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Synthesis of potential inhibitors of the protein ATase

by

Céline S. BLAIS

A thesis submitted to the University of Dublin

For the degree of Doctor of philosophy
This thesis has not been submitted as an exercise for a degree at this or any other university. Except when otherwise indicated, the work described herein has been carried out by the author alone.

Céline S. Blais

October 2000
Dedicated to my parents and sisters.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition/Description</th>
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<tbody>
<tr>
<td>Ac₂O</td>
<td>Acetic anhydride</td>
</tr>
<tr>
<td>m.p.</td>
<td>melting point</td>
</tr>
<tr>
<td>Na₂CO</td>
<td>Sodium carbonate</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>Sodium bicarbonate</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>Phosphorus pentoxide</td>
</tr>
<tr>
<td>POCl₃</td>
<td>Phosphorus oxychloride</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>Rₚ</td>
<td>Retention factor</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
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<tr>
<td>tD</td>
<td>triplet of doublet</td>
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<tr>
<td>Hz</td>
<td>Herzt</td>
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<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>Magnesium sulphate</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole</td>
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Thin layer chromatography was carried out using Merck Kieselgel 60 F254 0.2 mm silica gel plates. Visualisation was by means of ultra-violet light at 254 nm. Flash column chromatography was carried out using Merck Kieselgel 60 (mesh 230-400 ASTM) silica gel.

Evaporation under reduced pressure refers to the use of a Büchi rotary evaporator.

Dry tetrahydrofuran was obtained by first drying over potassium hydroxide pellets for 24 hours, then distilling from lithium aluminium hydride and finally distilling from sodium benzophenone ketyl.

Melting points are uncorrected and were measured in unsealed capillary tubes using either Stuart Scientific SMP2 digital apparatus or Electrothermal IA9100 melting point apparatus.

Infrared spectra were recorded as Nujol mulls using either Perkin-Elmer 883 or Perkin-Elmer Paragon 1000 spectrophotometer.

Nuclear magnetic resonance spectra were recorded using Bruker WP-80, Bruker MSL 300 or Bruker DPX 400 spectrometers. Chemical shifts of $^1$H and $^{13}$C NMR spectra were measured in deuterated dimethyl sulfoxide. Coupling constants (J) are quoted in Hertz.

Elemental analyses were carried out at the Microanalytical Laboratory, University College Dublin.
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CONCLUSIONS
INTRODUCTION
INTRODUCTION

1- The cellular basis of cancer

The 30 trillion cells of the normal, healthy body live in a complex, interdependent biosystem, regulating each other’s proliferation. Indeed, normal cells reproduce only when instructed to do so by other cells nearby, ensuring that each tissue maintains a size and architecture appropriate to the body’s needs.

Cancer cells, in contrast, violate this norm; they become indifferent to the usual controls on proliferation and follow their own agenda for reproduction. They also possess an even more insidious property, known as metastasis: the ability to migrate from the site where they began, invading nearby tissues and forming masses at distant sites in the body. Tumours composed of such malignant cells become lethal when they disrupt the tissues and organs needed for the survival of the organism as a whole.

The malignant transformation of a cell is the result of an accumulation of mutations in specific classes of genes in that cell. Genes are carried in the DNA molecules of the chromosomes in the cell nucleus. A gene specifies a sequence of amino acids that must be linked together to make a particular protein; the protein then carries out the work of the gene. When a gene is switched on, the cell responds by synthesising the encoded protein. Mutations in a gene can perturb a cell by changing the amount or the activities of the protein product.

Two genes classes, which together constitute only a small proportion of the full genetic set, play major roles in triggering cancer. In their normal configuration, they ensure the normal development of the cell. Proto-oncogenes encourage growth, whereas tumour suppressor genes inhibit it. When mutated, proto-oncogenes can become carcinogenic oncogenes, which drive excessive multiplication. Tumour
suppressor genes, in contrast, contribute to cancer when they are inactivated by mutation.

2- Causes and therapy

Cancer seems to arise from the effects of two different kinds of carcinogens. One of these categories comprises agents that damage genes involved in controlling cell proliferation and migration. Another category includes agents that do not damage genes but instead selectively enhance the growth of tumour cells or their precursors. Environmental agents such as radiation (e.g., X-rays), chemicals (e.g., tobacco smoke) and some viruses (e.g., hepatitis B virus) are known or suspected to cause most human cancers.

2.1- Three principal approaches to therapy

2.1.1- Surgery

The earliest therapy established for cancer, and still the most widely used approach, is surgery. Surgical removal of a tumour is both quick and effective, and it accounts for the largest number of cures. Unfortunately, this form of treatment is not fully reliable. Removal of the tumour mass visible to the surgeon does not guarantee elimination of the microscopic extensions that so often characterise cancer, and to do so a surgeon may be forced to cut out a large amount of healthy tissue, resulting in permanent damage to the patient’s functioning or appearance. In some instances, cancer grips vital structures, which cannot be surgically removed. And finally, the most crucial limitation of surgery is that it cannot treat an advanced stage of cancer in which metastasis has occurred throughout the body.
2.1.2- Radiation

Another form of therapy, which is preferable to surgery for many reasons, is radiation treatment. In this method, powerful x-rays or gamma rays irradiate the region of the patient's cancerous tumour. Radiation treatments act either by inflicting genetic damage sufficient to kill cells directly or by inducing cellular suicide, a process called apoptosis. Because healthy tissues can recover from radiation exposure more readily than cancerous cells, radiation therapy can preserve the anatomical structures that surround a cancerous growth, thus curing the cancer without sacrificing the patient's ability to function. As another advantage over surgical removal of a tumour, radiation can destroy microscopic extensions of cancerous tissues. Nevertheless, radiation therapy at times proves inadequate, because, like surgery, it sometimes fails to kill all the cancer cells of a tumour and, like surgery, radiation cannot treat widespread metastasis. Whole-body radiation exposure sufficient to kill widely dispersed cancer cells would destroy some delicate vital tissues.

2.1.3- Chemotherapy

In such cases, a patient must make use of chemotherapy, the systematic administration of anti-cancer drugs that travel throughout the body via the blood circulatory system. These drugs typically operate on human cells, in a manner similar to which some antibiotics act on bacteria, i.e. they prevent cells from multiplying, often by interfering with their ability to replicate DNA. Some of the main families of chemotherapeutic agents for treatment of cancer are:

(a) Antimetabolites: anticancer compounds that act as false substances in the biochemical reactions of a living cell (e.g., methotrexate, which is a chemical
analogue of the nutrient folic acid). These drugs prevent cells from dividing by incapacitating their ability to construct new DNA.

(b) Topoisomerase inhibitors: Drugs that inhibit the action of the topoisomerase enzymes (Topase I and Topase II) and cause cells to die. There are few Topase I inhibitors (e.g., camptothecin and derivatives), most of them are Topase II inhibitors (e.g., doxorubicin). Replication of a cell’s genetic material requires a means to pull the DNA double helix apart into two strands. This separation is accomplished with the aid of a special “topoisomerase” enzyme that temporarily cleaves one strand (Topase I), or both strands (Topase II), passes the other strand through the break and then reattaches the cut ends together.

(c) Plant alkaloids with various mechanism of action: (e.g., vinblastin) substances derived from plants that can prevent successful cell division by binding to the protein tubulin. Tubulin forms microtubular fibres that help to orchestrate cell division.

(d) Alkylating agents: (e.g., cyclophosphamide) compounds that form chemical bonds with a wide variety of biologically important nucleophiles. When it happens with particular DNA building blocks, e.g., guanine, it produces defects in the normal double helical structure of the DNA molecule. Damage caused by these chemicals will trigger cellular suicide.

Many different compounds are currently in use as anticancer agents, and additional ones are constantly being synthesised, screened and tested for potential activity. This project deals with an aspect of chemotherapy, and in particular the synthesis and evaluation of new alkylating agents.
The first chemotherapeutic drugs, developed during the 1940s, often proved inadequate when administrated individually. During the 1960s, however, physicians discovered that chemotherapy could cure some cancers when several drugs were given simultaneously. Many malignancies, e.g. leukemia, lymphomas and testicular cancer, are now successfully treated with such combination chemotherapy.

The currently available chemotherapeutic drugs often fail patients because they kill many healthy cells and thus bring on serious side effects. Damage to the rapidly growing cells of the bone marrow, for instance, causes anemia, an inability to fight infection and a propensity for internal bleeding. Other side effects of chemotherapy include diarrhea, nausea, vomiting, and hair loss.

3- Folate analogs as antimetabolites

Folate metabolism represents an attractive target for chemotherapy, since it plays a key role in the biosynthesis of nucleic acid precursors. Therefore, it is of interest to note that, since the identification of folic acid as a vitamin, agents that antagonise its metabolic role have been discovered that are effective, not only against proliferation disorders such as cancer and psoriasis, but also against a variety of microbial diseases. In 1948, Mowat successfully identified the structure of folic acid, and following on from this, the compound, and a number of related derivatives were prepared. Of these, only 4-amino-4-deoxyfolic acid (aminopterin) and its $N^{10}$-methyl derivative (methotrexate) showed significant biological activity when assayed for the inhibition of growth of Streptococcus faecalis R.
Considerable interest in these antimetabolites was generated by the finding that aminopterin produced temporary remission in acute leukemia in children. This interest has resulted in the elucidation of pathways of one-carbon metabolism involving the tetrahydrofolates and in the understanding that the primary mechanism by which these amino compounds inhibit cell growth is by the inhibition of dihydrofolate reductase (DHFR).

Because the only enzymatic step in the one-carbon transfer that involves oxidation of the tetrahydrofolate to the dihydrofolate is the conversion of deoxyuridylate to thymidylate by thymidylate synthase (TS), it appears that this conversion is the most sensitive to depletion of tetrahydrofolate by the inhibition of DHFR, although the indirect inhibition of other enzymatic transformations may also be important. Later it was established that the 3', 5'-dichloro derivative of methotrexate (MTX) and MTX
itself are hydroxylated by aldehyde oxidase of the liver to give (1) and (2) respectively. This oxidation inactivates these compounds with respect to their inhibition of DHFR and, consequently, their anti-cancer activity.

\[
\begin{align*}
(1) & \quad X = \text{Cl} \\
(2) & \quad X = \text{H}
\end{align*}
\]

Most of the anti-folates that have been synthesised resulted from systematic modification, first of the structure of folic acid and later of both folic acid and MTX with different objectives in mind. These alterations are examined with reference to the components of the molecule.
3.1- Modifications at the pteridine moiety

One of the simplest modifications of the pteridine ring of aminopterin or methotrexate is reduction of the pyrazine ring. Horwitz and Kisliuk separated the reduced forms of methotrexate and found that they are both less potent inhibitors of DHFR but more potent inhibitors of TS.

In an effort to prevent the metabolic inactivation of methotrexate mentioned earlier, by hydroxylation at C-7, Farquhar and Loo prepared 7-methylaminopterin (3) and 7-methylmethotrexate (4) and found that these compounds are inactive against leukemia L1210. Substitution of C-7 by a nitrogen led to the synthesis of 7-azafolic acid (5) and 7-azaaminopterin (6), neither of which inhibited DHFR. Fully reduced pyrazine ring derivatives were also found inactive.

\[
\begin{align*}
\text{(3)} & \quad R = H \\
\text{(4)} & \quad R = \text{Me}
\end{align*}
\]

\[
\begin{align*}
\text{(5)} & \quad R = \text{OH} \\
\text{(6)} & \quad R = \text{NH}_2
\end{align*}
\]
In a program designed to establish the importance of the nitrogens of the pyrazine ring, a number of quinazoline analogs of folic acid (7) and aminopterin (8) were synthesised.

![Chemical structure of folic acid analogs](attachment:image.png)

(7) \( R = \text{OH} \)

(8) \( R = \text{NH}_2 \)

The diaminoquinazolines, particularly those in which the L-glutamate moiety is replaced by L-aspartate (8, \( n = 1 \)), are quite active against leukemia L1210 and several of its sublines. They are potent inhibitors of DHFR but not of TS.

Both the 5-\(^{16}\) and the 8-deaza\(^{17}\) analogs of folic acid (9 and 12 respectively), aminopterin (10 and 13 respectively) and methotrexate (11 and 14 respectively) have been prepared. The diamino derivatives (10 and 11) of the 5-deaza compounds are potent inhibitors of DHFR and are active against leukemia L1210.\(^{16}\) 8-Deazafolic acid (12) is reported to be a poor inhibitor of DHFR and TS but was active against some bacteria.\(^{17}\)
Elliott et al.\textsuperscript{18} described the synthesis and biological evaluation of 1- and 3-deazamethotrexate (15, 16) and a number of related compounds.

The activity of the 3-deaza compound against leukemia L1210 \textit{in vivo} approached that of MTX, but high doses of the drug were required for both activity and toxicity, presumably a reflection of the fact that it is a less effective inhibitor of DHFR. These data established the importance of \textit{N}-1 but not \textit{N}-3 in the binding of MTX to DHFR.
Attachment of the MTX side chain to C-7 rather than C-6 of 2,4-diaminopteridine (17) resulted in a complete loss of activity.\textsuperscript{19}

\begin{center}
\includegraphics[width=0.8\textwidth]{17.png}
\end{center}

(17)

3.2- The C\textsubscript{9}-N\textsubscript{10} bridge

Many variations have been made in the C\textsubscript{9}-N\textsubscript{10} bridge of both folic acid and aminopterin, but few have resulted in improved anti-cancer activity, relative to MTX. A number appear to be at least as active as MTX in the L1210 leukemia system,\textsuperscript{20} however clear superiority has not been established.

3.3- The benzene ring.

An early modification of the benzene ring of folic acid and MTX resulted from chlorination of the parent structure.\textsuperscript{4} The report that this crude 3',5'-dichloromethotrexate (18) was more active than MTX in the treatment of advanced leukemia L1210 led to the preparation of pure material for clinical testing. In the event, this trial showed that the compound showed no advantage over MTX. Additional halo derivatives have also been prepared (19) and tested but have found no application in human patients. Alkylbenzenes (20) and a wide variety of heterocycles were substituted for benzene in the folic acid system\textsuperscript{21}. These modifications resulted in only marginal activity. More drastic changes, such as substituting an alkyl group for the benzene ring, were also unproductive.
3.5- The glutamate Moiety

A number of amino acids and/or additional groups have been substituted for the glutamic acid moiety of aminopterin (27) or MTX (28). None of these analogs have proven to be superior to the parent compounds, in in vivo model systems, with many of them being considerably less active. More success has been achieved with derivatives of aminopterin or MTX. For example, esters of MTX (29-31), although less potent inhibitors of DHFR, are highly active compounds against leukemia L1210.26

In addition to inhibiting DHFR, the dibutyl ester (29, R1 = R2 = nBu) interferes with the incorporation of thymidine into DNA, perhaps by blocking its transport across the cell membrane.27
There are many products found in nature that have shown potential biological properties. The discovery of many active compounds very often occurs via empirical studies. The products are only uncovered when biological activities of the organisms containing them are observed.

Taxol (Paclitaxel)\textsuperscript{28, 29} is a plant alkaloid of major clinical importance with significant activity in the treatment of refractory ovarian cancer, as well as breast cancer, and potential activity in the treatment of lung cancer.
The study of products from microbial fermentation also yielded some potential compounds. Adriamycin is a natural antibiotic derived from bacteria, which also act as an anti-tumour drug.\(^{30}\) This product has many modes of action including Topase II inhibition and DNA alkylation.

![Adriamycin structure](image)

Adriamycin

Dolastin 10\(^{31}\) is a natural product derived from the peptide of the Indian Ocean mollusc *Dolabella auricularia*. It has been found to be the most potent anti-neoplastic* and tubulin inhibitory substances known.

![Dolastin 10 structure](image)

Dolastin 10

* Inhibiting or preventing the development of tumours.
5- Alkylating agents

5.1- Principal sites of attack

Chemotherapeutic chloroethylnitrosoureas (e.g. carmustine (32)) and methylating agents (e.g. temozolomide (33)) are presently used to treat neoplastic diseases, particularly lymphomas*, brain neoplasms, and gastrointestinal carcinomas.

\[ \text{Cl-CH}_2-\text{N=NO}_2 \]  

1,3-Bis(2-chloroethyl)-1-nitrosourea  
(BCNU)

Chloroethylnitrosoureas and methylating agents destroy tumour cells by reacting covalently with DNA. Alkylating agents are electrophilic centres, which will react with at least twelve nucleophilic sites on DNA, but particularly at guanine. The order of reactivity of the different nucleophilic centres in guanine is \( N^7 \gg N3 \gg O6 \). Alkylation of the 7-position of guanine gives rise to an unstable quaternary nitrogen, which may detach from the DNA chain, resulting in a gap which may prevent replication of the strand, or incorporation of the wrong base. However, the chances of the \( N^7 \)-alkylated guanine detaching are low, therefore alkylation at the \( O^6 \)-position, even if occurring to a smaller extent, is much more important in cancer chemotherapy. Alkylation of DNA has long been associated with the initiation and progression of

* A general term applied to any neoplastic disorder of the lymphoid tissues (fluids of the body).
cancer, with the major promutagenic lesions identified as $O^6$-alkylguanine (34) and, to a lesser degree, $O^4$-alkylthymine (35).

If the alkylating agent has more than one alkyl group, then rather than only simple monoadducts being formed, there is formation of cross-linkages in the DNA strand. The alkylation of two bases on the opposite strands of a DNA molecule via a bifunctional alkylating agent is described as an interstrand cross-link. On the other hand, the alkylation of two bases on the same strand of DNA is called an intrastrand cross-link.

Alkylation of guanine at the 7-position does not normally result in the miscoding of the DNA base. However, the presence of $O^6$-alkylguanine causes G-T mispairing, and subsequent A-T mutation lead to toxic mutagenic effects.\textsuperscript{35, 36} The chloroethylnitrosoureas form a number of adducts on DNA, including an interstrand cross-link between guanine and cytosine on the opposite strand of DNA.\textsuperscript{37, 38} Formation of this lesion has been postulated to occur as follows. Initial attack at the $O^6$-position of guanine (36) by the chloroethylnitrosourea (37) to produce 2-chloroethylguanine (38), followed by an intramolecular rearrangement to form 1,$O^6$-ethanoguanine (39). Within about 10-12 hours, this adduct reacts with the opposite cytosine to form the 1-(3-cytosinyl)-2-(1-guanyl)ethane crosslink (40). There is a strong correlation between the number of crosslinks formed and cell kill.\textsuperscript{38}
5.2- Principal types of alkylating agents

Agents that alkylate DNA include the nitrogen mustards,\textsuperscript{28} nitrosoureas\textsuperscript{28} (e.g. BCNU (32)), temozolomide (33)\textsuperscript{39} and dacarbazine (DTIC).\textsuperscript{28} Those agents contain
at least one alkyl group. The nitrogen mustards are an example of dialkylating agents.

\[ \text{RN(CH}_2\text{CH}_2\text{Cl)}_2 \]

Nitrogen mustards 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU)

\[ \text{DTIC} \]

\[ \text{Temozolomide} \]

5.2.1- The nitrogen mustards

These are analogues of the mustard gas (the sulphide S(CH\text{2}CH\text{2}Cl)_2), of which the toxic effects on blood and bone marrow have been found, after World War I, to be the result of alkylation of DNA. The replacement of sulphur by nitrogen allowed the synthesis of a wide range of compounds. In the late 1940’s the therapeutic and clinical evaluation of these nitrogen mustards effectively launched the cancer chemotherapy era. Since then, many active compounds have been produced, some of which owe their activity to crosslinking of the DNA.
5.2.2- Nitrosoureas

1-Methyl-1-nitroso- as well as 1-methyl-3-nitro-1-nitrosoguanine was found to be active against mice leukemia. The testing and evaluation of related compounds showed that 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) (32) has a high degree of efficiency against experimental tumours. The nitrosoureas can cause alkylation of the base, interstrand cross-linkages and single strand breakage. The interstrand cross-link prevents the replication and transcription of the DNA strand, causing the death of the cell.

\[ \text{1-methyl-1-nitrosourea} \quad \text{1-methyl-3-nitro-1-nitrosourea} \]

5.2.3- Temozolomide

Temozolomide is used as a prodrug, which undergoes base-catalysed hydrolytic ring opening followed by loss of a molecule of carbon dioxide to generate 5-(3-methyltriazene-1-yl)imidazole-4-carboxamide (MTIC). This further fragments to the methyldiazonium species at the proximal DNA-methylating agent. Thus temozolomide can be considered as a small molecular weight drug-delivery device able to transfer an electrophilic methyl group to vulnerable sites within tumour cells. The chemical mechanism underlying the antitumour properties of temozolomide is shown below.
5.3- **Resistance to alkylnitrosourea**

Tumour cells display a variety of mechanisms of resistance to many anti-cancer agents. Mechanisms of alkylating agent resistance include alterations in drug transport, enzymatic detoxification and repair of lesions generated by alkylation.\textsuperscript{36, 41}

Because DNA is a critical target of alkylating agents, the repair of DNA has been considered an important mechanism of resistance to these agents. The DNA repair protein, $O^6$-alkylguanine-DNA alkyltransferase (ATase), plays a major role in the resistance to alkylnitrosourea therapy.\textsuperscript{36, 42, 43} The protein provides a natural repair mechanism in many cells, by removing alkyl groups from the $O^6$-position of guanine residues and directly transferring the offending alkyl group to one of its own cysteine residues. The ATase protein protects cells by correcting alkylation damage to the $O^6$-position of guanine, and therefore reduces the effectiveness of some of the alkylating drugs in their treatment of cancer cells.
The mechanism of action of ATase is represented as an $S_n2$ reaction, in which the active cysteine, probably its thiolate anion, attacks the $\alpha$-carbon of the $O^\alpha$-alkyl group of $O^\alpha$-alkylguanine, which may be activated by proton transfer from the protein to $O^\alpha$, $N$-1, or $N$-3.\textsuperscript{44}

In cancer chemotherapy based on alkylating drugs, the level of ATase in targeted cells can be a significant determinant of the effectiveness of certain agents. A lower level of ATase activity is associated with a greater risk of tumour development,\textsuperscript{45} but such tumours are correspondingly more sensitive to alkylating agent chemotherapy, a property shown in particular by tumours cells devoid of ATase (the Mer\textsuperscript{-} phenotype).\textsuperscript{46} Clinical evidence suggests that the reduction of high ATase levels in target cells could have potential benefits in alkylating agent cancer chemotherapy.

