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Synthesis of Some 8-Aza-7-deazaguanines as Potential Inactivators of ATase

by

Úna Hanafin

A thesis presented to the University of Dublin for the degree of Doctor of Philosophy

Chemistry Department
Trinity College
Dublin

September 2003
Declaration

This thesis has not been submitted as an exercise for a degree at any other university. Except where stated, the work described therein was carried out by me alone.

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September 2003
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Abstract

Certain cancer chemotherapeutic agents act by alkylating the $O^6$-position of guanine residues in DNA. This lesion can ultimately cause the death of the targeted cancer cells. However, the efficacy of the cytotoxic effect of these alkylating agents may be greatly reduced by the action of the ubiquitous repair protein, ATase ($O^6$-alkylguanine-DNA alkyltransferase). ATase transfers the damaging alkyl group from the alkylated guanine to one of its cysteine residues in an autoinactivating reaction. Pretreatment with a pseudosubstrate of ATase prior to the administration of the alkylating agent would knock out the ATase and can improve the cytotoxic effect of the chemotherapy.

$O^6$-(4-Bromothenyl)guanine (or PaTrin-2) has been found to be an excellent inactivator of ATase. Phase 2 trials of PaTrin-2 with temozolomide are currently in progress. However, PaTrin-2 shows poor solubility in aqueous media and has limited selectivity for cancerous tissue. In an attempt to overcome these problems, derivatives of PaTrin-2 have been investigated for their ATase inhibiting properties.

The synthesis of compounds based on the 8-aza-7-deazaguanine ring system has been investigated. In Chapter 2, the unambiguous synthesis of $O^6$-(alkoxy)-8-aza-7-deazaguanines bearing alkyl substituents in the 9-position is described. The synthesis of some of the corresponding 8-aza-7-deazapurine compounds (the 2-amino group is replaced by a proton) is also described. Comparison of the biological activities of both sets of compounds offers a good indication of the importance of the 2-NH$_2$ to the mechanism of inactivation of ATase.

Chapter 3 deals with the synthesis of alkoxycarbonyl derivatives of $O^6$-(4-bromothenyl)-8-aza-7-deazaguanine (or alloPaTrin). Depending on the alkoxycarbonylating reagent used, substitution of alloPaTrin can occur at the 8- or 9-positions. Potentially these derivatives may act as prodrugs of alloPaTrin, by breaking down gradually to release free alloPaTrin and thereby possibly lengthening the duration of action of the parent drug.
The syntheses of mono- and di-alkylcarbamoyl derivatives of alloPaTrin are discussed in Chapter 4. Reaction conditions for selective substitution at the 8- or 9-positions, or in some cases, at the 2-amino group, are discussed.

The biological activities of all the alloPaTrin derivatives are outlined in Chapter 5. The relative stabilities of the some acylated derivatives of alloPaTrin are also explored.
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Abbreviations

ATase $O^6$-Alkylguanine-DNA-alkyltransferase
d doublet
DABCO 1,4-diazabicyclo[2.2.2]octane
DBU 1,5-diazadicyclo[5.4.0]undec-5-ene
DCM dichloromethane
DMAP 4-dimethylaminopyridine
DMF N, N-dimethylformamide
DMSO methyl sulfoxide
DNA deoxyribonucleic acid
EtOAc ethyl acetate
EtOH ethanol
g gram
HMBC Heteronuclear Multiple Bond Correlation
HMQC Heteronuclear Multiple Quantum Coherence
Hz Hertz
IR infrared
J coupling constant
m multiplet
MeOH methanol
ml millilitre
mol mole
mp melting point
MS (ES) mass spectrometry (electron spray)
NMR Nuclear Magnetic Resonance
nOe Nuclear Overhauser Effect
q quaternary carbon
quart quartet
quin quintet
Rf retention factor
s singlet
t triplet
THF tetrahydrofuran
TLC thin layer chromatography
uv ultraviolet
Experimental

Nuclear magnetic resonance spectra were recorded by a Brüker DPX 400 MHz spectrometer, operating at 400.13 MHz (proton) and 100.62 MHz (carbon) using the solvent signal as an internal lock. Chemical shifts were quoted in parts per million and coupling constants (J) were quoted in Hertz (Hz). Infrared spectra were recorded by a Mattson Genesis II FTIR spectrophotometer, using either nujol mulls or liquid films. Ultraviolet spectra were recorded, using solutions of the compounds in methanol, by a Unicam UV4 spectrometer. Accurate mass was determined by a Micromass LCT TOF mass spectrometer, using solutions in acetonitrile. Elemental analyses were carried out at the Microanalytical Laboratory, University College Dublin. Melting points were determined using a Stuart Scientific SMP1 melting point apparatus and are uncorrected.

Thin layer chromatography was carried out using Merck Kieselgel 60 F254 silica gel plates, which were visualised by ultraviolet light (254 nm). Flash chromatography was carried out using Merck Kieselgel 60 silica gel (230-400 mesh). Dry DMF (99.8% anhydrous) was purchased from Aldrich. THF was dried by treatment with sodium hydroxide pellets, followed by sequential distillations from calcium hydride and sodium wire/benzophenone.
Chapter 1

Introduction
1.1 Introduction

Cancer is a word used to describe a group of illnesses, which all share particular characteristics. In very general terms, all cancers stem from a lack of regulated cell growth in the body. Cancerous or neoplastic* cells can grow out of control and consequently can invade or destroy normal healthy tissue. Cancer is a systemic disease. Over two hundred different types of cancer have been identified. Each cancer has a different name and each is treated using a specific treatment regimen. Some respond readily to treatment, while many others result in death, even after the most vigorous treatment. Figure 1.1 shows the ten most common cancers diagnosed in the UK in 1999.

![Figure 1.1 The ten most common cancers diagnosed in the UK in 1999.](image)

Every year in Ireland, approximately twenty thousand people are diagnosed with cancer, while seven and a half thousand people die from the disease. It is estimated that one in three people will develop some form of cancer during their lifetimes. These statistics highlight the need for a better understanding of the causes and

* Neoplasm is a general term for an abnormal new growth of tissue or tumour.
mechanisms of the various cancers, and the subsequent improvement in the treatment of the diseases.

1.2 What is Cancer? 1,3

Cancer is a disease of our genes. Every cell in the body has a specific function. When cells are damaged or die, they are usually replaced by an exact replica. This replication process is controlled by genes. When these genes become damaged, or more specifically when the DNA or genetic code becomes damaged, an error can occur in this replication process. Cancer is caused by this error. The cell, which has become cancerous as a result of this error, does not heed the body’s normal control mechanisms which stop growth. The continuous and rapid production of these abnormal cells can give rise to the growth of cancerous tumour, which may invade and destroy other healthy tissues.

Cancer cells are abnormal in many ways besides their lack of self-regulation over cell division. They are all descendants of a single cell, grow without regard to the function of the tissue of this parent cell, and they reproduce much faster than normal cells.

Figure 1.2 Cancerous tissue showing physical abnormalities, which are absent from the ordered healthy tissue.
Due to abnormalities in the surfaces of the cancer cells, they may lose their attachments to neighbouring cells, which enables them to spread to other tissues surrounding the original tumour. If they enter the circulatory system, the cancer cells may spread beyond their original sites, to form cancerous tissue in distant parts of the body, in a process known as metastasis.

### 1.3 How is a Normal Cell Transformed into a Cancer Cell? 1,3

The development of cancer appears to involve multiple causes. These causes include both hereditary and environmental factors. The risk of cancer increases with age. An older person has had more exposure to environmental factors, and may have a diminished immune response. The majority of cancers are caused by genetic alterations in proto-oncogenes. These are the normal cellular genes, which are responsible for the regulation of cell growth, cell division and cell adhesion. Proto-oncogenes become cancerous when they mutate to oncogenes (*onco* is the Greek word for tumour). A mistake in the genetic code of the cell can cause the cell to grow without control and cease to function in any constructive way.

Many cancers are triggered by environmental factors. Exposure to cancer causing agents or carcinogens, together with age and lifestyle factors all can contribute to the development of cancer. Carcinogens are agents, which cause DNA to mutate. Cancer develops when this mutation causes the cell to grow without control and to become immortal.

Carcinogens 1,4 can be subdivided into three different classes:

(a) Physical carcinogens: The physical mutation of DNA can be caused by ionising radiation, ultraviolet radiation and by mineral fibres. Ionising radiation (such as from X-rays or solar radiation) causes the formation of gaps in the genetic sequence. Ultraviolet radiation (from sunlight) causes mutations by binding certain portions of DNA together.

(b) Chemical carcinogens: Chemical carcinogens cause mutation by binding to the DNA, which causes mismatches in the DNA sequence. Examples of known chemical carcinogens are 2-naphthylamine, vinyl chloride and benzo[a]pyrene.
(c) Biological carcinogens: Biological carcinogens may be viral or bacterial. They can cause a cell to become cancerous through a series of complex mechanisms. Examples include the papilloma virus, which has been linked to the cancer of the cervix, while the hepatitis B virus appears to cause liver cancer in individuals with chronic infections.

1.4 Cancer Therapy
The main types of cancer therapy are:

(a) Surgery\(^{1,5}\), if feasible, is often the first and most successful treatment for a tumour, which has not undergone metastasis. It is frequently followed by other forms of cancer treatment.

(b) Radiotherapy\(^{1,5}\) involves the treatment of cancerous tissue by high energy beams of radiation (x-ray, proton beams, radioisotopes). The radiation causes mutations to occur in the fast growing tumour cells, which cause the cells to die. This is only useful on tumours which have not undergone metastasis.

(c) Immunotherapy\(^{1,5}\): The principle behind anti-cancer immunotherapy involves the improvement of the body’s own ability to seek out and destroy rogue cancer cells. The therapy stimulates the immune system through various mechanisms to fight cancer. Cancer immunotherapy can be subdivided into a number of categories. These include biological response modifiers such as the proteins, interferon and interleukin, which stimulate the immune system to act more effectively. Colony-stimulating factors are substances, which boost the production of blood cells. In some cases, increased levels of blood cells can improve the body’s tolerance of other cancer therapies. Vaccines, which may assist the patient’s immune system to recognise cancer cells, are being investigated. Monoclonal antibodies are also in development. These antibodies can react with proteins, which are associated with the neoplasm, and may aid tumour detection or direct drug delivery specifically to the site of the tumour.

(d) Chemotherapy\(^{1,5,1,6}\): In cases where surgery or radiotherapy is insufficient to treat the cancer, chemotherapy is required. Current chemotherapy consists mainly of cytotoxic or cell-killing agents, which prevent the cancer cells from reproducing.
Thousands of compounds are known which have excellent antineoplastic properties, however relatively few are in clinical use due to their lack of selectivity. The clinical success of the majority of chemotherapy drugs depends on the rapid nature of cell division of cancer cells. However, healthy cells in bone marrow, the gastrointestinal tract and hair follicles divide at least as rapidly as cancer cells. Therefore, the lack of selectivity of some chemotherapeutic agents frequently causes significant side-effects such as anaemia, internal bleeding, nausea, diarrhoea and hair loss. In most cases, these side-effects limit the dosage of the chemotherapeutic drug which can be administered to the patient. Consequently the efficacy of these drugs is restricted.

To combat these problems, an oncologist frequently treats a cancer patient with cycles of the antineoplastic agent. Theoretically this would allow the non-cancerous tissue, which was damaged as a result of the chemotherapy, to recover in between cycles. However, this strategy appears to cause an increase in the resistance of the cancer to the anti-cancer agent. In practice, an early response to chemotherapy may be followed by a relapse. The progressing or recurring tumour may display a greater resistance to chemotherapy. In general these problems apply to the vast majority of chemotherapy agents.

Chemotherapy drugs vary dramatically in their structures and respective modes of action. Their structures range from analogues of substances, which are found in the body to very complex natural products and their derivatives, and even inorganic complexes such as cis-platin. Some inhibit the synthesis of nucleic acids (e.g. methotrexate and 5-fluorouracil). Many attack the cancer cell during cell division. Vinblastine, found in the periwinkle plant, attacks the microtubules during cell division, causing their dissolution and ultimately cell death. The use of Taxol as a chemotherapeutic agent has been a significant development in the treatment of breast cancer; however its precise mode of action is still not fully understood. Cancer chemotherapeutic agents which act as alkylating agents will be discussed in more detail later (Section 1.6, p.9).
1.01

1.02

5-Fluorouracil
1.03

Vinblastine
1.04a
In most cases, patients are given a combination of treatments. This may involve surgery or radiotherapy followed by chemotherapy. It is usual for chemotherapy to consist of a “cocktail” of different drugs, rather than a single anti-neoplastic agent.

1.5 Chemotherapy in the Future

Recent developments in genetics have led to rapid advances in the understanding of the genetic processes, which cause cancer. While gene therapy remains very much the holy grail of all chemotherapy, a greater knowledge of cancer at a molecular level has led to the discovery of new types of cancer chemotherapy drug.

Among these new classes of drugs are kinase inhibitors.\textsuperscript{1,12} Protein kinases are the principle component of the signal transduction pathway. This pathway plays a major role in a wide range of biological processes including the control of cell growth, metabolism, differentiation, and apoptosis. The cells of some types of cancer have abnormalities in these kinase proteins of the signalling pathways. Hence it may be possible to target the proteins for selective inhibition, and therefore disrupt the cancer cells sufficiently to cause the death of the cell. It is hoped that a kinase protein of individual cancers can be identified, which would act as an extremely specific target for chemotherapy, thereby drastically increasing the efficacy of the cancer treatment and avoiding major unwanted side-effects.
The first drug of this family of signal transduction inhibitors to reach patients was Gleevec 1.06, which was released by Novartis in late 2001. This heterocycle, which is based on a phenylaminopyrimidine moiety, is used with much success to treat a type of blood cancer called chronic myeloid leukaemia (CML).

\[
\text{CH}_3
\]

1.06

CML is caused by the constant production of abnormal white blood cells. This phenomenon has been attributed to a kinase protein, which controls white blood cell production and is not found in other parts of the body. Gleevec inhibits this protein, and therefore stops the production of the cancer-causing cells. More recently Gleevec has been used for the treatment of malignant gastrointestinal stromal tumours.

In May 2003, the US Food and Drug Administration (FDA) approved the use of another kinase inhibitor, Iressa 1.07, to treat non-small cell lung cancer, the most common form of lung cancer, in patients who have failed to respond to platinum-based and Taxol chemotherapies.

\[
\text{H}_3\text{C}
\]

1.07

Recently questions have been raised over the potential side-effects of the drug. There is growing evidence that Iressa may trigger an aggressive and sometimes fatal form of
pneumonia called interstitial lung disease (ILD).\textsuperscript{1,15} Despite this possible set-back in the development of this new family of drugs, over twenty protein kinase inhibitors are known to be undergoing human clinical trials.

1.6 Alkylating Agents

While the development of new generations of cancer chemotherapy is very welcome, the use of these new families of drugs to treat cancer sufferers is very much in its infancy and it remains to be seen how successful a part they will come to play in the treatment of cancer. Until these therapies are more advanced, clinicians must rely on standard chemotherapeutic cytotoxic agents. Hence, research into the improvement of this approach is ongoing.

Alkylating agents, their use in chemotherapy and their use in conjunction with “chemoenhancers” are of particular interest to us. Alkylating agents are the oldest class of anti-cancer drug.\textsuperscript{1,16} They originate from mustard gas \textbf{1.08}, which was used as an antipersonnel weapon during World War 1. During an investigation of potential war gases during World War 2, researchers noticed that the mustard gas analogue, mustine \textbf{1.09}, caused a reduction in the white blood cell count, and was subsequently used as an anti-leukaemia agent in the 1940s.

\begin{center}
\begin{tabular}{cc}
\textbf{1.08} & \textbf{1.09} \\
\end{tabular}
\end{center}

In general alkylating agents used in cancer treatment can be divided into two categories, chloroethylating compounds and methylating agents. They act by targeting the oxygen and nitrogen atoms in the bases of DNA and the phosphodiester bonds between the bases, which are all potential sites of alkylation.\textsuperscript{1,17} Figure 1.3 shows the alkylation sites of the DNA bases and the relative amounts of the products, when \textit{N}-methyl-\textit{N}-nitrosourea is used as the alkylating agent.\textsuperscript{1,18} These percentages can vary depending on the alkylating agent used.
Figure 1. 3\textsuperscript{1,18} Alkylation sites in DNA base-pairs. The sites of attack are shown in blue. The relative amounts of the products are given for the methylating agent, $N$-methyl-$N$-nitrosourea. Alkylation of the phosphodiester accounts for the remaining ~12%.

The introduction of an alkyl group into the $O^6$-position of guanine gives the most lethal of these adducts, even though it does not form in high yields. This resulting adduct, which is strongly miscoding, can pair with thymine instead of cytosine during DNA replication.\textsuperscript{1,19} This transition mutation can cause the death of the cell. The $O^6$-MeG:T mispair is recognised by a repair mechanism, the so-called post-replication mismatch repair pathway (MMR), as a mistake. However instead of removing the species responsible for the mispair (i.e. $O^6$-methylguanine), the MMR pathway removes the thymine residue from the DNA strand. This in turn is replaced by another thymine residue resulting in a futile cycle of mis-match and repair. The retention of $O^6$-methylguanine in the DNA strand ultimately leads to the death of the cell.\textsuperscript{1,20}
1.6.1 Nitrosourea Alkylating Agents

Alkylating agents damage DNA by either methylation (for example temozolomide 1.10) or chloroethylation (for example N-(2-chloroethyl)-N-nitrosoureas 1.11). Agents belonging to the latter category cause the formation of an interstrand cross-link between guanine and cytosine on the opposite strand of DNA (see Scheme 1.1). The chloroethyl diazonium ion (from the nitrosourea) reacts with guanine to produce O\(^6\)-(2-chloroethyl)guanine 1.12. This initial product is unstable and undergoes an intramolecular rearrangement giving the 1,O\(^6\)-ethanoguanine 1.13. This adduct can react with a cystosine residue to form an \(N^{'H}\)-guanine, \(N^3\)-cytosine interstrand cross link 1.14.
Scheme 1. 1 The formation of a DNA-interstrand crosslink caused by a \( N-(2\text{-chloroethyl})-N\text{-nitrosourea} \).\(^\text{1,21}\)

### 1.6.2 Methylating Agents

Methylating agents can give rise to the formation of \( O^6\)-methylguanine as mentioned previously. This adduct can mispair with thymine during DNA replication. An example of an antineoplastic methylating agent is temozolomide \( 1.10 \).\(^\text{22}\) This imidazotetrazine compound converts to the reactive methylating agent MTIC (methyltriazenylimidazole-4-carboxamide) \( 1.15 \), which in turn degrades to release the transient methyl diazonium cation. This cation methylates various sites on the DNA.
bases.\textsuperscript{123} $O^6$-Methylguanine \textbf{1.16} accounts for only about 5\% of the total adduct formed by temozolomide, but it has been shown that this adduct is the principle cause of toxicity of temozolomide to cancer cells.\textsuperscript{24}

\begin{equation}
\begin{aligned}
\text{O\textsuperscript{6}-Methylguanine} &\quad \text{ accounts for only about 5\% of the total adduct formed by temozolomide, but } \\
\text{it has been shown that this adduct is the principle cause } &\quad \text{of toxicity of temozolomide to cancer cells.}
\end{aligned}
\end{equation}

\begin{equation}
\begin{aligned}
\text{NH} &\quad \text{O} \\
\text{O} &\quad \text{pH>7} \\
\text{O} &\quad +\text{H}_2\text{O} \\
\text{CH}_3 &\quad -\text{CO}_2
\end{aligned}
\end{equation}

\textbf{Scheme 1. 2}

\textbf{1.7 Resistance to Alkylating Agents}
Many types of cancer cells have a marked resistance to the cytocidal effects of alkylating agents. Studies have shown that there is a direct correlation between the sensitivity of tumour cells to alkylating agents, and the activity of the DNA repair protein, $O^6$-alkylguanine alkyltransferase or ATase.\textsuperscript{125}

\textbf{1.7.1 ATase}\textsuperscript{126}
$O^6$-Alkylguanine-DNA-alkyltransferase (ATase) is an important DNA repair protein, which repairs specifically the damaged nucleotide, $O^6$-alkylguanine, in DNA. This premutagenic and potentially lethal lesion may be induced by many environmental chemicals and its repair by ATase is vital to maintain healthy tissue. However,
because of its role in DNA repair, the presence of ATase limits the effectiveness of certain anti-cancer alkylating agents.

Figure 1. 4 Pathways of the biological effects of methylating agents. $S^1$ and $S^2$ are first and second rounds of DNA replication after methylating agent damage.

The protein repairs $O^6$-alkylguanine by acting as an alkyl group acceptor. The alkyl of the $O^6$-alkylguanine species transfers stoichiometrically to a sulfhydryl group of a cysteine residue of ATase (see Scheme 1.3). This alkyl group transfer produces a neutral guanine species either by protonation of the liberated guanine anion or by protonation of the $O^6$-alkylguanine residue before its reaction with the cysteine residue to produce a zwitterionic neutral guanine residue. Whichever the exact mechanism sequence, the net result is guanine at the original site of the $O^6$-alkylguanine residue, and an S-alkylcysteine residue is produced at the protein’s active site. This S-alkylcysteine residue is very stable and there is no apparent mechanism to restore the normal active-site cysteine. Once its primary function of DNA repair by “self-alkylation” is carried out, ATase has no way of regenerating itself and can therefore be termed a “suicide protein”.

\[
\begin{align*}
\text{methylating agent} & \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad 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\quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad 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1.8 Strategies to Overcome This Resistance

Therapeutic strategies have been designed to combat this *de novo* resistance to cytotoxic alkylating agents. ATase provides an important mode of resistance to chemotherapeutic alkylating agents, and many tumour types have a particularly high concentration of ATase in their cells. This problem may be addressed by altering the schedule of administration of the anti-neoplastic alkylating agent. It was thought that if subsequent doses of the drug were administered at the ATase nadir following the previous dose, it would potentiate the efficacy of the alkylating agent as an anti-cancer drug. The damaging alkyl group cannot be removed from the $O^6$-position of guanine until sufficient fresh ATase has been synthesised to carry out the repair reaction. However, it was found that this scheduling caused a significant and unacceptable increase in the toxicity of the chemotherapy drug. Alternatively in order to maximise the effectiveness of the alkylating agent as a chemotherapeutic drug, it might be administered in conjunction with an ATase inhibiting “chemoenhancer”, which could reverse the mechanism of resistance in the tumour.

However this strategy of ATase inhibition to enhance the effects of alkylating agents as anti-neoplastic agents is not without its problems. ATase is a ubiquitous protein. It is found in all human organs and tissue, and is necessary for the repair of damaged DNA. The depletion of ATase in order to increase the sensitivity of cancer cells to the chemotherapeutic effects of alkylating agents must be achieved in such a manner so as not to increase significantly the toxicity of alkylating agents to healthy cells. A
significant depletion of ATase in normal cells would necessitate a reduction in the dosage of chemotherapeutic agent.

1.9 ATase Inactivators

In 1985, Pegg reported that $O^\delta$-methylguanine 1.16 acts as a pseudosubstrate for ATase and can inactivate ATase from human cancer cells.\textsuperscript{1,28} ATase was found to demethylate $O^\delta$-methylguanine in an autoinactivating irreversible reaction, to give free guanine and an $S$-methylcysteine residue in the active site of the protein. $O^\delta$-Methylguanine had limited clinical potential because of the high dose of the drug which would have been required, due to its low solubility in aqueous media, low affinity for ATase, poor uptake in cells and the lack of selectivity for ATase depletion.\textsuperscript{1,29} However, it did act as a lead compound in the search for a suitable inactivator of ATase and led to an investigation into the ability of a wide range of $O^\delta$-alkylguanines to inhibit ATase. Of these, $O^\delta$-benzylguanine 1.17 was found to inactivate the protein 2000 times more effectively than $O^\delta$-methylguanine.\textsuperscript{30} $O^\delta$-Benzylguanine was a better candidate for clinical use than $O^\delta$-methylguanine as it deactivated ATase at a much faster rate than the methyl compound. It also had improved solubility and a superior cellular uptake.

\[ \text{Diagram of } O^\delta\text{-Benzylguanine} \]

$O^\delta$-Benzylguanine 1.17 is not an ideal choice of ATase inhibitor to go into clinical use. Even though it is more soluble than $O^\delta$-methylguanine, it still has limited solubility in water, which makes its formulation difficult for clinical use in humans. Clinical trials of $O^\delta$-benzylguanine with the alkylnitrosourea carmustine 1.18 (also called BCNU) have produced some discouraging results.\textsuperscript{1,31}
Pretreatment with \( O^\beta \)-benzylguanine causes an increase in the cytotoxicity of carmustine in healthy tissue. Consequently, the maximum tolerated dose of the alkylating agent had to be lowered significantly compared to the maximum tolerated dose, which could be safely used when the patient was not pretreated with an ATase inhibitor. The usual dose of carmustine is 200 mgm\(^{-2}\) when it is used without an ATase inhibitor. However, when \( O^\beta \)-benzylguanine is used as a pre-treatment before the alkylating agent, clinical trials have shown a marked reduction in the maximum tolerated dose of carmustine (40 mgm\(^{-2}\)). It remains to be seen as to whether this reduction of the maximum tolerated dose of chemotherapy drug due to the inactivation of ATase will prevent \( O^\beta \)-benzylguanine from going into long-term clinical use.

The synthesis of additional ATase inhibitors with greater potency, solubility and specificity towards cancer cells is required. Attempts to improve on \( O^\beta \)-benzylguanine have led to the production of a vast array of substituted guanine derivatives. Certain trends have emerged from analysis of the biological activities of these compounds (see figure 1.5).

The ATase inhibiting abilities of these compounds is largely retained by substitution at the \( O^\beta \), N-9 and C-8 positions. Substitution at the N-7 position or at the exocyclic amino group is not well tolerated as it causes a substantial decrease in biological activity, while altering the oxygen atom at the six position or the atoms of the pyrimidine ring also has a detrimental effect on the ATase inhibiting properties of these compounds. This is in agreement with the proposed mechanism of alkyl group transfer from \( O^\beta \)-alkylguanine to the cysteine residue of ATase. The oxygen
<table>
<thead>
<tr>
<th>1.17</th>
<th>X=H</th>
<th>$I_{50}^*$ (μM)</th>
</tr>
</thead>
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<tr>
<td>1.19a</td>
<td>X=F</td>
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</tr>
<tr>
<td>1.19b</td>
<td>X=Cl</td>
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</tr>
<tr>
<td>1.19c</td>
<td>X=CH$_3$</td>
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<tr>
<td>1.19d</td>
<td>X=Ph</td>
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</tr>
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</tr>
<tr>
<td>1.20b</td>
<td>R=CH$_2$CH(OH)Et</td>
<td>2</td>
</tr>
<tr>
<td>1.20c</td>
<td>R=CH$_2$CH(OH)CH$_2$NHCH(CH$_3$)$_2$</td>
<td>0.8</td>
</tr>
<tr>
<td>1.21a</td>
<td>R=CH$_2$CN</td>
<td>inactive</td>
</tr>
<tr>
<td>1.21b</td>
<td>R=CH$_2$CH(OH)Et</td>
<td>inactive</td>
</tr>
<tr>
<td>1.21c</td>
<td>R=CH$_2$CH(OH)CH$_2$NHCH(CH$_3$)$_2$</td>
<td>inactive</td>
</tr>
<tr>
<td>1.22a</td>
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<td>0.06</td>
</tr>
<tr>
<td>1.22b</td>
<td>X=NH$_2$</td>
<td>2</td>
</tr>
<tr>
<td>1.22c</td>
<td>X=CH$_3$</td>
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</tr>
<tr>
<td>1.22d</td>
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</tr>
<tr>
<td>1.23</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>1.24</td>
<td></td>
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</tr>
</tbody>
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---

Figure 1. $I_{50}^*$ Values of Some $O^6$-Benzyguanine Derivatives (incubated at 37 °C for 30 minutes).

---

*I$_{50}$ is the concentration of the substrate to produce a 50% inactivation of ATase in cells (quoted in μM).
atom in the six position, the two nitrogen atoms in the pyrimidine ring and the exocyclic amino group are all involved in the transfer of the alkyl group.\textsuperscript{1,33}

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {\text{His}};
\node (B) at (0.5,0) {\text{N}};
\node (C) at (1,0) {\text{N}};
\node (D) at (1.5,0) {\text{H}};
\node (E) at (2,0) {\text{H}};
\node (F) at (2.5,0) {\text{ATase}};
\node (G) at (3,0) {\text{Cys}};
\node (H) at (3.5,0) {\text{H}};
\node (I) at (4,0) {\text{R}};
\node (J) at (4.5,0) {\text{A}_1};
\node (K) at (5,0) {\text{N}};
\node (L) at (5.5,0) {\text{N}};
\node (M) at (6,0) {\text{H}};
\node (N) at (6.5,0) {\text{dR}};
\node (O) at (7,0) {\text{A}_2};
\node (P) at (0,-1) {\text{Asn}};
\node (Q) at (0.5,-1) {\text{O}};
\node (R) at (1,-1) {\text{N}};
\node (S) at (1.5,-1) {\text{H}};
\node (T) at (2,-1) {\text{O}};
\node (U) at (2.5,-1) {\text{R}};
\node (V) at (3,-1) {\text{H}};
\node (W) at (3.5,-1) {\text{A}_1};
\node (X) at (4,-1) {\text{N}};
\node (Y) at (4.5,-1) {\text{N}};
\node (Z) at (5,-1) {\text{H}};
\node (AA) at (5.5,-1) {\text{dR}};
\node (BB) at (6,-1) {\text{A}_2};
\end{tikzpicture}
\end{center}

\textbf{Scheme 1.} 4 Schematic of alkylated guanine in binding site of ATase\textsuperscript{1,33}

Since this investigation began into the potential of these guanine derivatives as viable alternatives to \(O^6\)-benzylguanine, Moody \textit{et al.} have published the crystal structure of human ATase with \(O^6\)-benzylguanine in the binding site.\textsuperscript{1,34} The crystal structure shows the sulphhydryl group of the cysteine in the correct position to facilitate alkyl transfer from the \(O^6\)-alkylguanine. It also shows that the imino ring from a nearby proline residue provides a favourable hydrophobic packing surface for the benzyl group of \(O^6\)-benzylguanine. The hydrophobic binding pocket appears large enough to facilitate substituted benzyl groups and substituents located at the C-8 and N-9 position of the guanine ring. \(O^6\), C-8 and N-9 substituents appear to be directed towards the protein surface. Consequently there is little constraint on the size of lipophilic substituents, which can be accommodated at these positions.
1.10 PaTrin-2

The deactivation of ATase by the reaction of $O^\alpha$-alkylguanine with a sulfhydryl group of a cysteine residue is an $S_n2$ bimolecular displacement reaction, where guanine is the leaving group X, and the sulfhydryl group acts as the nucleophile (Nuc$^-$).

\[
\text{RX} + \text{NucH}^+ \rightarrow H^+X^+ + \text{RNuc}
\]

Eqn. 1.1

In $S_n2$ reactions of the type in equation 1.1, the order of reactivity of some heterocyclic examples of R is 3-thienylmethyl $1.25 <$ benzyl $1.26 <$ thenyl $1.27 <$ furfuryl $1.28$. $^{35}$

Bearing this in mind, our group have explored the viability of replacing the benzyl group of Pegg's $O^6$-benzylguanine with a hetarylmethyl group to produce an alternative ATase inhibitor for clinical use. $^{136}$ $O^6$-Thenylguanine $1.29$ was found to be a very efficient inhibitor of ATase. The introduction of a halogen atom into the 4- or the 5-position of the thiophene ring of $1.29$ caused a notable increase in the deactivation of ATase. Thus, $O^6$-(4-bromothenyl)guanine $1.30$ proved to be a potent and efficient inactivator, and further appears to be inherently non-toxic. $^{137}$
In the screening test, it is an order of magnitude more effective against ATase than $O^6$-benzylguanine. Currently, the feasibility of its use in combination with the methylating agent, temozolomide, against metastatic melanoma and colorectal cancer is being investigated in clinical trials. $O^6$-(4-Bromothenyl)guanine is usually called PaTrin-2 (the title PaTrin-1 was given to its thenyl analogue). The name comes from a combination of the two institutions whose researchers have developed the project - the Paterson Institute in Manchester and Trinity College. $O^6$-(4-Bromothenyl)guanine will be called PaTrin-2 routinely throughout this report.

Phase 1 clinical trials of PaTrin-2 with the methylating agent temozolomide have been completed in the Christie Hospital, Manchester and University College Hospital, London. It was found that the maximum tolerated dose of temozolomide, which can be used in conjunction with PaTrin-2 is 75% that of the recommended dosage, when it is used on its own. This is in contrast with the findings of the clinical trials of the $O^6$-benzylguanine/carmustine combination, which showed that the tolerated dosage of carmustine by patients dropped significantly when $O^6$-benzylguanine was given in combination.

1.11 PaTrin-2 Derivatives

Even though PaTrin-2 appears to be a promising candidate for clinical use as an ATase inhibitor, attempts to improve both its solubility and selectivity are ongoing. Over 200 derivatives or analogues of PaTrin-2 have been synthesised in this laboratory, and their abilities to inactivate ATase have been evaluated. Numerous PaTrin-2 derivatives with alkyl groups in the 9-position have been prepared.
corresponding isomers bearing the alkyl group in the 7-position were found to be relatively inactive against ATase, which is in keeping with the biological activities of the alkylated $O^b$-benzylguanine derivatives.

Currently under investigation are PaTrin-2 derivatives, which could potentially be more soluble than the parent compound, while retaining potent bioactivity.

The use of a modified form of an active drug, which will ultimately release the parent drug in vivo, is favoured for a number of reasons. Modifications to the active drug may be required

- to increase the absorption of a drug
- to eliminate or mask unwanted physical properties of the drug
- to improve the selectivity of the drug
- to increase the stability of the drug
- or as in our case, to increase the solubility and duration of action of the drug.\textsuperscript{140}

At present, PaTrin-2 (and $O^b$-benzylguanine) is administered prior to the cytotoxic alkylating agent in order to achieve maximum inactivation of ATase. However, this regimen may not produce sustained ATase depletion and fresh ATase may have begun to form before the alkylating agent has completed its mode of action. Ideally, persistence of PaTrin-2 in sufficient concentrations is required to ensure that freshly formed ATase molecules are inactivated, otherwise this ATase would react with the alkylating agent, thereby limiting the efficacy of the cytotoxic agent.

Derivatives of PaTrin-2 are being investigated, which would hydrolyse in vivo to release gradually free PaTrin-2 (Scheme 1.5). The rate of the hydrolysis reaction dictates the concentration of free PaTrin-2 present in vivo at any particular time.
As the rate of the hydrolysis reaction is dependent on the nature of X, the use of different groups as the side-chain will influence the rate of release of the active drug. The 9-methoxycarbonyl derivative of PaTrin-2 1.31 has acted as a lead compound in this investigation.

Its $I_{50}$ value of 0.0048 μM compares favourably with that of its parent compound and the compound can undergo hydrolysis under physiological conditions to release free PaTrin-2. Consequently the synthesis and rates of hydrolysis of derivatives with alkoxy and primary and secondary amino groups linked to PaTrin-2 via a carbonyl group are currently being investigated (Scheme 1.5).
Not only have substituted PaTrin-2 derivatives been prepared, but also numerous compounds have been synthesised where there are alterations to the PaTrin-2 skeleton (see Figure 1.6).\(^1\)\(^3\)\(^9\)

![Figure 1.6 PaTrin-2 analogues, which have been investigated as potential ATase inactivators.](image)

\(^{1}\)\(^3\)\(^9\)
1.12 8-Aza-7-deazaguanines

Of particular interest were the series of $O^6$-hetaryl-8-aza-7-deazaguanine compounds, as their abilities to inhibit ATase compared very favourably with those of the analogous guanine compounds. The 8-aza-7-deazaguanines were the compounds, which produced such a consistent pattern of biological activities.$^{139,141}$

![8-aza-7-deazaguanine derivative](image_url)

![guanine derivative](image_url)

<table>
<thead>
<tr>
<th>$R$</th>
<th>ATase activity <em>in vivo</em> $I_{50}$ ($\mu$M) for 8-aza-7-deazaguanine derivatives$^{141}$</th>
<th>ATase activity <em>in vivo</em> $I_{50}$ ($\mu$M) for guanine derivatives</th>
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<tbody>
<tr>
<td>$O^6$-benzyl</td>
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<td>0.04</td>
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<td>-</td>
</tr>
<tr>
<td>$O^6$-(4-fluorobenzyl)</td>
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<td>-</td>
</tr>
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<td>$O^6$-piperonyl</td>
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</tr>
<tr>
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</table>

Figure 1. $7 I_{50}$ values of $O^6$-alkoxy-8-aza-7-deazaguanine and guanine derivatives (incubated at 37 °C for one hour).

$O^6$-Alkoxy-8-aza-7-deazaguanines differ from $O^6$-alkoxyguanines at the 7 and 8-positions.$^{142}$ The nitrogen atom in the 7-position and carbon atom in the 8-position of guanine “exchange places” in the 8-aza-7-deazaguanine. While this difference is subtle, it affects the chemistry of 8-aza-7-deazaguanines in key areas.
The 9-NH of the 8-aza-7-deazaguanine compound should be less acidic than the corresponding 9-NH of the guanine series, as the anion corresponding to the latter is more "symmetrical". Consequently, the 8-aza-7-deazaguanine anion 1.32 is more basic than the guanine anion 1.33. The 8-aza-7-deazaguanine anion will react more quickly with electrophilic species than its guanine analogue, with consequences that will be discussed in Chapters 3 and 4.

