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The Synthesis of Prodrugs Derived From PaTrin-2

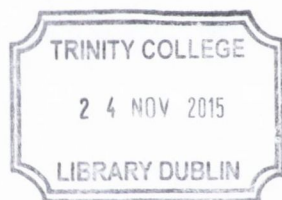
By

Dawn Bridget Ronan

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

Chemistry Department
Trinity College
Dublin

October 2002



Thesis 7310

Declaration

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Acknowledgements

I wish to thank my supervisor Prof. T.B.H. McMurry for his guidance and direction these past few years. I am also very grateful to Dr. R.S. McElhinney for all his help and encouragement. Many thanks also to everyone in the top lab, as without their company it may not have been possible to endure the leaky ceiling and all those freezing days at altitude. A special thanks also to Dr. Dorothy J. Donnelly, for being the source of all knowledge, and for brightening up all my days.

I am thankful to everyone in the Chemistry Department, especially to the technical staff who have always been there to help me out. I am extremely grateful to Dr. John O'Brien for supplying me with endless numbers of "interesting" NMR spectra and for all those carbons at coffee.

I wish to express my gratitude to Dr. G.P. Margison and his colleagues at the Paterson Institute in Manchester for all their work on our PaTrin-2 prodrugs. I would also like to thank Kudos Pharmaceuticals Ltd., Cambridge, for generously supplying us with 10 grams of PaTrin-2, thereby allowing me to frivolously scale up my reactions (from 0.1 to 0.2 mmol!) in the final months.

Finally, I would like to thank all those who are dear to me; my family, especially my parents, for their love, support and encouragement, and for putting up with me when the joys of chemistry did not make me too joyful. And thank you to Pierre, for being my own personal computer advisor, and more importantly for loving me.

Abstract

Chemotherapeutic alkylating agents, like triazenes and nitrosoureas, whose mechanism of action involves the modification of guanine residues at the O^6 -position in DNA are often rendered ineffective by the repair enzyme ATase. Found in all cells, ATase (O^6 -alkylguanine-DNA alkyltransferase) is responsible for the removal of the introduced alkyl groups from the O^6 -atom of guanine. Inactivation of ATase by the administration of pseudosubstrates prior to chemotherapy has been shown to enhance the cytotoxic effects of subsequently administered alkylating agents.

PaTrin-2, O^6 -(4-bromophenyl)guanine, is a potent ATase inactivator and has recently completed a successful Phase I clinical trial. However, PaTrin-2 has major disadvantages, exhibiting poor water-solubility and limited selectivity for neoplastic tissue. In an effort to alter the pharmacokinetics of this drug we have prepared a series of prodrugs of PaTrin-2 through the bioreversible derivatization of the N-9 position in the purine ring system. These prodrugs have advantages for drug formulation and enhancing selectivity.

The synthesis of these prodrugs has required a comprehensive investigation of the most efficient routes and effective reagents. As a result, we have established procedures that have made possible the preparation of prodrugs for this current pharmacological investigation and will facilitate the synthesis of further prodrugs of this type, as and if required.

In addition, this investigation has provided an insight into mechanism, particularly with regards to the role of PaTrin-2 as a chemical entity in acylating reactions. Difficulties with the use of this reactant are associated with the unique characteristics of the purine ring system. This current work has increased our understanding of PaTrin-2 as a reactant, aiding current and related PaTrin-2 research.

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Appendix 1: Glossary

Appendix 2: NMR Spectra

Abbreviations

<i>t</i>-BuMe₂SiCl	<i>t</i> -Butyldimethylsilyl chloride
(C₂H₅)₃N	Triethylamine
(CH₃)₃N	Trimethylamine
DBU	Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl acetate
EtOH	Ethanol
HCl	Hydrochloric acid
Hünig's Base	Ethyl-diisopropylamine {NEt(<i>i</i> -Pr) ₂ }
K₂CO₃	Potassium carbonate
LiAlH₄	Lithium aluminium hydride
MgSO₄	Magnesium sulfate
NaBH₄	Sodium borohydride
NaCl	Sodium chloride
NaH	Sodium hydride
NaHCO₃	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
NH₄Cl	Ammonium chloride
P₂O₅	Phosphorus pentoxide
THF	Tetrahydrofuran

CHAPTER 1

Chapter 1: Introduction^{*}

1.1 Cancer – definition^{1,2}

Cancer is one of the most feared diseases in the world. Tumours, abnormal tissue growths can be benign or malignant, the difference being that a primary malignant tumour does not remain localized. Malignant tumour cells often lack the adhesion devices that bind normal cells into the extracellular matrix, therefore they are easily transported by blood and lymphatic systems to secondary locations. The spreading of cancerous tumour cells is known as metastasis. There are many types of cancer, but all are characterized by having cells that proliferate without constraint.

1.1.1 Causes^{1,2}

The conversion of a normal cell into a cancer cell is usually the result of a mutation (irreversible genetic change) within a cell. The establishment of a tumour cell line, where cells divide uncontrollably, is not the result of a single mutation, but the accumulation of a number of different changes within a cell. Even then there is no certainty that the tumour will become cancerous rather than remaining a localised non-malignant growth. Mutations in the DNA of a cell may be spontaneous or the result of external agents. There are many types of cancer-promoting agents. Cancers may be caused by ionizing radiation (X-rays, γ -rays), viruses (Epstein-Barr virus, which is one of the herpes viruses), UV radiation (sunlight), chemical carcinogens or even diet.

^{*} A glossary of biological terms and phrases can be found in the Appendix.

1.2 Damage to DNA^{2,3}

Deoxyribonucleic acid (DNA), the double helix, provides the genetic code and therefore the blueprint for life. DNA consists of two intertwining polynucleotide chains, with the nucleotide residues within the chain interconnected by phosphate groups that are esterified to both the 3'-OH group of one 2-deoxy-D-ribose sugar and the 5'-OH of another (Fig. 1.1). Each nucleotide residue is a phosphate ester of a nucleoside. Nucleosides in DNA are composed of one of four heterocyclic bases connected to the sugar 2-deoxy-D-ribose.

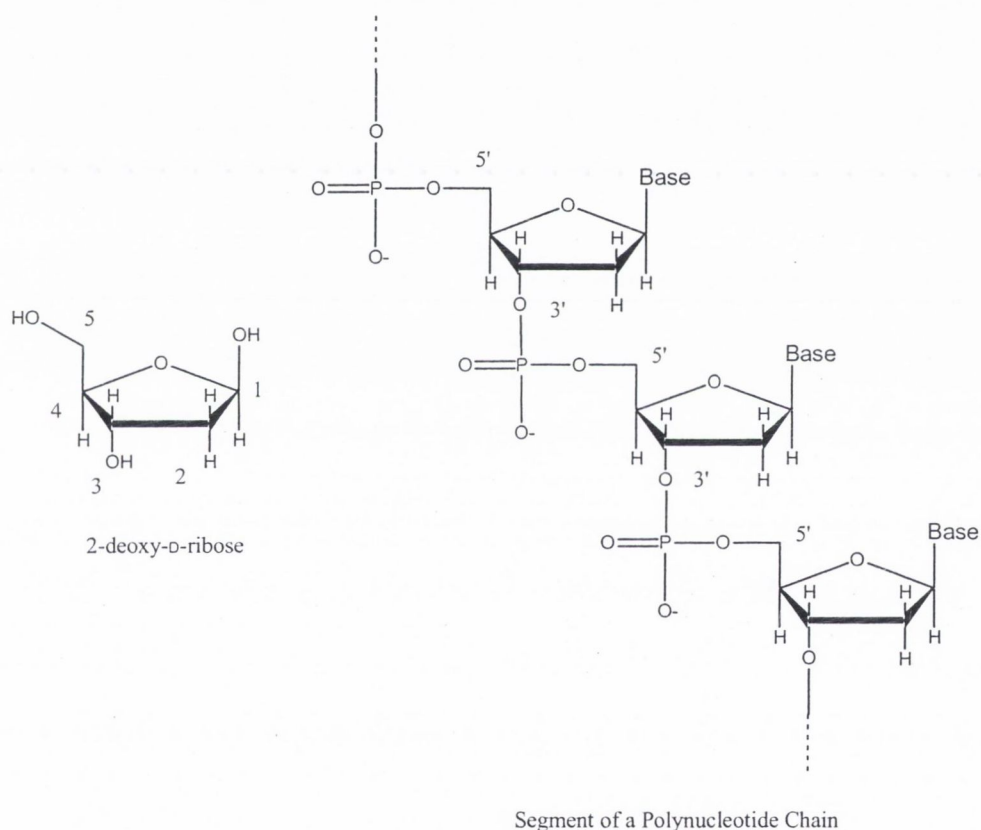


Fig. 1.1

These heterocyclic bases are of two types, the pyrimidines (cytosine and thymine) and the purines (adenine and guanine). The two polynucleotide chains of the double helix are held together through hydrogen bonding between a purine in one chain and a pyrimidine in the other chain. In these base pairs adenine (A) is always paired with