5.4- Inhibition of Alkyltransferase

Because successful cancer treatment involves inactivation of the protein, thereby increasing the sensitivity to alkylnitrosoureas, efforts to design potent ATase
substrates would have considerable potential in chemotherapy. The first inhibition experiments used a combination of methylating and chloroethylating agents.\textsuperscript{47,48} The reason for this combination is that pre-treatment of cells with methylating agents decreases alkyltransferase levels by introducing $O^6$-methylguanine residues (41) in DNA, which are then repaired by the alkyltransferase. This mechanism totally inactivates the protein, which has then no regenerative path available.

\begin{center}
\includegraphics{41.png}
\end{center}

More recently, direct substrates for the alkyltransferase protein have been designed and tested for their ability to effectively inactivate the protein. Exposure of cells or cell extracts to millimolar amounts of the free base, $O^6$-methylguanine, for 4h, resulted in a loss of ATase activity and subsequent increase in the sensitivity of tumour cells to alkylating agents.\textsuperscript{49,50} Although preliminary results in cells looked promising, there was no enhancement of the therapeutic index of BCNU when combined with $O^6$-methylguanine to treat mice carrying human tumour xenografts. Most likely, this was due to poor solubility of the drug, only partial inhibition of the ATase activity and poor affinity of the ATase for the substrate. As a result $O^6$-methylguanine never entered clinical trials.
5.5- Design and testing of $O^6$-Benzylguanine

Effective inhibition of the alkyltransferase protein, resulting in an increase in the therapeutic index of BCNU, required a more potent and selective substrate. $O^6$-Benzylguanine was designed based on an understanding of the bimolecular displacement reaction between the ATase protein and the leaving group at the $O^6$-position of guanine. Benzyl groups are known to enter more readily into bimolecular reactions compared to alkyl groups because the electron charge stabilises the benzyl group in the transition state. Administration to human tumour cells of micromolar concentrations of $O^6$-benzylguanine for 2 minutes resulted in a complete depletion of the alkyltransferase protein, rendering cells more sensitive to agents that alkylate at the $O^6$-position of guanine.

5.6- Analogues of $O^6$-benzylguanine

Since the discovery of the potent inactivation of ATase by $O^6$-benzylguanine, a considerable number of additional compounds have been tested for their potential ATase inactivation and in some cases for the ability to render cells more sensitive to the effects of chloroethylating agents. A wide range of activities has been reported, with some compounds being more potent than $O^6$-benzylguanine and others being much less active. Quite minor changes such as the addition of methyl groups to the N-2 or N-7 position of $O^6$-benzylguanine leads to a huge loss of ATase inactivating activity, whereas other changes, such as the addition of an 8-bromo or 8-aza substituent increase potency. Many compounds have now been made that inactivate ATase at levels that suggest they would be useful for chemotherapeutic inactivation.
In 1992, \(^{51}\) \(\alpha\)-benzyl-2'-deoxyguanosine (42) was found much more soluble in aqueous solutions than \(\alpha\)-benzylguanine. And despite the 10-fold lower potency of \(\alpha\)-benzyl-2'-deoxyguanosine compared to \(\alpha\)-benzylguanine \emph{in vitro}, these compounds were equally effective \emph{in vivo}. As another example, \(\alpha\)-benzyl-8-oxoguanine\(^{53}\) (43) has been described as the primary plasma metabolite in humans, monkeys, rats and mice. Direct administration of this compound would be expected to reduce the variability of ATase inactivation observed upon administration of \(\alpha\)-benzylguanine. Other 8-substituted \(\alpha\)-benzylguanine derivatives, such as 8-aza-\(\alpha\)-benzylguanine (44) or \(\alpha\)-benzyl-8-bromoguanine (45) are more active than \(\alpha\)-benzylguanine and will differ in metabolism.\(^{53}\) \(\alpha\)-allylguanine (46)\(^{51}\) is able to inactivate some of the ATases that are resistant to \(\alpha\)-benzylguanine. The most potent ATase inhibitors described by Pegg and co-workers\(^{53}\) are 2,4-diamino-6-benzylpyrimidines, substituted at the 5-position with a strong electron-withdrawing group, such as nitro (47) or nitroso (48).
In 1975, Arcoria et al. reported that the order of reactivity of the $S_N$2 reactions of furfuryl, 2-thenyl and benzyl chloride with aniline is benzyl<2-thenyl<furfuryl. McElhinney et al. decided then to undertake the synthesis of $O^6$-hetarylalkylguanines such as $O^6$-furfurylguanine (49), $O^6$-thenylguanine (50), $O^6$-(4-bromothenyl)guanine (51) (PaTrin 2). The latter was found to be 10-fold more active than $O^6$-benzylguanine. In 1996, ‘PaTrin 2’ (‘Pa’ for Paterson Institute, Manchester and ‘Trin’ for Trinity College, Dublin) was accepted for clinical trial and in 1999 was proved to enhance the anti-tumour efficacy of temozolomide against human melanoma xenografts.

6- Purpose of this project

In addition to the guanine derivatives described above, McMurry et al. have also prepared pterin derivatives (52-54), in an effort to investigate the role of the
pyrazine ring in the inhibition of the protein ATase. These were found to be very active compounds.

The aim of the present project was to synthesise a number of substituted deazapterins as potential inhibitors of the protein ATase, in order to determine the importance of the N-atoms in the pyrazine ring.

The first series involved the preparation of four $O^t$-substituted 5-deazapterins (2-amino-4-aryloxypyrido[2,3-$d$]pyrimidine) namely, $O^t$-benzyl-5-deazapterin (55), $O^t$-thenyl-5-deazapterin (56), $O^t$-piperonyl-5-deazapterin (57) and $O^t$-(4-bromothenyl)-5-deazapterin (58). (Chapter One)

This was followed by the four corresponding $O^t$-substituted 5,8-dideazapterins (2-amino-4-aryloxyquinoxaline): $O^t$-benzyl-5,8-dideazapterin (59), $O^t$-thenyl-5,8-dideazapterin (60), $O^t$-piperonyl-5,8-dideazapterin (61), and $O^t$-(4-bromothenyl)-5,8-dideazapterin (62). (Chapter Two)

Then came the four $O^t$-substituted 8-deazapterins (2-amino-4-aryloxypyrido[3,2-$d$]pyrimidine): $O^t$-benzyl-8-deazapterin (63), $O^t$-thenyl-8-deazapterin (64), $O^t$-piperonyl-8-deazapterin (65), and $O^t$-(4-bromothenyl)-8-deazapterin (66). (Chapter Three)
And finally, we investigated the 7-aza-5,8-deazapterin ring system (2-amino-4-aryloxy[3,4-d]pyrimidine) (67), followed by 1-methyl-5,8-dideazapterin (68) and studied the synthesis of 2-amino-6-chloropurine (69). (Chapter Four)
7- References


58 Unpublished results.
CHAPTER ONE
CHAPTER ONE: Synthesis of 5-deazapterin derivatives

1- Introduction

2-Amino-4-hydroxypteridines or pterins are some of the principal naturally occurring derivatives of pteridines. The origin of pteridine chemistry is associated with wing (Greek, pteron) pigments of butterflies studied for the first time by Frederick G. Hopkins more than 100 years ago. From 1924 to 1926, two of these pigments were further purified by Clemens Schöpf and have been named according to their colours and appearance in nature, xanthopterin (2) and leucopterin (3). In 1933 a third component, isoxanthopterin (4), was isolated. All three compounds turned out to be derivatives of the pyrazino[2,3-d]pyrimidine ring-system termed by Wieland “pteridine”, which is numbered according to the figure (1).

![Diagram of pteridine ring system]

Examination of the pyrazino[2,3-d]pyrimidine structure of pteridines suggests two principal pathways for the synthesis of this ring system, namely, formation of a pyrimidine ring on a pyrazine derivative (pathway a), or formation of a pyrazine ring on a pyrimidine derivative (pathway b) (Scheme 1). Since pyrimidines are more easily accessible, the latter pathway is the more important. Less important methods
include degradations of more complex substances and ring transformations of structurally related bicyclic nitrogen heterocycles.

The most straightforward synthesis of a pteridine is the Gabriel-Isay reaction involving the condensation of a 4,5-diaminopyrimidine with a 1,2-dicarbonyl compound (R'COCR'). When the pyrimidine has an oxygen function in the 6-position and a 2-amino substituent, a "pterin" results (Scheme 2, Pathway a). Analogous condensation reactions with 5-amino-4-(monosubstituted amino)pyrimidines lead to N(\delta)-substituted pteridines (Scheme 2, Pathway b).

2- Synthesis of 5-deazapterin

By a logical extension of the synthesis of pterin where a 4,5-diaminopyrimidine is condensed with an \( \alpha \)-dicarbonyl compound, the most
obvious route to 5-deazapterins (2-amino-(3H)-pyrido[2,3-å]pyrimidin-4-one) is the condensation of a 4-aminopyrimidine with a β-dicarbonyl compound.

Using the method of Bernetti et al., we were able to synthesise 5-deazapterin by condensation of 2,4-diamino-6-hydroxypyrimidine (5) with malonaldehyde bis(dimethylacetal). In this case, the primary product appeared to be the dianil (6), which cyclised to yield 5-deazapterin (7) on treatment with hot concentrated sulfuric acid.

\[
\begin{align*}
\text{(5)} & \quad + \quad \text{CH}_2[\text{CH(OMe)}_2]_2 \\
\begin{array}{c}
\begin{array}{c}
\text{H}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{H}_2\text{N}
\end{array}
\end{array} \\
\rightarrow \\
\begin{array}{c}
\begin{array}{c}
\text{H}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{H}_2\text{N} \\
\text{N} \\
\text{H}_2\text{N}
\end{array}
\end{array}
\end{align*}
\]

\[
\begin{align*}
\text{(6)} & \quad \text{H}_2\text{SO}_4 \\
& \quad 160^\circ\text{C} \\
\begin{array}{c}
\begin{array}{c}
\text{H}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{H}_2\text{N}
\end{array}
\end{array} \\
\rightarrow \\
\begin{array}{c}
\begin{array}{c}
\text{H}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{H}_2\text{N}
\end{array}
\end{array}
\end{align*}
\]

5-Deazapterin was synthesised in good yield (crude 99%), and its structure confirmed by the UV spectrum in 0.1 M HCl, which showed \(\lambda_{\text{max}}\) values of 235, 264 and 343 nm (lit.: 236, 272, 343 nm). The low solubility of this compound in appropriate solvents, did not allow easy investigation by \(^1\text{H} \text{NMR}\), but we still managed to obtain characteristic values such as a broad singlet at \(\delta=7.06\) ppm (NH\(_2\))
group) and three double doublets at δ=8.23 (5-H, J_{5,6}=7.7, J_{5,7}=2.0 Hz), δ=8.61 (7-H, J_{7,5}=2.0, J_{7,6}=4.6 Hz) and δ=7.12 ppm (6-H, J_{6,5}=7.7, J_{6,7}=4.6 Hz) representative of the pyridine ring protons.

In 1993, Ivery et al. improved the reaction conditions used by Bernetti et al. As mentioned before, the above procedure is carried out in two steps with the formation of the dianil intermediate (6). Ivery et al. noted that in the presence of hydrochloric acid and sodium bisulfite at 70°C, the reaction becomes a 'one-pot' synthesis. The acid was added initially to hydrolyse the acetal and start the condensation. Unidentified coloured intermediates started to form. They were removed by addition of sodium bisulfite. More acid was then added, followed by bisulfite, until the reaction was complete. The mixture was acidified (pH 1-2) to yield 65% of 5-deazapterin. The UV spectrum of the sample was identical to that reported before.

3- Synthesis of 2-amino-4-chloro-5-deazapteridine

The next stage of the investigation was the conversion of the 4-oxygen substituent into a chlorine, using a phosphorus halide, POCl₃, which is known to be a very effective reagent in heterocyclic chemistry. In 1987, Seela et al. synthesized 2-amino-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (9) from 2-amino-7H-pyrrolo[2,3-d]pyrimidine-4(3H)-one (8) using POCl₃ and dimethylaniline.

\[
\begin{align*}
\text{(8)} & \quad \xrightarrow{\text{POCl}_3, \text{PhNMMe}_2} \quad \text{reflux for 2h at 110°C} \quad \text{(9)}
\end{align*}
\]
In the present work, using the same conditions but with 5-deazapterin (7) as starting material, some 2-amino-4-chloro-5-deazapteridine (10) could be identified in the almost insoluble crude product. The $^1$H NMR of (10) showed very similar features to those reported for 5-deazapterin, with a broad singlet at $\delta=7.07$ ppm for the amino group and three double doublets at $\delta=7.13$, 8.22, and 8.63 ppm for the pyridine ring protons. The difference is a missing peak at $\delta=7.06$ ppm which was present in the spectrum of 5-deazapterin. This broad singlet corresponded to the $N^4$-proton and its disappearance confirmed the presence of a tertiary nitrogen and a chloro substitution at the 4-position.

\[
\begin{align*}
\text{(7)} & \quad \text{POCl}_3, \text{PhNMMe}_2 \\ 
\text{reflux for 2h at 110°C} & \quad \text{(10)}
\end{align*}
\]

4- Synthesis of $O^4$-substituted-5-deazapterin

4.1- Starting from 2-amino-4-chloro-5-deazapteridine

Crude (10), benzyl alcohol and sodium hydride in a ratio of 1:3:1.1 were heated at 110°C in DMSO and the reaction progress followed by UV spectral measurements in methanol. Even after 6 hours, no change in the UV data was observed, therefore suggesting that no reaction was taking place. Even with the very insoluble nature of the chloro compound in DMSO or other organic solvents, we were, nevertheless, expecting some reaction. Attention was then focused on finding a better leaving group than chloride in this addition-elimination mechanism leading to displacement of the 4-substituent by a nucleophile.
Ashwell et al.\textsuperscript{8} reported a two-stage procedure for the preparation of guanines from 2-amino-6-chloropurines. The 6-chloro group was displaced with trimethylamine at 0°C to give a 2-amino-6-trimethylammonium purine salt (Scheme 3).

![Scheme 3]

Trimethylammonium is a better leaving group than chloride. However, the use of trimethylamine is problematic, especially on a large scale, because of its volatility, toxicity and unpleasant odour. Furthermore, the nucleophilic displacement of the trimethylammonium group (\textit{S}_\text{N}2 type) has the disadvantage of competing with the degradation of this group into a dimethylamino group (Scheme 4).

![Scheme 4]

In 1997, Lembicz et al.\textsuperscript{9} found that trimethylamine could be replaced by 1,4-diazabicyclo-[2.2.2]octane (DABCO) and reported a few reactions with 6-chloropurines. The resulting compounds, 'DABCO-purines' (Scheme 5), were
found to undergo facile displacement reactions with alkoxides to afford 6-alkoxypurines.

![Reaction Scheme](image)

(a) $X = NH_2$, $Y = H$
(b) $X = Y = H$
(c) $X = Cl$, $Y = H$
(d) $X = NH_2$, $Y = \text{ribose}$

Scheme 5

The reaction with 2-amino-4-chloro-5-deazapteridine (10) was carried out in DMF. In the first attempt the chloro compound failed to dissolve in the amount of solvent used, even after heating. Nevertheless, assuming that some of the compound went into solution, 1 eq. of DABCO was then added to the suspension and the reaction followed by UV spectral measurements. No changes, whatsoever, were noticeable even after 12 hours.

4.2- Using the Mitsunobu reaction

4.2.1 The pivaloyl group

Attention was next turned to the synthesis of a 5-deazapterin derivative, which would be more soluble in organic solvents.

In 1987, Taylor and Ray\textsuperscript{10} reported that 6-chloropterin (11) was too insoluble in organic solvents to be used in certain reactions. However, they found that pivaloylation of (11) yields $N^2$-pivaloyl-6-chloropterin (12), which is readily soluble
in a variety of organic solvents. The derivatisation of (11) was carried out by heating with pivalic anhydride in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP).

![Chemical structure of (11) and (12)]

Application of this procedure to 5-deazapterin (7) yields 74% of \(N^2\)-pivaloyl-5-deazapterin (13), which, too, was found to be soluble in many organic solvents. \(^1\)H and \(^{13}\)C NMR in d$_6$-DMSO confirmed its structure.

![Chemical structure of (7) and (13)]

The next stage of the investigation was to find an efficient method for the \(O^\prime\)-alkylation. Himmelsbach and his team\textsuperscript{11} conducted a series of unsuccessful attempts to displace the 6-substituent in 6-chloro- and 6-methylsulfonyl-2-amino-9-β-D-ribofuranosylpurine nucleophilically by \(p\)-nitrophenylethanol. He found that the "Mitsunobu reaction"\textsuperscript{12, 13} offered a good chance of \(O^\prime\)-alkylation if the 2-amino group is acylated. This substituent hinders a possible reaction on the adjacent \(N\)-1 ring atom.
4.2.2- Introduction to the Mitsunobu reaction

Alkyl-and aryl-phosphines react with compounds having weak heteroatom-heteroatom bonds, such as S-S or O-O or with azo compounds to form reactive phosphonium salts. These salts in turn promote “redox” condensation reactions with compounds having active hydrogens. The condensation reaction of alcohols using the redox couple of a triaryl- or trialkylphosphine and dialkyl azodicarboxylate is known as the Mitsunobu reaction.12, 13 The overall reaction is summarised below (Scheme 6). The alcohol (R-OH) and the acidic compound (H-X) are condensed to form the product (R-X), while triphenylphosphine is oxidised to triphenylphosphine oxide and the azodicarboxylate is reduced to the hydrazo-ester.

Scheme 6

The Mitsunobu reaction proceeds in four steps:

Step 1: Formation of a quaternary phosphonium salt
Step 2: Protonation of the salt

\[
\begin{array}{c}
\text{HX} + \left[ (\text{CH}_3)_2\text{CHO} - \text{C} = \text{N} - \text{N} = \text{C} - \text{OCH}(\text{CH}_3)_2 \right] \text{Bu}_3\text{P}^\ominus \\
\rightarrow \left[ (\text{CH}_3)_2\text{CHO} - \text{C} = \text{N} - \text{N} = \text{C} - \text{OCH}(\text{CH}_3)_2 , \text{X}^\ominus \right] \text{Bu}_3\text{P}^\ominus
\end{array}
\]

Step 3: Formation of an alkoxy phosphonium salt

\[
\begin{array}{c}
\left[ (\text{CH}_3)_2\text{CHO} - \text{C} = \text{N} - \text{N} = \text{C} - \text{OCH}(\text{CH}_3)_2 , \text{X}^\ominus \right] \text{Bu}_3\text{P}^\ominus \\
\rightarrow \text{ROH} \rightarrow \left[ (\text{CH}_3)_2\text{CHO} - \text{C} = \text{NH} \right]_2 + \left[ \text{Bu}_3\text{P}^\ominus - \text{OR} , \text{X}^\ominus \right]
\end{array}
\]

Step 4: S_N2 type displacement of the resulting species

\[
\left[ \text{Bu}_3\text{P}^\ominus - \text{OR} , \text{X}^\ominus \right] \rightarrow \text{Bu}_3\text{P} = \text{O} + \text{R} - \text{X}
\]

Treatment of \(N^2\)-pivaloyl-5-deazapterin with 1.5 molecular equivalents each of diisopropyl azodicarboxylate (cheaper and safer than the original diethyl azodicarboxylate\(^{11, 13}\)), tributylphosphine (more soluble than the phenyl derivative\(^{11}\)) and thenyl alcohol, in THF at room temperature led after 2h to the thenyl derivative (14) in 47 % yield. The corresponding benzyl (15), piperonyl (16) and 4-bromothenyl (17) derivatives were formed in a similar manner. The yield, \(\lambda_{\text{max}}\) and melting point of the four compounds are reported in (Table 1). 

\(^{1}\)H and \(^{13}\)C NMR confirmed the four structures.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Yield (%)</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(14)</td>
<td>47</td>
<td>272, 311</td>
<td>107-108</td>
</tr>
<tr>
<td>(15)</td>
<td>48</td>
<td>276, 312</td>
<td>144-145</td>
</tr>
<tr>
<td>(16)</td>
<td>46</td>
<td>276, 313</td>
<td>110-112</td>
</tr>
<tr>
<td>(17)</td>
<td>37</td>
<td>273, 313</td>
<td>100-101</td>
</tr>
</tbody>
</table>

| Table 1 |

4.2.3- Removal of the pivaloyl group

4.2.3.1- Using aqueous sodium hydroxide

The $N^2$-pivaloyl group was readily removed by hydrolysis with 3 M aqueous sodium hydroxide and ethanol in a ratio 2:1 at 90°C to yield after 24h 51, 55, 53 and 27 % of depivaloylated products. These were expected to be $O'$-thenyl-5-deazapterin (18), $O'$-benzyl-5-deazapterin (19), $O'$-piperonyl-5-deazapterin (20) and $O'$-(4-bromothenyl)-5-deazapterin (21) respectively.
The yield, $\lambda_{\text{max}}$ and melting point of the latter compounds are reported in (Table 2).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Yield (%)</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(22)</td>
<td>51</td>
<td>248, 309</td>
<td>215-216</td>
</tr>
<tr>
<td>(23)</td>
<td>55</td>
<td>248, 308</td>
<td>201-202</td>
</tr>
<tr>
<td>(24)</td>
<td>53</td>
<td>243, 292, 309</td>
<td>220-221</td>
</tr>
<tr>
<td>(25)</td>
<td>27</td>
<td>248, 308</td>
<td>229-230</td>
</tr>
</tbody>
</table>

Table 2

The carbon on the 2-position of the ring system, surrounded by three electron-withdrawing nitrogen atoms, is more electrophilic than the carbon of the carbonyl group of the pivaloyl group. Therefore the nucleophilic attack from the hydroxide ion is more likely to happen on C-2. The pivaloyl group, due to steric hindrance,
becomes a good leaving group. The mechanism of the reaction is shown below (Scheme 7).

Attention was then focused on finding milder conditions for the removal of the pivaloyl group.

Some experiments were undertaken in ethanol using decreasing concentrations of sodium hydroxide (1.5 M, 0.75 M, 0.1 M). The reactions were followed by TLC with DCM and methanol in a 9:1 ratio as solvent system. The formation of a mixture (3 components) was detected after one hour. The latter was identified as, e.g., a
mixture of the amino compound (18), the oxo compound (22) and the remaining starting material (14).

Other attempts at hydrolysing the amide bond using 1 M $\text{K}_2\text{CO}_3$ in ethanol, either at reflux or at room temperature, failed to deliver the desired amino compounds. Attempts in aqueous ammonia at different concentrations (2 %, 4 % and 10 %) gave only slight conversion to the amine.