Calculations carried out by Dr. Dónall Mac Dónaill on $O^\delta$-methoxy-8-aza-7-deazaguanine gave different results depending on the method used, but they suggest that the 9-nitrogen is more basic than the 8-nitrogen both in the neutral species and the anion.

1.13 Allopurinol

Allopurinol 1.34 (or 6-hydroxy-8-aza-7-deazapurine), which is the standard treatment for gout, is an example of a drug containing the 8-aza-7-deazapurine ring system.

Gout is a painful condition caused by the deposition of crystalline sodium urate in joints due to an abnormally high concentration of uric acid 1.36 in the blood. Uric acid is a product of guanine degradation in the body. Initially, guanine is hydrolysed to the 2, 6-dihydroxy compound, xanthine 1.35, which in turn is converted by xanthine oxidase into uric acid (Scheme 1.6).
Scheme 1.6

Allopurinol treats this condition by inhibiting the enzyme xanthine oxidase, which is responsible for the production of uric acid. Allopurinol acts as a substrate for xanthine oxidase. It binds to the enzyme, but does not react any further, thereby causing the inactivation of the enzyme. Consequently xanthine is not converted to uric acid.

1.14 Aim

To date only the ATase inhibiting properties of 8-aza-7-deazaguanines with substituents at the 6-position have been investigated. Bearing in mind the consistently excellent biological activated of these compounds, we thought it would be rewarding to investigate the synthesis and biological activities of a series of substituted derivatives of $O^\delta$-alkyl-8-aza-7-deazaguanine.

The aim of this project is to prepare some substituted derivatives of $O^\delta$-alkyl-8-aza-7-deazaguanine. This investigation will deal mainly with derivatives of $O^\delta$-(4-bromothenyl)-8-aza-7-deazaguanine 1.37, the analogue of PaTrin-2. For convenience, this compound shall be called alloPaTrin (the abbreviation “allo” comes from the Greek word *allos* meaning “other”), by analogy with “allopurinol”.

1.35

1.36

1.37
In Chapter 2, the synthesis of alkyl derivatives of alloPaTrin will be discussed. Chapters 3 and 4 deal with the preparation of alkoxy carbonyl and carbamoyl derivatives of alloPaTrin. Both the alkoxy carbonyl and carbamoyl derivatives of alloPaTrin are potential prodrugs of alloPaTrin. Chapter 5 will deal with the biological activity of these compounds.
1.15 References

1.1 Statistics from www.cancerresearchuk.org

1.2 Statistics from National Cancer Registry, Ireland, 1998.


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Chapter 2

Synthesis of 9-Alkylated $O^6$-Alkoxy 8-Aza-7-deazaguanines and 8-Aza-7-deazapurines
2.1 Introduction

Previous studies by Pegg and McElhinney have shown that analogues of $O^6$-benzylguanine and PaTrin-2, which were alkylated in the 9-position largely retain the biological activity of their respective mother compounds. Both research groups have prepared a series of 9-alkylated guanines, with the alkyl groups ranging from methyl to sugars and steroidal groups.

![Structures 2.01 and 2.02](image)

Moody's crystal structure of human ATase complemented these results. In a model experiment, he placed $O^6$-benzylguanine into the active site of ATase and observed that alteration at the $O^6$- and 9-positions of the molecule could be tolerated. Substituents at these positions are orientated such that their size does not affect the binding of the molecule to the protein.

To date only analogues of alloPaTrin with different $O^6$-alkoxide groups have been investigated. In this chapter, we will discuss the synthesis of some 9-alkyl-8-aza-7-deazaguanines. We will also discuss the synthesis of the corresponding 8-aza-7-deazapurine compounds, analogues of the anti-gout drug, allopurinol.

Two different systems can be used to name and discuss such compounds. We will use the purine nomenclature, as this is more in keeping with the current literature trends regarding guanine based ATase inhibitors, though the systematic numbering is more correct, but also more cumbersome.
2.2.1 Established Synthesis of 8-Aza-7-deazapurines

The proton in the 9-position of both the purine and the 8-aza-7-deazapurine systems is acidic, and therefore can be easily removed with a strong base. Treatment of the anion with an alkyl halide gives the desired alkylated product. Two methods are favoured by those working with PaTrin-2. Sodium alkoxide can be used to create the sodium salt of the purine, which is subsequently treated with an alkyl halide. Alternatively lithium hydride can be used as the strong base.

Similarly, synthetic strategies have been used to produce a vast array of N-9 alkylated 8-aza-7-deazapurines and N-alkylated compounds of related systems.

Unfortunately, this method gives relatively poor yields of the desired 9-substituted compound due to its lack of regioselectivity. When an 8-aza-7-deazapurine is attacked by a strong base, the subsequent anion possesses canonical forms as shown below (Scheme 2.1). When this anion is treated with an alkyl halide, two products are formed, the N-9 alkylated compound and the N-8 alkylated compound. Similar results are observed in the 8-aza-7-deazaguanine system. In the guanine and purine series, this type of reaction produces also a mixture of products, namely the N-9 and N-7 species.
Whilst the obtaining of both isomers may be valuable to those pursuing other interests, we are only concerned with the regioselective synthesis of 9-alkylated 8-aza-7-deazaguanines. Their biological activities may be directly compared to their guanine analogues. The regioselective synthesis of the 8-alkylated 8-aza-7-deazaguanines, and their abilities to inactivate ATase may be worthy of further study in the future.
2.2.2 Proposed Synthesis of 9-Alkyl-8-aza-7-deazaguanine

The non-regioselective nature of these alkylating reactions is undesirable. Not only does the mixture of the two isomers have to be separated, but also the yield of the desired N-9 isomer is greatly reduced. For example, in the case where the alkyl group is methyl, Seela treated 2-amino-6-chloro-8-aza-7-deazapurine \(2.03\) with benzylammonium chloride in aqueous sodium hydroxide followed by methyl iodide.\(^{2,7}\) The resultant crude mixture had to be chromatographed to give 2-amino-6-chloro-9-methyl-8-aza-7-deazapurine \(2.04\) (41%) and 2-amino-6-chloro-8-methyl-8-aza-7-deazapurine \(2.05\) (17%) (Scheme 2.2).

We propose to by-pass these problems by incorporating the alkyl group into the building of the 8-aza-7-deazaguanine and 8-aza-7-deazapurine ring systems.

The parent 8-aza-7-deazaguanine can be constructed from either ring component. A synthesis from a pyrazole derivative is known, however it requires a ten-step synthesis resulting in yields of less than 10%.\(^{2,8}\) Alternatively, hydrazine has been used to build the pyrazole ring onto the pyrimidine ring. We aim to use an alkylhydrazine to make the pyrazole ring of a 9-alkyl-8-aza-7-deazaguanine as Bhuyan has done.\(^{2,9}\) The use of a monosubstituted hydrazine with the pyrimidine \(2.06\) will lead to the formation of one regioisomer only, the N-9 substituted compound (Scheme 2.3).
2.3 Alkylhydrazines

Some simple alkylhydrazines such as methylhydrazine and hydroxyethylhydrazine are commercially available and are relatively inexpensive. However, these would not give end-products which would be of biological interest to us. Studies have shown that compounds with either large or small lipophilic N-9 substituents in the guanine series retain the biological activity of their parent guanine compound, while more polar alkyl substituents generally reduce activity.\textsuperscript{2,1,2,2} We have chosen 1-phenoxypropyl and 1-(2-naphthoxy)propyl as the alkyl groups which we ultimately want in the 9-position of our 8-aza-7-deaza guanines and purines.

\[ 
\begin{align*}
\text{H}_2\text{N} & \quad \text{Cl} \\
\text{H}_2\text{N} & \quad \text{Cl} \\
\text{R} & \quad \text{N} \\
\end{align*} 
\]

The chemical and biological activities of these compounds can then be compared to their purine analogues. In the guanine series both these compounds showed a good
ability to inhibit ATase. The $I_{50}$ value of the phenoxypropyl derivative of PaTrin-2 was 0.036 $\mu$M whilst the 2-naphthoxypropyl PaTrin-2 derivative produced a value of 0.026 $\mu$M, both of which compared favourably to the 0.0034 $\mu$M value of free PaTrin-2. Interestingly, the corresponding 1-naphthoxypropyl compound ($I_{50}$ value of 0.27 $\mu$M) was vastly inferior to its $\beta$ analogue and it would be interesting and informative to see if the 8-aza-7-deazaguanine $\beta$ compound also shows good biological activity.

2.3.1 Synthesis of Alkyl bromide

Neither 1-phenoxypropylhydrazine or 1-(2-naphthoxy)propylhydrazine are commercially available. They can be made from the propyl bromide and hydrazine. 1-Phenoxypropyl bromide $^{2.09}$ was made from the displacement of bromide ion from 1,3-dibromopropane by phenolate anion. In the literature, this type of reaction can be carried out in two ways. Potassium carbonate in acetone can be used to generate the phenolate ion, which can then react with 1,3-dibromopropane to give the desired compound $^{2.09}$. However, under these conditions, we sometimes found that another phenolate ion attacked the bromide of the newly formed alkyl bromide to give the "dimeric" compound, 1,3-diphenoxypropane $^{2.11}$. Trace amounts (<5%) of the allylic side product $^{2.12}$ also formed through the base catalysed elimination of hydrogen bromide from 1-phenoxypropyl bromide $^{2.09}$. Similar unsatisfactory results were observed when 2-naphthol was used in place of phenol.

To avoid this phenomenon, the amount of 1,3-dibromopropane could have been increased, however we chose to adopt the alternative method reported in the literature.
Reflux of a bi-phasic mixture of the phenol in aqueous sodium hydroxide and a 50% excess of 1,3-dibromopropane consistently gave the required product.\textsuperscript{2,12} Both 1-phenoxypropylbromide \textbf{2.09} and 1-(2-naphthoxy)propylbromide\textsuperscript{3,14} \textbf{2.10} were isolated in good yields using excess 1,3-dibromopropane. The products were isolated by fractional distillation.

### 2.3.2 Synthesis of Alkylhydrazine

The alkyl bromide can be converted to an alkylhydrazine \textit{via} nucleophilic attack by a hydrazine molecule. The initial product is the hydrobromide of the monoalkylhydrazine from which the free base is formed by hydrazine.\textsuperscript{2,13}

\[
\begin{align*}
H_2N-NH_2 & \quad RBr & \quad RH_2N^+NH_2Br & \quad RHN-NH_2 \\
R_2N-NH_2 & & RHN-NHR & \\
(R_3N^+-NH_2)Br- & \quad (R_3N^+-NHR)Br- & \quad (R_3N^+-NR_2)Br-
\end{align*}
\]

The monoalkylhydrazine can then be attacked by a further molecule of alkyl halide either on the alkylated nitrogen atom or on the unsubstituted nitrogen, resulting in a 1,1-dialkylhydrazine or a 1,2-dialkylhydrazine. These in turn can undergo further alkylations (see Scheme 2.4).

To avoid the formation of these polyalkylhydrazines, a solution of the bromide was added slowly to a dilute solution of hydrazine, which was in excess. Osei-Twum used
a 500% excess of hydrazine when making phenoxypropylhydrazine 2.13 in 75% yield.\textsuperscript{2,14} 1-(2-Napthoxy)propylbromide 2.10 was treated similarly to give a good yield of 1-(2-napthoxy)propylhydrazine 2.14, which was converted to the crystalline and more stable hydrochloride salt.\textsuperscript{2,15}

\[ R\text{Br} \quad \xrightarrow{5\text{NH}_2\text{NH}_2\text{H}_2\text{O}} \quad R\text{NHNH}_2 \]

2.09 R = phenoxy  
2.10 R = 2-naphthoxy  
2.13 R = phenoxy  
2.14 R = 2-naphthoxy

Scheme 2.5

2.4 Synthesis of 8-Aza-7-deazaguanine Ring System

2.4.1 The Pyrimidine Ring

The alkylhydrazines form the N-N section of the pyrazole ring of 8-aza-7-deazaguanine. The rest of the skeleton comes from a commercially available pyrimidine, which requires modification. 2-Amino-4,6-dihydroxypyrimidine 2.15 was converted to 2-amino-4,6-dichloropyrimidine-5-carboxaldehyde 2.06 via a Vilsmeier reaction (Scheme 2.6).\textsuperscript{2,7,2,16} These modifications introduce the aldehyde group into the 5-position of the pyrimidine, which will eventually be the carbon in the 7-position of the 8-aza-7-deazaguanine ring system while the hydroxy functions are replaced by two chlorides, which are convenient leaving groups.

Seela \textit{et al.} found that this reaction is dependent on the ratio of the different reagents used.\textsuperscript{2,7} Phosphorus oxychloride (5 equivalents), DMF (2 equivalents) and the pyrimidine (1 equivalent) gave inconsistent results. It is reported that a ratio of 8:2.2:1 is much more satisfactory, consistently resulting in yields in excess of 70%. Seela \textit{et al.} also isolated the amidine 2.16 intermediate, thereby supporting the proposed mechanism for the reaction (Scheme 2.6). This shows the formation of a dimethylaminoethyleneamino group on the exocyclic amine, which effectively acts as a protecting group for the \(-\text{NH}_2\) during the reaction. The amino function is regenerated when the reaction is worked up with water at 50 °C. Higher temperatures could lead to the oxidation of aldehyde group, yielding the carboxylic acid.
2.4.2 Construction of 8-Aza-7-deazaguanine Ring

The 8-aza-7-deazaguanine ring system is generated from the reaction of the pyrimidine 2.06 and a hydrazine. The reaction occurs by the initial attack of the
unsubstituted nitrogen of alkylhydrazine at the aldehyde group in the 5-position of the pyrimidine 2.06 to from a hydrazone 2.17, followed by cyclisation at the 6-position to afford the required 8-aza-7-deazaguanine 2.04 (Scheme 2.7).

Seela used two equivalents of hydrazine monohydrate when making 2-amino-6-chloro-8-aza-7-deazapurine 2.03 from the same starting pyrimidine 2.06. The second equivalent of hydrazine was used as a base to neutralise the hydrogen chloride generated in the cyclisation reaction. In a model experiment, two equivalents of methylhydrazine were reacted with the pyrimidine (Seela’s conditions). This gave the 9-substituted 8-aza-7-deazaguanine, 2-amino-6-chloro-9-methyl-8-aza-7-deazapurine 2.04, in 90% yield. However, when only one equivalent of methylhydrazine was used, the yield of the bicyclic compound 2.04 was less than 20%, with 70% of the starting pyrimidine remaining untouched. These results showed that Seela’s conditions would only work when two equivalents of alkylhydrazine were used. This is not desirable when using substituted hydrazines, which are not commercially available and would be expensive and difficult to make. Consequently, we modified Seela’s method and used one equivalent of alkylhydrazine, with one equivalent of another base, which would neutralise the hydrogen chloride formed.

There is one report in the literature to support this synthetic approach. Bhuyan reacted one equivalent of triethylamine together with the 6-chloro-5-formyluracil 2.18 and a monosubstituted hydrazine 2.19 in methanol at room temperature for one hour followed by reflux for fifteen minutes to give exclusively the N-9 substituted bicyclic product 2.20 in 80% yield (Scheme 2.8).
Scheme 2. 8

Disappointingly, when we applied these conditions to the pyrimidine 2.06 and one equivalent of methyl hydrazine, the yield of the bicyclic compound (35%) was similar to the corresponding reaction using no base. The use of pyridine as the base produced similar results. When more nucleophilic bases were used in place of triethylamine, namely 4-dimethylaminopyridine (DMAP) and sodium methoxide, the 4- and 6-chlorides of the pyrimidine 2.06 were both displaced giving rise to a mixture of the 4,6-di(4-dimethylaminopyridinio) 2.21 (~50%) or the 4,6-dimethoxy 2.22 (~50%) pyrimidine and the unreacted 4,6-dichloropyrimidine (~50%), with only trace amounts of the required compound 2.04.

We then decided to react the dichloropyrimidine aldehyde 2.06 with just one equivalent of DMAP by itself. This resulted in the formation of the 4,6-di(4-dimethylaminopyridinio) substituted pyrimidine 2.21 (~50%) and the unreacted pyrimidine 2.06 (50%). Once the chloride in the four position had been replaced, it is kinetically more feasible to displace the chloride in the six position of the mono-(4-dimethylaminopyridinio) intermediate than to continue displacing the 4-chloride. The use of two equivalents of DMAP produced the 4,6-di(4-dimethylaminopyridinio) substituted pyrimidine 2.21 in a 92% yield. Similar experiments were carried out with sodium methoxide using methanol as the solvent. Whilst both chlorides were
displaced by the base, the reaction took significantly longer and required heating. The mono-methoxide pyrimidine 2.23 was easily isolated, and it took a further four equivalents of sodium methoxide for the substitution reaction to go to completion.

When one equivalent of methylhydrazine was reacted with the di-(dimethylaminopyridino) substituted pyrimidine 2.21 in water at room temperature, the 8-aza-7-deazaguanine salt 2.24 precipitated from the aqueous solution after five minutes in a 65% yield. Due to the insolubility of the salt in most solvents, it proved difficult to displace the DMAP with an alkoxide. Upon reaction with one equivalent of methylhydrazine, the dimethoxy pyrimidine 2.22 formed a hydrazone 2.25 in the 5-position of the pyrimidine ring. As expected the methoxide in the 4-position was not considered to be a good enough leaving group to facilitate the ring closure of the pyrazole ring. Hence, the use of further bases, DBU and sodium acetate were investigated. DBU gave similar results to triethylamine. Sodium acetate was the base, which gave the most successful results. The reaction of the dichloropyrimidine aldehyde 2.06, one equivalent of methylhydrazine and two equivalents of sodium acetate in aqueous tetrahydrofuran at 80 °C for two hours gave 9-methyl-8-aza-7-deazaguanine 2.04 in a 78% yield. These reaction conditions were adopted on the syntheses using 3-(phenoxy)propyl hydrazine 2.26 to give the 9-phenoxypropyl compound 2.27 (82%) and 3-(2-naphthoxy)propyl hydrazine 2.14 to give the 9-(2-naphthoxy)propyl compound 2.27 (94%).
2.4.3 Replacement of Chloride by Alkoxide

Having created the 8-aza-7-deazaguanine ring system in good yield, the final step of the reaction sequence is to make the PaTrin-2 and benzylguanine analogues by displacing the chloride of 2.26 and 2.27 with 4-bromothenyl and benzyl groups. We did consider substituting the 6-chloride of the dichloropyrimidine 2.06 with an alkoxide to give 6-alkoxy-4-chloro-pyrimidine and reacting this with an alkylhydrazine to give the final product. Having already obtained the 6-methoxypyrimidine 2.23 (see Section 2.3.2), methylhydrazine was successfully reacted with it to give O^6,9-dimethyl-8-aza-7-deazaguanine 2.28 in 70% yield.

![Scheme 2.9](image)

However, we could not make other 6-alkoxypyrimidines cleanly using only a five-fold excess of the corresponding alcohol (or less) and DMF as the solvent. The use of the alcohol as the solvent (as in the synthesis of the monomethoxypyrimidine 2.23) for the reaction is not practical as the alcohol would be too expensive and difficult to remove.
during work-up. 4-Bromothenyl alcohol has to be made from the reduction of the corresponding aldehyde by sodium borohydride.\textsuperscript{2.17}

Seela converted 2-amino-6-chloro-8-aza-7-deazapurine \textbf{2.03} to its 6-methoxy derivative in a 90\% yield by reflux of the chloride and 2.5 equivalents of sodium methoxide in methanol.\textsuperscript{2.7} These conditions were repeated using the 9-methyl derivative \textbf{2.04} to give the \(O^6\)-methoxy compound \textbf{2.28} (72\%).

Murray used a range of solvents (DMF, DMSO and sulfolane) when making several different \(O^6\)-alkoxy-8-aza-7-deazaguanines.\textsuperscript{2.4} Of these, DMF produced the most consistent results.\textsuperscript{2.18} Three equivalents of the sodium alkoxides of 4-bromothenyl and benzyl alcohol were reacted with the chloride \textbf{2.26} and \textbf{2.27} in DMF at 80 °C for three hours giving the \(O^6\)-alkoxy compounds \textbf{2.29-2.34}.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>(R)</th>
<th>(R')</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textbf{2.29}</td>
<td>phenyl</td>
<td>4-bromothenyl</td>
</tr>
<tr>
<td>\textbf{2.30}</td>
<td>phenyl</td>
<td>benzyl</td>
</tr>
<tr>
<td>\textbf{2.31}</td>
<td>phenyl</td>
<td>piperonyl</td>
</tr>
<tr>
<td>\textbf{2.32}</td>
<td>phenyl</td>
<td>thenyl</td>
</tr>
<tr>
<td>\textbf{2.33}</td>
<td>2-naphthyl</td>
<td>4-bromothenyl</td>
</tr>
<tr>
<td>\textbf{2.34}</td>
<td>2-naphthyl</td>
<td>benzyl</td>
</tr>
</tbody>
</table>

The yields were low as the reaction produced a dark brown tar, which separated from the reaction mixture during work-up. This may be due to the occurrence of side-reactions, e.g. ring opening.
2.5 9-Alkyl-8-Aza-7-deazapurines

Having successfully prepared a number of 8-aza-7-deazaguanine compounds, which possess an alkyl group in the 9-position, we thought it would be worthwhile to prepare some of the corresponding 8-aza-7-deazapurine compounds. It has been widely reported that the amino group in the 2-position of a guanine based substrate plays a crucial role mechanism of inactivation of ATase, and the absence of this group in any potential ATase inhibitors in the guanine series causes a marked decrease in the ATase inhibiting properties of these compounds.\(^2\) We investigated whether a similar pattern can be observed in the 8-aza-7-deazapurine series.

2.5.1 Established Synthesis of 8-Aza-7-deazapurines

A synthesis of 1-alkyl-8-aza-7-deazapurine, the 9-alkylated analogue of allopurinol, is known. Robins made the pyrazole ring from a monosubstituted hydrazine and ethoxymethylene malononitrile \(2.35\) to give the corresponding 1-substituted-5-amino-4-cyanopyrazole \(2.36\) (see Scheme 2.10).\(^2\)\(^9\)

![Scheme 2.10](image)

Robins investigated the possibility that the alternative structure, 1-substituted-3-amino-4-cyanopyrazole \(2.37\), could be formed (Scheme 2.11). This tautomer would ultimately have lead to the 8-substituted-8-aza-7-deazapurine \(2.38\). For the second tautomer to form, the alkyl bearing nitrogen atom of the alkylhydrazine would have to react with ethoxymethylene malononitrile \(2.35\) in the initial step of the reaction. While this reaction would be disfavoured both electronically and sterically, it is possible theoretically and therefore must be considered.
He devised an unambiguous synthesis of a pyrazole of this type 2.39 (Scheme 2.12).

The alternative isomer was made by isolating an intermediate 2.40 of the reaction between phenylhydrazine and ethoxymethylenemalonic ester. Hydrolysis of 2.40 followed by decarboxylation gave 2.41 (Scheme 2.13).
Comparison of the ultraviolet absorption spectra of these isomers showed significant differences. At pH 1, \textbf{2.41} had a $\lambda_{\text{max}}$ of 219 $\mu$m, whilst its other isomer \textbf{2.39} had a $\lambda_{\text{max}}$ of 275 $\mu$m. The ultraviolet adsorption spectra of the pyrazole produced by a monosubstituted hydrazine and ethoxymethylene malononitrile resembles the spectrum produced by \textbf{2.41}. This suggests that the correct isomer is formed, which will ultimately lead to desired $N$-alkyl substituted 8-aza-7-deazapurine.

Having established the structure of the pyrazole compound, Robins treated it with cold concentrated sulfuric acid to give the corresponding 1-alkyl-5-amino-4-pyrazole carboxamide \textbf{2.2}.$^{2,19,2,20}$ This was then converted to the 9-alkyl-8-aza-7-deazapurine \textbf{2.43} using boiling formamide. Finally, a chloride was introduced into the 6-position by reflux in phosphorus oxychloride, which was readily displaced by a range of alkoxides.
2.5.2 Synthesis of 9-Alkyl-8-aza-7-deazapurine

The synthesis of the 8-aza-7-deazapurine ring system outlined in the previous section uses formamide to close the pyrimidine ring. As formamide is a well known teratogen, we wish to avoid using it. We propose to follow the synthetic route outlined in Scheme 2.15. In the first step, treatment of the commercially available ethoxy(ethoxymethylene)cyanoacetate 2.45 with a monosubstituted alkylhydrazine gives the 1-alkyl-5-amino-pyrazole-4-carboxylic acid ethyl ester 2.46.

The reaction of 2.46 with ethyl orthoformate and acetic anhydride introduces an ethoxymethylene group on the amino hydrogen of the pyrazole giving 2.47. Treatment of 2.47 by alcoholic ammonia should result in cyclisation of the pyrimidine ring to give the 8-aza-7-deazapurine ring system 2.48.
2.5.3 Synthesis of 8-Aza-7-deazapurine Ring System

In this case, we chose to begin from the pyrazole ring, when making the 8-aza-7-deazapurine skeleton, unlike the 8-aza-7-deazaguanine skeleton, which was made by building a pyrazole ring onto a commercially available pyrimidine. Methylhydrazine was again used as a model alkylhydrazine.

When an alkylhydrazine was reacted with ethoxy(ethoxymethylene)cyanoacetate in refluxing ethanol for two hours, the pyrazole, 1-alkyl-5-amino-pyrazole-4-carboxylic acid ethyl ester formed in good yield (81% for R = methyl, 2.51a, 61% for R = phenoxypropyl 2.51b).
The pyrazole rings 2.51a-b were further modified with the introduction of ethoxymethylene groups onto their 5-amino groups by refluxing the pyrazoles in a mixture of ethyl orthoformate and acetic anhydride to give 2.52a^-b in a quantitative yield both as brown oils. The ethoxymethylene group would ultimately form the carbon atom in the two position of the 8-aza-7-deazapurine system. (An advantage to this reaction is the product does not have to be purified before going on to the next stage of the reaction sequence. Both a purified sample and a sample in which the mixture of ethyl orthoformate and acetic anhydride was merely concentrated were used in the next stage with no significant change in yield). Wamhoff has reported such a reaction using only ethyl orthoformate to give the pyrazole 2.52a in a 70% yield. However, similar reactions have been reported using a mixture of ethyl orthoformate and acetic anhydride. We found that the latter conditions produced a quantitative yield of the required pyrazole.
Finally, the pyrazoles 2.52a-b were dissolved in a vast excess of ethanolic ammonia and left at room temperature for several days.\textsuperscript{2,24} The 9-alkyl-8-aza-7-deazapurines 2.53a-b precipitated from the solution. The ammonia reacted with the pyrazole in a double condensation reaction to close the pyrimidine ring and produce the bicyclic compound (85% for R = methyl 2.53a\textsuperscript{25}, 58% for R = phenoxypropyl 2.53b).

Having obtained the 8-aza-7-deazapurine ring system, further modification was required to introduce an alkoxy group in the 6-position. Initially a chloride leaving group was placed into this position. The base dimethylaniline is believed to be required as well as with phosphorus oxychloride to achieve this displacement (Scheme 2.17).\textsuperscript{2,18}

![Scheme 2.17](image)

However, we found the use of a base was not necessary as the 6-position was sufficiently activated by the presence of the alkyl group in the 9-position. The chloride was introduced by refluxing the 9-alkyl-8-aza-7-deazapurine 2.53a-b in phosphorus oxychloride alone (59% for R = methyl 2.54a\textsuperscript{25}, 90% for R = phenoxypropyl 2.54b).

**2.5.4 Replacement of Chloride by Alkoxide**

The conversion of the 6-chloro-8-aza-7-deazapurine compounds 2.54a-b to their O\textsuperscript{6}-alkoxy analogues 2.55-2.56 was carried out using the conditions outlined in Section 2.4.3, pp 47-48.
2.54b

2.54b

\[ \text{PhO} \]

\[ \xrightarrow{\text{NaOR}} \]

\[ \text{OR} \]

\[ \text{PhO} \]

2.55 \( R = 4\)-bromothenyl

2.56 \( R = \text{benzyl} \)

2.6 Chapter 2 – An Overview

Prior to this study, only 8-aza-7-deazaguanine compounds bearing various \( O^6 \)-alkoxy groups but without 9-substituents have been investigated as potential inhibitors of the protein ATase. In this chapter, the syntheses of some 9-alkyl-8-aza-7-deazaguanines and 9-alkyl-8-aza-7-deazapurines were discussed.

The 9-alkyl-8-aza-7-deazaguanine ring system was prepared from a suitable pyrimidine and an alkylhydrazine. This regioselective synthesis resulted in the alkyl group being positioned in the 9-position (Section 2.4.2, pp.43-47). Other syntheses of such compounds involve the reaction of an alkyl halide with a pre-formed 9-alkyl-8-aza-7-deazaguanine. This routinely results in a mixture of 8- and 9-alkylated compounds. Our unambiguous synthesis has obvious advantages over this synthetic strategy, where 9-alkylated compounds are required and the alkylhydrazine is readily available or can be conveniently prepared. Having obtained the 9-alkyl-2-amino-6-chloro-8-aza-7-deazaguanines, a series of \( O^6 \)-alkoxy derivatives were synthesised by replacement of the 6-chloride by alkoxide (pp.47-48).

A synthetic route was also developed for the preparation of a series of 9-alkyl-8-aza-7-deazapurines, starting with the pyrazole ring. This was constructed using an alkylhydrazine and ethyl(ethoxymethylene)cyanoacetate (p.53). The reaction gave the alkylated pyrazole 2-alkyl-3-amino-4-carbethoxy-pyrazole, which had the alkyl group in the correct position to ultimately give the required 9-substituted compound. The pyrazole was derivatised further to produce an ethoxymethyleneamino side-chain in the 3-position (p.54). Reaction with aqueous ammonia resulted in closure of the
pyrazole ring to give the desired ring system (p.54). A chloride group was introduced into the 6-position, which facilitated the synthesis of a number of $O^6$-alkoxide compounds (p.55). Their biological activities could then be compared to those of their guanine analogues (see Chapter 5), and the effect of replacement of an amino group by a proton on the inhibiting abilities of these compounds could be assessed.
2.7 Experimental

3-(2'-Naphthoxy)propyl hydrazine 2.14

A hot solution of 1-bromo-3-(2'-naphthoxy) propane\(^2\)\(^1\)\(^4\) 2.10 (8.4 g, 0.032 mol) in ethanol (70 ml) was added drop-wise to a refluxing solution of 98% hydrazine monohydrate (15.8 ml, 0.32 mol) in ethanol (40 ml). After refluxing for 18 hours, the ethanol was removed leaving a yellow oil. This was dissolved in water. A white solid precipitated upon addition of aq. KOH. This was filtered and dried (4.3 g, 62%).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 2.05 (quin, 2H, J 6.5, 2-CH\(_2\)), 2.75 (broad s., 3H, -NH, -NH\(_2\)), 3.00 (t, 2H, J 6.5, 1-CH\(_2\)), 4.16 (t, 2H, J 6.5, 3-CH\(_2\)), 7.14 (m, 2H, 1', 3'-CH), 7.33 (td, 1H, J 7.0, 1.0, 6'-CH), 7.43 (td, 1H, J 5.5, 1.5, 9'-CH), 7.73 (m, 3H, 4', 5', 8'-CH).

\(^1\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 29.1 (2-CH\(_2\)), 42.1 (1-CH\(_2\)), 65.9 (3-CH\(_2\)), 106.4 (1'-CH), 119.0 (3'-CH), 123.5 (6'-CH), 126.6 (7'-CH), 126.8 (8'-CH), 127.5 (5'-CH), 128.6 (q, 4a'-C), 129.3 (4'-CH), 135.2 (q, 8a'-C), 156.2 (q, 2'-C).

\(v_{\text{max}}\) 1496, 1601, 2931, 3338 cm\(^{-1}\).

mp 53 °C

2-Amino-4,6-di(4'-dimethylaminopyridinium)pyrimidine-5-carboxaldehyde dichloride 2.21

2-Amino-4,6-dichloropyridine-5-carboxaldehyde\(^2\)\(^1\)\(^6\) 2.06 (192 mg, 1 mmol) was dissolved in DMF (2 ml). 4-Dimethylaminopyridine (244 mg, 2 mmol) was added and the reaction mixture was stirred at room temperature for 30 minutes. A pale yellow precipitate was filtered and dried (401 mg, 92.0%).

\(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 3.40 (s, 12H, N-CH\(_3\)), 7.30 (d, 4H, J 9.5, 3', 5'-CH), 8.83 (d, 4H, J 9.5, 2', 6'-CH), 8.95 (s, 2H, -NH\(_2\)), 9.40 (s, 1H, -CHO).

\(^1\)C NMR (100 MHz, DMSO-d\(_6\)) \(\delta\) 40.6 (N-CH\(_3\)), 104.5 (q, 5-C), 107.3 (3'-CH), 140.9 (2'-CH), 157.4 (q, 4'-C), 162.5 (q, 2 or 4, 6-C), 162.8 (q, 2 or 4, 6-C), 181.9 (-CHO).

\(v_{\text{max}}\) (C=O) 1518, 1597, 1651 cm\(^{-1}\).

mp >200 °C

2-Amino-4-chloro-6-methoxypyrimidine-5-carboxaldehyde 2.23

Sodium methoxide (59 mg, 1.1 mmol) was added to a solution of 2-amino-4,6-dichloropyrimidine-5-carboxaldehyde\(^2\)\(^1\)\(^6\) 2.06 (96 mg, 0.5 mmol) in refluxing methanol (15 ml) and refluxing was continued for 2 hours. The methanol was

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removed leaving a cream coloured solid, which was washed with water, filtered and
dried (84 mg, 89.6%).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.94 (s, 3H, 6-CH$_3$), 8.07 (s, 2H, -NH$_2$), 10.00 (s, 1H, -CHO).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 53.7 (O-CH$_3$), 93.4 (q, 5-C), 163.3 (q, 2-C), 164.5 (q, 4-C), 171.2 (q, 6-C), 182.3 (-CHO).

$\nu_{max}$ 1514, 5540, 1570, 1665 cm$^{-1}$

mp 100-102 °C

2-Amino-4,6-dimethoxypyrimidine-5-carboxaldehyde 2.22

(a) Sodium methoxide (162 mg, 3 mmol) was added to a solution of 2-amino-4-chloro-6-methoxypyrimidine-5-carboxaldehyde 2.23 (188 mg, 1 mmol) in refluxing methanol (15 ml) and refluxing was continued for 4 hours. The methanol was removed leaving a cream coloured solid, which was washed with water, filtered and dried (162 mg, 84.7%).

(b) Sodium methoxide (162 mg, 3 mmol) was added to a solution of 2-amino-4,6-dichloropyrimidine-5-carboxaldehyde$^{2,16}$ 2.06 (96 mg, 0.5 mmol) in refluxing methanol (15 ml) and the reflux was continued for 4 hours. The methanol was removed leaving a cream coloured solid, which was washed with water, filtered and dried (62 mg, 67.7%).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.89 (s, 6H, 4, 6-CH$_3$), 7.61 (s, 2H, -NH$_2$), 9.92 (s, 1H, -CHO).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 53.9 (O-CH$_3$), 92.3 (q, 5-C), 163.4 (q, 2-C), 171.2 (q, 4, 6-C), 182.3 (-CHO).

$\nu_{max}$ 1511, 1540, 1575, 1661 cm$^{-1}$

mp 240 °C (decomp.)

2-Amino-9-methyl-6-(4'-dimethylaminopyridinium)-8-aza-7-deazapurine chloride 2.24

Methylhydrazine (47 µl, 0.88 mmol) was added to the pyrimidine 2.21 (381 mg, 0.87 mmol) in water (4 ml). A precipitate formed after 5 minutes, which was filtered and dried (172 mg, 64.6%).
\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 2.30 (s, 6H, N-CH\(_3\)), 3.88 (s, 3H, 9-CH\(_3\)), 7.25 (d, 2H, J 7.5, 3', 5'-CH), 7.38 (s, 2H, -NH\(_2\)), 8.31 (s, 1H, 7-CH), 8.90 (d, 2H, J 7.5, 2', 6'-CH).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 33.5 (9-CH\(_3\)), 40.6 (N-CH\(_3\)), 97.5 (q, 5-C), 108.05 (3', 5'-CH), 131.6 (7-CH), 138.3 (2', 6'-CH), 151.9 (q, 4'-C), 157.4 (q, 4-C), 158.11 (q, 2-C), 161.2 (q, 6-C).

mp >200 °C

Procedure 2.1

2-Amino-4,6-dichloropyrimidine-5-carboxaldehyde\(^2\)\(^6\) 2.06 (768 mg, 4 mmol) was added to a solution of a monosubstituted alkylhydrazine (4 mmol) and sodium acetate (382 mg, 4 mmol) in THF (6 ml) and water (4 ml). The reaction mixture was heated at 80 °C for 2 hours. The THF was removed leaving a pale yellow suspension in water. This was filtered and dried.

2-Amino-6-chloro-9-methyl-8-aza-7-deazapurine\(^2\)\(^7\) 2.04

This synthesis was performed according to procedure 2.1 using methylhydrazine (27 \(\mu\)l, 0.5 mmol) to give a yellow solid (72 mg, 78%).

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 3.80 (s, 3H, -CH\(_3\)), 7.27 (s, 2H, -NH\(_2\)), 7.97 (s, 1H, 7-CH).

