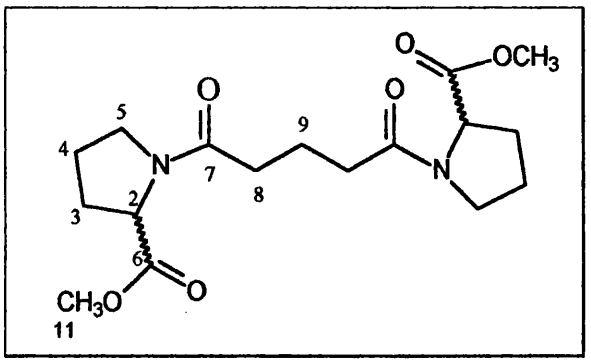
***N,N'*-(1,4-Dioxo-1,4-butanediyl)bis(2RS-2-methoxycarbonylpyrrolidine) 36**



Bispyrrolidine **36** was prepared as in general method (a) using **35** (1.50 g, 9.05 mmol) in chloroform (10 ml), dry triethylamine (2.29 g, 23 mmol) and freshly distilled succinyl chloride (0.70 g, 4.5 mmol) . The product was obtained as a light pink oil (1.57 g, 78 %);υ_{max} (thin film) 1169, 1440, 1635, 1735, 2885 and 2948 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.82-2.00 (8H, m, $C\underline{H}_2CHN$, $C\underline{H}_2CH_2N$), 2.43-2.79 (4H, m, NCH_2), 3.45-3.60 (4H, m, $COCH_2$), 3.65 (6H, s, CH_3) and 4.07-4.41 (2H, m, CHN); δ_C (50

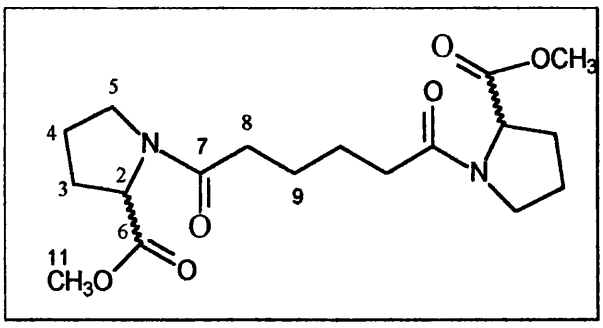
MHz, $CDCl_3$) 25.1, 29.49, 29.6, 29.7, 29.8, 47.2, 47.3, 52.2, 52.2, (C-3), (C-4), (C-5), (C-8), (C-10), 59.0 and 57.1 (C-2), 171.2 and 171.3 (C-6), 173.2 and 173 3 (C-7); (*m/z*) (EI^+) 340.1 (M^+, 100 %) (found: M^+ 340.1634 $C_{16}H_{24}N_2O_6$ requires 340.1634).

_N,N'_-(1,5-Dioxo-pentanediyl)bis(2RS-2-methoxycarbonylpyrrolidine) 37



Bispyrrolidine **37** was prepared by general method (a) using **35** (1.50 g, 9.0 mmol) in chloroform (30 ml), dry triethylamine (2.29 g, 23 mmol) and freshly distilled glutaryl dichloride (0.76 g, 4.5 mmol). The product was afforded as an off white solid (1.44 g, 68 %); υ_{max} (thin film) 1203, 1639, 1738, 28812, 2958 and 2980 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.80-2.05 (10H, m, $C\underline{H}_2CHN$, $C\underline{H}_2CH_2N$, $COCH_2C\underline{H}_2$), 2.18-2.34 (4H, m, NCH_2), 3.48-3.60 (4H, m, $COCH_2$), 3.58 (6H, m, CH_3), 4.30-4.38 (2H, m, CHN); δ_C (50 MHz, $CDCl_3$) 19.7, 19.9, 24.7, 29.1, 31.2, 32.9 (C-3), (C-4), (C-8), (C-9), 46.1 and 465.8 (C-5), 51.98 and 52.4 (C-11), 58.4 and 59.1 (C-2), 171.4 and 172.6 (C-6), 172.8 and 173.2 (C-7); *m/z* (EI^+) 354.1 (M^+, 100 %) (found: M^+ 354.1719. $C_{17}H_{26}N_2O_6$ requires 354.1719).

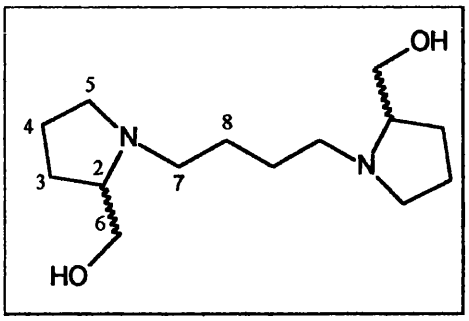
_N,N'_-(1,6-Dioxo-1,6-hexanediyl)bis(2RS-2-methoxycarbonylpyrrolidine) 38



Bispyrrolidine **38** was prepared using general method (a) with **35** (1.50 g, 9.05 mmol) in chloroform (30 ml), dry triethylamine (2.29 g, 23 mmol) and freshly distilled

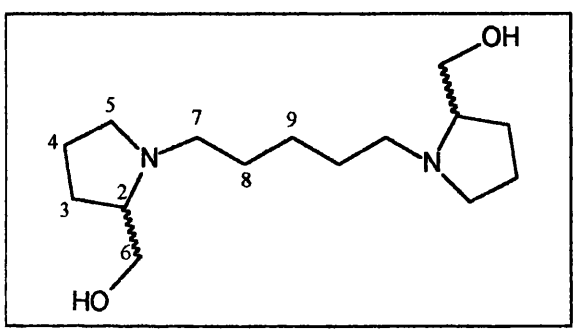
adipoyl dichloride (0.83 g, 4.5 mmol). The product was obtained as a white oil (1.92 g, 87 %);υ_{max} (thin film) 1173, 1435, 1632, 1742, 2874, 2950 and 2980 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.59-1.69 (4H, m, $COCH_2C\underline{H_2}$), 1.90-2.17 (8H, m,$C\underline{H_2}CHN$, $C\underline{H_2}CH_2N$), 2.27 (4H, s, $COCH_2$), 3.42-3.77 (4H, M, CH_2N), 3.59 (6H, s, CH_3) and 4.33-4.40 (2H, m, CHN); δ_C (50 MHz, $CDCl_3$) 22.9, 24.6, 24.9, 29.6, 34.6, (C-3), (C-4), (C-8), (C-9), 46.3, 46.7 (C-5), 52.5, 52.9 (C-11), 58.9 and 59.7 (C-2), 172.1 and 173.3 (C-6), 174.1 (C-7); *m/z* (EI^+) 368.2 (MH^+, 100 %) (found: MH^+ 368.1947. $C_{18}H_{28}N_2O_6$ requires 368.1947).

N,N'-(1,4-Butanediyl)bis(2RS-2-hydroxymethylpyrrolidine) 39



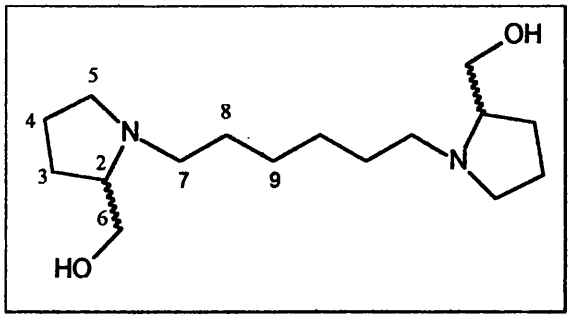
Diol **39** was synthesised using general method (b) with **36** (1.00 g, 2.7 mmol) in dry THF (10 ml) and lithium aluminium hydride (0.82 g, 20 mmol) in dry THF (15 ml) as a clear oil (0.73 g, 82 %); υ_{max} (thin film) 1074, 1397, 3422 and 3612 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.36-1.46, 1.47-1.71, 1.76-1.83 and 2.11-2.21 (12H, m, $C\underline{H_2}CH_2N$ ring and chain), 2.24-2.47 (2H, m, CH_2N), 2.49-2.69 (2H, m, CH_2N), 3.07-3.10 (2H, m, CHN), 3.31 (2H, bs, OH), 3.25-3.34 (4H, m, CH_2N) and 3.55-3.76 (4H, m, $C\underline{H_2}OH$); δ_C (50 MHz, $CDCl_3$) 23.1, 26.6, 27.2 (C-3), (C-4), (C-8), 54.2 (C-5), 54.9 (C-7), 63.0 (C-6), 65.5 (C-2); *m/z* (CI^+) 257.0 (MH^+, 100%), 313.2 (M^+ C_4H_9, 18 %) (found: MH^+ 257.2226. $C_{14}H_{29}N_2O_2$ requires 257.2229).

N,N'-(1,5-Pentanediyl)bis(2RS-2-hydroxymethylpyrrolidine) 40



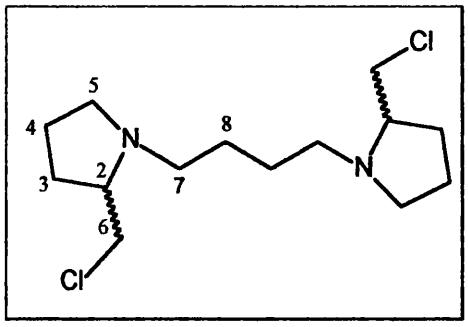
Diol **40** was synthesised by general method (b) using **37** (.40 g, 6.8 mmol) in dry THF (30 ml) and lithium aluminium hydride (1.01 g, 30 mmol) in dry THF (30 ml) as a yellow oil (1.01 g, 55 %); υ_{max} (thin film) 1071, 1404, 3429 and 3627 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.17-1.81 (10H, m, $C\underline{H_2}CH_2CH_2N$, $C\underline{H_2}CH_2N$ ring and chain), 2.11-2.19 (4H, m, $C\underline{H_2}CHN$), 2.46-2.48 and 2.49-2.66 (4H, m, NCH_2 chain), 3.02-3.10 and 3.29-3.32 (4H, m, CH_2N ring) and 3.33-3.74 (8H, m, $C\underline{H_2}OH$, OH); δ_C (50 MHz, $CDCl_3$) 23.6, 25.5, 27.5, 28.5 (C-3), (C-4), (C-8), (C-9), 54.1 (C-5), 54.6 (C-7), 62.2 (C-6), 64.9 (C-2); *m/z* (Cl^+) 271.26 (MH^+, 100%), 327.2 ($M^+ C_4H_9$, 18 %) (found: MH^+ 271.2381. $C_{15}H_{31}N_2O_2$ requires 271.2386).

_N,N'_-(1,6-Hexanediyl)bis(2RS-2-hydroxymethylpyrrolidine) 41



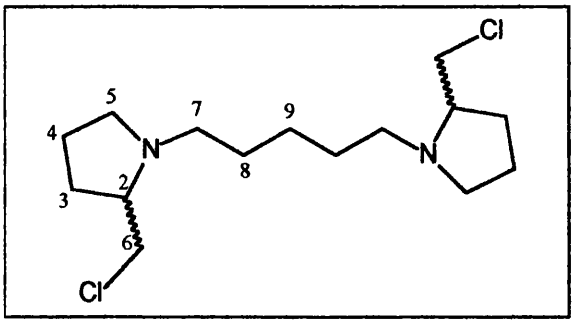
Diol **41** was formed using general method (b) with **38** (2.40 g, 6.5 mmol) in dry THF (30 ml) and lithium aluminium hydride (1.10 g, 29 mmol) in dry THF (30 ml) as a yellowish oil (1.07 g, 75 %); υ_{max} (thin film) 1074, 1408, 3422 and 3627 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.21-1.82 (16H, m, $C\underline{H_2}CHN$, $C\underline{H_2}CH_2N$ ring and chain), 2.11-2.19 (4H,m, CH_2N), 2.45-2.49 (2H, m, CH_2N ring), 2.58-2.66 (2H, m, CH_2N ring), 3.11-3.31 (2H, m, CHN), 3.31-3.66 (6H, m, $C\underline{H_2}O\underline{H}$); δ_C (50 MHz, $CDCl_3$) 27.1, 27.5, 28.7, 32.6 (C-3), (C-4), (C-8), (C-9), 54.1 (C-5), 54.7 (C-7), 62.1 (C-6), 64.9 (C-2); *m/z* (Cl^+) 285.23 (MH^+, 100 %), 341.2 ($M+C_4H_9$, 18 %) (found: MH^+ 285.2539. $C_{16}H_{33}N_2O_2$ requires 285.2542).

_N,N'_-(1,4-Butanediyl)bis(2RS-2-chloromethylpyrrolidine) 42



Chloride **42** was prepared by general method (c) using **39** (0.85 g, 3.3 mmol) and thionyl chloride (20 ml). **42** Was obtained as an off white solid which was recrystallised from EtOH and diethyl ether (0.40 g, 41 %); m.p. 245-247 °C as the HCl sat; υ_{max} (KBr disc) 752, 2947 and 2994 cm^{-1}; δ_H (200 MHz, D_2O) 1.78-2.28 (12H, m, $C\underline{H}_2CH_2N$ ring and chain, $C\underline{H}_2CHN$), 3.14-3.21 (4H, m, CH_2N chain), 3.31-3.54 (4H, m, CH_2N ring), 3.59-3.69 (2H, m, CHN) and 3.82-3.98 (4H,m, CH_2Cl); δ_C (50 MHz, $CDCl_3$) 22.7, 23.0, 27.9 (C-3), (C-4), (C-8), 42.9 (C-5), 54.9 (C-7), 55.6 (C-6), 69.3 (C-2); *m/z* (Cl^+) 293.1 (*MH*$^+$, 100 %) (found: *MH*$^+$ 293.1551. $C_{14}H_{27}N_2{}^{35}Cl_2$ requires 293.1551).

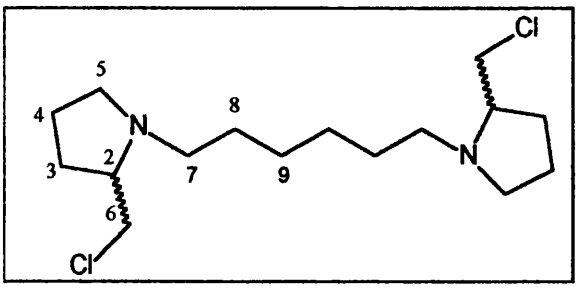
_N,N'_-(1,5-Pentanediyl)bis(2RS-2-chloromethylpyrrolidine) 43



Chloride **43** as the free base was afforded by general method (c) using **40** (1.00 g, 3.7 mmol) and thionyl chloride (20 ml). The product was obtained as an off white/ yellow solid and recrystallised from EtOH and diethyl ether (0.81 g, 71 %); υ_{max} (KBr disc) 750, 2874 and 2940 cm^{-1}; δ_H (200 MHz, D_2O) 1.25-1.44 (2H, m, CH_2 chain),1.70-1.79 (4H, m, CH_2 chain), 1.79-2.08 (6H,m, $C\underline{H}_2CHN$, $C\underline{H}_2CH_2N$ ring), 2.22-2.29 (2H, m, $C\underline{H}_2CHN$), 3.04-3.20 (4H, m, CH_2N chain), 3.35-3.46 and 3.62-3.68 (4H, m, CH_2N ring) and 3.82-3.97 (6H, m, CH_2Cl, CHN); δ_C (50 MHz, D_2O) 22.7, 23.8,

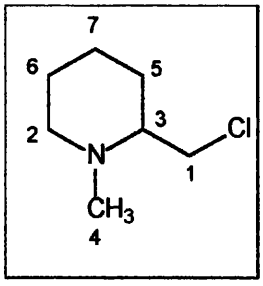
25.3, 27.9 (C-3), (C-4), (C-8), (C-9), 43.0 (C-5), 55.3 (C-7), 55.5 (C-6) and 69.2 (C-2); *m/z* (Cl^+) 307.19 (*MH*$^+$, 100 %) (found: *MH*$^+$ 307.1680. $C_{15}H_{29}N_2{}^{35}Cl_2$ requires 307.1680).

N,N'-(1,6-Hexanediyl)bis(2RS-2-chloromethylpyrrolidine) 44



Chloride **44** was prepared by general method (c) using **41** (1.00 g, 3.5 mmol) and thionyl chloride (20 ml). The product was obtained as an off white solid and recrystallised from EtOH and diethyl ether (0.85 g, 76 %); m.p. 232-234 °C; υ_{max} (KBr disc) 741, 2871 and 2940 cm^{-1}; δ_H (200 MHz, D_2O) 1.10-1.36 (8H, m, CH_2 chain), 1.67-2.09 (6H, m, $C\underline{H}_2CHN$, $C\underline{H}_2CH_2N$ ring), 2.22-2.29 (2H, m, $C\underline{H}_2CHN$). 3.33-3.40 and 3.61-3.67 (8H, m, CH_2N) and (6H, m, CH_2Cl, CHN); δ_C (50 MHz, D_2O) 22.6, 25.5, 26.1, 27.9 (C-3), (C-4), (C-8), (C-9), 43.0 (C-5), 55.5 (C-7), 55.8 (C-6) and 69.1 (C-2); *m/z* (Cl^+) 321.23 (*MH*$^+$, 50%) (found: *MH*$^+$ 321.1862. $C_{16}H_{31}N_2{}^{35}Cl_2$ requires 321.1864).

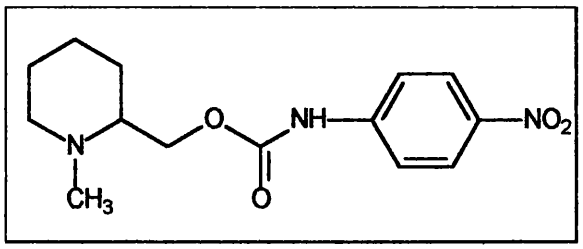
2-Chloromethyl-*N*-methyl piperidine 46



Chloride **46** was prepared by general method (c) using 2-hydroxymethyl-*N*-methylpiperidine (1.4 g, 10.8 mmol) and thionyl chloride (20 ml) as a yellow solid and recrystallised from EtOH and diethyl ether (1.34 g, 84 %), m.p. 161-163 °C; υ_{max} (KBr disc) 750, 2802, 2870, 2945 and 3001 cm^{-1}; δ_H (200 MHz, D_2O) 1.01-1.12 (1H, m, $C\underline{H}_2CHN$), 1.22-1.44 (5H, m, CH_2 ring), 1.69-1.94 (2H, m, NCH_2), 2.02 (3H, s, CH_3),

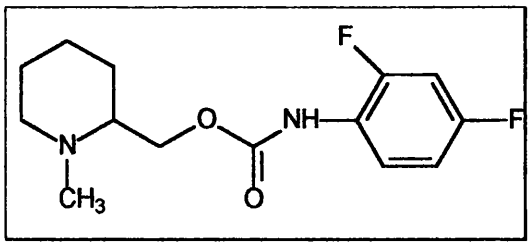
2.57-2.62 (1H, m, CHN) and 3.22-3.44 (2H, dd, CH_2Cl); δ_C (50 MHz, D_2O) 23.3 (C-7), 25.1 (C-6), 28.9 (C-5), 42.2 (C-4), 46.1 (C-2), 53.2 (C-1) and 63.4 (C-3); *m/z* (EI^+) 147.06 (*MH^+*, 100 %).

2-(*p*-Nitrophenylaminocarbonyloxymethyl)-*N*-methylpiperidine 47



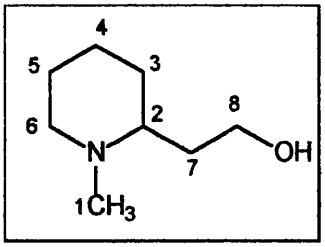
Carbamate **47** was obtained by general method (d) as a yellow solid using **46** (0.5 g, 3.87 mmol), *p*-nitrophenylisocyanate (0.95 g, 5.8 mmol), DCM (30 ml) and dibutyltin diacetate (3 drops). (0.46 g, 40 %); m.p. 165-167 °C; υ_{max} (KBr disc) 1110, 1333, 1508, 1559, 1598, 1739 and 3426 cm^{-1}; δ_H (200 MHz, DMSO) 1.42-1.48 (2H, m, CH_2 ring), 1.81-1.96 (5H, m, CH_2 ring), 2.51-2.59 (1H, dd, CH_2 ring), 2.65 (3H, s, CH_3), 3.30-3.36 (1H, d, CHN), 4.27-4.46 (2H, m, CH_2O), 5.05 (1H, bs, NH) and 7.61-8.21 (4H, AA'BB' system, Ar-H).

2-(2,4-Difluorophenylcarbonyloxymethyl)-*N*-methylpiperidine 48



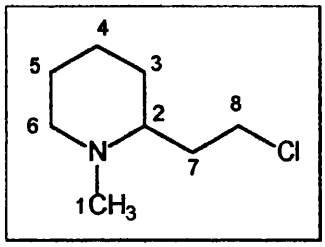
Carbamate **48** was prepared by general method (d) using **46** (0.5 g, 3.87 mmol), 2,4-difluorophenylisocyanate (0.90 g, 5.8 mmol), DCM (30 ml) and dibutyltin diacetate (3 drops). The product was obtained as a white solid (0.20 g, 18 %); m.p. 165-167 °C; υ_{max} (KBr disc) 728, 1376, 1536, 1612, 1730, 2788, 2858, 2938, 3074 and 3218 cm^{-1}; δ_H (200 MHz, DMSO) 1.40-1.46 (1H, m, CH_2 ring), 1.52-1.71 (5H, m, CH_2 ring), 2.55-2.59 (1H, dt, NCH_2 ring), 2.56 (3H, s, CH_3), 3.13-3.28 (1H, d, CH_2), 4.07-4.40 (2H, m, CH_2O), 6.73-6.91 and 7.29-7.41 (3H, m, Ar-H).