thymine (T) through two hydrogen bonds, and guanine (G) with cytosine (C) through three hydrogen bonds (Fig. 1.2). It is the sequence of these base pairs that provides the genetic code.

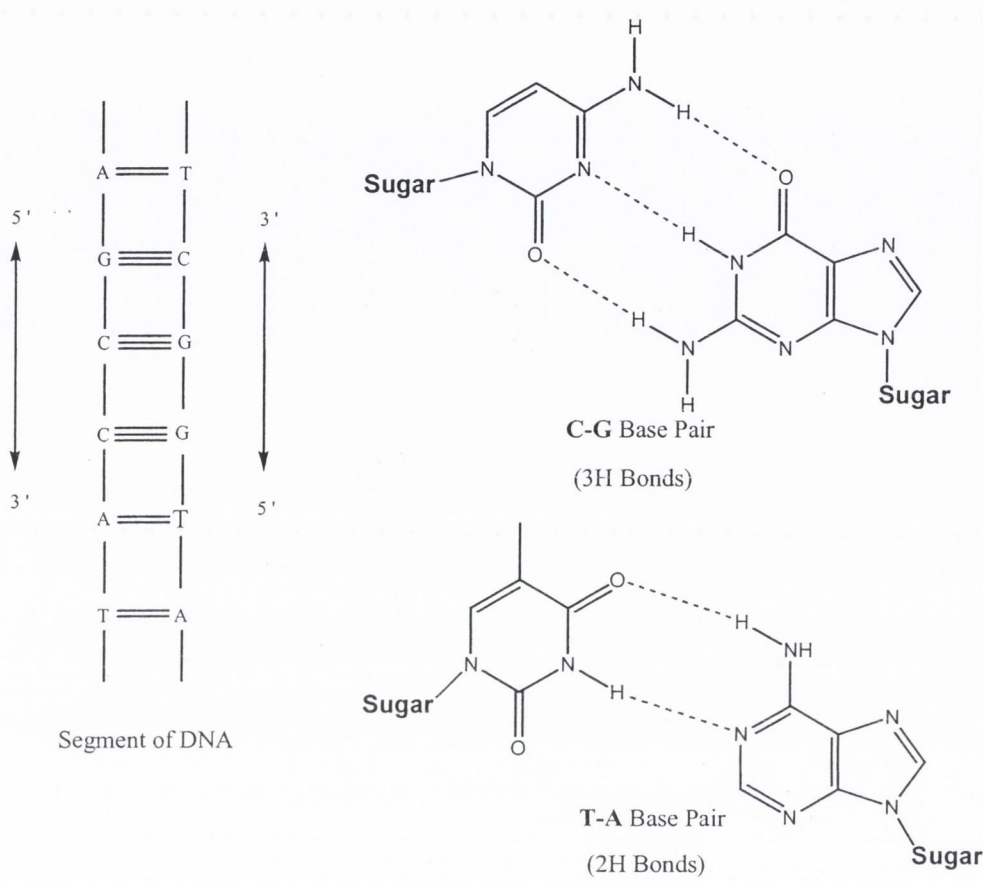


Fig. 1.2

During mitosis, the process of cell reproduction, mistakes can occur initiating events that can convert a normal cell into a rogue cell and possibly a cancer cell. Mutation causing agents, such as chemical carcinogens or ionizing radiation may induce breaks in DNA. Such breaks may be repaired by specific repair enzymes within a cell. However during the process of repair, mistakes such as the omission of a nucleotide pair can occur. Such a deletion may then be transcribed by messenger RNA (ribonucleic acid) and translated into a new strand of DNA that also carries the deletion. A transcribed strand of messenger RNA from such a gene (segment of DNA) will also direct the synthesis of a specific protein that will in turn show the result of

the deletion through an abnormal amino acid sequence (Fig. 1.3). In messenger RNA, uracil takes the place of thymine, but lacks the methyl group.

Each cell division involves the replication of approximately 3 billion nucleotides. Mutagens are also responsible for the mispairing of base pairs, which is estimated to occur in normal cells at a frequency of one in every 2 million base pairs.⁴ An example of one such point mutation directs the incorrect incorporation of thymine during DNA replication, resulting in GC to AT transition mutations. Again, the error is transcribed and translated by mRNA and leads to a different amino acid sequence being inserted in the protein coded for by the mutated gene (Fig. 1.4).