2-Amino-6-chloro-9-(3'-phenoxypropyl)-8-aza-7-deazapurine 2.26

This synthesis was performed according to procedure 2.1, using 3-phenoxypropyl hydrazine\(^2\)\(^1\)\(^3\), (0.66 g, 4 mmol) to give a yellow solid (1.00 g, 82% crude), which was recrystallised from ethyl acetate (0.78 mg, 65%).

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 2.23 (quin, 2H, J 6.0, 2'-CH\(_2\)), 3.99 (t, 2H, J 6.0, 1'-CH\(_3\)), 4.35 (t, 2H, J 6.0, 3'-CH\(_2\)), 6.88 (m, 3H, 2", 4", 6"-CH), 7.26 (m, 4H, 3", 5"-CH, -NH\(_2\)), 7.98 (s, 1H, 7-CH).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 28.0 (2'-CH\(_2\)), 42.7 (1'-CH\(_2\)), 65.8 (3'-CH\(_2\)), 101.2 (q, 5-C), 129.6 (2", 6"-CH), 130.8 (4"-CH), 131.8 (3", 5"-CH), 132.5 (7-CH), 154.1 (q, 1"-C), 157.4 (q, 4-C), 161.0 (q, 2-C), 162.3 (q, 6-C).

\(\nu\)\(_{\text{max}}\) 1567, 1609, 1645, 3203, 3318, 3433 cm\(^{-1}\)

mp 165-166 °C
2-Amino-6-chloro-9-[3'-(2''-naphthoxy)propyl]-8-aza-7-deazapurine 2.27

This synthesis was performed according to procedure 2.1, using 3-(2''-naphthoxy)propyl hydrazine 2.14 (0.86 g, 4 mmol), to give a yellow solid (1.34 g, 94% crude), which was recrystallised from ethyl acetate (0.87g, 61%).

\(^1\)H NMR (400 MHz, DMSO-d6) \(\delta\) 2.33 (quin, 2H, J 6.5, 2'-CH\(_2\)), 4.13 (t, 2H, J 6.5, 1'-CH\(_2\)), 4.41 (t, 2H, J 6.5, 3'-CH\(_2\)), 7.10 (dd, 1H, J 2.5, 6.5, 1''-CH), 7.24 (s, 1H, 3''-CH), 7.26 (s, 2H, -NH\(_2\)), 7.33 (td, 1H, J 5.5, 1.5, 6''-CH), 7.44 (t, 1H, J 7.0, 7''-CH), 7.79 (m, 3H, 4'', 5'', 8''-CH), 8.00 (s, 1H, 7-CH).

\(^13\)C NMR (100 MHz, DMSO-d6) \(\delta\) 28.5 (2'-CH\(_2\)), 43.6 (1'-CH\(_2\)), 65.0 (3'-CH\(_2\)), 106.3 (q, 5-\(\mathcal{C}\)), 106.8 (1''-\(\mathcal{C}\)), 118.6 (3''-\(\mathcal{C}\)), 123.6 (6''-\(\mathcal{C}\)), 126.3 (7''-\(\mathcal{C}\)), 126.6 (8''-\(\mathcal{C}\)), 127.5 (5''-\(\mathcal{C}\)), 128.5 (q, 4a''-\(\mathcal{C}\)), 129.2 (4''-\(\mathcal{C}\)), 132.1 (7-\(\mathcal{C}\)), 134.2 (q, 8a''-\(\mathcal{C}\)), 153.4 (q, 2''-\(\mathcal{C}\)), 155.7 (q, 4-\(\mathcal{C}\)), 156.3 (q, 2-\(\mathcal{C}\)), 161.4 (q, 6-\(\mathcal{C}\)).

\(\nu_{\text{max}}\) 1556, 1606, 1645, 3213, 3315, 3423 cm\(^{-1}\).

mp 158-160 °C.

\(\text{O}^6\)-9-Dimethyl-8-aza-7-deazaguanine 2.28

(a) The chloride 2.04 (184 mg, 1 mmol) was refluxed in 1M NaOCH\(_3\)/methanol (2.5 ml, 2.5 mmol) for 1 hour. Upon cooling, Et\(_2\)O was added and the precipitate was filtered off. The filtrate was neutralised with acetic acid and evaporated. The residue was dissolved in methanol and the product precipitated by the addition of iced water (136 mg, 76%).

(b) Methylhydrazine (27 \(\mu\)l, 0.5mmol) was added to the pyrimidine 2.23 (94 mg, 0.5 mmol) and sodium acetate (20 mg, 0.5 mmol) in THF (5 ml). The reaction mixture was heated at 80 °C for 2 hours. The THF was removed, leaving an off-white solid, which was washed with water, filtered and dried (63 mg, 70%).

\(^1\)H NMR (400 MHz, DMSO-d6) \(\delta\) 3.76 (s, 3H, 9-CH\(_3\)), 3.98 (s, 1H, \(O^6\)-CH\(_3\)), 6.73 (s, 2H, -NH\(_2\)), 7.78 (s, 1H, 7-CH).

\(^13\)C NMR (100 MHz, DMSO-d6) \(\delta\) 33.1 (9-CH\(_3\)), 53.5 (O-CH\(_3\)), 95.9 (q, 5-\(\mathcal{C}\)), 131.1 (7-\(\mathcal{C}\)), 158.5 (q, 4-\(\mathcal{C}\)), 161.9 (q, 2-\(\mathcal{C}\)), 163.1 (q, 6-\(\mathcal{C}\)).

mp 168-170 °C.
**Procedure 2.2**

Sodium hydride (60 mg of 60% oil suspension in mineral oil, 1.5 mmol) was added to a solution of the alcohol (1.5 mmol) in dry DMF (2 ml) and stirred at room temperature for 30 minutes. The chloro compound (2.26, 2.27, 2.54a) (0.5 mmol) was added and the reaction mixture was stirred for 3 hours at 80 °C. Dry ether (10 ml) and acetic acid (0.25 ml) was added to the cooled solution, and the precipitate was filtered off. The filtrate was evaporated leaving a brown residue. This was dissolved in methanol. Upon the drop wise addition of ice-cold water, a solid precipitated. This was collected and dried.

\[ \text{O}^6-(4'-\text{Bromothenyl})-9-(3''-\text{phenoxypropyl})-8-\text{aza}-7-\text{deazaguanine} \ 2.29 \]

The synthesis of 2.29 was performed according to procedure 2.2, using 4-bromothenyl alcohol (76 mg, 1.2 mmol) and 2-amino-6-chloro-9-(3'-phenoxypropyl)-8-aza-7-deazapurine 2.26 (125 mg, 0.4 mmol), to give an off-white solid (49 mg, 26.6%).

\[ ^1H \text{ NMR} (400 \text{ MHz}, \text{DMSO-d}_6) \delta 2.22 \text{ (quin, 2H, J 6.5, 2''-CH}_2), 3.98 \text{ (t, 2H, J 6.5, 1''-CH}_2), 4.33 \text{ (t, 2H, J 6.5, 3''-CH}_2), 5.64 \text{ (s, 2H, O''-CH}_2), 6.87 \text{ (m, 5H, 2'', 4'', 6''-CH, NH}_2), 7.26 \text{ (m, 2H, 3'', 5''-CH)}, 7.35 \text{ (s, 1H, 3'-CH)}, 7.69 \text{ (s, 1H, 5'-CH)}, 7.82 \text{ (s, 1H, 7-CH)}. \]

\[ ^{13}C \text{ NMR} (100 \text{ MHz}, \text{DMSO-d}_6) \delta 28.7 \text{ (2''-CH}_2), 43.3 \text{ (1''-CH}_2), 60.8 \text{ (O''-CH}_2), 64.8 \text{ (3''-CH}_2), 95.8 \text{ (q, 5-C), 108.1 \text{ (q, 4'-C), 114.5 \text{ (2'', 6''-CH), 120.6 \text{ (4''-CH), 125.3 \text{ (5''-CH), 129.4 \text{ (3''}, 5''-CH)}, 131.0 \text{ (3'-CH), 131.4 \text{ (7-CH), 140.2 \text{ (q, 2'-C), 157.3 \text{ (q, 1''-C), 158.5 \text{ (q, 4-C), 161.6 \text{ (q, 2-C), 162.4 \text{ (q, 6-C)}.}}}} \]

\[ \nu_{\max} 1573, 1610, 1645, 3195, 3320, 3444 \text{ cm}^{-1} \]

mp 165 °C

\[ \lambda_{\max} 266, 282 \text{ nm} \]

% Calculated for C_{19}H_{18}BrN_{5}O_{2}S·1/8CH_{3}OH : C 49.47, H 4.02, N 15.08

% Found: C 49.87, H 4.14, N 14.74.

**O^6-\text{Benzyl}-9-(3''-\text{phenoxypropyl})-8-\text{aza}-7-\text{deazaguanine} \ 2.30**

The synthesis of 2.30 was performed according to procedure 2.2, using benzyl alcohol (155 µl, 1.5 mmol) and 2-amino-6-chloro-9-(3'-phenoxypropyl)-8-aza-7-deazapurine 2.26 (151 mg, 0.5 mmol), to give a pale yellow solid which was recrystallised from methanol (61 mg, 32.6%).
$^1$H NMR (400 MHz, DMSO-$d_6$) δ 2.25 (quin, 2H, J 6.5, 2''-CH$_2$), 3.99 (t, 2H, J 6.5, 1''-CH$_2$), 4.31 (t, 2H, J 6.5, 3''-CH$_2$), 5.51 (s, 2H, O$_6$-CH$_2$), 6.71 (m, 5H, 2'', 4'', 6''-CH, -NH$_2$), 7.39 (m, 7H, 2', 3', 4', 5', 6', 3'', 5''-CH), 7.80 (s, 1H, 7-CH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 28.8 (2''-CH$_2$), 43.2 (1''-CH$_2$), 64.8 (O$_6$-CH$_2$), 67.0 (3''-CH$_2$), 96.0 (q, 5-Ç), 114.5 (2'', 6''-CH), 120.6 (4''-CH), 128.1 (2', 6'-CH), 128.4 (4'-CH), 128.5 (3', 5'-CH), 129.4 (3'', 5''-CH), 131.1 (7-CH), 136.4 (q, 1'-Ç), 157.2 (q, 1''-Ç), 158.5 (q, 4-Ç), 161.9 (q, 2-Ç), 163.1 (q, 6-Ç).

$\nu_{max}$ 1576, 1610, 1643, 3201, 3323, 3446 cm$^{-1}$

mp 165 °C

$\lambda_{max}$ 266, 280 nm

% Calculated for C$_{22}$H$_{21}$N$_5$O$_4$: C 65.61, H 5.76, N 18.22

% Found: C 65.75, H 5.62, N 18.20.

9-(3''-Phenoxypropyl)-O$_6$-piperonyl-8-aza-7-deazaguanine 2.31

The synthesis of 2.31 was performed according to procedure 2.2, using piperonyl alcohol (228 mg, 1.5 mmol) and 2-amino-6-chloro-9-(3'-phenoxypropyl)-8-aza-7-deazapurine 2.26 (151 mg, 0.5 mmol), to give a pale yellow solid, which was recrystallised from methanol (72 mg, 34.6%).

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 2.22 (quin, 2H, J 6.5, 2''-CH$_2$), 3.98 (t, 2H, J 6.5, 1''-CH$_2$), 4.31 (t, 2H, J 6.5, 3''-CH$_2$), 5.39 (s, 2H, O$_6$-CH$_2$), 6.02 (s, 2H, O-CH$_2$-O), 6.78 (s, 2H, -NH$_2$), 6.91 (m, 4H, 2', 5', 2'', 6''-CH), 7.02 (m, 1H, 6'-CH), 7.11 (s, 1H, 4''-CH), 7.26 (t, 2H, J 8, 3'', 5''-CH), 7.81 (s, 1H, 7-CH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 28.8 (2''-CH$_2$), 43.2 (1''-CH$_2$), 64.8 (O$_6$-CH$_2$), 67.0 (3''-CH$_2$), 95.9 (q, 5-Ç), 101.1 (O-CH$_2$-O), 108.1 (2'-CH), 109.4 (5'-CH), 114.5 (2'', 6''-CH), 120.6 (4''-CH), 122.7 (6'-CH), 129.4 (3'', 5''-CH), 130.0 (q, 1'-Ç), 131.1 (7-CH), 147.2 (q, 3'-Ç), 147.3 (q, 4'-Ç), 157.2 (q, 1''-Ç), 158.5 (q, 4-Ç), 161.8 (q, 2-Ç), 163.0 (q, 6-Ç).

$\nu_{max}$ 1572, 1610, 1643, 3195, 3321, 3440 cm$^{-1}$

mp 159-160 °C

$\lambda_{max}$ 268, 278 nm

% Calculated for C$_{22}$H$_{21}$N$_5$O$_4$: C 63.00, H 5.05, N 16.70

% Found: C 62.70, H 5.06, N 16.52.
9-(3'-Phenoxypropyl)-O^-thenyl-8-aza-7-deazaguanine 2.32

The synthesis of 2.32 was performed according to procedure 2.2, using thenyl alcohol (171 mg, 1.5 mmol) and 2-amino-6-chloro-9-(3'-phenoxypropyl)-8-aza-7-deazapurine 2.26 (151 mg, 0.5 mmol), to give a pale yellow solid, which was recrystallised from methanol (47 mg, 24.6%).

\^H NMR (400 MHz, DMSO-d_6) δ 2.22 (quin, 2H, J 6.5, 2'-CH_2), 3.99 (t, 2H, J 6.5, 1'-CH_2), 4.32 (t, 2H, J 6.5, 3'-CH_2), 5.68 (s, 2H, O^-CH_2), 6.81 (m, 2H, -NH_2), 6.91 (m, 3H, J 8.0, 2'', 4'', 6''-CH), 7.04 (t, 1H, J 4.0, 3'-CH), 7.26 (m, 2H, J 8.0, 3'', 5''-CH), 7.32 (d, 1H, J 4.0, 2'-CH), 7.57 (d, 1H, J 4.0, 5'-CH), 7.80 (s, 1H, 7-CH).

\^C NMR (100 MHz, DMSO-d_6) δ 28.8 (2''-CH_2), 43.2 (1''-CH_2), 61.3 (O^-CH_2), 64.8 (3''-CH_2), 95.9 (q, 5'-C), 114.5 (2'', 6''-CH), 120.6 (4''-CH), 127.7 (3'-CH), 129.1 (5'-CH), 129.4 (3'', 5''-CH), 131.0 (7-CH), 136.8 (4'-CH), 138.1 (q, 2'-C), 158.4 (q, 1''-C), 161.7 (q, 4-C), 161.7 (q, 2-C), 162.6 (q, 6-C).

\( \lambda_{max} \) 1576, 1612, 1645, 3197, 3320, 3448 cm\(^{-1}\)

mp 188-190 °C

% Calculated for C_{19}H_{19}N_{5}O_{2}: C 59.83, H 5.02, N 18.36.

% Found: C 59.53, H 4.96, N 18.25.

O^-{(4'-Bromothenyl)-9-[3''-(2''-naphthoxy)propyl]-8-aza-7-deazaguanine 2.33

The synthesis of 2.33 was performed according to procedure 2.2, using 4-bromothenyl alcohol (281 mg, 1.5 mmol) and 2-amino-6-chloro-9-[3''-(2''-naphthoxy)propyl]-8-aza-7-deazapurine 2.27 (178 mg, 0.5 mmol), to give a white solid, which was recrystallised from methanol (131 mg, 45.2%).

\^H NMR (400 MHz, DMSO-d_6) δ 2.30 (quin, 2H, J 6.5, 2''-CH_2), 4.12 (t, 2H, J 6.5, 1''-CH_2), 4.37 (t, 2H, J 6.5, 3''-CH_2), 5.64 (s, 2H, O^-CH_2), 6.85 (s, 2H, -NH_2), 7.12 (dd, 1H, J 2.5, 6.5, 1''-CH), 7.24 (s, 1H, 3''-CH), 7.35 (m, 2H, 3', 6''-CH), 7.44 (m, 1H, 7''-CH), 7.68 (s, 1H, 5'-CH), 7.78 (m, 4H, 4'', 5'', 8'', 7-CH).

\^C NMR (100 MHz, DMSO-d_6) δ 28.7 (2''-CH_2), 43.3 (1''-CH_2), 60.9 (3''-CH_2), 65.0 (O^-CH_2), 95.6 (q, 5'-C), 106.8 (1''-CH), 108.1 (q, 4'-C), 118.7 (3''-CH), 123.5 (7''-CH), 125.3 (5'-CH), 126.3 (7''-CH), 126.6 (8''-CH), 127.5 (5''-CH), 128.5 (q, 4a''-C), 129.2 (4''-CH), 131.0 (7-CH), 134.2 (q, 8a''-C), 140.1 (q, 2''-C), 156.3 (q, 2''-C), 157.3 (q, 4-C), 161.6 (q, 2-C), 162.4 (q, 6-C).

\( \lambda_{max} \) 1520, 1614, 1647, 3195, 3323, 3446 cm\(^{-1}\)
mp 196 °C
λ<sub>max</sub> 268, 280 nm
% Calculated for C<sub>23</sub>H<sub>20</sub>BrN<sub>5</sub>O<sub>2</sub>S: C 54.12, N 13.72, H 3.95
% Found: C 54.38, N 13.71, H 4.01.

O<sup>6</sup>-Benzy-9-[3''-(2''-naphthoxy)propyl]-8-aza-7-deazaguanine 2.34

The synthesis of 2.34 was performed according to procedure 2.2, using benzyl alcohol (155 µl, 1.5 mmol) and 2-amino-6-chloro-9-[3'-2''(naphthoxy)propyl]-8-aza-7-deazapurine 2.27 (178 mg, 0.5 mmol), to give a white flaky solid, which was recrystallised from methanol (87 mg, 41.7%).

1<sup>H</sup> NMR (400 MHz, DMSO-<i>d</i><sub>6</sub>) δ 2.31 (quin, 2H, J 6.5, 2''-CH<sub>2</sub>), 4.12 (t, 2H, J 6.5, 1''-CH<sub>2</sub>), 4.36 (t, 2H, J 6.5, 3''-CH<sub>2</sub>), 5.50 (s, 2H, O<sup>6</sup>-CH<sub>2</sub>), 6.78 (s, 2H, -NH<sub>2</sub>), 7.12 (dd, 1H, J 6.5, 2.5, 1''-CH), 7.25 (d, 1H, J 2, 3''-CH), 7.40 (m, 5H, 2', 4', 6', 6''', 7'''-CH), 7.50 (m, 2H, 3', 5'-CH), 7.81 (m, 4H, 4'', 5'', 8'', 7-CH).

1<sup>3</sup>C NMR (100 MHz, DMSO-<i>d</i><sub>6</sub>) δ 28.7 (2''-CH<sub>2</sub>), 42.2 (1''-CH<sub>2</sub>), 65.0 (3''-CH<sub>2</sub>), 67.0 (O<sup>6</sup>-CH<sub>2</sub>), 100.0 (q, 5-C), 106.8 (1''-CH), 118.7 (3''-CH) 123.5 (6''-CH), 126.3 (7''-CH), 126.6 (8''-CH), 127.5 (5''-CH), 128.1 (2', 6'-CH), 128.4 (4''-CH), 128.5 (3', 5'-CH), 128.7 (q, 4a''-C), 129.2 (4''-CH), 131.1 (7-CH), 134.2 (q, 8a''-C), 136.4 (q, 1'-C), 156.4 (q, 2''-C), 157.3 (q, 4-C), 161.9 (q, 2-C), 163.1 (q, 6-C).

υ<sub>max</sub> 1579, 1614, 1645, 3194, 3320, 3452 cm<sup>-1</sup>
mp 184 °C
λ<sub>max</sub> 268, 280 nm
% Calculated for C<sub>23</sub>H<sub>22</sub>N<sub>5</sub>O<sub>2</sub>: C 67.57, N 16.46, H 5.29
% Found: C 67.71, N 16.01, H 5.23.

3-Amino-4-carbethoxy-2-methylpyrazole<sup>25</sup> 2.51a

Methylhydrazine (537 µl, 10 mmol) was added to ethyl (ethoxymethylene)cyanoacetate 2.45 (1.69 g, 10 mmol) in ethanol (20 ml). The reaction mixture was refluxed for 2 hour. The ethanol was removed leaving a brown oil which crystallised from diethyl ether to give fine pale yellowed coloured crystals (1.37 g, 81.0%).

1<sup>H</sup> NMR (400 MHz, DMSO-<i>d</i><sub>6</sub>) δ 1.24 (t, 3H, J 7.0, 3'-CH<sub>3</sub>), 3.54 (s, 3H, N-CH<sub>3</sub>), 4.17 (quart, 2H, J 7.0, 2'-CH<sub>2</sub>), 6.18 (s, 2H, -NH<sub>2</sub>), 7.42 (s, 1H, 3-CH).
3-Amino-4-carbethoxy-2-(3'-phenoxypropyl)pyrazole 2.51b

The hydrazine 2.16 (1.66 g, 10 mmol) and ethyl (ethoxymethylene)cyanoacetate 2.45 (1.69 g, 10 mmol) were refluxed in ethanol (10 ml) for 2 hours. The ethanol was removed leaving a brown oil which was crystallised from petroleum ether to give pale yellow needles (1.76 g, 61.0%).

\[ \text{H NMR (400 MHz, DMSO-d}_6\text{)} \delta 1.22 (t, 3H, J 7.0, 2''-CH}_3), 2.09 (2H, J 6.5, 2'-CH\text{)}, 3.95 (t, 2H, J 6.5, 1'-CH\text{)}, 4.04 (t, 2H, J 7.0, 1''-CH\text{)}, 4.15 (quart, 2H, J 7.0, 1''-CH\text{)}, 6.22 (broad s, 2H, -NH\text{)}, 6.90 (m, 3H, 2'', 4'', 6''-CH\text{)}, 7.26 (t, 2H, J 8.0, 3'', 5''-CH\text{)}, 7.45 (s, 1H, 5-CH). \]

\[ \text{C NMR (100 MHz, DMSO-d}_6\text{)} \delta 14.5 (CH\text{)}, 28.3 (2''-CH\text{)}, 43.4 (1''-CH\text{)}, 58.6 (1'-CH\text{)}, 64.6 (3''-CH\text{)}, 93.9 (q, 4-C), 114.5 (2'', 6''-CH\text{)}, 120.6 (4''-CH\text{)}, 129.5 (3'', 5''-CH\text{)}, 138.5 (5-CH\text{)}, 149.8 (q, 1''-C), 158.5 (q, 3-C), 163.4 (C=O). \]

\[ \text{\boldsymbol{\nu}}_{\text{max}} \text{ 1619, 1672, 1714, 1943 cm}^{-1} \]

\[ \text{mp 90-92 }^\circ\text{C} \]

4-Carbethoxy-3-ethoxymethyleneamino-2-methylpyrazole 2.52a

The pyrazole 2.51a (0.670 g, 4 mmol) was refluxed in a mixture of ethyl orthoformate (2.35 ml) and acetic anhydride (2.2 ml) for 2 hours. The excess ethyl orthoformate and acetic anhydride was removed leaving a brown tar (0.827 g, 99.2%).

\[ \text{H NMR (400 MHz, DMSO-d}_6\text{)} \delta 1.24 (t, 3H, J 7.0, 3''-CH\text{)}, 1.35 (t, 3H, J 7.0, 3''-CH\text{)}, 3.53 (s, 3H, N-CH\text{)}, 4.16 (quart, 2H, J 7.0, 2'-CH\text{)}, 4.38 (quart, 2H, J 7.0, 2''-CH\text{)}, 7.72 (s, 1H, 3-CH\text{)}, 8.35 (s, 1H, 1''-CH). \]

9-Methyl-8-aza-7-deazapurine 2.53a

The pyrazole 2.52a was dissolved in a 1M solution of ammonia in ethanol (4 ml). The solution was allowed to stand at room temperature for 7 days, during which the product precipitated as an off-white solid (55 mg, 85%).

\[ \text{H NMR (400 MHz, DMSO-d}_6\text{)} \delta 3.91 (s, 3H, N-CH\text{)}, 8.04 (s, 1H, 7-CH\text{}), 8.07 (s, 1H, 2-CH\text{)}, 11.77 (s, 1H, -NH). \]

9-(3'-Phenoxypropyl)-8-aza-7-deazapurine 2.53b

The pyrazole 2.51b (1.40 g, 4.84 mmol) was refluxed in a mixture of ethyl orthoformate (3.14 ml, 18.86 mmol) and acetic anhydride (2.23 ml, 23.57 mmol) for 2
hours. The excess ethyl orthoformate and acetic anhydride were removed leaving a brown oil (1.64 g). The oil was dissolved in a solution of aq. ammonia in ethanol and left to stand at room temperature for 4 days. A white solid precipitated which was filtered and dried (0.76 g, 57.9%).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.26 (quin, 2H, J 6.5, 2'-CH$_2$), 3.98 (t, 2H, J 6.5, 1'-CH$_2$), 4.47 (t, 2H, J 6.5, 3'-CH$_2$), 6.85 (d, 2H, J 7.5, 2'', 6''-CH), 6.91 (t, 1H, J 7.5, 4''-CH), 7.25 (t, 2H, J 7.5, 3'', 5''-CH), 8.04 (s, 1H, 2-CH), 8.08 (s, 1H, 7-CH), 12.13 (s, 1H, -NH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 28.9 (2'-CH$_2$), 44.1 (1'-CH$_2$), 64.7 (3'-CH$_2$), 105.8 (q, 5-C), 114.4 (2'', 6''-CH), 120.6 (4''-CH), 129.4 (3'', 5''-CH), 134.3 (7-CH), 147.8 (2-CH), 151.8 (q, 1''-C), 157.3 (q, 4-C), 158.4 (q, 6-C).

$\nu_{\text{max}}$ (C=O) 1648 cm$^{-1}$

mp 209-212 °C

4-Chloro-9-(3'-phenoxypropyl)-8-aza-7-deazapurine 2.54b

9-(3'-Phenoxypropyl)-8-aza-7-deazapurine 2.53b (725 mg, 2.7 mmol) was refluxed in phosphorus oxychloride (3.5 ml) for 2.5 hours. Removal of phosphorus oxychloride gave a brown residue, which was added to crushed ice (~50 g). The subsequent sticky solid was extracted with diethyl ether leaving a pale yellow solid (701 mg, 89.9%), which was recrystallised from EtOAc/hexane to give (510 mg).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.34 (quin, 2H, J 6.5, 2'-CH$_2$), 4.01 (t, 2H, J 6.5, 1'-CH$_2$), 4.66 (t, 2H, J 6.5, 3'-CH$_2$), 6.80 (d, 2H, J 7.5, 2'', 6''-CH), 6.90 (t, 1H, J 7.5, 4''-CH), 7.23 (t, 2H, J 7.5, 3'', 5''-CH), 8.48 (s, 1H, 7-CH), 8.82 (s, 1H, 2-CH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 28.6 (2'-CH$_2$), 44.6 (1'-CH$_2$), 64.7 (3'-CH$_2$), 113.1 (q, 5-C), 114.3 (2'', 6''-CH), 120.5 (4''-CH), 129.4 (3'', 5''-CH), 132.1 (7-CH), 152.9 (q, 1''-C), 153.6 (q, 4-C), 154.5 (2-CH), 158.3 (q, 6-C).

$\nu_{\text{max}}$ 1589, 1685 cm$^{-1}$

mp 207-209 °C

O''-(4'-Bromothenyl)-9-(3''-phenoxypropyl)-8-aza-7-deazapurine 2.55

The synthesis of 2.55 was performed according to procedure 2.2, using 4-bromothenyl alcohol (434 mg, 2.25 mmol) and 6-chloro-9-(3'-phenoxypropyl)-8-aza-7-deazapurine
2.54b (217 mg, 0.75 mmol), to give a white solid (142 mg, 42.5%), which was recrystallised from EtOAc/hexane.

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.30 (quin, 2H, J 6.1, 2$''$-CH$_2$), 3.97 (t, 2H, J 6.1, 1$''$-CH$_2$), 4.60 (t, 2H, J 6.1, 3$''$-CH$_2$), 5.79 (s, 2H, $O^6$-CH$_2$), 6.82 (d, 2H, J 8.2, 2$''$, 6$''$-CH), 6.90 (t, 1H, J 8.2, 4$''$-CH), 7.2 (t, 2H, J 8.2, 3$'''$, 5$'''$-CH), 7.36 (s, 1H, 3$'$-CH), 7.72 (s, 1H, 5$'$-CH), 8.27 (s, 1H, 7-CH), 8.63 (s, 1H, 2-CH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 28.8 (2$''$-CH$_2$), 44.1 (1$''$-CH$_2$), 61.9 ($O^6$-CH$_2$), 64.6 (3$''$-CH$_2$), 101.8 (q, 5- C), 108.2 (q, 4'-C), 114.3 (2$''$, 6$''$-CH), 120.5 (4$'''$-CH), 125.5 (5$'$-CH), 129.4 (3$'''$, 5$'''$-CH), 131.1 (3$'$-CH), 131.2 (7-CH), 139.8 (q, 2'-C), 154.4 (q, 1$''$-C), 154.7 (2-CH), 158.3 (q, 4-C), 162.2 (q, 6-C).

$\nu_{max}$ 1563, 1600, 1686 cm$^{-1}$

mp 97-98 °C

λ$_{max}$ 266, 282 nm

% Calculated for C$_{19}$H$_{17}$BrN$_4$O$_2$: C 51.24, H 3.85, N 12.58

% Found: C 51.33, H 3.86, N 12.79.

O$^6$-Benzyl-9-(3$'''$-phenoxypropyl)-8-aza-7-deazapurine 2.56

The synthesis of 2.56 was performed according to procedure 2.2, using benzyl alcohol (233 µl, 2.25 mmol) and 6-chloro-9-(3$'$-phenoxypropyl)-8-aza-7-deazapurine 2.54b (217 mg, 0.75 mmol) to give a white solid (94 mg, 27.8%), which was recrystallised from EtOAc/hexane.

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.30 (quin, 2H, J 6.5, 2$''$-CH$_2$), 3.98 (t, 2H, J 6.5, 1$''$-CH$_2$), 4.60 (t, 2H, J 6.5, 3$''$-CH$_2$), 5.64 (s, 2H, $O^6$-CH$_2$), 6.82 (d, 2H, J 8.0, 2$''$, 6$''$-CH), 6.90 (t, 1H, J 8.0, 4$''$-CH), 7.39 (m, 7H, 2', 3', 4', 5', 6', 3$'''$, 5$'''$-CH), 8.29 (s, 1H, 7-CH), 8.59 (s, 1H, 2-CH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 28.9 (2$''$-CH$_2$), 44.2 (1$''$-CH$_2$), 64.7 ($O^6$-CH$_2$), 68.1 (3$''$-CH$_2$), 102.0 (q, 5- C), 114.4 (2$''$, 6$''$-CH), 120.6 (4$'''$-CH), 128.3 (2', 6'-CH), 128.4 (4$'$-CH), 128.6 (3', 5$'$-CH), 129.4 (3$'''$, 5$'''$-CH), 131.2 (7-CH), 136.0 (q, 1$''$-C), 154.4 (q, 1$''$-C), 155.0 (2-CH), 158.4 (q, 4-C), 162.9 (q, 6-C).

$\nu_{max}$ 1569, 1598, 1655, 1691 cm$^{-1}$

mp 80-82 °C

λ$_{max}$ 266, 280 nm

% Calculated for C$_{21}$H$_{20}$N$_4$O$_2$: C 69.98, H 5.59, N 15.55

% Found: C 69.69, H 5.55, N 15.63.

68
2.8 References


2.21 See Formamide Material Safety Data Sheet, Aldrich Chemical Co., Inc.

2.23  (a) E. C. Taylor and W. A. Ehrhart, *J. Am. Chem. Soc.*, 1960, **82**, 3138-3146,  


Chapter 3

Synthesis of Alkoxy carbonyl Derivatives of AlloPaTrin
3.1 Introduction

In 1994, Pegg et al. published the synthesis of the 9-acetyl derivative of O'-benzylguanine 3.01. Its biological activity was similar to that of its parent compound, O'-benzylguanine 1.17. This was attributed to the conversion of 3.01 to O'-benzylguanine 1.17 by hydrolysis in vitro.

Similarly a higher than expected I₅₀ value was found for the 9-methoxycarbonyl derivative of PaTrin-2 1.31, which was stable under physiological conditions (pH 7.4 at 37 °C), and decomposes to give free PaTrin-2 at pH 8.3. The potential of 1.31 to act as a prodrug of PaTrin-2 has lead us to prepare a series of alkoxycarbonyl derivatives of alloPaTrin.

3.2 Carbazates

In Chapter 2, we discussed the synthesis of 9-alkyl substituted 8-aza-7-deazaguanines. These compounds were made by the reaction of the pyrimidine 2.06 with a monosubstituted alkylhydrazine (see Scheme 2.3, p.39). The alkylhydrazine not only forms part of the pyrazole ring, but it also facilitates the formation of the 9-alkylated product only. Other routes to such compounds routinely produce a mixture of the 8- and 9-alkylated compounds, which require separation and reduce the yields of the N-9 isomer. In this chapter, we explore the use of this synthetic strategy to produce a range of N-9 substituted alkoxycarbonyl 8-aza-7-deazaguanines.

To produce 9-substituted alkoxycarbonyl 8-aza-7-deazaguanines via the synthetic approach outlined in Chapter 2, carbazates (or alkoxycarbonylhydrazines) 3.02, NH₂NHCO₂R, were reacted with the pyrimidine 2.06 (Scheme 3.1) instead of
alkylhydrazine used in the earlier study. As in Chapter 2, the great advantage of this strategy is that the alkoxy carbonyl substituent should occur in the 9-position only. None of the 8-substituted compound should be formed, which results in higher yields of the 9-substituted isomer and avoiding the need for lengthy purification measures.

While some carbazates are available commercially, the synthesis of many of our target molecules would require the synthesis of non-commercial carbazates. This can be achieved in excellent yields by the reaction of an excess of hydrazine with

- a chloroformate 3.03
- an unsymmetrical carbonate with one substituent acting as a leaving group 3.04
- a pyrocarbonate 3.05.

Initially methylcarbazate 3.06, NH₂NHCO₂CH₃, was used as a model carbazate to find the optimal reaction conditions for the pyrazole ring forming step.
3.2.1 Reaction of Methylcarbazate with a Pyrimidine

When methylcarbazate \( 3.06 \) was reacted with the pyrimidine \( 2.06 \) and sodium acetate in aqueous THF, a single product was formed. Analysis of its \(^1\text{H} \) NMR spectrum showed peaks at 3.70 ppm corresponding to the methyl group, at 7.82 ppm corresponding to the \(-\text{NH}_2\) and at 8.04 ppm corresponding to the proton in the 7-position. An additional peak occurred at 11.24 ppm. A D\(_2\)O exchange experiment showed that this peak was assigned to an \(-\text{NH}\). Its presence indicated that the reaction of the carbazate and the pyrimidine \( 2.06 \) did not give the bicyclic product \( 3.08 \) as required.

\[
\begin{align*}
\text{Cl} & \quad \text{O} & \quad \text{NH}_2 \quad \text{H}_2\text{N} \quad \text{CO}_2\text{CH}_3 \\
\text{H}_2\text{N} & \quad \text{C} & \quad \text{O} & \quad \text{X} & \quad \text{H} \\
\end{align*}
\]

\( 2.06 \)

\[
\begin{align*}
\text{Cl} & \quad \text{NH} \quad \text{CO}_2\text{CH}_3 \\
\text{H}_2\text{N} & \quad \text{N} & \quad \text{C} & \quad \text{O} & \quad \text{X} \\
\end{align*}
\]

\( 3.07 \)

\[
\begin{align*}
\text{Cl} & \quad \text{N} & \quad \text{H} & \quad \text{CO}_2\text{CH}_3 \\
\text{H}_2\text{N} & \quad \text{N} & \quad \text{C} & \quad \text{O} & \quad \text{X} \\
\end{align*}
\]

\( 3.08 \)

The reaction stopped after the initial reaction of the carbazate with the aldehyde group of the pyrimidine \( 2.06 \), yielding the hydrazone \( 3.07 \), which failed to cyclise to give the pyrazole ring (Scheme 3.3). The structure of the compound was determined by nOe spectroscopy. Irradiation of the 1'-proton signal produced a positive response from the signal corresponding to the \(-\text{NH}\).
Figure 3. $^1$H NMR and nOe spectra of the hydrazone $3.07$ (DMSO-$d_6$).

The result shows that the -CH and -NH are relatively close to each other spatially. This indicates that $3.09$, with its (E) geometry around the C=N double bond, is the true structure of the hydrazone and not $3.10$, its (Z) isomer.

![Figure 3. $^1$H NMR and nOe spectra of the hydrazone $3.07$ (DMSO-$d_6$).](image)

For ring closure to occur, the hydrazone would have to adopt the (Z) conformation as in $3.10$. The $3'$-NH is also less nucleophilic than its alkyl counterpart due to the proximity of the electron attracting carbonyl group. This factor would militate against the closure of the pyrazole ring even if the hydrazone possessed the correct configuration.
3.2.2 Attempted Closure of the Pyrazole Ring

A number of approaches were adopted to see if we could overcome these difficulties. The mechanism by which the hydrazone can cyclise shows that an equivalent of hydrogen chloride is produced during the reaction (Scheme 3.3). Theoretically, as in the corresponding alkyl case (see pp. 43-44), addition of one equivalent of base should assist in the formation of hydrogen chloride, and consequently closure of the pyrazole ring.\(^3\)\(^3\)

As stated earlier, the use of sodium acetate had no effect on the progress of the reaction. In the alkyl series, we showed that the cyclisation reaction is particularly sensitive to the type of base used as the “acid scavenger” (Section 2.4.2, pp. 43-47). Bearing this in mind, we persisted with our investigation of the use of base to promote the formation of the bicyclic ring system. The pyrimidine 2.06 was reacted with methylcarbazate 3.02 and a selection of bases (pyridine, triethylamine, DMAP, DABCO, DBU) under a variety of reaction conditions. In each experiment, the hydrazone chain formed but failed to react further to produce the required pyrazole ring. As an alternative approach, the hydrazone 3.09 was treated with base (either pyridine, triethylamine, DMAP, DABCO, DBU), but the hydrazone chain remained intact.