2-Hydroxyethyl-*N*-methylpiperidine 50



Alcohol **50** was obtained from 2-hydroxyethylpiperidine (3.0 g, 23 mmol), formaldehyde 37 % (3.84 g, 0.126 mol) [3.56 ml], formic acid 90 %(4.8 ml) and H_2O (3 ml). The solution was heated overnight at reflux before being cooled to room temperature and basified with sodium hydroxide solution. The aqueous solution was extracted with chloroform (4 x 60 ml). The extracts were washed with saturated brine (3 x 30 ml) before being dried over MgSO4, filtered and concentrated under reduced pressure to leave a clear oil. This was purified by column chromatography on alumina with EtOAc and chloroform, (3.0 g, 90 %); Rf= 0.3 (EtOAc); υ_{max} (thin film) 1075, 1373, 2790, 2855 and 2933 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.17-1.39 (5H, m, CH_2 ring), 1.64-1.84 (1H, m, CH_2 ring), 1.88-1.90 (2H, dt, $C\underline{H_2}COH$), 2.23 (3H, s, CH_3), 2.76-2.79 (1H, m, CHN), 3.44-3.48 and 3.59-3.65 (4H, m, CH_2OH, CH_2N) and 4.21 (1H, bs, OH); δ_C (50 MHz, $CDCl_3$) 22.2, 23.8 (C-3), (C-4), 28.9 (C-5), 32.9 (C-6), 41.6 (C-1), 57.3 (C-7), 58.1 (C-2), 64.2 (C-8)

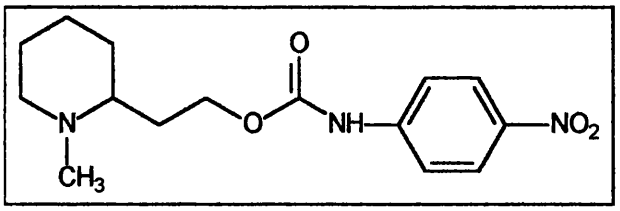
2-Chloroethyl-*N*-methylpiperidine 51



Chloride **51** was obtained by stirring **50** (0.5 g, 3.5 mmol) in thionyl chloride (20 ml) overnight, as in general method (c), before being concentrated under reduced pressure to leave a light brown solid which was recrystallised from EtOH and diethyl ether (0.40 g, 70 %); υ_{max} (KBr disc) 740 cm^{-1}; δ_H (200 MHz, D_2O) 1.34-1.42, 1.43-1.63, 1.72-1.91 and 1.92-2.01 (7H, $C\underline{H_2}CH_2N$, $C\underline{H_2}CH_2CH_2N$, $C\underline{H_2}CH_2Cl$), 2.26-2.33 (1H, m, $C\underline{H_2}CHN$), 2.75 (3H, s, CH_3), 2.94-2.99 (2H, m, CH_2N) and

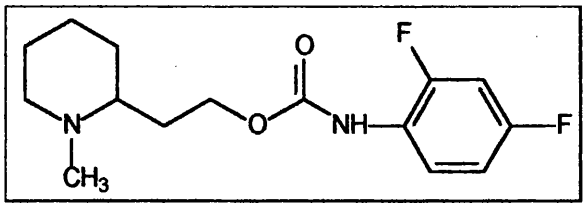
3.47-3.70 (3H, m, CH_2Cl, CHN); δ_C (50 MHz, D_2O) 21.2, 23.2 (C-3), (C-4), 27.3 (C-5), 32.8 (C-6), 40.5 (C-7), 41.1 (C-1), 56.8 (C-1) and 57.6 (C-2); *m/z* (CI^+) 162.1 (*MH*$^+$, 15 %) (found: *MH*$^+$ 162.1050. $C_8H_{17}N^{35}Cl$ requires 162.1050).

2-(*p*-Nitrophenylcarbonyloxyethyl)-*N*-methylpiperidine 52



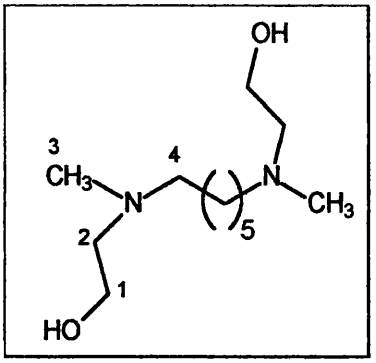
Carbamate **52** was obtained as in general method (d) using **50** (0.5 g, 3.5 mmol), DCM (30 ml) and dibutyltin diacetate (3 drops) as a bright yellow solid (0.25 g, 23 %); Rf = 0.6 (EtOAc); mp 143-147 °C; υ_{max} (KBr disc) 1350, 1506, 1599 and 1741 cm^{-1};δ_H (200 MHz, DMSO) 1.19-1.41 (5H, m, ring CH_2), 1.70-2.03 (1H, m, ring CH_2), 2.10 (3H, s, CH_3), 3.25-3.34 (3H, m, CHN), 3.65-4.05 (4H, m, CH_2O, CH_2N), 7.67-7.79 and 8.09-8.20 (4H, AA'BB' system, Ar-H); *m/z* (EI^+) 308 (*MH*$^+$, 100 %).

2-(2,4-Difluorophenylcarbonyloxyethyl)-*N*-methylpiperidine 53



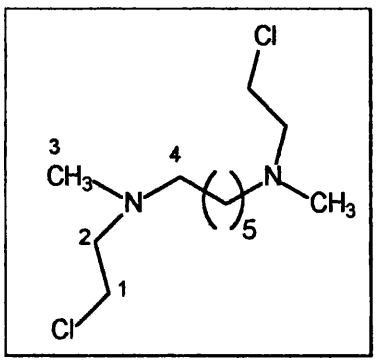
Difluorophenylcarbamate **53** was made as in general method (d) using **50** (1.0 g, 6.9 mmol), 2,4-difluorophenyl isocyanate (1.08 g, 7.0 mmol), DCM (30ml) and dibutyltindiacetate (3 drops). The product was obtained as a white solid (0.20 g, 20 %); Rf= 0.8 (EtOAc); υ_{max} (KBr disc) 730, 1381, 1526, 1520, 1612, 1734, 2869, 2952 and 3430 cm^{-1}; δ_H (200 MHz, DMSO) 1.22-1.37 (1H, m, CH_2 ring), 1.55-1.90 (5H, m, CH_2 ring), 2.15 (3H, s, CH_3), 3.05-3.27 (4H, m, CH_2O, CH_2N), 3.58-3.69 (1H, m, CHN), 4.06-4.29 (2H, dd, $C\underline{H}_2CHN$), 4.42 (1H, bs, NH), 6.73 (2H, m, Ar-H) and 7.27-7.39 (1H, m, Ar-H); δ_C (50 MHz, DMSO) 22.0, 22.8 (C-7), (C-4), 28.3, 29.9 (C-3), (C-5), 39.8 (C-1), 55.6 (C-6), 62.2 (C-2), 104.8, 111.2, 111.6, 122.4, 124.1 and 124.2 (aromatic) and 154.2 (C-9).

N,N'-Dihydroxyethyl-N,N'-dimethylhexane-1,6-diamine 55



Diol **55** was obained as in general method (e) using *N,N'*-dimethyl-1,6-hexanediamine (2.0 g, 13.9 mmol) in EtOH (10 ml) and ethylene oxide (1.32 g, 30.0 mmol) as a white solid (2.0 g, 63 %); υ_{max} (thin film) 1040, 1467, 2934 and 3387 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.12-1.39 (8H, m, CH_2 chain), 3.47 (6H, s, CH_3), 3.44-3.58 (8H, m, CH_2N) and 3.59-3.64 (4H, m, CH_2O); δ_C (50 MHz, $CDCl_3$) 45.9 (C-6), 50.9 (C-5), 54.11 (C-4), 56.1 (C-3), 56.9 (C-2) and 61.8 (C-1); *m/z* (EI^+) 233.2 (*MH*$^+$, 10 %), (found *MH*$^+$ 233.2229. $C_{12}H_{29}N_2O_2$ requires 233.2229).

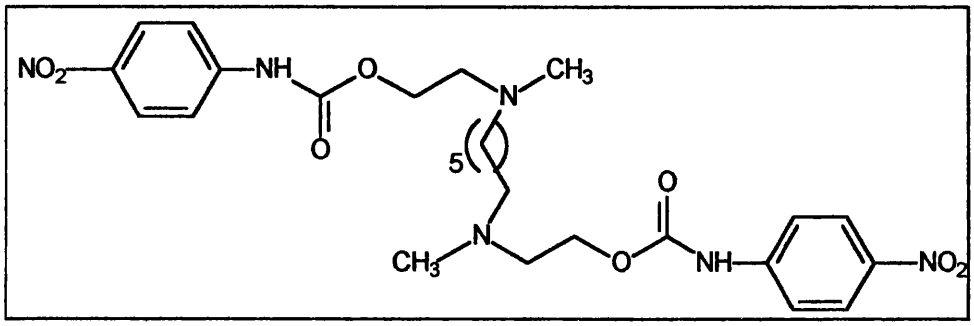
N,N'-Dichloroethyl-N,N'-dimethylhexane-1,6-diamine 56



Chloride **56** was obained using general method (c) with *N,N'*-dihydroxyethyl-*N,N'*-dimethylhexane-1,6-diamine (1.5 g, 6.4 mmol) and thionyl chloride (15 ml) as a white/ yellow solid which was recrystallised from EtOH and diethyl ether (1.05 g, 60 %);m.p. >212 °C; υ_{max} (KBr disc) 619, 2805, 2861 and 2939 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.36-1.70 (8H, m, CH_2 chain), 2.86 (6H, s, CH_3), 3.12-3.21, 3.47-3.70 and 3.87-3.89 (12H, m, CH_2N, CH_2Cl); δ_C (50 MHz, $CDCl_3$) 23.9

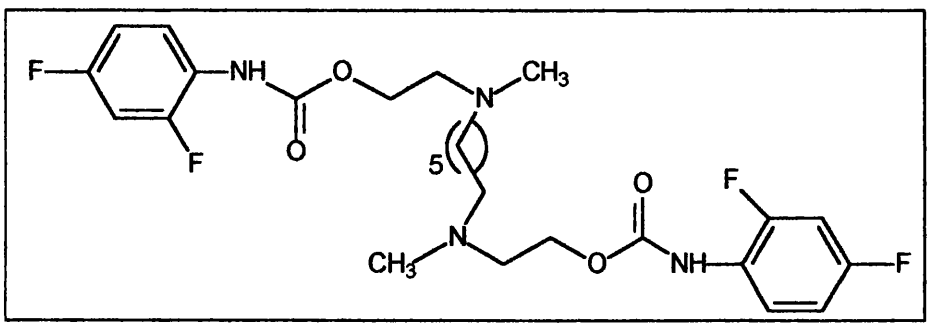
(C-6), 25.9 (C-5), 38.4 (C-1), 40.7 (C-2), 56.6 (C-4) and 57.2 (C-3); *m/z* (Cl^+) 269.1 (MH^+, 65 %) (found: M^+ 269.1551. $C_{12}H_{26}N_2{}^{35}Cl_2$ requires 269.1551).

N,N'-p-(Nitrophenylcarbonyloxyethyl)-N,N'-dimethylhexane-1,6-diamine 57



Carbamate **57** was obtained as in general method (d) with **55** (1.0 g, 4.4 mmol) in DCM (20 ml) with *p*-nitrophenyl isocyanate (1.96 g, 12 mmol) and three drops of dibutyltin diacetate as a yellow solid (1.0 g, 41 %); υ_{max} (KBr disc) 1328, 1498, 1554, 1598, 1733, 2950, 3077 and 3365 cm^{-1}; δ_H (200 MHz, D_2O) 1.10-1.61 (8H, m, CH_2 chain), 2.97 (6H, s, CH_3), 3.14-3.58 (8H, m, CH_2N), 3.75-3.91 (4H, m, CH_2O), 6.55-6.60 and 7.87-7.92 (8H, AA'BB' system, Ar-H).

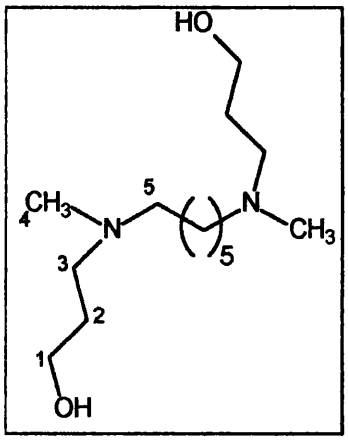
N,N'-(2,4-Difluorophenylcarbonyloxyethyl)-N,N'-dimethylhexane-1,6-diamine 58



Bisdifluorophenyl carbamate **58** was obtained as in general method (d) with **55** (1.0 g, 4.4 mmol) in DCM (20 ml) 2,4-difluorophenyl isocyanate (1.86 g, 12 mmol) and three drops of dibutyltin diacetate as white crystals (0.99 g, 42 %); m.p. 179-180 °C; υ_{max} (KBr disc) 760, 1381, 1520, 1526, 1626, 1734, 2870, 2953 and 3441 cm^{-1}; δ_H (200 MHz, DMSO) 1.34-1.85 (8H, m, CH_2 chain), 2.67 (6H, s, CH_3), 2.80-3.13 (4H, m, CH_2N), 3.24-3.32 (4H, m, $C\underline{H_2}CH_2OH$), 4.04 (4H, m, CH_2O), 6.72-6.90 (4H, m, ar-H) and 7.26-7.38 (2H, m, Ar-H); δ_C (50 MHz, DMSO) 27.7 (C-4),28.3 (C-3), 29.4

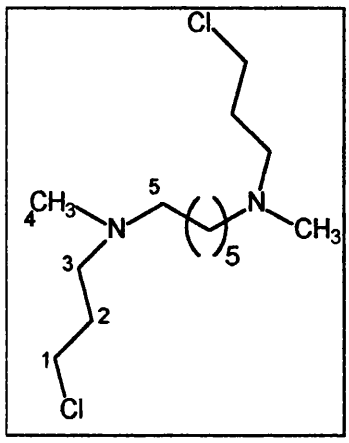
(C-2), 39.8 (C-1), 55.5 (C-5), 62.2 (C-6), 104.3, 104.5, 104.8, 111.4, 111.6, 122.9 (aromatic) and 154.2 (C-7).

_N,N'_-Dihydroxypropyl-_N,N'_-dimethylhexane-1,6-diamine 60



Diol **60** was obained as in general method (e) using _N,N'_-dimethyl -1,6-hexanediamine (2.0 g, 13.9 mmol) in acetonitrile (50 ml) and bromopropanol (3.89 g, 2.53 ml, 28.0 mmol); (1.10 g, 30 %); υ_{max} (thin film) 1096, 1162, 2826, 2884 and 3642 cm^{-1}; δ_H (200 MHz, D_2O) 0.91-1.28 (8H, m, CH_2 chain), 1.32-1.44 (4H, m, $C\underline{H}_2CHO$), 1.77 (6H, s, CH_3), 1.94-2.36 (8H, m, CH_2N) and 3.70-3.81 (4H, m, CH_2O); δ_C (50 MHz, D_2O) 28.5 (C-7), 29.3 (C-6), 35.2 (C-2), 41.9 (C-4), 54.5 (C-5), 57.6 (C-3) and 60 9 (C-1).

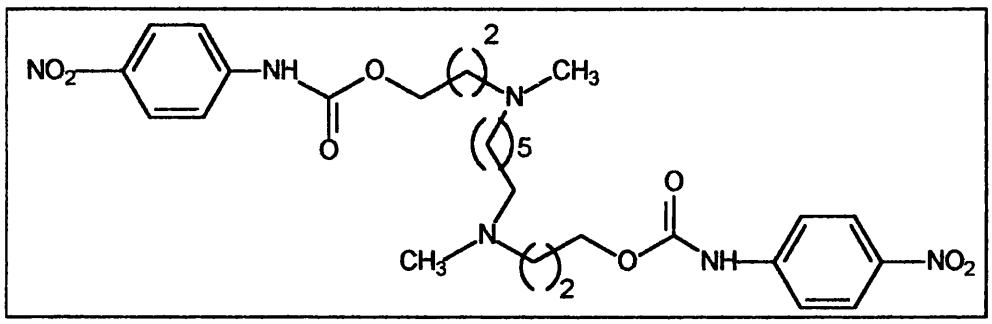
_N,N'_-Dichloropropyl-_N,N'_-dimethylhexane-1,6-diamine 61



Dichloride **61** was obained as the free base using general method (c) with **60** (0.5 g, 1.9 mmol) and thionyl chloride (15 ml) as a white/ yellow solid which was

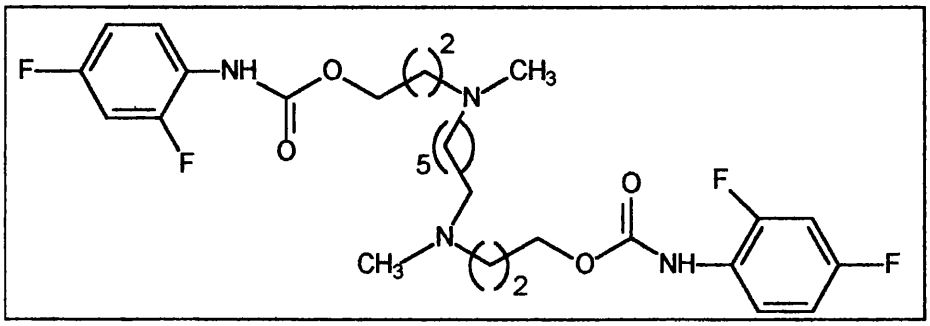
recrystallised from EtOH and diethyl ether (0.35 g, 60 %); Rf=0.3 (CH_3OH:NH_3); υ_{max} (KBr disc) 750, 2825 and 2950 cm^{-1}; δ_H (200 MHz, D_2O) 1.21-1.58 (8H, m, CH_2 chain), 1.61-1.85 (4H, m, $NCH_2C\underline{H}_2CH_2Cl$), 2.04 (6H, s, CH_3), 2.16-2.70 (8H, m, CH_2N) and 3.25-3.52 (4H, m, CH_2Cl); δ_C (50 MHz, D_2O) 25.9 (C-7), 26.4 (C-6), 28.2 (C-2), 38.9 (C-4), 53.9 (C-5), 55.2 (C-3) and 56.7 (C-1); *m/z* (CI/NH_3) 120 (*M*$^+$ C_5H_9NCl, 97 %), 152 (*M*$^+$ $C_7H_{15}NCl$, 6 %), 112 (*M*$^+C_5H_9N$, 31 %) (found: *MH*$^+$ 297.1864. $C_{14}H_{31}N_2{}^{35}Cl_2$ requires 297.1864).

N,N'-(*p*-Nitrophenylcarbonyloxypropyl)-N,N'-dimethylhexane-1,6-diamine 62



Biscarbamate **62** was obained using general method (d) with **60** (2.0 g, 0.77 mmol), DCM (20 ml), *p*-nitrophenyl isocyanate (0.28 g, 1.7 mmol) and dibutyltindiacetate (3 drops) (2.26 g, 58 %), Rf= 0.87 (EtOAc); υ_{max} (KBr disc) 1301, 1503, 1508, 1591, 1628, 1736, 2924, 3371 and 3483 cm^{-1}; δ_H (200 MHz, DMSO) 0.90-1.00 and 1.20-1.25 (12H, m, $CH_2CH_2CH_2$), 1.65 (8H, m, CH_2N), 2.25-2.30 (4H, m, CH_2O), 3.05 (6H, s, CH_3), 4.37 (2H, bs, NH), 6.60-6.65 and 8.10-8.18 (8H, AA'BB' system, Ar-H); *m/z* (EI$^+$) 295 (1.7 %) and 138 (80 %).

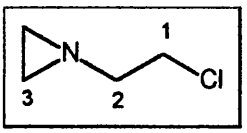
N,N'-(2,4-Difluorophenylcarbonyloxypropyl)-N,N'-dimethylhexane-1,6-diamine 63



Bisdifluorophenyl carbamate **63** was obained as in general method (d) using **60** (1.0 g, 3.84 mmol), DCM (20 ml), 2,4-difluorophenylisocyanate (1.86 g, 12.0 mmol) and dibutyltindiacetate (3 drops) (0.22 g, 6 %); υ_{max} (KBr disc) 750, 1205, 1512, 1612, 1728, 2934 and 3083 cm^{-1}.

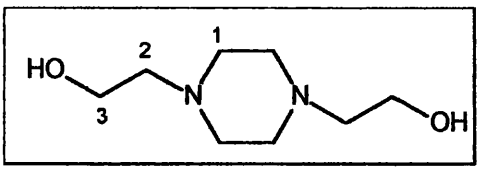
8.4.1 Experimental to Chapter 4

N'-Chloroethylaziridine 64



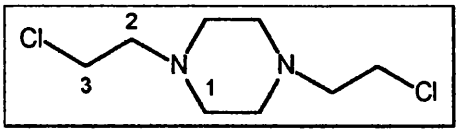
Chloride **64** was obtained from *N*-hydroxyethylaziridine **63** (2.0 g, 23 mmol) and thionyl chloride (10 ml) using general method (c) and recrystallised from EtOH and diethyl ether (0.87 g, 36 %); υ_{max} (KBr disc) 779, 2981 and 3466 cm^{-1}; δ_H (200 MHz, D_2O) 2.90-3.43 and 3.66-4.09 (8H, m, NCH_2, $ClCH_2$); δ_C (50 MHz, D_2O) 28.2 (C-1), 48.8 (C-2) and 55.07 (C-3).

N,N'-Di(hydroxyethyl)piperazine 66



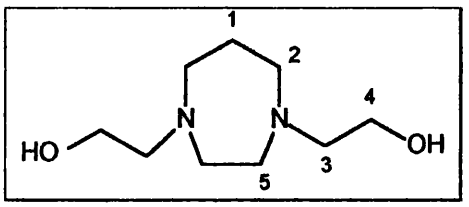
N,N'-Di(hydroxyethyl)piperazine **56** was obtained as in general method (e) using piperazine **65** (4.0 g, 46 mmol) in dry EtOH (10 ml) and ethylene oxide (4.05 g, 4.5 ml, 92 mmol) as a white solid. This was recrystallised using EtOH and diethyl ether (3.16 g, 47 %); m.p. 128-128.5 °C; δ_H (200 MHz, $CDCl_3$) 2.26-2.32 (12H, t, CH_2N), 3.39-3.49 (4H, t, CH_2OH); δ_C (50 MHz, $CDCl_3$) 53.3 (C-1), 57.9 (C-2), 59.5 (C-3); lit[69] 53.13, 58.4 and 60.2; *m/z* (EI^+) 175.14 (*MH*$^+$, 100 %) (found: *MH*$^+$ 175.1446. $C_8H_{19}N_2O_2$ requires 175.1447).

N,N'-Di(chloroethyl)piperazine 67



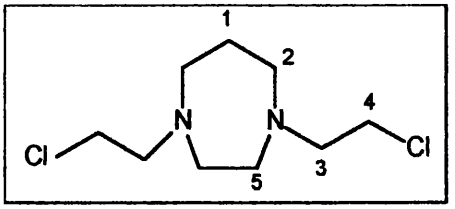
Dichloride **67** was obtained as a white solid from **66** (1.50 g, 8.5 mmol) and thionyl chloride (15 ml) using general method (c). The resulting white precipitate was filtered and washed with diethyl ether and recrystallised from hot EtOH (1.47 g, 81 %); 197-198.5 °C; υ_{max} (KBr disc) 625, 2850, 2927 and 2987 cm^{-1}; δ_H (200 MHz, D_2O) 3.23-3.28 (4H, t, NCH_2CH_2N), 3.57 (4H, s, CH_2Cl) and 3.71-3.76 (8H, t, NCH_2CH_2Cl); δ_C (50 MHz, D_2O) 49.3 (C-2), 55.6 (C-1) and 58.9 (C-3); *m/z* (Cl^+) 213 (*MH*$^+$, 8 %).

N,N'-Di(hydroxyethyl)-1,4-diazacycloheptane 69



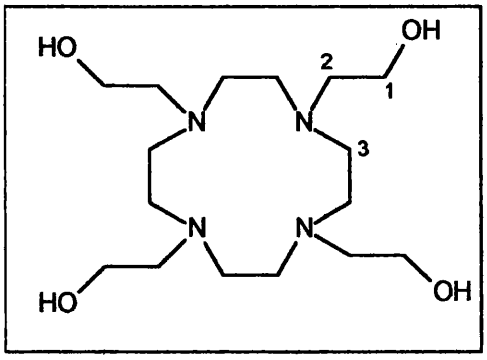
Diol **69** was synthesised with 1,4-diazacycloheptane **68** (2.0 g, 20 mmol) and ethylene oxide (2.65 g, 60 mmol) in EtOH (10 ml) using general method (e) as an orange oil (1.14 g, 30 %); υ_{max} (Nujol mull) 1052, 1252 and 2828 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.66-1.79 (2H, m, $CH_2C\underline{H_2}CH_2$), 2.56-2.87 (12H, dt, NCH_2), 3.49-3.61 (4H, m, OCH_2) and 4.05 (2H, bs, OH); δ_C (50 MHz, $CDCl_3$) 27.5 (C-1), 54.12 (C-2), 54.6 (C-3), 58.6 (C-5) and 59.5 (C-4); *m/z* (Cl^+) 189.19 (*MH*$^+$, 100 %), 245.26 (*MH*$^+$ $C_4H_9^+$, 20 %) (found: *MH*$^+$ 189.1603. $C_9H_{20}N_2O_2$ requires 189.1603).