It seemed then, that the pivaloyl group could not easily be removed without hydrolytic deamination.

4.2.3.2- Using hydrazine monohydrate

In 1990, Espie et al.\textsuperscript{14} reported that reaction with hydrazine monohydrate in ethanol at room temperature leads to rapid and quantitative removal of the acetyl group in acetamidopyrimidines.

Application of this method to the pivaloyl derivatives (14-17) resulted in an immediate reaction. After 30 seconds crystals started to form, and within 10 minutes the conversion to the amino derivatives (18-21) was completed in a 32 % average yield (Table 3).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Yield (%)</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>m.p. (°C)</th>
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<tr>
<td>(18)</td>
<td>31</td>
<td>231, 268, 303</td>
<td>313-314</td>
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<tr>
<td>(19)</td>
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<td>323-325</td>
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<tr>
<td>(20)</td>
<td>33</td>
<td>231, 262, 292</td>
<td>304-305</td>
</tr>
<tr>
<td>(21)</td>
<td>30</td>
<td>230, 267, 302</td>
<td>308-309</td>
</tr>
</tbody>
</table>

Table 3
The mechanism of the reaction is shown in (Scheme 8). The proton NMR spectra showed a broad singlet around $\delta=7.60$ ppm characteristic of the amino group, and the microanalysis results confirmed their structures.
Those samples (18-21) were found inactive as inhibitors of the protein ATase (IC$_{50}$ > 3000 μM) when sent to the Paterson Institute (Manchester).

5- Experimental

**Preparation of 5-deazapterin (2-amino-(3H)-pyrido[2,3-d]pyrimidin-4-one)**

(a) A hot solution of 2,4-diamino-pyrimidin-6-one (5.6 g, 44.40 mmol) in water (200 ml) and acetic acid (0.5 ml), filtered to remove impurities, was treated with malonaldehyde bis(dimethylacetal) (3.8 g, 23.14 mmol) and water (50 ml), and the mixture heated for 3h at 95°C. The reaction mixture was allowed to cool down. The resulting precipitate was filtered and washed with aqueous sodium hydrogen carbonate (10 %) to give white crystals of the dianil (6.21 g, 97 %).

The dianil (3.0 g, 10.42 mmol) and concentrated sulphuric acid (30 ml) were heated for 2h at 160°C, cooled, and poured onto crushed ice. The mixture was diluted with water to 450 ml. Gradual neutralisation with sodium hydrogen carbonate gave 5-deazapterin as a brown solid (1.70 g, 99 %).

m.p. >360°C (lit. $>360°C$).

$\lambda_{\text{max}}(0.1N \text{ HCl})$ 235, 268, 343 nm (lit. $236, 272, 343$ nm).

NMR(DMSO-d$_6$): $\delta_H (300MHz)$ 7.06(2H, s, NH$_2$), 7.12(1H, dd, 6-H), 8.23(1H, dd, 5-H), 8.61(1H, dd, 7-H), (J$_{6-H, 5-H}$ 7.7, J$_{6-H, 7-H}$ 4.7, J$_{5-H, 7-H}$ 2.0Hz), 11.44(1H, s, NH).

(b) A mixture of 2,4-diamino-pyrimidin-6-one (0.2 g, 1.59 mmol) and hydrochloric acid (0.01 M; 20 ml) was treated with malonaldehyde bis(dimethylacetal) (0.26 g, 1.58 mmol) and heated at 70°C. After 5 min the solution

* The concentration required to produce 50 % inactivation of ATase.
turned pink. Sodium metabisulfite was then added to discharge the colour. The solution was re-acidified and heated until the pink colour reappeared (30 min). The reaction was followed by UV spectral measurements in 0.01 M HCl and needed four cycles of metabisulfite addition, re-acidification and heating for completion. The pH was brought to 1-2 and the solution cooled to give 5-deazapterin as a light-pink solid (0.17 g, 65%).

m.p. >360°C (lit.6 >360°C).

λ\text{max} (0.1\text{M NaOH}) 240, 264, 330 nm (lit.6 239, 264, 329 nm).

Preparation of 2-amino-4-chloro-5-deazapteridine (2-amino-4-chloropyrido[2,3-d]pyrimidine) (10)

A solution of 5-deazapterin (1.4 g, 8.64 mmol) in POCI₃ (11 ml) and \(N,N\)-dimethylaniline (0.44 ml) was heated under reflux for 2 h at 110°C. The reaction mixture was then allowed to cool and the excess of POCI₃ removed by distillation. The remaining black tar was added slowly to ice, gently heated for 30 min and then allowed to cool to room temperature. The insoluble material was removed by filtration and the filtrate brought to pH 12 with concentrated NH₄OH. The precipitate formed, was collected and washed with water to give a cream-coloured solid (1.09 g). Most of this was insoluble in organic solvents, but a little of the desired chloride (10) was extracted into DMSO.

m.p. 325-327°C.

NMR(DMSO-d₆): \(\delta\)H (300MHz) 7.07(2H, s, NH₂), 7.13(1H, dd, 6-H), 8.22(1H, dd, 5-H), 8.63(1H, dd, 7-H), (J₆-H,5-H 7.7Hz, J₆-H,7-H 4.7Hz, J₅-H,7-H 2.0Hz).
Preparation of $N^2$-pivaloyl-5-deazapterin (2-pivaloylamino-(3H)-pyrido[2,3-d]pyrimidin-4-one) (13)

A mixture of 5-deazapterin (2.0 g, 12.35 mmol), 4-dimethylaminopyridine (0.22 g, 1.80 mmol) and pivalic anhydride (12 ml) was heated under nitrogen for 5h at 165°C. Excess pivalic anhydride was removed by distillation. The residue was dissolved in dichloromethane and the solution applied to a pad of silica gel, which was eluted with dichloromethane-methanol (49:1). Evaporation and recrystallisation of the product from ethanol gave shiny cream-coloured crystals (2.25 g, 74 %) of the pivaloyl derivative.

m.p. 258-259°C.

(Found: C, 58.58; H, 5.77; N, 22.58 %. C$_{12}$H$_{14}$N$_4$O$_2$ requires: C, 58.53; H, 5.69; N, 22.76 %).

$\lambda_{\text{max}}$(MeOH) 277 nm.

NMR(DMSO-d$_6$): $\delta$H(300MHz) 1.28(9H, s, 3CH$_3$), 7.44(1H, dd, 6-H), 8.43(1H, dd, 5-H), 8.88(1H, dd, 7-H), (J$_{6-H,5-H}$ 7.8, J$_{6-H,7-H}$ 4.6, J$_{5-H,7-H}$ 2.0Hz), 11.40(1H, s, $N^2$-H), 12.31(1H, s, $N^2$-H).

$\delta$C(100MHz) 26.2(CH$_3$), 114.8(q-C$_2$), 120.7(C$_6$-H), 135.6(C$_5$-H), 150.3(q-C$_4$), 151.4(q-C$_8$), 154.0(C$_7$-H), 161.2(q-C$_4$), 182.1(C=O).

Preparation of $N^2$-pivaloyl-O$_4$-thenyl-5-deazapterin (2-pivaloylamino-4-thenyloxypyrido[2,3-d]pyrimidine) (14)

A suspension of $N^2$-pivaloyl-5-deazapterin (0.492 g, 2mmol) in tetrahydrofuran (8 ml) was stirred for 10 min, and tributylphosphine (0.606 g, 3 mmol), thenyl alcohol (0.342 g, 3 mmol) and diisopropyl azodicarboxylate (0.590 ml, 3 mmol) were added in succession. The reaction was allowed to proceed
for 2h at room temperature. Removal of the solvent gave an oil. Hexane was added to induce crystallisation. Filtration and recrystallisation from hexane gave bright yellow crystals of the thenyl derivative (0.32 g, 47 %).

m.p. 107-108°C.

(Found: C, 59.82; H, 5.35; N, 16.22; S, 9.25 %. C₁₇H₁₈N₄O₂S requires: C, 59.65; H, 5.26; N, 16.37; S, 9.36 %).

λₘₐₓ(MeOH) 272, 311 nm.

NMR(DMSO-d₆): δ_H(300MHz) 1.28(9H, s, 3CH₃), 5.86(2H, s, CH₂), 6.98(1H, dd, 4'-H), 7.28(1H, dd, 3'-H), 7.43(1H, dd, 5'-H); (J₄'-H, 3'-H 3.5, J₄'-H, 5'-H 5.3, J₃'-H, 5'-H 1.1 Hz), 7.52(1H, dd, 6-H), 8.46(1H, dd, 5-H), 8.89(1H, dd, 7-H); (J₆-H, 5-H 7.8, J₆-H, 7-H 4.6, J₅-H, 7-H 2.0 Hz), 13.25(1H, s, NH).

δ_C(75.5MHz) 27.5(CH₃), 41.9(CH₂), 112.6(q-C₂), 116.0(C₄-H), 120.6(C₆-H), 126.3(C₃'-H), 128.1(C₅'-H), 137.0(C₅-H), 138.1(q-C₂'), 150.4(q-C₄a), 152.2(q-C₈a), 154.6(C₇-H), 156.2(C=O), 158.6(q-C₄).

Preparation of O'-benzyl-N'-pivaloyl-5-deazapterin (4-benzyloxy-2-pivaloylamino-pyrido[2,3-d]pyrimidine) (15)

The same procedure was followed, but using benzyl alcohol (0.324 g, 3 mmol) instead of thenyl alcohol. Filtration and recrystallisation from hexane gave bright yellow crystals of the O'-benzyl derivative (0.324 g, 48 %).

m.p. 144-145°C.

(Found: C, 67.81; H, 6.07; N, 16.48 %. C₁₉H₂₀N₄O₂ requires: C, 67.86; H, 5.95; N, 16.67 %).

λₘₐₓ(MeOH) 276, 312 nm.
NMR(DMSO-d6): $\delta_H(300 \text{MHz})$ 1.12(9H, s, 3CH$_3$), 5.72(2H, s, CH$_2$), 7.29(3H, m, Ph), 7.39(2H, m, Ph), 7.49(1H, dd, 6-H), 8.48(1H, dd, 5-H), 8.81(1H, dd, 7-H), (J$_{6-H, 5-H}$ 7.7, J$_{6-H, 7-H}$ 4.7, J$_{5-H, 7-H}$ 2.0Hz), 13.24(1H, s, NH).

NMR(DMSO-d6): $\delta_H(300 \text{MHz})$ 1.12(9H, s, 3CH$_3$), 5.72(2H, s, CH$_2$), 7.29(3H, m, Ph), 7.39(2H, m, Ph), 7.49(1H, dd, 6-H), 8.48(1H, dd, 5-H), 8.81(1H, dd, 7-H), (J$_{6-H, 5-H}$ 7.7, J$_{6-H, 7-H}$ 4.7, J$_{5-H, 7-H}$ 2.0Hz), 13.24(1H, s, NH).

Preparation of $O^t$-piperonyl-N$^2$-pivaloyl-5-deazapterin (4-piperonyloxy-2-pivaloylamino-pyrido[2,3-d]pyrimidine) (16)

The same procedure was followed, using piperonyl alcohol (0.228 g, 1.5 mmol). Filtration and recrystallisation from hexane gave bright yellow crystals of the $O^t$-piperonyl derivative (0.176 g, 46 %).

m.p. 110-112°C.
(Found: C, 63.11; H, 5.43; N, 14.52 %. C$_{26}$H$_{20}$N$_4$O$_3$ requires C, 63.16; H, 5.26; N, 14.74 %).

$\lambda_{\text{max}}$ (MeOH) 276, 313 nm.

Preparation of $O^t$-(4-bromothenyl)-N$^2$-pivaloyl-5-deazapterin (4-(4-bromothenyloxy-2-pivaloylamino-pyrido[2,3-d]pyrimidine) (17)

The same procedure using 4-bromothenyl alcohol (0.579 g, 3 mmol) was followed. Removal of the solvent gave a yellow oil. Separation by column
(hexane/ethyl acetate, 9:1) and evaporation gave a colourless oil. Hexane was then added to induce crystallisation.

Filtration and recrystallisation from hexane gave white crystals of the 4-bromothenyl derivative (0.31 g, 37%).

m.p. 100-101°C.

(Found: C, 48.36; H, 4.28; N, 13.61; S, 9.05 %. C_{17}H_{17}BrN_{2}O_{2}S requires: C, 48.46; H, 4.04; N, 13.30; S, 9.50 %).

$\lambda_{\text{max}}$ (MeOH) 273, 313 nm.

**NMR (DMSO-d$_6$):** $\delta$ (400 MHz) 1.24 (9H, s, 3CH$_3$), 5.79 (2H, s, CH$_2$), 7.21 (1H, s, 5'-H), 7.50 (1H, dd, 6-H), 7.55 (1H, s, 3'-H), 8.44 (1H, dd, 5-H), (J$_{6-5, 7}$ 7.7 Hz, J$_{6-5, 7}$ 4.7 Hz, J$_{5-7}$ 1.9 Hz), 8.85 (1H, dd, 7-H), 13.18 (1H, s, NH).

$\delta_c$ (100 MHz) 27.4 (CH$_3$), 40.5 (CH$_2$), 108.4 (q-C$_2$), 112.7 (q-C$_3$), 120.7 (C$_6$-H), 123.9 (C$_5$-H), 130.0 (C$_5$-H), 137.0 (C$_7$-H), 140.2 (q-C$_2'$), 150.1 (q-C$_{4a}$), 152.3 (q-C$_{8a}$), 154.6 (C$_7$-H), 156.2 (C=O), 158.4 (q-C$_4$).

**Preparation of O'-thenyl-5-deazalumazine (4-thenyloxy-(1H)-pyrido[2,3-d]pyrimidin-2-one) (22)**

$N^2$-pivaloyl-O'$'$-thenyl-5-deazapterin (0.28 g, 0.82 mmol) was heated 24 h under reflux with aqueous NaOH (3M; 2 ml) and ethanol (1 ml). The solvent was removed by evaporation and the residual solid dissolved in water. Acidification with acetic acid gave a white precipitate. Filtration and recrystallisation from ethanol gave white crystals of O'$'$-thenyl-5-deazalumazine (0.107 g, 51 %).

m.p. 215-216°C.

(Found: C, 55.49; H, 3.86; N, 15.55; S, 12.41 %. C$_{12}$H$_6$N$_3$O$_2$S requires: C, 55.59; H, 3.50; N, 16.21; S, 12.36 %).
$\lambda_{\text{max}}$(MeOH) 248, 309nm.

NMR(DMSO-d$_6$): $\delta_{\text{H}}$(300MHz) 5.54(2H, s, CH$_2$), 6.97(1H, dd, 4'-H), 7.17(1H, dd, 3'-H), 7.38(1H, dd, 5'-H), (J$_{4'-H}$, 3'-H 3.5, J$_{4'-H}$, 5'-H 5.3, J$_{3'-H}$, 5'-H 1.1Hz), 7.41(1H, dd, 6-H), 8.39(1H, dd, 5-H), (J$_{6-H}$, 5-H 7.5, J$_{6-H}$, 7-H 4.7, J$_{5-H}$, 7-H 2.0Hz), 8.80(1H, dd, 7-H), 11.87(1H, s, NH).

Synthesis of O'-benzyl-5-deazalumazine (4-benzyloxy-(1H)-pyrido[2,3-d]pyrimidin-2-one) (23)

The same procedure with O'-benzyl-N$^2$-pivaloyl-5-deazapterin (0.26 g, 0.77 mmol) was followed. Recrystallisation from ethanol gave white crystals of O'-benzyl-5-deazalumazine (0.107 g, 55 %).

m.p. 201-202°C.

(Found: C, 66.49; H, 4.37; N, 16.51 %. C$_{14}$H$_{11}$N$_3$O$_2$ requires: C, 66.40; H, 4.38; N, 16.59 %).

$\lambda_{\text{max}}$(MeOH) 248, 308nm.

NMR(DMSO-d$_6$): $\delta_{\text{H}}$(300MHz) 5.41(2H, s, CH$_2$), 7.26(3H, m, Ph), 7.30(2H, m, Ph), 7.25(1H, dd, 6-H), 8.40(1H, dd, 5-H), 8.70(1H, dd, 7-H), (J$_{6-H}$, 5-H 7.8, J$_{6-H}$, 7-H 4.7, J$_{5-H}$, 7-H 2.0Hz), 11.87(1H, s, NH).

$\delta_{\text{C}}$(100MHz) 43.9(CH$_2$), 111.3(q-C$_2$), 119.1(C$_6$-H), 127.0(3×CH, Ph), 128.3(2×CH, Ph), 137.0(q-C$_1$), 137.3(C$_5$-H), 150.6(q-C$_4$), 151.6(q-C$_8$), 154.1(C$_7$-H), 161.2(q-C$_4$).
Synthesis of \(O^\prime\)-piperonyl-5-deazalumazine (4-piperonyloxy-(1H)-pyrido2,3-d|pyrimidin-2-one) (24)

The same procedure with \(O^\prime\)-piperonyl-N\(^\prime\)-pivaloyl-5-deazapterin (0.12 g, 0.32 mmol) was followed. Recrystallisation from ethanol gave white crystals of \(O^\prime\)-piperonyl-5-deazalumazine (0.049 g, 53 %).

\textbf{m.p.} 220-221°C.

(Found: C, 60.32; H, 3.65; N, 14.00 %. \(\text{C}_{15}\text{H}_{11}\text{N}_{3}\text{O}_{4}\) requires: C, 60.60; H, 3.73; N, 14.14 %).

\(\lambda_{\text{max}}\) (MeOH) 243, 292, 309 nm.

\textbf{NMR(DMSO-d6):} \(\delta_{\text{H}}\) (400MHz) 5.28(2H, s, CCH\(_2\)O), 5.95(2H, s, OCH\(_2\)O), 6.812(1H, d, 2'-H), 6.815(1H, d, 3'-H), (J\(_{2'-H, 3'-H}\) 8.0Hz), 6.92(1H, s, 6'-H), 7.33(1H, dd, 6-H), 8.36(1H, dd, 5-H), 8.69(1H, dd, 7-H), (J\(_{6-H, 5-H}\) 7.7, J\(_{6-H, 7-H}\) 4.6, J\(_{5-H, 7-H}\) 2.0Hz), 11.83(1H, s, NH).

Synthesis of \(O^\prime\)-(4-bromothenyl)-5-deazalumazine (4-(4-bromothenyl)oxy-(1H)-pyrido2,3-d|pyrimidin-2-one) (25)

The same procedure with \(O^\prime\)-(4-bromothenyl)-N\(^\prime\)-pivaloyl-5-deazapterin (0.12 g, 0.285 mmol) was followed. Recrystallisation from ethanol gave white crystals of \(O^\prime\)-(4-bromothenyl)-5-deazalumazine (0.026 g, 27 %).

\textbf{m.p.} 229-230°C.

(Found: C, 42.68; H, 2.71; N, 12.87; S, 8.64 %. \(\text{C}_{12}\text{H}_{8}\text{BrN}_{3}\text{O}_{2}\text{S}\) requires: C, 42.62; H, 2.38; N, 12.42; S, 9.48 %).

\(\lambda_{\text{max}}\) (MeOH) 248, 308nm.
NMR(DMSO-d$_6$): $\delta_H$(400MHz) 5.49(2H, s, CH$_2$), 7.12(1H, s, 5'-H), 7.37(1H, dd, 6-H), 7.53(1H, s, 3'-H), 8.37(1H, dd, 5-H), 8.76(1H, dd, 7-H), (J$_{6-H}$, 5-H 7.6, J$_{6-H}$, 7-H 4.7, J$_{5-H}$, 7-H 1.8Hz), 11.85(1H, s, NH).

$\delta_C$(100MHz) 41.9(CH$_2$), 107.8(q-C$_4$), 111.4(q-C$_2$), 119.3(C$_6$-H), 123.6(C$_5$-H), 129.3(C$_3$-H), 137.1(C$_5$-H), 141.0(q-C$_2$), 150.2(q-C$_{4a}$), 150.9(q-C$_{8a}$), 153.9(C$_7$-H), 161.1(q-C$_4$).

**Synthesis of O'-thenyl-5-deazapterin (2-amino-4-thenyloxypyrido[2,3-d]pyrimidine)**

(18)

Hydrazine monohydrate (0.568 ml, 11.6 mmol) was added to a solution of $N^2$-pivaloyl-O'thenyl-5-deazapterin (0.200 g, 0.58 mmol) in ethanol (6 ml). The reaction was allowed to proceed for 10 min at room temperature. Filtration and recrystallisation from ethanol gave O'-thenyl-5-deazapterin as a white solid (0.046 g, 31%).

m.p. 313-314°C.

(Found: C, 55.61; H, 4.05; N, 21.47 %. C$_{12}$H$_{10}$N$_4$O requires: C, 55.81; H, 3.88; N, 21.71 %).

$\lambda_{max}$(MeOH) 231, 268, 303 nm.

NMR(DMSO-d$_6$): $\delta_H$(400MHz) 5.73(2H, s, CH$_2$), 6.97(1H, dd, 4'-H), 7.22(1H, dd, 3'-H), 7.38(1H, dd, 5'-H), (J$_{4'-H}$, 3'-H 3.5, J$_{4'-H}$, 5'-H 5.3, J$_{3'-H}$, 5'-H 1.1Hz), 7.40( 1H, dd, 6-H), 7.67(2H, s, NH$_2$), 8.30(1H, dd, 5-H), 8.69(1H, dd, 7-H), (J$_{6-H}$, 5-H 7.6, J$_{6-H}$, 7-H 5.0, J$_{5-H}$, 7-H 2.0 Hz).

$\delta_C$(100MHz) 40.0(CH$_2$), 113.7(q-C$_2$), 120.0(C$_6$-H), 126.1(C$_5$-H), 126.5(C$_4$-H), 127.2(C$_3$-H), 136.4(C$_5$-H), 138.1(q-C$_2$), 150.6(q-C$_{4a}$), 151.8(q-C$_{8a}$), 156.1(C$_7$-H), 167.1(q-C$_4$).
Synthesis of \(O^\prime\)-benzyl-5-deazapterin (2-amino-4-benzylloxypyrido[2,3-d]pyrimidine) (19)

The same procedure with \(O^\prime\)-benzyl-\(N^\prime\)-pivaloyl-5-deazapterin (0.200 g, 0.60 mmol) was followed. Filtration and recrystallisation from ethanol gave white crystals of \(O^\prime\)-benzyl-5-deazapterin (0.051 g, 34 %).

\textit{m.p.} 324-325°C.