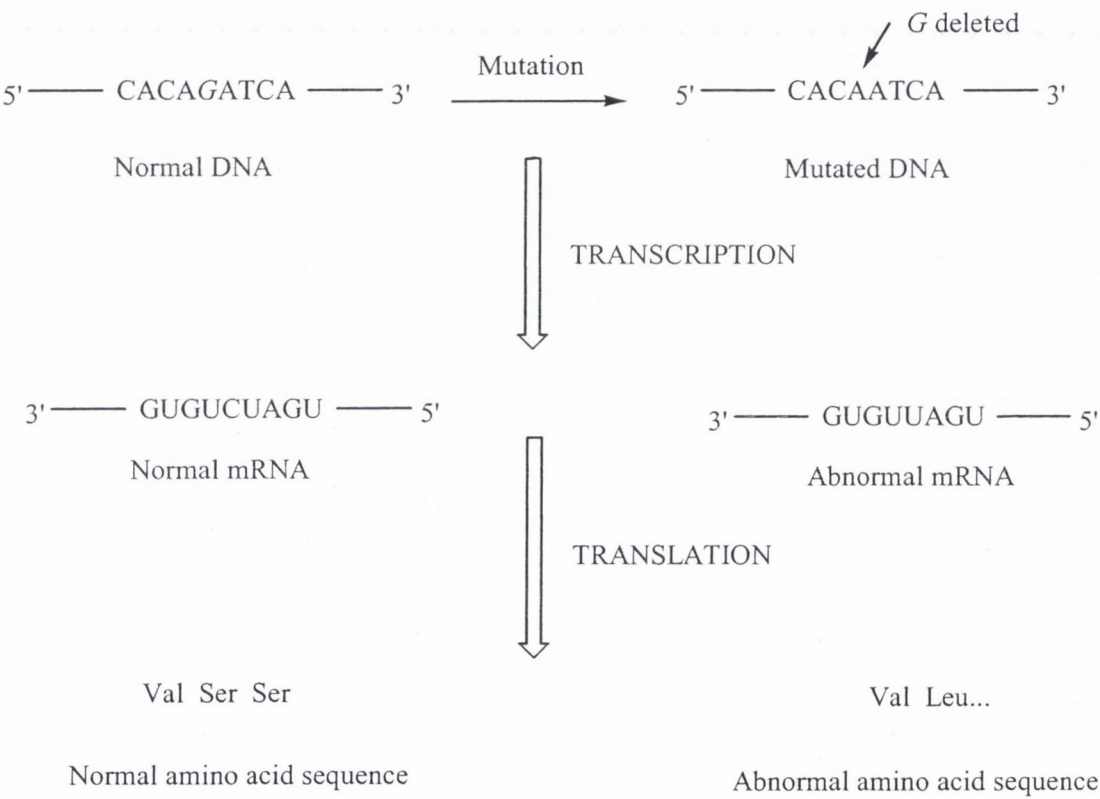


Fig. 1.3: Example of deletion

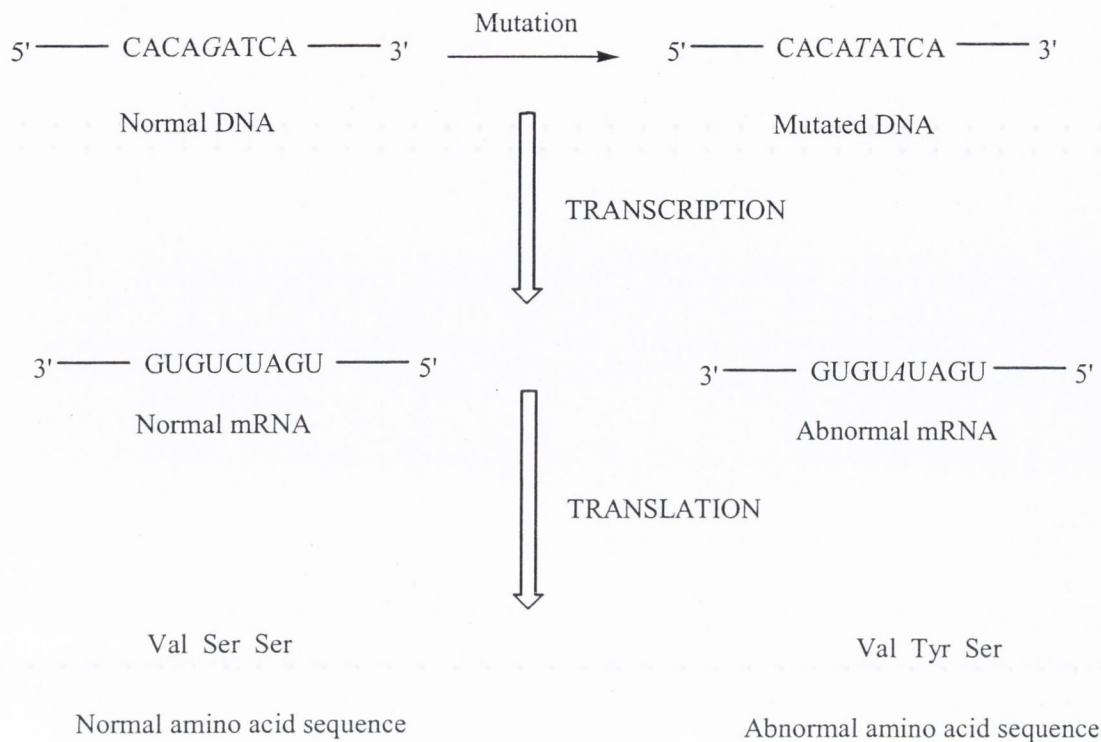


Fig. 1.4: Example of a point mutation

DNA transcription and translation relies on the specific base-pairing complementarity (Fig. 1.2) to regulate normal processes within a cell. There are two classes of genes that are responsible for controlling the life cycle of cells. The proto-oncogenes control growth, differentiation and proliferation, while the tumour suppressor genes code for proteins that control DNA transcription, DNA repair and the cell cycle. Damage by mutagens to proto-oncogenes can convert them to oncogenes, encouraging excessive cell growth. Tumour suppressor genes, such as the p53 gene which is found to be mutated in about half of all tumours, normally respond to DNA damage by embarking on programmed cell death or apoptosis, however once mutated they no longer have the ability to destroy such rogue cells. It is a combination of such mutations,

spontaneous or external, that upsets the precise complementarity within DNA that normally preserves the genetic code and the function of protein biosynthesis. It is these mutations that contribute to the uncontrolled state of cell division known as cancer.

1.2.1 Damage to DNA – the effects

The 18th century saw the first link between cancer and the environment. Percivall Pott, a surgeon at St. Bartholomew's Hospital in London, observed a high incidence of scrotal cancer amongst chimney sweeps, which he attributed to soot. In 1933, the first chemical carcinogen benzo[*a*]pyrene, a constituent of soot, cigarette smoke and car exhaust fumes was isolated.² Although many aromatic hydrocarbons have been found to be responsible for DNA damage, it is actually their metabolized form that is usually the true carcinogen.⁵ Such is true for benzo[*a*]pyrene which is metabolized in living tissue to give the benzo[*a*]pyrene diol epoxide (Fig. 1.5).⁵ It is this epoxide that reacts with nucleophilic sites on DNA. Among the reactions that occur is the alkylation of the guanine base at the 2-amino position (Fig. 1.5).⁵⁻⁸ This nitrogen is involved in the hydrogen bonding interaction of guanine with cytosine (Fig. 1.2), but once alkylated by the diol-epoxide it can no longer participate and instead mispairs (Fig. 1.4) during DNA replication, leading to the mutation being carried over into the newly synthesized strand. Such mutations may be responsible for the conversion of normal cells into rogue cells and possibly cancer cells.