As the use of organic bases mentioned above failed to give ring closure, a much stronger base, NaH, was employed, using the dichlorohydrazone 3.09 as substrate. Not only could this potentially promote the elimination of the chloride from the 4-position of the pyrimidine ring, but it is possible that the –NH of the hydrazone would be deprotonated. The subsequent negative charge would be stabilised by the formation of a resonance hybrid, thereby lowering the energy barrier to rotation of the C= N double bond (Scheme 3.4). This might allow ring closure to take place. In practice, we were unable to secure ring closure using this approach.
We also attempted to reduce the bond order of the C=N bond by treating the hydrazone 3.09 with acid. In theory, the hydrazone should be protonated by the acid. The positive charge would be spread throughout the molecule in a resonance hybrid (Scheme 3.5). This would potentially weaken the C=N bond and allow the side-chain to adopt the correct orientation to facilitate ring closure. Concentrated sulfuric acid, p-toluenesulfonic acid and trifluoroacetic acid were reacted with a solution of the hydrazone 3.09 in DMF. The hydrazone decomposed giving a black tar upon treatment with concentrated sulphuric acid, while p-toluenesulfonic acid and trifluoroacetic acid gave no reaction.
In the pyrimidine 2.06, the 4- and 6-chlorine substituents are equivalent and one of them might have acted as a leaving group in the cyclisation reaction. We investigated the effect of changing both chlorine substituents for other groups, which might lead to easier cyclisation. As mentioned earlier (p.45), once the first chlorine is displaced, the second chloride is displaced more easily. We were never able to obtain a molecule with only one replacement group.

Both the 4- and 6-chlorides of the hydrazone were displaced by a tosyl group\textsuperscript{3,8} and a 2,4,5-trichlorophenoxy group\textsuperscript{3,9} to give the pyrimidines 3.11 and 3.12. These derivatives were then treated with a variety of bases (triethylamine, pyridine, DBU, DABCO, DMAP, sodium methoxide). However, none of the experiments using these approaches led to ring closure.
The use of carbazates to build the pyrazole ring of 9-alkoxycarbonyl substituted 8-aza-7-deazaguanines would have been of great synthetic use. Novel carbazates could have been made following well-established methods, which would have eventually provided a wide range of 9-alkoxycarbonyl substituents in the final compounds. Another advantage to this unambiguous synthesis is the alkoxycarbonyl substituent would automatically have been in the 9-position. Unfortunately, the reaction of a carbazate and the pyrimidine 2.06 only gave a hydrazone 3.09 which we could not cyclise to give the required bicyclic ring system.
3.3 AlloPaTrin

As all our attempts to synthesise an 8-aza-7-deazaguanine with an alkoxycarbonyl substituent in the 9-position using the carbazate-pyrimidine method failed, our focus turned to O(\textsuperscript{6})-(4'-bromothenyl)-8-aza-7-deazaguanine 1.37, or alloPaTrin, the 8-aza-7-deazaguanine analogue of PaTrin-2. In the PaTrin-2 series, substituents are placed in the 9-position by the direct displacement of the N-9 proton. This reaction can also potentially produce 7-substituted compounds and in some cases did so.\textsuperscript{3,2}

Adapting this approach to alloPaTrin has advantages and disadvantages. We would be able to capitalise on the vast pool of knowledge that our co-workers have obtained with regard to the displacement of the 9-proton of PaTrin-2. However, this approach would give rise to an ambiguous synthesis, with the possible production of 8- and 9-substituted compounds possible (Scheme 3.6). It is known that 2-amino-6-chloro-8-aza-7-deazapurine 2.03 reacts with methyl iodide to afford both 8- and 9-methyl derivatives 2.04 and 2.05 (see p.38).\textsuperscript{3,10} The occurrence of two products from the acylation reaction will cause complications on work up and purification. The yield of individual isomers will be reduced. However, the biological activity of the two isomers can be assessed. The activities of a set of isomers may not differ greatly, as the side-chain could be removed before the compound reaches its site of action. Finally, the chemistry of both the guanine and 8-aza-7-deazaguanine systems can now be compared directly, as the reactions both systems undergo should be broadly similar.

![Scheme 3.6](image-url)
3.3.1 Synthesis of AlloPaTrin

AlloPaTrin has been made before in this laboratory.\textsuperscript{3,11} The pyrimidine 2.06 was treated with hydrazine monohydrate resulting in the formation of 2-amino-6-chloro-8-aza-7-deazapurine 2.03, which was then reacted with 4-bromothenyl alcohol and sodium hydride in DMSO at 80 °C for one hour to form alloPaTrin (Scheme 3.7). This synthesis is based on the method used by Seela to make the corresponding O\textsuperscript{6}-methoxy derivative of alloPaTrin.\textsuperscript{3,10} That displacement reaction used excess methanol as solvent. However this approach would not be desirable for the more expensive and commercially unavailable 4-bromothenyl alcohol. Murray adopted Seela’s method using three equivalents of the alcohol and using DMSO as solvent.\textsuperscript{3,11}

Previously, only small quantities of alloPaTrin had been synthesised, as it was only required for biological evaluation. We wished to use alloPaTrin as starting material for alkoxy carbonyl derivatives, and consequently needed a method, which could produce it consistently on a much larger scale. When Murray's synthesis of alloPaTrin was repeated, problems emerged. Large amounts of a sticky dark brown substance formed during the work-up, which led to a reduction in the yield of the
reaction. AlloPaTrin itself was difficult to isolate from the reaction mixture and the crude product required several recrystallations to purify. However, the biggest problem with this scaled-up reaction was that it was not reproducible, either in its progress and work-up, leading to variable yields. Frequently no product could be isolated. The relative amounts of reagent and solvent, and changes in reaction time, solvent and reaction temperature were to no avail.

The failure of this reaction was attributed to the formation of a polymeric type species (Scheme 3.8). An anion of 2-amino-6-chloro-8-aza-7-deazapurine 2.03 is formed through the attack on the acidic N-9 proton by sodium hydride. This anion at either 8- or 9-position displaces chloride from the 6-position of another molecule of 2.03. This sequence is repeated several times giving the “polymer”. Attack may also occur at the exocyclic –NH₂. This could account for the huge amount of unidentifiable by-products, which formed during the reaction.
Scheme 3. 8
3.4. Alternative Syntheses of AlloPaTrin

3.4.1 Synthesis of AlloPaTrin from $O^6$-Alkoxypyrimidine

Because of these difficulties, we decided to concentrate on alternative methods of making alloPaTrin. In Chapter 2, the synthesis of monoalkoxypyrimidines 3.14 was briefly discussed (Section 2.4.3, p.47). The monoalkoxypyrimidine 3.14 could have reacted with hydrazine monohydrate to give the $O^6$-alkoxy-8-aza-7-deazaguanine. This method would have put the alkoxy group in place before the pyrazole ring was completed, thereby avoiding the problems outlined in Scheme 3.8. However, we could only make one $O^6$-alkoxypyrimidine, the $O^6$-methyl 2.24 derivative using methanol ($R = CH_3$) as both a reactant and a solvent (Scheme 3.9).

![Scheme 3.9](image)

We failed to make the corresponding $O^6$-(4-bromothenyl)pyrimidine 3.15. No compound could be isolated from the reaction of the dichloropyrimidine 2.06 and 4-bromothenyl alcohol with sodium hydride. TLC analysis of the reaction mixture showed the presence of several compounds. We thought that perhaps the aldehyde group of the pyrimidine 2.06 was reacting with the sodium alkoxide. Attempts to protect the aldehyde group by converting it to an acetal group using ethyl orthoformate or ethylene glycol failed. Both the syntheses of the diethyl acetal derivative 3.16 and the cyclic acetal derivative 3.17 were investigated, but decomposition appeared to take place in both cases before acetalisation occurred. This was possibly due to the high temperatures (>150 °C) required for the reactions to proceed. We did prepare the corresponding oxime 3.18 by the reaction of the aldehyde 2.06 with hydroxylamine hydrochloride and acetic acid. However again, we could only isolate the monomethoxy compound 3.19, and not the 4-bromothenyl.
derivative, from the reaction of the dichloro-5-pyrimidinecarboxaldehyde oxime 3.18 and sodium alkoxide.

\[
\begin{align*}
3.16 & : \quad \text{Cl} \quad \text{OEt} \\
3.17 & : \quad \text{Cl} \quad \text{O} \\
3.18 & : \quad \text{Cl} \quad \text{N} \quad \text{OH} \\
3.19 & : \quad \text{Cl} \quad \text{N} \quad \text{OH} \\
3.20 & : \quad \text{Cl} \quad \text{N} \quad \text{OCH}_3 \\
3.21 & : \quad \text{Cl} \quad \text{N} \quad \text{OCH}_3 \\
3.22 & : \quad \text{Cl} \quad \text{N} \quad \text{OCH}_3
\end{align*}
\]

The potential use of a methoxime group as a protecting group for the aldehyde of the pyrimidine 2.06 was also investigated. The methoxime compound 3.21 was readily prepared from the reaction of the aldehyde 2.06 with methoxylamine hydrochloride and sodium acetate, however the yield was low (20%). This was due to the formation of the mono-chloro compound 3.22 (23%) through the displacement of the 6-Cl of the desired product 3.21 by methoxylamine. The use of more methoxylamine hydrochloride or sodium acetate only lead to the increased formation of 3.22, the unwanted side-product.

3.4.2 Protection of 2-Amino-6-chloro-8-aza-7-deazapurine

Having failed to produce alloPaTrin from a mono-alkoxy pyrimidine, our attention returned to 2-amino-6-chloro-8-aza-7-deazapurine 2.03. We considered how to stop the unwanted side-reactions (see Scheme 3.8), by blocking the sites of 2.03, namely the 9-NH, which we considered to be causing the problems. Common protecting
groups for -NH groups are the methoxycarbonyl group, the pivoyl group and the t-butyloxycarbonyl group (or Boc).

In our first attempt, the -NH of 2.03 was initially protected by a methoxycarbonyl group to give 3.08 (potentially the 8-isomer 3.23 could have formed also). This compound was prepared by the reaction of 2.03 with a two-fold excess of methylpyrocarbonate and triethylamine to give a single product in a yield of 71% (see Scheme 3.10).\(^{3,16}\) When the protected compound 3.23 was treated with sodium alkoxide, the starting material 2.03 was obtained in a yield of over 90%. Instead of displacing the chloride in the 6-position of 3.23, the sodium alkoxide acts as a base and cleaves the methoxycarbonyl group.

\[\text{H,N} \quad \text{H} \quad 2.03 \quad \text{(CH}_3\text{CO}_2\text{)}_2\text{O} \quad \text{Et}_3\text{N} \quad \text{NaOR} \quad \text{H}^\text{N} \quad \text{CO}_2\text{CH}_3 \]

\[\text{CO}_2\text{CH}_3 \quad \text{3.08} \quad \text{H}_2\text{N} \quad \text{N-CO}_2\text{CH}_3 \quad \text{3.23} \]

Scheme 3.10

In retrospect, we were mistaken in our attempts to make the bicyclic ring system with an inbuilt 9-alkoxycarbonyl group using a carbazate (Section 3.2). If we had succeeded in closing the hydrazone side-chain to give the pyrazole ring, we might not have been able to complete the final stage of the reaction sequence (displacement of the 6-chloride with an alkoxide group). We would merely have removed the 9-alkoxycarbonyl group from the heterocycle. For the carbazate/pyrimidine strategy to have worked, we would have had to react the carbazate with an \(O^\phi\)-alkoxypyrimidine (Scheme 3.11).

\(^*\) See Section 3.6 for discussion of structural assignment of such compounds.
Having failed to protect the -NH of 2.03 with a methoxycarbonyl group, it was decided to try the use of the pivoyl group as a protecting group for the 9-NH. Its increased bulkiness when compared to the methoxycarbonyl group may provide more resistance to cleavage by sodium alkoxide.
The protected compound 3.27 was prepared from 2.03, pivalic anhydride and triethylamine in DMF.\textsuperscript{3,17} (In contrast to the synthesis of the 9-methoxycarbonyl compound 3.08, this reaction required heating (60 °C) for three hours). The reaction yielded the mono-protected species 3.27 in a yield of 71%. Using these reaction conditions there was no evidence of the formation of other products (e.g. bis- or tris-protected compound, or the 8-pivaloyl compound – see Section 3.6.2-3.6.4 for discussion of structural assignment). Treatment of 3.27 with NaH and 4-bromothenyl alcohol produced a complex reaction mixture, from which no product was isolated. TLC showed the presence of the unreacted pivoyl compound 3.27, the unprotected 6-chloro compound 2.03 and alloPaTrin, as well as several unidentified compounds. Even though we were able to produce the pivoyl protected heterocycle 3.27 in good yields, it appeared to decompose when reacted with sodium alkoxide.

Our attention then turned to the use of the \textit{tert}-butoxycarbonyl group (Boc) as a protecting group for the 9-NH of 2-amino-6-chloro-8-aza-7-deazapurine 2.03. Boc is widely regarded as one of the most common acid labile protecting groups for amines.\textsuperscript{3,18} The synthesis of the 9-Boc-protected 2-amino-6-chloro-8-aza-7-deazapurine 3.29 proved difficult due to the lack of selectivity in the \textit{t}-butylcarboxylation reaction. The starting heterocycle 2.03 has three potential sites onto which the Boc group can attach itself, and two Boc groups can attach themselves to the 2-NH\textsubscript{2}. Thus, many Boc protected compounds 3.29, 3.29a-f are possible (Scheme 3.13).
Treating the chloride 2.03 with Boc₂O and a catalytic amount of DMAP in DMF produced a complicated reaction mixture, from which only 22% of the desired 9-Boc protected compound 3.29 was obtained. As this yield could not be improved upon by the change of solvent, reaction time or reaction temperature, it was decided that it was not worthwhile to continue the reaction sequence by treating the protected chloride 3.29 with sodium alkoxide.

The use of the tris-Boc protected chloride 3.29c as the protected intermediate was also considered. The formation of this compound is favoured by the use of THF as solvent in place of DMF.

Scheme 3.13
Treatment of the tris- substituted compound with sodium hydride and 4-bromothenyl alcohol would give the protected alloPaTrin derivative 3.30. However, the literature indicates that the Boc groups would have to be removed sequentially. The 9-Boc of 3.30 could be readily removed using sodium bicarbonate, and the second Boc group (on the exocyclic amino group) could be cleaved using sodium hydroxide.
The third Boc group would almost certainly require very vigorous condition to be displaced, and consequently the 4-bromothenyl group would probably be cleaved. Zinc bromide has been used in our group for a similar reaction in the guanine series, however residual zinc was left in the final compound, which was very difficult to remove.\textsuperscript{3,21} As an improved method of making alloPaTrin, this reaction sequence was deemed too long, with a low yield of alloPaTrin anticipated and was therefore abandoned.

### 3.4.3 Synthesis of PaTrin-2

As our approaches to the synthesis of alloPaTrin had been unsuccessful thus far, we examined how PaTrin-2 is made. The trimethylamine salt 3.34 is made by treating the commercial 2-amino-6-chloropurine 3.33 with trimethylamine.\textsuperscript{3,22} The trimethylamine group acts as an excellent leaving group, and is readily displaced by 4-bromothenyl alcohol to give PaTrin-2 (Scheme 3.15).\textsuperscript{3,23}

![Scheme 3.15](image)

This reaction sequence was repeated using 2-amino-6-chloro-8-aza-7-deazapurine 2.06. As in the corresponding purine reaction, addition of a solution of excess
trimethylamine to a solution of 2.06 in DMF resulted in a precipitate. Unlike the quaternary salt of the analogous purine, this precipitate was largely insoluble in water. Analysis of the proton NMR of the precipitate showed the presence of two trimethylamino groups. One trimethylamino group had displaced the 6-chloride. However, another trimethylamine molecule had attached itself to the molecule. The crude salt could not be purified by recrystallisation due to the insolubility of the salt, and the second trimethylamine group could not be removed by refluxing the crude salt in various solvents. The bis(trimethylamino) 8-aza-7-deazaguanine salt 3.35 was reacted with sodium alkoxide using the same procedure as that used in the synthesis of PaTrin-2. Unfortunately, the reaction was no better than the initial reaction of the 2-amino-6-chloro-8-aza-7-deazapurine 2.06 and sodium alkoxide. Either the second trimethylamine group of 3.35 was interfering with the progress of the reaction or the “polymeric” species (see p.83) was forming during the reaction. The latter explanation is more likely since the trimethylamino group is a significantly better leaving group than chloride, the formation of the “polymer” may be promoted by the change of leaving group.

3.4.4 1,4-Diazadicyclo[2.2.2]octane (DABCO) as Leaving Group

An alternative leaving group is 1,4-diazadicyclo[2.2.2]octane (DABCO) 3.36. In the purine series, the rate of displacement of trimethylamine is approximately ten times greater than that of DABCO. This slower rate of displacement and the increased steric bulk of the DABCO group may discourage the formation of the “polymer” 3.13a-b during the final stage of the reaction sequence (Scheme 3.16). DABCO has other significant advantages over trimethylamine. Trimethylamine is difficult to work with as it is a gas at room temperature, is toxic and has an unpleasant smell. The crystalline DABCO has been promoted as an alternative to trimethylamine in this type of displacement reaction.

The DABCO salt 3.37 was made by the reaction of the chloride 2.06 to give the quaternary ammonium salt as a precipitate. The DABCO group was readily displaced by alkoxide in DMSO giving alloPaTrin, which was precipitated from the reaction mixture by the very slow addition of water. Yields varied from 20% to 50%. The yield was further reduced by the purification of the crude product, which routinely contained large amounts of impurities, which were insoluble in organic solvents and
removed from the crude product by solvation of the pure alloPaTrin. These impurities may again be due to the formation of the “polymer”. While the DABCO method of making alloPaTrin is far from ideal, we found it to be the most reliable and reproducible method of preparing alloPaTrin.

Scheme 3.16
3.5 Alkoxy carbonylation of AlloPaTrin

3.5.1 Reaction of AlloPaTrin with Methylpyrocarbonate

Our initial target for synthesis in this series was the methoxycarbonyl derivative of alloPaTrin 3.38. This compound is the direct 8-aza-7-deazaguanine analogue of the original lead compound in the PaTrin-2 series. The methoxycarbonyl compound 3.38 was prepared from alloPaTrin, methylpyrocarbonate and triethylamine.*

![Scheme 3.17](image_url)

The progress and yield of this reaction was similar to that of the parallel PaTrin-2 reaction, which suggested that the same synthetic strategies could be used for both the guanine and 8-aza-7-deazaguanine systems to create their alkoxy carbonyl derivatives.32

* See Sections 3.6.2-3.6.4 for discussion of structural assignment of such compounds.
3.5.2 General Methods of Alkoxy carbonylation of AlloPaTrin

Having shown that alloPaTrin behaves similar to PaTrin-2 when reacted with a pyrocarbonate, we investigated two general methods, which have been developed simultaneously in the analogous guanine series.*

Route 1

\[
\text{alloPaTrin} \xrightarrow{\text{CO(L)}_2} \text{3.38} \xrightarrow{\text{HX}} \text{LCOX} \xrightarrow{\text{alloPaTrin}} \text{3.40}
\]

Route 2

\[
\text{alloPaTrin} \xrightarrow{\text{HX}} \text{3.41}
\]

Scheme 3.18

Route 1\textsuperscript{2.26}

-Route 1 involves the reaction of an activated carbonic acid derivative, \(\text{COL}_2\) \text{3.38} where \(L\) is a leaving group, with alloPaTrin, to give \text{3.39}. Treatment of \text{3.39} with HX, where \(X\) is an alkoxide group, should result in the removal of the leaving group and the addition of the alkoxide group in its place to give \text{3.40}, the required alkoxy carbonyl substituted alloPaTrin compound.

Route 2\textsuperscript{2.27}

-Alternatively Route 2 could be used. \(\text{COL}_2\) \text{3.38} is reacted with the alcohol, HX, to give the unsymmetrical carbonate, LCOX \text{3.41}, where \(L\) is again a good leaving group.

\* Except where mentioned, all the guanine reactions, which will be referred to, have been carried out by Dawn Rowan as part of her Ph.D. studies and are discussed in her Ph.D. thesis (2002, University of Dublin).
The reaction of alloPaTrin with LCOX, should cause the displacement of the leaving group L and the subsequent formation of 3.40.

The principle variable in both the Route 1 and Route 2 reaction sequences is the identity of L, the leaving group. The carbonyl group of the carbonic acid derivatives, LCOL 3.38 and LCOX 3.41, is the activated by the electron withdrawing nature of L.\textsuperscript{3.28} For example, the carbonyl group of phosgene, COCl\textsubscript{2}, is highly activated by the electron withdrawing chloride groups.\textsuperscript{3.29} The reactivity of compounds LCOL and LCOX are determined by the difference in the electron withdrawing abilities of L. A large range of potential leaving groups for these types of reactions already exist.\textsuperscript{3.30} We shall confine ourselves to the investigation of the use of chloride, 2,4,5-trichlorophenol and 4-nitrophenol as leaving groups for Route 1 and Route 2 type reactions. Of these three groups, the chloride is the best leaving group, and by extension, phosgene, COCl\textsubscript{2} and chloroformates, ROCOCl, should be the most reactive compounds of their respective categories.\textsuperscript{3.29} The 2,4,5-trichlorophenoxy group is a marginally better leaving group than the 4-nitrophenoxy group, and in theory its reaction should produce better results than the corresponding reactions using 4-nitrophenol.

3.6 Chloroformates

Initially, we investigated the suitability of chloroformates as alkoxy carbonylation reagents for alloPaTrin. The chloroformate would act as a LCOX type compound in the Route 2 reaction sequence (L is the chloride leaving group and X is an alkoxide group - see Scheme 3.18).
Chloroformates were used with considerable success in the PaTrin-2 series to make a range of alkoxycarbonyl derivatives. Both commercial and non-commercial chloroformates were used, the latter being made from the corresponding alcohol and triphosgene as a source of phosgene. Typically the reaction of chloroformate and PaTrin-2 with base in DMF gave the 9-substituted compound 3.42 (80%) and trace amounts of the 7-isomer 3.43 (15%) (Scheme 3.20).

3.6.1 Reaction of Chloroformates with AlloPaTrin

The reaction of alloPaTrin and one equivalent of triethylamine with one equivalent of propyl chloroformate at room temperature gave rise to an immediate precipitate from the DMF solution. After two hours of stirring, the precipitate was collected. NMR analysis showed the precipitate to be a pure mono-substituted propyloxycarbonyl 8-aza-7-deazaguanine species (41%). For convenience, we shall call this precipitate A. TLC analysis of the filtrate showed the presence of A, unreacted alloPaTrin and a third faster migrating species. This compound was isolated chromatographically and NMR spectroscopy indicated that it was a second pure mono-substituted propyloxycarbonyl 8-aza-7-deazaguanine species (14%). We shall name this second compound B. A had to be either the 8- or 9-propyloxycarbonyl derivative, 3.44 or 3.45, while B had to be the other isomer. The NMR spectra rule out other alternatives, such as an 2-N-propyloxycarbonyl derivative 3.46.
Scheme 3.21
Figure 3. 2 $^1$H NMR of A in DMSO-d$_6$.

Figure 3. 3 $^1$H NMR of B in DMSO-d$_6$. 
Figure 3. 5. $^{13}$C NMR of B in DMSO-d$_6$.

Figure 3. 4. $^{13}$C NMR of A in DMSO-d$_6$. 
3.6.2 Structural Assignment of 8- and 9-Substituted 8-aza-7-deazaguanines in the Literature

The problem of structural assignment of N-8 and N-9 alkyl-8-aza-7-deazaguanines has been addressed in the literature.

As discussed in Chapter 2 (p.38), Seela synthesised the pair of isomers 2.04 and 2.05 by the methylation of 2-amino-6-chloro-8-aza-7-deazapurine 2.03.\textsuperscript{3,10} Comparison of the proton NMR spectra of the compounds show that the signals representing the –NH\textsubscript{2} and 7-CH differ considerably for the two isomers. Seela assigned the 8-methyl structure 2.05 to the isomer with an –NH\textsubscript{2} signal at 6.87 ppm and a 7-H signal at 8.41 ppm. In contrast, the –NH\textsubscript{2} signal and the 7-H signal of the 9-methyl compound occur at 7.28 ppm and 7.93 ppm respectively.

![Diagram](image)

Table 3. 1\textsuperscript{1}H NMR in DMSO-d\textsubscript{6} (ppm)\textsuperscript{3,10}

<table>
<thead>
<tr>
<th></th>
<th>2.03</th>
<th>2.04</th>
<th>2.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>-CH\textsubscript{3}</td>
<td>-</td>
<td>3.80</td>
<td>3.99</td>
</tr>
<tr>
<td>-NH\textsubscript{2}</td>
<td>7.14</td>
<td>7.28</td>
<td>6.87</td>
</tr>
<tr>
<td>7-CH</td>
<td>7.94</td>
<td>7.93</td>
<td>8.41</td>
</tr>
</tbody>
</table>

The \textsuperscript{13}C NMR data for the methylated derivatives also show differences. N-8 substitution produces a signal for the 4-carbon (163.5 ppm), which has shifted downfield compared to the corresponding signal of the unsubstituted species (157.4 ppm). In contrast, the analogous signal of the 9-methyl compound 2.04 shifts upfield (155.6 ppm). The positions of the 7-carbon signals of the two isomers and 2.03 differed by 6 ppm (131.6 ppm in the 9-substituted compound and 125.2 ppm in the 8-substituted compound).
Seela noted that the spectroscopic data for one isomer resembled that of the unsubstituted compound \(2.03\), while the spectra of the other isomer displayed differences in the key areas involving the 4- and 5-carbons, and both the 7-hydrogen and carbon. Previous studies argued that the proton on the five membered ring of \(2.03\) occurs at the N-9 position, and not in the N-8 position. This is based on the fact that in \(2.03\), both the 5- and 6-membered rings possess aromatic sextets, while the alternative structure would have a six-membered ring with a quininoid structure and would be less stable as a result. Seela deduced that the methylated compound, whose spectroscopic data most resembled those of the starting material was the 9-methylated species \(2.04\), whilst the second methylated isomer had the alternative structure, the N-8 compound \(2.05\). This assignment of structures to the isomers was further supported by the \(^3\)J-coupling pattern of the proton coupled carbon spectrum of the 8-methyl isomer \(2.05\). A large \(^1\)J of 196 Hz was observed for the 7-CH signal of both compounds, however the 8-substituted isomer showed a \(^3\)J (7-C, -CH\(_3\)) coupling of 3.2 Hz, while the other isomer did not. These findings support the location of the methyl group on the 8-position, and subsequently the structural assignment of the pair of isomers. Further evidence for the correct assignment of structures to \(2.04\) and \(2.05\) was produced by Rosemeyer, who used a similar rationale to assign structures to the 8- and 9-methylated 8-aza-7-deazapurines \(3.47\) and \(3.48\) and the corresponding allopurinol compounds \(3.49\) and \(3.50\). Lichtenthaler also assigned structures to the isomers \(3.49\) and \(3.50\) using uv data.

<table>
<thead>
<tr>
<th></th>
<th>(2.03)</th>
<th>(2.04)</th>
<th>(2.05)</th>
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<tr>
<td></td>
<td></td>
<td>N-9</td>
<td>N-8</td>
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<tr>
<td>-CH(_3)</td>
<td>-</td>
<td>33.3</td>
<td>40.8</td>
</tr>
<tr>
<td>5-C</td>
<td>105.7</td>
<td>106.0</td>
<td>108.6</td>
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<tr>
<td>7-CH</td>
<td>132.5</td>
<td>131.6</td>
<td>125.2</td>
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<tr>
<td>6-C</td>
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<td>153.3</td>
<td>156.5</td>
</tr>
<tr>
<td>4-C</td>
<td>157.4</td>
<td>155.6</td>
<td>163.5</td>
</tr>
<tr>
<td>2-C</td>
<td>161.4</td>
<td>161.3</td>
<td>161.1</td>
</tr>
</tbody>
</table>

Table 3. \(^{13}\)C NMR in DMSO-d\(_6\) (ppm)

\(^{3}\)a Lichtenthaler also assigned structures to the isomers \(3.49\) and \(3.50\) using uv data.
Earl reported some general rules for the assignment of structures to N-8 and N-9 methyl 6-amino-8-aza-7-deazapurines 3.51, 3.52 and 3.53. He noted that for each pair of isomeric compounds, the N-8 isomer shows an absorption maximum at a longer wavelength than the N-9 isomer. He concluded that this difference in the absorption maxima of the isomers is a general trend and is most likely due to the different type of electronic structure of the two isomers. The uv data for Seela’s pair of isomers are in agreement with Earl’s findings. 2.03 and its 9-methyl derivative 2.04 possess almost identical lowest energy maxima (305 nm and 306 nm respectively) while 2.05, the 8-methyl derivative, shows a maximum at 322 nm.
Table 3. 3 UV absorption maxima (nm) (MeOH)\textsuperscript{3,10,34}

Earl also noted that if one compares the $^{13}$C NMR spectrum of a nitrogen heterocycle with an $N$-alkylated derivative of the heterocycle, differences in the chemical shifts can be observed, which reflect the displacement of a proton and alkylation of a specific nitrogen atom. In general, the signal for the carbon atom adjacent to the site of nitrogen substitution moves upfield.

Table 3. 4 $^{13}$C NMR in HMPT (ppm)

Again, Seela's assignment of structures to the 8- and 9-methyl derivatives of 2-amino-6-chloro-8-aza-7-deazapurine 2.04 and 2.05 supports this argument.

3.6.3 Assignment of Structures to A and B

Having outlined trends in the literature, we shall now return to our pair of unassigned isomers, A and B. Earl claimed that generally, the $^{13}$C NMR signal of a carbon atom $\alpha$
to the site of alkylation moves upfield compared to the corresponding value of the unsubstituted –NH species.

<table>
<thead>
<tr>
<th></th>
<th>AlloPaTrin</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>-CH₃</td>
<td>-</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>-CH₂CH₃</td>
<td>-</td>
<td>1.74-1.81</td>
<td>1.70-1.79</td>
</tr>
<tr>
<td>-OCH₂CH₂CH₃</td>
<td>-</td>
<td>4.37</td>
<td>4.32</td>
</tr>
<tr>
<td>O⁵-CH₂</td>
<td>5.63</td>
<td>5.68</td>
<td>5.65</td>
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<tr>
<td>-NH₂</td>
<td>6.74</td>
<td>7.07</td>
<td>7.33</td>
</tr>
<tr>
<td>3'-CH</td>
<td>7.36</td>
<td>7.41</td>
<td>7.39</td>
</tr>
<tr>
<td>5'-CH</td>
<td>7.71</td>
<td>7.74</td>
<td>7.73</td>
</tr>
<tr>
<td>7'-CH</td>
<td>7.78</td>
<td>8.81</td>
<td>8.11</td>
</tr>
<tr>
<td>9'-NH</td>
<td>12.89</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.5 \(^1\text{H} \text{NMR data of alloPaTrin, A and B (}\delta, \text{DMSO-d}_6\)
The $^{13}$C signal of the carbon in the 7-position of alloPaTrin occurs at 131.6 ppm, while the equivalent signal of A occurs at 126.9 ppm, and B 136.3 ppm. The 4-carbon of alloPaTrin produces a signal at 161.6 ppm, while A shows a signal at 163.5 ppm, and B produces a signal at 160.2 ppm. The upfield shift of the 7-CH carbon signal of A compared to that of alloPaTrin suggests that substitution has occurred in the 8-position. Similarly, the signal of the quaternary carbon in the 4-position of B has moved slightly upfield compared to the analogous alloPaTrin signal, which suggests substitution in the 9-position. The $^1$H spectra also support the structures assigned. In particular, the 7-H signal should appear more downfield in the 8-alkoxycarbonyl derivative than in its 9-isomer.

In addition, the absorption maxima of alloPaTrin (278 nm) and B (266 nm) are similar, while the absorption maximum of A (324 nm) occurs at a longer wavelength. The bathochromic shift of A is much greater than that found in the N-alkyl case, but this could be due to the increased conjugation caused by the presence of the carbonyl group. These findings point towards A being the 8-propyloxycarbonyl 8-aza-7-deazaguanine species 3.44, and B being the 9-propyloxycarbonyl 8-aza-7-deazaguanine species 3.45.

We attempted to gain further evidence for these structural assignments by obtaining proton-coupled carbon spectra for A and B. Seela reported that the proton coupled 7-C signal of 2-amino-6-chloro-8-methyl-8-aza-7-deazapurine 2.05 not only shows a $^1$J value of 196 Hz, but also a $^3$J coupling constant of 3.2 Hz can be observed. This is due to the proximity of the methyl group to the carbon atom in the 7-position. This $^3$J coupling pattern is absent from the N-9 methylated species 2.04. In our case, it was not expected to find a long range coupling between the 7-C and the O-CH$_2$ in the propyloxy side-chain as they are separated by too many atoms. When the experiment was repeated with either the N-8 or the N-9 propyloxycarbonyl 8-aza-7-deazaguanines, a large $^1$J value was observed for both compounds, but no long range coupling.

We also applied heteronuclear multiple bond correlation (HMBC) spectroscopy to the pair of isomers. The HMBC NMR technique is another method of detecting long range coupling and is routinely used to study two or three bond coupling correlations.
We hoped that the HMBC would show coupling between the carbon of the carbonyl group of the side-chain and the proton in the 7-position of the heterocyclic ring. Initially the experiment was carried out on solutions of A and B in deuteriated dimethyl sulfoxide. In both compounds the carbonyl carbon showed only coupling to the O-CH₂ of the propyloxy side-chain. We concluded that the signal, for which we were searching was very weak, and perhaps may not have been detected because of the viscosity of the deuteriated dimethyl sulfoxide.3,35

nOe studies of the pair of isomers were also carried out in the hope that the 7-proton would be close enough in space to the protons of the propyloxy side-chain of the 8-substituted isomer to produce a response in an nOe experiment. However the required signal was not observed in nOe experiments on either A or B.

The HMBC experiment was repeated for both compounds, using deuteriated acetone in place of DMSO.3,35 A very weak coupling appeared between the carbon of the carbonyl group and the signal of the proton in the 7-position of B, though the intensity of the signal varied between experiments. In an initial experiment using a solution of A, no such coupling was observed, but repetition cast doubt on the validity of this observation. Nevertheless, if the conclusions from our initial experiments in deuteriated acetone were valid, the results are contrary to what we had expected. We had thought that coupling might occur between the 8-carbonyl and the 7-proton but not between the 9-carbonyl and 7-proton, as the former are separated by three atoms. However, having compared our initial carbon and uv data with those in the literature, we had provisionally assigned the 8-alkoxycarbonyl structure 3.44 to A and the 9-alkoxycarbonyl structure 3.45 to B. At this point, our experimental evidence appeared to be in direct conflict.

However, the HMBC spectra (deuterated acetone) of both compounds show no coupling (weak) between the 7-hydrogen and the 6-carbon. If the geometry between these two atoms is such that no coupling is observed, then the same situation occurs between our 8-carbonyl carbon and the 7-hydrogen, and no coupling should be observed. On the other hand, the 9-carbonyl carbon and the 7-hydrogen possess a W-shaped geometry, and might be expected to show a weak 1H-coupling.
In conclusion, we have assigned the 8-propyloxycarbonyl 8-aza-7-deazaguanine 3.44 to A and the 9-propyloxycarbonyl 8-aza-7-deazaguanine 3.45 structure to B. Our HMBC results are ambiguous, but the other evidence is conclusive. The comparison between our case of the acyl derivatives with the literature values^{3,29,3,30,3,31} for alkyl derivatives provides a sound foundation for our assignments. Furthermore, we can rationalise the formation of mainly the 8-isomer from a chloroformate (see pp. 109-110) while the 4-nitrophenyl active ester 3.54 gives mainly the 9-derivative (see pp. 122-125).

![Image](3.54)

It should be pointed out that the geometry of the 7-carbon and the 8-methyl hydrogens (which show long range coupling) in 2.05, and which is used by Seela to predict its structures, is not the same as the geometry of the 7-hydrogen and the 8-carbonyl carbon in A. No comparison between the results of the two experiments is valid.

### 3.6.4 Summary of Structural Assignment of 8- and 9-Substituted 8-Aza-7-deazaguanines

From our investigation of the structural assignment of the 8- and 9-isomers of the propyloxycarbonyl derivative 3.44 and 3.45, patterns have emerged in both the NMR and uv spectra, which allow for quick assignment of structures to the 8- and 9-substituted derivatives of alloPaTrin.

- The absorption maximum of the 8-isomer occurs at a longer wavelength than the corresponding measurement of its 9-isomer (experiment carried out using a solution of the compound in methanol).

- Comparison of the $^1$H NMR of both isomers show differences in the signals of the 7-H and the –NH$_2$. The 7-H signal of the 9-isomer typically occurs at 8.1 ppm (experiment carried out using a solution of the compound in deuteriated dimethyl sulfoxide), while the corresponding signal of an 8-isomer occurs at 8.8 ppm. The signal of the exocyclic amino group occurs at 7.1 ppm for a 9-
substituted compound, while this signal is further downfield in the 8-isomer (7.3 ppm).