(Found: C, 66.57; H, 4.98; N, 21.70 %. \(\text{C}_{14}\text{H}_{12}\text{N}_{4}\text{O}\) requires: C, 66.67; H, 4.76; N, 22.22 %).

\(\lambda_{\text{max}}(\text{MeOH})\) 231, 268, 303 nm.

\textit{NMR(DMSO-d_6)}: \(\delta_H(400\text{MHz})\) 5.61(2H, s, CH\_2), 7.24(5H, m, Ph), 7.35(1H, dd, 6-H), 7.51(2H, s, NH\_2), 8.32(1H, dd, 5-H), 8.58(1H, dd, 7-H), (J\_6-H, 5-H 7.6, J\_6-H, 7-H 5.0, J\_5-H, 7-H 2.0 Hz).

\(\delta_C(100\text{MHz})\) 44.5(CH\_2), 113.6(q-C\_2), 119.9(C\_6-H), 126.7(3\times\text{CH}, Ph), 128.5(2\times\text{CH}, Ph), 136.2(q-C\_1\_), 136.3(C\_5-H), 151.2(q-C\_4\_a), 152.1(q-C\_8\_a), 156.8(C\_7-H), 167.2(q-C\_4).

Synthesis of \(O^\prime\)-piperonyl-5-deazapterin (2-amino-4-piperonyloxypyrido[2,3-d]pyrimidine) (20)

The same procedure with \(O^\prime\)-piperonyl-\(N^\prime\)-pivaloyl-5-deazapterin (0.200 g, 0.53 mmol) was followed. Filtration and recrystallisation from ethanol gave white crystals of \(O^\prime\)-piperonyl-5-deazapterin (0.051 g, 33 %).

\textit{m.p.} 304-305°C.

(Found: C, 59.93; H, 4.33; N, 18.05 %. \(\text{C}_{15}\text{H}_{12}\text{N}_{4}\text{O}_3\) requires: C, 60.81; H, 4.05; N, 18.92 %).

\(\lambda_{\text{max}}(\text{MeOH})\) 231, 262, 292 nm.
NMR(DMSO-d$_6$): $\delta_{H}(400\text{MHz})$ 5.50(2H, s, CCH$_2$O), 5.97(2H, s, OCH$_2$O), 6.65(1H, d, 2'-H), 6.81(1H, d, 3'-H), (J$_{2'}$-$H_1$, 3'-H 8.0 Hz), 6.84(1H, s, 6'-H), 7.36(1H, dd, 6-H), 7.50(2H, s, NH$_2$), 8.31(1H, dd, 5-H), 8.62(1H, dd, 7-H), (J$_{6-H_1}$, 5.4 Hz 7.8, J$_{6-H_1, 7-H}$ 4.8, J$_{5-H_1, 7-H}$ 2.0 Hz).

$\delta_C(100\text{MHz})$ 44.1(CCH$_2$O), 100.9(OCH$_2$O), 107.4(C$_3$-H), 108.2(C$_6$-H), 113.6(q-C$_2$), 119.6(C$_2$-H), 119.9(C$_6$-H), 129.8(q-C$_4$), 136.3(C$_5$-H), 146.3(q-C$_5$), 147.3(q-C$_4$), 151.1(q-C$_{4a}$), 152.0(q-C$_{8a}$), 156.7(C$_7$-H), 167.2(q-C$_4$).

**Synthesis of O'-{(4-bromothenyl)-5-deazapterin (2-amino-4-(4-bromothenyl)oxypyrido[2,3-d]pyrimidine) (21)}**

The same procedure with O'-(4-bromothenyl)-$N'$-pivaloyl-5-deazapterin (0.170 g, 0.4 mmol) was followed. Filtration and recrystallisation from ethanol gave white crystals of O'-(4-bromothenyl)-5-deazapterin (0.041 g, 30 %).

m.p. 308-309°C.

(Found: C, 43.06; H, 2.94; N, 15.99 %. C$_{12}$H$_9$BrN$_4$OS requires: C, 42.73; H, 2.67; N, 16.62 %).

$\lambda_{\text{max}}(\text{MeOH})$ 230, 267, 302 nm.

NMR(DMSO-d$_6$): $\delta_{H}(400\text{MHz})$ 5.69(2H, s, CH$_2$), 7.23(1H, s, 5'-H), 7.40(1H, dd, 6-H), 7.54(1H, s, 3'-H), 7.68(2H, s, NH$_2$), 8.31(1H, dd, 5-H), 8.69(1H, dd, 7-H), (J$_{6-H_1, 5-H}$ 7.8, J$_{6-H_1, 7-H}$ 4.8, J$_{5-H_1, 7-H}$ 2.0 Hz).

$\delta_C(100\text{MHz})$ 40.4(CH$_2$), 107.8(q-C$_4$), 113.8(q-C$_2$), 120.2(C$_6$-H), 123.9(C$_5$-H), 129.3(C$_3$-H), 136.5(C$_7$-H), 140.3(q-C$_2$), 150.5(q-C$_{4a}$), 151.8(q-C$_{8a}$), 156.1(C$_7$-H), 167.0(q-C$_4$).
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31, 1423.
CHAPTER TWO: Synthesis of 5,8-dideazapterin derivatives

1- Introduction

1.1- Nomenclature of quinazoline

Quinazoline has also been called phenmiazine, benzyleneamidine, benzo-1,3-diazone, 5,6-benzopyrimidine and 1,3-diazanaphthalene. The term phenmiazine was used by Widman and later by Bischler et al., and the positions in the pyrimidine ring were designated by a, b, c, and d (1). A second system of numbering is shown in (2). The name quinazoline (German: chinazoline), which is now universally adopted, was first proposed by Weddige because he observed that his compounds were isomeric with the then known cinnoline and quinoxaline. It probably arose from the fact that it was an aza derivative of quinoline, hence quinazoline. The numbering shown in (3) was suggested by Paal et al. and is the one in current use.

![Diagram](image)

1.2- The dual character of quinazolines

Quinazolines can be divided into two main groups according to their characteristic properties. The first group includes all the quinazolines in which the two ring systems are fully aromatic. These do not behave entirely as pyrimidines. The benzene ring has a profound effect on the properties of the pyrimidine ring: it delocalises the \( \pi \) electrons of the 3,4-double bond making its reactivity like that of an...
isolated double bond. As a consequence of this, quinazoline is very reactive towards nucleophiles, which readily add across the 3,4-double bond.

The second group includes the quinazolines, which lack the full complement of six π electrons in either the pyrimidine or the benzene ring. These compounds can be divided into the quinazolines with tautomeric groups in the pyrimidine ring and the reduced quinazolines. 5,8-Dideazapterin (2-amino-(3H)-quinazolin-4-one) is part of the second group of quinazolines and has two tautomeric forms (4) and (5), the lactam form (4) being the more stable.

2- Synthesis of 5,8-dideazapterin

2.1- Using anthranilic acid and guanidine

5,8-Dideazapterin (2-amino-(3H)-quinazolin-4-one) (4) was first synthesised in 1869 by Griess from ethanolic anthranilic acid (6) and cyanogen.
In 1984, Hynes et al. synthesized 2-amino-6-nitroquinazolin-4-one (8) from the condensation of 5-nitrosatoic anhydride (7) with guanidine carbonate in DMF. The reaction yielded 75% of (8) after 2 days refluxing.

Earlier, Kunckell had synthesised 5,8-dideazapterin (4) by a direct fusion of anthranilic acid (6) with guanidine carbonate. In order to synthesise 5,8-dideazapterin (4), we applied this method using DMF as solvent. The reaction was followed by TLC (DMF:EtOAc, 1:2) and UV spectroscopy in DMF. The reaction was completed after 5 days at 155°C in DMF.

Considering the slow rate of this reaction, we decided to change the solvent, DMF, for sulfolan, which has a higher boiling point (285°C). As expected, the reaction was faster and 5,8-dideazapterin was obtained after 16 hours at 200°C in a somewhat better crude yield. Finally, in an effort to improve the rate and yield of our reaction, we decided to revert to the fusion conditions without solvent. A product
containing the expected compound was obtained after 8 hours. Because of the very low solubility of this material, we were unable to obtain a conclusive $^1$H NMR. Nevertheless, the UV spectra (0.1 M HCl) of the crude products from the three procedures were exactly the same with a $\lambda_{\text{max}}$ around 310 nm.

The next stage of this work was pivaloylation of 5,8-dideazapterin (from the fusion reaction without solvent). Following the same procedure as for 5-deazapterin,\(^8\) $N^\beta$-pivaloyl-5,8-dideazapterin was obtained in 27 % yield, based on anthranilic acid. As expected, it is soluble in many organic solvents. The $^1$H NMR confirmed the expected structure and the UV spectrum ($\lambda_{\text{max}}$ 231 and 276 nm) was almost identical to that of $N^2$-pivaloyl-5-deazapterin ($\lambda_{\text{max}}$ 277 nm).

The low overall yield was no doubt, due to the poor quality of the sample of 5,8-dideazapterin. We therefore felt that we had to find a better way of synthesising and purifying 5,8-dideazapterin.

2.2- Using ethyl anthranilate and guanidine

2.2.1- Synthesis of ethyl anthranilate

Ethyl anthranilate (7) was synthesised in good yield (78 %) from anthranilic acid (6), ethanol and concentrated sulphuric acid, following the procedure of Oakes et al.\(^9\) for the synthesis of ethyl 3-aminopicolinate, and was used in the next stage.

\[
\begin{align*}
\text{HO}_2\text{C} & \quad \text{EtOH} \quad \text{conc. H}_2\text{SO}_4 \\
\text{H}_2\text{N} & \quad \rightarrow \\
\text{EtO}_2\text{C} & \quad \text{(6)} \quad \text{(7)}
\end{align*}
\]
2.2.2- Synthesis of 5,8-dideazapterin

In a first attempt, we refluxed a mixture of ethyl anthranilate (7) and guanidine carbonate (1 eq.) in ethanol. The progress of the reaction was followed by TLC (DCM:MeOH, 9:1). After 6h, no reaction had yet taken place. So, we investigated other experimental conditions.

In 1975, Acharya et al.\textsuperscript{10} synthesised 6-methyl-5,8-dideazapterin (2-amino-6-methyl-(3\(H\))-quinazolin-4-one) (9) in good yield (79-82\% ) by treating ethyl 2-amino-5-methylbenzoate (8) with guanidine hydrochloride and sodium ethoxide.

In 1964, Mulvey et al.\textsuperscript{11} reported that the monoester (10) gave 2-amino-(3\(H\))-6-pyrido[2,3-\(d\)]pyrimidin-4-one carboxylic acid (11) by condensation with guanidine hydrochloride and sodium ethoxide.
Application of this procedure to unsubstituted ethyl anthranilate (7) gave a mixture of very insoluble by-products.

So far, all our attempts at the preparation of 5,8-dideazapterin (4) by condensation or fusion of anthranilic acid (6) or its ethyl ester (7) with guanidine gave multiple, high-melting products, which proved difficult to separate and purify. This route was therefore abandoned.

2.3- Using methyl anthranilate and isothiourea derivative

In recent years, a number of reagents have been devised for converting amines into guanidines.\textsuperscript{12, 13}

In 1987, Bergeron \textit{et al.},\textsuperscript{12} in a study of the synthesis of (+)-15-deoxyspergualin (12), a potent antitumour and antibiotic spermidine alkaloid, condensed the guanidine reagent $N,N'$-bis(\textit{tert}-butoxycarbonyl)-$S$-methylisothiourea (13) with 7-aminoheptanamide (14) to give the \textit{bis(\textit{tert}-butoxycarbonyl)}guanidinoamide (15).
By analogy, treatment of methyl anthranilate (16), which is commercially available, with the isothiourea (13) should generate compound (17), which would then cyclise to the N\(^2\)-Boc-5,8-dideazapterin (18).

The reaction, in THF, was followed by TLC (Hexane:EtOAc, 2:1). After 3h at 52°C no reaction had occurred, presumably because of the weakness of the
aromatic amine, methyl anthranilate (16), as a base. Stronger reaction conditions were investigated using DMF instead of THF. This allowed an increase in reaction temperature. Unfortunately, the attempts in aqueous DMF (2%) or dry DMF at 100°C led only to the decomposition of the isothiourea compound. The addition of DMAP as a base catalyst, and the use of dry THF as solvent, were also unsuccessful. Thus, this approach was also abandoned.

2.4- Using methyl anthranilate and benzoylcyanamide

Maguire et al. reported that the condensation of benzoylcyanamide (N-cyanobenzamide) with either methyl anthranilate (16), ethyl anthranilate (7) or anthranilic acid (6), gave the isomer 2-amino-3-benzoyl-quinazolin-4-one (20), which is readily hydrolysed in alkali to give 5,8-dideazapterin (4).

\[
\begin{align*}
(6) \text{ R= H} \\
(7) \text{ R= Et} \\
(16) \text{ R= Me}
\end{align*}
\]

81
2.4.1- Synthesis of benzoylcyanamide

Benzoylcyanamide (22) was synthesised from benzoyl chloride (21) and sodium hydrogen cyanamide in 43 % yield following the procedure of Diels et al.\textsuperscript{16} The NMR data confirmed its structure.

\[
\begin{align*}
\text{COCl} & \quad \text{CONHCN} \\
\text{(21)} & \quad \text{alkali} \\
& \quad \text{(22)}
\end{align*}
\]

2.4.2- Synthesis of 5,8-dideazapterin

Our approach using the Maguire et al.\textsuperscript{14,15} procedure, with methyl anthranilate (16), was successful. After 48h at 95°C, in DMF, and 12h at room temperature, in aqueous ethanol, we obtained compound (20) in 48 % yield. The NMR data and the microanalysis results confirmed this structure.

The hydrolysis of (20) was performed under gentle heating for 30 minutes in 0.5 N NaOH, and gave the expected 5,8-dideazapterin (4) in good yield (94 %).

The next stage of the investigation was to increase the rate and, if possible, the yield of the condensation reaction. Introduction of an electron-withdrawing group on the benzene ring of benzoylcyanamide would render the nitrile group of the molecule more electrophilic and therefore increase the rate of the reaction. We chose to use the electron-withdrawing nitro group. It is known that the effect of any group, whether activating or deactivating, is strongest at the ortho and para positions (Scheme 1).
Keeping this in mind, we introduced, in a first stage, one nitro group in the para position (less steric effect).

2.5- Using methyl anthranilate and 4-nitrobenzoyl cyanamide

2.5.1- Synthesis of 4-nitrobenzoyl cyanamide

In 1966, Howard et al.\textsuperscript{17} synthesised 4-nitrobenzoyl cyanamide (24) from 4-nitrobenzoyl chloride (23) and sodium hydrogen cyanamide (2 eq.) at room temperature.
Following the latter procedure, we obtained 4-nitrobenzoyl cyanamide in good yield (69%).

2.5.2- Synthesis of 5,8-dideazapterin

As for the synthesis of 2-amino-3-benzoyl-quinazolin-4-one (20), we used the Maguire et al.\textsuperscript{14, 15} procedure. The condensation of 4-nitrobenzoyl cyanamide (24) with methyl anthranilate (16) (24h at 95°C, in DMF), gave pure 2-amino-3-(4-nitrobenzoyl)-quinazolin-4-one (25) in 56 % yield.

The NMR data confirmed the structure of compound (25), and furthermore the UV spectrum has the same features as for compound (20) with $\lambda_{\text{max}}$ 234 and 311 nm (236 and 290 nm for compound (20)).
The hydrolysis was carried out under the same conditions as for (20), using 0.5 N NaOH, and afforded 98% yield of 5,8-dideazapterin. Its structure was confirmed by its UV spectrum, which was identical to the one obtained from the sample of (25) synthesised from benzoylcyanamide ($\lambda_{\text{max}}$ 234, 264, 325 nm in both cases).

As expected, the presence of the nitro group did improve the rate and the yield of the reaction. After this success, we decided to introduce a second nitro group on the ortho position, to see if we could improve the yield and rate further.

2.6- Using methyl anthranilate and 2,4-dinitrobenzoyl cyanamide

2.6.1- Synthesis of 2,4-dinitrobenzoyl chloride

In 1947, Challis et al.,$^{18}$ in their study of some amines and amides derived from vanillin, mentioned the preparation of acyl chlorides from the corresponding acids and thionyl chloride (1.5 eq.), by refluxing in ether for 1h. We applied this method to 2,4-dinitrobenzoic acid, and followed the progress of the reaction by TLC (DCM:MeOH, 4:1). After 2h refluxing, no reaction was taking place. We decided then to follow the procedure of Norris et al.,$^{19}$ which involves neat thionyl chloride and no solvent. After 1h at 79°C, the conversion of 2,4-dinitrobenzoic acid (26) into 2,4-dinitrobenzoyl chloride (27) was completed. The latter was obtained in 97% yield. The NMR data, as well as its IR spectrum with the characteristic peak at 1781 cm$^{-1}$ for acid chlorides (lit.$^{20}$ 1750-1820 cm$^{-1}$), confirmed its structure. The melting point of (27), 45-46°C also agreed with the literature value$^{21}$ (42-46°C).
2.6.2- Synthesis of 2,4-dinitrobenzoylcyranamide

As with the synthesis of 4-nitrobenzoylcyranamide, we followed the procedure of Howard et al.\textsuperscript{17} Treatment of 2,4-dinitrobenzoyl chloride with sodium hydrogen cyanamide (2 eq.) resulted in an unusual orange-pink colouration, almost immediately. A TLC (DCM:MeOH, 5:1) check showed the presence of three components, two new products and 2,4-dinitrobenzoyl chloride. After 1h stirring at room temperature, the solution turned yellow, but no change on TLC was noted. The reaction was stirred overnight.

In their publication, Howard et al.\textsuperscript{17} mentioned that the sodium salts of the 3-nitro-, 4-nitro- and 4-chlorobenzoylcyranamide precipitated during the reaction period and were separated by filtration. The salts were then dissolved in water and acidified with ice-cold HCl. As in the case of benzoylcyranamide and 4-methylbenzoylcyranamide, we obtained no solid sodium salt and therefore, the reaction mixture itself was acidified. Surprisingly, no precipitate was obtained, so we extracted the acidified mixture with ethyl acetate. Evaporation to dryness of the organic layer gave a yellow oil containing the same two products as mentioned above. The proton NMR shows the presence of the three expected aromatic protons, $\delta= 8.11$, 8.59 and 8.78 ppm, also present in the carbon NMR, $\delta= 119.6$, 128.1 and 131.2ppm.

Attention was focused on to an unusual broad singlet, $\delta= 3.72$ ppm, which should
correspond to the expected NH group (δ = 6.96 ppm for 4-nitrocyanamide). This could be the result of hydrogen bonding between the amine and the ortho nitro group (Scheme 2).

![Scheme 2](image)

The nitro group on the ortho position could also have interacted with the cyanamide bearing side chain to give one of two possible by-products shown on (Scheme 3), and this would correspond to the additional components detectable on TLC. Furthermore, this would explain the colouration during the reaction, due to the presence of a nitroso group.

The infrared spectrum confirmed the presence of a cyano group at 2255 cm⁻¹ (lit.²⁰ 2200-2260 cm⁻¹) and a NH group at 3107 cm⁻¹ (lit.²⁰ 3100-3500 cm⁻¹). The next stage of this investigation was therefore the purification of the latter mixture to allow the identification of the products. Purification by passing through a column of silica, using DCM:MeOH (5:1) as solvent, resulted in total decomposition of the mixture.
Howard et al.\textsuperscript{17} also synthesised \textit{3-N,N-} (28) and \textit{4-N,N-} dimethylaminobenzoylcyanamide (29) under dry conditions, using dry DMF and 50\% sodium hydride-mineral oil dispersion.

Application of this procedure to 2,4-dinitrobenzoyl chloride gave the same inseparable mixture, as mentioned above. This proved that the by-product is the one
obtained from route (b) (Scheme 3), instead of route (a) where water is needed to complete the reaction.

In 1974, Feiccabrino et al.,\textsuperscript{22} in their study for the preparation of some triethylammonium(organocynanoamino)chlorotriphenylstannates (30), discovered that 4-nitrobenzoyl chloride, when reacting with (triphenylstannyl)cyanamide and triethylamine, gave the triethylammonium salt of the corresponding acylcyanamide.

\[
\begin{align*}
&\text{Cl} \\
&\text{Ph} \\
&\text{Sn} \\
&\text{Ph} \\
&\text{RNCN} \\
\end{align*}
\]

(30)

2.6.3- Synthesis of triethylammonium 4-nitrobenzoyl cyanamide

Application of the Feiccabrino et al.\textsuperscript{22} method to 4-nitrobenzoyl chloride, using cyanamide and triethylamine, afforded triethylammonium 4-nitrobenzoyl cyanamide in 40 % yield.

The NMR data confirmed its structure and the melting point 127-128°C agreed with the literature value\textsuperscript{22} (126-133°C).

For comparison, we also synthesised the same triethylammonium salt, by stirring at room temperature for 10 min, a solution of 4-nitrobenzoyl cyanamide and triethylamine in THF. The salt was obtained in 68 % yield and its melting point found to be 131-133°C. The NMR data of both salts were identical.

The next stage of the investigation was, therefore, to apply this method to 2,4-dinitrobenzoyl chloride.
2.6.4- Synthesis of triethylammonium 2,4-dinitrobenzoylcyanamide

As for the synthesis of triethylammonium 4-nitrobenzoylcyanamide, 2,4-dinitrobenzoyl chloride was treated with cyanamide and triethylamine in THF. In contrast with the 4-nitrobenzoyl chloride reaction, there was no precipitation of triethylamine hydrochloride. Instead, after the usual work up, we obtained a mixture of two compounds. The NMR data helped to recognise a mixture of triethylamine hydrochloride and triethylammonium 2,4-dinitrobenzoylcyanamide in a 4:1 ratio. This mixture was found impossible to separate. Nevertheless, knowing the ratio, we decided to go ahead with the synthesis of 2-amino-3-(2,4-dinitrobenzoyl)-quinazolin-4-one (31).

\[ \text{Scheme 4} \]

2.6.5- Synthesis of 5,8-dideazapterin

2.6.5.1- Using triethylammonium 4-nitrobenzoylcyanamide

Once again, we applied the procedure of Maguire et al.\textsuperscript{14,15} to triethylammonium 4-nitrobenzoylcyanamide. The reaction progress was followed by TLC (DCM:MeOH, 9:1). After 4h at 95°C, no reaction had yet taken place. Addition of p-toluenesulfonic acid (1 eq.) to the reaction mixture helped catalyse it. Indeed, p-toluenesulfonic acid was readily converted into its triethylammonium salt (Scheme 4), allowing the resulting 4-nitrobenzoylcyanamide to react, as expected, with methyl anthranilate. 2-Amino-3-(4-nitrobenzoyl)-quinazolin-4-one (25) was
synthesised in 30 % yield, after 2h reaction at 95°C. The NMR data confirmed its structure.