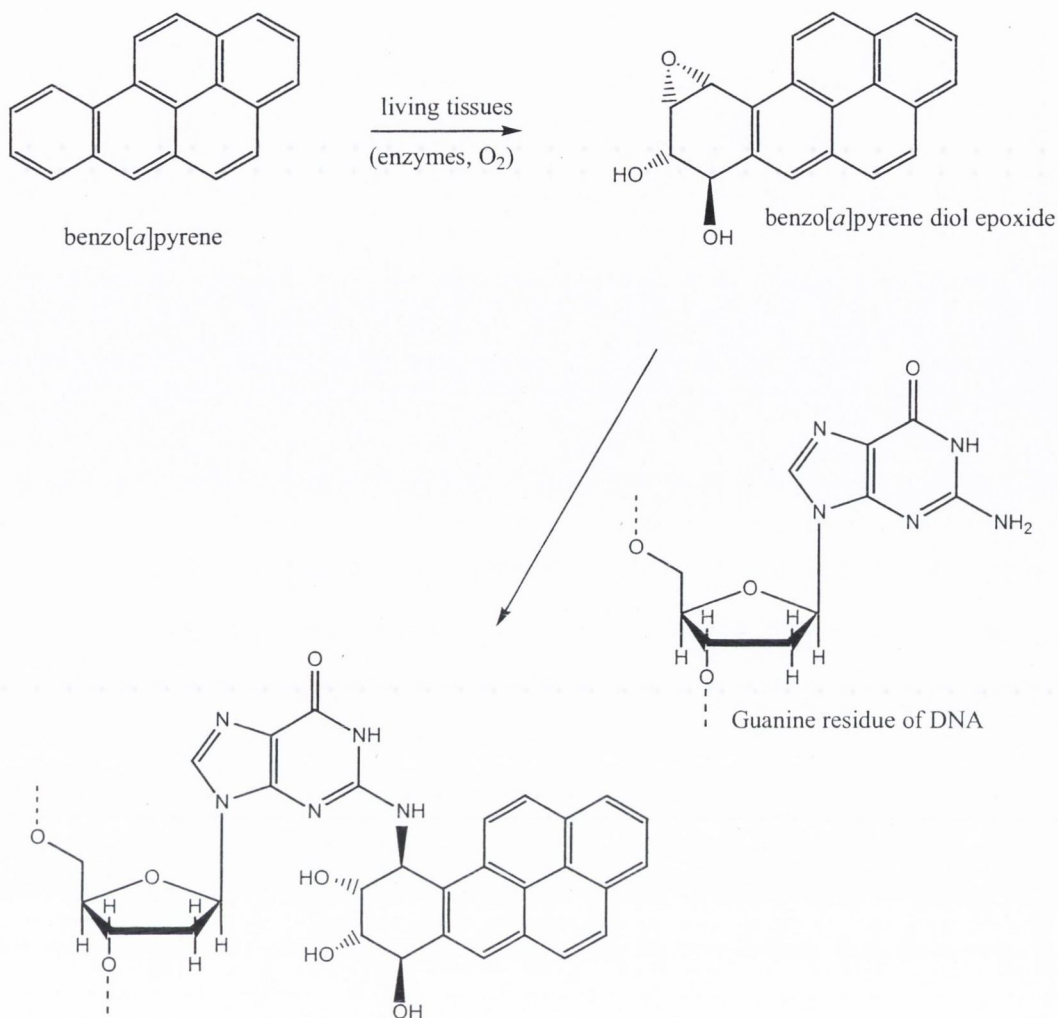


Fig. 1.5

1.3 Cancer therapy

1.3.1 Treatment of cancer

Surgery and radiotherapy are usually successful treatments of a localised and accessible solid tumour. A surgeon can excise the tumour, and a radiotherapist can irradiate the tumour killing cells with lethal doses of X-rays, or radiation from radioactive substances such as cobalt-60. However these treatments are no longer successful once a tumour has metastasized. Effort has been devoted to developing

new treatments like genetic and immune therapy, but for the moment, chemotherapy remains the treatment of choice for a metastasized tumour.⁹

1.3.2 Chemotherapy

Tumours most susceptible to chemotherapy are those with a high percentage of cells undergoing mitosis. The faster the cells of the tumour are undergoing division, the larger is the window of opportunity for treatment. Cancers that are most successfully treated are cancers like Hodgkin's lymphoma, whose cells undergo mitosis every three to four days. Less vulnerable to anticancer drugs are breast, prostate, lung and colon cancers, characteristically undergoing cell division every eighty to a hundred days. Cancer cells are not the only cells in the human body with a high rate of proliferation. Normal tissues such as bone marrow, hair follicles and cells of the gastrointestinal tract undergo rapid division, and so are also subject to damage by the cytotoxic drugs used in chemotherapy. It is this toxicity that limits the usefulness of these drugs.^{2,10}

There are four main types of chemotherapeutic agents commonly used in the treatment of carcinomas (examples in Fig. 1.6)¹¹:

- Antimetabolites *e.g.* analogues of folic acid (methotrexate), pyrimidines (5-fluorouracil) and purines (6-mercaptopurine).
- Natural products *e.g.* vinca alkaloids (vincristine and taxol) and antibiotics (doxorubicin).
- Signal transduction inhibitors¹²⁻¹⁴ *e.g.* cyclin dependent kinase and tyrosine kinase inhibitors (flavopiridol and Glivec).
- Alkylating agents (these are the drugs most relevant to the contents of this thesis, and they are described in the following sections).

1.4 Alkylating agents

The cytotoxicity of alkylating agents towards rapidly proliferating cells provides the basis for their therapeutic applications and also for many of their toxic side effects. Their sometimes advantage over other antineoplastic agents is due to physical and chemical properties, such as lipophilicity, stability in aqueous solutions and their ability to cross biological membranes (*e.g.* the blood-brain barrier).¹⁰

Five major types of alkylating agent are used in the chemotherapeutic treatment of neoplastic disease (examples in Fig. 1.7)^{10,11}:

- (1) Nitrogen mustards *e.g.* melphalan
- (2) Ethylenimines *e.g.* thiotepa
- (3) Alkyl sulfonates *e.g.* busulfan
- (4) Nitrosoureas *e.g.* lomustine (CCNU) and carmustine (BCNU)
- (5) Triazenes *e.g.* temozolomide and dacarbazine

Each of these types have in common the ability to become strong electrophiles, which react with nucleophilic moieties such as phosphate, amino, sulfhydryl, hydroxyl, carboxyl and imidazole groups. The cytotoxic effects of many of these drugs are a consequence of their ability to alkylate DNA.¹⁰

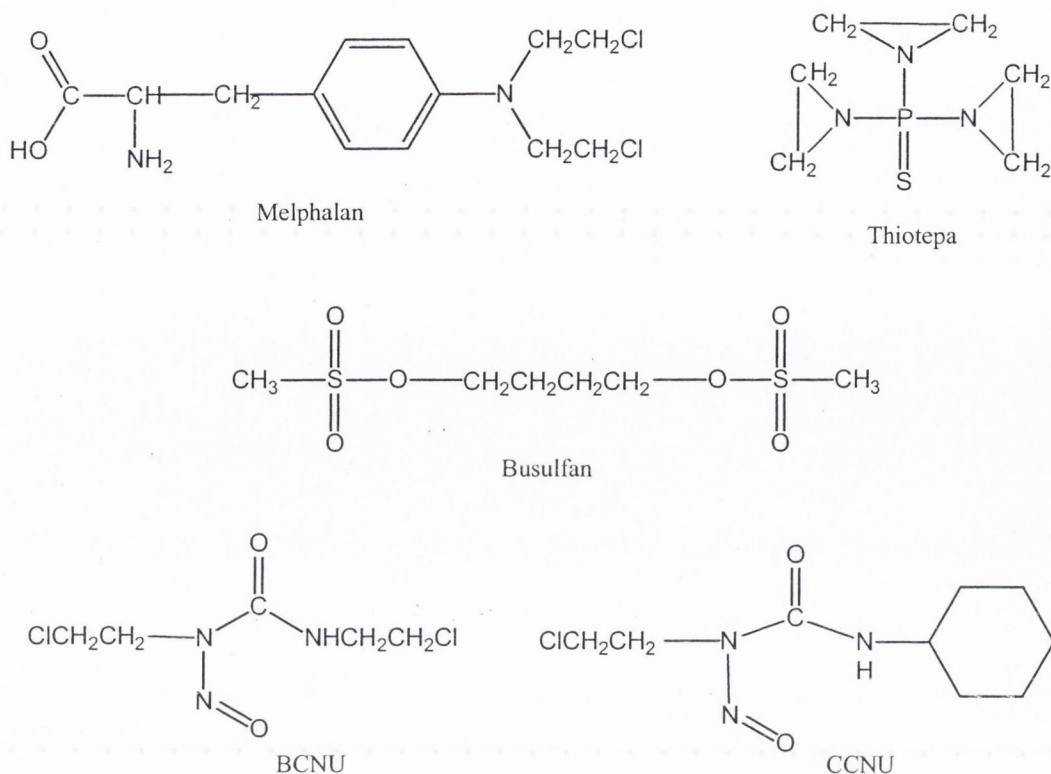


Fig. 1.7: structures of some commonly used alkylating agents

The actual alkylating moieties of these drugs are generated *in vivo*, with activation being the result of biological metabolism of the prodrug or simply activation at physiological pH. The triazenes temozolomide and dacarbazine are prodrugs of the same active methylating agent, MTIC (5-[3-methyltriazene-1-yl]imidazole-4-carboxamide) (Fig. 1.8). While temozolomide is rapidly hydrolysed to give MTIC at pH > 7, dacarbazine requires metabolic demethylation by the cytochrome P450 mixed-function oxidase system of the liver to become active.¹⁵⁻¹⁷