- The \(^1\text{H}\) NMR signal of the 7-C of a 9-substituted compound typically occurs at 136 ppm, while the equivalent signal of an 8-substituted compound occurs at 125 ppm. The 4-C of the 9-isomer produces a signal at 163 ppm, while the analogous signal of the 8-isomer occurs further upfield at 160 ppm.

3.6.5 Formation of a Mixture of Isomers
The formation of a mixture of 8- and 9-substituted isomers from the reaction of alloPaTrin with propyl chloroformate may be explained by the mechanism below (Scheme 3.22). Treatment of alloPaTrin with base gives an anion, which may be represented as the pair of canonical forms 3.55a and 3.55b. Addition of the chloroformate causes a reaction to occur between the highly activated carbonyl group of the chloroformate and the newly formed anion. The rate of reaction between the carbonyl and the anion at the 8-position 3.55b should be much faster than the corresponding reaction at the 9-position 3.55a as it is much less hindered. The tetrahedral intermediate 3.56b has a choice of eliminating chloride (red arrows) to give the 8-alkoxycarbonyl derivative 3.57b, or alternatively, breaking the C-N bond (blue arrows) and returning to the starting anion 3.55b. Because chloride is a good leaving group, the former reaction is preferred. The 9-position is more hindered, and the reaction of 3.55a with the chloroformate is slower, but the tetrahedral intermediate 3.56a will also preferentially eliminate chloride to give the 9-alkoxycarbonyl derivative 3.57a. This rationale may explain the lower yield of the 9-substituted species (14%) compared to that of the corresponding 8-substituted species (42%). Later we shall discuss other alkoxycarbonylating agents whose reaction with alloPaTrin favours the formation of the 9-substituted compound (Section 3.8).
Scheme 3.22
3.6.6 Reaction of Phenyl Chloroformate with AlloPaTrin

Having established that the reaction between alloPaTrin and propyl chloroformate produces two products, the 8- and 9-propyloxycarbonyl 8-aza-7-deazaguanine 3.44 and 3.45, we wished to expand our range of alkoxy groups linked to alloPaTrin via a carbonyl group, by reacting alloPaTrin with other commercially available and non-commercial chloroformates using the same reaction conditions. This could provide an array of alloPaTrin derivatives whose biological activity and rates of hydrolysis could supply valuable information on potential prodrugs of alloPaTrin. With this in mind, alloPaTrin was reacted with phenyl chloroformate and triethylamine. It was found that the reaction of alloPaTrin and a chloroformate only worked if the chloroformate was added to a solution of alloPaTrin and the base.* As in the case of the propyl chloroformate reaction, a precipitate formed almost immediately, which was isolated, to give a mono-substituted phenoxycarbonyl 8-aza-7-deazaguanine 3.58 or 3.59 (38%) (Scheme 3.23).

![Scheme 3.23](image)

Its $^1$H NMR spectrum showed the -NH$_2$ signal at 7.15 ppm, while the 7-proton signal occurred at 9.04 ppm, a downfield shift of 1.26 ppm compared to the corresponding alloPaTrin signal. The $^{13}$C NMR signal of the carbon in the 7-position occurred at 128.0 ppm. This upfield shift of the 7-carbon signal compared to the alloPaTrin 7-C

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* Addition of triethylamine to a solution of alloPaTrin and a chloroformate consistently gave rise to a complex reaction mixture, from which pure products were very difficult to isolate
signal points towards substitution in the 8-position. The precipitation of the 8-phenoxycarbonyl species **3.58** shows similar behaviour to the reaction of propyl chloroformate with alloPaTrin.

The phenyl chloroformate reaction was repeated on a larger scale, so that we could investigate the products remaining in the filtrate after the initial isolation of the N-8 substituted species. The reaction conditions were identical, but in this case no precipitate formed in the reaction mixture. After two hours of stirring at room temperature, diethyl ether was added to the solution. A precipitate formed which was isolated (22%). NMR analysis showed that the precipitate was not the same compound as the product from the initial smaller scale reaction. The $^1$H NMR signal for the 7-H occurred at 8.24 ppm. The $^{13}$C NMR signal for the quaternary carbon in the 4-position occurred at 162.2 ppm, an upfield shift of 1 ppm compared to the corresponding signal of alloPaTrin. This NMR data suggests that the initial product from the second reaction is the N-9 substituted phenoxy carbonyl alloPaTrin compound **3.59**.

Both these reactions of alloPaTrin and phenyl chloroformate were carried out using identical reaction conditions. However, two different products were formed. Consequently, different reaction conditions were investigated in an attempt to introduce reproducibility into the progress of the reaction.

In the literature Kingsbury *et al.* found that in general the reaction of indazoles and chloroformates at low temperatures leads to predominant substitution at the 2-position **3.61** (equivalent to the 8-position in the 8-aza-7-deazaguanine system), while the 1-isomer **3.62** (equivalent to the 8-position in the 8-aza-7-deazaguanine system) could be generated by carrying out the reaction at ambient temperature (Scheme 3.24).
Consequently we repeated the reaction of alloPaTrin and phenyl chloroformate at different temperatures (-20 °C, 0 °C, RT, 40 °C). However, our attempts to improve the reaction conditions only led to the formation of a mixture of the various products and alloPaTrin, with the amount of unreacted alloPaTrin increasing as the reaction temperature was lowered. Kingsbury also found that with some bulkier chloroformates (benzyl and 2-furoyl chloride), the 2-substituted indazole 3.61 was produced, even when the reaction was carried out at room temperature. This implies that steric factors are also important in determining the outcome of these reactions. This may provide another explanation for the difference in behaviour of phenyl and propyl chloroformates in their reactions with alloPaTrin.
3.7 2,4,5-Trichlorophenyl Carbonates

As we could not find reproducible reaction conditions for the reaction between chloroformates and alloPaTrin, we began investigating other alkoxycarboxylating agents. In the PaTrin-2 series, some success was achieved through the use of the aryl group, 2,4,5-trichlorophenoxy, as the leaving group.

The Route 1 and Route 2 pathways (p. 95) require the synthesis of a carbonate with 2,4,5-trichlorophenoxy substituents. Bis(2,4,5-trichlorophenyl) carbonate 3.66 is not commercially available was made from the reaction of 2,4,5-trichlorophenol 3.65 with \( N,N \)-dimethylaniline and triphosgene 3.64 (or bis (trichloromethyl) carbonate) (Scheme 3.25).\(^3\)\(^3\)\(^7\)

![Scheme 3.25](image)

Triphosgene 3.63 has been frequently used in organic synthesis as a phosgene (\( \text{COCl}_2 \)) precursor.\(^3\)\(^3\)\(^8\) This crystalline stable solid has the advantage of being much easier to handle than the highly toxic and gaseous phosgene, while retaining the
chemical properties of phosgene. One third of a mole of triphosgene with a nucleophile react similarly to one mole of phosgene (Scheme 3.26).

![Chemical reaction diagram]

Scheme 3.26

3.7.1 Reaction of Bis(2,4,5-trichlorophenyl) Carbonate with AlloPaTrin

The symmetrical diaryl carbonate 3.66 was reacted with alloPaTrin and Hünig’s base in an attempt to make the substituted alloPaTrin derivative 3.67. The success of a Route 1 type synthesis required a clean synthesis of this intermediate compound in good yield. The 2,4,5-trichlorophenoxy group could then be displaced by alcohols giving an efficient synthesis of a range of alloPaTrin derivatives. Unfortunately, we could not make the substituted alloPaTrin 2,4,5-trichlorophenoxy carbonyl complex 3.67 (Scheme 3.27).
The final crude product always contained large amounts of free alloPaTrin (up to 40%). The large amounts of alloPaTrin not only caused a low yield of the product, but proved very difficult to remove. In contrast, the corresponding PaTrin-2 compound 3.68 was readily prepared via the reaction of PaTrin-2 and the symmetrical carbonate 3.66 in a yield of 92%. 9-Alkoxycarbonyl derivatives of PaTrin-2 1.31 and 3.69 were successfully prepared by heating a DMF solution of the PaTrin-2 derivative 3.68 with a vast excess of alcohol. However, a similar substitution reaction with 3.68 would not proceed to completion with lower concentrations of alcohol (Scheme 3.28).
3.7.2 Mixed Carbonates of 2,4,5-Trichlorophenol

As our attempted synthesis of 9-(2,4,5-trichlorophenoxy)carbonyl derivative of alloPaTrin 3.67 failed, we examined the use of 2,4,5-trichlorophenol as a leaving group in a Route 2 type reaction. This involves the formation of an activated carbonic acid derivative, LCOX, where L is the 2,4,5-trichlorophenoxy leaving group and X is the desired alkoxy substituent, and its reaction with alloPaTrin to give the 9-alkoxycarbonyl derivative of alloPaTrin 3.57a.

This synthetic strategy has worked well in the guanine series, producing PaTrin-2 derivatives 3.70a and 3.70b in yields of 76% and 44% respectively.
The unsymmetrical carbonates 3.71a-b were prepared from the reaction of the diaryl carbonate 3.66 with either benzyl or piperonyl alcohol and Hünig’s base. These mixed carbonates were then reacted with alloPaTrin and Hünig’s base. The tetrahedral intermediate 3.72a-b should have formed with the subsequent loss of 2,4,5-trichlorophenol and the formation of the final product 3.73a-b (Scheme 3.29).
However, in the reaction, considerable quantities of alloPaTrin (>50%) remained at the end of the reaction. Due to the large amounts of unreacted alloPaTrin present in the crude product, the alloPaTrin derivatives 3.73a-b could not be isolated and purified. This contrasts with the clean reaction of the reagents with PaTrin-2.

This synthetic pathway successfully produced some 9-alkoxycarbonyl derivatives of PaTrin-2, but it failed when the same reaction sequence was applied to alloPaTrin. This may be explained by the difference in reactivity of the –NH of the pyrazole ring of alloPaTrin and the –NH of the imidazole ring of PaTrin-2. Staab found that the formation of 1-acetyl-3,5-dimethylpyrazole from 3,5-dimethylpyrazole and acetyl chloride took significantly longer than the analogous imidazole reaction. As this is in keeping with our findings concerning the ease of displacement of the 9-NH of the guanine and 8-aza-7-deazaguanine ring systems, it could be argued that alloPaTrin is less reactive than PaTrin-2, and perhaps this could account for the difficulties which have been encountered when trying to acylate the 9-position of alloPaTrin.

3.8 4-Nitrophenyl Carbonates

Having failed to make alloPaTrin derivatives by a Route 2 pathway using 2,4,5-trichlorophenoxide as a leaving group, the use of 4-nitrophenol as an alternative phenol was investigated. Even though the electron withdrawing properties of both phenoxide anions are similar, considerably more success has been achieved in the guanine series using 4-nitrophenol in place of 2,4,5-trichlorophenol. A range of PaTrin-2 derivatives were synthesised using carbonates (LCOX) containing a 4-nitrophenyl substituent as the leaving group, L, via a Route 2 type reaction (Scheme 3.30). No evidence for the formation of the 7-substituted PaTrin-2 derivative 3.43 was found.
3.8.1 9-(4-Nitrophenoxycarbonyl) Derivative of AlloPaTrin

We investigated the viability of using a Route 1 type reaction, using the 4-nitrophenoxy leaving group, to synthesise alloPaTrin derivatives.

The 9-(4-nitrophenoxy)carbonyl alloPaTrin intermediate \(3.75\) was prepared by treating a solution of alloPaTrin in DMF with the commercially available symmetrical carbonate \(3.76\) and Hüning's base (Scheme 3.31). The 9-substituted product \(3.76\) conveniently precipitates from the reaction mixture and therefore can be easily isolated in good yields (78%). This is in marked contrast to the corresponding reaction using trichlorophenoxy as leaving group reaction, from which no product could be isolated. The reaction also differs considerably from the parallel PaTrin-2 reaction. As mentioned earlier (p.116), the 9-(2,4,5-trichlorophenoxy)carbonyl derivative of PaTrin-2 \(3.68\) was readily obtained from PaTrin-2 and \(bis-(2,4,5-\)
trichlorophenyl) carbonate 3.66. In contrast the same reaction using bis-(4-nitrophenyl) carbonate 3.76 failed to produce the required guanine compound.

![Chemical structures](image)

Scheme 3. 31

Treatment of a solution of the intermediate 3.75 in DMF with a vast excess of propanol yielded a mixture of the unreacted ester derivative, the required 9-propyloxy compound 3.45 and free alloPaTrin. An identical reaction but with one equivalent of base caused the ester derivative to decompose giving free alloPaTrin (74%). The initial product 3.45 reacts with the propoxide anion to give dipropyl carbonate and alloPaTrin.

While the intermediate ester derivative of alloPaTrin 3.75, which is required for a synthesis via a Route 1 reaction sequence, is relatively easy to prepare, where L = 4-NO2C6H4O, we found that its transformation to an alkoxy carbonyl derivative of
alloPaTrin was problematic. Even the use of a vast excess of alcohol did not cause complete reaction of the 4-nitrophenoxy group of the intermediate 3.75 with the alkoxide, and in a side reaction the intermediate gave alloPaTrin.

### 3.8.2 Mixed Carbonates of 4-Nitrophenol

Since the Route 1 synthesis of alkoxy carbonyl derivatives of alloPaTrin, using 4-nitrophenoxide as the leaving group was unsuccessful, we investigated the Route 2 pathway. This involves the reaction of a mixed carbonate containing a 4-nitrophenyl group with alloPaTrin. Considerable success has been achieved in the guanine series using this strategy.

**Scheme 3.32**

The initial step of the reaction sequence is the synthesis of the mixed carbonate, 4-NO$_2$C$_6$H$_4$OCOOR, **3.54a-d**, by the reaction of *bis*(4-nitrophenyl) carbonate **3.76** with

\[
\text{NO}_2
\]

\[
\text{ROH}
\]

\[
\text{OR}
\]

\[
3.76
\]

\[
\text{Br}
\]

\[
\text{alloPaTrin}
\]

\[
\text{H}_2\text{N}
\]

\[
\text{RO}
\]

\[
3.77\text{b-e}
\]

- a R = benzyl,
- b R = piperonyl,
- c R = 4-picolyl,
- d R = N-methylpiperidine

The initial step of the reaction sequence is the synthesis of the mixed carbonate, 4-NO$_2$C$_6$H$_4$OCOOR, **3.54a-d**, by the reaction of *bis*(4-nitrophenyl) carbonate **3.76** with
an alcohol. The chosen alcohols were benzyl\textsuperscript{3,40}, piperonyl, 4-picolyl\textsuperscript{3,41} and N-methylpiperidin-4-ol. These R groups could potentially provide compounds whose biological activities and rates of hydrolysis could be directly compared to their analogous guanine compounds.

The reaction of alloPaTrin with the mixed carbonate of 4-nitrophenol \textbf{3.54a-d} proceeded much more readily than the corresponding 2,4,5-trichlorophenylcarbonate \textbf{3.71a-b} reaction. Even so the reaction was slow (2-3 days), but most of the alloPaTrin reacted. This facilitated the isolation and purification of the final product (59-72\%). Only a single product was obtained from each of the four reactions. In all four cases, proton NMR of the product showed the 7-H peak in the 8.1-8.2 ppm region, while the 7-C peak of the carbon NMR occurred around 136 ppm. According to our structural assignments of 8- and 9-substituted isomers of substituted alloPaTrin derivatives, the 9-substituted compound had been formed in all four instances.

\textbf{3.8.3 Formation of the 9-Substituted Derivative}

Earlier (Section 3.6.5, pp. 109-110) we discussed a possible explanation as to why the reaction of propyl chloroformate with alloPaTrin produces a large amount of the 8-substituted compound \textbf{3.44}. We attributed this to the loss of chloride from the tetrahedral intermediates \textbf{3.56a-b} in preference to the loss of the alloPaTrin anion \textbf{3.55a-b}. In this case, the 4-nitrophenoxide anion is the leaving group. As in the reaction of the chloroformate with alloPaTrin, addition of a 4-nitrophenyl carbonate (symmetrical or mixed) to the anion, represented by the canonical forms \textbf{3.55a} and \textbf{3.55b}, gives the tetrahedral intermediates \textbf{3.78a} and \textbf{3.78b}. However, in this instance, the rate of formation of the 8-substituted tetrahedral intermediate \textbf{3.78b} should be slower than that of its chloroformate analogue. This is due to the carbonyl of the carbonate being less electrophilic than the chloroformate carbonyl, as the 4-nitrophenyl group has inferior electron withdrawing abilities than the chloride group. Because of the 4-nitrophenoxide's inferiority as a leaving group relative to the leaving ability of chloride, the tetrahedral intermediate \textbf{3.79b} has a longer life-time than the corresponding chloro intermediate \textbf{3.56b} and decomposition of the intermediate is
Scheme 3.33
more likely in this case (i.e. the alloPaTrin anion \textbf{3.55b} is a better leaving group than the 4-nitrophenoxide anion). The breaking down of the tetrahedral intermediate \textbf{3.78b} to release alloPaTrin encourages the formation of the 9-tetrahedral intermediate \textbf{3.78a}, which ultimately gives the 9-substituted compound as the final product. Once formed, the tetrahedral intermediate \textbf{3.78a} can either lose nitrophenoxide to give the product or revert to starting alloPaTrin. Because the 4-nitrophenoxide is a poorer leaving group than chloride, the latter reaction is favoured.

\textbf{3.9 Chapter 3 – an Overview}

This chapter dealt with the synthesis of alkoxy carbonyl derivatives of $O^\beta$-(4-bromothenyl)-8-aza-7-deazaguanine (or alloPaTrin). Initially their synthesis from a pyrimidine and an alkylcarbazate was investigated. However the reaction only produced a di-chloropyrimidine with a hydrazone side-chain, which failed to cyclise to form the pyrazole ring of the 8-aza-7-deazaguanine skeleton (Section 3.2).

Our attention then turned towards the direct acylation of alloPaTrin by a variety of acylating agents. Initially the synthesis of alloPaTrin had to be improved upon (Section 3.4). The existing synthesis involved the displacement of chloride from 2-amino-6-chloro-8-aza-7-deazapurine by alkoxide. We found that instead of direct displacement of the chloride, replacement by DABCO, and subsequent reaction of the quaternary salt formed with alkoxide gave higher yields, even so the yield was routinely below 50%.

The 9-methoxycarbonyl derivative of alloPaTrin was prepared by the reaction of alloPaTrin with methyl pyrocarbonate (p.94). Treatment of alloPaTrin with a chloroformate produced a mixture of 8- and 9-alkoxycarbonyl derivatives of alloPaTrin. The ratio of isomers formed differed depending on the chloroformate used in the reaction (Section 3.6.2). Unsymmetrical carbonates containing an aryloxide leaving group (2,4,5-trichlorophenoxy or 4-nitrophenoxide) were also investigated as acylating agents. Initially the 9-(4-nitrophenoxy)carbonyl derivative of alloPaTrin was prepared (we were unable to synthesise the corresponding 9-(2,4,5-trichlorophenoxy) derivative). However we failed to replace the 4-nitrophenoxide leaving group with alkoxide \textit{via} a "Route 1" type reaction (p.121).
The "Route 2" pathway was then investigated. A series of carbonates were prepared from the appropriate alcohol and bis-(4-nitrophenyl) carbonate. These unsymmetrical carbonates were reacted with alloPaTrin to give the required 9-alkoxy carbonyl product in each case (p.122).
### 3.10 Experimental

**2-Amino-4,6-dichloro-5-formylpyrimidine 2’-(methoxycarbonyl)hydrazone 3.09**

Methyl carbazate (144 mg, 1.6 mmol) was added to a suspension of 2-amino-4,6-dichloropyrimidine-5-carboxaldehyde 2.06 (307 mg, 1.6 mmol) in THF (5 ml) and water (3 ml). The mixture was stirred vigorously at 50 °C for 30 minutes. After removal of the THF, the pale yellow product was filtered and dried (402 mg, 95%).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.70 (s, 3H, -CH$_3$), 7.82 (s, 2H, -NH$_2$), 8.04 (s, 1H, N=CH), 11.24 (s, 1H, -NH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 51.9 (CH$_3$), 112.2 (q, 5-C), 137.1 (N=CH), 153.6 (C=O), 159.8 (q, 4,6-C), 160.7 (q, 2-C).

$\nu_{max}$ 1712 cm$^{-1}$

mp 156 °C

**2-Amino-5-formyl-4,6-ditosylpyrimidine 2’-(methoxycarbonyl)hydrazone 3.11**

The hydrazone 3.09 (132 mg, 0.5 mmol) and the anhydrous sodium salt of p-toluene-4-sulfinic acid (178 mg, 1 mmol) were stirred in DMSO (1.5 ml) at 100 °C for 5 hours. The DMSO was removed leaving an orange residue, which was crystallised from dichloromethane, filtered and dried (182 mg, 72%).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.30 (s, 6H, -CH$_3$), 3.68 (s, 3H, O-CH$_3$), 7.11 (d, 4H, J 7.5, 2" CH), 7.49 (d, 4H, J 7.5, 3", 5"-CH), 8.09 (s, 1H, N=CH), 8.66 (s, 2H, -NH$_2$), 10.59 (s, 1H, -NH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 20.1 (Ar-CH$_3$), 53.1 (OCH$_3$), 114.0 (q, 5-C), 126.0 (2", 6"-CH), 128.7 (3", 5"-CH), 136.5 (N=CH), 137.8 (q, 1"-C), 145.9 (q, 4"-C), 152.6 (C=O), 159.0 (q, 2-C), 160.8 (q, 4, 6-C).

$\nu_{max}$ 1689 cm$^{-1}$

mp >200 °C

**2-Amino-5-formyl-4,6-di(2,4,5-trichlorophenoxy)pyrimidine**

2’-(methoxycarbonyl)hydrazone 3.12

2-Amino-4,6-dichloro-5-formylpyrimidine 2’-(methoxycarbonyl)hydrazone 3.09 (132 mg, 0.5 mmol) was added to 2,4,5-trichlorophenol (197 mg, 1 mmol) and potassium carbonate (138 mg, 1 mmol) in refluxing dry acetone (4 ml). The reaction mixture was refluxed for 2 hours. The hot mixture was filtered. The acetone filtrate was
evaporated to dryness leaving a yellow solid, which was washed with water, filtered and dried (168 mg, 62%).

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 3.62 (s, 3H, O-CH$_3$), 7.11 (s, 2H, -NH$_2$), 7.89 (s, 2H, 6'-CH), 8.04 (s, 2H, 3'-CH), 8.31 (s, 1H, N=CH), 10.90 (s, 1H, -NH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 51.7 (O-CH$_3$), 89.5 (q, 5-C), 126.2 (6'-CH), 126.3 (q, 2'-C), 128.9 (q, 4'-C), 130.6 (q, 5'-C), 131.1 (3'-CH), 137.4 (N=CH), 148.2 (q, 1'-C), 160.5 (q, 2-C), 167.2 (q, 4, 6-C).

$\nu_{\text{max}}$ 1712 cm$^{-1}$

mp > 200 °C

2-Amino-4,6-dichloro-5-pyrimidinecarboxaldehyde methoxime 3.21 and 2-amino-6-chloro-4-methoxylamino-5-pyrimidinecarboxaldehyde methoxime 3.22

Sodium acetate (3.28 g, 40 mmol) in water (20 ml) was added to a refluxing solution of the pyrimidine 2.06 (3.84 g, 20 mmol) and methoxylamine hydrochloride (1.83 g, 22 mmol) in MeOH (250 ml). The solution was refluxed for 20 hours. After cooling to room temperature, water (50 ml) was added. The MeOH was removed leaving an aqueous suspension of the product, which was filtered, dried (2.13 g) and chromatographed (EtOAc/hexane : 4/1) to give the dichloropyrimidine 3.21 (0.85 g, 20%) and the mono-chloropyrimidine 3.22 (0.94 g, 20%).

3.21 : $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 3.89 (s, 3H, -OCH$_3$), 7.84 (s, 2H, -NH$_2$), 8.12 (s, 1H, N=CH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 61.8 (OCH$_3$), 109.9 (q, 5-C), 142.6 (N=CH), 160.0 (q, 2-C), 161.0 (q, 4, 6-C).

$R_f$ (EtOAc/hexane : 4/1) 0.81

$\nu_{\text{max}}$ 1633 cm$^{-1}$

mp 124-126 °C

3.22 : $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 3.76 (s, 3H, -NH-OCH$_3$), 3.91 (s, 3H, -OCH$_3$), 7.25 (s, 2H, -NH$_2$), 8.30 (s, 1H, N=CH), 10.40 (s, 1H, -NH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 61.8 (OCH$_3$), 63.7 (-NH-OCH$_3$), 92.6 (q, 5-C), 145.2 (N=CH), 159.3 (q, 4-C), 160.4 (q, 2-C), 161.5 (q, 6-C).

$R_f$ (EtOAc/hexane : 4/1) 0.48

$\nu_{\text{max}}$ 1639 cm$^{-1}$

mp 192-195 °C
2-Amino-6-chloro-9-(methoxycarbonyl)-8-aza-7-deazapurine 3.08

Triethylamine (209 μl, 1.5 mmol) was added to a suspension of 2-amino-6-chloro-8-aza-7-deazapurine 2.03 (84 mg, 0.5 mmol) and methylpyrocarbonate (268 mg, 2 mmol) in DMSO (1 ml). The reactants dissolved. After leaving the reaction mixture at room temperature for two hours, the solvent was removed leaving a brown residue. Ice was added, causing the product to precipitate, which was filtered and dried (88 mg, 75%).

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 3.99 (s, 3H, CH$_3$), 7.73 (s, 2H, -NH$_2$), 8.28 (s, 1H, 7-CH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 54.5 (CH$_3$), 107.4 (q, 5-C), 137.1 (7-CH), 148.9 (C=O), 154.0 (q, 4-C), 158.4 (q, 2-C), 162.8 (q, 6-C).

$\nu_{\text{max}}$ 1639, 1762 cm$^{-1}$

mp 178-180 °C

2-Amino-6-chloro-9-pivaloyl-8-aza-7-deazapurine 3.27

Triethylamine (140 μl, 1 mmol) was added to a solution of 2-amino-6-chloro-8-aza-7-deazapurine 2.03 (84 mg, 0.5 mmol) and pivalic anhydride (406 μl, 2 mmol) in DMF (2 ml). After heating at 60 °C for three hours, the DMF was removed leaving a yellow residue, which was crystallised with diethyl ether to afford the product (85 mg, 71.4%).

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 1.43 (s, 9H, 3CH$_3$), 7.68 (s, 2H, -NH$_2$), 8.27 (s, 1H, 7-CH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 26.9 (CH$_3$), 41.6 (q, C(CH$_3$)$_3$) 106.7 (q, 5-C), 135.63 (7-CH), 154.09 (C=O), 159.2 (q, 4-C), 162.9 (q, 2-C), 175.4 (q, 6-C).

$\nu_{\text{max}}$ 1639, 1733 cm$^{-1}$

mp 164-165 °C

2-Amino-9-(butoxycarbonyl)-6-chloro-8-aza-7-deazapurine 3.29

N, N-Dimethylaminopyridine (12 mg, 0.1 mmol) was added to a solution of 2-amino-6-chloro-8-aza-7-deazapurine 2.03 (84 mg, 0.5 mmol) and Boc$_2$O (218 mg, 1 mmol) in DMF (3 ml). The reaction mixture was stirred at room temperature for 20 hours. The DMF was removed leaving a brown residue. Addition of acetonitrile caused a precipitate to form, which was filtered (32 mg, crude mixture). The acetonitrile
filtrate was evaporated and crystallised from DCM/hexane giving three crops (i) 33 mg (crude mixture), (ii) 29 mg (pure 3.29), (iii) 9 mg (pure 3.29) (38 mg, 28%).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.44 (s, 9H, 3CH$_3$), 7.68 (s, 2H, -NH$_2$), 8.20 (s, 1H, 7-CH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 27.6 (CH$_3$), 84.4 (q, C-(CH$_3$)$_3$), 107.3 (q, 5-C) 136.36 (7-CH), 146.6 (C=O), 153.8 (q, 4-C), 158.4 (q, 2-C), 162.6 (q, 6-C).

$\nu_{\text{max}}$ 1637, 1766 cm$^{-1}$

mp 156-159 °C

Reaction of 2-Amino-6-chloro-8-aza-7-deazapurine 2.06 with trimethylamine

A solution of trimethylamine (3 ml) in DMF (6 ml) at 0 °C was added to a solution of 2-amino-6-chloro-8-aza-7-deazapurine 2.06 (339 mg, 2 mmol) in DMF (20 ml) at 0 °C. After stirring at room temperature for one hour, diethyl ether (30 ml) was added. After 30 minutes, the suspension was filtered, washed with diethyl ether and dried (366 mg).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.11 (s, 1OH), 3.66 (s, 9H), 7.46 (broad s, 2H), 8.44 (s, 1H), 13.68 (broad s, 1H).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 53.9 (CH$_3$), 54.3 (CH$_3$), 96.4 (q-C), 132.9 (CH), 158.2 (q-C), 160.2 (q-C), 160.4 (q-C).

O$^6$-(4-Bromothenyl)-8-aza-7-deazaguanine 1.30

4-Bromothenyl alcohol (2.80 g, 14.5 mmol) and sodium hydride (580 mg of a 60% suspension in mineral oil, 14.5 mmol) were stirred in DMSO (20 ml) for 20 minutes. The DABCO salt 3.37 (2.04 g, 7.25 mmol) was added and stirring was continued for 20 hours. Water (50 ml) was added very slowly, and the crude product filtered, washed with diethyl ether and dried (1.42 g, 60%). The crude product was heated to 70°C in acetonitrile (40 ml). The hot solution was filtered, and the filtrate was evaporated to give the pure product (1.05g, 44%).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 5.63 (s, 2H, O$^6$-CH$_2$), 6.74 (s, 2H, -NH$_2$), 7.36 (s, 1H, 3'-CH), 7.71 (s, 1H, 5'-CH), 7.78 (s, 1H, 7-CH), 12.89 (s, 1H, 9-NH).

Procedure 3.1

Triethylamine (42 µl, 0.3 mmol) was added to O$^6$-substituted 8-aza-7-deazaguanine in DMF (0.4 ml). Methyl pyrocarbonate (43 µl, 0.6 mmol) was added and the reaction
mixture was stirred at room temperature for 1.5 hours. The mixture was poured into ice (~5 g)/acetic acid (0.5 ml). The precipitate was filtered and dried.

**O<sup>6</sup>-(4'-Bromothenyl)-9-(methoxycarbonyl)-8-aza-7-deazaguanine 3.38**

The synthesis of **3.38** was performed according to procedure 3.1, using alloPaTrin (98 mg, 0.3 mmol), to give a pale yellow solid (85 mg, 74%), which was recrystallised from ethanol (42 mg, 36.4%).

\[ ^1H \text{ NMR (400 MHz, DMSO-}\text{d}_6\text{)} \delta 3.96 (s, 3H, O-CH}_3) , 5.66 (s, 2H, O<sup>6</sup>-CH<sub>2</sub>), 7.33 (s, 2H, -NH<sub>2</sub>), 7.38 (s, 1H, 3'-CH), 7.72 (s, 1H, 5'-CH), 8.11 (s, 1H, 7-CH).

\[ ^13C \text{ NMR (100 MHz, DMSO-}\text{d}_6\text{)} \delta 54.2 (O-CH}_3) , 61.2 (O<sup>6</sup>-CH<sub>2</sub>), 96.9 (q, 5-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 131.3 (3'-CH), 136.4 (7-CH), 139.7 (q, 2'-C), 149.2 (C=O), 160.1 (q, 4-C), 162.5 (q, 2-C), 163.1 (q, 6-C).

\[ \nu_{max} 1772 \text{ cm}^{-1} \]

\[ \text{mp 212 °C} \]

\[ \lambda_{max} 266 \text{ nm} \]

% Calculated for C<sub>12</sub>H<sub>10</sub>BrN<sub>5</sub>O<sub>3</sub>S·1/2EtOH : C 38.34, N 17.20, H 3.22.

% Found : C 38.61, N 17.31, H 3.12.

**O<sup>6</sup>-Benzyl-9-(methoxycarbonyl)-8-aza-9-deazaguanine 3.38a**

The synthesis of **3.38a** was performed according to procedure 3.1, using O<sup>6</sup>-benzyl-8-aza-7-deazaguanine **1.37a** (121 mg, 0.5 mmol), to give a pale yellow solid (118 mg, 79%), which was recrystallised from ethanol (66 mg, 44%).

\[ ^1H \text{ NMR (400 MHz, DMSO-}\text{d}_6\text{)} \delta 3.96 (s, 3H, O-CH}_3) , 5.92 (s, 2H, O<sup>6</sup>-CH<sub>2</sub>), 7.25 (s, 2H, -NH<sub>2</sub>), 7.37 (m, 3H, 2', 4', 6'-CH), 7.54 (d, 2H, J 7.0, 3', 5'-CH), 8.12 (s, 1H, 7-CH).

\[ ^13C \text{ NMR (100 MHz, DMSO-}\text{d}_6\text{)} \delta 54.2 (O-CH}_3) , 67.4 (O<sup>6</sup>-CH<sub>2</sub>), 97.0 (q, 5-C), 128.2 (2', 4'-CH), 128.5 (3'-CH), 136.0 (q, 1'-C), 136.5 (7-CH), 149.2 (C=O), 160.1 (q, 4-C), 163.2 (q, 2-C), 163.4 (q, 6-C).

\[ \nu_{max} 1763 \text{ cm}^{-1} \]

\[ \text{mp 215 °C} \]

\[ \lambda_{max} 272 \text{ nm} \]

% Calculated for C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>N<sub>5</sub>·0.3EtOH : C 56.00, N 22.37, H 4.96.

% Found : C 56.66, N 22.38, H 4.87.
O\(^6\)-(4'-Bromothenyl)-8-(propyloxy carbonyl)-8-aza-7-deazaguanine 3.44 and 
O\(^6\)-(4'-Bromothenyl)-9-(propyloxy carbonyl)-8-aza-7-deazaguanine 3.45

Propyl chloroformate (56 µl, 0.5 mmol) was added to a solution of alloPaTrin (163 mg, 0.5 mmol) and triethylamine (70 µl, 0.5 mmol) in DMF (0.5 ml). After stirring at room temperature for two hours, diethyl ether (5 ml) was added, the precipitate was filtered, washed with water and dried to give the 8-isomer 3.44 (86 mg, 41.7%). The DMF/diethyl ether filtrate was evaporated to dryness and the residue was chromatographed (EtOAc/hexane : 9/1) to afford the 9-isomer 3.45 (29 mg, 14%).

8-Isomer 3.44: \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 0.97 (t, 3H, J 7.0, 3''-CH\(_3\)), 1.77 (sextet, 2H, J 7.0, 2''-CH\(_2\)), 4.37 (t, 2H, J 7.0, 1''-CH\(_2\)), 5.68 (s, 2H, O\(^6\)-CH\(_2\)), 7.07 (s, 2H, -NH\(_2\)), 7.41 (s, 1H, 3'-CH), 7.74 (s, 1H, 5'-CH), 8.81 (s, 1H, 7-CH).

\(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)) \(\delta\) 10.1 (3''-CH\(_3\)), 21.4 (2''-CH\(_2\)), 61.5 (O\(^6\)-CH\(_2\)), 70.0 (1''-CH\(_2\)), 100.7 (q, 5'-C), 108.2 (q, 4'-C), 125.6 (5'-CH), 126.9 (7-CH), 131.5 (3'-CH), 139.4 (q, 2'-C), 149.1 (C=O), 160.2 (q, 2-C), 162.5 (q, 4-C), 163.1 (q, 6-C).

\(v_{\text{max}}\) 1637, 1777 cm\(^{-1}\)

mp 160-162 °C

\(\lambda_{\text{max}}\) 224, 266, 282 (sh), 324 nm

% Calculated for C\(_{14}\)H\(_{14}\)BrN\(_5\)O\(_3\)S: C 40.43, H 3.49, N 16.84

% Found : C 40.20, H 3.44, N 16.85

\(R_f\) (EtOAc/hexane : 9/1) 0.23.

9-Isomer 3.45: \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 0.98 (t, 3H, J 7.0, 3''-CH\(_3\)), 1.74 (sextet, 2H, J 7.0, 2''-CH\(_2\)), 4.32 (t, 2H, J 7.0, 1''-CH\(_2\)), 5.65 (s, 2H, O\(^6\)-CH\(_2\)), 7.33 (s, 2H, -NH\(_2\)), 7.39 (s, 1H, 3'-CH), 7.73 (s, 1H, 5'-CH), 8.11 (s, 1H, 7-CH).

\(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)) \(\delta\) 10.2 (3''-CH\(_3\)), 21.5 (2''-CH\(_2\)), 61.2 (O\(^6\)-CH\(_2\)), 68.6 (1''-CH\(_2\)), 96.9 (q, 5'-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 131.3 (3'-CH), 136.3 (7-CH), 139.7 (q, 2'-C), 148.6 (C=O), 160.2 (q, 4-QC), 162.5 (2-QC), 163.1 (6-QC).