N,N'-Di(chloroethyl)-1,4-diazacycloheptane 70



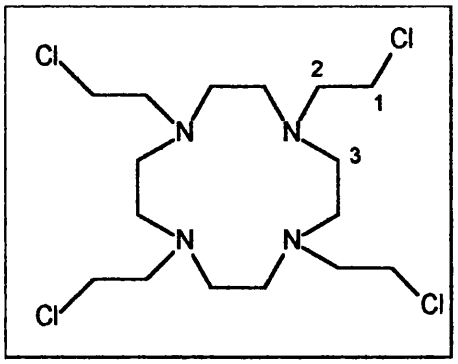
N,N'-Di(chloroethyl)-1,4-diazacycloheptane **70** was prepared from **69** (0.80 g, 4.2 mmol) and thionyl chloride (20 ml) in the manner of general method (c). A beige solid formed after 48 h stirring at room temperature. This was filtered and washed with diethyl ether (3 x 20 ml) (0.90 g, 96 %); m.p. 205-207 °C; υ_{max} (KBr disc) 750 and 2852 cm^{-1}; δ_H (200 MHz, D_2O) 2.16-2.21 (2H, m, CH_2CH_2), 3.23-3.58 (8H, m, CH_2N,) and 3.73-3.79 (8H, m, $ClCH_2$, $NC\underline{H_2}CH_2Cl$); δ_C (50 MHz, D_2O) 20.8 (C-1), 38.0 (C-4), 49.2 (C-3), 54.2 (C-2) and 58.8 (C-5); *m/z* (Cl^+) 225.07 (*MH*$^+$, 100 %) (found: *MH*$^+$ 225.0926. $C_9H_{18}N_2{}^{35}Cl_2$ requires 225.0928).

N,N',N'',N'''-Tetra(hydroxyethyl)-1,4,7,10-tetraazacyclododecane 72



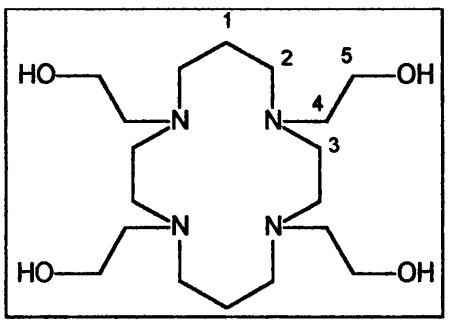
Tetraol **72** was synthesised from 1,4,7,10-tetraazacyclododecane **71** (1.94 g, 88 mmol) and an excess of ethylene oxide (4.70 g, 360 mmol) in water (20 ml) using general method (e) as white crystals (2.23 g, 57 %); δ_H (200 MHz, $CDCl_3$) 2.16-2.23 (24H, m, NCH_2), 3.27-3.31 (8H, m, OCH_2) 4.87 (4H, bs, OH) ; δ_C (50 MHz, $CDCl_3$) 48.8 (C-2), 54.1 (C-3) and 60.1 (C-1).

N,N',N",N"'-Tetra(chloroethyl)-1,4,7,10-tetraazacyclododecane 73



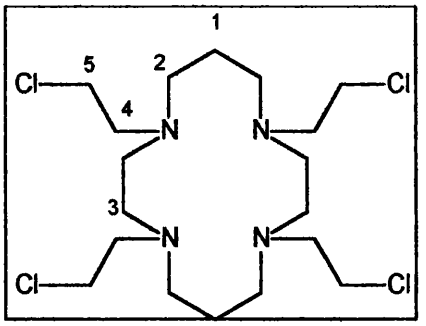
Tetrachloride **73** was prepared using **72** (2.41 g, 6.9 mmol) and thionyl chloride (80 ml) in the manner of general method (c). A light beige solid formed after 48 h stirring at room temperature which was filtered off and washed with diethyl ether (2.28 g, 67 %); υ_{max} (KBr disc) 727, 2852 and 2965 cm^{-1}; δ_H (200 MHz, D_2O) 3.17-3.25 (24H, m, NCH_2, CH_2Cl), 3.65-3.76 (8H, OCH_2); δ_C (50 MHz, D_2O) 39.3 (C-1), 49.1 (C-2), 54.9 (C-3); (found: C, 38.65; H, 7.09; N, 11.10 %. $C_{16}H_{32}N_4Cl_4$ requires C, 38.81;H, 6.92; N, 11.31 %).

N,N',N",N"'-Tetra(hydroxyethyl)-1,4,8,11-tetraazacyclotetradecane 75



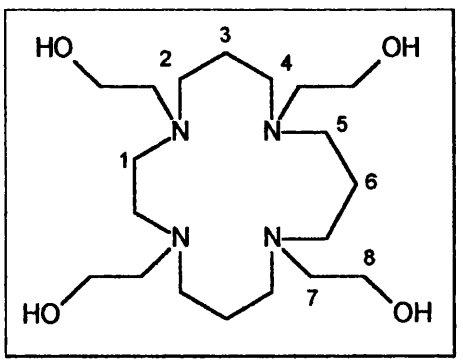
Tetraol **75** was synthesised from 1,4,8,11-tetraazacyclododecane **74** (1.0 g, 5.0 mmol) and excess ethylene oxide (1.5 ml) in EtOH (10 ml) using general method (e) as colourless crystals (1.84 g, 98 %); δ_H (200 MHz, $CDCl_3$) 1.48-1.61 (4H, m, $CH_2C\underline{H}_2CH_2$), 2.48-2.59 (24H, m, NCH_2), 3.52-3.58 (8H, m, CH_2OH); δ_C (50 MHz, $CDCl_3$) 25.1 (C-1), 49.1 (C-4), 51.9, 55.6 (C-2)(C-3) and 59.3 (C-5).

***N,N',N'',N'''*-Tetra(chloroethyl)-1,4,8,11-tetraazacyclotetradecane 76**



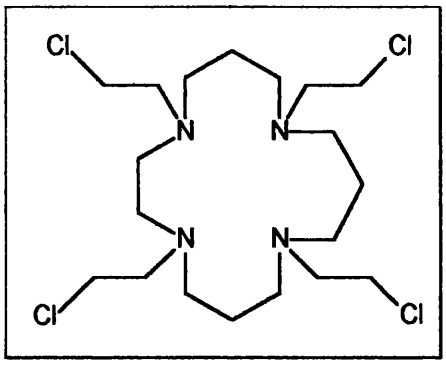
Tetrachlorinated cyclam derivative, **76** was prepared from **75** (0.4 g, 1.0 mmol), chloroform (10 ml) and thionyl chloride (10 ml) in the manner of general method (c). A beige solid formed after 36 h stirring at room temperature and the tetrahydrochloride salt was afforded on treatment with ethereal hydrochloric acid (0.14 g, 30 %); υ_{max} (KBr disc) 766, 2893 and 2925 cm^{-1}; δ_H (200 MHz, D_2O) 1.92-2.18 (4H, m, CH_2), 3.13-3.37 (16H, m, NCH_2), 3.72-3.79 (16H, m, NCH_2, $ClCH_2$);δ_C (50 MHz, D_2O) 17.5 (C-1), 43.5 (C-5), 47.9 (C-3), 56.1 (C-7) and 57.9 (C-3); (found: C, 36.42; N, 9.45 %. $C_{18}H_{40}N_4Cl_8$ requires C, 36.26; N, 9.40 %).

***N,N',N'',N'''*-Tetra(hydroxyethyl)-1,4,8,12-tetraazacyclopentadecane 78**



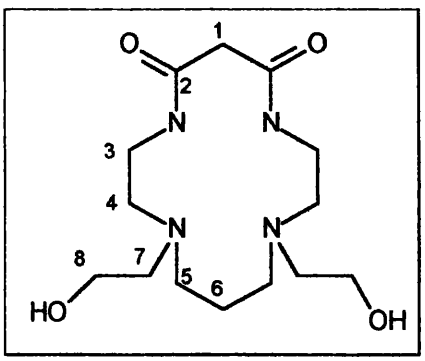
N,N',N'',N'''-Tetra(hydroxyethyl)-1,4,8,12-tetraazacyclopentadecane **78** was formed using 1,4,8,12-tetraazacyclopentadecane **77** (0.2 g, 0.9 mmol), EtOH (15 ml) and excess ethylene oxide (0.2 g, 5.0 mmol) as in general method (e) (0.30 g, 86 %), δ_H (200 MHz, $CDCl_3$) 1.02-1.33 (6H, m, $NCH_2C\underline{H}_2$), 1.98-2.70 (24H, m, NCH_2), 3.00-3.20 (8H, m, OCH_2); δ_C (50 MHz, $CDCl_3$) 25.0 (C-6), 26.6 (C-3), 53.4, 54.4, 54.7 (C-2)(C-4)(C-5), 58.9, 59.6 (C-1)(C-4) and 63.8 (C-8); *m/z* (Cl^+) 391.3 (*MH*$^+$, 100 %) (found: *MH*$^+$ 391.3284 $C_{19}H_{27}N_2O_4$ requires 391.3284).

N,N',N",N"'-Tetra(chloroethyl)-1,4,8,12-tetraazacyclopentadecane 79



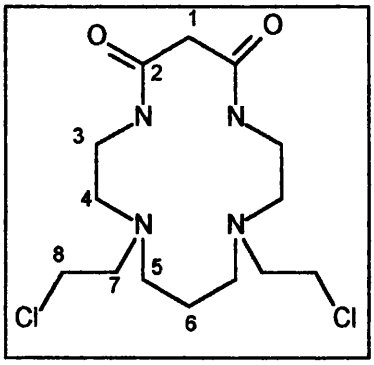
N,N',N",N"'-Tetra(chloroethyl)-1,4,8,12-tetraazacyclopentadecane **79** was formed on stirring **78** (0.30 g, 0.8 mmol) in thionyl chloride (20 ml) as in general method (c) (0.26 g, 74 %); υ_{max} (KBr disc) 730, 2840 and 2975 cm^{-1}; δ_H (200 MHz, D_2O) 1.81-2.10 (6H, m, NCH_2CH_2), 2.85-3.55 (24H, m, NCH_2), 3.62-3.81 (8H, m, CH_2Cl); δ_C (50 MHz, D_2O) 25.5 (C-6), 26.9 (C-3), 41.2 (C-8), 53.7, 54.8, 54.9 (C-2)(C-4)(C-5), 59.3 and 59.9 (C-1)(C-4); *m/z* 463.2 (*MH*$^+$, 100 %) (found: *MH*$^+$ 463.1927. $C_{19}H_{39}N_4{}^{35}Cl_4$ requires 463.1929).

1,4-Di(hydroxyethyl)-1,4,8,11-tetraazacyclododecane-5,7-dione 81



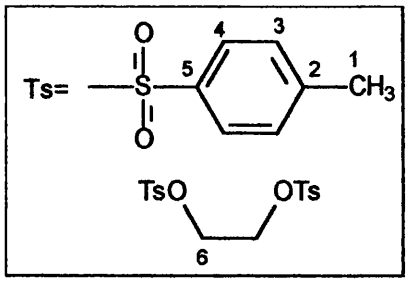
1,4,8,11-Tetraazacyclododecane-5,7-dione **80** (0.36g, 1.6 mmol) was used with ethylene oxide (0.5 ml) and EtOH (8 ml) to form 1,4-di(hydroxyethyl)-1,4,8,11-tetraazacyclododecane-5,7-dione **81** according to general method (e) (0.3 g, 60 %); υ_{max} (KBr disc) 1370, 1556, 1685 and 3224 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.90-097 (2H, m, NCH_2CH_2), 2.32 (2H, s, CH_2CO), 3.03-3.44 (16H, m, NCH_2), 3.71-3.92 (4H, m, OCH_2) and 5.46 (2H, bs, OH);δ_c (50 MHz, $CDCl_3$) 25.0 (C-6), 38.7 (C-1), 52.8, 54.1, 57.4, 59.2 (C-7)(C-3)(C-4)(C-5), 63.5 (C-8), 167.7 (C-2); *m/z* (EI$^+$) 317 (MH^+, 10 %) (found: MH^+ 315.1558. $C_{14}H_{28}N_4O_4$ requires 315.1558).

1,4-Di(chloroethyl)-1,4,8,11-Tetraazacyclododecane-5,7-dione 82



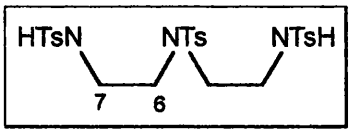
1,4-Di(chloroethyl)-1,4,8,11-tetraazacyclododecane-5,7-dione **82** was prepared as in general method (c) by stirring **81** (0.7 g, 2.2 mmol) in thionyl chloride (30 ml) and chloroform (10 ml). The off white solid obtained was recrystallised from EtOH and diethyl ether (0.75 g, 54 %); υ_{max} (KBr disc) 745, 1686, 2960 and 2980 cm^{-1}; δ_c (50 MHz, D_2O) 27.5 (C-6), 38.9 (C-1), 42.9 (C-8), 52.9, 54.3, 57.4, 59.3 (C-7) (C-3)(C-4)(C-5) and 167.9 (C-2); *m/z* (EI^+) 353 (MH^+, 40 %).

***O,O'*-Di(*p*-toluenesulfonyl)-1,2-ethanediol 84**



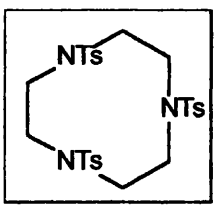
Tosylated compound *O,O'*-di(*p*-toluenesulfonyl)-1,2-ethanediol **84** was formed using general method (h) with *p*-toluenesulfonyl chloride (190 g, 1.0 mol), 1,2-ethanediol **83** (6.2 g, 0.1 mol), 30% sodium hydroxide, TEBA (0.50 g, 0.002 mmol) and DCM (200 ml) as white crystals (27.2 g, 73 %); υ_{max} (KBr disc) 1037, 1373, 1452, 1495 and 1597 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.45 (6H, s, CH_3), 4.18 (4H, s, OCH_2), 7.69-7.74, 7.31-7.36 (8H, dd, AA'BB' system Ar-H); δ_C (50 MHz, D_2O) 21.6 (C-1), 66.7 (C-6), 127.5, 129.6 (C-3)(C-5), 132.2 (C-4) and 145.3 (C-2); lit[152] 21.6, 66.9, 128.1, 130.2, 132.7, 145.5.

N,N',N''- Tri(*p*-toluenesulfonyl)diethylene triamine 86



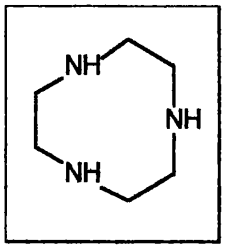
N,N',N''- Tri(*p*-toluenesulfonyl)diethylene triamine **86** was obtained using general method (f) using diethylenetriamine **85** (15.0 g, 0.15 mol), *p*-toluenesulfonyl chloride (83.6 g, 0.44 mol), sodium hydroxide solution and diethyl ether (250 ml). The white solid was obtained in quantitative yield (75.0 g, 93 %); m.p. 180-183 °C; υ_{max} (KBr) 1036, 1445, 1494 and 1598 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.58 (9H, s, Ar-CH_3), 2.97 (4H, t, tsNCH_2), 3.10 (4H, t, NCH_2), 7.45 (12H, dd, AA'BB system Ar-H), δ_C (50 MHz, $CDCl_3$) 21.5 (C-1), 42.5 (C-8), 50.3 (C-9), 127.3 (C-4), 129.9 (C-2), 136.5 (C-3), 143.5 (C-5); lit[153] 21.8, 42.7, 50.8, 127.6, 130.3, 130.5, 135.2, 137.1, 144.1, 144.6.

N,N',N''-Tri(*p*-toluenesulfonyl)-1,4,7-triazacyclononane 88



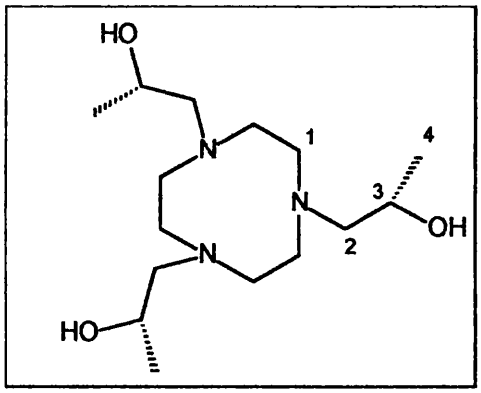
N,N',N''-Tri(*p*-toluenesulfonyl)-1,4,7-triazacyclononane **88** was obtained using general method (j) as a white solid from the disodium salt of **87** (230 g, 0.38 mol), dry DMF (1.4 l) and *O,O'*-di(*p*-toluenesulfonyl)-1,2-ethanediol **84** (140 g, 0.38 mol) (195 g, 87%); υ_{max} (KBr) 1376, 1451, 1494 and 1597 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.42 (9H, s, Ar-CH_3), 3.50 (12H, s, NCH_2), 7.70, 7.33 (12H, dd, AA'BB' system Ar-H); δ_C (50 MHz, $CDCl_3$) 21.5 (C-1), 51.8 (C-8), 127.5 (C-4), 129.8 (C-3), 135.2 (C-2), 143.2 (C-5); lit[114] 21.5, 51.8, 127.4, 129.7, 134.5, 143.9 ; *m/z* (FAB^+) 614 (*MNa*$^+$, 60 %) (found: C, 54.19; H, 5.63; N, 6.96 %. $C_{27}H_{33}N_3S_3O_6$ requires C, 54.87 ;H, 5.63; N, 7.11%).

1,4,7-Triazacyclononane 89



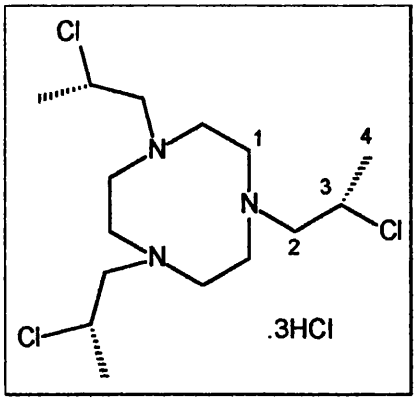
1,4,7-Triazacyclononane **89** was prepared using a literature method[113] with **88** (10.0 g, 17 mmol) and concentrated sulfuric acid (75 ml). The white solid was recrystallised from chloroform (1.2 g, 20 %); δ_H (200 MHz, $CDCl_3$) 1.75 (3H, bs, NH), 2.47 (12H, s, CH_2); δ_C (50 MHz, $CDCl_3$) 47.0; lit[114] δ_C (50 MHz, $CDCl_3$) 45.9.

***N,N',N''*-Tri[(S)-2-hydroxypropyl]-1,4,7-triazacyclononane 90**



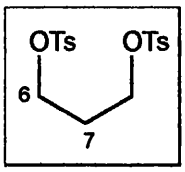
N,N',N''-Tri[(S)-2-hydroxypropy]-1,4,7-triazacyclononane **90** was formed from **89** (0.19 g, 1.5 mmol) and (S)-propylene oxide (0.38 g, 6.6 mmol) using literature method.[154] The product **90** was obtained as a yellow oil which crystallised on cooling (0.43 g, 96 %); δ_H (200 MHz, $CDCl_3$) 1.14-1.16 (9H, s, CH_3), 2.41-2.55 (18H, m, NCH_2), 3.71-3.90 (3H, m, CH); δ_C (50 MHz, $CDCl_3$) 50.0, 50.8, 54.1 (C-1)(C-2)(C-4) and 64.3 (C-3); *m/z* (EI^+) 303 (MH^+, 0.5 %).

N,N',N"-Tri[(S)-2-chloropropyl]-1,4,7-triazacyclononane 91



N,N',N"-Tri[(S)-2-chloropropyl]-1,4,7-triazacyclononane **91** was formed using **90** in chloroform (40 ml) and cooled in an ice bath. Dry HCl was bubbled through for 30 min to afford a white precipitate. Chloroform (40 ml) was added to the slurry, followed by thionyl chloride (80 ml) slowly in portions. The ice bath was removed and the mixture stirred at room temperature for 10 d. The solution was concentrated *in vacuo* and the residue was taken up in water (30 ml) and washed with ethyl acetate (3 x 80 ml). The aqueous layer was evaporated to dryness to yield a yellow/ brown solid (3.15 g, 85 %); υ_{max} (KBr disc) 780 and 2969 cm^{-1}; δ_H (200 MHz, D_2O) 1.26-1.30 (9H, d, CH_3), 3.02-3.38 (18H, m, NCH_2), 4.26-4.33 (3H, m, CH); δ_C (200 MHz, D_2O) 23.0 (C-4), 50.9 (C-1), 53.7 (C-3) and 64.3 (C-2); (found: C, 38.48; H, 7.38; N, 8.80 % requires C, 38.48 ;H, 7.20; N, 8.98 %).

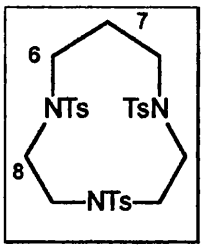
O,O'-Di(*p*-toluenesulfonyl)-1,3-propanediol 93



O,O'-Di(*p*-toluenesulfonyl)-1,3-propanediol **93** was formed using general method (h) with 1,3-propane diol **92** (11.41 g, 0.15 mol), *p*-toluenesulfonyl chloride (69.23 g, 0.30 mol) sodium hydroxide solution, TEBA (0.92 g, 4 mmol) and DCM (100 ml). The product was afforded as a white solid (52 g, 91 %); δ_H (200 MHz, $CDCl_3$) 1.58-1.71 (2H, m, CH_2CH_2), 2.45 (6H, s, CH_3), 3.97 (4H, m, OCH_2) and 7.30-7.34, 7.71-7.75 (8H, dd, AA'BB' system); δ_C (50 MHz, $CDCl_3$) 22.0 (C-1), 25.6 (C-7), 66.3 (C-6), 127.4, 130.7 (C-3)(C-4), 138.5 (C-2), 147.3 (C-5); lit[80] 21.6, 28.7, 66.0, 128.1,

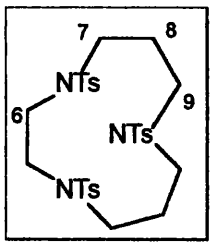
130.1, 132.9 and 145.3; *m/z* (Cl^+) 385.1 (MH^+, 54 %) (found: MH^+ 385.0780 $C_{17}H_{21}O_6S_2$ requires 385.0780).

N,N',N"-Tri(*p*-toluenesulfonyl)-1,4,7-triazacyclodecane 94



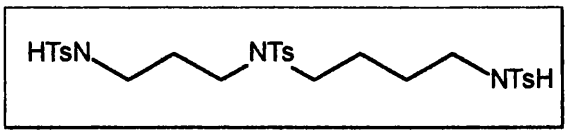
Compound **94** was formed using general method (e) with the disodium salt of **87** (2.5 g, 4.05 mmol) and **93** (1.55 g, 4.05 mmol). The product was obtained as a white solid (0.89 g, 36 %); δ_H (200 MHz, $CDCl_3$) 1.62-1.74 (2H, m, CH_2CH_2), 2.45 (9H, s, CH_3), 3.45-3.70 (12H, m, NCH_2), 7.36-7.73 (12H, dd, AA'BB' aromatic system); δ_C (50 MHz, $CDCl_3$) 21.5 (C-1), 25.4 (C-7), 51.3, 51.8 (C-6)(C-8), 127.6, 129.7, 135.4 (C-2)(C-3)(C-4) and 144.1 (C-5).