The hydrolysis of the latter sample, under the usual conditions, afforded 5,8-dideazapterin (4) in 98 % yield. The UV spectrum was identical to those of the previous samples (λ_max 234, 264 and 325 nm).

![Scheme 4](image)

2.6.5.2- Using the (4:1) mixture of triethylamine hydrochloride and triethylammonium 2,4-dinitrobenzoylcyranamide

The mixture of triethylammonium 2,4-dinitrobenzoylcyranamide and triethylamine hydrochloride was treated under the same conditions as for triethylammonium 4-nitrobenzoylcyranamide, using the procedure of Maguire et al. The progress of the reaction was followed by TLC (DCM:MeOH, 9:1).
After 5h at 95°C, the reaction was complete. The precipitate, which formed during the reaction period, was collected. The NMR data proved it to be triethylamine hydrochloride. The melting point of the sample, 252-254°C, agreed with the literature value\(^2\) (253-254°C).

The usual work up of the filtrate in aqueous EtOH, gave 70% yield of a brown solid, expected to be 2-amino-3-(2,4-dinitrobenzoyl)-quinazolin-4-one (31). The proton NMR confirmed the structure with the seven aromatic protons (\(\delta = 7.44-8.78\) ppm) and the broad singlet, \(\delta = 12.61\) ppm, characteristic of the amino group on the 2-position. The carbon NMR also agrees with the expected feature. The low solubility of this compound in organic solvents did not allow further purification. Microanalysis data were therefore compromised.

The hydrolysis was carried out under the usual conditions, using 0.5 N NaOH. The reaction progress was followed by TLC (DCM:MeOH, 9:1). The reaction mixture became purple immediately, instead of yellow as for the other derivatives. After 20 min, compound (31) was converted into a new product, with a very different \(R_f\) value. It was less polar than the expected 5,8-dideazapterin.

In 1964, Loudon et al.\(^2\) reported a few reactions resulting from substituent interactions, such as the interaction between a nitro and an amine group. For example, they noted that 3-aminobenzotriazole-1-oxide (33) is formed rapidly when 2-nitrophenylguanidine (32) is warmed with dilute alkali, and the 2-nitrophenylacetamide (34) similarly cyclises to the cinnoline-1-oxide (35).
The same type of interaction could have happened with compound (31), and would explain the deep purple colouration of the solution, characteristic of the presence of an azoxy group. A possible mechanism of the reaction is shown below (Scheme 5).

Acidification with acetic acid afforded 78 % yield of red-brown solid, which was insoluble in organic solvents. No further purification was possible. The NMR data were very inconclusive. This approach was therefore abandoned.

Still keeping in mind that the presence of two nitro groups would improve the rate and yield of the condensation reaction, we decided to apply it to 3,5-dinitrobenzoylcyanamide (37).
2.7- Using methyl anthranilate and 3,5-dinitrobenzoylcyanamide

2.7.1-Synthesis of 3,5-dinitrobenzoylcyanamide

We applied the Howard et al.\textsuperscript{17} procedure to 3,5-dinitrobenzoyl chloride (36), which is commercially available. Acidification of the reaction mixture, after 2h stirring at room temperature, afforded in 79 % yield the expected 3,5-dinitrobenzoylcyanamide (37)
The proton NMR confirmed the presence of the two equivalent protons 2-H and 6-H, $\delta = 9.08$ ppm, and the proton on the 4-position, $\delta = 9.0$ ppm. We also have a broad singlet, $\delta = 7.85$ ppm, for the NH group. The carbon NMR also agrees with the structure, and the IR spectrum recorded the expected peaks for the amino ($\nu_{\text{max}} 3177$ cm$^{-1}$) and the cyano ($\nu_{\text{max}} 2260$ cm$^{-1}$) groups.

The next stage of the investigation was therefore the synthesis of 2-amino-3-(3,5-dinitrobenzoyl)-quinazolin-4-one (38).

2.7.2- Synthesis of 2-amino-3-(3,5-dinitrobenzoyl)-quinazolin-4-one

The Maguire et al.$^{14,15}$ procedure was then applied to 3,5-dinitrobenzoylcyanamide (37). The reaction progress was followed by TLC (DCM:MeOH, 9:1). After 5h at 95°C, the condensation reaction between 3,5-dinitrobenzoylcyanamide (37) and methyl anthranilate (16) was complete. Filtration of the resulting precipitate gave pure 2-amino-3-(3,5-dinitrobenzoyl)-quinazolin-4-one (38) in 58 % yield.
compound. After 10 min, the orange suspension turned into a brown solution, but there was no change on TLC. Another 10 minutes heating was required for complete removal of the starting material. Unfortunately, it seems that 5,8-dideazapterin has been converted into the unknown compound. Acidification of the reaction mixture resulted in a brown precipitate, which decomposed upon filtration. Several other hydrolysis experiments, using aqueous ammonia (5%), aqueous HCl (1 M) or aqueous Na₂CO₃, led to the same result. The use of 3,5-dinitrobenzoylcyanamide was therefore abandoned.

3- Pivaloylation of 5,8-dideazapterin

As for the synthesis of N²-pivaloyl-5-deazapterin, we applied the Taylor et al. procedure to 5,8-dideazapterin (4). N²-Pivaloyl-5,8-dideazapterin (39) was synthesised in good yield (78%).

![Diagram]( attachment)

Both the proton and carbon NMR confirmed its structure. The UV spectrum was identical to the sample previously synthesised, with λ_max 231, 275 nm (231, 277 from crude 5,8-dideazapterin).
4- The Mitsunobu reaction

As for the synthesis of the four \(N^2\)-pivaloyl-5-deazapterin derivatives,\textsuperscript{25, 26} treatment of \(N^2\)-pivaloyl-5,8-dideazapterin with 1.5 molecular equivalents each of diisopropyl azodicarboxylate, tributylphosphine and the corresponding alcohols, in THF at room temperature, led after 2h to the four \(N^2\)-pivaloyl-5,8-dideazapterin derivatives (40-43). The respective yields, \(\lambda_{\text{max}}\) and melting point values are reported in Table 3. The NMR spectra and the microanalytical data confirmed the structure of all four compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Yield (%)</th>
<th>(\lambda_{\text{max}}) (nm)</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(40)</td>
<td>52</td>
<td>251, 278</td>
<td>122-123</td>
</tr>
<tr>
<td>(41)</td>
<td>50</td>
<td>249, 278</td>
<td>127-128</td>
</tr>
<tr>
<td>(42)</td>
<td>48</td>
<td>280</td>
<td>137-138</td>
</tr>
<tr>
<td>(43)</td>
<td>51</td>
<td>228, 277</td>
<td>155-156</td>
</tr>
</tbody>
</table>

Table 3
5- Depivaloylation using hydrazine monohydrate

Using the Espie et al.\textsuperscript{27} procedure, we treated the four $N^2$-pivaloyl-5,8-dideazapterin derivatives (40-43) in ethanol with hydrazine monohydrate (10 eq.). In this case the reaction was slower than the corresponding reaction with the 5-deazapterin derivatives (10 min at 20°C). The conversion to the free amine derivatives (44-47) was completed after 3 h at 78°C, in an average yield of 69 \% (Table 4). The NMR spectra and analysis data confirmed the four structures.

\begin{table}
\begin{center}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Compounds} & \textbf{Yield (\%)} & \textbf{$\lambda_{\text{max}}$ (nm)} & \textbf{m.p. (°C)} \\
(44) & 83 & 238, 265, 316 & 200-201 \\
(45) & 43 & 234, 267, 328 & 169-170 \\
(46) & 67 & 238, 271, 324 & 229-230 \\
(47) & 84 & 234, 268, 333 & 187-188 \\
\hline
\end{tabular}
\end{center}
\textbf{Table 4}
\end{table}
These compounds were also found to be inactive as inhibitors of the protein ATase at the Paterson Institute in Manchester (IC$_{50}^*$ > 2500 μM). They thus resemble the 5-deazapterin derivatives, and contrasted markedly with the pterin analogues.

6- Experimental

*Preparation of ethyl anthranilate* (7)

Anthranilic acid (4 g, 29.2 mmol) was heated under reflux with ethanol (8 ml) and sulphuric acid (4.26 ml) for 4h. The resulting solution was allowed to cool, poured onto ice and basified with aqueous ammonia. The mixture was extracted with ether (× 3) and the combined organic layers dried over MgSO$_4$. Evaporation of the filtrate and recrystallisation from hexane gave ethyl anthranilate (3.76 g, 78 %) as an oil.

(lit.,$^{23}$ m.p.13-15°C).

**NMR(DMSO-d$_6$):** δ$_H$(400MHz) 1.30 (3H, t, CH$_3$), 4.25 (2H, q, CH$_2$), 6.52 (1H, td, 5-H), 6.61 (2H, s, NH$_2$), 6.77 (1H, dd, 6-H), 7.23 (1H, td, 4-H), 7.71 (1H, dd, 3-H).

(J$_{3-H,4-H}$, J$_{5-H,6-H}$ 8.0, J$_{4-H,5-H}$, J$_{4-H,6-H}$ 1.5, J$_{3-H,6-H}$ 1.0 Hz).

δ$_C$(100MHz) 14.1 (CH$_3$), 59.7 (CH$_2$), 109.0 (q-C$_2$), 114.7 (C$_3$-H), 116.5 (C$_5$-H), 130.6 (C$_6$-H0, 133.9 (C$_4$-H), 151.3 (q-C$_1$), 167.4 (C=O).

*Preparation of benzoylcyanamide* (22)

A mixture of benzoyl chloride (66.8 g, 0.48 mol), NaOH (30 %; 48ml) and water (300 ml) was added to a solution of cyanamide (20 g, 0.48 mol) in NaOH (30 %; 48 ml) and water (800 ml). The suspension was vigorously stirred for 30 min in ice-

* The concentration required to produce 50 % inactivation of ATase.
cold bath. The resulting clear solution was then acidified with concentrated HCl at 0°C. The white precipitate was filtered off and washed with ether to give benzoylcyanamide (30 g, 43%).

m.p. 138-139°C (lit.16 141-142°C).

λ_max (MeOH) 235 nm.

NMR(DMSO-d_6): δH(400MHz) 3.87(1H, br. s, NH), 7.57(1H, td, 3-H), 7.70(1H, td, 4-H), 7.93(1H, dd, 2-H). (J_{2-H, 3-H}, J_{3-H, 4-H} 7.9, J_{2-H, 4-H} 1.0 Hz).

δ_C(100MHz) 108.8(CN), 128.3(2xC_3-H), 128.8(C_4-H), 130.2(q-C_1), 133.7(2xC_2-H), 166.8(C=O).

IR(nujol, cm^{-1}): 3231(NH), 2253(CN), 1676(C=O).

Preparation of 4-nitrobenzoylcyanamide\textsuperscript{17} (24)

4-Nitrobenzoyl chloride (20 g, 0.11 mol) was added to a solution of cyanamide (9 g, 0.22 mol) in 1M NaOH (220 ml). The mixture was stirred overnight at room temperature. The resulting white precipitate was filtered, dissolved in water and acidified with ice-cold concentrated HCl. Filtration gave 4-nitrobenzoylcyanamide (14.18 g, 69%).

m.p. 156-157°C (lit.\textsuperscript{17} 158-159°C).

λ_max (MeOH) 261 nm.

NMR(DMSO-d_6): δH(400MHz) 6.96(1H, br. s, NH), 8.12(2H, d, 2-H), 8.36(2H, d, 3-H). (J_{2-H, 3-H} 8.5 Hz).

δ_C(100MHz) 123.8(2xC_2-H), 129.8(2xC_3-H), 136.6(q-C_1), 150.1(C_4-NO\textsubscript{2}), 166.2(C=O).

IR(nujol, cm^{-1}): 3114(NH), 2264 (CN), 1706 (C=O), 1540 (NO\textsubscript{2}), 1350 (NO\textsubscript{2}).
Preparation of triethylammonium 4-nitrobenzoylcyanamide

(a)- From 4-nitrobenzoyl chloride

Triethylamine (1.64 ml, 11.8 mmol) was added to a solution of cyanamide (0.24 g, 5.71 mmol) in THF (45 ml). To this was added slowly a solution of 4-nitrobenzoyl chloride (1 g, 5.71 mmol) in THF (30 ml). The mixture was stirred at 26°C for 2h. The solvent was then evaporated and the residue refluxed in ethyl acetate (120 ml) for 1h. Triethylamine hydrochloride was filtered and the filtrate evaporated to dryness. Recrystallisation from EtOH/EtOAc gave triethylammonium 4-nitrobenzoylcyanamide (0.69 g, 40 %).

m.p. 127-128°C (lit. 126-133°C).

NMR(DMSO-d6): δ_H(400MHz) 1.19(9H, t, 3CH₃), 3.10(6H, q, 3CH₂), 8.13(2H, d, 2-H), 8.18(2H, d, 3-H). (J₂₋₃-H 8.5 Hz).

δ_C(100MHz) 8.6(CH₃), 45.8(CH₂), 122.8(C₂-H), 129.2(C₃-H), 144.6, 148.4, 172.6(q-C).

(b)- from 4-nitrobenzoylcyanamide

Triethylamine (0.14 ml, 1 mmol) was added to a solution of 4-nitrobenzoyl cyanamide (0.19 g, 1 mmol) in THF (5 ml). The mixture was stirred at room temperature for 10 min. The resulting white precipitate was filtered to give triethylammonium 4-nitrobenzoylcyanamide (0.20 g, 68 %).

m.p. 131-133°C (lit. 126-133°C).

NMR(DMSO-d6): δ_H(400MHz) 1.18(9H, t, 3CH₃), 3.11(6H, q, 3CH₂), 8.13(2H, d, 2-H), 8.17(2H, d, 3-H). (J₂₋₃-H 8.5 Hz).

δ_C(100MHz) 8.7(CH₃), 45.9(CH₂), 122.8(C₂-H), 129.2(C₃-H), 144.6, 148.4, 172.6(q-C).
**Preparation of 2,4-dinitrobenzoyl chloride (27)**

2,4-Dinitrobenzoic acid (15.53 g, 73.21 mmol) and freshly distilled thionyl chloride (10 ml) was refluxed for 1 h. The excess thionyl chloride was distilled off and the resulting residue co-distilled with ether (x3). The residue was washed with hot hexane and then, left in the freezer for 2 days to give 2,4-dinitrobenzoyl chloride (16.45 g, 97 %) as cream-coloured crystals.

m.p. 45-46°C (lit.\textsuperscript{21} 42-46°C).

**NMR(DMSO-d\textsubscript{6}):** $\delta_{H}(400\text{MHz})$ 8.09(1H, dd, 6-H), 8.57(1H, dd, 5-H), 8.75(1H, d, 3-H). ($J_{5-H, 6-H}$ 8.5, $J_{5-H, 3-H}$ 2.0 Hz).

$\delta_{C}(100\text{MHz})$ 119.4(C\textsubscript{5}-H), 127.9(C\textsubscript{6}-H), 131.4(C\textsubscript{3}-H), 132.6(q-C\textsubscript{i}), 147.8(q-C\textsubscript{4}), 148.7(q-C\textsubscript{2}), 164.6(C=O).

**IR(nujol, cm\textsuperscript{-1}):** 1781(C=O), 1545(NO\textsubscript{2}), 1344(NO\textsubscript{2}).

**Preparation of 3,5-dinitrobenzoylcyanamide (35)**

3,5-Dinitrobenzoyl chloride (5 g, 21.7 mmol) was added to a solution of cyanamide (1.82 g, 43.4 mmol) in 1M NaOH (43.4 ml). The mixture was stirred at room temperature for 2 h. The resulting solution was acidified with ice-cold concentrated HCl. The white precipitate that formed was filtered and washed with ether to give 3,5-dinitrobenzoylcyanamide (4.04 g, 79 %).

m.p. 155-156°C.

$\lambda_{\text{max}}$(MeOH) 232 nm.

**NMR(DMSO-d\textsubscript{6}):** $\delta_{H}(400\text{MHz})$ 9.0(1H, t, 4-H), 9.08(2H, d, 2-H and 6-H), 7.85(1H, s, NH), ($J_{4-H, 1-H}$, $J_{4-H, 6-H}$, $J_{2-H, 6-H}$ 2.0 Hz).

$\delta_{C}(100\text{MHz})$ 112.6(CN), 121.5(C\textsubscript{4}-H), 128.2(2xC\textsubscript{2}-H), 136.3(q-C), 148.1(C\textsubscript{3}-H, C\textsubscript{5}-H), 166.1(C=O).
IR(nujol, cm⁻¹): 3177(NH), 2260(CN), 1719(C=O), 1545(NO₂), 1351(NO₂).

Preparation of 5,8-dideazapterin (2-amino-(3H)-quinazolin-4-one) (4)

(a) Anthranilic acid (16 g, 0.117 mol) and guanidine carbonate (21 g, 0.117 mol) were thoroughly mixed together and heated at 95°C until the mixture liquefied. The temperature was then increased to 170°C. The residue formed a liquid foam and NH₃ evolved. The reaction was kept at this temperature for 1h, and finally heated to 200°C until no more NH₃ was evolved. The mixture solidified. The reaction was then allowed to cool down and aqueous ethanol (1:1) was added. The crude product (18.62 g) was filtered off and washed with ether, and contained 5,8-dideazapterin.

m.p. >300°C.

λ_max (0.1 N HCl) 223, 310 nm.

(b) From benzoylcyanamide

(i) 2-amino-3-benzoyl-quinazolin-4-one¹⁴,¹⁵ (20)

Benzoylcyanamide (14.6 g, 0.1 mol) was added to a solution of methyl anthranilate (15.12 g, 0.1 mol) in dry DMF (200ml). The mixture was heated at 95°C for 48h and the solution poured into ethanol (300 ml). The ethanolic solution was in turn poured into ice-water (1.5 l) and the resulting suspension was stirred overnight. The solid was filtered off, washed with water and then ether. Recrystallisation from 70 % ethanol gave the benzoyl derivative (20) as a white solid (9.07 g, 48 %).

m.p. 178-179°C (lit.¹⁴,¹⁵ 188.5-190°C).

λ_max (MeOH) 236, 290 nm.
NMR(DMSO-d$_6$): $\delta$H(400MHz) 7.41(1H, td, 6-H), 7.55(1H, dd, 5-H), 7.56(2H, m, Ph), 7.64(1H, td, 7-H), 7.78(1H, dd, 8-H), 8.11(3H, m, Ph), 12.24(2H, br. s, NH$_2$). (J$_{5-H}$,6-H, J$_{6-H}$,7-H, J$_{7-H}$,8-H, J$_{5-H}$,7-H 1.5, J$_{5-H}$,8-H 1.0 Hz).

$\delta$C(100MHz) 124.9(C$_6$-H), 126.4(C$_5$-H), 128.4(C$_2$-H), 128.6(C$_3$-H), 132.8(C$_2$-H), 134.9(C$_8$-H), 160.6(q-C$_4$), 167.6(C=O).

(ii) Hydrolysis

2-Amino-3-benzoyl-quinazolin-4-one (6 g, 0.02 mol) was added to 0.5 N NaOH (300 ml) and the suspension gently heated for 30 min. The solution was then filtered and acidified with acetic acid to give 5,8-dideazapterin (4) (3.42 g, 94 %) as a white solid.

m.p. >300°C.

$\lambda_{max}$ (0.1N NaOH) 234, 264, 325 nm.

NMR(DMSO-d$_6$): $\delta$H(400MHz) 6.44(2H, br. s, NH$_2$), 7.10(1H, td, 6-H), 7.21(1H, dd, 5-H), 7.56(1H, td, 7-H), 7.89(1H, dd, 8-H), 11.07(1H, br. s, NH). (J$_{5-H}$,6-H, J$_{6-H}$,7-H, J$_{7-H}$,8-H 7.5, J$_{6-H}$,8-H, J$_{5-H}$,7-H 1.5, J$_{5-H}$,8-H 1.0 Hz).

(c) From 4-nitrobenzoylcyanoamide

The same procedure was followed, using 4-nitrobenzoylcyanoamide (1.26 g, 6.62 mmol) instead of benzoylcyanoamide. The reaction was heated at 95°C for 24h and then allowed to cool. The precipitate was filtered and washed with ether to give pure 2-amino-3-(4-nitrobenzoyl)-quinazolin-4-one (25) (1.15 g, 56 %). The filtrate was diluted with ethanol (20 ml) and then poured into ice-water (90 ml). The resulting suspension was stirred overnight, filtered and washed with ether to give an additional fraction of less pure material (0.43 g, 21 %).
m.p. >300°C.

$\lambda_{\text{max}}$ (MeOH) 234, 311 nm

(Found: C, 57.98; H, 3.11; N, 17.87%. C$_{15}$H$_{10}$N$_{4}$O$_{4}$ requires: C, 58.06; H, 3.23; N, 18.06%).

NMR(DMSO-$d_6$): $\delta_{H}(400\text{MHz})$ 7.44(1H, td, 6-H), 7.61(1H, dd, 5-H), 7.81(1H, td, 7-H), 8.06(1H, dd, 8-H), 8.35(4H, m, Ph), 12.54 (2H, br s, NH$_2$) (J$_{5-H, 6-H}$, J$_{6-H, 7-H}$, J$_{7-H, 8-H}$ 7.5, J$_{6-H, 8-H}$, J$_{5-H, 7-H}$ 1.5, J$_{5-H, 8-H}$ 1.0 Hz).

$\delta_{C}(100\text{MHz})$ 121.0(q-C$_2$), 123.5(C$_2$-H), 124.6(C$_6$-H), 125.1(C$_5$-H), 126.6(C$_7$-H), 130.0(C$_3$-H), 135.4(C$_8$-H), 140.4 (q-C$_1$), 149.6 (q-C$_4$).

Hydrolysis of this product (0.363 g, 2.25 mmol) gave 5,8-dideazapterin (4) (0.685 g, 98%).

m.p. >300°C.

$\lambda_{\text{max}}$ (0.1N NaOH) 234, 264, 325 nm.