\(v_{\text{max}}\) 1576, 1643, 1757 cm\(^{-1}\)

mp 169-170 °C

\(\lambda_{\text{max}}\) 216, 266, 282\(^{\text{sh}}\) nm

% calculated for C\(_{14}\)H\(_{14}\)BrN\(_5\)O\(_3\)S: C 40.79, H 3.42, N 16.99

% found : C 40.71, H 3.31, N 16.73

\(R_f\) (EtOAc/hexane : 9/1) 0.69.
The synthesis of 358 and 359 was carried out using a similar procedure to that outlined above for the reaction of alloPaTrin with propyl chloroformate. However, it varied as to whether the 8- or 9-phenoxycarbonyl compounds formed. Typical procedures for the formation of both isomers are outlined below.

N-8 isomer 3.58: Phenyl chloroformate (38 μl, 0.3 mmol) was added to a solution of alloPaTrin (98 mg, 0.3 mmol) and triethylamine (42 μl, 0.3 mmol) in DMF (0.3 ml). After 30 minutes stirring at room temperature, during which time a solid precipitated, ice was added. The product was isolated, dried to give pale yellow solid (79mg, 59.0%) and recrystallised from acetonitrile.

N-8 isomer 3.58:  
\[ ^1H\text{ NMR (400 MHz, DMSO-d}_6)\delta 5.71 (s, 2H, O^6-CH\_2), 7.18 (s, 2H, -NH\_2), 7.41 (m, 6H, 3'^-CH, phenyl Ar-CH), 7.74 (s, 1H, 5'-CH), 9.04 (s, 1H, 7-CH). \]  
\[ ^13C\text{ NMR (100 MHz, DMSO-d}_6)\delta 61.6 (O^6-CH\_2), 101.3 (q, 5-C), 108.2 (q, 4'-C), 121.5 (2'', 6''-CH), 125.6 (5'-CH), 126.8 (4''-CH), 128.0 (7-CH), 129.8 (3'', 5''-CH), 131.4 (3'-CH), 139.4 (q, 2'-C), 147.8 (q, 1'-C), 150.1 (C=O), 162.3 (q, 2-C), 163.7 (q, 4-C), 164.6 (q, 6-C). \]

\[ \nu_{\text{max}} 1580, 1643, 1770\text{cm}^{-1} \]

\[ \text{mp 265 }^\circ\text{C (decomp.)} \]

\[ \lambda_{\text{max}} 222, 250 (\text{sh}), 276, 324 \text{nm} \]

% Calculated for C\textsubscript{17}H\textsubscript{12}BrN\textsubscript{3}O\textsubscript{2}S: C 45.75, H 2.71, N 15.69  
% Found: C 45.39, H 2.67, N 15.31.

N-9 isomer 3.59: Phenyl chloroformate (64 μl, 0.5 mmol) was added to a solution of alloPaTrin (163 mg, 0.5 mmol) and triethylamine (70 μl, 0.5 mmol) in DMF (0.5 ml). After 2 hours stirring at room temperature, no precipitate had formed. Diethyl ether was added. The resultant precipitate was isolated and washed with water to give the 9-isomer 3.59 (39 mg, 22%). The product was recrystallised from acetonitrile.

N-9 isomer 3.59:  
\[ ^1H\text{ NMR (400 MHz, DMSO-d}_6)\delta 5.68 (s, 2H, O^6-CH\_2), 7.40 (m, 8H, -NH\_2, 3'^-CH, phenyl Ar-CH), 7.74 (s, 1H, 5'-CH), 8.24 (s, 1H, 7-CH). \]  
\[ ^13C\text{ NMR (100 MHz, DMSO-d}_6)\delta 61.3 (O^6-CH\_2), 97.0 (q, 5-C), 108.2 (q, 4'-C), 121.7 (2'', 6''-CH), 125.6 (5'-CH), 126.5 (4''-CH), 129.8 (3'', 5''-CH), 131.4 (3'-CH), 136.0 (7-CH), 139.7 (q, 2'-C), 147.2 (q, 1'-C), 150.2 (C=O), 162.2 (q, 4-C), 162.6 (q, 2-C), 163.2 (q, 6-C). \]
\( \nu_{\text{max}} \) 1576, 1639, 1773 cm\(^{-1}\)

mp 210-212 °C

\( \lambda_{\text{max}} \) 222, 276 nm

% Calculated for C\(_{17}\)H\(_{12}\)BrN\(_5\)O\(_2\)S : C 45.76, H 2.71, N 15.69

% Found : C 45.53, H 2.64, N 15.39.

**Procedure 3.2**

Bis(2,4,5-trichlorophenyl) carbonate\(^{3,37} \) **3.66**, (421 mg, 1 mmol) was added to Hünig’s base (171 \( \mu \)l, 1 mmol) and alcohol (1 mmol) in DMF (4 ml) and the reaction mixture was stirred at room temperature for 18 hours. The DMF was removed leaving an oil which was extracted with petroleum ether.

**Benzyl(2,4,5-trichlorophenyl) carbonate 3.71a**

The synthesis of **3.71a** was performed according to procedure 3.2 using benzyl alcohol (104 \( \mu \)l, 1 mmol). The oily product was crystallised from petroleum ether to give fine needle-shaped white crystals (246 mg, 78%).

\(^1\)H NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 5.34 (s, 2H, O-CH\(_2\)), 7.43 (m, 5H, benzyl Ar-H), 7.97 (s, 1H, 6-CH), 8.05 (s, 1H, 3-CH).

\(^{13}\)C NMR (100 MHz, DMSO-\( d_6 \)) \( \delta \) 70.2 (O-CH\(_2\)), 125.6 (6-CH), 125.7 (q, 2-C), 128.4 (2', 6'-CH), 128.6 (4'-CH), 128.7 (3', 5'-CH), 129.0 (q, 4-C), 130.6 (q, 5-C), 131.1 (3-CH), 134.5 (q, 1'-C), 145.8 (q, 1-C), 151.5 (C=O).

\( \nu_{\text{max}} \) 1753 cm\(^{-1}\)

\( \text{mp} \) 70 °C

**Piperonyl(2,4,5-trichlorophenyl) carbonate 3.71b**

The synthesis of **3.71b** was performed according to procedure 3.2 from piperonyl alcohol (152 mg, 1 mmol). The oily product was crystallised from acetonitrile to give a white solid (262 mg, 70%).

\(^1\)H NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 5.22 (s, 2H, O-CH\(_2\)), 6.05 (s, 2H, O-CH\(_2\)-O), 6.93 (s, 1H, 6'-CH), 6.95 (s, 1H, 2'-CH), 7.02 (s, 1H, 4'-CH), 7.96 (s, 1H, 6-CH), 8.05 (s, 1H, 3-CH).

\(^{13}\)C NMR (100 MHz, DMSO-\( d_6 \)) \( \delta \) 70.9 (O-CH\(_2\)), 101.2 (O-CH\(_2\)-O), 108.2 (2'-CH), 109.2 (5'-CH), 122.9 (6'-CH), 125.7 (6-CH), 128.7 (q, 2-C), 129.9 (q, 4-C, 5-C), 130.6 (q, 1'-C), 131.1 (3-CH), 145.8 (q, 4'-C), 147.4 (q, 3'-C), 147.7 (q, 1-C), 151.4 (C=O).
O\textsuperscript{6}-(4'-Bromothenyl)-9-(4"-nitrophenoxycarbonyl)-8-aza-7-deazaguanine 3.75

Hünig's base (648 μl, 4 mmol) was added to a solution of alloPaTrin (652 mg, 2 mmol) and bis(4-nitrophenyl) carbonate 3.76 (608 mg, 2 mmol) in DMF (6 ml). After stirring at room temperature for 3 hours, the product was filtered, washed with diethyl ether and dried (764 mg, 77.8%).

\textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}) δ 5.69 (s, 2H, O\textsuperscript{6}-CH\textsubscript{2}), 7.40 (s, 1H, 3'-CH), 7.44 (s, 2H, -NH\textsubscript{2}), 7.73 (m, 3H, 2", 6"-CH, 5'-CH), 8.26 (s, 1H, 7-CH), 8.37 (d, 2H, J 9.0, 3", 5"-CH).

\textsuperscript{13}C NMR (100 MHz, DMSO-d\textsubscript{6}) δ 61.4 (O\textsuperscript{6}-CH\textsubscript{2}), 97.1 (q, 5-C), 108.2 (q, 4'-C), 115.9 (4"-CH), 123.2 (3"-CH, 5"-CH), 125.2 (5'-CH), 125.5 (2"-CH, 6"-CH), 131.3 (3'-CH), 137.6 (7-CH), 139.7 (q, 2'-C), 148.3 (C=O), 154.8 (q, 1'-C), 160.8 (q, 4-C), 162.7 (q, 2-C), 163.34 (q, 6-C).

Procedure 3.3

Bis(4-nitrophenyl) carbonate 3.76 (304 mg, 1 mmol), an alcohol (1 mmol) and Hünig's base (1 mmol) were stirred in dichloromethane (10 ml) for 18 hours at room temperature. The dichloromethane solution was washed with sodium bicarbonate solution (10 ml x 5) and brine (10 ml x 2) and dried with MgSO\textsubscript{4}. The dichloromethane was evaporated leaving the product.

\textit{4-Nitrophenyl(piperonyl) carbonate 3.54b}

The synthesis of 3.54b was performed according to procedure 3.3, using piperonyl alcohol (152 mg, 1 mmol), to give a white solid (142 mg, 45%), which was recrystallised from hexane (142 mg, 45%).

\textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}) δ 5.75 (s, 2H, O-CH\textsubscript{2}), 5.97 (s, 2H, O-CH\textsubscript{2}-O), 6.82 (m, 3H, 2'-CH, 5'-CH, 6'-CH), 7.73 (d, 2H, J 7.5, 2-CH, 6-CH), 8.37 (d, 4H, J 7.5, 3-CH, 5-CH).
$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 70.4 (O-CH$_2$), 101.2 (O-CH$_2$-O), 108.2 (2'-CH), 109.3 (5'-CH), 122.6 (2'-CH, 6'-CH), 122.9 (6'-CH), 125.4 (3'-CH, 4'-CH), 128.2 (q, 1'-C), 145.2 (q, 4'-C), 147.4 (q, 4''-C), 147.7 (q, 3'-C), 151.9 (C=O), 155.3 (q, 1-C).

$\nu$ max 1594, 1656, 1745 cm$^{-1}$

mp 132-134 $^\circ$C

N-Methyl-4-piperidinyl 4-nitrophenylcarbonate 3.54d

The synthesis of 3.54d was performed according to procedure 3.3, using N-methylpiperidin-4-ol (115 mg, 1 mmol), to give a pale yellow solid (139 mg, 55.8%), which was recrystallised from EtOAc/hexane (124 mg, 49.7%).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.69 (2H, br s, 3$\alpha$'-, 5$\alpha$'-CH), 1.94 (2H, br s, 3$\epsilon$', 5$\epsilon$'-CH), 2.20 (m, 5H, -NCH$_3$, 2$\alpha$', 6$\alpha$'-CH), 2.52 (br s, 2H, 2$\epsilon$', 6$\epsilon$'-CH), 4.70 (br s, 1H, 4''-CH), 7.56 (d, 2H, J 9.0, 2-CH, 6-CH), 8.31 (d, 2H, J 9.0, 3-CH, 5-CH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 30.4 (3', 5$\epsilon$'-CH), 45.8 (CH$_3$), 52.3 (2', 6$\epsilon$'-CH), 75.4 (4''-CH), 122.6 (2, 6-CH), 125.4 (3, 5-CH), 145.6 (q, 1-C), 150.9 (C=O), 155.6 (q, 4-C).

$\nu$ max 1580, 1736 cm$^{-1}$

mp 116-117 $^\circ$C

Procedure 3.4

The mixed carbonate 3.54a-d (0.3 mmol), alloPaTrin (98 mg, 0.3 mmol) and Hüning’s base (107 $\mu$l, 0.6 mmol) were stirred in DMF (0.4 ml) at room temperature for 72 hours. The DMF was removed leaving a yellow residue, which was crystallised from acetonitrile.

9-(Benzyloxy carbonyl)-O$^6$-(4'-bromothenyl)-8-aza-7-deazaguanine 3.77a

The synthesis of 3.77a was performed according to procedure 3.4, using 4-nitrophenyl(benzyl) carbonate$^{340}$ 3.54a (82 mg, 0.3 mmol), to give a pale yellow solid (99 mg, 72%), which was recrystallised from acetonitrile.

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 5.45 (s, 2H, Ph-CH$_2$), 5.66 (s, 2H, O$^6$-CH$_2$), 7.41 (m, 6H, -NH$_2$, 3'-CH, 2'', 4'', 6''-CH), 7.51 (m, 2H, 3'', 5''-CH), 7.72 (s, 1H, 5'-CH), 8.11 (s, 1H, 7-CH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 61.3 (O$^6$-CH$_2$), 68.4 (Ph-CH$_2$), 97.0 (q, 5-C), 108.39 (q, 4'-C), 125.3 (5'-CH), 128.0 (2''-CH, 6''-CH), 128.4 (4''-CH), 128.6 (3''-CH,
$5^\prime$-CH), 131.4 ($3^\prime$-CH), 136.7 (7-CH), 139.6 (q, 2'-C), 148.4 (C=O), 160.3 (q, 1''-C), 162.4 (q, 4-C), 163.5 (q, 2-C), 164.9 (q, 6-C).

$\nu_{\text{max}}$ 1755 cm$^{-1}$

mp 188-190 $^\circ$C

$\lambda_{\text{max}}$ 268 nm

% Calculated for C$_{18}$H$_{14}$BrO$_3$N$_5$S·0.3CH$_3$CN : C 47.27, H 3.20, N 15.71

% Found : C 47.48, H 3.25, N 15.48.

O$^6$-(4'-Bromothenyl)-9-(piperonyloxycarbonyl)-8-aza-7-deazaguanine 3.77b

The synthesis of 3.77b was performed according to procedure 3.4, using 4-nitrophenyl(piperonyl) carbonate 3.54b (95 mg, 0.3 mmol), to give a pale yellow solid (107 mg, 71%), which was recrystallised from acetonitrile.

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 5.34 (s, 2H, Ph-CH$_2$), 5.66 (s, 2H, O$^6$-CH$_2$), 6.03 (s, 2H, O-CH$_2$-O), 6.95 (m, 2H, 2'', 5''-CH), 7.03 (m, 1H, 6''-CH), 7.31 (s, 2H, -NH$_2$), 7.37 (s, 1H, 3'-CH), 7.71 (s, 1H, 5'-CH), 8.09 (s, 1H, 7-CH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 61.3 (O$^6$-CH$_2$), 68.4 (Ph-CH$_2$), 97.0 (q, 5-C), 101.1 (O-CH$_2$-O), 108.1 (q, 4'-C), 108.2 (2''-CH), 109.0 (5''-CH), 122.5 (6''-CH), 125.4 (5'-CH), 128.9 (q, 1''-C), 131.2 (3'-CH), 136.4 (7-CH), 139.7 (q, 2'-C), 147.4 (q, 4''-C), 147.4 (q, 3'-C), 148.4 (C=O), 160.6 (q, 4-C), 162.5 (q, 2-C), 163.2 (q, 6-C).

$\nu_{\text{max}}$ 1751 cm$^{-1}$

mp 168-170 $^\circ$C

$\lambda_{\text{max}}$ 280 nm

% Calculated for C$_{19}$H$_{14}$BrN$_5$O$_5$S·1/2CH$_3$CN : C 45.77, H 2.98, N 14.69.

% Found : C 45.58, H 2.98, N 14.48.

O$^6$-(4'-Bromothenyl)-9-(4''-picolyloxycarbonyl)-8-aza-7-deazaguanine 3.77c

The synthesis of 3.77c was performed according to method 3.4, using the 4-nitrophenyl salt of 4-nitrophenyl(4-picoly) carbonate$^{3,4,1}$ 3.54c (124 mg, 0.3 mmol), to give a pale yellow solid (91 mg, 65.9%), which was recrystallised from acetonitrile.

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 5.52 (s, 2H, picolyl-CH$_2$), 5.68 (s, 2H, O$^6$-CH$_2$), 7.36 (s, 2H, -NH$_2$), 7.39 (s, 1H, 3'-CH), 7.54 (d, 2H, J 6.0, 2''-CH, 6''-CH), 7.73 (s, 1H, 5'-CH), 8.17 (s, 1H, 7-CH), 8.63 (d, 2H, J 6.0, 3''-CH, 5''-CH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 61.2 (O$^6$-CH$_2$), 66.5 (picolyl-CH$_2$), 96.9 (q, 5-C), 108.1 (q, 4'-C), 121.6 (2''-CH, 6''-CH), 125.4 (5'-CH), 131.2 (3'-CH), 136.7 (7-CH), 137.1...
139.7 (q, 2'-\(\text{C}\)), 144.3 (3''-\(\text{CH}\), 5''-\(\text{CH}\)), 149.8 (\(\text{C}=\text{O}\)), 160.3 (q, 4'-\(\text{C}\)), 162.6 (q, 2'-\(\text{C}\)), 163.1 (q, 6'-\(\text{C}\)).

\(v_{\text{max}}\) 1755 cm\(^{-1}\)

mp 180 °C

\(\lambda_{\text{max}}\) 258 nm

% Calculated for C\(_{17}\)H\(_{13}\)BrN\(_6\)O\(_3\)S: C 43.42, H 3.00, N 17.86

% Found: C 43.59, H 2.84, N 18.22.

\(O^6-(4'-\text{Bromothenyl})-9-(\text{N-methyl-4''-piperidyloxycarbonyl})-8-\text{aza-7-deazaguanine}\)

3.77d

The synthesis of 3.77d was performed according to procedure 3.4, using N-methyl-4-piperidinyl(4-nitrophenyl) carbonate 3.54d (84 mg, 0.3 mmol), to give a pale yellow solid (83 mg, 59%), which was recrystallised from acetonitrile.

\(^1\text{H NMR (400 MHz, DMSO-}d_6\text{)} \delta 1.75 (m, 2H, 2ax'', 6ax''-\text{CH}), 1.95 (m, 2H, 2eq'', 6eq''-\text{CH}), 2.18 (m, 5H, 3ax'', 5ax''-\text{CH}, -\text{NCH}_3), 2.61 (m, 2H, 3eq'', 5eq''-\text{CH}), 4.92 (\text{broad} \ s, 1H, 1''-\text{CH}), 5.65 (s, 2H, \(O^6\)-\text{CH}_2), 7.30 (s, 2H, -\text{NH}_2), 7.39 (s, 1H, 3''-\text{CH}), 7.73 (s, 1H, 5''-\text{CH}), 8.10 (s, 1H, 7-\text{CH}).

\(^{13}\text{C NMR (100 MHz, DMSO-}d_6\text{)} \delta 30.3 (2'', 6''-\text{CH}_2), 45.8 (-\text{NCH}_3), 52.1 (3'', 5''-\text{CH}_2), 61.2 (\(O^6\)-\text{CH}_2), 73.4 (1''-\text{CH}), 96.9 (q, 5'-\text{C}), 108.2 (q, 4''-\text{C}), 125.5 (5'-\text{CH}), 131.3 (3''-\text{CH}), 136.2 (7-\text{CH}), 139.7 (q, 2''-\text{C}), 147.9 (\text{C}=\text{O}), 160.3 (q, 4'-\text{C}), 162.5 (q, 2'-\text{C}), 163.1 (q, 6'-\text{C}).

\(v_{\text{max}}\) 1574, 1644, 1753 cm\(^{-1}\)

mp 185-186 °C

\(\lambda_{\text{max}}\) 268 nm

% Calculated for C\(_{17}\)H\(_{19}\)BrN\(_6\)O\(_3\)S: C 43.69, H 4.10, N 17.98

% Found: C 43.39, H 4.10, N 17.68.
3.11 References


3.2 R. S. McElhinney, T. B. H. McMurry and D. J. Donnelly, unpublished work.


3.35 The change of solvent was suggested by Dr. J. E. O’Brien.


Chapter 4

Synthesis of Alkylcarbamoyl Derivatives of AlloPaTrin
4.1 Introduction

In Chapter 3, we have already discussed the synthesis of a series of alkoxycarbonyl derivatives of alloPaTrin. These new compounds could potentially act as prodrugs of alloPaTrin, depending on their stabilities under physiological conditions. In this chapter we will address the synthesis of mono-4.01-4.02 and dialkylcarbamoyl 4.03-4.04 derivatives of alloPaTrin. Their biological activities, stabilities under physiological conditions and overall potential as prodrugs of alloPaTrin can be assessed. These compounds were chosen as targets for synthesis, as they should be more resistant to hydrolysis than the alkoxycarbonyl derivatives of Chapter 3, due to the greater stability of the carbamoyl side-chain. Comparing the properties of the three different kinds of alloPaTrin derivatives could give an insight into which, if any, of these species could act as a good prodrug of alloPaTrin.
$N$-Alkoxycarbonyl $4.05$, mono-$4.07$ and dialkylcarbamoyl $4.08$ derivatives of 5-fluorouracil $1.03$ have been investigated in a similar manner, as potential prodrugs of 5-fluorouracil.\textsuperscript{4,1} The cancer chemotherapy drug, 5-fluorouracil has also been used in the treatment of certain skin diseases, but because of its low lipophilicity, it does not react at its target site in optimal levels.\textsuperscript{4,1c} Prodrugs of 5-fluorouracil have been developed which show increased lipophilicity and their biological activities and stabilities have been investigated.\textsuperscript{4,1} $N$-Alkoxycarbonyl derivatives of 5-fluorouracil $4.05$ were found to hydrolyse more readily than mono-alkylcarbamoyl derivatives $4.06$ or $N, N$-dialkylcarbamoyl derivatives $4.07$. The latter proved to be highly stable (see Section 5.6 for a more detailed discussion of this study).

\[ \text{Figure 1.03} \]

\[ \text{Figure 4.05a, 4.05b, 4.05c} \]

\[ \text{Figure 4.06, 4.07} \]

### 4.2 Reaction of AlloPaTrin with an Isocyanate

Initially, the carbamoylation of alloPaTrin was attempted using an isocyanate.\textsuperscript{4,2} In the guanine series, a number of 9-alkylcarbamoyl derivatives of PaTrin-2 have been produced in high yields from the base-catalysed reaction of an alkylisocyanate with PaTrin-2 in DMF. When this synthesis was replicated, using alloPaTrin in place of PaTrin-2, hexyl isocyanate and a 0.1 equivalent of triethylamine, a mixture formed consisting of the required 9-hexylcarbamoyl compound $4.08$ and alloPaTrin in an
equal ratio. The mixture could not be separated through recrystallisation or chromatography. A change of base to DBU, DABCO, DMAP or Hünig’s base did not alter the result of the reaction. Heating the reaction mixture, increasing the reaction time or increasing the amount of the various bases only led to the isocyanate reacting with itself to give the symmetrical dihexyl urea, \((\text{CH}_3\text{(CH}_2)_5\text{NH})_2\text{CO}\).

![Scheme 4.1](image)

We thought that perhaps a change from the organic bases to the much stronger sodium hydride may effect the progress of the reaction. When alloPaTrin was reacted with hexyl isocyanate and sodium hydride, a product was isolated in good yields (84%). The \(^1\text{H} \text{NMR spectrum of this product showed the absence of the 2-NH}_2 \text{ group and the presence of peaks representing three different -NHs. The signal corresponding to the 7-C in the } ^{13}\text{C NMR spectrum occurred at 131.9 ppm. The analogous signal of unsubstituted alloPaTrin occurs at 131.0 ppm. We have already shown in Section 3.6}
that substitution in the 8-position causes the 7-C signal to shift upfield significantly (the signals of 8-substituted compounds are observed at ~125 ppm), while a 9-substituent causes a downfield shift of the signal to approximately 136 ppm. The uv spectrum of the product showed a maximum at 270 nm, and resembled neither the typical uv spectra of an 8- or 9- substituted derivative of alloPaTrin. This pointed towards substitution at the exocyclic amino group to give 4.10 rather than the 8- or 9-substituted species 4.09 or 4.08. The experiment was repeated using cyclohexyl isocyanate in place of hexyl isocyanate. Substitution again occurred at the amino group to give 4.11.

![Diagram](image)

Initially, sodium hydride deprotonates alloPaTrin to give a mixture of the anions 3.55a-b and 4.12. The anion 4.12 forms, in this instance, as the deprotonating agent, sodium hydride, is a much stronger base than the organic bases which have been used previously. Alkyl isocyanate reacts with the mixture of anions to give the tetrahedral intermediates 4.13a-c. Potentially, these intermediates could produce the final compounds 4.01-4.02, 4.14. The high yield of the derivative bearing the substituent on the exocyclic amino group 4.14 would suggest that either the intermediates 4.13a-b revert back to their parent anions in a reversible reaction, or alternatively, the initial anion 4.12 may form in a far greater quantity than either of its anionic isomers 3.55a-b. The greater basic strength of sodium hydride may contribute to the altered progress of the reaction when compared to similar reactions using weaker organic bases.
Scheme 4.2
Alternatively, as the initial reaction of sodium hydride with alloPaTrin resulted in the evolution of hydrogen gas, the reaction system contains no constant supply of an acidic proton, which would stabilise the intermediates 4.13a-c. This factor may contribute to the preferential formation of the 2-substituted product.

4.3 Mixed Carbamates of 4-Nitrophenol
As the reaction of alloPaTrin with an isocyanate failed to produce the desired derivatives of alloPaTrin, the usefulness of carbamates as carbamoylating agents were investigated. In Chapter 3, we discussed the synthesis of alkoxy carbonyl derivatives of alloPaTrin using carbonates containing the leaving group, 4-nitrophenoxide (see p. 122). We wondered whether a similar strategy could be employed to make carbamoyl derivatives of alloPaTrin. This synthetic pathway has been used to good effect in the guanine series to prepare mono-alkylcarbamoyl derivatives of alloPaTrin, whose corresponding isocyanate is not commercially available.

To prepare the carbamate 4.15, 3-ethoxypropylamine was reacted with bis-(4-nitrophenyl) carbonate 3.76. The carbamate 4.15 was then reacted with alloPaTrin and one equivalent of Hünig's base. However, the reaction produced a mixture of compounds from which no pure species was isolated. The quantity of base used in the reaction was doubled but the outcome of the reaction was not significantly affected. A clean product was only isolated when both the quantities of base and carbamate were doubled relative to the amount of alloPaTrin. The $^1$H NMR spectrum showed the peak representing 7-H occurring at 8.68 ppm, while the signal of the 7-C was observed at 123.3 ppm in the $^{13}$C NMR spectrum. An absorption maximum of 318 nm was observed in the uv spectrum of the compound (solution in methanol). According to our structural assignment of 8- and 9-substituted 8-aza-7-deazaguanines, the product of the reaction between alloPaTrin and 4-nitrophenyl N-(3-ethoxypropyl) carbamate 4.15 was the 8-substituted species 4.17 in a yield of 45%. Traces of the corresponding 9-isomer 4.16 were observed by TLC of the crude reaction mixture, but was never isolated.
Scheme 4.3

4.3.1 Formation of the 8-Substituted Derivative

In Chapter 3, we discussed the formation of 9-alkoxycarbonyl derivatives of alloPaTrin from the reaction of alloPaTrin with a carbonate containing a 4-nitrophenyl substituent (see p. 122). The lack of formation of the 8-substituted compound was attributed to the preferential loss of the alloPaTrin anion from the tetrahedral intermediate 3.79b. In contrast, the 4-nitrophenoxide group acted as the leaving group in the 9-tetrahedral intermediate 3.79a to give the 9-substituted compound. In this case, the reaction of alloPaTrin with a 4-nitrophenyl carbamate 4.15 produced predominantly the 8-substituted compound 4.17. The difference in the outcomes of the reactions can be attributed to the difference in the electronic properties of the alkoxycarbonyl and carbamoyl side-chains. The carbamoyl side-chain is stabilised by the inductive effect of the alkyl group attached to the –NH. This should increase the
electron availability of the nitrogen atom, and consequently increase the stability of
the carbonyl group to which it is attached. Due to the stability of the carbonyl group
of the carbamate, the rate of formation of both the 8- and 9- tetrahedral intermediates
4.18 and 4.19 should be slower than that of their alkoxy analogues. As in the
corresponding carbonate case, the 8-substituted tetrahedral intermediate 4.19 can
either lose the 4-nitrophenoxide group or return to alloPaTrin. The loss of the 4-
nitrophenoxide group is favoured in this case due to the stabilising effect of the
alkylamino side-chain, to give the 8-isomer. The 9-isomer also forms, but is less
favoured due to the hindered nature of the 9-position.
Scheme 4.4
4.4 Reaction of the 9-(4-Nitrophenoxy)carbonyl Derivative of AlloPaTrin with a Primary Amine

The reaction between alloPaTrin and a 4-nitrophenyl carbamate produces predominantly an 8-substituted compound in a Route 2 type reaction. This involves the displacement of a 4-nitrophenoxide group by a secondary amine (alloPaTrin). As we had previously prepared the 9-(4-nitrophenoxy)carbonyl derivative of alloPaTrin 3.75 (see p.120), we decided to investigate the potential of displacing its 4-nitrophenoxide group with a primary amine to give the 9-carbamoyl derivative of alloPaTrin.

As the 4-nitrophenoxy group is already in the 9-position, the 9-substituted carbamoyl compound is the only potential product. Previously, we have attempted to displace the 4-nitrophenoxide group by an alcohol to give the 9-alkoxycarbonyl derivative of alloPaTrin (see p.121). However, the reaction only gave an inseparable mixture of the unreacted 4-nitrophenoxy compound 3.75, the required alloPaTrin derivative and free alloPaTrin, even when a huge excess of alcohol was used. In the reaction of 3.75 with a primary amine, it was hoped that the basicity of the amine would facilitate the displacement of the 4-nitrophenoxide group.

When the 9-(4-nitrophenoxy)carbonyl derivative of alloPaTrin 3.75 in DMF was treated with one equivalent of hexylamine, an inseparable mixture formed. Doubling the quantity of hexylamine or varying the reaction conditions did not improve the outcome of the reaction.
Wörle et al.\textsuperscript{4,4} have reported a similar displacement of a 4-nitrophenoxide group by a primary amine using pyridine as the solvent, while Hay et al.\textsuperscript{4,5} have reported the displacement of a 4-nitrophenoxide group by a secondary amine using the same reaction conditions. Even though the starting (4-nitrophenoxy)carbonyl compound 3.75 is largely insoluble in pyridine, we found that the addition of two equivalents of hexylamine to a suspension of the alloPaTrin derivative 3.75 in pyridine causes instantaneous solvation. Immediate work-up of the reaction produced the required 9-hexylcarbamoyl product 4.08 in a 77\% yield. When less than two equivalents of the amine were used, some of the starting material remained in suspension. Undissolved starting material was also observed when one equivalent of the amine was used in conjunction with varying quantities of triethylamine.

The reaction of 3.75 with two equivalents of a primary amine was repeated with 3-ethoxypropylamine, cyclohexylamine and 4-(3-aminopropyl)morpholine, to give the 9-carbamoyl derivatives of alloPaTrin 4.16, 4.20 and 4.21 in yields of 53\%, 80\% and 51\% respectively.\textsuperscript{*} In all cases, the progress of the reactions were identical to that of

\textsuperscript{*} The hydrochloride salt 4.22 of 4.21 was prepared by the method discussed in Section 4.8.
the initial hexylamine reaction, and solvation appeared to provide a convenient indicator of the completion of the reaction.

The reaction of the 4-nitrophenoxy intermediate 3.75 with the less basic primary amine aniline also gave the required 9-carbamoyl product 4.23. However, the addition of two equivalents of aniline to the suspension of 3.75 in pyridine did not cause the immediate solvation of the alloPaTrin derivative 3.75. Solvation, and the completion of the reaction, occurred after 24 hours. A longer reaction time was necessary due to the decreased basicity of aniline compared to the alkylamines, which were used earlier.

When 3-aminobenzamide was added to a suspension of 4-nitrophenoxy intermediate 3.75 in pyridine, solvation did not occur after several days. Heating the suspension did result in solvation, but the work-up only produced free alloPaTrin and unreacted 3-aminobenzamide. In this case the amine was not sufficiently basic to cause the
displacement of the 4-nitrophenoxy group, and heating the reaction caused the decomposition of 3.75 to give free alloPaTrin.

This series of reactions show that while 4-nitrophenoxy intermediate 3.75 is an extremely convenient intermediate in the preparation of 9-monoalkylcarbamoyl derivatives of alloPaTrin, its usefulness is limited by the base strength of the amine used to displace the 4-nitrophenoxy group. In addition, the necessary use of two equivalents of amine is not ideal in cases where the use of valuable or non-commercial amines is required.
4.6 Reaction of N, N-Dialkylcarbamoyl Chloride with AlloPaTrin

Having successfully prepared a number of monoalkylcarbamoyl derivatives of alloPaTrin, our focus turned to the synthesis of dialkylcarbamoyl derivatives.\(^4,6\)

In the PaTrin-2 series, a number of compounds with 9-dialkylcarbamoyl substituents were prepared using \(N, N\)-dialkylcarbamoyl chlorides. Bearing in mind the result of the reaction of a chloroformate with alloPaTrin (see Section 3.6), we predicted the application of these reaction conditions to alloPaTrin would give predominantly the 8-dialkylcarbamoyl compound.

As we had expected, when alloPaTrin was treated with an \(N, N\)-dialkylcarbamoyl chloride and DBU, the 8-substituted compound \(425a-d\) was isolated in each case (45-73%). TLC of each reaction mixture showed the probable presence of the 9-isomer, but they had formed in such low yields that they were never isolated.

\[ \text{DBU was used as base in preference to either triethylamine or Hünig's base. In the PaTrin-2 series, the reaction of PaTrin-2, dimethylcarbamoyl chloride and} \]

![Scheme 4.8](image-url)
triethylamine failed to yield a product. This may have been due to the sterically hindered nature of the intermediate 4.26 formed by the base and the carbamoyl chloride, which would prevent the attack of the anion of PaTrin-2.

\[
\begin{align*}
4.26 & \quad 4.27
\end{align*}
\]

We assume that similar difficulties would have been encountered when using alloPaTrin in place of PaTrin-2. Buur et al. have supported these findings by attributing the decreased susceptibility of 1-(N,N-dimethyl)carbamoyl-5-fluorouracil to hydrolysis to the steric hindrance caused by the two methyl groups. The use of DBU as base is successful in this type of reaction, as the intermediate 4.27 formed from its reaction with dimethylcarbamoyl chloride possesses a less hindered carbonyl group.

The formation of the 8-isomer in preference to the 9-isomer can be explained through the mechanism in Scheme 4.9. The reaction of a carbamoyl chloride with the anion of alloPaTrin gives rise to the two tetrahedral intermediates 4.28 and 4.29. The formation of the 8-substituted tetrahedral intermediate 4.29 is favoured as the 9-position is more hindered. Chloride is eliminated from both intermediates, as it is a good leaving group, to give the 8- and 9-dialkylcarbamoyl derivatives of alloPaTrin.
Scheme 4.9
4.7 Reaction of the 9-(4-Nitrophenoxy)carbonyl Derivative of AlloPaTrin with a Secondary Amine

As only 8-dialkylcarbamoyl derivatives were isolated from the reaction of a carbamoyl chloride with alloPaTrin, we required another synthetic strategy to obtain 9-dialkylcarbamoyl derivatives of alloPaTrin. In Section 4.4, we discussed the synthesis of a number of 9-monoalkylcarbamoyl compounds 4.08, 4.16, 4.20-4.22 from the displacement of the 4-nitrophenoxide of the intermediate 3.75 by a primary amine. In this instance, a secondary amine was used in place of the primary amine.

Initially N-methylpiperazine was chosen as the secondary amine. This side-chain should facilitate the conversion of the final product 4.30a to a water soluble salt. In this experiment, when just one equivalent of the amine was added to a suspension of 3.75 in pyridine, the ester dissolved almost immediately. Previously when we had used a primary amine such as hexylamine in this reaction, we found that all of the starting material would not dissolve until two equivalents of the amine was added to the suspension. We also found that solvation of 3.75 was a reliable indication of the completion of the reaction. In this case, only one equivalent of the amine was required as N-methylpiperazine has two sites of basicity, the \(-\text{NH}\) and the \(-\text{NCH}_3\).

Work-up of the reaction involved the removal of pyridine and extraction of the 4-nitrophenol group produced as a by-product in the reaction by aqueous sodium hydroxide to give the 9-(N-methylpiperazino)carbamoyl compound 4.30a in a yield of 40.3%. The reaction was repeated using morpholine, 1-benzylpiperazine and 4-piperidinopiperidine as secondary amines in place of N-methylpiperazine, and the 9-substituted compounds 4.30b-d were isolated in yields of 67%, 61% and 88% respectively.\*\*

\* The hydrochloride salt of the 4-piperidinopiperidino derivative 4.30d, 4.31 was prepared by the method outlined in Section 4.8, as was the citrate salt of 1-benzylpiperazino derivative 4.30c, 4.32.
The piperazino and piperidino based groups were chosen as side-chains for the dialkylcarbamoyl derivatives of alloPaTrin, as many important biologically active compounds contain such substituents. For example, sildenafil 4.33 (more commonly known as Viagra®), which has received a huge amount of attention since its launch onto the market place, contains a $N$-methylpiperazino group. Programs to investigate the derivatisation of biologically active compounds using these and related side-chains have been extensively reported in the literature.4.8
4.8 Water Soluble Derivatives of AlloPaTrin

The alloPaTrin derivatives 4.22, 4.30c and 4.30d all possess a basic centre in their carbamoyl side-chain, and consequently are all good candidates for conversion to water soluble salts. This conversion was achieved by the addition of a molar equivalent of citric acid or aqueous hydrochloric acid to a solution of the alloPaTrin derivative in methanol. In each experiment, the solvent was removed after two hours to give a “glass”. The “glass” was crystallised from diethyl ether to give the product, which was water soluble in each case.