N,N',N"-Tri(*p*-toluenesulfonyl)-1,4,7-triazacyclounadecane 95



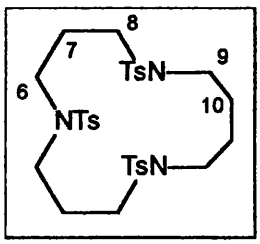
Compound **95** was formed using general method (e) with the disodium salt of *N,N',N"*-tri(*p*-toluenesulfonyl)-dipropyl-1,5,8-triamine (2.5 g, 4.05 mmol) and **84** (1.40 g, 4.05 mmol). The product was obtained as a white solid (0.67 g, 28 %); δ_H (200 MHz, $CDCl_3$) 1.52-1.69 (4H, m, CH_2CH_2), 2.48 (9H, s, CH_3), 3.35-3.67 (12H, m, NCH_2), 7.40-7.82 (12H, dd, AA'BB' aromatic system); lit[155] 6.56 (t, 4H), 6.71 (s, 4H), 6.98 (t, 4H), 8.10 (m 4H); δ_C (50 MHz, $CDCl_3$) 21.8 (C-1), 24.9 (C-7), 51.8, 52.4, 52.6 (C-6)(C-7)(C-9), 27.4, 129.3, 135.4 (C-2)(C-3)(C-4) and 145.3 (C-5).

N,N',N''-Tri(*p*-toluenesulfonyl)spermidine 97



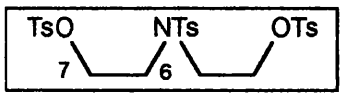
N,N',N''-Tri(*p*-toluenesulfonyl)spermidine **97** was formed by general method (f) using spermidine **96** (1.0 g, 7.0 mmol), sodium hydroxide solution, diethyl ether (15 ml), and *p*-toluenesulfonyl chloride (3.81 g, 21 mmol) to afford the product as a white solid (2.52 g, 60 %); δ_H (200 MHz, $CDCl_3$) 1.32 (4H, m, $CH_2C\underline{H_2}CH_2$), 1.54-1.60 (2H, t, $NCH_2C\underline{H_2}$), 2.24 (9H, s, CH_3), 2.68-2.97 (8H, m, NCH_2), 7.09-7.14, 7.54-7.59 (12H, dd, AA'BB' aromatic system); *m/z* (FAB$^+$) 608.1 (*MH*$^+$, 100 %) (found: *MH*$^+$ 608.1926. $C_{28}H_{39}N_3O_6S_3$ requires 608.1923).

N,N',N''-Tri(*p*-toluenesulfonyl)-1,5,9-triazacyclotridecane 99



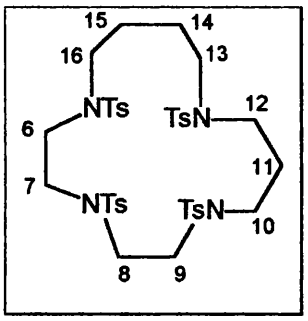
N,N',N''-Tri(*p*-toluenesulfonyl)-1,5,9-triazacyclotridecane **99** was formed by general method (j) using **93** (0.29 g, 0.90 mmol) and the disodium salt of **98** (0.50 g, 0.75 mmol) in dry DMF (30 ml) (0.2, 41 %); υ_{max} (KBr disc) 1168, 1380, 1501, 1604 and 3300 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.32-1.76 (8H, m, CH_2CH_2), 2.29 (9H, s, CH_3), 3.34-3.87(12H, m, NCH_2) and 7.28-7.67 (12H, m, Ar-H) ; δ_C (50 MHz, $CDCl_3$) 21.6 (C-1), 23.3 (C-10), 26.5 (C-7), 51.0, 53.2, 53.5 (C-6)(C-8)(C-9), 126.5, 128.6 (C-3) (C-4), 133.5 (C-2) and 145.0 (C-5).

N,O,O'-Tri(*p*-toluenesulfonyl)diethanolamine 101



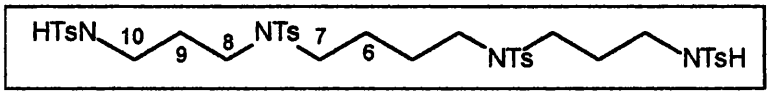
N,O,O'-Tri(*p*-toluenesulfonyl)diethanolamine **101** was obtained as white crystals by general method (f) from diethanolamine (5.26 g, 50 mmol), 30 % sodium hydroxide solution (35 ml), *p*-toluenesulfonyl chloride (31.5 g, 150 mmol), TEBA (0.46 g, 20 mmol) and DCM (50 ml) (26 g, 93%); υ_{max}(KBr) 1164, 1350, 1500 and 1600 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.45 (9H, s, Ar-CH_3), 3.34 (4H, t, NCH_2), 4.06-4.17 (4H, t, OCH_2), 7.65-7.91, 7.91-7.56 (12H, dd, AA'BB' system Ar-H); δ_C(50 MHz, $CDCl_3$) 21.7 (C-1), 48.4 (C-6), 66.7 (C-7), 126.9, 129.97 (C-3)(C-4), 132.4 (C-2), 143.4 (C-5); lit[152] δ 21.6, 21.7, 48.5, 68.4, 127.5, 128.2, 130.3, 132.8, 135.7 and 144.4; *m/z* (Cl^+) 568.2 (*MH*$^+$, 90 %) (found: *MH*$^+$ 568.1130. $C_{25}H_{30}NO_9S_3$ requires 568.1134).

N,N',N'',N'''-Tetra(*p*-toluenesulfonyl)-1,4,7,11-tetraazacyclopentadecane 102



N,N',N'',N'''-Tetra(*p*-toluenesulfonyl)-1,4,7,11-tetraazacyclopentadecane **102** was formed by general method (e) using **101** (0.25 g, 0.44 mmol) and the disodium salt of **98** (0.28 g, 0.44 mmol) in dry DMF (20 ml) (0.18, 50 %); δ_H (200 MHz, $CDCl_3$) 2.32-2.40 (6H, m, $C\underline{H}_2CH_2N$), 2.75 (6H, s, Ar-CH_3), 2.84 (6H, s, Ar-H), 3.18-3.31, 3.55-3.62 (16H, dt, NCH_2), 7.16-7.26 (8H, m, AA'BB' system Ar-H), 7.50-7.69 (8H, m, Ar-H); δ_C (50 MHz, $CDCl_3$) 21.3 (C-1), 31.1 (C-14)(C-15), 36.3 (C-11), 41.6, 42.0, 45.8, 49.3, 49.4 (C-10)(C-12)(C-13 (C-16)(C-6), 51.4 , 65.7, 69.6 (C-7)(C-8)(C-9), 127.7, 129.8 (C-3)(C-4), 135.3 (C-2), and 143.8 (C-5).

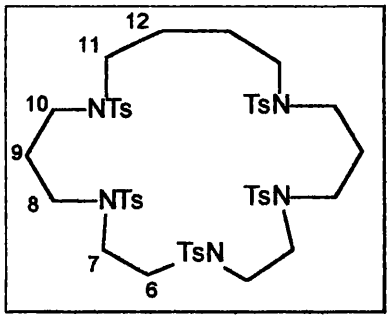
N,N',N",N"'-Tetra(*p*-toluenesulfonyl)spermine 104



N,N',N",N"'-Tetra(*p*-toluenesulfonyl)spermine **104** was formed using general amine tosylation method (d) to afford the product as a white solid using spemine **103** (1.0 g, 5.0 mmol), 30 % sodium hydroxide solution, diethyl ether (15 ml) and *p*-toluenesulfonyl chloride (3.81 g, 20 mmol) (1.84 g, 46 %); δ_H (200 MHz, $CDCl_3$) 1.47-1.60 (4H, m, $NCH_2CH_2C\underline{H}_2$), 1.68-1.79 (4H, m, $NCH_2C\underline{H}_2$), 2.42 (12H, s, Ar-CH_3), 2.95 (12H, m, NCH_2), 7.25-7.30, 7.62-7.74 (16H, m, Ar-H); δ_C (50 MHz, $CDCl_3$) 21.4 (C-1), 26.3 (C-6), 28.9 (C-9), 42.4 (C-7), 45.9 (C-10), 48.6 (C-8), 128.7, 129.6 (C-3)(C-4), 135.9 (C-2), 136.8 (C-5).

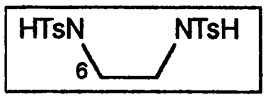
N,N',N",N"',N""

-Penta(*p*-toluenesulfonyl)-1,4,7,11,16-pentaazacyclononadecane 105



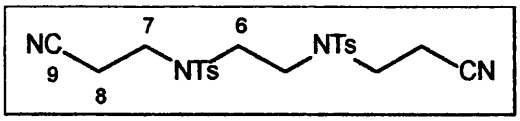
N,N',N",N"',N""-Penta(*p*-toluenesulfonyl)-14,7,11,16-pentaazacyclononadecane **105** was formed using general method (g) with **104** (1.0 g, 1.2 mmol), caesium carbonate (0.20 g, 0.6 mmol), dry DMF (30 ml) and **86** (0.70 g, 1.2 mmol) (0.30 g, 23 %); δ_H (200 MHz, $CDCl_3$) 2.31-2.50 (8H, m, $C\underline{H}_2CH_2N$), 2.74 (15H, s, Ar-CH_3), 2.94-3.19 (6H, m, NCH_2), 3.26-3.62 (10H, m, NCH_2), 3.99-4.12 (4H, m, NCH_2), 7.17-7.29, 7.48-7.67 (20H, m, Ar-H); δ_C (50 MHz, $CDCl_3$) 21.4 (C-1), 25.6 (C-15), 28.9 (C-18), 40.1, 41.6 (C-11), 45.9, 48.5 (C-8)(C-10), 51.4, 51.4 (C-6)(C-7), 126.9, 129.9 (C-3)(C-4), 135.9 (C-2) and 143.3 (C-5).

N,N'-Di(*p*-toluenesulfonyl)ethylenediamine 107



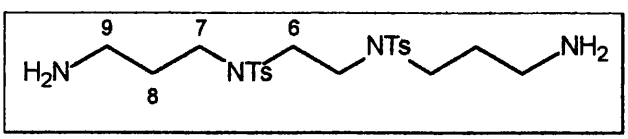
N,N'-Di(*p*-toluenesulfonyl)ethylenediamine **107** was prepared by general method (f) as white crystals using ethylendiamine (9.61 g, 0.16 mol), water (100 ml), sodium hydroxide (12.8 g, 0.32 mol), diethyl ether (100 ml) and *p*-toluenesulfonyl chloride (61.07 g, 0.32 mol) (58.46 g, 99%); mp 163-165 °C; lit[152] 162-164 °C; υ_{max}(KBr) 1165, 1340, 1460, 1510 and 1610 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.41 (6H, s, Ar-CH_3), 2.77 (4H, t, CH_2), 3.47 (2H, s, NH), 7.47 (8H, 2 x AA'BB', Ar-H); δ_C (50 MHz, $CDCl_3$) 21.0 (C-1), 40.7 (C-6), 126.6, 129.7 (C-3)(C-4), 137.4 (C-2), 142.8 (C-5); lit[152] 21.6, 43.2, 127.4, 130.1, 135.1, 144.2; *m/z* (CI^+) 369.1 (*MH*$^+$, 100 %) (found: *MH*$^+$ 369.0943, C, 52.10; H, 5.46; N, 7.33 % $C_{16}H_{20}N_2O_4S_2$ requires 369.0942, C, 51.72; H, 5.47; N, 7.60 %).

N', N''-Di(*p*-toluenesulfonyl)-4,7-diazadecanedinitrile 108



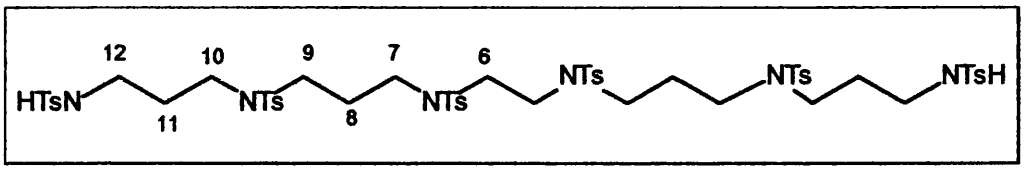
N',N''-Di(*p*-toluenesulfonyl)-4,7-diazadecanedinitrile **108** was formed from **107** (19.50 g, 53 mmol), potassium carbonate (21.68 g, 0.21 mol) and acrylonitrile (13.8 ml, 0.21 mol) in dry DMF (160 ml) as in the literature[95] (19.7 g, 78 %); υ_{max} (KBr disc) 1140, 1339, 1496, 1599 and 2250 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.38 (6H, s Ar-CH_3), 2.65-2.68 (4H, t, CH_2), 3.31-3.38 (4H, t, CH_2N) 3.42 (4H, s, CH_2N), 7.29-7.31, 7.66-7.68 (8H, dd, AA'BB' system); δ_C (50 MHz, $CDCl_3$) 19.5 (C-8), 21.9 (C-1), 47.3 (C-7), 50.8 (C-6), 118.2 (C-9), 127.8, 130.6 (C-3)(C-4), 134.7 (C-2), 145.0 (C-5).

N', N''-Di(*p*-toluenesulfonyl)-4,7-diazadecanedinitrile-1,10-diamine 109



N',N''-Di(*p*-toluenesulfonyl)-4,7-diazadecanedinitrile-1,10-diamine **109** was formed by reducing **108** (19.0 g, 40 mmol) using 1 M borane/ THF solution (300 ml) according to the literature[95] (9.07 g, 47 %); υ_{max} (KBr disc) 2258, 1340, 1491, 1594, 2258 and 3376 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.55 (4H, bs, NH), 1.60-1.66 (4H, m, $C\underline{H_2}CH_2N$), 2.37 (6H, s, Ar-CH_3), 2.68-2.70 (4H, t, CH_2N), 3.10-3.14 (4H, t, CH_2N), 3.26 (4H, s, CH_2N), 7.24-7.26, 7.64-7.66 (8H, dd, AA'BB' system); δ_C (50 MHz, $CDCl_3$) 21.9 (C-1), 32.6 (C-8), 39.2 (C-9), 48.0 (C-7), 49.3 (C-6), 127.6, 130.2 (C-3)(C-4), 135.9 (C-2), 144.0 (C-5).

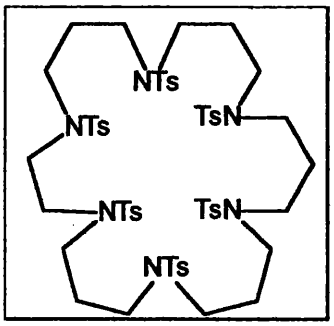
N,N',N'',N''',N'''',N'''''-Hexa(*p*-toluenesulfonyl)-1,5,9,12,16,20-hexaazaisocosane 110



N,N',N'',N''',N'''',N'''''-Hexa(*p*-toluenesulfonyl)-1,5,9,12,16,20-hexaazaisocosane **110** was formed by a linear extension using the last three steps repeated as a white solid by reported procedures[95] using the amine obtained on repeating the previous two steps (3.30 g, 3.65 mmol) in water (15 ml), sodium hydroxide (0.30 g, 7.3 mmol), diethyl ether (20 ml) and *p*-toluenesulfonyl chloride (1.40 g, 7.3 mmol). (4.40 g, 99 %); υ_{max} (KBr disc) 1158, 1333, 1493, 1597 and 3285 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.72-1.75, 1.82-1.86 (8H, t, $CH_2CH_2CH_2$), 2.30, 2.36 (18H, s, Ar-CH_3), 2.81-2.91 (4H, m, CH_2N), 3.01 (12H, m, CH_2N), 3.21 (4H, s, NCH_2CH_2N), 5.23 (2H, bs, NH), 7.14-7.26, 7.58-7.74 (24H, dd, AA'BB' system); δ_C (50 MHz, $CDCl_3$) 21.9, 22.0 (C-1), 29.0, 29.9 (C-8), (C-11), 40.6 (C-12), 47.1, 47.9, 48.7 (C-7)(C-9)(C-10), 49.9 (C-6), 127.4, 127.5, 127.6, 130.3, 130.4 (C-3)(C-4), 135.3, 135.8, 137.3, 138.5 (C-2), 143.5, 144.0 and 144.3 (C-5).

N,N',N",N"',N"",N""'

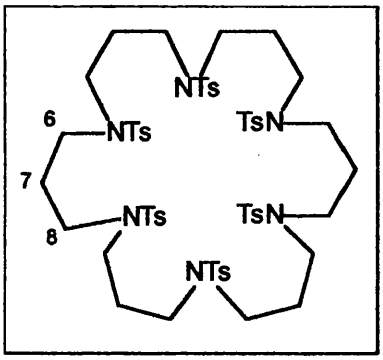
-Hexa(*p*-toluenesulfonyl)1,4,8,12,16,20-hexaazacyclotriisocosane 112



N,N',N",N"',N"",N""'-Hexa(*p*-toluenesulfonyl)-1,4,8,12,16,20-hexaazacyclotri isocosane **112** was formed using **110** (2.0 g, 1.6 mmol) in dry DMF (60 ml) and **93** (0.60 g, 1.6 mmol) in DMF (40 ml) by general method (e). The product was obtained as a light beige solid (1.45 g, 73 %); υ_{max} (KBr disc) 1159, 1339, 1494, 1597 and 3289 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.70-1.82 (10H, dt, CH_2CH_2N), 2.36 (18H, s, Ar-CH_3), 2.88-2.91 (4H, m, NCH_2), 3.06-3.21 (14H, m, NCH_2), 3.29 (4H, m, NCH_2CH_2N), 7.14-7.26, 7.56-7.67 (24H, dd, AA'BB' system).

N,N',N",N"',N"",N""'

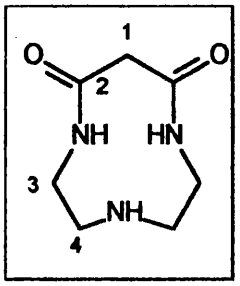
-Hexa(*p*-toluenesulfonyl)-1,5,9,13,17,21-hexaazacyclotetraisocosane 113



N,N',N",N"',N"",N""'-Hexa(*p*-toluenesulfonyl)-1,5,9,13,17,21-hexaazacyclotetra isocosane **113** was formed by general method (e) with *N',N",N"',N"",N""'*-hexa (*p*-toluenesulfonyl)-1,5,9,13,17,21-hexaazacyclounaisocosane (2.38 g, 2.0 mmol) in dry DMF and **93** (0.74 g, 2.0 mmol); δ_H (200 MHz, $CDCl_3$) 1.60-1.90 (12H, m, $CH_2CH_2CH_2$), 2.45 (18 H, s, CH_3), 3.05-3.30 (24H, m, NCH_2), 7.35-7.92 (24H, m,

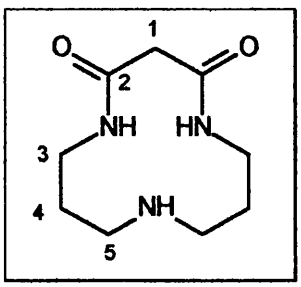
AA'BB' system Ar-H); δ_C (50 MHz, $CDCl_3$) 21.9 (C-1), 29.9, 30.4 (C-7), 47.6, 47.9 (C-6)(C-8), 125.5, 127.6 (C-3), 130.2, 130.4 (C-4), 136.2 (C-2) and 144.0 (C-5).

1,5,8-Triazacyclodecane-2,4-dione 115



1,5,8-Triazacyclododecane-2,4-dione **115** was prepared as in the literature[97] by heating diethyl malonate **114** (16.0 g, 0.1 mol), diethylenetriamine **85** (10.3 g, 0.1 mol) and EtOH (1000 ml) at reflux for 5 d under nitrogen with a drying tube attached. The solvent was removed under reduced pressure to leave a deep red viscous oil which solidified on standing at room temperature (10.2 g, 64 %); υ_{max} (KBr disc) 1550, 1690, 2830, 2950 and 3320 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.61 (2H, s, $COCH_2$), 2.59-2.65 (4H, t, NCH_2), 3.15-3.24 (4H, t, NCH_2); δ_C (50 MHz, $CDCl_3$) 39.1 (C-1), 43.4, 47.1 (C-3)(C-4), 170.0 (C-2).

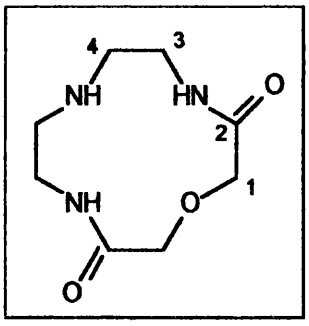
1,5,9-Triazacyclododecane-2,4-dione 117



1,5,9-Triazacyclododecane-2,4-dione **117** was prepared by heating *N*-(3-aminopropyl)-1,3-propanediamine **116** (5.0 g, 38 mmol), diethylmalonate **114** (7.30 g, 46 mmol) and MeOH (200 ml) at reflux for 4 d under nitrogen. The solvent was removed under reduced pressure to leave a light brown oil which solidified on standing at room temperature (7.2 g, 78 %); υ_{max} (KBr disc) 1449, 1684, 2850, 2900 and 3400 cm^{-1}; δ_H (200 MHz, D_2O) 1.58-1.79 (4H, m, NCH_2CH_2), 2.27 (2H, s,

$COCH_2$), 2.58-2.79 (4H, m, NCH_2), 3.16-3.24 (4H, m, NCH_2); lit[97] 1.66, 1.85, 2.76, 3.13, 3.40, 8.56; δ_C (50 MHz, D_2O) 28.4 (C-4), 36.9 (C-1), 45.7, 45.9 (C-3)(C-5), 170.0 (C-2).

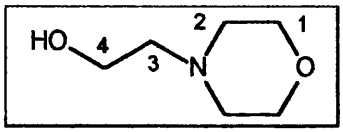
1-Oxa-4,7,10-triazacyclododecane-3,11-dione 119



1-Oxa-4,7,10-triazacyclododecane-3,11-dione **119** was prepared by heating diglycolic dimethylate **118** (4.0 g, 25 mmol), **85** (2.06 g, 20 mmol) and dry MeOH (150 ml) at reflux for 3 d under nitrogen. The solvent was removed *invacuo* to leave a light beige oil which solidified on standing at room temperature. The solid was taken up in DCM (50 ml) and washed with water (3x 50 ml). The DCM was dried over $MgSO_4$, filtered and concentrated to a yellow oil (0.34 g, 8 %); υ_{max} (KBr disc) 1125, 1330, 1552, 1680, 2800, 2925, and 3380 cm^{-1}; δ_H (200 MHz, D_2O) 2.74 (3H, bs, NH), 3.34 (8H, m, NCH_2), 4.07 (4H, m, OCH_2); δ_C (50 MHz, D_2O) 38.3 (C-3), 47.4 (C-3), 69.9 (C-1), 177.6 (C-2).