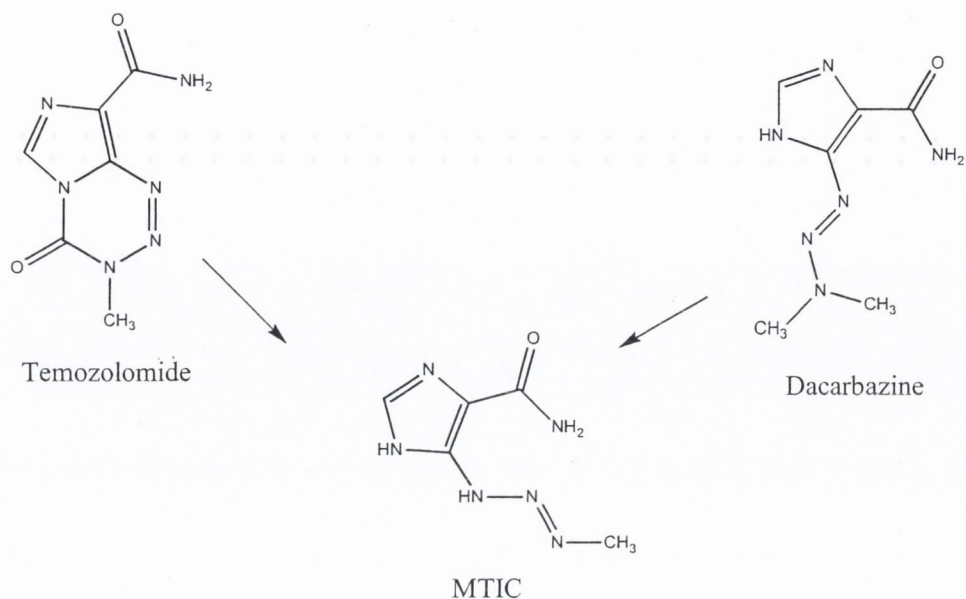
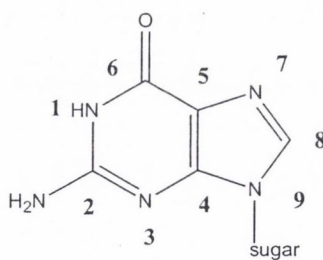


Fig. 1.8

1.4.1 Alkylating agents – mechanism of action

The therapeutic effectiveness of alkylating agents in rapidly proliferating tissues directly results from their ability to alkylate DNA. The 7-nitrogen (N-7) and oxygen (O^6) atoms of guanine (Fig. 1.9) are particularly susceptible to attack by these compounds.^{10,18-20}

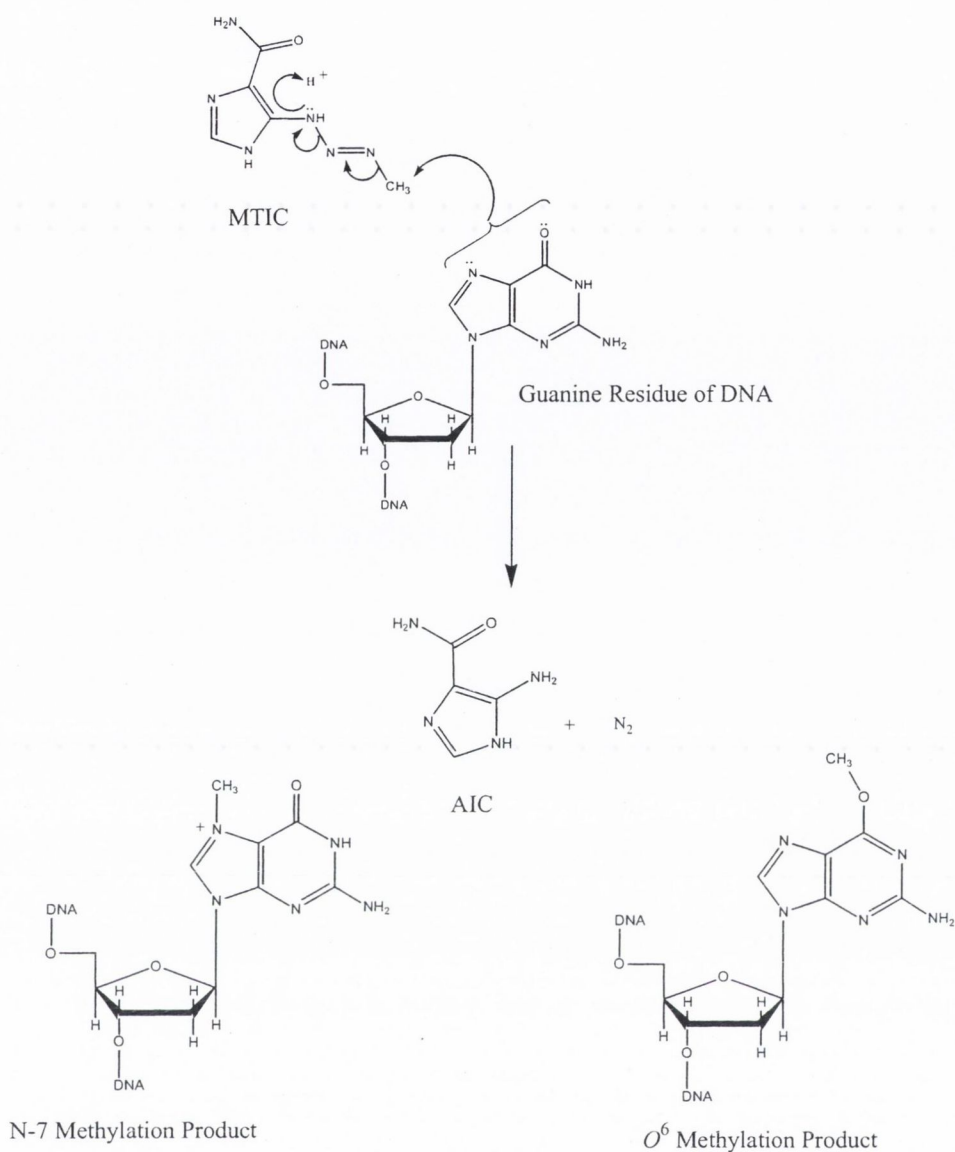


Guanine residue of DNA

Fig. 1.9

Generally the major site of the alkylation of guanine is at N-7. Although O^6 is a less frequent target for alkylating agents, the resulting lesion is mainly responsible for the cytotoxic effects of nitrosoureas and triazenes. Other atoms in the purine and pyrimidine bases of DNA may be alkylated to a lesser extent – for example, the 1- and 3-nitrogen atoms of adenine, as well as the 3-nitrogen of cytosine.¹⁰ The mechanism of action of two alkylating agents is discussed below.

Temozolomide, a triazene, undergoes hydrolytic activation to generate the active methylating agent, MTIC (Fig. 1.8). MTIC subsequently transfers a methyl group principally to the N-7 position, but also to the O^6 -position (Scheme 1.1).²⁰



Scheme 1.1

In this reaction the leaving groups are molecular nitrogen and 5-aminoimidazole-4-carboxamide (AIC).¹⁷ Methylation of these nucleophilic moieties in DNA upsets the precise base pairing complementarity. *O*⁶-Methylguanine can no longer coordinate in the same way to cytosine (Fig. 1.10), and *O*⁶-methylguanine-thymine pairing (Fig. 1.10) becomes competitive with the pairing involving cytosine. This results in GC to AT transition mutations.^{4,15}