4.9 Conclusion

In this chapter, the syntheses of carbamoyl derivatives of alloPaTrin were discussed. The site of substitution varies depending on the carbamoylating agent and the reaction conditions used.

We have found that treatment of alloPaTrin by an alkylisocyanate and sodium hydride produces substitution exclusively at the exocyclic amino group (pp.146-148), while the use of a carbamate with a 4-nitrophenyl substituent results in substitution at the 8-position predominantly (pp. 148-151). 9-Substituted compounds were prepared from the displacement of the 4-nitrophenoxide group from the 9-(4-nitrophenoxycarbonyl) derivative of alloPaTrin 3.75 by a primary amine (pp.152-155).

Treatment of alloPaTrin with a dialkylcarbamoyl chloride resulted in selective substitution at the 8-position of alloPaTrin (pp. 156-157). AlloPaTrin derivatives containing dialkylcarbamoyl substituents at the 9-position were prepared in a similar
way to the 9-(monoalkyl)carbamoyl derivatives (displacement of a 4-nitrophenoxy group of 3.75 by a secondary amine) (pp. 159-160).
\[ \text{4.10 Experimental} \]

**O}^{6-(4'-Bromothenyl)-2-(N-hexylcarbamoyl)-8-aza-7-deazaguanine 4.10**

Sodium hydride (14 mg of a 60% suspension in mineral oil, 0.35 mmol) was added to a solution of alloPaTrin (98 mg, 0.3 mmol) and hexyl isocyanate (44 \( \mu l \), 0.3 mmol) in DMF (0.3 ml). After two days at room temperature, the DMF was removed leaving a residue, which solidified in water to give the crude product (114 mg, 84%). The crude product was refluxed in acetonitrile (4 ml). The undissolved pure product was filtered from the hot suspension.

\[ ^1H \text{ NMR (400 MHz, DMSO-\text{d}_6)} \delta 0.86 (m, 3H, 6"-CH_3), 1.28 (m, 6H, 3", 4", 5"-CH_2), 1.50 (m, 2H, 2"-CH_2), 3.25 (m, 2H, 1"-CH_2), 5.72 (s, 2H, O}^{6-CH_2}), 7.53 (s, 1H, 3'-CH), 7.72 (s, 1H, 5'-CH), 8.04 (s, 1H, 7-CH), 9.01 (s, 1H, 1"-NH), 9.78 (s, 1H, 2-NH), 13.59 (broad s, 1H, 9-NH). \]

\[ ^{13}C \text{ NMR (100 MHz, DMSO-\text{d}_6)} \delta 13.9 (6"-CH_3), 22.0 (5"-CH_2), 26.2 (3"-CH_2), 29.5 (2", 4"-CH_2), 31.0 (1"-CH_2), 61.4 (O}^{6-CH_2}), 97.4 (q, 5-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 131.7 (3'-CH), 131.9 (7-CH), 139.5 (q, 2'-C), 153.8 (C=O), 155.7 (q, 2-C), 156.6 (q, 4-C), 162.4 (q, 6-C). \]

\( \nu_{\text{max}} \) 1594, 1638, 1706 cm\(^{-1}\)

mp 248-250 °C

\( \lambda_{\text{max}} \) 228, 256 (sh), 270 nm

% Calculated for C\(_{17}\)H\(_{27}\)BrN\(_6\)O\(_2\)S-0.2H\(_2\)O: C 44.68, H 4.72, N 18.39.

% Found: C 44.63, H 4.61, N 18.40.

**O}^{6-(4'-Bromothenyl)-2-(N-cyclohexylcarbamoyl)-8-aza-7-deazaguanine 4.11**

Sodium hydride (14 mg of a 60% suspension in mineral oil, 0.35 mmol) was added to a solution of alloPaTrin (98 mg, 0.3 mmol) and cyclohexyl isocyanate (38 \( \mu l \), 0.3 mmol) in DMF (0.3 ml). After two days at room temperature, the DMF was removed leaving a residue, which was solidified in water to give the crude product (99 mg, 73%), which was recrystallised from acetonitrile.

\[ ^1H \text{ NMR (400 MHz, DMSO-\text{d}_6)} \delta 1.24-1.38 (m, 5H, 2ax", 3ax", 4ax", 5ax", 6ax"-CH), 1.59 (m, 1H, 4eq"-CH), 1.70 (m, 2H, 3eq"-CH), 1.91 (m, 2H, 2eq"-CH), 3.65 (m, 1H, 1"-CH), 5.72 (s, 2H, O}^{6-CH_2}), 7.36 (s, 1H, 3'-CH), 7.71 (s, 1H, 5'-CH), 8.05 (s, 1H, 7-CH), 9.02 (d, 1H, J 7.5, 1"-CHNH), 9.73 (s, 1H, 2-NH), 13.58 (s, 1H, 9-NH). \]

\[ ^{13}C \text{ NMR (100 MHz, DMSO-\text{d}_6)} \delta 24.2 (3", 5"-CH_2), 25.3 (4"-CH_2), 32.7 (2", 6"-CH_2), 47.9 (1"-CH), 61.5 (O}^{6-CH_2}), 97.4 (q, 5-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 163.0 (C=O). \]
131.8 (3'-CH), 131.9 (7-CH), 139.4 (q, 2'-C), 153.0 (C=O), 155.9 (q, 4-C), 156.4 (q, 2-C), 162.5 (q, 6-C).

$\nu_{max}$ 1680 cm$^{-1}$

mp 260 °C (decomp.)

$\lambda_{max}$ 258, 272 nm

% Calculated for C$_{17}$H$_{19}$BrN$_6$O$_2$S : C 45.24, H 4.24, N 18.62.

% Found : C 45.11, H 4.20, N 18.73.

4-Nitrophenyl N-(3-ethoxypropyl)carbamate 4.15

3-Ethoxypropylamine (240 µl, 2 mmol) was added to a solution of bis(4-nitrophenyl) carbonate 3.76 (608 mg, 2 mmol) in DCM (5 ml). After stirring at room temperature for 2 hours, the DCM solution was washed with saturated NaHCO$_3$ solution (10 ml x 3), water and brine, dried over MgSO$_4$ and evaporated to give the carbamate 4.15 (365 mg, 68.0%).

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 1.12 (t, 3H, J 7.0, -CH$_3$), 1.71 (m, 2H, 2'-CH$_2$), 3.13 (m, 2H, 1'-CH$_2$), 3.40 (m, 4H, 1", 3'-CH$_2$), 7.39 (d, 2H, J 9.0, 2, 6-CH), 8.03 (broad s, 1H, -NH), 8.25 (d, 2H, J 9.0, 3, 5-CH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 15.0 (-CH$_3$), 29.4 (2'-CH$_2$), 65.8 (1",CH$_2$), 67.7 (1'-CH$_2$), 122.5 (2, 6-CH), 125.3 (3, 5-CH), 144.1 (q, 1-C), 153.4 (C=O), 156.4 (q, 4-C).

$\nu_{max}$ 1594, 1613, 1720 cm$^{-1}$

mp 84-85 °C

O$^6$-(4'-Bromothenyl)-8-[N-(3-ethoxypropyl)carbamoyl]-8-aza-7-deazaguanine 4.17

Hünig’s base (107 µl, 0.6 mmol) was added to alloPaTrin (98 mg, 0.3 mmol) and 4-nitrophenyl N-(3-ethoxypropyl)carbamate 4.15 (160 mg, 0.6 mmol) in DMF (0.3 ml) and the reaction mixture was stirred at room temperature for 48 hours during which time the product precipitated from solution. The DMF was removed leaving a yellow solid, which was washed with diethyl ether, filtered and dried (62 mg, 45.4%).

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 1.11 (t, 3H, J 4.5, 5"-CH$_3$), 1.78 (m, 2H, J 4.5, 2"-CH$_2$), 3.40 (m, 6H, J 4.0, 1", 2", 3"-CH$_2$), 5.67 (s, 2H, O$^6$-CH$_2$), 6.93 (s, 2H, -NH$_2$), 7.40 (s, 1H, 3'-CH), 7.72 (s, 1H, 5'-CH), 8.68 (s, 1H, 7-CH), 8.75 (s, 1H, -NH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 15.1 (5"-CH$_3$), 29.1 (2"-CH$_2$), 38.1 (1"-CH$_2$), 61.4 (O$^6$-CH$_2$), 65.4 (4"-CH$_2$), 67.8 (3'-CH$_2$), 99.7 (q, 5-C), 108.1 (q, 4'-C), 123.3 (7-CH), 164
125.4 (5'-CH), 131.2 (3'-CH), 139.7 (q, 2'-C), 149.3 (C=O), 162.0 (q, 2-C), 163.0 (q, 4-C), 164.5 (q, 6-C).

$\nu_{max}$ 1526, 1619, 1726 cm$^{-1}$

mp 128-130 °C

$\lambda_{max}$ 232, 270, 282 (sh), 318 nm

% Calculated for C$_{16}$H$_{15}$BrN$_6$O$_7$S : C 42.21, H 4.21, N 18.46.

% Found : C 42.09, H 4.21, N 18.31.

### Procedure 4.1

The amine (0.4 mmol) was added to a suspension of $O^6$-(4'-bromothenyl)-9-(4''-nitrophenoxycarbonyl)-8-aza-7-deazaguanine 3.75 (98 mg, 0.2 mmol) in pyridine (2 ml). All the solid dissolved, and the clear solution was stirred at room temperature for 30 minutes. The solid was dissolved leaving a brown residue, which was dissolved in DCM (8 ml). The DCM solution was washed with 2M NaOH (10 ml x 2), water and brine, dried over MgSO$_4$, and evaporated to give the product.

$O^6$-(4'-Bromothenyl)-9-(N-hexylcarbamoyl)-8-aza-7-deazaguanine 4.08

The synthesis of 4.08 was performed according to procedure 4.1, using hexylamine (43 µl, 0.6 mmol) and $O^6$-(4'-bromothenyl)-9-(4''-nitrophenoxycarbonyl)-8-aza-7-deazaguanine 3.75 (147 mg, 0.3 mmol), to give a pale brown solid (105 mg, 77.2%), which was recrystallised twice from acetonitrile.

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 0.86 (m, 3H, 6''-CH$_3$), 1.30 (m, 6H, 3'', 4'', 5''-CH$_2$), 1.57 (m, 2H, 2''-CH$_2$), 3.29 (m, 2H, 2''-CH$_2$), 5.67 (s, 2H, $O^6$-CH$_2$), 7.38 (m, 3H, 3''-CH, -NH$_2$), 7.73 (s, 1H, 5''-CH), 8.05 (s, 1H, 7-CH), 8.80 (t, 1H, J 6.0, -NH).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 13.9 (6''-CH$_3$), 22.0 (5''-CH$_2$), 26.0 (3''-CH$_2$), 29.1 (2'', 4''-CH$_2$), 30.9 (1''-CH$_2$), 61.3 ($O^6$-CH$_2$), 96.7 (q, 5-C), 108.1 (q, 4'-C), 125.5 (5'-CH), 131.3 (3''-CH), 134.2 (7-CH), 139.6 (q, 2'-C), 149.6 (C=O), 158.0 (q, 4'-C), 162.0 (q, 2-C), 162.6 (q, 6-C).

$\nu_{max}$ 1581, 1618, 1723 cm$^{-1}$

mp 150-152 °C

$\lambda_{max}$ 226, 260, 280 (sh) nm

% Calculated for C$_{17}$H$_{23}$BrN$_6$O$_7$S·0.4NaCl : C 43.22, H 4.48, N 17.79.

% Found : C 42.78, H 4.53, N 18.07.
O⁶-(4'-Bromothenyl)-9-[N-(3''-ethoxypropyl)carbamoyl]-8-aza-7-deazaguanine 4.16

The synthesis of 4.16 was performed according to procedure 4.1, using 3-ethoxypropylamine (48 µl, 0.4 mmol) and O⁶-(4'-bromothenyl)-9-(4''-nitrophenoxycarbonyl)-8-aza-7-deazaguanine 3.75 (98 mg, 0.2 mmol), to give a white solid (48 mg, 52.7%), which was recrystallised from acetonitrile.

¹H NMR (400 MHz, DMSO-d₆) δ 1.10 (t, 3H, J 4.5, 5''-CH₃), 1.80 (quin, 2H, J 4.5, 2''-CH₂), 3.42 (m, 6H, J 4.0, 1'', 2'', 3''-CH₂), 5.67 (s, 2H, O⁶-CH₂), 7.38 (m, 3H, -NH₂, 3''-CH), 7.73 (s, 1H, 5'-CH), 8.05 (s, 1H, 7-CH), 8.80 (t, 1H, J 5.5, -NH).

¹³C NMR (100 MHz, DMSO-d₆) δ 15.1 (5''-CH₃), 29.4 (2''-CH₂), 37.4 (1''-CH₂), 61.3 (O⁶-CH₂), 65.4 (4''-CH₂), 67.5 (3''-CH₂), 96.7 (q, 5-C), 108.2 (q, 4'-C), 125.6 (5'-CH), 131.4 (3'-CH), 134.3 (7-CH), 139.6 (q, 2'-C), 149.3 (C=O), 158.0 (q, 4-C), 162.1 (q, 2-C), 162.7 (q, 6-C).

v_max 1579, 1618, 1739 cm⁻¹
mp 144-145 °C
λ_max 228, 262, 278 nm
% Calculated for C₁₆H₁₀BrN₆O₃S : C 42.21, H 4.21, N 18.46.
% Found : C 41.90, H 4.10, N 18.17.

O⁶-(4'-Bromothenyl)-9-(N-cyclohexylcarbamoyl)-8-aza-7-deazaguanine 4.20

The synthesis of 4.20 was performed according to procedure 4.1, using cyclohexylamine (51 µl, 0.4 mmol) and O⁶-(4'-bromothenyl)-9-(4''-nitrophenoxycarbonyl)-8-aza-7-deazaguanine 3.75 (98 mg, 0.2 mmol), to give an off-white solid (72 mg, 80.0%), which was recrystallised from acetonitrile.

¹H NMR (400 MHz, DMSO-d₆) δ 1.22 (m, 1H, 4ax''-CH), 1.39 (m, 4H, 2ax'', 3ax'', 5ax'', 6ax''-CH), 1.61 (m, 1H, 4eq''-CH), 1.73 (m, 2H, 3eq'', 5eq''-CH), 1.94 (m, 2H, 2eq'', 6eq''-CH), 3.69 (m, 1H, 1''-CH), 5.67 (s, 2H, O⁶-CH₂), 7.39 (s, 1H, 3'-CH), 7.44 (s, 2H, -NH₂), 7.73 (s, 1H, 5'-CH), 8.04 (s, 1H, 7-CH), 8.80 (d, 1H, J 8.0, -NH).

¹³C NMR (100 MHz, DMSO-d₆) δ 24.4 (3'', 5''-CH₂), 25.1 (4''-CH₂), 32.4 (2'', 6''-CH₂), 48.8 (1''-CH), 61.4 (O⁶-CH₂), 96.7 (q, 5-C), 108.2 (q, 4'-C), 125.6 (5'-CH), 131.4 (3'-CH), 134.3 (7-CH), 139.6 (q, 2'-C), 148.3 (C=O), 157.9 (q, 4-C), 161.9 (q, 2-C), 162.7 (q, 6-C).

v_max 1584, 1610, 1721 cm⁻¹
mp 200-201 °C
λ_max 262, 278 nm
% Calculated for C<sub>17</sub>H<sub>19</sub>BrN<sub>6</sub>O<sub>2</sub>S : C 45.24, H 4.24, N 18.62.
% Found : C 45.08, H 3.99, N 18.50.

O<sup>6</sup>-(4'-Bromothenyl)-9-(N-(3''-morpholinopropyl)carbamoyl)-8-aza-7-deazaguanine 4.21

The synthesis of 4.21 was performed according to procedure 4.1, using 4-(3-aminopropyl)morpholine (60 µl, 0.4 mmol) and O<sup>6</sup>-(4'-bromothenyl)-9-(4''-nitrophenoxy carbonyl)-8-aza-7-deazaguanine 3.75 (98 mg, 0.2 mmol), to give a white solid (51 mg, 51.4%), which was recrystallised from acetonitrile.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 1.73 (quin, 2H, J 7.0, 2''-CH<sub>2</sub>), 2.35 (m, 4H, 2''-CH<sub>2</sub>), 3.39 (m, 4H, 3''-CH<sub>2</sub>), 3.55 (m, 4H, 1''-CH<sub>2</sub>), 5.67 (s, 2H, O<sup>6</sup>-CH<sub>2</sub>), 7.39 (s, 3H, 3''-CH<sub>2</sub>), 7.73 (s, 1H, 5''-CH<sub>2</sub>), 8.06 (s, 1H, 7-CH), 8.83 (t, 1H, J 5.5, -NH).  

<sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 25.9 (2''-CH<sub>2</sub>), 38.4 (1''-CH<sub>2</sub>), 53.3 (2''-CH<sub>2</sub>), 56.0 (3''-CH<sub>2</sub>), 61.3 (O<sup>6</sup>-CH<sub>2</sub>), 66.2 (3'', 5''-CH<sub>2</sub>), 96.7 (q, 5-C), 108.2 (q, 4'-C), 125.6 (5''-CH), 131.3 (3''-CH), 134.3 (7-CH), 139.7 (q, 2'-C), 149.3 (C=O), 158.1 (q, 4-C), 162.1 (q, 2-C), 162.7 (q, 6-C).

ν<sub>max</sub> 1570, 1611, 1727 cm<sup>-1</sup>  
mp 193-195 °C  
λ<sub>max</sub> 230, 262, 280 nm

% Calculated for C<sub>18</sub>H<sub>22</sub>BrN<sub>7</sub>O<sub>3</sub>S : C 43.55, H 4.47, N 19.75.  
% Found : C 43.80, H 4.40, N 19.54.

O<sup>6</sup>-(4'-Bromothenyl)-9-(N-phenylcarbamoyl)-8-aza-7-deazaguanine 4.23

Aniline (36 µl, 0.4 mmol) was added to a suspension of O<sup>6</sup>-(4'-bromothenyl)-9-(4''-nitrophenoxy carbonyl)-8-aza-7-deazaguanine 3.75 (98 mg, 0.2 mmol) in pyridine (2 ml). The mixture was stirred at room temperature overnight, during which the mixture became clear. The pyridine was removed leaving a cream-coloured solid, which was washed with DCM, filtered and dried (76 mg, 85%).

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 5.70 (s, 2H, O<sup>6</sup>-CH<sub>2</sub>), 7.16 (t, 1H, J 7.3, 4''-CH), 7.40 (m, 3H, 3''-CH, 3'', 5''-CH), 7.59 (broad s, 2H, -NH<sub>2</sub>), 7.78 (m, 3H, 5''-CH, 2'', 6''-CH), 8.16 (s, 1H, 7-CH), 11.17 (s, 1H, -NH).

<sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 61.5 (O<sup>6</sup>-CH<sub>2</sub>), 96.8 (q, 5-C), 108.2 (q, 4'-C), 119.8 (3'', 5''-CH), 124.1 (4''-CH), 125.6 (5''-CH), 129.0 (2'', 6''-CH), 131.5 (3''-CH), 135.1 (3'-CH), 135.1
(7-CH), 137.6 (q, 1'-C), 139.5 (q, 2'-C), 146.6 (C = O) 158.0 (q, 4-C), 161.9 (q, 2-C), 162.8 (q, 6-C).

$\nu_{max}$ 1600, 1731 cm$^{-1}$

mp 200-202 °C

$\lambda_{max}$ 212, 252, 268 (sh), 278 (sh) nm

% Calculated for C$_{17}$H$_{13}$BrN$_{6}$O$_{5}$S : C 45.85, H 2.94, N 18.87.

% Found : C 45.68, H 2.86, N 18.79.

O$^6$-(4'-Bromothenyl)-9-[N-(3''-morpholinopropyl)carbamoyl]-8-aza-7-deazaguanine hydrochloride 4.22

1M HCl (70 µl, 0.07 mmol) was added to a solution of O$^6$-(4'-bromothenyl)-9-[N-(3''-morpholinopropyl)carbamoyl]-8-aza-7-deazaguanine 4.21 (32 mg, 0.07 mmol) in methanol (10 ml). After 2 hours stirring at room temperature, the methanol was removed leaving a "glass", which was crystallised from a 50/50 mixture of acetonitrile and diethyl ether to give the hydrochloride (14 mg, 37.5%).

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.02 (m, 2H, 2''-CH$_2$), 3.06 (m, 4H, 2'', 6''-CH$_2$), 3.43 (m, 4H, 3'', 5''-CH$_2$), 3.75 (t, 2H, J 11.7, 3''-CH$_2$), 3.94 (m, 2H, 1''-CH$_2$), 5.67 (s, 2H, O$^6$-CH$_2$), 7.43 (s, 3H, 3'-CH, -NH$_2$), 7.74 (s, 1H, 5'-CH), 8.08 (s, 1H, 7-CH), 8.92 (m, 1H, -NH), 10.62 (broad s, 1H, -HCl).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 23.4 (2''-CH$_2$), 37.0 (1''-CH$_2$), 51.0 (2'', 6''-CH$_2$), 53.7 (3''-CH$_2$), 61.3 (O$^6$-CH$_2$), 63.2 (3'', 5''-CH$_2$), 96.7 (q, 5-C), 108.2 (q, 4'-C), 125.6 (5'-CH), 131.4 (3'-CH), 134.5 (7-CH), 139.6 (q, 2'-C), 149.5 (C = O), 158.2 (q, 4-C), 162.2 (q, 2-C), 162.7 (q, 6-C).

$\nu_{max}$ 1614, 1718 cm$^{-1}$

mp decomposes with evolution of gas at 100 °C

$\lambda_{max}$ 226, 262, 278 (sh) nm

% Calculated for C$_{18}$H$_{23}$BrClN$_7$O$_5$S·0.5H$_2$O : C 39.89, H 4.46, N 18.09.

% Found : C 39.90, H 4.38, N 18.57.

Procedure 4.2

A dialkylcarbamyl chloride (0.3 mmol) was added to alloPaTrin (98 mg, 0.3 mmol) and DBU (90 µl, 0.6 mmol) in DMF (0.4 ml). The reaction mixture was stirred at room temperature for 18 hours. Removal of DMF left a brown residue, from which the product was crystallised.
**O^6-(4'-Bromothenyl)-8-(N, N-dimethylcarbamoyl)-8-aza-7-deazaguanine 4.25a**

The synthesis of **4.25a** was performed according to procedure 4.2, using dimethylcarbamyl chloride (37 µl, 0.4 mmol), DBU (60 µl, 0.4 mmol) and alloPaTrin (65 mg, 0.2 mmol), to give a cream coloured solid (36 mg, 45.0%), which was recrystallised from acetonitrile.

`1H NMR (400 MHz, DMSO-d6) δ 3.09 (s, 6H, -CH3), 5.67 (s, H, O^6-CH2), 6.85 (s, 2H, -NH2), 7.39 (s, 1H, 3'-CH), 7.72 (s, 1H, 5'-CH), 8.61 (s, 1H, 7-CH).`

`13C NMR (100 MHz, DMSO-d6) δ 38.2 (CH3), 39.8 (CH3), 61.2 (O^6-CH2), 98.6 (q, 5'-C), 108.1 (q, 4'-C), 125.5 (5'-CH), 126.3 (7-CH), 131.3 (3'-CH), 139.7 (q, 2'-C), 151.7 (C=O), 161.7 (q, 2-C), 162.7 (q, 4-C), 164.3 (q, 6-C).`

v_max 1544, 1639, 1702 cm^-1
mp 170-172 °C
λ_max 214, 230, 258 (sh), 278 (sh), 314 nm
% Found : C 39.21, H 3.43, N 21.06.

**O^6-(4'-Bromothenyl)-8-(N, N-phenylcarbamoyl)-8-aza-7-deazaguanine 4.25b**

The synthesis of **4.25b** was performed according to procedure 4.2, using diphenylcarbamyl chloride (93 mg, 0.4 mmol), DBU (60 µl, 0.4 mmol) and alloPaTrin (65 mg, 0.2 mmol), to give a yellow coloured solid (51 mg, 48.9%), which was recrystallised from acetonitrile.

`1H NMR (400 MHz, DMSO-d6) δ 5.63 (s, H, O^6-CH2), 6.83 (s, 2H, -NH2), 7.39 (m, 11H, 3'-CH, phenyl CH), 7.73 (s, 1H, 5'-CH), 8.63 (s, 1H, 7-CH).`

`13C NMR (100 MHz, DMSO-d6) δ 61.3 (O^6-CH2), 99.0 (q, 5-C), 108.2 (q, 4'-C), 125.6 (5'-CH), 126.6 (2'', 6''-CH), 126.8 (7-CH), 127.0 (4''-CH), 129.3 (3'', 5''-CH), 131.4 (3'-CH), 139.6 (q, 2'-C), 142.8 (q, 1''-C), 151.0 (C=O), 161.6 (q, 2-C), 162.4 (q, 4-C), 164.2 (q, 6-C).`

v_max 1545, 1650, 1706 cm^-1
mp 195-198 °C
λ_max 212, 230, 262 (sh), 324nm
% Calculated for C23H17BrN6O2S·0.33H2O : C 52.44, H 3.37, N 15.95.
% Found : C 52.35, H 3.31, N 15.63.
O^-{(4'-Bromothenyl)-8-(morpholinocarbonyl)-8-aza-7-deazaguanine 4.25c

The synthesis of 4.25c was performed according to procedure 4.2, using 4-morpholinocarbonyl chloride (70 μl, 0.6 mmol), DBU (60 μl, 0.4 mmol) and alloPaTrin (98 mg, 0.3 mmol), to give a cream coloured solid (84 mg, 63.8%), which was recrystallised from acetonitrile.

\[ \text{H NMR (400 MHz, DMSO-d}_6] \delta 3.68 (m, 8H, 2", 3", 5", 6"-CH}_2), 5.68 (s, 2H, O^-CH}_2), 6.84 (s, 2H, -NH}_2), 7.39 (s, 1H, 3"-CH), 7.72 (s, 1H, 5"-CH), 8.64 (s, 1H, 7-CH).

\[ \text{C NMR (100 MHz, DMSO-d}_6] \delta 45.1 (2" or 6"-CH}_2), 47.3 (2" or 6"-CH}_2), 56.5 (3" or 5"-CH}_2), 61.3 (O^-CH}_2), 65.9 (3" or 5"-CH}_2), 98.8 (q, 5-CH), 108.1 (q, 4'-CH), 125.5 (5'-CH), 126.8 (7-CH), 131.3 (3'-CH), 139.7 (q, 2'-CH), 150.6 (C=O), 161.8 (q, 2-C), 162.9 (q, 4-C), 164.3 (q, 6-C).

\[ \nu_{\text{max}} 1545, 1644, 1645 \text{ cm}^{-1}

mp 144 °C

\[ \text{C}_{15}\text{H}_{13}\text{BrN}_6\text{O}_5\text{S} \cdot 0.2\text{H}_2\text{O} : \text{C} 40.68, \text{H} 3.51, \text{N} 18.98.

\% Found : C 40.47, H 3.38, N 19.18.

O^-{(4'-Bromothenyl)-8-(N-pyrrolidinocarbamoyl)-8-aza-7-deazaguanine 4.25d

The synthesis of 4.25d was performed according to procedure 4.2, using 1-pyrrolidinecarbonyl chloride (44 μl, 0.4 mmol), DBU (60 μl, 0.4 mmol) and alloPaTrin (65 mg, 0.2 mmol), to give a cream coloured solid (62 mg, 73%), which was recrystallised from acetonitrile.

\[ \text{H NMR (400 MHz, DMSO-d}_6] \delta 1.87-1.93 (m, 4H, 3", 4"-CH}_2), 3.54-3.81 (m, 4H, 2", 5"-CH}_2), 5.67 (s, h, O^-CH}_2), 6.86 (s, 2H, -NH}_2), 7.40 (s, 1H, 3"-CH), 7.72 (s, 1H, 5"-CH), 8.65 (s, 1H, 7-CH).

\[ \text{C NMR (100 MHz, DMSO-d}_6] \delta 23.5 (3" or 4"-CH}_2), 26.1 (3" or 4"-CH}_2), 48.5 (2" or 5"-CH}_2), 49.8 (2" or 5"-CH}_2), 61.3 (O^-CH}_2), 98.6 (q, 5-CH), 108.1 (q, 4'-CH), 125.5 (5'-CH), 125.6 (7-CH), 131.3 (3'-CH), 139.7 (q, 2'-CH), 149.2 (C=O), 161.7 (q, 2-C), 162.8 (q, 4-C), 164.4 (q, 6-C).

\[ \nu_{\text{max}} 1542, 1642, 1677 \text{ cm}^{-1}

mp 188-190 °C

\[ \lambda_{\text{max}} 228, 258 (\text{sh}), 280 (\text{sh}), 318\text{nm}.

\% Calculated for C_{15}\text{H}_{13}\text{BrN}_6\text{O}_2\text{S} \cdot 0.4\text{H}_2\text{O} : \text{C} 41.85, \text{H} 3.70, \text{N} 19.52.

\% Found : C 42.13, H 3.67, N 19.06.
O\(^6\)-(4'-Bromothenyl)-9-(4''-methyl-1''-piperazinylcarbonyl)-8-aza-7-deazaguanine

4.30a

The synthesis of 4.30a was performed according to procedure 4.1, using 1-methylpiperazine (36 µl, 0.4 mmol) and O\(^6\)-(4'-bromothenyl)-9-(4''-nitrophenoxycarbonyl)-8-aza-7-deazaguanine 3.75 (196 mg, 0.4 mmol), to give a cream coloured solid (81 mg, 44.8%). The crude product was recrystallised from acetonitrile.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 2.21 (s, 3H, -CH\(_3\)), 2.30-2.40 (m, 4H, 3", 5"-CH\(_2\)), 3.56-3.63 (m, 4H, 2", 6"-CH\(_2\)), 5.66 (s, 2H, O\(^6\)-CH\(_2\)), 7.18 (s, 2H, -NH\(_2\)), 7.39 (s, 1H, 3'-CH), 7.72 (s, 1H, 5'-CH), 8.04 (s, 1H, 7-CH).

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 44.2 (2" or 6"-CH\(_2\)), 47.3 (2" or 6"-CH\(_2\)), 56.6 (3" or 5"-CH\(_2\)), 61.1 (O\(^6\)-CH\(_2\)), 65.9 (3" or 5"-CH\(_2\)), 95.8 (q, 5-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 131.3 (3'-CH), 134.3 (7-CH), 139.8 (q, 2'-C), 149.5 (C =O), 159.0 (q, 4-C), 162.4 (q, 2-C), 162.5 (q, 6-C).

\(\nu\)\textsubscript{max} 1569, 1625, 1706 cm\(^{-1}\)

mp 196-198 °C

% Calculated for C\(_{16}\)H\(_{18}\)BrN\(_7\)O\(_2\)S: C 42.48, H 4.01, N 21.68.

% Found: C 42.18, H 3.99, N 21.63.

O\(^6\)-(4'-Bromothenyl)-9-(N-morpholinocarbamoyl)-8-aza-7-deazaguanine 4.30b

The synthesis of 4.30b was performed according to procedure 4.1, using morpholine (35 µl, 0.4 mmol) and O\(^6\)-(4'-bromothenyl)-9-(4''-nitrophenoxycarbonyl)-8-aza-7-deazaguanine 3.75 (98 mg, 0.2 mmol), to give a white solid (59 mg, 67.1%), which was recrystallised from acetonitrile.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 2.21 (s, 3H, -CH\(_3\)), 2.30-2.40 (m, 4H, 3", 5"-CH\(_2\)), 3.56-3.63 (m, 4H, 2", 6"-CH\(_2\)), 5.66 (s, 2H, O\(^6\)-CH\(_2\)), 7.18 (s, 2H, -NH\(_2\)), 7.39 (s, 1H, 3'-CH), 7.72 (s, 1H, 5'-CH), 8.04 (s, 1H, 7-CH).

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 61.0 (O\(^6\)-CH\(_2\)), 61.7 (N-CH\(_2\)Ph), 95.8 (q, 5-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 131.3 (3'-CH), 134.0 (7-CH), 139.8 (q, 2'-C), 149.8 (C =O), 159.8 (q, 4-C), 162.4 (q, 2-C), 164.5 (q, 6-C).

\(\nu\)\textsubscript{max} 1574, 1647, 1713 cm\(^{-1}\)

mp 178-180 °C

% Calculated for C\(_{15}\)H\(_{15}\)BrN\(_6\)O\(_3\)S-0.5NaCl: C 38.45, H 3.23, N 17.94.
% Found : C 38.45, H 3.12, N 17.80.

9-(4"-Benzyl-1"'-piperazinylcarbonyl)-O^6-(4'-bromothenyl)-8-aza-7-deazaguanine 4.30c

The synthesis of 4.30c was performed according to procedure 4.1, using 1-benzylpiperazine (70 μl, 0.4 mmol) and O^6-(4'-bromothenyl)-9-(4"-nitrophenoxycarbonyl)-8-aza-7-deazaguanine 3.75 (98 mg, 0.2 mmol), to give a yellow oil, which was crystallised from EtOAc/hexane to give the product as a white solid (64 mg, 60.6%). The crude product was recrystallised from acetonitrile.

^1H NMR (400 MHz, DMSO-d_6) δ 2.41-2.46 (m, 4H, 3", 5"-CH_2), 3.34 (m, 2H, 6"-CH_2), 3.52 (s, 2H, N-CH_2Ph), 3.62 (m, 2H, 2"-CH_2), 5.65 (s, 2H, O^6-CH_2), 7.18-7.38 (m, 8H, -NH_2, 2", 3", 4", 5", 6"-CH, 3'-CH), 7.72 (s, 1H, 5'-CH), 8.04 (s, 1H, 7-CH).

^13C NMR (100 MHz, DMSO-d_6) δ 43.8 (2"-CH_2), 46.9 (6"-CH_2), 51.9 (3" or 5"-CH_2), 52.6 (3" or 5"-CH_2), 61.0 (O^6-CH_2), 61.7 (N-CH_2Ph), 95.8 (q, 5-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 127.1 (4"-CH), 128.2 (3", 5"-CH), 128.9 (2", 6"-CH), 131.3 (3'-CH), 134.0 (7-CH), 137.7 (q, 1'-C), 139.8 (q, 2'-C), 149.8 (C=O), 158.9 (q, 4-C), 162.4 (q, 2-C), 164.5 (q, 6-C).

v_max 1572, 1607, 1622, 1692 cm⁻¹

mp 155-156 °C

λ_max 210, 224, 258, 280 (sh) nm

% Calculated for C_{22}H_{22}BrN_{7}O_{2}S : C 50.01, H 4.20, N 18.55.

% Found : C 49.72, H 4.14, N 18.44.

O^6-(4'-Bromothenyl)-9-[4"-(N-piperidino)piperidinocarbonyl]-8-aza-7-deazaguanine 4.30d

The synthesis of 4.30d was performed according to procedure 4.1, using 90% 4-piperidinopiperidine (37 mg, 0.2 mmol) and O^6-(4'-bromothenyl)-9-(4"-nitrophenoxycarbonyl)-8-aza-7-deazaguanine 3.75 (98 mg, 0.2 mmol), to give a yellow oil, which was crystallised from EtOAc/hexane to give the product as a white solid (92 mg, 88.4%). The crude product was recrystallised from acetonitrile.

^1H NMR (400 MHz, DMSO-d_6) δ 1.37 (m, 2H, 4"-CH_2), 1.48 (m, 4H, 3", 5"-CH_2), 1.69 (m, 2H, 3'_ax", 5'_ax"-CH), 1.83 (m, 2H, 3'_eq", 5'_eq"-CH), 2.45 (m, 5H, 4"-CH, 2", 6"-CH_2), 3.00 (m, 2H, 2'_ax", 6'_ax"-CH), 3.54 (s, 1H, 2'_eq"-CH), 4.28 (m, 1H, 6'_eq"-CH), 5.66
(s, 2H, O<sup>δ</sup>-CH<sub>2</sub>), 7.17 (s, 2H, -NH<sub>2</sub>), 7.39 (s, 1H, 3'-CH), 7.72 (s, 1H, 5'-CH), 8.03 (s, 1H, 7-CH).

<sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 24.5 (4''-CH<sub>2</sub>), 26.0 (3'', 5''-CH<sub>2</sub>), 27.2 (3'' or 5''-CH<sub>2</sub>), 28.0 (3'' or 5''-CH<sub>2</sub>), 43.8 (6''-CH<sub>2</sub>), 46.4 (2''-CH<sub>2</sub>), 49.6 (2'', 6''-CH<sub>2</sub>), 61.0 (O<sup>δ</sup>-CH<sub>2</sub>), 61.2 (4''-CH), 95.7 (q, 5'-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 131.3 (3'-CH), 133.8 (7'-CH), 139.8 (q, 2'-C), 149.7 (C=O), 158.8 (q, 4'-C), 162.4 (q, 2'-C), 162.5 (q, 6'-C).

ν<sub>max</sub> 1572, 1609, 1640, 1705 cm<sup>-1</sup>

mp 204-206 °C

λ<sub>max</sub> 212, 230, 258, 278 (sh) nm

% Calculated for C<sub>29</sub>H<sub>26</sub>BrN<sub>7</sub>O<sub>2</sub>S·0.25NaCl : C 47.14, H 4.90, N 18.32.

% Found : C 47.17, H 4.80, N 18.34.