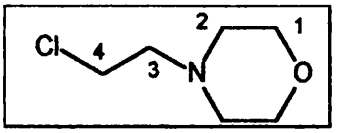
8.5.1 Experimental to Chapter 5

***N*-Hydroxyethylmorpholine 121**



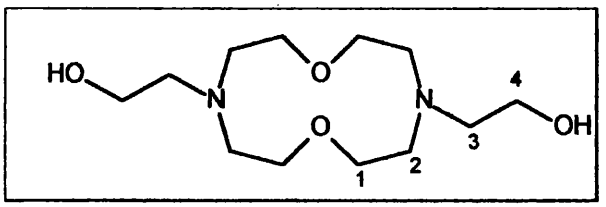
Alcohol **121** was obtained as a light yellow oil from morpholine **120** (2.0 g, 23 mmol) and ethylene oxide (1.41 g, 32 mmol) in EtOH (5 ml) using general method (e) (2.04 g, 68 %); δ_H (200 MHz, $CDCl_3$) 1.96-2.01 (6H, m, CH_2N), 3.08-3.19 (6H, m, CH_2O) and 3.47 (1H, s, OH); δ_C (50 MHz, $CDCl_3$) 53.4 (C-2), 57.7 (C-4), 60.3 (C-3) and 67.4

N-Chloroethylmorpholine 122



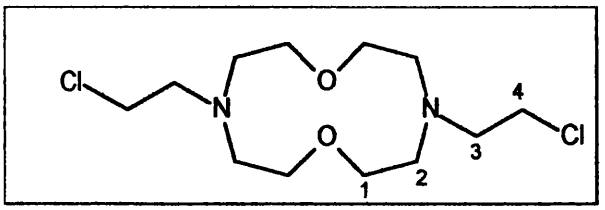
Chloride product **122** was obtained from **121** (2.0 g, 15 mmol) in chloroform (15 ml) and thionyl chloride (20 ml) by general method (c). The cream coloured precipitate which occurred after stirring overnight at room temperature was filtered and washed with diethyl ether (1.95 g, 86 %); υ_{max} (KBr disc) 659, 2870 and 3011 cm^{-1}; δ_H (200 MHz, D_2O) 2.98-3.15 (2H, m, $NC\underline{H_2}CH_2Cl$), 3.33-3.52 (4H, m, $NC\underline{H_2}CH_2O$) and 3.58-3.98 (6H, m, CH_2O, CH_2Cl); δ_C (50 MHz, D_2O) 37.3 (C-4), 52.4 (C-3),58.5 (C-2) and 64.2 (C-1); *m/z* (Cl^+) 150.06 (*MH*$^+$, 100 %) (found: *MH*$^+$ 150.0685 $C_6H_{13}NO^{35}Cl$ requires 150.0686).

N,N'-Di(hydroxyethyl)-1,7-dioxa-4,10-diazacyclododecane 124



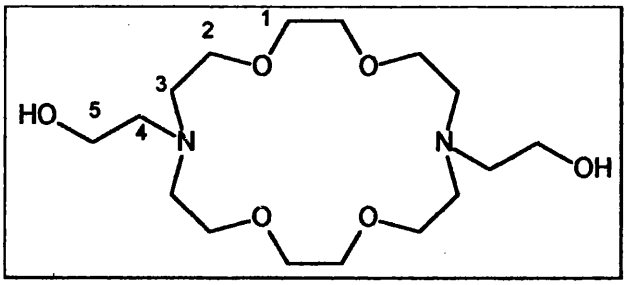
1,7-Dioxa-4,10-diazacyclododecane **123** (0.1 g, 0.57 mmol) was treated with ethelyene oxide (0.18 g, 4 mmol) in EtOH (4 ml) according to general method (e) to afford *N,N'*-di(hydroxyethyl)-1,7-dioxa-4,10-diazacyclododecane **124** as an orange oil (0.12 g, 92 %); υ_{max} (KBr disc) 1095, 1105, 1267 and 2865 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.58-2.63 (12H, m, NCH_2), 3.33-3.53 (12H, m, OCH_2), 4.78 (2H, bs, OH); δ_C (50 MHz, $CDCl_3$) 55.3, 56.3 (C-2) (C-3), 59.1 (C-4), 65.1 (C-1).

N,N'-Di(chloroethyl)-1,7-dioxa-4,10-diazacyclododecane 125



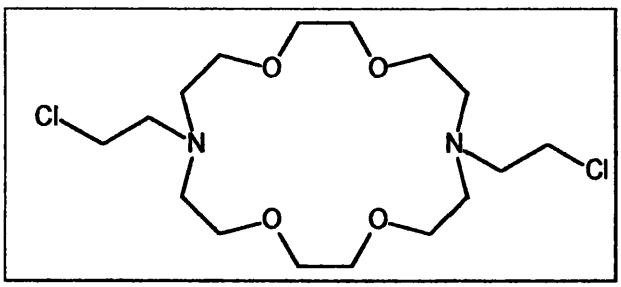
124 (0.40 g, 1.5 mmol) was taken up in chloroform (10 ml) and added to thionyl chloride as in general method (c). The chloride **125** was afforded as a light brown solid (0.20 g, 80 %); υ_{max} (KBr disc) 729, 2970 and 3100 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.43-2.65 (12H, m, NCH_2), 3.20-3.78 (12H, m, OCH_2, CH_2Cl); δ_C (50 MHz, $CDCl_3$) 39.2 (C-4), 55.4, 56.6 (C-2) (C-3), 65.0 (C-1); *m/z* (Cl^+) 269 (*MH*$^+$, 100 %).

N,N'-Di(hydroxyethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane 127



N,N'-Di(hydroxyethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane **127** was formed according to general method (e) using 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane **126** (0.05 g, 0.14 mmol), ethylene oxide (0.05g, 1.2 mmol) and EtOH (10 ml) (0.06 g, 55 %); υ_{max} (KBr disc) 1065, 1098, 1250 and 2880 cm^{-1}; δ_C (50 MHz, D_2O) 53.7, 56.3, 59.1 (C-3) (C-4) (C-5), 68.8 (C-2) and 70.1 (C-1); *m/z* (Cl^+) 351.5 (*MH*$^+$, 100 %).

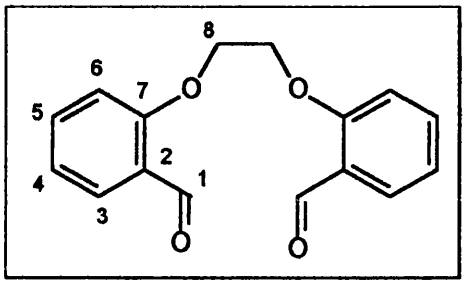
N,N'-Di(chloroethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane 128



127 (2.63 g, 7.5 mmol) was taken up in DCM (30 ml) and cooled to 0 °C. Thionyl chloride (15 mmol) was slowly added to the stirring solution. The solution was then heated at 60 °C for 6 h. Solvent and excess thionyl chloride were removed *invacuo* to leave a brown liquid. The residue was taken up in water (30 ml). Sodium bicarbonate was added until an oily layer occurred. The oily layer was removed and extracted with diethyl ether (5 x 50 ml). The combined organic were dried over

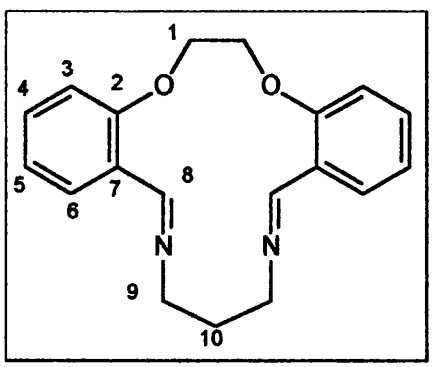
$MgSO_4$, filtered and concentrated *invacuo* to leave a yellow solid which when taken up in chloroform afforded the product **128** as a white solid (2.80 g, 62 %); υ_{max} (KBr disc) 713, 2920 and 2870 cm^{-1}; δ_H (200 MHz, D_2O) 1.65-2.41 (12H, m, NCH_2), 3.40-3.85 (20H, m, OCH_2, $ClCH_2$); δ_C (50 MHz, D_2O) 43.1 (C-5), 44.9 (C-4), 62.3 (C-3), 70.9, 71.7 (C-1) (C-2); *m/z* (Cl^+) 356.2 (*MH*$^+$, 45 %).

1,4-Bis(*O,O'*-2'-formylphenyl)-1,2-ethanediol 130



130 Was obtained as in the literature method[101] using salicylaldehyde **129** (24.4 g, 0.2 mol) in EtOH (20 ml), sodium hydroxide (8.0 g, 0.2 mol) in water (400 ml) and 1,2-dibromoethane (18.4 g, 8.45 ml, 0.1 mol) as a beige solid (13.34 g, 25 %); υ_{max} (KBr disc) 1484, 1597, 1696 and 2866 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 4.56 (4H, s, OCH_2), 7.59-7.62, 7.86-7.88 (8H, m, Ar-H) and 10.48 (2H, s, COH); lit[101] 4.53, 7-8, 10.38; δ_C (50 MHz, $CDCl_3$) 67.4 (C-8), 113.1, 125.6,129.1, 136.3 (C-3) (C-4) (C-5) (C-6), 161.1 (C-7), 189.7 (C-1); *m/z* (EI^+) 307.1 (*MH*$^+$, 86 %).

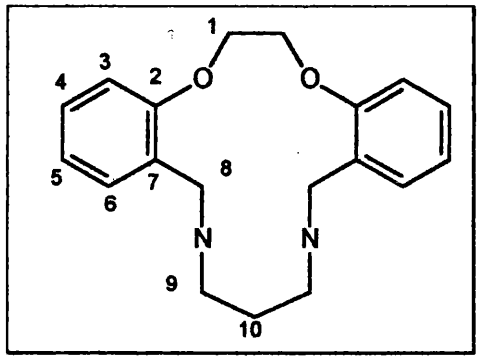
5,6:14,15-Dibenzo-1,12-diaza-1,4-dioxacyclopentadecane-7,12-diene 131



5,6:14,15-Dibenzo-1,12-diaza-1,4-dioxacyclopentadecane-7,12-diene **131** was obtained as in the literature[101] using **130** (10.0 g, 0.037 mol) in MeOH (500 ml) and 1,3-diaminopropane (2.7 g, 0.037 mol) in MeOH (115 ml). The product was obtained

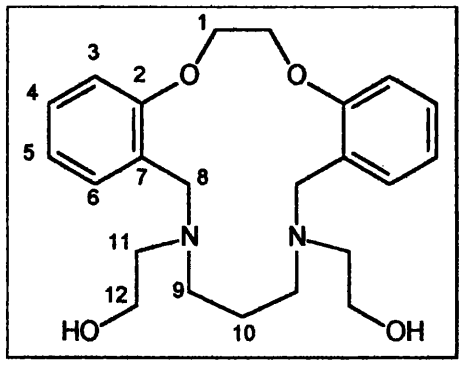
as fluffy beige needles (5.60 g, 48 %); υ_{max} (KBr disc) 1451, 1599, 1633 and 2872 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.28-2.34 (2H, quintet, $CH_2\underline{CH_2}$), 3.61-3.66 (4H, t, NCH_2), 4.42 (4H, s, OCH_2), 6.98-7.99 (8H, m, Ar-H), 8.81 (2H, s, CHN); lit[101] 2.29, 3.63, 4.35, 6.8-8.0, 8.7; δ_C (50 MHz,$CDCl_3$) 28.9 (C-10), 58.3 (C-1), 68.4 (C-8), 113.9, 122.2, 127.5 , 132.1, 158.9, 159.2 (C-2)(C-3) (C-4)(C-5)(C-6)(C-7).

5,6:14,15-Dibenzo-1,12-diaza-1,4-dioxacyclopentadecane 132



5,6:14,15-Dibenzo-1,12-diaza-1,4-dioxacyclopentadecane **132** was obtained by the literature method.[101] using **131** (1.0 g, 3.25 mmol) in dry THF (20 ml) and 1M borane THF (30 ml) as a white solid (0.57 g, 56 %); δ_H (200 MHz, $CDCl_3$) 2.03 (2H, s, NH), 2.54-2.57 (2H, m, $NCH_2\underline{CH_2}$), 3.67 (4H, m, NCH_2), 4.26 (4H, s, OCH_2), 5.19 (4H, s, Ar-CH_2), 6.77-6.84, 7.10-7.19 (8H, dt, Ar-H); δ_C (50 MHz, $CDCl_3$) 28.2 (C-10), 46.4 (C-9), 50.6 (C-8), 64.7 (C-1), 109.1, 119.6, 127.2, 127.5, 130.2, 156.0 (C-2)(C-3)(C-4)(C-5)(C-6)(C-7).

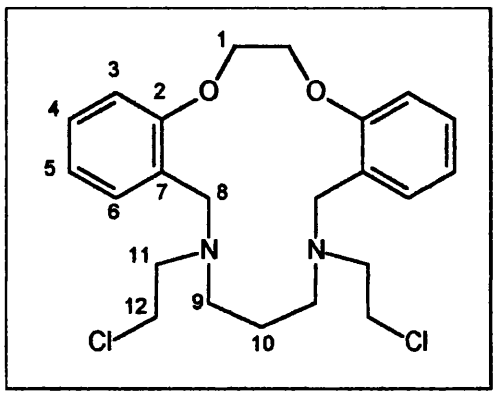
***N,N'*-Di(hydroxyethyl)-5,6:14,15-dibenzo-1,12-diaza-1,4-dioxacyclopentadecane 133**



Using **132** (0.5 g, 1.25 mmol), ethylene oxide in EtOH (15 ml) as in general method (c), the product **133** was afforded on refrigeration overnight as a white solid (0.45 g, 81 %); υ_{max} (KBr disc) 1050, 1270, 1490, 1598, 2950 and 3400 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.50-1.64 (2H, m, $NCH_2C\underline{H_2}$), 2.46-2.62 (8H, m, NCH_2), 3.34-3.46 (8H, m, NCH_2), 3.61 (4H, m, CH_2OH), 4.27-4.36 (4H, m, OCH_2), 5.22 (2H, s, OH), 7.15-7.25, 7.53-7.69 (8H, m, Ar-H); lit[100] 1.65, 2.53-2.57, 3.56, 4.37, 6.91-7.3; δ_C (50 MHz, $CDCl_3$) 24.4 (C-10), 50.5 (C-9), 52.2 (C-8), 53.2 (C-11), 58.2 (C-12), 65.9 (C-1), 110.9, 120.5, 125.7, 128.7, 132.6, 157.0 (C-2)(C-3)(C-4)(C-5)(C-6)(C-7).

_N,N'_-Di(chloroethyl)-5,6:14,15-dibenzo-1,12-diaza-1,4-dioxacyclopentadecane

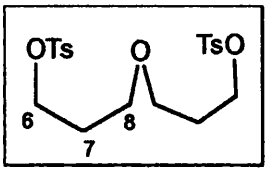
134



133 (0.50 g, 1.25 mmol) was added to thionyl chloride (15 ml) as in general method (c) in DCM (8 ml). The solution was returned to room temperature and stirred overnight followed by heating at reflux for 2 h. The solution was returned to room temperature and EtOH (20 ml) was added. No further gas was evolved and the mixture was stirred for 2 h. The product **134** precipitated at 0 °C overnight as a white solid; (0.21 g, 40 %); δ_H (200 MHz, D_2O) 1.12-1.18 (2H, m, $NCH_2C\underline{H_2}$), 1.59-1.61 (4H, m, NCH_2), 2.36 (4H, s, OCH2), 3.04-3.11 (4H, m, NCH_2), 3.60-3.66 (4H, m, CH_2Cl), 4.27-4.36 (4H, m, OCH_2), 7.15-7.25, 7.53-7.69 (8H, m, Ar-H); lit[102] 1.60, 2.49, 2.79, 3.45, 3.69, 4.35, 6.88-7.29; δ_C (50 MHz, D_2O) 21.9(C-10), 30.1 (C-12), 42.9, 43.5 (C-9)(C-11), 50.9 (C-8), 63.2 (C-1), 127.5, 127.7, 130.2, 130.4, 138.5, 144.0 (C-2)(C-3)(C-4)(C-5) (C-6) (C-7).

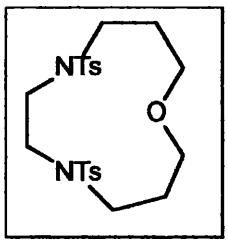
The hydrochloride salt was obtained on treatment with ethereal hydrochloric acid as a white solid (0.86 g, 78%); υ_{max} (KBr) 780, 1496 and 1600 cm^{-1}; (found: C, 51.03, H, 5.95 , N, 5.21 % $C_{23}H_{32}N_2O_2Cl_4$ requires C, 51.3, H, 5.99, N, 5.20 %).

O,O"Di(*p*-toluenesulfonyl)dipropylene glycol 136



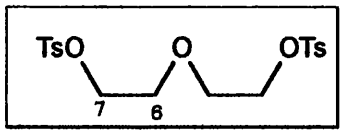
Tosylated compound O,O"-di(*p*-toluenesulfonyl)dipropylene glycol **136** was formed using general method (h) with *p*-toluenesulfonyl chloride (190.6 g, 1.0 mol), dipropylene glycol **135** (10.0 g, 0.075 mol), 30% sodium hydroxide, TEBA (0.50 g, 0.002 mmol) and DCM (200 ml) as white crystals (25.4 g, 76 %); υ_{max} (KBr) 1198, 1352, 1497 and 1599 cm^{-1}; δ_H (200 MHz, D_2O) 1.80 (4H, m, CH_2CH_2), 2.46 (6H, s, CH_3), 3.26 (8H, m, OCH_2), 7.34-7.79 (8H, dd, AA'BB' system Ar-H); δ_C (50 MHz, D_2O) 21.4 (C-1), 27.5 (C-7), 42.1, 42.3 (C-6), (C-8), 127.5, 129.6 (C-3) (C-4), 134.9 (C-2) and 145.3 (C-5).

N,N'-Di(*p*-toluenesulfonyl)-5,8-diaza-1-oxacyclounadecane 137



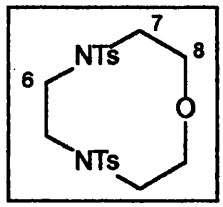
N,N'-Di(*p*-toluenesulfonyl)-1-oxa-4,7-diazacyclononane **137** was obtained by general method (j) using the disodium salt of **107** (1.0 g, 2.4 mmol) in dry DMF (50 ml) and O,O"-di(*p*-toluenesulfonyl)dipropylene glycol **136** (1.06 g, 2.4 mmol) in dry DMF (50 ml) as a beige solid (0.25 g, 22 %); mp 195-197 °C; lit[105] 199-201 °C; υ_{max} (KBr disc) 1160, 1337, 1450, 1501, 1601 and 2940 cm^{-1}; δ_H (200 MHz, D_2O) 1.80 (4H, m, CH_2CH_2), 2.45 (6H, s, CH_3), 3.15-3.33 (4H, m, CH_2N), 3.40 (4, $NC\underline{H_2}CH_2O$), 3.03-3.83 (4H, m, CH_2O), 7.74-7.78, 7.47-7.51 (8H, dd, AA'BB' system Ar-H); δ_C (50 MHz, D_2O) 21.9 (C-1), 25.4 (C-8), 52.3 (C-6), 52.7 (C-7), 67.6 (C-9), 127.5 , 129.3 (C-2)(C-3)(C-4) and 145.1 (C-5).

O,O''-Di(*p*-toluenesulfonyl)diethylene glycol 139



O,O''-Di(*p*-toluenesulfonyl)diethylene glycol **139** was formed using general method (h) using *p*-toluenesulfonyl chloride (190.6 g, 1.0 mol), diethylene glycol **138** (50.06 g, 0.5 mol), 30% sodium hydroxide (350 ml), TEBA (4.6 g, 20 mmol) and DCM (500 ml) as white crystals (165.34 g, 80 %); mp 86-87 °C; lit[156] 87-87.5 °C; υ_{max} (KBr) 1192, 1358, 1494, 1597 and 3429 cm^{-1}; δ_H (200 MHz, $CDCl_3$), 2.36 (6H, s, $C\underline{H}_3$), 3.49 (4H, t, tsO$C\underline{H}_2CH_2O$), 4.00 (4H, t, O$C\underline{H}_2CH_2O$),7.20-7.84 (8H, 2 x AA'BB', Ar-H); δ_C (50 MHz, $CDCl_3$) 21.7 (C-1), 68.5 (C-6), 69.2 (C-7), 127.8 129.8 (C-3) (C-4), 132.6 (C-2) and 144.9 (C-5); *m/z* (Cl^+) 414 (*MH*$^+$, 100 %) (found: C,52.71; H, 4.89; N, 17.65 % requires C, 52.74; H, 4.97; N,17.60 %).

N,N'-Di(*p*-toluenesulfonyl-1-oxa-4,7-diazacyclononane 141

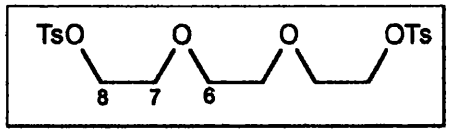


N,N'-Di(*p*-toluenesulfonyl)-1-oxa-4,7-diazacyclononane **141** was obtained by general method (j) using the disodium salt of **140** (1.0 g, 2.4 mmol) in dry DMF (50 ml) and **139** (1.0 g, 2.4 mmol) in dry DMF (50 ml) as a light yellow solid (0.66 g, 62 %); υ_{max} (KBr disc) 1119, 1157, 1335, 1447, 1495, 1598 and 2924 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.43 (6H, s, Ar-CH_3), 3.20-3.31 (4H, s, NCH_2), 3.37 (4H, s, NCH_2CH_2O), 3.00-3.78 (4H, m, CH_2O), 7.30-7.46, 7.77-7.73 (8H, dd, AA'BB' system Ar-H); δ_C (50 MHz, $CDCl_3$) 21.9 (C-1), 52.3 (C-6), 52.5 (C-7), 72.3 (C-8), 127.7, 130.2 (C-3) (C-4), 135.3 (C-2), 144.0 (C-5); *m/z* (Cl^+) 439.1 (*MH*$^+$, 100 %) (found: *MH*$^+$ 439.1361. $C_{20}H_{27}N_2O_5S_2$ requires 439.1361).

The [1+1] was prepared according to general cyclisation method (i): Caesium carbonate (8.26 g, 25.4 mmol) was added to *O,O''*-di (*p*-toluenesulfonyl)ethylene glycol **139** (5.01 g 12.1 mmol) in dry DMF (50 ml) along with

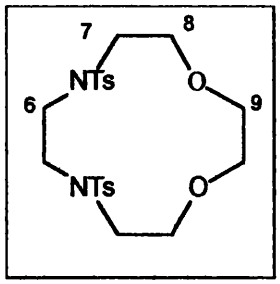
N,N'-di(*p*-toluenesulfonyl)ethylene diamine **107** (4.44 g,12.1 mmol) in dry DMF (50 ml). The product was afforded as a white solid (1.00 g, 57%).