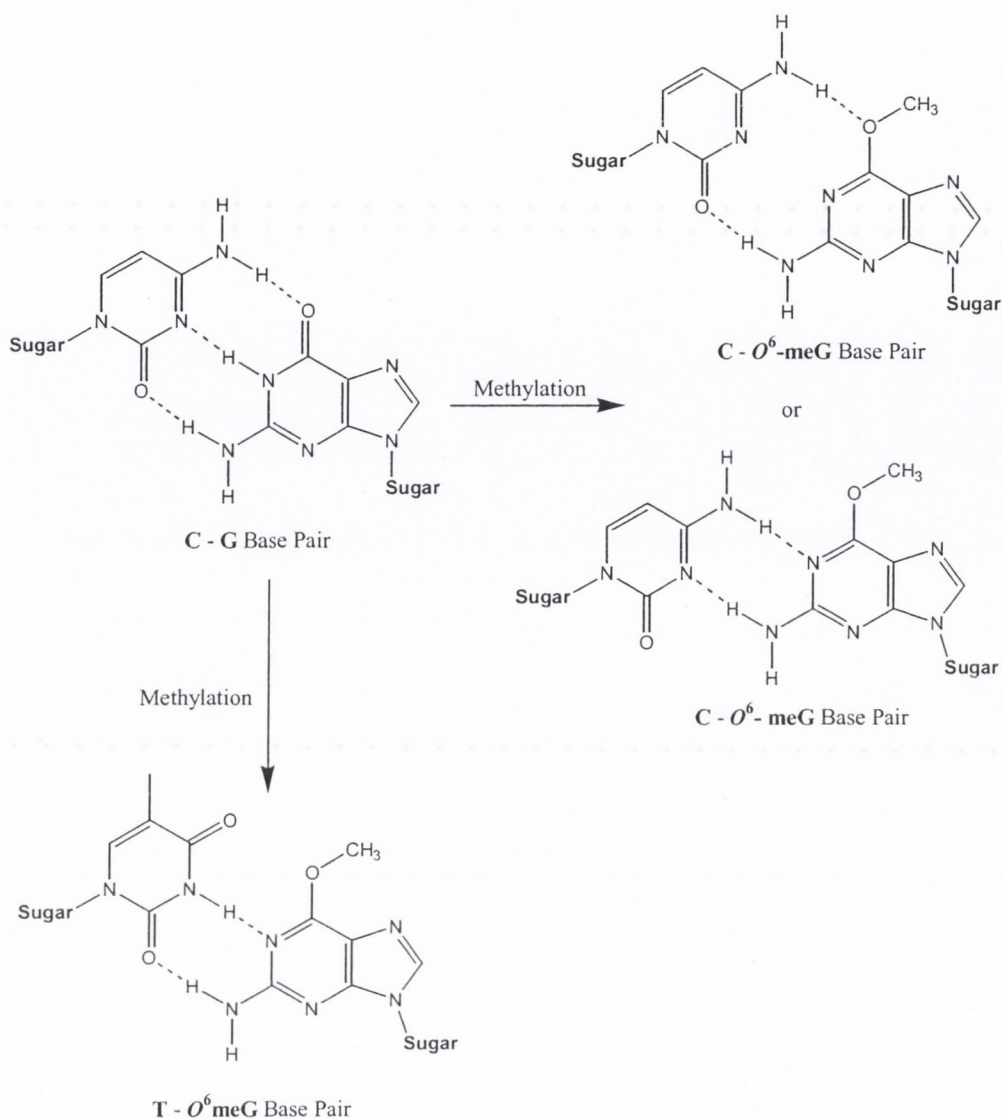
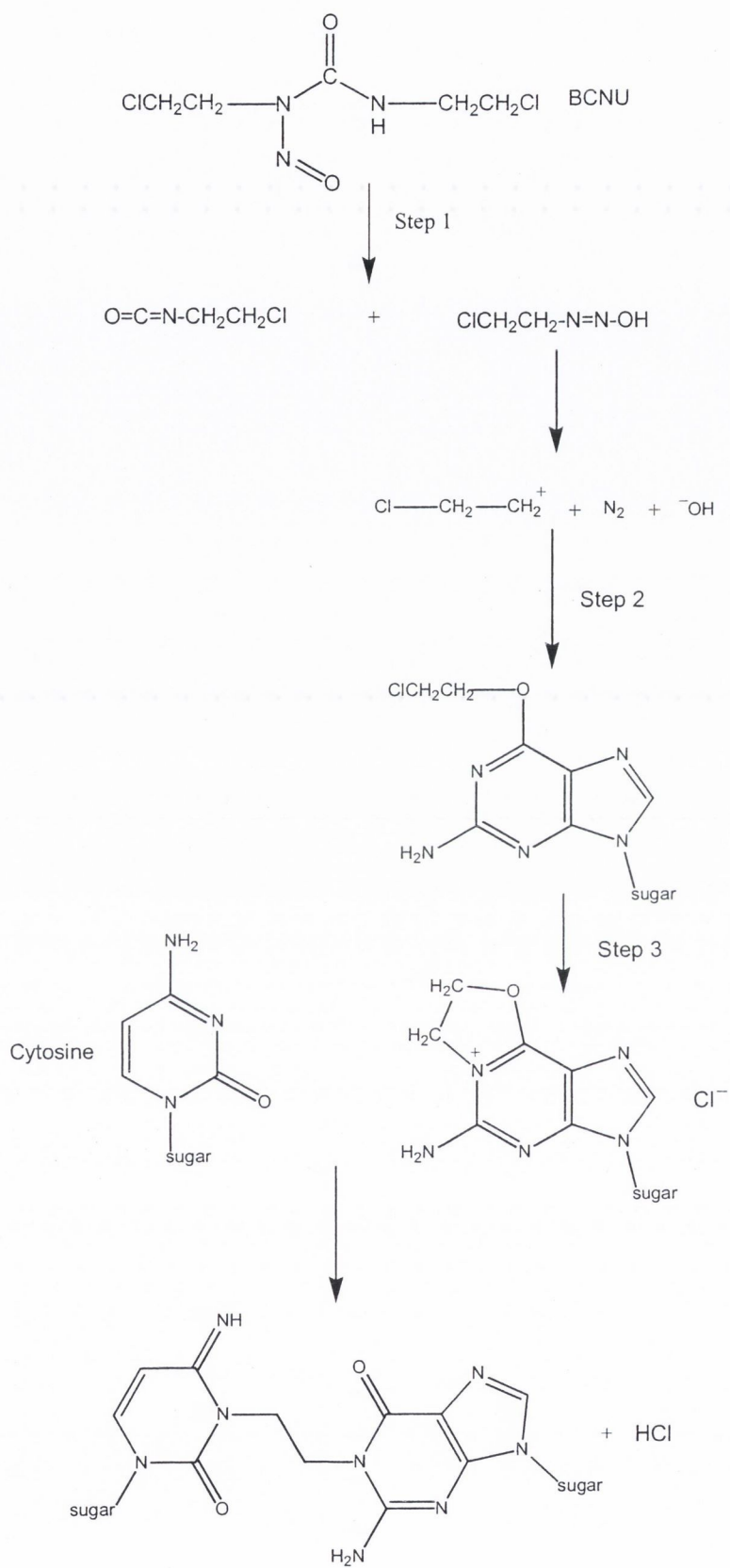


Fig. 1.10

The chloroethylating agent carmustine (BCNU), a nitrosourea, undergoes spontaneous, non-enzymatic degradation with the formation of a 2-chloroethyl carbonium ion (Scheme 1.2, Step 1).¹⁰ This strong electrophile can alkylate a variety of positions, including the O⁶-position in guanine (Scheme 1.2, Step 2). The displacement of the chlorine atom from the resulting O⁶-chloroethylguanine can lead to inter-strand crosslinking (Scheme 1.2, Step 3), which is responsible for the cytotoxicity associated with such nitrosoureas.^{21,22}



Scheme 1.2

The methylation of the N-7 of guanine is also responsible for similar cytotoxic effects. The guanine residue can undergo keto-enol tautomerism (Fig. 1.11), but it exists mostly as the keto tautomer. Once alkylated at N-7, it becomes more acidic and the enol tautomer is favoured.¹⁰ The base-pairing complementarity is again upset, and guanine can no longer hydrogen-bond with cytosine.