(d) From triethylammonium 4-nitrobenzoylcyanamide

The same procedure as above was followed, using triethylammonium 4-nitrobenzoylcyanamide (0.292 g, 2 mmol) and $p$-toluenesulfonic acid hydrate (0.38 g, 2 mmol). The reaction was heated at 95°C for 2h and then allowed to cool. The precipitate was filtered and washed with ether to give pure 2-amino-3-(4-nitrobenzoyl)-quinazolin-4-one (25) (0.189 g, 30%).

m.p. >300°C.

$\lambda_{\text{max}}$ (MeOH) 234, 311 nm.

NMR(DMSO-$d_6$): $\delta_{H}(400\text{MHz})$ 7.43(1H, td, 6-H), 7.60(1H, dd, 5-H), 7.80(1H, td, 7-H), 8.05(1H, dd, 8-H), 8.33(4H, m, Ph), 12.51 (2H, br s, NH$_2$) (J$_{5-H, 6-H}$, J$_{6-H, 7-H}$, J$_{7-H, 8-H}$ 7.5, J$_{6-H, 8-H}$, J$_{5-H, 7-H}$ 1.5, J$_{5-H, 8-H}$ 1.0 Hz).
$\delta_c(100\text{MHz})$ 121.0(q-C$_2$), 123.5(C$_2$-H), 124.6(C$_6$-H), 125.1(C$_5$-H), 126.6(C$_7$-H), 130.0(C$_3$-H), 135.4(C$_8$-H), 140.4(q-C$_4$), 149.6(q-C$_4$).

Hydrolysis of (0.14 g, 0.45 mmol) of this product gave 0.623 g (98%) of 5,8-dideazapterin (4).

m.p. >300°C.

$\lambda_{\text{max}}$ (0.1 N NaOH) 234, 264, 325 nm.

(e) From 3,5-dinitrobenzoylcyanamide

The same procedure was followed using 3,5-dinitrobenzoylcyanamide (0.236 g, 1 mmol) instead of 4-nitrobenzoylcyanamide. The reaction was heated at 95°C for 5 h, and then allowed to cool. The precipitate was filtered and washed with ether to give pure 2-amino-3-(3,5-dinitrobenzoyl)-quinazolin-4-one (36) (0.412 g, 58%). An additional 10% of crude product was obtained by following the same procedure as outlined for 4-nitrobenzoylcyanamide above.

m.p. >300°C.

(Found: C, 50.71; H, 2.54; N, 19.51%. C$_{15}$H$_9$N$_5$O$_6$ requires: C, 50.70; H, 2.54; N, 19.72%).

$\lambda_{\text{max}}$ (MeOH) 222, 311 nm.

NMR(DMSO-$d_6$): $\delta_H(400\text{MHz})$ 7.39(1H, td, 6-H), 7.58(1H, dd, 5-H), 7.78(1H, td, 7-H), 7.97(1H, dd, 8-H), (J$_{5\text{-}H}$, 6-H, J$_{6\text{-}H}$, 7-H, J$_{7\text{-}H}$, 8-H 7.5, J$_{6\text{-}H}$, 8-H, J$_{5\text{-}H}$, 7-H 1.5, J$_{5\text{-}H}$, 8-H 1.0 Hz), 8.92(1H, d, 4'-H), 9.11(2H, d, 2'-H, 6'-H), (J$_{2\text{-}H}$, 4'-H, J$_{2\text{-}H}$, 6'-H, J$_{4\text{-}H}$, 6'-H 2.0 Hz), 12.72 (2H, br s, NH$_2$).
Preparation of $N^2$-pivaloyl-5,8-dideazapterin (2-pivaloylamino- (3H)- quinazolin-4-one) (39)

(a) From crude 5,8-dideazapterin

The same procedure as for the preparation of $N^2$-pivaloyl-5-deazapterin was followed but using an aliquot (0.16 g, 1 mmol) of the crude 5,8-dideazapterin (18.62 g) obtained from anthranilic acid and guanidine carbonate ((a), p. 31). Filtration and recrystallisation from ethanol gave $N^2$-pivaloyl-(5,8-dideazapterin) (0.067 g, 27 %) as shiny white needles.

$\lambda_{\text{max}}$ (MeOH) 231, 277 nm.

NMR(DMSO-d$_6$): $\delta_H$(400MHz) 1.26(9H, s, 3CH$_3$), 7.40(1H, td, 6-H), 7.52(1H, dd, 5-H), 7.77(1H, td, 7-H), 8.06(1H, dd, 8-H), (J$_5$-H, 6-H, J$_6$-H, 7-H, J$_7$-H, 8-H 7.5, J$_6$-H, 8-H, J$_5$-H, 7-H 1.7, J$_5$-H, 8-H 1.0 Hz), 11.43(1H, s, $N^2$-H), 12.35(1H, s, $N^2$-H).

(b) The above procedure was followed using purified 5,8-dideazapterin (3.0 g, 18.63 mmol). Filtration and recrystallisation from ethanol gave shiny white crystals of $N^2$-pivaloyl-5,8-dideazapterin (3.47 g, 78 %).

m.p. 203-205°C.

(Found: C, 63.40; H, 6.10; N, 16.98 %. C$_{13}$H$_{15}$N$_3$O$_2$ requires: C, 63.73; H, 6.17; N, 17.15 %).

$\lambda_{\text{max}}$(MeOH) 231, 275 nm.

NMR(DMSO-d$_6$): $\delta_H$(400MHz) 1.26(9H, s, 3CH$_3$), 7.39(1H, td, 6-H), 7.51(1H, dd, 5-H), 7.76(1H, td, 7-H), 8.05(1H, dd, 8-H), (J$_5$-H, 6-H, J$_6$-H, 7-H, J$_7$-H, 8-H 7.5, J$_6$-H, 8-H, J$_5$-H, 7-H 1.7, J$_5$-H, 8-H 1.0 Hz), 11.06(1H,br s, $N^2$-H), 12.12(1H,br s, $N^2$-H).

$\delta_C$(100MHz) 26.3(CH$_3$), 119.7(q-C$_2$), 124.8(C$_6$-H), 125.5(C$_5$-H), 126.2(C$_7$-H), 134.7(C$_8$-H), 147.5(q-C$_{4a}$), 148.7(q-C$_{8a}$), 160.4(q-C$_4$), 182.1(C=O).
Preparation of \( \text{O}^1\text{-benzyl-N}^2\text{-pivaloyl-5,8-dideazapterin (4-benzyloxy-2-pivaloylamino-quinazoline)} \) (40)

A suspension of \( \text{N}^2\text{-pivaloyl-5,8-dideazapterin (0.400 g, 1.63 mmol)} \) in tetrahydrofuran (8 ml) was stirred for 10 min and tributylphosphine (0.495 g, 2.45 mmol), benzyl alcohol (0.265 g, 2.45 mmol) and diisopropyl azodicarboxylate (0.495 ml, 2.45 mmol) were added in succession. The reaction was allowed to proceed for 2h at room temperature. Removal of the solvent gave a yellow oil.

Separation by column (hexane/ethyl acetate, 9:2) and evaporation gave a colourless oil. Hexane was then added to induce crystallisation.

Filtration and recrystallisation from hexane gave white crystals of the benzyl derivative (0.284 g, 52 %).

m.p. 122-123°C.

(Found: C, 71.11; H, 6.32; N, 12.42 %. \( \text{C}_{20}\text{H}_{21}\text{N}_{3}\text{O}_{2} \) requires: C, 71.71; H, 6.32; N, 12.54 %).

\( \lambda_{\text{max}}(\text{MeOH}) \) 251, 278 nm.

\( \text{NMR(DMSO-d}_6): \) \( \delta_{\text{H}}(400\text{MHz}) \) 1.13(9H, s, \( \text{SCH}_3 \)), 5.36(2H, s, \( \text{CH}_2 \)), 7.27(5H, m, Ph), 7.40(1H, td, 6-H), 7.68(1H, dd, 5-H), 7.78(1H, td, 7-H), 8.06(1-H, dd, 8-H), 13.35(1H, s, NH). (\( J_{5-\text{H},6-\text{H}}, J_{6-\text{H},7-\text{H}}, J_{7-\text{H},8-\text{H}} \) 7.5, \( J_{6-\text{H},8-\text{H}}, J_{5-\text{H},7-\text{H}} \) 1.7, \( J_{5-\text{H},8-\text{H}} \) 1.0 Hz).

\( \delta_{\text{C}}(100\text{MHz}) \) 27.4 (\( \text{CH}_3 \)), 44.7 (\( \text{CH}_2 \)), 116.9 (q-\( \text{C}_2 \)), 120.4 (\( \text{C}_6\text{-H} \)), 124.5 (\( \text{C}_5\text{-H} \)), 127.0 (Ph), 127.2 (C\( \gamma\)-H), 128.2 (Ph), 135.4 (\( \text{C}_8\text{-H} \)), 137.1 (q-\( \text{C}_1\)).

Preparation of \( \text{O}^1\text{-benzyl-5,8-dideazapterin (2-amino-4-benzyloxyquinazoline)} \) (44)

Hydrazine hydrate (0.22 ml, 4.5 mmol) was added to a solution of \( \text{O}^1\text{-benzyl-N}^2\text{-pivaloyl-5,8-dideazapterin (0.150 g, 0.45 mmol)} \) in ethanol (4.5 ml). The solution was heated under reflux for 3h. The reaction mixture was then allowed to cool.
Filtration and recrystallisation of the solid from ethanol gave pale yellow crystals (0.093 g, 83 %) of \( O'^{i}\)-benzyl-5,8-dideazapterin.

m.p. 200-201\(^\circ\)C.

(Found: C, 69.0; H, 5.40; N, 16.09 %. \( \text{C}_{15}\text{H}_{13}\text{N}_{3}\text{O}_{0.5}\text{H}_{2}\text{O} \) requires: C, 69.23; H, 5.38; N, 16.15 %).

\( \lambda_{\text{max}} \) (MeOH) 238, 265, 316 nm.

NMR (DMSO-\( d_{6} \)): \( \delta_{\text{H}} \) (400MHz) 5.30(2H, s, CH\( \text{2} \)), 6.95(2H, s, NH\( \text{2} \)), 7.12(1H, td, 6-H), 7.25(5H, m, Ph), 7.33(1H, dd, 5-H), 7.58(1H, td, 7-H), 7.95(1-H, dd, 8-H).

Preparation of \( O'^{i}(4\text{-bromothenyl})\)-\( N'^{-}\)-pivaloyl-5,8-dideazapterin (4-(4-bromothenyl)-oxy-2-pivaloylamino-quinazoline) (41)

The same procedure was followed, but using 4-bromothenyl alcohol (0.6 g, 2.44 mmol) instead of benzyl alcohol. Recrystallisation from ethanol gave white crystals of the \( O'^{i}(4\text{-bromothenyl}) \) derivative (0.505 g, 50 %).

m.p. 127-128\(^\circ\)C.

(Found: C, 51.40; H, 4.31; N, 9.89; S, 7.61; Br, 19.25 %. \( \text{C}_{18}\text{H}_{18}\text{N}_{3}\text{O}_{2}\text{BrS} \) requires: C, 51.48; H, 4.32; N, 10.00; S, 7.63; Br, 19.02 %).

\( \lambda_{\text{max}} \) (MeOH) 249, 278 nm.

NMR (DMSO-\( d_{6} \)): \( \delta_{\text{H}} \) (400MHz) 1.23(9H, s, 3CH\( \text{3} \)), 5.46(2H, s, CH\( \text{2} \)), 7.16(1H, s, 3'-H), 7.38(1H, td, 6-H), 7.56(1H, s, 5'-H), 7.68(1H, dd, 5-H), 7.76(1H, td, 7-H),
8.04(1H, dd, 8-H), 13.34(1H, s, NH), (J5-H, 6-H, J6-H, 7-H, J7-H, 8-H 7.5, J6-H, 8-H, J5-H, 7-H 1.7, J5-H, 8-H 1.0 Hz).

δc(100MHz) 27.5(CH3), 39.6(CH2), 107.7(q-C4'), 113.5(q-C2'), 117.4(C6-H), 123.9(C5-H), 124.8(C3'-H), 127.0(C7-H), 130.1(C5'-H), 135.6(C8-H), 140.3(q-C2'), 148.1(q-C4a), 149.3(q-C8a), 160.1(q-C4).

**Preparation of O′-(4-bromothenyl)-5,8-dideazapterin (2-amino-4-(4-bromothenyloxy-quinazoline) (45)**

The same procedure as with the O′-benzyl derivative with O′-pivaloyl-5,8-dideazapterin (0.250 g, 0.6 mmol) was followed. Recrystallisation from ethanol gave white crystals (0.085 g, 43 %) of O′-(4-bromothenyl)-5,8-dideazapterin.

m.p. 169-170°C.

(Found: C, 46.66; H, 3.56; N, 11.52; S, 9.19; Br, 23.15 %. C11H10BrN2OS requires: C, 46.47; H, 2.99; N, 12.50; S, 9.52; Br, 23.78 %).

λ<sub>max</sub> (MeOH) 234, 267, 328 nm.

NMR(DMSO-d6): δ<sub>H</sub>(400MHz) 5.38(2H, s, CH2), 7.11(2H, s, NH2), 7.12(1H, td, 6-H), 7.19(1H, dd, 5-H), 7.23(1H, s, 3'-H), 7.54(1H, s, 5'-H), 7.58(1H, td, 7-H), 7.94(1H, dd, 8-H), (J5-H, 6-H, J6-H, 7-H, J7-H, 8-H 7.5, J6-H, 8-H, J5-H, 7-H 1.7, J5-H, 8-H 1.0 Hz).

δc(100MHz) 39.7(CH2), 107.6(q-C4'), 115.9(q-C2'), 121.6(C6-H), 123.87(C5-H), 123.91(C3'-H), 126.6(C7-H), 129.6(C5'-H), 134.5(C8-H), 140.4(q-C2'), 149.7(q-C4a), 151.1(q-C8a), 161.7(q-C4).
Preparation of $O^t$-piperonyl-$N^2$-pivaloyl-5,8-dideazapterin (4-piperonyloxy-2-pivaloylamino-quinazoline) (42)

The usual standard procedure was followed, but using piperonyl alcohol ($0.557 \text{ g, 3.66 mmol}$) instead of benzyl alcohol. Recrystallisation from ethanol gave white crystals ($0.438 \text{ g, 48%}$) of the $O^t$-piperonyl derivative.

**m.p.** 137-138°C.

(Found: C, 65.90; H, 5.49; N, 10.90 %. $\text{C}_{21}\text{H}_{21}\text{N}_{3}\text{O}_3$ requires: C, 66.55; H, 5.58; N, 11.09 %).

$\lambda_{\text{max}}$ (MeOH) 280 nm.

NMR(DMSO-$d_6$): $\delta_{\text{H}}$(400MHz) 1.18(9H, s, $3\text{CH}_3$), 5.27(2H, s, CCH$_2$O), 5.95(2H, s, OCH$_2$O), 6.82(1H, d, 2'-H), 6.89(1H, d, 3'-H), (J$_{2'}$-$H$, 3'-H 8.0Hz), 6.98(1H, s, 6'-H), 7.36(1H, td, 6-H), 7.64(1H, dd, 5-H), 7.75(1H, td, 7-H), 8.03(1H, dd, 8-H), (J$_{6}$-$H$, 5-H, J$_{6}$-$H$, 7-H 1.7, J$_{8}$-$H$, 5-H 1.0Hz), 13.42(1H, s, NH).

$\delta_{\text{c}}$(100MHz) 27.5(CH$_3$), 44.3(CCH$_2$O), 100.9(OCH$_2$O), 108.4(C$_3$-$H$), 108.81(C$_6$-$H$), 115.6(q-C$_2$), 117.2(C$_2$-$H$), 121.7(C$_6$-$H$), 124.7(C$_5$-$H$), 127.1(C$_7$-$H$), 130.9(q-C$_1$-$H$), 135.4(C$_8$-$H$), 137.1(q-C$_5^-$), 146.3(q-C$_4$), 147.1(q-C$_4^-$), 152.6(q-C$_8^-$), 160.6(q-C$_4$).

Preparation of $O^t$-piperonyl-5,8-dideazapterin (2-amino-4-piperonyloxyquinazoline) (46)

The procedure, as outlined above for the benzyl derivative, with $O^t$-piperonyl-$N^2$-pivaloyl-5,8-dideazapterin (0.250 g, 0.7 mmol) was followed. Recrystallisation from ethanol gave white crystals of $O^t$-piperonyl-5,8-dideazapterin (0.130 g, 67 %).

**m.p.** 229-230°C.

(Found: C, 64.89; H, 4.42; N, 14.16 %. $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_3$ requires: C, 65.14; H, 4.44; N, 14.24 %).
\( \lambda_{\text{max}}(\text{MeOH}) \) 238, 271, 324 nm.

**NMR(DMSO-\text{d}_6):\) \( \delta_{\text{H}}(400\text{MHz}) \) 5.19(2H, s, CCH\(_2\)O), 5.97(2H, s, OCH\(_2\)O), 6.76(1H, d, 2'-H), 6.84(1H, d, 3'-H), (J\(_{2'}-\text{H}, \text{3'}-\text{H}\) 8.0Hz), 6.87(1H, s, 6'-H), 6.93(2H, s, NH\(_2\)), 7.12(1H, td, 6-H), 7.20(1H, dd, 5-H), 7.59(1H, td, 7-H), 7.94(1H, dd, 8-H), (J\(_{6}-\text{H}, 5-\text{H}\), J\(_{6}-\text{H}, 7-\text{H}\), J\(_{7}-\text{H}, 8-\text{H}\) 7.5, J\(_{6}-\text{H}, 8-\text{H}\), J\(_{5}-\text{H}, 7-\text{H}\) 1.7, J\(_{8}-\text{H}, 5-\text{H}\) 1.0Hz).

\( \delta_{\text{C}}(100\text{MHz}) \) 43.6(CCH\(_2\)O), 100.9(OCH\(_2\)O), 107.8(C\(_3\)-H), 108.2(C\(_6\)-H), 116.1(q-C\(_2\)), 120.3(C\(_2\)-H), 121.6(C\(_6\)-H), 123.8(C\(_4\)-H), 126.6(C\(_7\)-H), 130.0(q-C\(_1\)'), 134.3(C\(_8\)-H), 146.4(q-C\(_5\)'), 147.3(q-C\(_4\)'), 149.7(q-C\(_{4a}\)), 151.8(q-C\(_{8a}\)), 162.0(q-C\(_4\)).

**Preparation of N\(^2\)-pivaloyl-O\(^4\)-thenyl-5,8-dideazapterin (2-pivaloylamino-4-thenyloxyquinazoline) (43)**

The usual procedure was followed, but using thenyl alcohol (0.418 g, 3.66 mmol) instead of benzyl alcohol. Recrystallisation from ethanol gave white crystals of the O\(^4\)-thenyl derivative (0.427g, 51%).

**m.p.** 155-156°C.

(Found: C, 62.73; H, 5.57; N, 12.14; S, 8.37 %. C\(_{18}\)H\(_{19}\)N\(_3\)O\(_2\)S requires: C, 63.40; H, 5.62; N, 12.32; S, 9.38 %).

\( \lambda_{\text{max}}(\text{MeOH}) \) 228, 277 nm.

**NMR(DMSO-\text{d}_6):\) \( \delta_{\text{H}}(400\text{MHz}) \) 1.27(9H, s, BCH\(_3\)), 5.52(2H, s, CH\(_2\)), 6.97(1H, dd, 4'-H), 7.20(1H, dd, 3'-H), 7.41(1H, dd, 5'-H), (J\(_{4'}-\text{H}, \text{3'}-\text{H}\) 3.3, J\(_{4'}-\text{H}, \text{s'}-\text{H}\) 5.5, J\(_{3'}-\text{H}, \text{s'}-\text{H}\) 1.0 Hz), 7.38(1H, td, 6-H), 7.66(1H, dd, 5-H), 7.76(1H, td, 7-H), 8.04(1H, dd, 8-H), (J\(_{5}-\text{H}, 6-\text{H}\), J\(_{6}-\text{H}, 7-\text{H}\), J\(_{7}-\text{H}, 8-\text{H}\) 7.5, J\(_{6}-\text{H}, 8-\text{H}\), J\(_{5}-\text{H}, 7-\text{H}\) 1.7, J\(_{8}-\text{H}, 5-\text{H}\) 1.0 Hz), 13.38(1H, s, NH).

\( \delta_{\text{C}}(100\text{MHz}) \) 27.5(CH\(_3\)), 41.5(CH\(_2\)), 115.4(q-C\(_2\)), 117.3(C\(_6\)-H), 124.7(C\(_5\)-H), 126.05(C\(_4\)-H), 126.24(C\(_3\)-H), 127.0(C\(_5\)-H), 127.9(C\(_7\)-H), 135.5(C\(_8\)-H), 137.0(q-C\(_2\)'), 138.2(q-C\(_{4a}\)), 152.0(q-C\(_{8a}\)), 160.0(q-C\(_4\)).
CHAPTER THREE
1.1.2- Route b

The cyclic hydroxamic acid (5) has been prepared by two methods,\textsuperscript{3} which exemplify well the variants possible within route b.

\[
\text{EtO}_2\text{C} \quad \text{NH}_2\text{OH} \quad \text{Ac}_2\text{O} \quad \text{HO} \quad \text{N} \quad \text{MeCONH} \quad \text{HO} \quad \text{N} \\
\text{IV} \quad \text{IC} \quad \text{I} \quad \text{IV} \quad \text{IC} \\
(5)
\]

1.2- From pyrimidines

The syntheses using pyrimidines as the starting materials, are completed either by an intramolecular electrophilic cyclisation of a pyrimidine with a vacant 4-position (route a) or by the addition of the C-5 and C-6 atoms to a 4-substituted 5-aminopyrimidine (route b).

1.2.1- Route a

The most satisfactory method\textsuperscript{4} involving this type of intramolecular electrophilic cyclisation was the thermal ring closure of aminomethylenemalonates (e.g. (6), R = CO\textsubscript{2}Et) to yield the pyrido[3,2-\textit{d}]pyrimidine-2,4,8(1\textit{H},3\textit{H},5\textit{H})-trione ((7), R = CO\textsubscript{2}Et).
1.2.2- Route b

An example is the formation of pyridopyrimidines (9) from the condensation of 5-amino-1,3,4-trimethyluracil (8) with α-keto esters, such as methyl pyruvate and ethyl mesoxalate, or diethyl oxalate.\(^5\)

2- Synthesis of 8-deazapterin

The most obvious route to the synthesis of 8-deazapterin (2-amino-(3\(H\))-pyrido[3,2-\(d\)]pyrimidin-4-one) occurs in three steps (Scheme 2).
2.1- Synthesis of quinolinimide

In 1925, Sucharda⁶ synthesised quinolinimide (3) from quinolinic acid (2,3-pyridinedicarboxylic acid) (1), via the intermediate anhydride (2). Following the same procedure we managed to synthesise (3) after 9h, in 73 % yield.