O,O'''-Di(*p*-toluenesulfonyl)triethyleneglycol 143



O,O'''-Di(*p*-toluenesulfonyl)triethyleneglycol **143** was formed using general method (h) with *p*-toluenesulfonyl chloride (31.5 g, 0.15 mol) in DCM (40 ml), triethylene glycol **142** (11.27 g, 0.075 mol), 30% sodium hydroxide solution [15 g NaOH in 35 ml water], DCM (50 ml) and TEBA (0.46 g, 2 mmol) at 0 °C as white crystals (27.7 g, 81%), υ_{max} (KBr) 1130, 1353, 1496 and 1596 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.45 (6H, s, CH_3), 3.42 (4H, s, $tsOCH_2$), 3.57 (4H, t, $tsCH_2C\underline{H_2}O$), 4.13 (4H,t, $OC\underline{H_2}CH_2O$), 7.60-7.64, 7.49-7.53 (8H, 2 x AA'BB', Ar-H); lit[106] 2.37, 3.50, 3.62, 4.06, 7.25, 7.72; δ_C (50 MHz, $CDCl_3$) 21.2 (C-1), 67.9 (C-6), 69. (C-7), 70.0 (C-8), 127.7, 130.2 (C-3) (C-4), 132.4 (C-2), 145.0 (C-5); *m/z* (Cl^+) 459.1 (*MH*$^+$, 100 %)(found: *MH*$^+$ 459.1148. $C_{20}H_{27}O_8S_2$ requires 459.1147).

N,N'-Di(*p*-toluenesulfonyl)-1,4-dioxa-7,10-diazacyclododecane 144

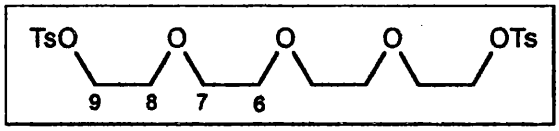


N,N'-Di(*p*-toluenesulfonyl)-1,4-dioxa-7,10-diazacyclododecane **144** was obtained by general method (j) using the disodium salt of **107** (2.0 g, 4.8 mmol) in dry DMF (50 ml) and **143** (2.22 g, 4.85 mmol) in dry DMF (50 ml) as a white solid (1.50 g, 63 %); δ_H (200 MHz, $CDCl_3$) 2.36 (6H, s, $Ar\text{-}CH_3$), 3.08-3.13 (4H, m, NCH_2), 3.45 (4H, s, OCH_2), 3.57-3.65 (4H, m, $NCH_2C\underline{H_2}O$), 7.24-7.26, 7.66-7.68 (8H, dd, AA'BB' system Ar-H); lit[105] 3.36, 3.50, 3.70, 3.73 (no aromatic moeities quoted); δ_C (50 MHz, $CDCl_3$) 21.9 (C-1), 49.9 (C-6), 50.2 (C-7), 70.3 (C-8), 72.9 (C-9), 127.8, 130.1 (C-3)

(C-4), 135.9 (C-2), 143.7 (C-5); *m/z* (Cl^+) 483.2 (MH^+, 100 %) (found: MH^+ 483.1624. $C_{22}H_{31}N_2O_8$ requires 483.1624).

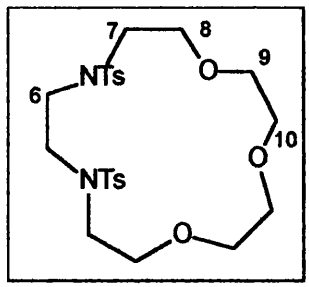
The [1+1] product was prepared according to general cyclisation method (i): Caesium carbonate (8.26 g, 25.4 mmol), **143** (5.84 g, 12.1 mmol) in dry DMF (50 ml) and **107** (4.44 g, 12.1 mmol) in dry DMF (50 ml). The filtrate was left at 0 °C overnight. The precipitatewas filtered off to give *N,N'*-di(*p*-toluenesulfonyl)-1,4-dioxa-7,10-diazacyclododecane; δ_C (200 MHz, $CDCl_3$) 21.20 (C-1), 41.4 (C-8), 62.4 (C-9), 63.4 (C-12), 64.6 (C-11), 119.9, 121.9 (C-3) (C-4), 128.2 (C-2), 137.4 (C-5).

O,O''''-Di(*p*-toluenesulfonyltetraethylene-1,4,7,10,13-tetraoxatridecane 146



O,O''''-Di(*p*-toluenesulfonyl)tetraethylene-1,4,7,10,13-tetraoxatridecane **146** was prepared using general method (h) with tetraethyleneglycol **145** (14.57 g, 75 mmol), *p*-toluenesulfonyl chloride (31.5 g, 150 mmol), TEBA (0.05 g, 2.0 mmol), 30 % sodium hydroxide solution (40 ml) and DCM (100 ml) as a clear viscous oil (21.3 g, 56 %); δ_H (200 MHz, $CDCl_3$) 2.30 (6H, s, Ar-CH_3), 3.42 (8H, s, OCH_2), 3.51-3.57 (4H, m, OCH_2), 4.02-4.05 (4H, t, OCH_2), 7.21-7.23, 7.64-7.66 (8H, dd, Ar-H); lit[106] 2.42, 3.55, 3.67, 4.16, 7.35, 7.80; δ_C (50 MHz, $CDCl_3$) 21.8 (C-1), 69.6 (C-8), 70.7 (C-7), 70.8 (C-6), 128.3, 130.5 (C-3) (C-4), 133.2 (C-2), 145.2 (C-5).

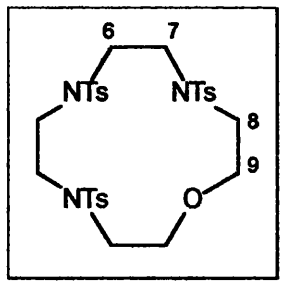
N,N'-Di(*p*-toluenesulfonyl)-1,4,7-trioxa-10,13-diazacyclopentadecane 147



N,N'-Di(*p*-toluenesulfonyl-1,4,7-trioxa-10,13-diazacyclopentadecane **147** was afforded as a white solid using general cyclisation method (j) with the disodium salt of **107** (2.0 g, 4.8 mmol) in dry DMF (50 ml) and **146** (2.44 g, 4.8 mmol) in dry DMF (50

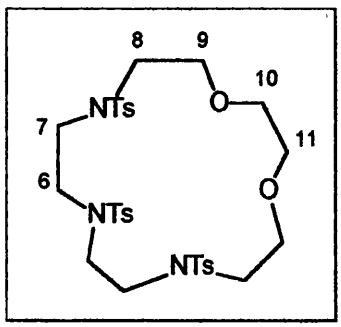
ml) (1.79 g, 70 %); υ_{max} (KBr disc) 1158, 1344, 1450, 1492, 1598 and 2935 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.36 (6H, s, Ar-CH_3), 3.09-3.15 (4H, m, NCH_2), 3.31 (4H, s, $NC\underline{H_2}CH_20$), 3.48 (8H, s, CH_2O), 3.61-3.64 (4H, m, CH_2O), 7.23-7.25, 7.69-7.71 (8H, dd, AA'BB' system Ar-H); δ_C (50 MHz, $CDCl_3$) 21.9 (C-1), 49.8 (C-6), 50.7 (C-7), 70.6 (C-8), 71.4 (C-9), 71.5 (C-10), 128.8, 130.1 (C-3) (C-4), 136.4 (C-2), 143.7 (C-5); *m/z* (Cl^+) 527.1 (*MH*$^+$, 100 %) (found: *MH*$^+$ 527.1886 $C_{24}H_{35}N_2O_7S_2$ requires 527.1886).

N,N',N''-Tri(*p*-toluenesulfonyl)-1-oxa-4,7,10-triazacyclododecane 148



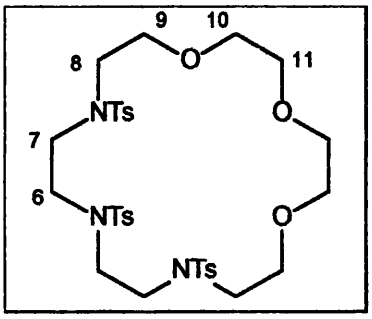
N,N',N''-Tri(*p*-toluenesulfonyl)-1-oxa-4,7,10-triazacyclododecane **148** was formed as a white solid using general cyclisation method (j) with the disodium salt of **86** (4.0 g, 6.6 mmol) in dry DMF (100 ml) and **139** (2.72 g, 6.6 mmol) in dry DMF (100 ml) (2.29 g, 55 %); δ_H (200 MHz, $CDCl_3$) 2.42 (9H, s, Ar-CH_3), 3.07-3.20 (8H, m, NCH_2), 3.48-3.54, 3.62-3.66 (8H, m, $NC\underline{H_2}C\underline{H_2}O$), 7.25-7.28, 7.60-7.65 (8H, dd, Ar-H), 7.32-7.37, 7.79-7.83 (4H, dd, AA'BB' systemAr-H); lit[108] 3.07 (12H, t, NCH_2), 3.43 (4H, t, OCH_2), 7.30 (12H, m, Ar-H); δ_C (50 MHz, $CDCl_3$) 21.9 (C-1), 42.9 (C-6), 50.9 (C-7), 58.8 (C-8), 72.4 (C-9), 127.5, 130.19 (C-3)(C-4), 137.1 (C-2), 144.5 (C-5); *m/z* (Cl^+) 718.1 (*MH*$^+$, 100 %).

N,N',N''-Tri(*p*-toluenesulfonyl)-1,4-dioxa-7,10,13-triazacyclopentadecane 149



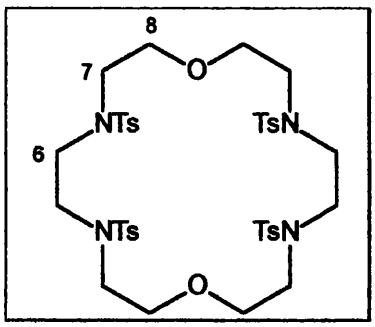
N,N',N"-Tri(*p*-toluenesulfonyl)-1,4-dioxa-7,10,13-triazacyclopentadecane **149** was afforded as a white solid using general cyclisation method (j) with the disodium salt of **86** (4.0 g, 6.6 mmol) in dry DMF (100 ml) and **143** (3.0 g, 6.6 mmol) in dry DMF (100 ml). The product **149** was afforded as a white solid (2.39 g, 54 %); υ_{max} (KBr disc) 1106, 1156, 1349, 1447, 1497,1597 and 2922 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.35 (9H, s, Ar-CH_3), 3.06-3.22 (8H, m, NCH_2), 3.20-3.22 (4H, m, $NC\underline{H_2}CH_2O$), 3.42 (4H, s, CH_2O), 3.46-3.53 (4H, m, CH_2O), 7.19-7.26, 7.66-7.76 (12H, m, Ar-H) lit[108] 2.27 (9H, s, CH_3), 3.30 (20H, m, OCH_2, NCH_2), 7.30 (12H, m, Ar-H); δ_C (50 MHz, $CDCl_3$) 21.8 (C-1), 40.4 (C-6), 47.4 (C-7), 48.3 (C-8), 56.3 (C-9), 56.3 (C-10) (C-11),129.8, 130.1 (C-3) (C-4), 136.4 (C-2), 143.7 (C-5);; *m/z* (Cl^+) 680 (*MH^+*, 15 %), 697 (*MNH_4^+*, 20 %).

N,N',N"-Tri(*p*-toluenesulfonyl)-1,4,7-trioxa-10,13,16-triazacyclooctadecane 150



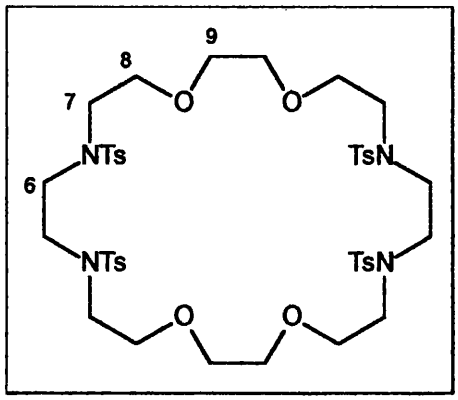
N,N',N"-Tri(*p*-toluenesulfonyl)-1,4,7-trioxa-10,13,16-triazacyclooctadecane **150** was afforded as a light beige solid using general cyclisation method (j) with the disodium salt of **86** (2.0 g, 2.2 mmol) in dry DMF (50 ml) and **146** (1.65 g, 3.3 mmol) in dry DMF (50 ml). The product was obtained as a white solid (1.44 g, 64 %); δ_H (200 MHz, $CDCl_3$) 2.35 (9H, s, Ar-CH_3), 3.01-3.12 (8H, m, NCH_2), 3.24-3.31 (4H, m, NCH_2), 3.26-3.52 (4H, m, OCH_2), 3.42-3.61 (8H, m, OCH_2), 7.20-7.28, 7.66-7.75 (12H, m, Ar-H) lit[108] 2.43 (9H, s, CH_3), 3.41 (24H, m, OCH_2, NCH_2), 7.40 (12H, m, Ar-H); δ_C (50 MHz, $CDCl_3$) 21.9 (C-1), 42.8 (C-6), 52.1 (C-7) (C-8), 69.7 (C-9), 69.8 (C-10), 71.2 (C-11), 127.6, 130.2 (C-3) (C-4), 135.1 (C-2), 143.2 (C-5); *m/z* (FAB^+) 724.2 (*MH^+*, 100 %) (found: *MH^+* 724.2396. $C_{33}H_{36}N_3O_9S_3$ requires 724.2396).

***N,N',N'',N'''*-Tetra(*p*-toluenesulfonyl)-1,10-dioxa-4,7,13,16-tetraazacyclooctadecane 151**



Caesium carbonate (8.26 g, 25.4 mmol) was added to **139** (5.01 g 12.1 mmol) in dry DMF (50 ml) as in general cyclisation method (i) along with **107** (4.44 g,12.1 mmol) in dry DMF (50 ml). The product **151** was afforded as a white solid (1.56 g, 44%); υ_{max}(KBr) 118, 1334, 1499 and 1597 cm^{-1} ; δ_H (200 MHz,$CDCl_3$) 2.36 (12H, s, Ar-CH_3) 3.16-3.20 (8H, m, NCH_2CH_2O), 3.20 (8H, s, NCH_2), 3.80-3.84 (8H, t, OCH_2), 7.22-7.26, 7.64-7.69 (16H, dd, AA'BB' system Ar-H); lit[94] 2.44, 3.22, 3.32, 3.54, 7.31, 7.71; δ_C (200 MHz,$CDCl_3$) 21.5 (C-1), 51.8 (C-6), 52.0 (C-7), 71.9 (C-8), 127.3, 129.8 (C-3) (C-4), 135.0 (C-2), 143.6 (C-5);*m/z* (FAB$^+$) 877 (*MH*$^+$, 8 %), 899 (*MNa*$^+$, 3 %).

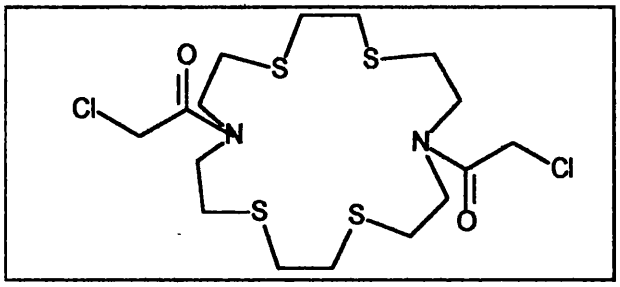
***N,N',N'',N'''*-Tetra(*p*-toluenesulfonyl)-1,4,13,16-tetraoxa-7,10,19,22-Tetraazacyclododecosane 152**



Caesium carbonate (8.26 g, 25.4 mmol), **143** (5.84 g, 12.1 mmol) in dry DMF (50 ml) and **107** (4.44 g, 12.1 mmol) in dry DMF (50 ml) were treated as in general cyclisation method (i). A light beige/yellow precipitate was found to be the desired **152** (5.39 g, 46 %); υ_{max} (KBr disc) 1160, 1345, 1500, 1598 and 2960 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.43 (12H, s, Ar-CH_3), 3.28-3.35 (8H, m, NCH_2), 3.51 (16H, s, OCH_2),

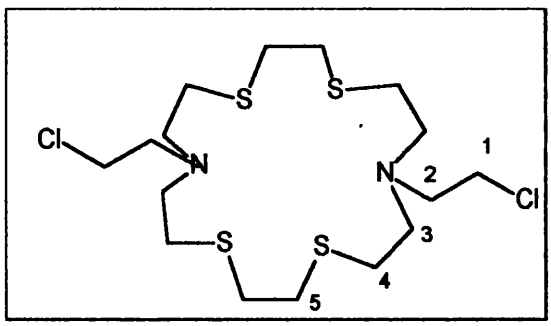
3.63-3.67 (8H, m, NCH_2), 7.30-7.34, 7.72-7.76 (16H, m, Ar-H); δ_C (50 MHz, $CDCl_3$) 21.5 (C-1), 49.5 (C-6), 49.8 (C-7), 69.9 (C-8), 72.5 (C-9), 127.3, 129.7 (C-3) (C-4), 135.6 (C-2), 143.0 (C-5); *m/z* (FAB$^+$) 965 (*MH*$^+$, 20 %) (found: C, 54.87; H, 6.36; N, 5.96 %. $C_{44}H_{60}N_4O_{12}S_4$ requires C, 54.75; H, 6.27; N, 5.80 %).

N,N'-Di(chloroacetyl)-1,4,10,13-tetrathia-7,13-diazacyclopentadecane 154



N,N'-Di(chloroacetyl)-1,4,10,13-tetrathia-7,13-diazacyclopentadecane **154** was afforded on the treatment of 1,4,10,13-tetrathia-7,13-diazacyclopentadecane (0.1 g, 0.31 mmol) with chloroacetyl chloride (0.087 g, 0.78 mmol) and triethylamine (0.078 g, 0.78 mmol) according to literature preperation.[109] The product was obtained as a pale yellow solid (0.12 g, 85 %); δ_H (200 MHz, $CDCl_3$) 2.63-2.86 (16H, m, CH_2S), 3.43-3.66 (8H, m, CH_2N), 4.17 ($COCH_2$); δ_C (50 MHz, $CDCl_3$) 41.5 (C-1), 47.3, 47.6 (C-3)(C-4), 49.4 (C-5) and 166.9 (C-2).

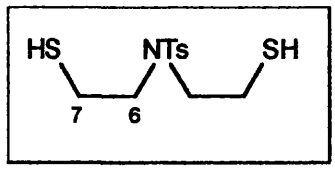
***N,N'*-Di(chloroethyl)-1,4,10,13-tetrathia-7,13-diazacyclopentadecane 155**



N,N'-Di(chloroethyl)-1,4,10,13-tetrathia-7,13-diazacyclopentadecane **155** was obtained by the reduction of **154** (0.12 g, 0.26 mmol) in dry DMF (8 ml) to a stirring solution of 1M borane THF (15 ml) under nitrogen at 0 °C. The solution was heated at reflux for 5 h before cooling to 0 °C. 6M Hydrochloric acid (3 ml) was slowly added

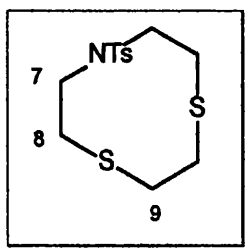
followed by water (10 ml). The solvents were distilled off and the residue was taken up in MeOH (20 ml). 3 Drops of concentrated hydrochloric acid were added to remove any methyl borate formed. The solution was concentrated *in vacuo* and the last three steps repeated until no further weight was noted. The orange oil was taken up in chloroform (10 ml) and triethylamine (2.5 ml) was added and the mixture was stirred for 30 min. The solution was concentrated under reduced pressure to a white/ yellow solid which was taken up in water (10 ml) and extracted with EtOAc (3 x 30 ml). The organic extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo* to leave the desired product as a viscous colourless oil (0.09 g, 77 %); υ_{max} (KBr disc) 775, 2520 and 2940 cm^{-1}; δ_C (50 MHz, D_2O) 29.0 (C-5), 29.8 (C-4), 35.6 (C-1) 54.7 (C-3), 54.8 (C-2); *m/z* (EI^+) 420.1 (*MH^+*, 55 %).

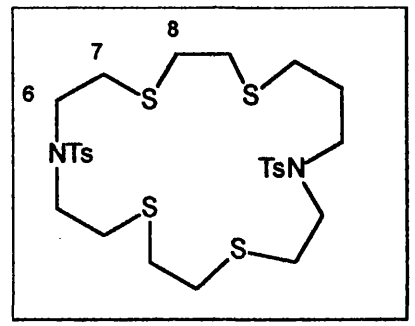
N-(p-Toluenesulfonyl)-4-azapentane-1,7-dithiol 156



The product **156** was obtained using a literature procedure[94] from **108** (25.0 g, 45 mmol), EtOH (170 ml) and thiourea (7.6 g, 100 mmol). The yellow oil obtained was purified by column chromatography on silica to leave the product **156** as a white solid (7.79 g, 59 %); *Rf* 0.5 (DCM:MeOH; 99:1) lit[94] 0.5; υ_{max}(KBr) 1164, 1332, 1598 and 2600 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.52 (4H, t, SCH_2), 2.41 (3H, s, CH_3), 3.62 (4H, t, NCH_2), 7.28-7.69 (4H, dd, Ar-H); δ_C (200 MHz, $CDCl_3$) 21.4 (C-1), 23.9 (C-7), 52.5 (C-6), 126.9 129.9 (C-3) (C-4), 136.4 (C-2), 143.6 (C-5); *m/z* (CI^+) 290 (*MH^+*, 80 %)

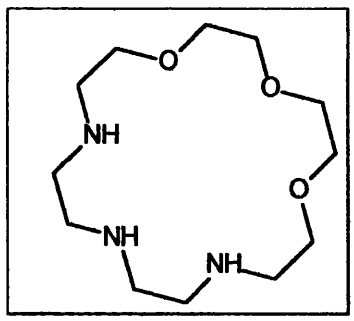
N-(p-Toluenesulfonyl)-1,4-dithia-7-azacyclononane 157 &
N,N'-Bis(p-toluenesulfonyl)-1,4,10,13-tetrathia-7,16-diazacyclooctadecane 158





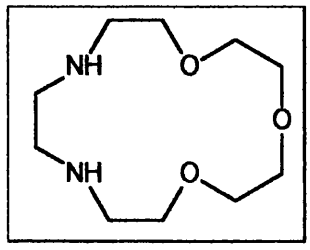
To caesium carbonate (1.8 g, 5.5 mmol) in dry DMF (250 ml) was added at 55 °C solutions of 1,2-dibromoethane (92.0 g, 5.0 mmol) in DMF (125 ml) and **156** (1.55 g, 5.32 mmol) in DMF (125 ml). The two solutions were added at equal rates over a period of 12 h with vigorous stirring. After the final addition the temperature was raised to 60 °C and the mixture was stirred for a further 3 h. The solvent was removed by distillation and the residue taken up in DCM (50 ml). The organic layer was washed several times with water, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The beige solid was taken up in warm toluene (10 ml) and hexane was added (2.5 ml). The solution was allowed to cool overnight. The brown solid was filtered off as *N,N'*-di(*p*-toluenesulfonyl)-1,4,10,13-tetrathia-7,16-diazacyclo octadecane. **158** mp. 123-125 °C; lit[94] 122-124 °C. Hexane was added for *N*-(*p*-toluenesulfonyl)-1,4-dithia-7-azacyclononane to precipitate out. **157** mp. 206-208 °C; lit[94] 206 °C.

1,4,7-Trioxa-10,13,16-triazacyclooctadecane 160



Procedure was carried out as in literature using *N,N',N''*-tri(*p*-toluenesulfonyl)-1,4,7-trioxa-10,13,16-triazacyclooctadecane **150** (1.50 g, 2.0 mmol), in dry THF (20 ml) and dimethylphenylsilyl lithium (10.4 ml, 11.4 mmol) and the mixture was stirred at 0 °C for 5 h. The white solid obtained on characterisation showed some detosylation but also impurities which proved to be difficult to remove.