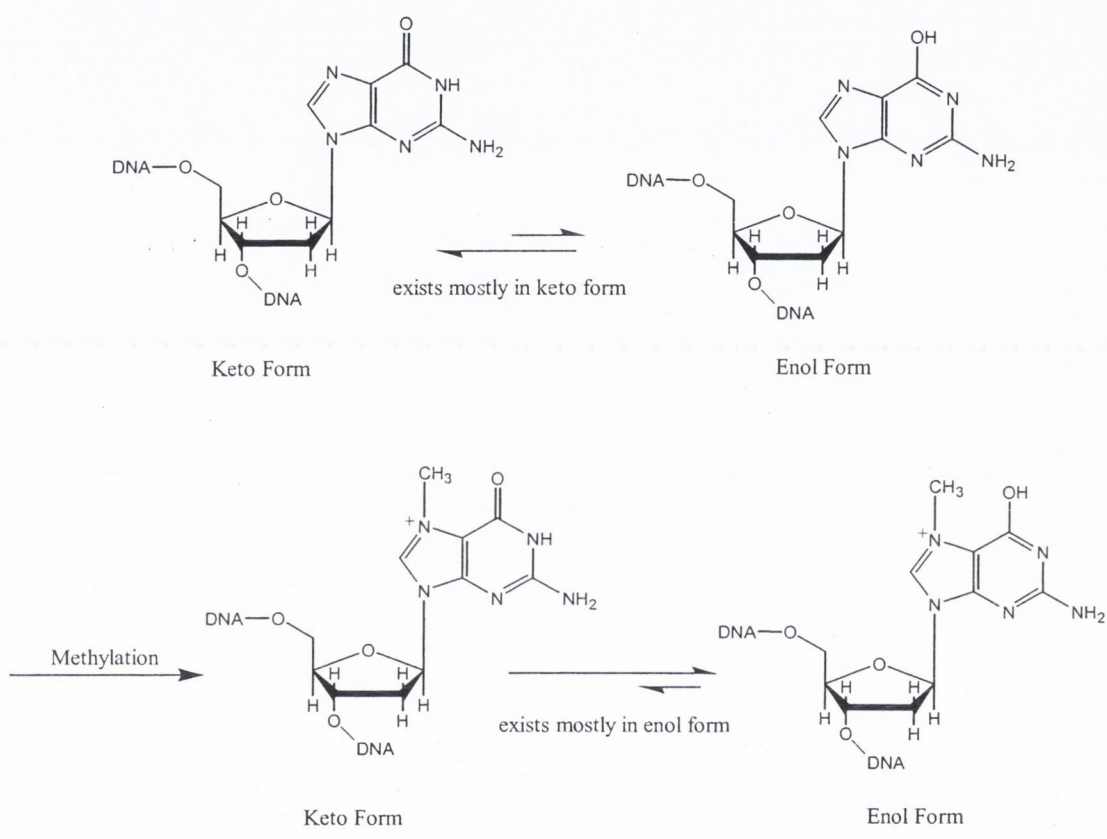


Fig. 1.11

When such mispairing of base pairs occurs, attention is directed by the mismatch repair (MMR) pathway towards the newly synthesized strand of DNA, leaving the alkylated guanine in the template strand free to mispair again with thymine. This futile attempt at repair by the MMR pathway may result in the accumulation of strand breaks and eventually apoptotic death.⁴

1.5 Resistance to alkylating agents

Cancer chemotherapy is often rendered ineffective as all cells possess repair mechanisms. Although detailed information is lacking on the biochemical reactions involved in clinical resistance, a number of events within a cell are associated with the development of resistance to alkylating agents. Among the events implicated are decreased permeation of actively transported drugs and increased levels of nucleophilic substances, like glutathione, which may compete with electron-rich sites on DNA for alkylation.¹⁰ A number of specific DNA repair processes have also been discovered.^{23,24} One example is base excision repair (BER) in which certain damaged bases are processed and the resultant single-strand breaks are re-joined, with the involvement of the enzyme poly(ADP-ribose) polymerase (PARP).²⁵ Another repair process, nucleotide excision repair (NER) works by removing the section of the DNA strand containing the damage.²⁶ Specific repair enzymes, are also associated with repairing DNA damage caused by alkylating agents.

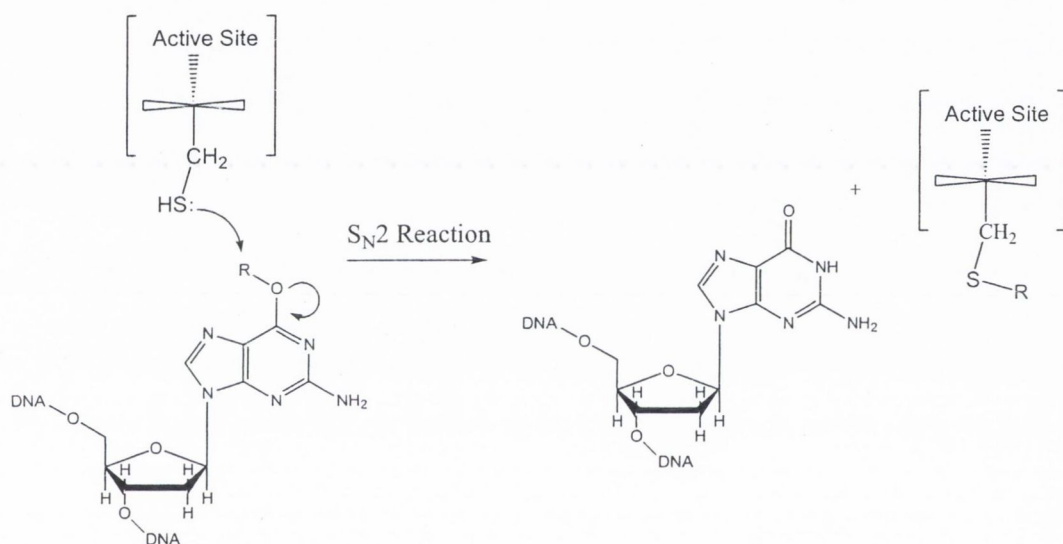
The repair enzyme ATase (*O*⁶-alkylguanine-DNA alkyltransferase)²⁷⁻³⁰ is responsible for the removal of alkyl groups from the *O*⁶-atom of guanine, while 3-methyladenine-DNA glycosylase removes alkyl groups from the 3-nitrogen of adenine.¹⁰

Much effort is being devoted to blocking the events that lead to chemotherapeutic drug resistance. This may be achieved by the depletion of repair enzymes, *via* the administration of pseudosubstrates for the enzymes prior to chemotherapy.

Our research has focused on the depletion of the repair enzyme ATase (*O*⁶-alkylguanine-DNA alkyltransferase) in order to enhance the therapeutic effectiveness of methylating agents like triazenes and nitrosoureas, whose mechanism of action involves the modification of guanine at the *O*⁶-position.

1.5.1 ATase

The cell protein, ATase (*O*⁶-alkylguanine-DNA alkyltransferase), repairs alkylation damage to the *O*⁶-position of guanine in DNA.²⁷⁻³⁰ It does so by transferring the alkyl group to a cysteine residue (Cys 145 in the human protein) at its own active site (Scheme 1.3).³¹ This is a bimolecular nucleophilic substitution (S_N2) reaction, in which guanine is the leaving group.³² The reaction is stoichiometric and auto-inactivating, with the resulting alkylated ATase inactivated and unable to participate in subsequent dealkylations.³⁰



Guanine residue of DNA, where R = alkyl group

Scheme 1.3

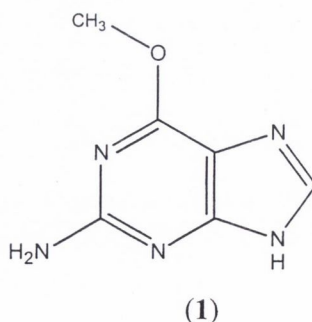
The ability of a tumour cell to repair lesions in DNA is dependent on the number of molecules of ATase present.²⁷ Cells with a higher concentration of ATase molecules can repair a greater amount of *O*⁶-alkylguanine damage, thereby exhibiting greater resistance to the cytotoxic effects of *O*⁶-alkylating agents. Cells with a lower concentration of ATase molecules are more sensitive to these chemotherapeutic drugs. Lowering the ATase content prior to administration of *O*⁶-alkylating agents

thus would enhance their cytotoxic effects. This is achieved by providing ATase with alternative deactivating substrates.