The NMR data confirmed the structure of the expected compound, and our melting point 242-243°C agreed with the literature value⁶ (233°C).
2.2- Synthesis of 3-aminopicolinic acid

We synthesised 3-aminopicolinic acid (4) from quinolinimide (3), in 43 % yield, following the procedure reported by Oakes et al.\textsuperscript{7} in 1956.

The melting point, 211°C (lit.\textsuperscript{7} 210°C) and the NMR spectra confirmed the structure of this compound.

\[
\begin{align*}
\text{(3)} & \quad \xrightarrow{\text{Br}_2 / \text{NaOH}} \quad \text{(4)} \\
\end{align*}
\]

2.3- Fusion reaction with guanidine carbonate

In 1952, Korte\textsuperscript{8} reported a method to synthesis 8-deazapterin (5). His procedure is described as a fusion reaction between 3-aminopicolinic acid (4) and guanidine carbonate. The two components were allowed to liquefy for 2h at 120°C and then kept at 140-170°C for 4h, to yield 40 % of 8-deazapterin (5).

\[
\begin{align*}
\text{(4)} & \quad \xrightarrow{\text{guanidine carbonate}} \quad \text{(5)} \\
\end{align*}
\]

Our approach using this procedure did give a product (37 % of crude material) containing 8-deazapterin (5). The very low solubility of this material did not allow further purification and conclusive NMR data.
The next stage of the investigation was therefore to improve the yield and purification of this synthesis. We decided to apply the Maguire et al.\textsuperscript{9,10} procedure as mentioned in Chapter Two for the successful synthesis of 5,8-dideazapterin.

By logical extension, the condensation of methyl 3-aminopicolinate (6) with 4-nitrobenzoylcyanamide (7) should give 3-(4-nitrobenzoyl)-8-deazapterin (8) (2-amino-3-(4-nitrobenzoyl)-pyrido[3,2-\textit{d}]pyrimidin-4-one), which should easily be hydrolysed to afford 8-deazapterin (5).

\begin{align*}
\text{(6)} & \quad \text{MeO}_2\text{C} \quad \text{CONHCN} \quad \text{DMF} \quad 24\text{h, 95°C} \\
\text{(7)} & \quad \text{CONHCN} \\
\rightarrow & \quad \text{CONH}_2\text{N} \quad \text{NO}_2 \\
\text{(8)} & \quad \text{H}_2\text{N} \quad \text{O}_2\text{N} \\
\rightarrow & \quad \text{HN} \quad \text{HN} \\
\text{(5)} & \quad \text{HN} \quad \text{N} \\
\end{align*}

2.4- Fusion reaction with 4-nitrobenzoylcyanamide

2.4.1- Synthesis of methyl 3-aminopicolinate

In 1956, Oakes \textit{et al.}\textsuperscript{7} synthesised ethyl 3-aminopicolinate (9) in 42 % yield, by refluxing 3-aminopicolinic acid (4) with ethanol and concentrated sulphuric acid for 4h. In 1966, Atkinson and Biddle\textsuperscript{11} reported that heating under reflux for 36h was necessary for a good yield of the ester (9).
Application of the Atkinson et al.\textsuperscript{11} procedure, using methanol instead of ethanol, afforded methyl 3-aminopicolinate (6) in 73\% yield. The NMR data confirmed its structure.

2.4.2- Synthesis of 3-(4-nitrobenzoyl)-8-deazapterin

Following the Maguire et al.\textsuperscript{9, 10} method, we obtained 3-(4-nitrobenzoyl)-8-deazapterin (8) in 36\% yield, after 24h at 95°C, by condensing methyl 3-aminopicolinate (6) with 4-nitrobenzoyl cyanamide (7). This compound was found to be very insoluble in organic solvents, therefore compromising any NMR or UV data. Its very high melting point, >300°C, is characteristic of highly insoluble materials. Nevertheless, the analytical data confirmed the presence of the expected product and therefore, we decided to proceed with the hydrolysis.

2.5- Hydrolysis of 3-(4-nitrobenzoyl)-8-deazapterin

The hydrolysis of 3-(4-nitrobenzoyl)-8-deazapterin (8) was carried out using the Maguire et al.\textsuperscript{9, 10} method, which involves gentle heating for 30 min in 0.5 N
The UV features are similar to those obtained for the 5-deazapterin and 5,8-dideazapterin derivatives (Table 2)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N^2$-pivaloyl-5-deazapterin</td>
<td>277</td>
</tr>
<tr>
<td>$N^2$-pivaloyl-5,8-dideazapterin</td>
<td>231, 275</td>
</tr>
<tr>
<td>$N^2$-pivaloyl-8-deazapterin</td>
<td>223, 277</td>
</tr>
</tbody>
</table>

Table 2

The proton NMR shows the expected peaks at 1.27 ppm representing the three methyls of the pivalic group, and at 7.75, 7.89, 8.67 ppm corresponding to the three aromatic protons. The similarity of the respective chemical shifts in the NMR spectra of the three ring systems is reported in Table 3.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>3CH$_3$</th>
<th>5-H</th>
<th>6-H</th>
<th>7-H</th>
<th>8-H</th>
<th>$N^2$-H</th>
<th>$N^2$-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N^2$-pivaloyl-5-deazapterin</td>
<td>1.28</td>
<td>8.43</td>
<td>7.44</td>
<td>8.88</td>
<td>–</td>
<td>11.40</td>
<td>12.31</td>
</tr>
<tr>
<td>$N^2$-pivaloyl-5,8-dideazapterin</td>
<td>1.26</td>
<td>7.52</td>
<td>7.40</td>
<td>7.77</td>
<td>8.06</td>
<td>11.43</td>
<td>12.35</td>
</tr>
<tr>
<td>$N^2$-pivaloyl-8-deazapterin</td>
<td>1.27</td>
<td>–</td>
<td>8.67</td>
<td>7.75</td>
<td>7.89</td>
<td>11.11</td>
<td>12.84</td>
</tr>
</tbody>
</table>

Table 3

4- The Mitsunobu reaction

As for the synthesis of the four $N^2$-pivaloyl-5-deazapterin and 5,8-dideazapterin derivatives, $^{13,14}$ $N^2$-pivaloyl-8-deazapterin (10) was treated with 1.5 molecular
equivalents each of diisopropyl azodicarboxylate, tributylphosphine and the corresponding alcohols, in THF at room temperature. The reaction mixture turned, almost immediately, into an unusual dark red solution, which showed a large mixture of products on TLC (DCM:MeOH, 9:1). The usual work-up led to a dark red oil containing the same multiple compounds, which because of similar polarity could not be separated by column chromatography. This approach was therefore abandoned.

The failure of the Mitsunobu reaction with the 8-deazapterin ring system may, perhaps, be connected with the tendency for the hydroxyl group to form hydrogen bonds with the ring nitrogen atom at position 5 (Scheme 3).

\[
\text{Scheme 3}
\]

5- Chloro substitution on position 4

5.1- The difference between 5-deazapterin and 8-deazapterin

In their study of the chemistry of the 1,3,5- and 1,3,8-triazanaphthalene, Oakes et al.\(^7\) reported that 2,4-dihydroxy-1,3,5-triazanaphthalene (11) was converted into its 2,4-dichloro derivative (12) in 53 % yield. The reaction was carried out with phosphorus oxychloride in the presence of triethylamine.
In the case of 1,3,8-triazanaphthalene, the 2,4-dihydroxy derivative (13) was converted into its 2,4-dichloro derivative (14) in poor yield (33 %). They also noticed that the addition of a tertiary base was without advantage in this case. This confirmed our trouble encountered during the synthesis of 2-amino-4-chloro-5-deazapteridine in Chapter one.

Furthermore, treatment of the dichloro compound (12) with sodium methoxide afforded the 2,4-dimethoxy-1,3,5-triazanaphthalene (15) in a good yield (63 %).

Keeping in mind the high insolubility of 8-deazapterin (5), the next stage of this investigation was to synthesise 4-chloro-\(N^2\)-pivaloyl-8-deazapteridine (16) from
$N^2$-pivaloyl-8-deazapterin (10). We could then convert (16) into 4-methoxy-$N^2$-pivaloyl-8-deazapteridine (17), as a trial, before undertaking the synthesis of the benzyl-, thienyl, piperonyl- and 4-bromothenyl-8-deazapterin derivatives.

5.2- Synthesis of 4-chloro-2-dimethylaminomethyleneamino-8-deazapteridine

In recent years, an improvement in the yield of chloro substitution has been reported when using dimethylchloromethylammonium chloride.\textsuperscript{15, 16, 17} This mild chlorinating agent is generated in situ from thionyl chloride and DMF.

In 1973, Robins and Basom\textsuperscript{15} synthesised (19) in 81 % yield, by treating (18) with dimethylchloromethylammonium chloride in refluxing dichloromethane for 7h. Baht \textit{et al.},\textsuperscript{16} in 1981, synthesised (21) in 97 % yield after 16h reaction.
In 1990, Nagahara et al.\textsuperscript{17} treated (22) under the same conditions to provide (23) in 84 % yield after 16h refluxing in dichloromethane.

We applied the procedure of Nagahara et al.\textsuperscript{17} to $N^2$-pivaloyl-8-deazapterin (10). After 16h at 60°C (reflux), instead of the expected 4-chloro-$N^2$-pivaloyl-8-deazapteridine (16), we obtained a compound whose NMR data confirmed it to be 4-chloro-2-dimethylaminomethyleneamino-8-deazapteridine (24).
Indeed, instead of the expected peak at 1.27 ppm for the three methyl groups, we noticed two peaks at 3.19 and 3.32 ppm, each of which integrated for three protons. The carbon NMR also confirmed the presence of two methyl groups (δ= 35.5 and 41.8 ppm). Another peak at 9.06 ppm (integration equal to one) triggered our attention. The chemical shift was too upfield to represent $N^2$-H (Table 3). In addition to this, the carbon NMR showed a CH group at 160.5 ppm. The chloro substitution at position 4 was confirmed by the absence of the singlet around 11.31 ppm corresponding to $N^2$-H (Table 3).
The mechanism of this reaction, in accordance with Helbert et al.\textsuperscript{18} reactions study of amides and dimethylchloromethylammonium chloride, is shown in Scheme 4.

The lack of time and material did not allow further investigations, which included the synthesis of 2-dimethylaminomethyleneamino-4-methoxy-8-deazapterin (25), using the procedure of Townsend and Robins.\textsuperscript{19} They readily prepared 2-amino-6-methoxy-3-methylpurine (27) from 2-amino-6-chloro-3-methylpurine (26) in less than 30 minutes with sodium methoxide at room temperature.

Upon the successful synthesis of (25), we would have treated (24) with the respective benzyl, thenyl, 4-bromothenyl and piperonyl alcohol and sodium hydride in DMSO, as reported in Chapter One (Section 4.1), in order to synthesise the corresponding 8-deazapterin derivatives.
6- The rather forgotten chloroformamidine hydrochloride

At an unfortunately late time, we came across a paper published by Bavetsias in 1999 about the synthesis of some quinazoline derivatives, among which, some 2-aminoquinazolin-4(3H)-ones. He reported that McNamara et al. synthesized 2-amino-6-methylquinazolin-4(3H)-one (28) from ethyl 2-amino-5-methyl benzoate (27) upon treatment with guanidine, in 80% yield (Chapter Two). The same transformation was achieved in a higher yield (99%) by simply using chloroformamidine hydrochloride (cyanamide dihydrochloride) instead of guanidine.

\[
\begin{align*}
\text{EtO} & \quad \text{guanidine (80%)} \\
\text{H}_2\text{N} & \quad \text{NH}_2\text{CCI}=\text{NH.HCl} \\
\text{dimethyl sulfone} & \quad \text{150°C, 1h} \\
\text{CH}_3 & \quad (99\%) \\
\end{align*}
\]

(27) → (28)

Similarly, Webber et al. employed chloroformamidine hydrochloride for the cyclisation of the methyl esters (29) to the corresponding 2-aminoquinazolin-4(3H)-ones (30).

\[
\begin{align*}
\text{O} & \quad \text{NH}_2\text{CCI}=\text{NH.HCl} \\
\text{Br} & \quad \text{diglyme} \\
\text{R=CH}_3 & \quad \text{R=OCH}_3 \\
\end{align*}
\]

(29) → (30)
7- Experimental

**Preparation of quinolinimide**\(^6\) (3)

A mixture of quinolinic acid (2,3-pyridinedicarboxylic acid) (22.5 g, 0.135 mol) and acetic anhydride (23 ml) was heated at 100°C until the acid had dissolved, and then at 125°C. Acetic acid and excess of acetic anhydride (11 ml) were removed at this temperature. The solution was allowed to cool down, acetamide (14 g, 0.237 mol) was added and the mixture heated for 8h at 125°C. The quinolinimide formed was filtered off, washed with glacial acetic acid and then water. The crude product was stirred with hot water (100 ml). Filtration and recrystallisation from acetic acid gave quinolinimide (14.5 g, 73 %) as a cream-coloured powder.

m.p. 242-243°C (lit.\(^6\) 233°C).

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

**NMR(DMSO-\text{d}_6):** \(\delta (400\text{MHz})\) 7.83(1H, dd, 6-H), 8.31(1H, dd, 5-H), 9.02(1H, dd, 7-H), (J\(_{6-H}\), J\(_{5-H}\), 7.7Hz, J\(_{6-H}\), J\(_{7-H}\) 4.9Hz, J\(_{5-H}\), 7-H 1.3Hz), 11.71(1H, s, NH).

\(\delta (100\text{MHz})\) 127.8(C\(_6\)-H), 128.1(C\(_5\)-H), 131.4(C\(_7\)-H), 152.1(q-C\(_{4a}\)), 154.9(q-C\(_{1a}\)), 167.3(C\(_2\)=O), 167.7(C\(_4\)=O).

**Preparation of 3-aminopicolinic acid** (3-amino-2-pyridinecarboxylic acid)\(^7\) (4)

To a solution of quinolinimide (3 g, 20.3 mmol) in ice-cold sodium hydroxide solution (10 %; 60 ml) was added aqueous sodium hypobromite [from bromine, (3.36 g) and ice-cold sodium hydroxide solution, (15 %; 21 ml)]. The mixture was stirred at room temperature for an hour, heated at 85°C for a further hour and then allowed to cool. The solution was brought to pH 5 with sulphuric acid (50 %) and stored at 0°C for 48h. The 2-aminonicotinic acid that precipitated was then removed by filtration. The filtrate containing 3-aminopicolinic acid was treated with a
solution of copper acetate (1.20 g) in hot water (24 ml) and acetic acid (0.6 ml). The precipitated copper salt was filtered off, washed with water and resuspended in water (24 ml). The suspension was then saturated with hydrogen sulphide and the copper sulphide filtered off. Evaporation of the filtrate and recrystallisation from ethanol gave a cream-coloured powder of 3-aminopicolinic acid (1.18 g, 44 %).

m.p. 211-212°C (lit.7 210°C).

λ_{max} (MeOH) 223, 257, 346 nm.

NMR(DMSO-d_{6}): δ_{H}(400MHz) 7.01(2H, s, NH_{2}), 7.31(1H, dd, 5-H), 7.37(1H, dd, 4-H), 7.85(1H, dd, 6-H), (J_{4-H},5-H 8.54 Hz, J_{4-H},6-H 4.02 Hz, J_{5-H},6-H 1.51 Hz).

δ_{C}(100MHz) 125.4(C_{5}-H), 126.21(q-C_{3}), 128.4(C_{4}-H), 135.0(C_{6}-H), 147.7(q-C_{2}), 167.6(CO_{2}H).

Preparation of ethyl 3-aminopicolinate\(^7\) (9)

3-Aminopicolinic acid (5 g, 36.2 mmol) was heated under reflux with ethanol (13 ml) and concentrated sulphuric acid (6 ml) for 4h. The resulting brown solution was allowed to cool, poured onto ice and basified (pH ~10) with aqueous ammonia. The alkaline mixture was then extracted with ether and the organic layer dried over MgSO_{4}. Evaporation of the filtrate and recrystallisation from hexane gave ethyl 3-aminopicolinate (2.42 g, 40 %) as pale yellow needles.

m.p. 130-131°C (lit.7 131-133°C).

λ_{max} (MeOH) 253, 341 nm.

NMR(DMSO-d_{6}): δ_{H}(400MHz) 1.31 (3H, t, CH_{3}), 4.28 (2H, q, CH_{2}), 6.64 (2H, s, NH_{2}), 7.21 (1H, dd, 5-H), 7.27 (1H, dd, 4-H), 7.86 (1H, dd, 6-H), (J_{4-H},5-H 8.54 Hz, J_{4-H},6-H 4.02 Hz, J_{5-H},6-H 1.51 Hz).
CHAPTER FOUR
The UV data, taken in methanol, were consistent with those reported for 3-aminopicolinic acid (Chapter Three) (Table 1), and the NMR data gave evidence of the expected compound.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-aminisoionicotinic acid</td>
<td>224, 248, 351</td>
</tr>
<tr>
<td>3-aminopicolinic acid</td>
<td>223, 257, 346</td>
</tr>
</tbody>
</table>

Table 1

We slightly increased the yield of the reaction to 49 % by treating (8) with sodium hypobromite, following the procedure of Oakes et al.\textsuperscript{19} for the synthesis of 3-aminopicolinic acid.

1.2.2- Synthesis of methyl 3-aminisoionicotinate

After 30h refluxing in methanol containing concentrated sulphuric acid, we synthesised methyl 3-aminisoionicotinate (10) in 65 % yield, following the procedure given in 1966 by Atkinson and Biddle.\textsuperscript{20} Our melting point, 84-86°C, agreed with the literature value\textsuperscript{1} (86-87°C). The NMR data show the presence of the expected
methyl group (δ=3.84 ppm), the amino group at the 2-position (δ=6.67 ppm) and the three aromatic protons (δ=7.47, 7.75 and 8.25 ppm).

\[
\text{HO,C.} \quad \text{MeOH} \quad \text{conc. H}_2\text{SO}_4
\]

1.2.3- Synthesis of 3-(4-nitrobenzoyl)-7-aza-5,8-dideazapterin

Following the well-established condensation reaction given in the previous chapters,\textsuperscript{4,5} we treated methyl 3-aminoisonicotinate (10) with 4-nitrobenzoyl-cyanamide (11) in dry DMF. After 24h at 95°C, 3-(4-nitrobenzoyl)-7-aza-5,8-dideazapterin (12) was synthesised in 32% yield.

\[
\begin{array}{c}
\text{MeO}_2\text{C} \quad \text{CONHCN} \\
\text{H}_2\text{N} \quad \text{DMF} \\
\text{H}_2\text{N} \quad 24\text{h, 95°C} \\
\end{array}
\]

The UV maxima, as well as the NMR data, were in accordance with the values obtained for the equivalent 5,8-dideazapterin derivative (Table 2). The high insolubility of the 8-deazapterin ring system in organic solvents did not allow such comparison.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-(4-nitrobenzoyl)-5,8-dideazapterin</td>
<td>234, 311</td>
</tr>
<tr>
<td>3-(4-nitrobenzoyl)-7-aza-5,8-dideazapterin</td>
<td>220, 268</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$2'-\text{H}$</th>
<th>$3'-\text{H}$</th>
<th>$5'-\text{H}$</th>
<th>$6'-\text{H}$</th>
<th>$7'-\text{H}$</th>
<th>$8'-\text{H}$</th>
<th>$\text{NH}_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-(4-nitrobenzoyl)-5,8-dideazapterin</td>
<td>8.35</td>
<td>8.35</td>
<td>7.61</td>
<td>7.44</td>
<td>7.81</td>
<td>8.06</td>
<td>12.54</td>
</tr>
<tr>
<td>3-(4-nitrobenzoyl)-7-aza-5,8-dideazapterin</td>
<td>8.28</td>
<td>8.36</td>
<td>7.91</td>
<td>8.58</td>
<td>-</td>
<td>8.98</td>
<td>12.46</td>
</tr>
</tbody>
</table>

Table 3

1.2.4- The hydrolysis of 3-(4-nitrobenzoyl)-7-aza-5,8-dideazapterin

The hydrolysis of 3-(4-nitrobenzoyl)-7-aza-5,8-dideazapterin (12) was carried out under the usual mild conditions$^4,5$ and gave 7-aza-5,8-dideazapterin (13) in 63 % yield, after 30 min of heating in 0.5 N NaOH.
Once again, the UV data (in 0.1 N NaOH) are similar to those reported for the previous ring systems (Table 4). No NMR data were available, due to the insolubility of this compound in organic solvents.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\lambda_{\text{max}} , (\text{nm})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-deazapterin</td>
<td>240, 264, 330</td>
</tr>
<tr>
<td>5,8-dideazapterin</td>
<td>234, 264, 325</td>
</tr>
<tr>
<td>8-deazapterin</td>
<td>231, 268, 329</td>
</tr>
<tr>
<td>7-aza-5,8-dideazapterin</td>
<td>225, 270, 335</td>
</tr>
</tbody>
</table>

Table 4

1.3- Importance of the ring nitrogen atom in the condensation reaction

Comparing the yield of the condensation reaction during the synthesis of our ring systems, it appears that the presence of a nitrogen atom or not, as well as its position on the ring of the ester, is of some importance (Table 5).

Maguire et al. reported that the condensation of ethyl 2-aminonicotinate (14, R=Et) and 2-aminonicotinic acid (14, R=H) with benzoylcyanamide failed to give the expected 3-benzoyl-5-deazapterin (15).
Our attempt using the more electrophilic agent, 4-nitrobenzoylcyanamide, also failed to give any result.

<table>
<thead>
<tr>
<th>Esters</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td>77</td>
</tr>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td>69</td>
</tr>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td>61</td>
</tr>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 5**

As expected, methyl anthranilate gave the highest yield, the amino group being the most nucleophilic. Indeed, the presence of a nitrogen, an electron-withdrawing atom, induced electron deficiency in the ring. This in consequence will diminish the nucleophilicity of the amino group. 2-Amino- (and presumably 4-amino-) pyridine derivatives show a non-nucleophilic character due to the positive charge directly present on the amino group on the 2-position of the ring (resonance of pyridines), in contrast to the 3-aminopyridine derivatives, where the positive charge is
only as close as the ortho position. The 3-amino esters in Table 5 would therefore have similar nucleophilic character.