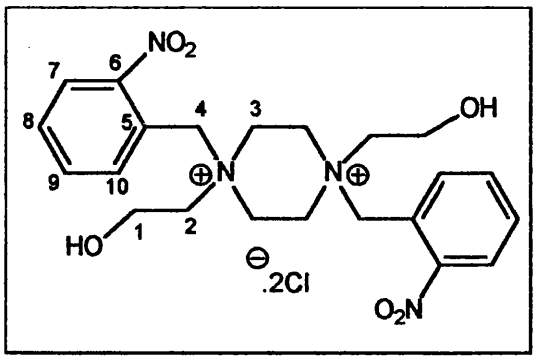
1,4,7-Trioxa-10,13-diazacyclopentadecane 161



Procedure was carried out as in the literature using *N,N'*-di(*p*-toluenesulfonyl)-1,4,7 trioxa-10,13-diazacyclopenadecane **147** (1.50 g, 2.85 mmol), in dry THF (20 ml) and dimethylphenylsilyl lithium (10.4 ml, 11.4 mmol) and the mixture was stirred at 0 °C for 5 h. The white solid obtained on crystallisation showed some detosylation but also impurities which proved to be difficult to remove.

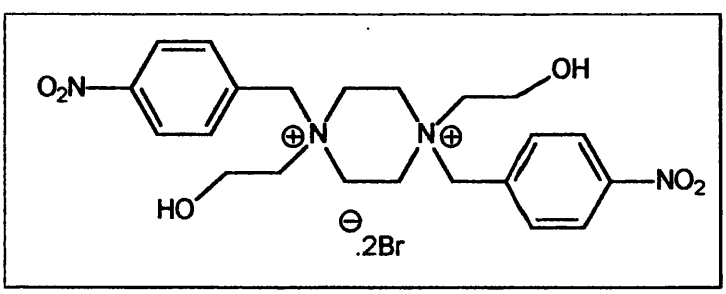
8.6.1 Experimental to Chapter 6

N,N'-Di(hydroxyethyl)-*N,N'*-di(*o*-nitrobenzyl)piperazinium dichloride 161a



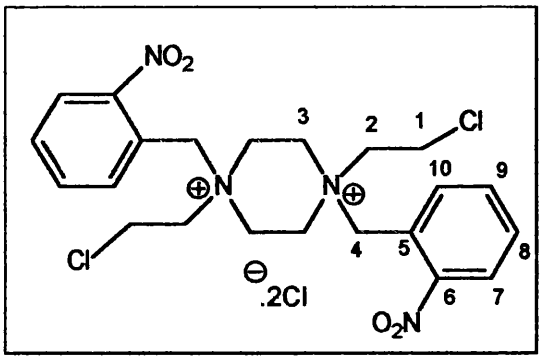
N,N'-dihydroxyethyl-*N,N'*-di(*o*-nitrobenzyl)piperazinium dichloride **161a** was obtained as in literature method[139] using **66** (0.25 g, 1.45 mmol) in acetonitrile (10 ml) and *o*-nitrobenzyl chloride (0.5 g, 2.91 mmol); υ_{max} (KBr) 1078, 1609, 2853, 2924 and 3387 cm^{-1}; δ_H (200 MHz, D_2O) 2.85-3.02 (12H, m NCH_2), 3.80-3.90 (4H, m, OCH_2), 4.92 (4H, s, CH_2Ar), 7.35-7.42, 7.55-7.77 (8H, m, Ar-H); δ_C (50 MHz, D_2O) 43.2 (C-3), 47.3 (C-2), 56.6 (C-4), 58.2 (C-1), 125.6, 129.8, 131.9, 132.8, 134.1 138.5 (C-5)(C-6)(C-7)(C-8)(C-9); *m/z* (FAB^+) 310.3 (*M*-NitrobenzylCl, 30 %), 445 2 (*M*-2Cl, 1 %), 481.4 (*M*-Cl, 1.6 %).

N,N'-Di(hydroxyethyl)-di(*p*-nitrobenzyl)-*N,N'*-piperazinium dibromide 161b



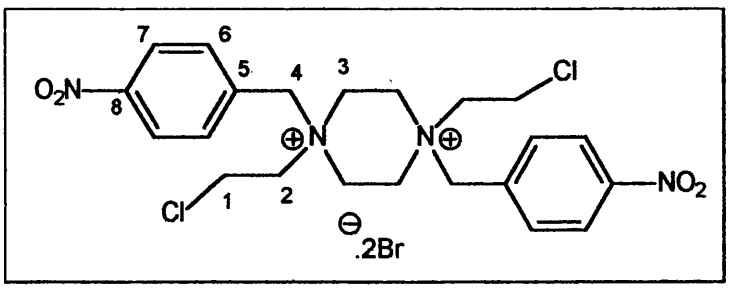
N,N'-Di(hydroxyethyl)-di(*p*-nitrobenzyl)-*N,N'*-piperazinium dibromide **161b** was obtained as in literature method[139] using **66** (3.0 g, 17.2 mmol) in EtOH (30 ml), *p*-nitrobenzyl bromide (3.72 g, 17.2 mmol) (0.7 g, 37 %); υ_{max} (KBr) 1082, 1350, 1496,1519, 1604, 2858, 2967, 3107, 3292 and 3546 cm^{-1}; δ_H (200 MHz, D_2O) 3.32-3.72 (16H, m, NCH_2), 4.74 (4H, m, Ar-CH_2), 7.44-8.20 (8H, AA'BB', Ar-H); *m/z* (Cl^+) 445.3 (*M-2Br*, 100 %).

N,N'-Di(chloroethyl)-N,N'-di(o-nitrobenzyl)piperazinium dichloride 162a



N,N'-Di(chloroethyl)-*N,N'*-di(*o*-nitrobenzyl)piperazinium dichloride **162a** was obtained as in literature method[139] **161a** (0.07 g, 0.14 mmol) in thionyl chloride (10 ml), as in general method (c) (0.04 g, 55 %); υ_{max} (KBr) 780, 1369, 1530 and 1631 cm^{-1};δ_H (200 MHz, D_2O) 2.97-3.85 (12H, m, NCH_2), 3.86-3.94 (4H, m, CH_2Cl), 4.78-4.85 (4H, s, Ar-CH_2), 7.51-7.78 (8H, m, Ar-H); δ_C (50 MHz, D_2O) 36.3 (C-1), 43.5 (C-3), 48.1 (C-2), 57.5 (C-4), 125.9, 130.2, 131.6, 133.0, 138.5 (C-5)(C-6)(C-7)(C-8)(C-9)(C-10).

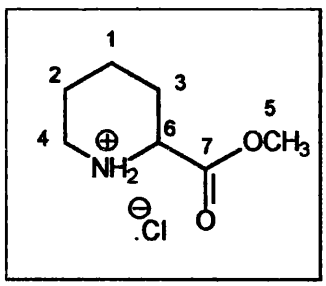
N,N'-Di(chloroethyl)-N,N'-di(p-nitrobenzyl)piperazinium dibromide 162b



N,N'-Di(chloroethyl)-*N,N'*-di(*p*-nitrobenzyl)piperazinium dibromide **162b** was obtained as in literature method[139] using **161b** (0.70 g, 1.15 mmol) and thionyl

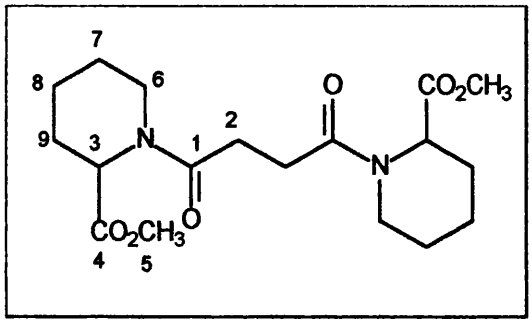
chloride (20 ml) as in general method (c). The product was obtained as a pale yellow solid and recrystallised twice from EtOH and diethyl ether (0.46 g, 62 %); υ_{max} (KBr) 752, 1351, 1496, 1525, 1608 and 2971 (br) cm^{-1}; δ_H (200 MHz, D_2O), 3.64-3.97 (12H, m, NCH_2), 4.04-4.09 (4H, m, CH_2Cl), 4.81-4.88 (4H, bs, Ar-CH_2), 7.66-7.70, 8.22-8.62 (8H, AA'BB', Ar-H); δ_C (50 MHz, D_2O) 35.7 (C-1), 46.4, 55.4, 58.4 (C-2)(C-3)(C-4), 125.4 (C-5), 132.8 (C-7), 135.4 (C-6), 150.0 (C-8); *m/z* (FAB^+) 729.4 (*M+Br*$_2$, 100 %).

Methyl DL-pipecolinate hydrochloride 164



Methyl DL-pipecolinate hydrochloride **164** was prepared as in the literature preparation[54] with DL-pipecolinic acid (5.0 g, 38.7 mmol), MeOH (50 ml) and thionyl chloride (5.13 ml) as a white solid. This was recrystallised from EtOH and diethyl ether (6.35 g, 91 %); δ_C (50 MHz, $CDCl_3$) 21.9 (C-1), 22.1 (C-2), 26.3 (C-3), 44.8 (C-4), 54.4 (C-5), 57.5 (C-6), 170.9 (C-7); lit[54] 22.0 (C-1), 22.1 (C-2), 26.5 (C-3), 44.9 (C-4), 54.4 (C-5), 57.6 (C-6), 171.1 (C-7).

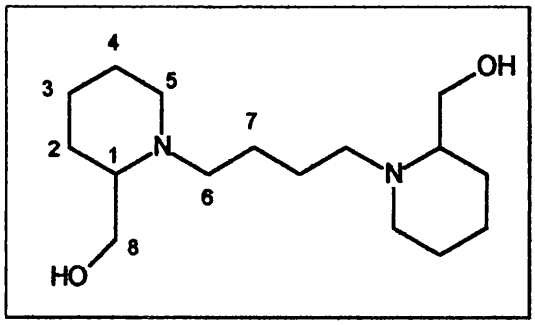
***N,N'*-(1,4-Dioxo-1,4-butanediyl)bis(2-methoxycarbonylpiperidine) 165**



N,N'-(1,4-Dioxo-1,4-butanediyl)bis(2-methoxycarbonyl piperidine) **165** was prepared by literature preparation[54] using **164** (5.0 g, 27.8 mmol), chloroform (30 ml), triethylamine (6.80 g, 66.7 mmol) and succinyl dichloride (1.44 ml, 13.0 mmol) as a colourless oil (4.67 g, 91 %); δ_C (50 MHz, $CDCl_3$) 20.8 (C-8), 25.1 (C-7), 27.0 (C-6),

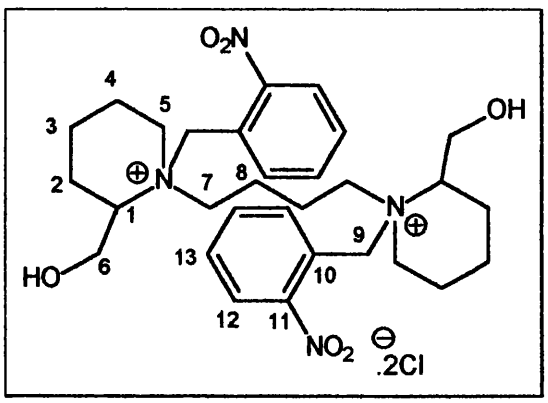
28.1 (C-2), 43.0 (C-9), 52.0 (C-5), 54.2 (C-3), 171.8, (C-1) 172.0 (C-4); lit[54] 20.8 (C-8), 25.0 (C-7), 27.0 (C-6) 28.2 (C-2), 42.9 (C-9), 52.0 (C-5), 53.8 (C-3), 167.2 (C-1),171.9 (C-4).

_N,N'_-(1,4-Butanediyl)bis(2-hydroxymethylpiperidine) 10



_N,N'_-(1,4-Butanediyl)bis (2-hydroxymethylpiperidine) **10** was prepared as in literature preparation[54] with **165** (5.0 g, 13.5 mmol) and lithium aluminium hydride (4.00 g, 0.11 mol) in dry THF (40 ml) as a clear viscous oil (1.68 g, 87 %); δ_C (50 MHz, $CDCl_3$) 23.5 (C-3), 24.0 (C-2), 24.3 (C-7), 27.5 (C-4), 51.1 (C-6), 53.0 (C-5), 61.0 (C-1), 62.4 (C-8); lit[54] 23.3 (C-3), 23.5 (C-2), 24.5 (C-7), 27.8 (C-4), 51.3 (C-6), 53.1 (C-5), 61.0 (C-1), 62.4 (C-8).

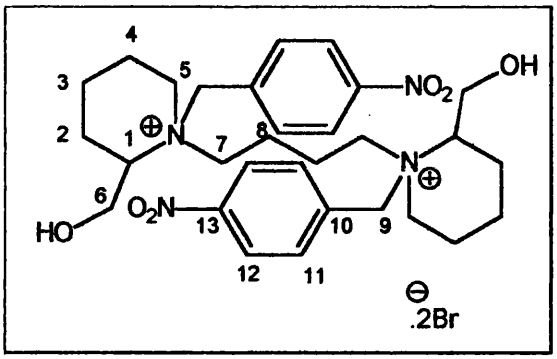
_N,N'_-Di(_o_-nitrobenzyl)-(1,4-butanediyl)bis(2-hydroxymethylpiperidinium) dichloride 166a



_N,N'_-Di(_o_-nitrobenzyl)-(1,4-butanediyl)bis(2-hydroxymethylpiperidinium) dichloride **166a** was obtained by literature method[139] using **10** (0.84 g, 2.95 mmol) in acetonitrile (30 ml) and _o_-nitrobenzyl chloride (1.30 g, 6.0 mmol) as a brown oil which was crystallised from EtOH and diethyl ether (1.2 g, 61 %); υ_{max} (KBr) 1040,1363,

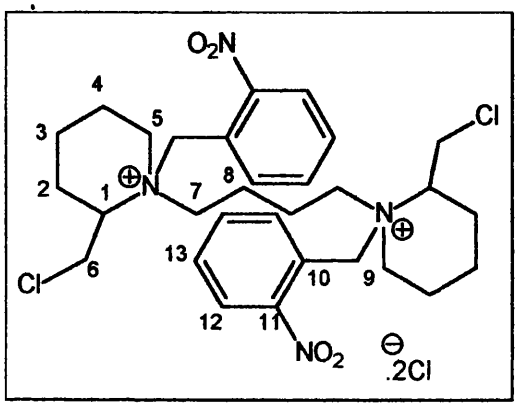
1528, 1618 and 3480 cm^{-1}; δ_H (200 MHZ, D_2O) 1.22-1.78 (18H, m, ring and chain CH_2CH_2), 2.72-3.74 (12H, m, ring and chain CH_2N, CHN), 4.56 (4H, s, CH_2-Ar), 7.66 (2H, bs, Ar-H), 7.47 (6H, bs, Ar-H);δ_C (50 MHZ, D_2O) 21.9 (C-3), 23.4 (C-4), 25.0 (C-2), 27.1 (C-8), 36.1 (C-1), 51.4 (C-6), 57.5 (C-5), 62.1 (C-7), 63.5 (C-9), 122.1, 128.5, 129.0, 133.0, 145.5 (C-9)(C-10)(C-11)(C-12)(C-13).

_N,N'_-Di(_p_-nitrobenzyl)-(1,4-butanediyl)bis(2-hydroxymethylpiperidinium) dibromide 166b



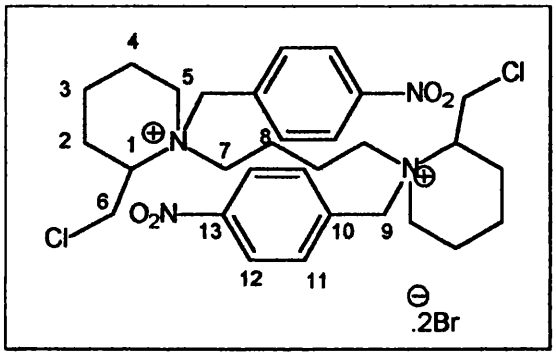
_N,N'_-Di(_p_-nitrobenzyl)-(1,4-butanediyl)bis(2-hydroxymethylpiperidinium) dibromide **166b** was obtained by literature method[139] using **10** (0.84 g, 2.95 mmol) in acetonitrile (30 ml), _p_-nitrobenzyl bromide (1.30 g, 6.0 mmol) as a brown oil which was crystallised from EtOH and diethyl ether (1.4 g, 63 %); υ_{max} (KBr) 1035, 1369, 1535, 1621 and 3400 cm^{-1}; δ_H (200 MHz, D_2O) 1.41-2.00 (18H, m, ring and chain CH_2CH_2), 2.83-3.59 (12H, m, ring and chain CH_2N, CHN), 4.11 (4H, s, CH_2-Ar), 7.12-7.18 and 7.64-7.70 (8H, AA'BB' system, Ar-H); δ_C (50 MHz, D_2O) 22.5 (C-3), 23.7 (C-4), 25.0 (C-2), 27.4 (C-8), 36.1 (C-7), 51.4 (C-6), 57.9 (C-5), 62.5 (C-1), 63.9 (C-9), 123.0 (C-10), 129.8 (C-12), 133.9 (C-11) 148.3 (C-13).

_N,N'_-Di(_o_-nitrobenzyl)-(1,4-butanediyl)bis(2-chloromethylpiperidinium) dichloride 167a



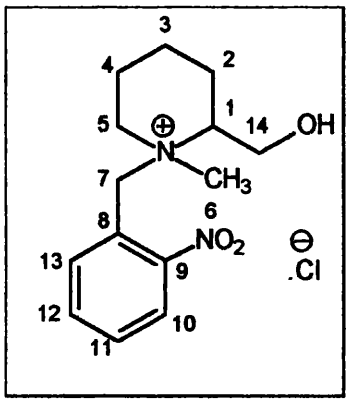
N,N'-Di(*o*-nitrobenzyl)-(1,4-butanediyl)bis(2-chloromethylpiperidinium) dichloride **167a** was obtained as in literature method[139] using **166a** (0.5 g, 1.7 mmol) in thionyl chloride (25 ml) as a solid which was recrystallised from EtOH and diethyl ether (0.35 g, 70 %), δ_H (200 MHz, D_2O) 1.50-2.98 (18H, m, ring and chain CH_2CH_2), 2.82-4.01 (12H, m, ring and chain CH_2N, CHN), 4.51 (4H, s, CH_2-Ar), 7.54 (2H, bs Ar-H), 8.03 (6H, bs, Ar-H);δ_C (50 MHZ, D_2O) 22.5 (C-3), 23.9 (C-4), 25.4 (C-2), 27.9 (C-8), 36.5 (C-1), 39.4 (C-6), 57.9 (C-5), 63.0 (C-7), 64.1 (C-9), 122.8, 128.6, 129.4, 133.4, 145.9 (C-10)(C-11)(C-12)(C-13)(C-14)(C-15).

N,N'-Di(*p*-nitrobenzyl)-(1,4-butanediyl)bis(2-chloromethylpiperidine) dibromide 167b



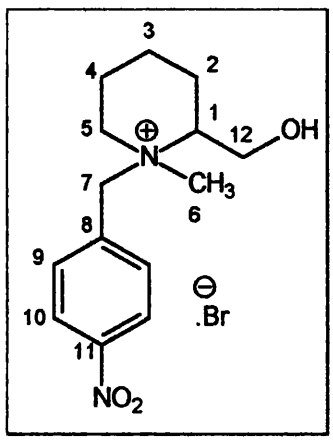
N,N'-Di(*p*-nitrobenzyl)-(1,4-butanediyl)bis(2-chloromethylpiperidine) dibromide **167b** was obtained as in literature preparation[139] using **166b** (0.5 g, 1.7 mmol) in thionyl chloride (20 ml) as a green crystalline solid which was recrystallised from EtOH and diethyl ether (0.23 g, 25 %); υ_{max} (KBr) 759, 1337, 1523 and 1636 cm^{-1}; δ_H (200 MHZ, D_2O) 1.54-2.05 (18H, m, ring and chain CH_2CH_2), 2.65-3.98 (12H, m, ring and chain CH_2N, CHN), 4.50 (4H, s, CH_2-Ar), 7.52 and 8.02 (8H, AA'BB' system, Ar-H); δ_C (50 MHZ, D_2O) 20.5 (C-3), 21.6 (C-4), 22.8 (C-2), 25.1 (C-8), 37.1 (C-6), 50.5 (C-7), 60.1 (C-5), 61.9 (C-1), 64.1 (C-9), 122.0, 128.1, 131.9, 146.9 (C-10) (C-11)(C-12)(C-13)(C-14)(C-15); *m/z* (FAB^+) 591.0 (*M-Br*, 1 %), 627.3, 629.3 (*M-Cl, Br*, 3 %), 647.3 (*M-Br*$^+$, 1.5 %).

2-Hydroxymethyl-*N*-methyl-*N*-(*o*-nitrobenzyl)piperidinium chloride 168a



2-Hydroxymethyl-*N*-methyl-*N*-(*o*-nitrobenzyl)piperidinium chloride **168a** was obtained as in literature preparation[139] using **45** (0.37 g, 2.91 mmol) in acetonitrile (20 ml), *o*-nitrobenzyl chloride (0.5 g, 2.91 mmol) as a light yellow solid (0.6g, 71 %); υ_{max} (KBr) 1064, 1341, 1527, 1604, 2871, 2945, 3231 and 3447 cm^{-1}; δ_H (200 MHz, D_2O) 1.40-1.86 (6H, m , ring CH_2), 2.80 (3H, s, CH_3N), 2.97-3.31 (2H, m, CH_2N), 3.48-3.59 (1H, m, CHN), 3.99 (2H, s, Ar-CH_2), 4.74-4.81, 5.34-5.41 (2H, d, CH_2O), 7.52-7.71, 7.99-8.04 (4H, m, Ar-H); δ_C (50 MHz, D_2O) 20.1 (C-3), 21.7 (C-4), 24.3 (C-2), 41.3 (C-6), 60.0 (C-5) 62.8 (C-7) 64.7 (C-14), 73.6 (C-1), 121.7 (C-10), 127.3 (C-12), 133.2 (C-9), 135.0 (C-8), 137.2 (C-13), 151.1 (C-11); *m/z* (FAB$^+$) 265.1 (*MH*+, 100 %).