O<sup>δ</sup>-[4''-(4'-Bromothenyl)][4''-(N-piperidino)piperidinocarbonyl]-8-aza-7-deazaguanine hydrochloride 4.31

1M HCl (70 μl, 0.07 mmol) was added to a solution of O<sup>δ</sup>-[4''-(4'-bromothenyl)][4''-(N-piperidino)piperidinocarbonyl]-8-aza-7-deazaguanine 4.30d (36 mg, 0.07 mmol) in methanol (10 ml). After 2 hours stirring at room temperature, the methanol was removed leaving a “glass”, which was crystallised from diethyl ether to give the hydrochloride (14 mg, 35.5%).

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 1.39 (m, 2H, 4''-CH<sub>2</sub>), 1.48 (m, 4H, 3'', 5''-CH<sub>2</sub>), 1.69 (m, 2H, 3<sub>ax</sub>'', 5<sub>ax</sub>''-CH), 1.83 (m, 2H, 3<sub:eq</sub>'', 5<sub:eq</sub>''-CH), 2.45 (m, 5H, 4''-CH<sub>2</sub>, 2'', 6''-CH<sub>2</sub>), 2.99 (m, 2H, 2<sub>ax</sub>'', 6<sub>ax</sub>''-CH), 3.54 (s, 1H, 2<sub:eq</sub>''-CH), 4.28 (m, 1H, 6<sub:eq</sub>''-CH), 5.66 (s, 2H, O<sup>δ</sup>-CH<sub>2</sub>), 7.20 (s, 2H, -NH<sub>2</sub>), 7.39 (s, 1H, 3'-CH), 7.73 (s, 1H, 5'-CH), 8.07 (s, 1H, 7-CH), 9.76 (s, 1H, -HCl).

<sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 24.5 (4''-CH<sub>2</sub>), 26.0 (3'', 5''-CH<sub>2</sub>), 27.2 (3'' or 5''-CH<sub>2</sub>), 28.0 (3'' or 5''-CH<sub>2</sub>), 43.8 (6''-CH<sub>2</sub>), 46.4 (2''-CH<sub>2</sub>), 49.6 (2'', 6''-CH<sub>2</sub>), 61.0 (O<sup>δ</sup>-CH<sub>2</sub>), 61.2 (4''-CH), 95.8 (q, 5'-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 131.3 (3'-CH), 134.3 (7'-CH), 139.8 (q, 2'-C), 149.6 (C=O), 159.0 (q, 4'-C), 162.4 (q, 2'-C), 162.5 (q, 6'-C).

ν<sub>max</sub> 1570, 1607, 1643, 1707 cm<sup>-1</sup>

mp 260 °C (decomp.)

λ<sub>max</sub> 212, 228, 258, 278 (sh) nm

% Calculated for C<sub>29</sub>H<sub>28</sub>BrClN<sub>7</sub>O<sub>2</sub>S·0.5H<sub>2</sub>O : C 44.57, H 4.99, N 17.32.

% Found : C 44.54, H 4.87, N 17.03.
9-(4'-Benzyl-1''-piperazinylcarbonyl)-O^6-(4'-bromothenyl)-8-aza-7-deazaguanine citrate 4.32

Citric acid (10 mg, 0.053 mmol) was added to a solution 9-(4''-benzyl-1''-piperazinylcarbonyl)-O^6-(4'-bromothenyl)-8-aza-7-deazaguanine 4.30c (28 mg, 0.053 mmol) in methanol (10 ml). After two hours at room temperature, the methanol was removed leaving a “glass” which was crystallised from a mixture of acetonitrile and diethyl ether to give the citrate salt as a white solid (30 mg, 78.6%).

^1H NMR (400 MHz, DMSO-d6) δ 2.41 (m, 6H, 3'', 5''-CH2, 2ax'', 6ax''-CH), 2.70 (m, 4H, citric acid-CH2), 3.52 (s, 2H, N-CH2Ph), 3.62 (m, 2H, 2eq'', 6eq''-CH), 5.65 (s, 2H, O^6-CH2), 7.18-7.38 (m, 8H, -NH2, 2'', 3'', 4'', 5'', 6''-CH, 3'-CH), 7.72 (s, 1H, 5'-CH), 8.04 (s, 1H, 7-CH), 12.12 (broad s, 1H, -OH).

^13C NMR (100 MHz, DMSO-d6) δ 43.0 (citric acid-CH2), 43.7 (2''-CH2), 46.7 (6''-CH2), 51.8 (3''-CH2), 52.5 (5''-CH2), 61.1 (O^6-CH2), 61.6 (N-CH2Ph), 95.8 (q, 5-C), 108.2 (q, 4'-C), 125.5 (5'-CH), 127.2 (4'-CH), 128.3 (3''', 5'''-CH), 129.0 (2'''', 6'''-CH), 131.3 (3'-CH), 134.0 (7-CH), 137.7 (q, 1'''-C), 139.8 (q, 2'''-C), 149.8 (C=O), 158.9 (q, 4-C), 162.4 (q, 2-C), 164.5 (q, 6-C), 171.3 (citric acid-COOH), 175.9 (citric acid-COO-).

υmax 1575, 1613, 1705 cm^-1

mp 128-132 °C (decomp.)

λmax 212, 232, 258, 276 (sh) nm

% calculated for C28H30BrN7O9S : C 46.67, H 4.20, N 13.61.

% found : C 46.69, H 4.28, N 13.98.
4.11 References


Chapter 5

Biological Activity and Stability Studies
5.1 Introduction
Thus far, we have discussed the synthesis of numerous potential inhibitors of ATase, whose structure is based on the 8-aza-7-deazaguanine ring system. The ability of each of these alloPaTrin derivatives to inhibit ATase has been determined by our collaborators, Dr. Geoffrey Margison and his co-workers, who are based in the Paterson Institute for Cancer Research, which is part of the Christie Hospital Trust in Manchester.

5.2 Determination of the Inactivating Ability \( I_{50} \) of an ATase Inhibitor
Researchers in the Paterson Institute have adopted a method, which was first described by Myrnes et al., to determine the inactivating ability of a potential ATase inhibitor. The method is based on the premise that ATase accepts an alkyl group from alkylated DNA or an alkylated pseudosubstrate irreversibly.

5.2.1 Outline of ATase Assay
Radiolabelled \(^{3}H\)-methyl DNA is added to a buffer solution containing ATase. This mixture is incubated at pH 8.3 and 37 °C for one hour, during which time the radiolabelled methyl groups are transferred from the DNA to a cysteine residue of ATase. After one hour, all the cysteine residues of ATase should be methylated. The \(^{3}H\)-methylated ATase is recovered and quantified.
The remaining $[^3]H$-methyl DNA is hydrolysed by acid and the subsequent radioactivity, which has been released into solution, can be measured. This represents the amount of methylated DNA, which ATase has not reacted with. The $[^3]H$-methylated ATase is also determined.

5.2.2 Outline of Assay to Determine the $I_{50}$ of an Inactivator

The $I_{50}$ value of an ATase inactivator is determined through a similar assay, in which, initially, the inactivator reacts with excess ATase. After a fixed period, $[^3]H$-methylated DNA is allowed to react with any remaining ATase. The assay procedure is similar to that of the original ATase assay. The difference is that the inhibitor and ATase are incubated together initially at 37 °C (pH 8.3) for one hour, during which time alkyl transfer can take place. Radiolabelled methylated DNA is then added and incubation is continued for another hour. The $[^3]H$-methylated DNA reacts with the remaining ATase which has not been inactivated by the pseudosubstrate. The amount of ATase which has reacted with the methylated DNA can be measured. As the total amount of ATase used in the experiment is known, the amount of ATase which has reacted with the inactivator can be calculated by subtracting the amount of $[^3]H$-
methyl ATase produced in the experiment from the amount of ATase used in the experiment. The rationale is similar to that of a back-titration.

The I_{50} value is determined by plotting the percentage of ATase which has not reacted with the inactivator versus the inactivator concentration (μM).

**Figure 5.2 Assay to Establish I_{50} of Inactivator**
Figure 5.3 Inactivation of ATase by $O^6$-(4'-bromothenyl)-8-(morpholinocarbonyl)-8-aza-7-deazaguanine 4.25c ($I_{50}$ 0.03 μM)

5.3 $I_{50}$ Values of AlloPaTrin Derivatives

The following tables show the $I_{50}$ values of the potential ATase inhibitors, whose syntheses were discussed in this report. The $I_{50}$ value of the corresponding PaTrin-2 derivative is also given, where available.
<table>
<thead>
<tr>
<th>R</th>
<th>$I_{50} (\mu M)$</th>
<th>$I_{50} (\mu M)$ of 9-substituted PaTrin-2 derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.29</td>
<td>-(CH$_2$)$_3$OPh</td>
<td>0.06</td>
</tr>
<tr>
<td>2.30</td>
<td>-(CH$_2$)$_3$OPh$^a$</td>
<td>0.25</td>
</tr>
<tr>
<td>2.31</td>
<td>-(CH$_2$)$_3$OPh$^b$</td>
<td>0.14</td>
</tr>
<tr>
<td>2.32</td>
<td>-(CH$_2$)$_3$OPh$^c$</td>
<td>0.04</td>
</tr>
<tr>
<td>2.33</td>
<td><img src="image1" alt="Image" /></td>
<td>0.013</td>
</tr>
<tr>
<td>2.34</td>
<td><img src="image2" alt="Image" /></td>
<td>0.135</td>
</tr>
<tr>
<td>2.55</td>
<td>-(CH$_2$)$_3$OPh$^d$</td>
<td>0.86</td>
</tr>
<tr>
<td>2.56</td>
<td>-(CH$_2$)$_3$OPh$^{a,d}$</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Table 5. 1 $I_{50}$ Values of 9-Alkyl Derivatives of AlloPaTrin and PaTrin-2

$^a$ 4-Bromothenyl is replaced by benzyl

$^b$ 4-Bromothenyl is replaced by piperonyl

$^c$ 4-Bromothenyl is replaced by thenyl

$^d$ 2-NH$_2$ of alloPaTrin replaced by -H
<table>
<thead>
<tr>
<th>R</th>
<th>I_{50} (µM)</th>
<th>I_{50} (µM) of 9-substituted PaTrin-2 derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>-H (alloPaTrin)</td>
<td>0.007</td>
<td>0.0034</td>
</tr>
<tr>
<td>3.38 9-COOCH₃</td>
<td>0.013</td>
<td>0.0048</td>
</tr>
<tr>
<td>3.38a 9-COOCH₃</td>
<td>0.042</td>
<td>0.15</td>
</tr>
<tr>
<td>3.44 8-COOCH₂CH₂CH₃</td>
<td>0.003</td>
<td>-</td>
</tr>
<tr>
<td>3.45 9-COOCH₂CH₂CH₃</td>
<td>0.006</td>
<td>0.0036</td>
</tr>
<tr>
<td>3.58 8-COOPh</td>
<td>0.0018</td>
<td>-</td>
</tr>
<tr>
<td>3.59 9-COOPh</td>
<td>0.004</td>
<td>0.005</td>
</tr>
<tr>
<td>3.77a 9-COOCH₂OPh</td>
<td>0.006</td>
<td>0.007</td>
</tr>
<tr>
<td>3.77b 9-COO</td>
<td>0.057</td>
<td>0.007</td>
</tr>
<tr>
<td>3.77c 9-COO</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td>3.77d 9-COO</td>
<td>0.003</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 5. 2 I_{50} Values of Alkoxycarbonyl of AlloPaTrin and PaTrin-2

^4-Bromothenyl is replaced by benzyl
9-substituted derivative 8-substituted derivative 9-substituted PaTrin-2 derivative

<table>
<thead>
<tr>
<th>R</th>
<th>$I_{50}$ (µM)</th>
<th>$I_{50}$ (µM) of 9-substituted PaTrin-2 derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>-H (alloPaTrin)</td>
<td>0.007</td>
<td>0.0034</td>
</tr>
<tr>
<td>2-NHCONH(CH$_2$)$_3$CH$_3$</td>
<td>0.32</td>
<td>-</td>
</tr>
<tr>
<td>2-NHCONH</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>8-CNH(CH$_2$)OEt</td>
<td>0.0041</td>
<td>-</td>
</tr>
<tr>
<td>9-CNH(CH$_2$)OEt</td>
<td>0.034</td>
<td>0.03</td>
</tr>
<tr>
<td>9-CNH(CH$_2$)$_3$CH$_3$</td>
<td>0.034</td>
<td>0.013</td>
</tr>
<tr>
<td>9-CNH</td>
<td>0.059</td>
<td>0.095</td>
</tr>
<tr>
<td>9-CNHPh</td>
<td>0.022</td>
<td>-</td>
</tr>
<tr>
<td>9-CNH.HCl</td>
<td>0.052</td>
<td>0.015</td>
</tr>
<tr>
<td>9-CNHPh</td>
<td>0.006</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5. $I_{50}$ Values of Alkylcarbamoyl Derivatives of AlloPaTrin and PaTrin-2

* substitution at 2-NH$_2$
<table>
<thead>
<tr>
<th>R</th>
<th>$I_{50}$ (μM)</th>
<th>$I_{50}$ (μM) of 9-substituted PaTrin-2 derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>-H (alloPaTrin)</td>
<td>0.007</td>
<td>0.0034</td>
</tr>
<tr>
<td>4.25a 8-CO(\text{N}(\text{CH}_3)_2)</td>
<td>0.014</td>
<td>0.05</td>
</tr>
<tr>
<td>4.25b 8-CO(\text{NPh}_2)</td>
<td>0.006</td>
<td>0.07</td>
</tr>
<tr>
<td>4.25c 8-CO(\text{N} \quad )</td>
<td>0.03</td>
<td>0.2</td>
</tr>
<tr>
<td>4.25d 8-CO(\text{N} \quad )</td>
<td>0.01</td>
<td>0.5</td>
</tr>
<tr>
<td>4.30a 9-CO(\text{N} \quad \text{CH}_3)</td>
<td>0.022</td>
<td>0.05</td>
</tr>
<tr>
<td>4.30b 9-CO(\text{N} \quad \text{O} \quad )</td>
<td>0.096</td>
<td>0.2</td>
</tr>
<tr>
<td>4.30c 9-CO(\text{N} \quad \text{CH}_2\text{Ph} \quad )</td>
<td>0.026</td>
<td>0.28</td>
</tr>
<tr>
<td>4.32 9-CO(\text{N} \quad \text{CH}_2\text{Ph} \quad \text{citrate} \quad )</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>
4.30d | 9-CO-N-N | 0.045 | 0.27\textsuperscript{f}  
4.31 | 9-CO-N-N.HCl | 0.03 | -  

Table 5. 4 (cont.) I\textsubscript{50} Values of Di-Alkylcarbamoyl Derivatives of AlloPaTrin and PaTrin-2

5.4 Analysis of I\textsubscript{50} Values

Table 5.1 shows the I\textsubscript{50} values (in \(\mu\text{M}\)) of 8-aza-7-deazaguanines and the corresponding guanine compounds with different alkyl groups in the 9-position. Thus far, no clear pattern has emerged in the comparison the two sets of values. However, it is encouraging that 8-aza-7-deazaguanines appear to be efficient inactivators of ATase.

Both the 8-aza-7-deazapurine derivatives, 2.55 and 2.56, have very high I\textsubscript{50} values compared to the I\textsubscript{50} values of the corresponding 8-aza-7-deazaguanine compounds. As the only difference between the two sets of compounds is the presence of a proton or an amino group in the two position of the heterocyclic skeleton, these results confirm that a free amino group in the 2-position of an alloPaTrin derivative is required for efficient inactivation of ATase.

The I\textsubscript{50} values of alloPaTrin and PaTrin-2 are 0.007 \(\mu\text{M}\) and 0.0034 \(\mu\text{M}\) respectively. While the I\textsubscript{50} values of the alkoxycarbonyl derivatives of PaTrin-2 are comparable with that of PaTrin-2, many of the corresponding alloPaTrin derivatives appear to be more efficient inactivators of ATase than their parent compound, alloPaTrin (Table 5.2). This would suggest that the observed I\textsubscript{50} value of the acylated compound is due to the ATase inactivating ability of the actual derivative and not to alloPaTrin, which potentially could be formed during the assay experiment. The alkoxycarbonyl series

\textsuperscript{f} I\textsubscript{50} of MeI salt of N-(piperidino)piperidinocarbonyl derivative of PaTrin-2
of compounds contain two pairs of isomers, the 8- and 9-propyloxy carbonyl derivatives, 3.44 and 3.45, and the 8- and 9-phenoxycarbonyl compounds, 3.58 and 3.59. In both cases, the 8-isomer was a better inactivator than the corresponding 9-isomer. At this stage, it would be premature to state that the 8-acyl derivatives of alloPaTrin produce a greater inactivation of ATase than their 9-acyl isomers. Further pairs of isomers would have to be prepared, and their biological activities analysed before any conclusions regarding a structure/activity relationship can be made.

In general, the $I_{50}$ values (Table 5.3) of the mono-alkylcarbamoyl derivatives of alloPaTrin are an order of magnitude greater than the compounds of the alkoxycarbonyl series (i.e. they are less efficient inhibitors of ATase than alkoxycarbonyl derivatives). The 8-phenylcarbamoyl derivative 4.23 is a notable exception. Its $I_{50}$ value of 0.006 $\mu$M is significantly better than any other mono-substituted carbamoyl derivatives of both alloPaTrin and PaTrin-2. The 8-ethoxypropylcarbamoyl derivative 4.17 is also a particularly good inactivator of ATase. It is ten times more efficient at inhibiting ATase than the corresponding 9-isomer 4.16. This is in keeping with the pattern of $I_{50}$ values produced by both pairs of alkoxycarbonyl isomers, which was discussed earlier. The very high $I_{50}$ values of both carbamoyl derivatives bearing the substituent in the 2-position confirm that a free amino group in the 2-position of an alloPaTrin derivative is required for efficient inactivation of ATase.

The biological activities (Table 5.4) of the di-substituted carbamoyl derivatives follow a similar pattern to those of the mono-substituted series. In general the di-substituted carbamoyl derivatives of alloPaTrin appear to be better inactivators of ATase than their guanine counterparts. The compound bearing phenyl substituents in the 8-position 4.25b is a better inactivator than the other alloPaTrin derivatives in the series, while the 8-morpholinocarbonyl compound is a better inhibitor of ATase than its 9-isomer 4.30b.

Very general trends and conclusions can be developed from the $I_{50}$ values of the potential prodrugs of alloPaTrin:

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In the alkoxy carbonyl series, the I\textsubscript{50} values of the 8-aza-7-deazaguanine compounds are comparable with those of the guanine compounds. In both the mono- and di-alkylcarbamoyl series, the 8-aza-7-deazaguanine compounds appear to be more efficient inactivators than their guanine analogues.

Four pairs of the 8- and 9-substituted derivatives of alloPaTrin have been prepared and their biological activities have been analysed. In each case, the 8-isomer was a better inactivator of ATase than its corresponding 9-isomer. Further isomeric pairs would have to be examined as inactivators of ATase before this could be confirmed as a general trend.

The compounds bearing a phenyl group (8- and 9-phenoxy carbonyl derivatives, 3.58 and 3.59, the 9-phenyl carbamoyl derivative 4.23 and the 8-di-phenyl carbamoyl derivative 4.25b) are better inactivators of ATase than the other compounds in their respective series. In particular, the 8-phenoxy carbonyl compound 3.58 produced a very low I\textsubscript{50} value (0.0018 \( \mu \text{M} \)) in the assay experiment. While the 9-phenoxy carbonyl and the di-phenyl carbamoyl derivatives of PaTrin-2 both possess excellent I\textsubscript{50} values, the trend of exceptionally low I\textsubscript{50} values produced by phenyl bearing derivatives is more noticeable in the alloPaTrin series than in the guanine series. This observation would require further examination before conclusions could be made.

In general, the acyl derivatives of alloPaTrin are compounds, which are more soluble than the parent compound, alloPaTrin, and they have excellent bioactivity against ATase. They were chosen as targets for synthesis as they could potentially act as prodrugs of alloPaTrin. However, some of the acyl derivatives of alloPaTrin (i.e. the compounds whose I\textsubscript{50} values are less than 0.007 \( \mu \text{M} \)) are more efficient inactivators of ATase than the parent compound, and as such, their application as prodrugs of alloPaTrin is not viable, though their activity merits their investigation as drugs in their own right.
5.5 Acyl Derivatives as ATase Inhibitors

The $I_{50}$ values of the alkylated derivatives whose synthesis was discussed in Chapter 2, represent the efficacy with which these alkyl compounds inhibit the protein. However, it is not certain whether the $I_{50}$ values of the alkoxy carbonyl and alkyl carbamoyl derivatives of alloPaTrin represent the inactivation by these compounds as the derivatives may have partially or wholly hydrolysed to release free alloPaTrin during the assay experiment. The liberated alloPaTrin may be responsible for the inactivation of ATase. This scenario is supported by Pegg et al., who have reported that the ATase inactivating ability of 9-acetyl-$O^6$-benzylguanine 3.01 can be attributed to the production of free $O^6$-benzylguanine 1.17 prior to the inactivation of the protein.\footnote{5.2}

Ultimately we wish the acyl derivatives of alloPaTrin to act as prodrugs. With this in mind, it would be useful to establish the relative susceptibilities to hydrolysis of these potential prodrugs of alloPaTrin.

In order to address these issues, researchers in the Paterson Institute have attempted to establish the stabilities of some of the acyl derivatives of alloPaTrin and PaTrin-2 under the conditions of the original assay experiment.

5.6 Similar Studies in the Literature

Buur et al. have reported the relative susceptibilities to hydrolysis of alkoxy carbonyl, and mono- and dialkyl carbamoyl derivatives of 5-fluorouracil.\footnote{5.3, 5.4}
They found that the 1-\(N\), 3-\(N\)-disubstituted alkoxy carbonyl derivatives, 4.05b, were found to degrade rapidly in aqueous solution to give the corresponding 3-\(N\) substituted derivative 4.05a. The 3-\(N\)-alkoxy carbonyl derivatives were reported to be much more stable under physiological conditions than their 1-\(N\)-substituted isomers 4.05c. This was attributed to the greater acidity of the 1-NH proton as compared with the 3-NH proton, and the consequent greater basicity of the corresponding counter anion.

The 1-\(N\)-mono-alkyl carbamoyl derivatives 4.06 were less susceptible to hydrolysis than the alkoxy carbonyl compounds. Intramolecular hydrogen bonding may influence the chemical behaviour of these derivatives, but the effect of this on rates of hydrolysis have never been determined.
Buur et al. reported two possible pathways for the hydrolysis of the mono-alkylcarbamoyl derivatives.\textsuperscript{5,4}

\[
\begin{align*}
\text{H} & \quad \text{N} & \quad \text{C} & \quad \text{O} \\
\text{H} & \quad \text{N} & \quad \text{C} & \quad \text{O} \\
\text{R} & \quad & \quad & \\
\text{H} & \quad \text{N} & \quad \text{C} & \quad \text{O} \\
\text{H} & \quad \text{N} & \quad \text{C} & \quad \text{O} \\
\text{R} & \quad & \quad & \\
\end{align*}
\]

\begin{align*}
& \xrightarrow{\text{E}_{1cb}} \\
& \xrightarrow{\text{B}_{AC2}} \\
& \xrightarrow{\text{R-NH}_2 + \text{CO}_2}
\end{align*}

Scheme 5.1

A reaction pathway involving an unstable isocyanate intermediate (E\textsubscript{1cb} mechanism) may be involved in the hydrolysis reaction. Alternatively, the reaction may proceed through a tetrahedral intermediate in a B\textsubscript{AC2} pathway. Buur et al. were unable to differentiate between the two pathways. Similarly, the hydrolysis of our mono-alkyl derivatives of alloPaTrin could follow either pathway (see Scheme 5.2), however we have currently no way of knowing the mechanism of the hydrolysis reaction.
The dialkylcarbamoyl derivatives 4.07 were found to be the most stable of the 5-fluorouracil derivatives under physiological conditions.

From these results, we would expect the alkoxy carbonyl derivatives of alloPaTrin to be the most susceptible to hydrolysis, while the dialkylcarbamoyl derivatives should be the most stable under the assay conditions. We expect the monoalkylcarbamoyl derivatives to be of intermediate stability under these conditions.

5.7 Method of Investigation of Stabilities of Acyl Derivatives
The original assay experiment to establish the I$_{50}$ of a potential ATase inactivator is carried out on a DMSO solution of the compound (with a buffer) at pH 8.3 and at 37 °C for one hour. The investigation of the stabilities of the acylated compounds
involved the initial analysis of a solution of the compound by HPLC (UV detection). The solution was of a similar concentration to that used in the assay experiment. After incubation at 37 °C, the solution was again analysed by HPLC. The percentage degradation was calculated for each compound by comparison of the peak heights of the acyl derivative recorded initially and after incubation for one hour. This technique may incorporate a large margin of error, but at the moment we have no method of estimating its value.

5.8 Degradation of Acyl Derivatives
Table 5.5 shows the percentages of degradation of some alloPaTrin derivatives under the assay conditions. The corresponding figures for the analogous PaTrin-2 derivatives are also given.

5.9 Analysis of Levels of Degradation
The acyl derivatives can be divided into three categories, the alkoxy carbonyl derivatives, the mono-alkyl carbamoyl derivatives and the di-alkyl carbamoyl derivatives.

Analysis of the HPLC traces of the alkoxy carbonyl series of derivatives before and after the one hour incubation period show varied levels of degradation. The 9-benzyloxycarbonyl derivative 3.59 showed no decomposition during the experiment, while the 8-phenoxycarbonyl 3.58 and the 4-picyloxy carbonyl compound 3.77c showed decomposition of 4% and 8% respectively. These results are unexpectedly low when compared to the levels of degradation of other alkoxy carbonyl derivatives of both alloPaTrin and PaTrin-2, and as such, the experiments should be repeated, so as to confirm these results. It would be particularly interesting if the 4% degradation of the 8-phenoxycarboxyl derivative 3.58 was accurate, as this compound produced an exceptionally low I50 value (0.0014 μM). Its I50 value suggests that the compound is five times more active against ATase than its parent, alloPaTrin, and these degradation results suggest it is a highly stable molecule.

The 9-phenoxycarbonyl derivative 3.59 also exhibits low levels of degradation (20%) during the assay experiment. Three of the remaining alkoxy carbonyl derivatives, the
Table 5.5 Percentage Degradation of Aeryl Derivatives Under Assay Conditions

<table>
<thead>
<tr>
<th>X</th>
<th>% degradation</th>
<th>% degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>alloPaTrin</td>
<td>9-substituted</td>
</tr>
<tr>
<td>3.38 -OCH$_3$</td>
<td>32</td>
<td>46</td>
</tr>
<tr>
<td>3.44 -OCH$_2$CH$_2$CH$_3$</td>
<td>54 8-isomer</td>
<td>35 9-isomer</td>
</tr>
<tr>
<td>3.45 -OCH$_2$CH$_2$CH$_3$</td>
<td>35 9-isomer</td>
<td>ND</td>
</tr>
<tr>
<td>3.58 -OPh</td>
<td>4 8-isomer</td>
<td>10</td>
</tr>
<tr>
<td>3.59 -OPh</td>
<td>20 9-isomer</td>
<td></td>
</tr>
<tr>
<td>3.77a -OCH$_2$Ph</td>
<td>ND</td>
<td>19</td>
</tr>
<tr>
<td>3.77b</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>3.77c</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>4.17 -NH(CH$_2$)$_3$OEt</td>
<td>46 8-isomer</td>
<td>61</td>
</tr>
<tr>
<td>4.16 -NH(CH$_2$)$_3$OEt</td>
<td>86 9-isomer</td>
<td></td>
</tr>
<tr>
<td>4.25c</td>
<td>ND 8-isomer</td>
<td></td>
</tr>
<tr>
<td>4.30b</td>
<td>ND 9-isomer</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Percentages quoted for 9-substituted derivatives of alloPaTrin except where stated.
9-methoxy 3.38, 9-propyloxy 3.45 and the 9-piperonyloxy 3.77b compounds all produce consistent levels of degradation (32-35%). We are unable to rationalise the much higher degree of degradation (54%) shown by the 8-propyloxy derivative 3.44.

Similarly, we were unable to rationalise the particularly low stability of the 9-ethoxypropylcarbamoyl compound 4.17 (86% degradation). This derivative is considerably less stable than its 8-isomer. Further compounds would have to be examined in a similar way to establish whether these results are representative of the mono-alkylcarbamoyl series of derivatives of alloPaTrin.

As expected, the dialkylcarbamoyl derivatives of alloPaTrin appear to be the most stable of the acylated compounds. While the 9-morpholinocarbonyl derivative 4.30b is relatively stable (22% degradation), its 8-isomer exhibited no degradation during the incubation period. This mirrors the results for the similar experiments on the dialkylcarbamoyl derivatives of PaTrin-2. In the guanine series, ten dialkylcarbamoyl derivatives were analysed in this way. All showed decomposition of less than 10%.

Overall, it is difficult to reach any conclusions from these results. No obvious difference between the 8-aza-7-deazaguanine and the guanine compounds has emerged. In the alkoxy carbonyl series, the rates of decomposition vary from 8% to 54%, and no clear pattern can be identified. Analysis of more monoalkylcarbamoyl compounds is required before conclusions can be developed. By combining results of the degradation of the dialkylcarbamoyl derivatives of both alloPaTrin and PaTrin-2, one can tentatively conclude that these derivatives are stable under conditions of the assay experiment.

The susceptibility to degradation of four pairs of 8- and 9-isomers has been examined. In three cases, the 9-substituted compound is more labile than its 8-isomer. The propyloxy carbonyl compounds 3.44 and 3.45 are an exception to this. Further analysis of pairs of isomers is required to establish whether the 8-substituted derivatives of alloPaTrin are generally more stable than their 9-isomers or whether the relative stabilities of the isomers is dependent on the identity of the side-chain, X.
It should be noted that while these results are an indication of the chemical stabilities of the acylated compounds, they may not be a true representation of the stabilities of the compounds \textit{in vivo}. The presence of esterases and amidases in the body may alter the chemical conditions sufficiently to change the behaviour of these compounds. Examination of the behaviour of these derivatives \textit{in vivo} is required before their potential as prodrugs of alloPaTrin can be established.
5.10 References


Chapter 6

Conclusion and Future Work
6.1 Conclusion

This study has dealt with the synthesis of substituted 8-aza-7-deazaguanines. The abilities of these derivatives to inactivate ATase have also been investigated.

In Chapter 2, an unambiguous synthesis of 9-alkyl derivatives of O^-alkoxy-8-aza-7-deazaguanine was described. The synthesis of the substituted 8-aza-7-deazaguanine involved the reaction of a pyrimidine 2.06 and an alkyl hydrazine to give the 8-aza-7-deazaguanine ring system bearing an alkyl group in the 9-position (see Scheme 6.1). Previous syntheses of such molecules involve the alkylation of a pre-formed 8-aza-7-deazaguanine. Potentially, this older synthetic strategy can produce 8- and 9-alkylated compounds.

The unambiguous synthesis of some of the corresponding alkylated 8-aza-7-deazapurines were also investigated. The biological activities of the alkylated 8-aza-7-deazapurines were markedly inferior, when compared to the biological results of the corresponding alkylated 8-aza-7-deazaguanines. This observation suggests that a free amino group in the 2-position of a potential inactivator of ATase is necessary for efficient inactivation of the protein.

The synthesis of alkoxy carbonyl derivatives of O^--(4-bromothenyl)-8-aza-7-deazaguanine (or alloPaTrin) is discussed in Chapter 3 (see Scheme 6.2). Initially, the
reaction of a pyrimidine aldehyde and an alkyl carbazate was investigated. However, this reaction only produced a pyrimidine bearing a hydrazone side-chain. All attempts to close the ring to form the pyrazole ring from the hydrazone side-chain and the pyrimidine failed. Our attention then turned to methods of direct acylation of alloPaTrin. During our investigations, we discovered we could control the site of substitution, depending on the alkoxy carbonylating agent used. Reagents containing good leaving groups (i.e. chloride) tended to form both the 8- and 9-substituted products 3.57a-b, while reagents with poorer leaving groups (i.e. 4-nitrophenoxy) generally produced the 9-substituted compound 3.57a. For example, treatment of alloPaTrin with an alkyl chloroformate produced a mixture of 8- and 9-substituted isomers, although the ratio of products varied, depending on the chloroformate used. The reaction of alloPaTrin with a pyrocarbonate or an unsymmetrical carbonate, bearing a 4-nitrophenoxy group and the required alkoxide, produced the 9-substituted product 3.57a predominantly, with only trace amounts of the 8-isomer 3.57b being detected.
Scheme 6. 2
Reaction conditions resulting in the selective derivatisation of alloPaTrin by mono- or di-alkylcarbamoyl groups were also developed (Chapter 4 and Scheme 6.3). Reaction of an alkyl isocyanate and sodium hydride with alloPaTrin produced carbamoylation exclusively at the 2-amino group. Treatment of alloPaTrin with either a mixed carbamate bearing a 4-nitrophenoxide leaving group, or a di-alkylcarbamoyl chloride resulted in the formation of an alloPaTrin derivative bearing a substituent in the 8-position. The 9-alkylcarbamoyl derivatives of alloPaTrin were produced by the displacement of the 4-nitrophenoxide leaving group from the 9-(4-nitrophenoxycarbonyl) derivative 3.75 by a primary or secondary amine.
Scheme 6.3
With the notable exceptions of the alloPaTrin derivatives without a free amino group in the 2-position, all the substituted 8-aza-7-deazaguanines possessed excellent ATase inhibiting abilities. While the 9-alkylated compounds were investigated as inactivators in their own right, the acylated derivatives of alloPaTrin were examined not only for their ATase inactivating abilities, but also as potential prodrugs of alloPaTrin. However, a number of the acyl derivatives produced greater inhibition of ATase than the parent compound, alloPaTrin. While we were surprised by these results, which makes the application of the prodrug strategy irrelevant for many of the alloPaTrin derivatives, their exceptional bioactivity (particularly of some of the alkoxy carbonyl derivatives) and their enhanced fat and water solubility compared to the parent compound may be of great value.
6.2 Future Work

While we have achieved our original aims of preparing 9-alkyl, alkoxy carbonyl and carbamoyl derivatives of alloPaTrin, there remains areas where further work would be useful. Further analysis of the stabilities under physiological conditions of the potential prodrugs, whose synthesis was discussed in Chapters 3 and 4, needs to be carried out.

Some 8- and 9-isomers were prepared in the acyl and carbamoyl series. The 8-isomer of each pair possessed a lower $I_{50}$ value (i.e. it was a better inhibitor of ATase) than the corresponding $I_{50}$ of the 9-isomer. To date, no 8-alkyl derivative of alloPaTrin 6.01 has been prepared. The synthesis of some 8-alkyl derivatives of alloPaTrin would be worthwhile, as their biological activities could be compared with those of their corresponding 9-isomers.

![Chemical Structure](image)

6.01

In the guanine series it is known that substitution in the 8-position by certain groups does not significantly affect their ATase inhibiting ability.\(^6\) It would be worthwhile to establish whether substitution at the 8-position of alloPaTrin by an alkyl group had a similar effect.

As mentioned above, four isomeric pairs of 8- and 9-substituted derivatives of alloPaTrin were prepared. In each case, the 8-isomer is a better inhibitor of ATase. To confirm this observation as a genuine structure/activity relationship, it would be useful to synthesise further pairs of 8- and 9-alkoxycarbonyl and alkyl carbamoyl derivatives of alloPaTrin, and analyse their respective abilities to inhibit ATase.
Ideally the actual synthesis of alloPaTrin could be improved upon. To date the best synthesis of alloPaTrin is displacement of a DABCO group of the quaternary ammonium salt 3.37 by alkoxide. Yields of alloPaTrin are routinely below 50%, which is undesirable considering alloPaTrin acts as starting material for all the acylation reactions discussed in this study.

It would also be worthwhile to synthesise alloPaTrin derivatives containing steroidal moieties. The presence of the steroid group could increase the targeting ability of the alloPaTrin-based ATase inhibitor. It has been shown that steroid receptors are over-expressed in steroid-dependent breast and testicular cancers. Breast cancers have been shown to have markedly increased expression or oestrogen receptors when compared to healthy tissue. Similarly, increased expression of testosterone receptors is a characteristic of testicular cancers. This property of these cancers has been exploited by other researchers who have made steroidal derivatives of biologically active compounds, with a view to increasing their selectivities. For example, derivatives of the topoisomerase-II inhibitor, ellipticine containing an oestradiol moiety have been prepared with a view to increasing the selectivity of the parent compound.

The dihydrotestosterone, 6.02 and 6.04, and oestradiol derivatives, 6.03 and 6.05, of PaTrin-2 have been prepared. All four compounds possess excellent ATase inhibiting, however any increase in selectivity due to the steroidal side-chain has yet to be evaluated. The synthesis of steroidal derivatives of alloPaTrin 6.06-6.09 would be a valuable exercise, since these compounds could be potentially very potent and selective inhibitors of ATase (Scheme 6.4).
Scheme 6.4
In the guanine series, the 8-oxo compound 6.10 has been identified as one of the principle metabolites of PaTrin-2. The corresponding 8-oxo derivative of $O^6$-benzylguanine has also been identified by Pegg et al.

![Chemical structures of 6.10 and 6.11](image)

The 8-oxo compound 6.10 retains potent biological activity of its parent compound, and may be responsible for the inactivation of ATase in vivo. The breakdown of alloPaTrin in vivo has yet to be fully examined. It would be interesting to prepare the 7-oxo derivative 6.11 of alloPaTrin, as it would facilitate its identification as a possible metabolite product of alloPaTrin. Its contribution, if any, to the biological activity of alloPaTrin could be assessed.
6.3 References