The hydrolysis reaction also followed this order of reactivity. (Table 6)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,8-dideazapterin</td>
<td>98</td>
</tr>
<tr>
<td>8-deazapterin</td>
<td>76</td>
</tr>
<tr>
<td>7-aza-5,8-dideazapterin</td>
<td>63</td>
</tr>
</tbody>
</table>

Table 6

2- A study of 1-methyl-5,8-dideazapterin (2-amino-1-methyl-(3H)-quinazolin-4-one)

2.1- Introduction

After the successful condensation of methyl anthranilate and 4-nitrobenzoyl cyanamide, leading to the synthesis of 5,8-dideazapterin (Chapter Two), we felt that a comparison with methyl N-methylanthranilate (17) would be very instructive in showing the effect of a methyl substituent during this reaction.

![Formula 17](image)

Indeed, as we have shown previously, the nucleophilic character of the amino group is of great importance. Introduction of a methyl group, which is slightly electron donating, might increase the nucleophilicity of the amine.
2.2- Synthesis of 1-methyl-5,8-dideazapterin

2.2.1- Synthesis of methyl N-methylantranilate

Following the usual procedure of esterification, we synthesised methyl
N-methylantranilate (17) from N-methylantranilic acid (16) in methanol and
concentrated sulphuric acid. After 4h at reflux, we obtained (17) in 54 % yield.

$$\text{CH}_3 \text{ CH}_2 \text{ } \text{H}_2 \text{SO}_4 \text{ MeOH}$$

The proton and carbon NMR spectra gave evidence of the presence of the
expected two methyl groups. The usual peaks at 3.79 and 51.4 ppm afforded proof of
the successful esterification (Table 7), and another one (2.85 and 29.2 ppm) for the
$N$-substituent.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$^1H$</th>
<th>$^{13}C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl 3-aminopicolinate</td>
<td>3.81</td>
<td>51.5</td>
</tr>
<tr>
<td>Methyl 3-aminonicotinate</td>
<td>3.84</td>
<td>51.9</td>
</tr>
<tr>
<td>Methyl 1-methylantranilate</td>
<td>3.79</td>
<td>51.4</td>
</tr>
</tbody>
</table>

Table 7
2.2.2- Synthesis of 1-methyl-3-(4-nitrobenzoyl)-5,8-dideazapterin

Following the standard conditions, \(^4\) methyl \(N\)-methylanthranilate (17) was condensed with 4-nitrobenzoylcyanamide (11) in dry DMF. After 24h at 95°C, we collected 1-methyl-3-(4-nitrobenzoyl)-5,8-dideazapterin (18) in 33% yield. This compound was found to be very insoluble in organic solvents, therefore compromising any relevant NMR data. The UV values of 221 and 267 nm agreed with the values obtained for the other ring systems (Table 2).

\[
\text{MeO}_2C\quad + \quad \text{CONHCN} \quad \xrightarrow{\text{DMF, 24h, 95°C}} \quad \text{O}_2N \quad \text{HN} \quad \text{CH}_3
\]

2.2.3- Synthesis of 1-methyl-3-(3,5-dinitrobenzoyl)-5,8-dideazapterin

1-Methyl-3-(3,5-dinitrobenzoyl)-5,8-dideazapterin (20) was synthesised in 30% yield, under the same conditions as for the synthesis of (18), using 3,5-dinitrobenzoylcyanamide (19) instead of 4-nitrobenzoyl cyanamide (11). This compound was also very insoluble in any organic solvents. Its UV maxima at 222 and 315 nm matches the values found for (18).
2.2.4- The hydrolysis reaction

2.2.4.1- Of 1-methyl-3-(4-nitrobenzoyl)-5,8-dideazapterin

The hydrolysis was carried out under the usual mild conditions, using 0.5 N NaOH. Even after 10 min of heating, dissolution was not complete and the solution was becoming darker. After the usual 30 min, the solution mixture was brown and some solid still remained. A TLC check of the solution using DCM:MeOH, 9:1 as solvent system, showed multiple products. The remaining solid was filtered off to give an impure brown material, very insoluble in organic solvents. Acidification of the filtrate with acetic acid did not give the required compound.

2.2.4.2- Of 1-methyl-3-(3,5-dinitrobenzoyl)-5,8-dideazapterin

The same decomposition as described above occurred during the hydrolysis of 1-methyl-3-(3,5-dinitrobenzoyl)-5,8-dideazapterin with 0.5 N NaOH. No further investigation of the 1-methyl derivatives was undertaken.
3- The 2-amino-6-chloropurine ring system

3.1- Introduction

The synthetic challenge for \( \phi^\prime \)-alkylguanine (23) and related 2-amino-6-hydroxy heterocycles is the construction of the \( CH_2-\phi^\prime \) bond. This very often occurs from guanine (21) via 2-amino-6-chloropurine (22).

\[
\begin{align*}
\text{(21)} & \quad \xrightarrow[]{} \quad \text{(22)} & \quad \xrightarrow[]{\text{ROH}} \quad \text{(23)}
\end{align*}
\]

But conversion of the 6-oxo function to the chloro moiety is complicated by the extremely low solubility of guanine. As for the pterin derivatives (Chapter Three) this is presumably due to intramolecular hydrogen bonding (Scheme 3).
All early attempts at conventional introduction of chlorine into guanine using the standard reagents phosphorus oxychloride and phosphorus pentachloride up to 150°C failed. In 1960, Montgomery et al. and Daves et al. resorted to the thiation of guanine followed by chlorinolysis. However, the direct conversion of guanine to 2-amino-6-chloropurine is so attractive for large-scale synthesis that determined attempts have been reported in recent years. Japanese firms used phosphorus oxychloride with either DMF or N,N-diethylaniline and obtained moderate yields (36 to 55 %). Beecham introduced phase transfer catalysts, such as triethylmethylammonium chloride, and warm acetonitrile as solvent. This afforded the chloro compound in 54 % yield. Boehringer, using prior acetylation or silylation of guanine, achieved the conversion (80-90 % yield) in refluxing acetonitrile with phosphorus oxychloride.

Since the price of the commercially available 2-amino-6-chloropurine is so high, any alternative method of synthesis has always been welcome. Following our successful conversion of 2-pivaloyl-8-deazapterin (10, Chapter Three) into 4-chloro-2-dimethylaminomethyleneamino-8-deazapteridine (25, Chapter Three) using the Robins and Basom procedure, we decided to apply the same reaction conditions to 2-pivaloylguanine. Subsequent deprotection would then yield the 2-amino-6-chloropurine (Scheme 4).
Scheme 4

3.2- Synthesis of N^2-pivaloylguanine

In 1991, Taylor et al.\textsuperscript{16} synthesised N^2-pivaloylguanine (26) as a precursor in the synthesis of [4-(2-guanine-8-ylethyl)benzoyl] glutamic acid (24), a guanine analogue of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (25).
Under the same reaction conditions, using pivaloyl chloride and pyridine, we obtained $N^2$-pivaloylguanine (26) after 5h in good yield (83 %). The NMR data confirmed the structure of the expected compound.

3.3- Synthesis of 2-dimethylaminomethyleneamino-6-chloropurine

3.3.1- From 2-amino-6-chloropurine

In 1994, in their preparation of some 2'-deoxyribonucleosides, Votruba et al.\textsuperscript{17} converted the amino group of adenine and guanine derivatives into an amidine group (the so-called dimethylaminomethylene derivative) by reaction with $N,N$-dimethylformamide dineopentyl acetal. Application of this procedure to 2-amino-6-chloropurine (22), gave 2-dimethylaminomethyleneamino-6-chloropurine (27) as a white solid in good 90 % yield.
The NMR data, with the two methyl groups showing at $\delta = 3.03$ and 3.14 ppm and the extra CH group at $\delta = 8.57$ ppm, confirmed its structure. This compound served as a reference for the following reaction.

3.3.2- From $N^2$-pivaloylguanine

Using the Nagahara et al.$^{18}$ procedure, we treated $N^2$-pivaloylguanine (26) with dimethylchloromethylammonium chloride (from thionyl chloride and DMF) in dry DMF since $N^2$-pivaloylguanine was insoluble in dichloromethane. The reaction was kept at 60°C for 16h. The usual work up, by extraction of the basified (aq. NaHCO$_3$) reaction mixture, gave three products (TLC, chloroform:acetone, 4:1). The expected dimethylaminomethyleneamino derivative was the minor component and the two principal products were less polar. We managed to isolate one of these by leaving it to crystallise at room temperature from the evaporated organic extract. We obtained this unknown as bright yellow crystals. Its insolubility did not permit any NMR data. The synthesis of 2-dimethylaminomethyleneamino-6-chloropurine via this route was abandoned.
4- Experimental

Preparation of 3-aminoisonicotinic acid\(^1,19\) (9)

(a) Ice-cold KOH (10 %; 17 ml) was added to a solution of 3,4-pyridinedicarboximide (8) (1.48 g, 10 mmol) in aqueous potassium hypobromite (29 ml) [from \(\text{Br}_2\) (1.10 ml) and ice-cold KOH (10 %; 60 ml)]. The solution was heated at 80°C for 1h. The reaction mixture was allowed to cool and acidified (~pH 5) with acetic acid. The crystals precipitated by glass scratching and were filtered, dried under vacuum over \(\text{P}_2\text{O}_5\) to give 3-aminoisonicotinic acid (0.617 g, 45 %) as a light pink solid.

m.p. >300°C (lit.\(^1\) 308-310°C).

\(\lambda_{\text{max}}\) (MeOH) 224, 248, 351 nm.

(b) Aqueous sodium hypobromite [from \(\text{Br}_2\) (0.9 ml) and ice-cold NaOH (15 %; 17.5 ml)] was added to a solution of 3,4-pyridinedicarboximide (2.5 g, 16.9 mmol) in ice-cold NaOH (10 %; 50 ml). The solution was stirred at room temperature for 1h and then at 85°C for another hour. The reaction was allowed to cool, acidified to pH~5 with 50 % sulphuric acid and kept at 0°C for 48h. The resulting precipitate was filtered and dried under vacuum over \(\text{P}_2\text{O}_5\) to give 3-aminoisonicotinic acid (1.15 g, 49 %) as a light pink solid.

m.p. >300°C.

\(\lambda_{\text{max}}\) (MeOH) 223, 247, 351 nm.

NMR(DMSO-\(d_6\)): \(\delta_{\text{H}}\) (400MHz) 6.85(2H, br s, \(\text{NH}_2\)), 7.47(1H, d, 5-H), 7.74(1H, d, 6-H), 8.21(1H, s, 2-H), (\(J_{5,6,H-H}\) 5.0 Hz).

\(\delta_{\text{C}}\) (100MHz) 114.5(q-C\(_3\)), 123.0(C\(_2\)-H), 135.2(C\(_6\)-H), 140.5(C\(_5\)-H), 145.7(q-C\(_4\)), 168.5(C=O).
Preparation of ethyl 3-aminoisonicotinate

A mixture of 3-aminoisonicotinic acid (1 g, 7.24 mmol), ethanol (4 ml) and concentrated sulphuric acid (1 ml) was heated under reflux for 30 h. The resulting brown solution was allowed to cool, poured on ice and basified (pH~10) with Na₂CO₃. Ether extraction of the basified mixture and recrystallisation of the residue from hexane, gave ethyl 3-aminoisonicotinate (0.767 g, 64 %) as yellow needles.

m.p. 63-64°C (lit.²⁰ 65°C).

λ_{max} (MeOH) 226, 240, 351 nm.

NMR(DMSO-d₆): δ_{H}(400MHz) 1.32(3H, t, CH₃), 4.30(2H, q, CH₂), 6.65(2H, NH₂), 7.4791H, d, 5-H), 7.75(1H, d, 6-H), 8.25(1H, s, 2-H), (J₅-H,₆-H 5.0 Hz).

δ_{C}(100MHz) 13.0(CH₃), 59.6(CH₂), 112.4(q-C₃), 121.2(C₂-H), 134.2(C₆-H), 139.8(C₅-H), 144.8(q-C₄), 165.4(C=O).

Preparation of methyl 3-aminoisonicotinate

The same procedure as above was followed but using methanol (26 ml) instead of ethanol. Extraction with chloroform of the basified mixture (Na₂CO₃) and recrystallisation from hexane gave methyl 3-aminoisonicotinate (5.21 g, 65 %) as yellow needles.

m.p. 84-86°C (lit.¹ 86-87°C).

λ_{max} (MeOH) 222, 248, 353 nm.

NMR(DMSO-d₆): δ_{H}(400MHz) 3.84(3H, s, CH₃), 6.67(2H, NH₂), 7.47(1H, d, 5-H), 7.75(1H, d, 6-H), 8.25(1H, s, 2-H), (J₅-H,₆-H 5.0 Hz).

δ_{C}(100MHz) 51.9(CH₃), 113.2(q-C₃), 122.3(C₂-H), 135.2(C₆-H), 140.9(C₅-H), 145.4(q-C₄), 166.9(C=O).
Preparation of 3-(4-nitrobenzoyl)-7-aza-5,8-dideazapterin (2-amino-3-(4-nitrobenzoyl)-pyrido-[3,4-d]pyrimidin-4-one) (12)

4-Nitrobenzoylcyanamide (1 g, 5.24 mmol), was added to a solution of methyl 3-aminoisonicotinate (0.796 g, 5.24 mmol) in dry DMF (5 ml). The mixture was heated at 95°C for 24 h and then allowed to cool down. The precipitate was filtered and washed with ether to give pure 3-(4-nitrobenzoyl)-7-aza-5,8-dideazapterin (0.52 g, 32%). The filtrate was diluted with ethanol (7.5 ml) and poured into ice water (35 ml). The resulting suspension was stirred overnight, filtered and then washed with ether to give an additional 0.47 g (29%) of crude material.

m.p. >300°C.

λ_{max} (MeOH) 220, 268 nm.

NMR(DMSO-d_6): δ_{H}(400 MHz) 7.91(1H, d, 5-H), 8.28(2H, d, 2'-H), 8.36(2H, d, 3'-H), 8.58(1H, d, 6-H), 8.98(1H, s, 8-H), (J_{5,6,6-H} 5.0 Hz), 12.46(2H, NH_2).

δ_{C}(100 MHz) 118.8(C_5-H), 123.8(C_2-H), 129.9(C_6-H), 130.4(C_3-H), 145.1(C_8-H), 149.5(q-C).

Preparation of 7-aza-5,8-dideazapterin (2-amino-(3H)-pyrido-[3,4-d]pyrimidin-4-one) (13)

3-(4-Nitrobenzoyl)-7-aza-5,8-dideazapterin (0.74 g, 2.38 mmol) was added to NaOH (0.5 M; 33 ml) and the mixture heated for 30 min after complete dissolution. The yellow solution was then filtered, allowed to cool and acidified to pH 5.5-6.0 with acetic acid. The resulting precipitate was filtered, washed with NaHCO_3, then ether to give 7-aza-5,8-dideazapterin (0.24 g, 63%).

m.p. >300°.

λ_{max} (0.1 M NaOH) 225, 270, 335 nm.
**Preparation of methyl N-methylantranilate**\(^{21}\) (17)

A mixture of \(N\)-methylantranilic acid (15.12 g, 0.1 mmol), methanol (30 ml) and concentrated sulphuric acid (15 ml) was heated under reflux for 4 h. The resulting brown solution was allowed to cool down, poured onto ice and basified with aqueous ammonia (25 %). The mixture was extracted with ether and the organic layer dried over MgSO\(_4\). Evaporation of the filtrate and purification of the resulting brown residue by passing through a column (hexane:EtOAc, 6:1) gave methyl \(N\)-methylantranilinate (8.84 g, 54 %) as an oil.

(lit.,\(^{21}\) m.p. 18.5-19 °C).

**NMR(DMSO-\(_d_6\))**: \(\delta_H(400\text{MHz})\) 2.85(3H, s, NHCH\(_3\)), 3.79(3H, s, OCH\(_3\)), 6.58(1H, td, 5-H), 6.71(1H, dd, 6-H), 7.39(1H, td, 4-H), 7.54(1H, br s, NH), 7.79(1H, dd, 3-H), (J\(_5\)-H, 6-H, J\(_6\)-H, 7-H, J\(_7\)-H, 8-H 7.5, J\(_6\)-H, 8-H, J\(_5\)-H, 7-H 1.5, J\(_5\)-H, 8-H 1.0 Hz).

\(\delta_C(100\text{MHz})\) 29.2(NHCH\(_3\)), 51.4(OCH\(_3\)), 109.2(q-C\(_1\)), 111.0(C\(_5\)-H), 114.1(C\(_6\)-H), 131.0(C\(_4\)-H), 134.8(C\(_3\)-H), 151.4(q-C\(_2\)), 168.1(C\(_=\text{O}\)).

**Preparation of 1-methyl-3-(4-nitrobenzoyl)-5,8-dideazapterin** (18)

4-Nitrobenzoylcyanamide (0.191 g, 1 mmol) was added to a solution of methyl \(N\)-methylantranilinate (0.165 g, 1 mmol) in dry DMF (1 ml). The mixture was heated at 95 °C for 24 h and then allowed to cool down. The precipitate was filtered and washed with ether to give pure 1-methyl-3-(4-nitrobenzoyl)-5,8-dideazapterin (0.107 g, 33 %).

m.p. 281-282 °C.

(Found: C, 59.13; H, 3.80; N, 17.16 %. C\(_{16}\)H\(_{12}\)N\(_4\)O\(_4\) requires: C, 59.26; H, 3.70; N, 17.28 %).

\(\lambda_{\text{max}}(\text{MeOH})\) 221, 267 nm.
Preparation of 1-methyl-3-(3,5-dinitrobenzoyl)-5,8-dideazapterin (20)

The same procedure as above is followed but using 3,5-dinitrobenzoylcyanoamide (0.236 g, 1 mmol) instead of 4-nitrobenzoylcyanoamide. The mixture was heated at 95°C for 5h. The precipitate was filtered and washed with ether to give pure 1-methyl-3-(3,5-dinitrobenzoyl)-5,8-dideazapterin (0.110 g, 30%).

m.p. 277-278°C.

(Found: C, 51.84; H, 2.98; N, 18.79 %. C_{16}H_{11}N_5O_6 requires: C, 52.03; H, 2.98; N, 18.97 %).

λ_max (MeOH) 222, 315 nm.

Preparation of N^2-pivaloylguanine (26)

Pivaloyl chloride (5 ml) was added to a suspension of guanine (1.51 g, 10 mmol) in pyridine (35 ml), and the mixture refluxed for 3h. The reaction mixture was then evaporated to dryness and ethanol (40 ml) was added. The reaction was refluxed for another 2h. The mixture was allowed to cool and aqueous ammonia (5 %; 12 ml) was added. The resulting precipitate was filtered, washed with water and dried under vacuum over P_2O_5 to give N^2-pivaloylguanine (1.94 g, 83 %) as a brown solid.

m.p. >300°C (lit. 315-318°C).

λ_max (MeOH) 221, 259 nm.

NMR(DMSO-d_6): δ (400MHz) 1.26(9H, s, CH_3), 8.03(1H, s, 8-H), 10.95(1H, s, N^2-H), 12.16(1H, s, N^2-H), 13.11(1H, br s, N^9-H).
Preparation of 2-dimethylaminomethyleneamino-6-chloropurine\textsuperscript{17} (27)

A mixture of 2-amino-6-chloropurine (0.169 g, 1 mmol), dry DMF (2.5 ml) and \textit{N},\textit{N}-dimethylformamide dineopentyl acetal (0.5 ml) was stirred at 80°C until complete dissolution, using a calcium chloride protecting tube. The solution was then stirred at room temperature overnight. The solvent was evaporated to dryness. The resulting white residue was co-distilled with toluene (2 × 2 ml) and then mixed with ethanol (1.7 ml). Ether (25 ml) was added, the resulting solid was filtered, washed with ether and dried under vacuum over \textit{P}_2\text{O}_5, to give 2-dimethylaminomethyleneamino-6-chloropurine (0.202 g, 90 %) as a white solid.

\textbf{m.p.} > 300°C (lit.\textsuperscript{17} > 280°C).

\textbf{\(\lambda_{max}(\text{MeOH})\)} 239, 281 nm.

**NMR(DMSO-\textit{d}_6):** \(\delta_{\text{H}}(400\text{MHz})\)

\begin{align*}
3.03(3\text{H, s, CH}_3), & 3.14(3\text{H, s, CH}_3), 8.31(1\text{H, s, 8-H}), 8.57(1\text{H, s, CH}), 13.11(1\text{H, br s, N}\textsuperscript{\phi}-\text{H}). \\
\delta_{\text{C}}(100\text{MHz}) & 34.6(\text{CH}_3), 40.8(\text{CH}_3), 143.6(\text{C}_8-\text{H}), 158.2(\text{CH}), 162.0(\text{q-C}).
\end{align*}

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**5- References**


CONCLUSIONS

As part of the ongoing research on the synthesis of potential inhibitors of the protein ATase, we have synthesised several new compounds. The latter are deaza derivatives of known active pterin lead compounds.

Four new $O^\prime$-aryl-5-deazapterin derivatives were prepared using the Mitsunobu reaction, which only proceeds when the pterin is suitably protected at the 2-amino position. The pivaloyl group used in this circumstance also imparts the necessary solubility. Initial deprotection reaction, using sodium hydroxide, gave in our case the corresponding $O^\prime$-lumazine derivatives. Hydrazine hydrate was used instead, and proved to be an efficient reagent for depivaloylation. We obtained the required 5-deazapterin derivatives. These latter compounds, however, were found to be inactive as inhibitors of ATase, therefore showing the importance of the nitrogen on the 5-position in the active pterins.

Four $O^\prime$-aryl-5,8-dideazapterin were synthesised via the same Mitsunobu reaction. These compounds were also proved to be inactive, confirming the importance of the nitrogen atoms in the lead compound. 5,8-Dideazapterin was obtained by the condensation of methyl anthranilate and benzoylcyanamide. The rate and yield of this ring formation was improved by the introduction of one or two nitro groups in the phenyl ring of benzoylcyanamide. Using this method, a series of 4-nitro-2,4- and 3,5-dinitrobenzoyl derivatives of 5,8-dideaza-, 1-methyl-5,8-dideaza- and 7-aza-5,8-dideazapterins were obtained, in improved yields and rates. Subsequent hydrolyses of the 4-nitro derivatives were successful, although the 2,4- and 3,5-dinitro compounds failed to give the desired deazapterins.