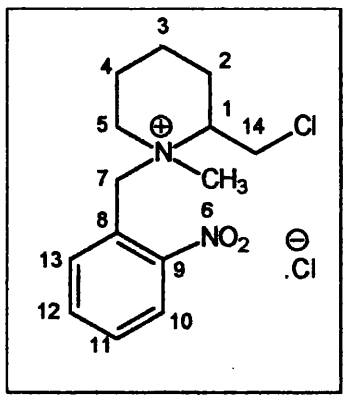
2-Hydroxymethyl-*N*-methyl-*N*- (*p*-nitrobenzyl)piperidinium bromide 168b



2-Hydroxymethyl-*N*-methyl-*N*-(*p*-nitrobenzyl)piperidinium bromide **168b** was obtained as in literature method[139] using **45** (4.0 g, 31 mmol) in acetonitrile (75 ml), *p*-nitrobenzyl bromide (6.69 g, 31 mmol) as a light yellow solid (11.4g, 106 %); υ_{max}

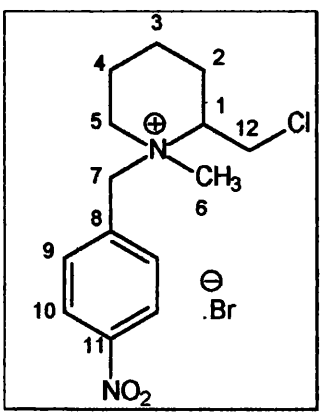
(KBr) 1045, 1330, 1352, 1518, 1604, 2869, 2943, 3338 and 3505 cm^{-1}; δ_H (200 MHz, D_2O) 1.62-1.80 (6H, m, ring CH_2), 2.95 (3H, s, CH_3), 3.14-3.19 (2H, m, CH_2N), 3.42 (1H, m, CHN), 4.79 (2H, s, Ar-CH_2), 4.36-4.63 (2H, m, CH_2O), 7.63-8.19 (4H, AA'BB' system, Ar-H); δ_C (50 MHz, D_2O) 20.2 (C-3), 21.6 (C-4), 24.1 (C-2), 43.7 (C-6), 60.7 (C-5), 63.3 (C-7), 68.3 (C-1), 72.0 (C-12), 124.7 (C-8), 134.8 (C-10), 135.4 (C-9),148.2 (C-11); *m/z* (Cl^+) 264 (*M-Br-H*$^+$, 8 %), 345 (*MH*$^+$,0.5 %).

2-Chloroethyl-*N*-methyl-*N*-(*o*-nitrobenzyl)piperidinium chloride 169a



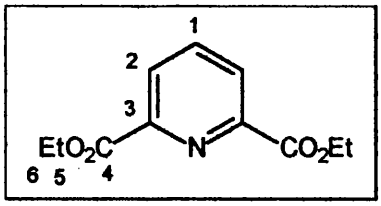
2-Chloroethyl-*N*-methyl-*N*-(*o*-nitrobenzyl)piperidinechloride **169a** was obtained as in literature preparation[139] using **168a** (0.5 g, 1.7 mmol) in thionyl chloride (10 ml), as in general method (c), as white crystals which were recrystallised from EtOH and diethyl ether (0.25 g, 46 %); υ_{max} (KBr) 749, 1347, 1533, 1633, 2872 and 2959 cm^{-1}; δ_H (200 MHz, D_2O) 1.20-1.61 (6H, m, ring CH_2), 2.43 (3H, s CH_3), 2.62-2.88 (2H, m, CHN), 3.35-3.49 (1H, m, CH), 3.62-3.75 (2H, m, CH_2Cl), 4.88 (2H, s, Ar-CH_2), 7.15-7.29, 7.56-7.61 (4H, m, Ar-H); δ_C (50 MHz, D_2O) 20.0 (C-3), 21.4 (C-4), 25.3 (C-2), 42.0 (C-6), 58.0 (C-9), 63.1 (C-5), 63.9 (C-7), 72.7 (C-1), 121.4, 127.1, 133.4, 135.2, 137.2, 150.7 (C-8)(C-9)(C-10)(C-11)(C-12)(C-13); *m/z* (Cl^+) 320.9 (*MH*$^+$, 1 %).

***N*-Methyl-*N*-(*p*-nitrobenzyl)piperidine-2-chloroethyl bromide 169b**



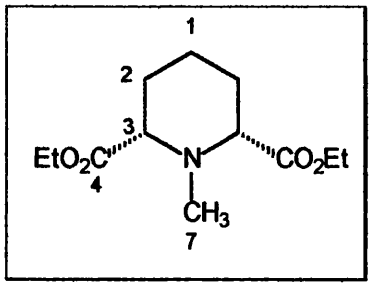
N-Methyl-*N*-(*p*-nitrobenzyl)piperidine-2-chloroethyl bromide **169b** was obtained as in literature method[139] using **168b** (3.0 g, 8.7 mmol) in thionyl chloride (30 ml), as in general method (c), as white crystals which were recrystallised from EtOH and diethyl ether (3.1 g, 98 %); υ_{max} (KBr) 750, 1351, 1492, 1523 and 1606 cm^{-1}; δ_H (200 MHz, D_2O) 1.62-1.80 (6H, m, ring CH_2), 2.99 (3H, s, CH_3), 3.14-3.19 (2H, m, CH_2N), 3.42 (1H, m, CHN), 3.65-3.85 (2H, m, CH_2Cl), 4.79 (2H, s, Ar-CH_2), 8.15-7.58 (4H, AA'BB' system, Ar-H); δ_C (50 MHz, D_2O) 20.2 (C-3) 21.5 (C-4), 25.2 (C-2), 41.9 (C-12), 42.5 (C-6), 63.5 (C-5), 67.8 (C-7), 70.1 (C-1), 124.9 (C-8), 134.3 (C-10), 135.3 (C-9), 149.8 (C-11); *m/z* (Cl^+) 148 (*M- Ar, Br*, 100 %), 283 (*M-Br*, 82 %), 365 (*MH+*, 0.2 %) (found: 283.1226 $C_{14}H_{20}N_2{}^{35}Cl$ requires 283.1213).

Diethyl-2,6-pyridinedicarboxylate 2



Diethyl-2,6-pyridinecarboxylate **2** was obtained as in previous literature preparation[141] using dipicolinic acid (5.0 g, 30 mmol) and thionyl chloride (15 ml) as white crystals afforded from MeOH (3.16 g, 47.2 %); δ_C (50 MHz, $CDCl_3$) 14.1 (C-6), 62.3 (C-5) 127.8 (C-2), 138.7 (C-1), 148.4 (C-3), 164.5 (C-4); lit[54] 14.1 (C-6), 62.2 (C-5), 127.7 (C-2), 138.1 (C-1), 148.4 (C-3), 164.4 (C-4).

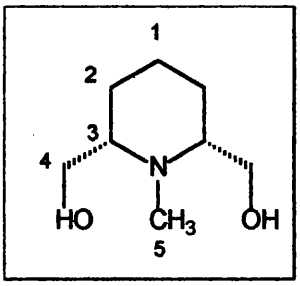
Diethyl *N*-methyl-*cis*-piperidinedicarboxylate



Diethyl *N*-methyl-*cis*-piperidinedicarboxylate was obtained as in literature preparation[54] as a white coloured oil using **2** (3.16 g, 14 mmol), glacial acetic acid (50 ml), platinum dioxide (0.2 g), 36 % formaldehyde solution (20 ml) and 10 % palladium

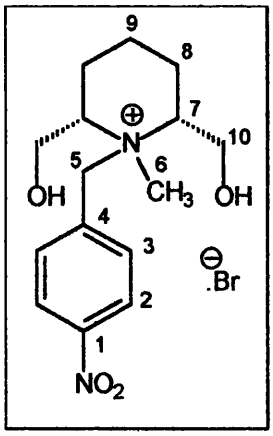
on carbon (0.20 g). (1.4 g, 44 %); δ_C (50 MHz, $CDCl_3$) 14.0 (C-6), 24.8 (C-1), 34.1 (C-2), 45.2 (C-3), 62.3 (C-5), 169.9 (C-4); lit[54] 13.9 (C-6), 25.0 (C-1), 33.9 (C-2), 45.3 (C-3), 62.1 (C-5), 169.6 (C-4).

_N_-Methyl-_cis_-2,6-bis(hydroxymethyl)piperidine 3



_N_-Methyl-_cis_-2,6-bis(hydroxymethyl)piperidine **3** was obtained as a white solid using literature preparation[54] from **2** (1.42 g, 6.22 mmol) in dry THF (20 ml) and lithium aluminium hydride (0.5 g, 13 mmol) in dry THF (20 ml) under nitrogen (0.2 g, 20 %); δ_C (50 MHz, $CDCl_3$) 23.6 (C-1),26.7 (C-2), 34.7 (C-5), 63.3 (C-4), 63.7 (C-3); lit[54] 23.7 (C-1), 26.7 (C-2), 34.9 (C-5), 63.5, 64.1 (C-3)(C-4).

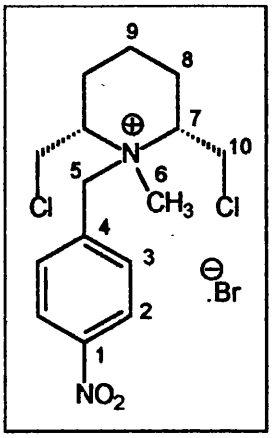
2,6-Di(hydroxymethyl)-_N_-methyl-_N_-(_p_-nitrobenzyl)piperidinium bromide 170



2,6-Di(hydroxymethyl)-_N_-methyl-_N_-(_p_-nitrobenzyl)piperidinium bromide **170** was obtained as in literature method[139] using _N_-methyl-_cis_-2 **3** (0.20 g, 1.25 mmol) in acetonitrile (30 ml) and _p_-nitrobenzyl bromide (0.27 g, 1.25 mmol) as a yellow/ orange solid residue (0.3 g, 64 %); υ_{max} (KBr) 1040, 1347, 1520, 1604 and 3421 cm^{-1}; δ_H (200 MHz, D_2O) 1.16-1.79 (6H, m, ring CH_2), 3.02 (3H, s, NCH_3), 3.45 (2H, dd, CH_2O), 3.86- 4.01 (2H, dd, CH_2O), 5.32 (2H, s, Ar-CH_2), 7.70-8.17 (4H, AA'BB'

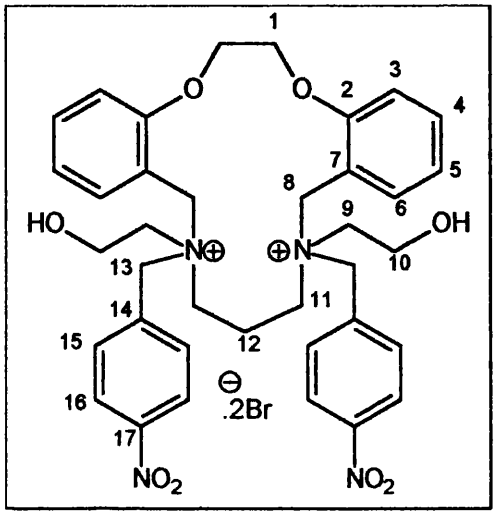
system, Ar-H); δ_C (50 MHz, D_2O) 24.1 (C-8), 26.9 (C-9), 56.3 (C-6), 67.0 (C-7), 68.0 (C-5), 69.1 (C-10), 122.1, 123.9, 135.0, 158.4 (C-1)(C-2)(C-3)(C-4).

2,6-Di(chloroethyl)-*N*-methyl-*N*-(*p*-nitrobenzyl)piperidinium bromide 171



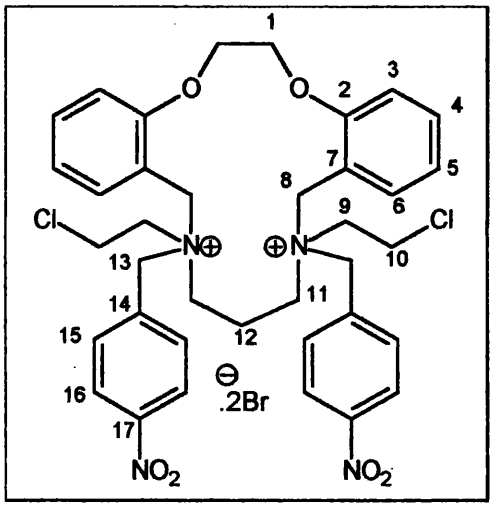
2,6-Di(chloroethyl)-*N*-methyl-*N*-(*p*-nitrobenzyl)piperidinium bromide **171** was obtained as in literature method[139] using **170** (0.30 g, 0.80 mmol) and thionyl chloride (20 ml), as in general method (c), as a pale yellow solid which was recrystallised twice from EtOH and diethyl ether (0.06 g, 20 %); υ_{max} (KBr) 704, 1348, 1473, 1522 and 1605 cm^{-1}; δ_H (200 MHz, D_2O) 1.54-1.90 (6H, m, CH_2 ring), 2.68 (3H, s CH_3), 2.80 (2H, s, Ar-CH_2), 3.27-3;39 (2H, m, CHN), 3.63-3.90 (4H, dd, CH_2Cl), 7.63-7.70, 8.07-8.15 (4H, AA'BB' system, Ar-H); δ_C (50 MHz, D_2O) 22.1 (C-8), 24.3 (C-9), 39.3 (C-10), 58.2 (C-6), 67.4 (C-7), 68.1 (C-5), 124.9 (C-4), 125.3 (C-2), 135.2 (C-3),159.4 (C-1); *m/z* (Cl^+) 205 (*MH*$^+$, 100 %).

***N,N'*-Di(hydroxyethyl)-5,6:14,15-dibenzo-1,12-diaza-1,4-dioxacyclopentadecane *N,N'*-(*p*-nitrobenzyl)piperidinium dibromide 172**



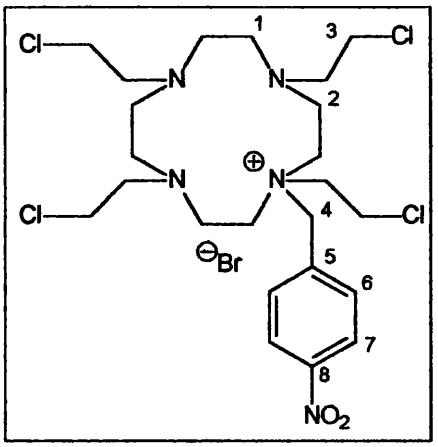
N,N'-Di(hydroxyethyl)-5,6:14,15-dibenzo-1,12-diaza-1,4-dioxacyclo pentadecane-*N,N'*-(*p*-nitrobenzyl)piperidinium dibromide **172** was obtained as in literature method[139] using **133** (0.20 g, 1.25 mmol) in acetonitrile (30 ml), *p*-nitrobenzyl bromide (0.75 g, 3.2 mmol) as a light yellow solid residue (1.15 g, 86 %); υ_{max} (KBr) 1051, 1360, 1498, 1598, 2958 and 3357 cm^{-1}; δ_H (200 MHz, D_2O) 1.73-1.83 (2H, m, CH_2CH_2), 3.0-3.65 (12H, m, NCH_2), 3.70 (4H, s, Ar-CH_2), 3.82-4.10 (8H, m, OCH_2), 7.08-7.45, 7.56-7.83 (16H, m, Ar-H); δ_C (50 MHz, D_2O) 21.9 (C-12), 30.0 (C-10), 42.9, 50.9, 57.4 (C-8) (C-9) (C-11), 64.5 (C-13), 68.2 (C-1), 127.0, 127.4, 131.2, 133.2, 136.5, 138.9, 149.1 (C-2)(C-3)(C-4) (C-5)(C-6)(C-7)(C-14)(C-15)(C-16)(C-17).

_N,N'_-Di(chloroethyl)-5,6:14,15-dibenzo-1,12-diaza-1,4-dioxacyclopentadecane _N,N'_-(_p_-nitrobenzyl)piperidinium dibromide 173



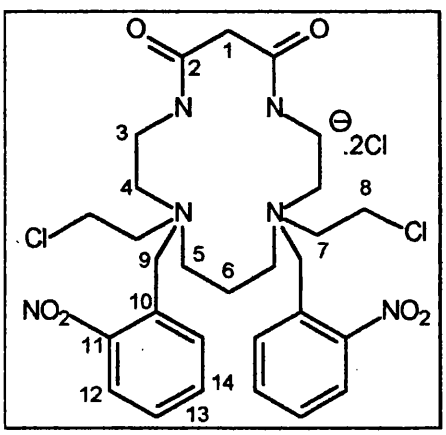
N,N'-Di(chloroethyl)-5,6:14,15-dibenzo-1,12-diaza-1,4-dioxacyclopentadecane *N,N'*-(*p*-nitrobenzyl)piperidinium dibromide **173** was obtained by general method (c) using **172** (1.15 g, 1.4 mmol) and neat thionyl chloride (30 ml). The product was obtained as a biege solid which was recrystallised from EtOH and diethyl ether (0.50 g, 43 %); υ_{max} (KBr) 758, 1348, 1494, 1525 and 1603 cm^{-1}; δ_H (200 MHz, D_2O) 1.59-1.61 (2H, m, CH_2CH_2), 3.04-3.11 (12H, m, NCH_2), 3.40 (2H, s, Ar-CH_2), 3.59-3.67 (8H, m, OCH_2, CH_2Cl), 7.00-7.25, 7.52-7.69 (16H, m, Ar-H); δ_C (50 MHz, D_2O) 21.9 (C-12), 30.0 (C-10), 42.9, 50.9, 55.2 (C-8) (C-9) (C-11), 64.5 (C-13), 68.2 (C-1), 127.5, 127.7, 130.2, 130.4, 136.3, 138.5, 144.0 (C-2)(C-3) (C-4)(C-5)(C-6)(C-7)(C-14)(C-15)(C-16)(C-17).

N,N',N'',N''' -Tetra(chloroethyl)-1,4,7,10-tetraazacyclododecane*N*-(*p*-nitrobenzyl) piperidinium bromide 175



N,N',N'',N'''-tetra(chloroethyl)-1,4,7,10-tetraazacyclododecane*N*-(*p*-nitrobenzyl) piperidinium bromide **175** was obtained by general method (c) using **174** (1.00 g, 1.0 mmol) and neat thionyl chloride (30 ml). The product was obtained as a white solid and recrystallised from EtOH and diethyl ether (0.32 g, 31 %); υ_{max} (KBr) 744, 1350, 1443, 1526 and 1608 cm^{-1}; δ_H (200 MHz, D_2O) 3.09-3.79 (24H, m, NCH_2), 3.52 (2H, s, Ar-CH_2), 3.74-3.81 (8H, m, $ClCH_2$), 7.62-8.22 (4H, dd, Ar-H); δ_C (50 MHz, D_2O) 39.2 (C-3), 48.9, 55.1, 62.2 (C-1)(C-2)(C-4), 125.1, 136.1, 137.3, 149.8 (C-5)(C-6)(C-7)(C-8) .

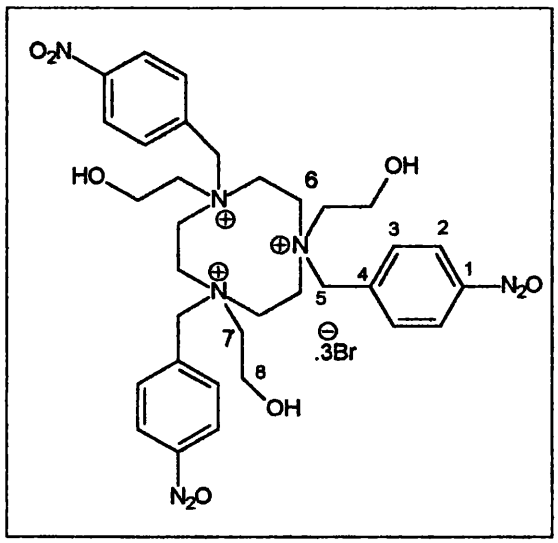
1,4-Di(hydroxyethyl)-1,4,8,11-tetraazacyclododecane-5,7-dione-*N, N'*-(*o*-nitrobenzyl) dichloride 177



1,4-Di(chloroethyl)-1,4,8,11-tetraazacyclododecane-5,7-dione-*N,N'*-(*o*-nitrobenzyl) dichloride **177** was obtained by general method (c) using **176** (0.1 g,

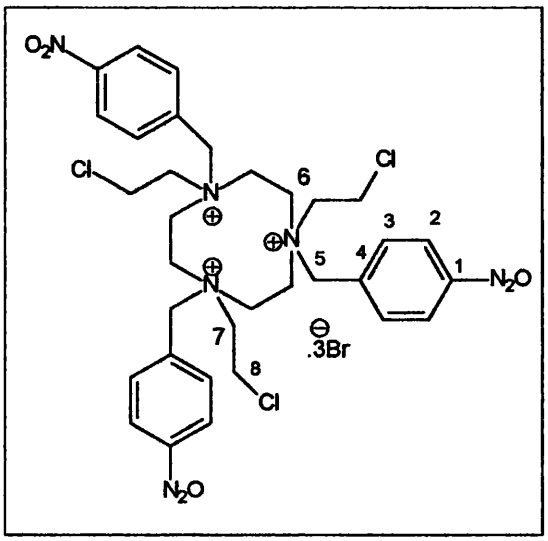
0.15 mmol) and neat thionyl chloride (20 ml). The product was obtained as an off white solid and recrystallised from EtOH and diethyl ether (0.05 g, 48 %); υ_{max} (KBr) 749, 1334, 1483, 152, 1605 and 1685 cm^{-1}; δ_C (50 MHz, D_2O) 28.2 (C-6), 39.2, 41.2 (C-1)(C-8),53.5, 55.5, 58.1, 58.9 (C-3)(C-4)(C-5)(C-7), 62.9 (C-9), 123.1, 127.5, 134.5, 135.9, 136.1, 137.6, 152.1 (C-10)(C-11)(C-12)(C-13)(C-14) (C-15) and 169.2 (C-2); *m/z* (FAB^+) 659.2 (M-Cl, 6 %).

N,N',N''-Tri(hydroxyethyl)-N,N',N''-tri(*p*-nitrobenzyl)-1,4,7-triazacyclononanium tribromide 179



N,N',N''-Tri(hydroxyethyl)-*N,N',N''*-tri(*p*-nitrobenzyl)-1,4,7-triazacyclononanium tribromide **178** was obtained by literature method[139] using **77** (0.43 g, 1.6 mmol) in acetonitrile (30 ml) and *p*-nitrobenzyl bromide (1.08 g, 5 mmol) as a off white solid (1.0 g, 69.4 %); υ_{max} (KBr) 1079, 1349, 1524, 1608, 2856, 2959 and 3368 cm^{-1}; δ_H (200 MHz, D_2O) 4.48 (6H, s, NCH_2), 4.31-4.61 (2H, s, OCH_2), 4.71 (2H, s, Ar-CH_2), 7.51-8.16 (4H, AA'BB' system, Ar-H); δ_C (50 MHz, D_2O) 50.0 (C-8), 58.0 (C-6), 58.6 (C-7), 59.2 (C-5)4, 123.9 (C-2), 129.9 (C-3), 144.8 (C-1).

N,N',N''-Tri(chloroethyl)-N,N',N''-tri(*p*-nitrobenzyl)-1,4,7-triazacyclononanium tribromide 180



N,N',N''-tri(chloroethyl)-*N,N',N''*-tri(*p*-nitrobenzyl)-1,4,7-triazacyclononane tribromide **179** was obtained as in literature method[139] using **178** (1.0 g, 1.1 mmol) in thionyl chloride (20 ml) as an off white solid which was recrystallised from EtOH and diethyl ether (3.16 g, 47.2 %); υ_{max} (KBr) 1111, 1299, 1349, 1503, 1524, 1606 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 3.57 (2H, CH_2Cl), 4.48 (4H, s, CH_2O), 4.31-4.38 (4H, s, NCH_2), 4.57 (4H, s, Ar-CH_2), 7.48-8.15 (4H, AA'BB' system, Ar-H); δ_C (50 MHz, $CDCl_3$) 44.4 (C-8), 57.7 (C-7), 58.0 (C-6), 64.0 (C-5), 123.8 (C-2) 124.1 (C-4), 129.3 (C-3), 144.4 (C-1); *m/z* (Cl^+) 759.2 (M^+-NO_2Ar, 100 %).

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