1.5.2 Early approaches to inactivation of ATase

Reduction of ATase activity was first approached by prior O^6 -methylation of guanine residues in cellular DNA, in a study of the cytotoxicity of *N*-(2-chloroethyl)-*N*-nitrosoureas (CNUs).³³ As discussed above, the transferral of the methyl group to the ATase active site is an autoinactivating reaction, leaving the methylated ATase unable to participate in further dealkylation reactions. The susceptibility of the cell to the cytotoxic effects of the subsequently administered 2-chloroethylating agent is greatly enhanced. However, the doses of methylating agent required to inactivate ATase adequately *in vivo* are extremely toxic and even more so in combination with chloroethylating agents.

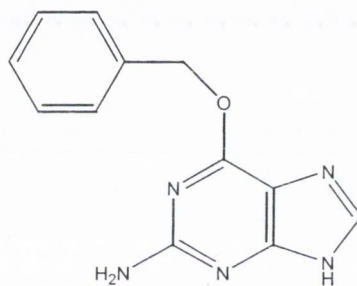
It was found that treatment of cells with O^6 -methylguanine (**1**) as a free base also effectively inactivated ATase.³⁴ Such pre-treatment with O^6 -methylguanine (**1**) at non-toxic doses was shown to enhance the cytotoxicity of 2-chloroethylating agents.^{35,36} However, this finding was not reproducible *in vivo* even when large doses of O^6 -methylguanine (**1**) were used.³⁷



Subsequent progress was made by replacing the O^6 -methyl group with various groups in an effort to produce an ATase inactivator superior to O^6 -methylguanine. The most effective of these pseudosubstrates are discussed in the following section.

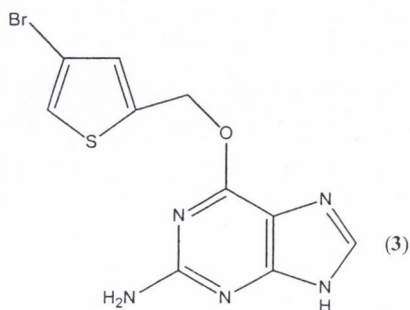
1.5.3 ATase inactivators

The impetus for research into the depletion of O^6 -alkylguanine-DNA alkyltransferase had been provided by the discovery that O^6 -methylguanine (1) was an ATase inactivator. Further investigations showed that O^6 -benzylguanine (2) was approximately two thousand times more effective at depleting ATase than O^6 -methylguanine.³⁸



(2)

Through structural modifications involving changes to the O^6 -substituent many more ATase inactivators have been designed, synthesized and assayed. Of these O^6 -(4-bromophenyl)guanine (3) (PaTrin-2) has been found to be the most effective ATase inactivator.³⁹



(3)

Activities of ATase inactivators are expressed as the I_{50} , the concentration of inactivator required to give a 50% reduction in ATase activity. O^6 -(4-Bromophenyl)guanine (3) has an I_{50} of 0.0034 μM , being over ten times more active than O^6 -benzylguanine (2), which has an I_{50} of 0.04 μM . To date, these are the only two ATase inactivators to have entered clinical trials.

1.5.4 Mechanism of action

ATase inactivators administered prior to treatment with chemotherapeutic alkylating agents act as alternative substrates for ATase. The reaction between ATase and the inactivator is a bimolecular substitution (S_N2) reaction in which the alkyl group (methyl, benzyl or 4-bromophenyl...) is transferred to the cysteine thiol group at the active site of the ATase protein.^{31,32} The reaction is analogous to that discussed in Section 1.5.1 (see Scheme 1.3), and as previously mentioned, is stoichiometric and auto-inactivating. By lowering the concentration of ATase molecules, through this irreversible reaction, the subsequent administration of the chemotherapeutic alkylating agent has been proven more effective.^{30,40}

1.6 ATase characterization and structure-based drug design

Much effort has been devoted to the structural characterization of ATase to enable the structure-based design of inactivators. Alkyltransferase is found in both eukaryotic and prokaryotic organisms. The alkyltransferase from *Escherichia coli* was the first of the O^6 -alkylguanine-DNA alkyltransferase family to be crystallized.³¹ Although similarities are found between *E. coli* ATase and human ATase, their substrate specificity indicates structural differences in their active sites. This diversity is illustrated by the resistance of *E. coli* ATase to O^6 -benzylguanine, but not O^6 -

methylguanine.^{41,42} Wibley *et al.*⁴³ reported the first homology model of human ATase based on the crystal structure of the *E. coli* protein. However, the X-ray crystal structures of the human protein⁴¹ (Protein Data Bank (PDB) entry 1QNT) and its benzylated (PDB entry 1EH8) and methylated (PDB entry 1EH7) products⁴⁴ have been determined recently. Although the homology model had suggested the active site to be deeply buried, the crystal structure shows it to be actually quite accessible to pseudosubstrates. This has enabled the determination of the amino acid residues that play a key role in the ATase reaction mechanism. Daniels *et al.*⁴⁴ have proposed a mechanism of substrate binding and the amino acid residues involved (Fig. 1.12).

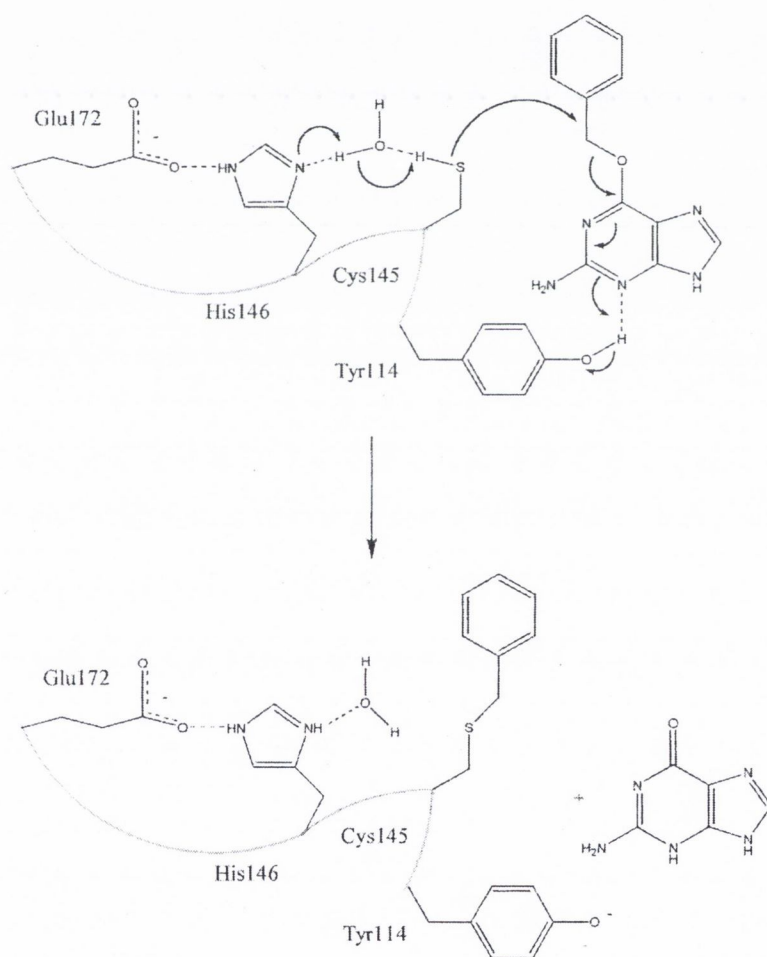
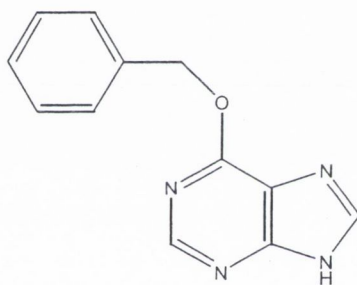


Fig. 1.12

The Cys 145 region of the protein exhibits an extensive hydrogen-bond network that is critical for protein stability and in driving the dealkylation reaction. In this hydrophobic pocket His 146 is believed to act as a water mediated base, deprotonating Cys 145 to facilitate nucleophilic attack on the O^6 -alkyl group. It is also proposed that the Tyr 114 side chain may act as a general acid to protonate guanine at N-3. This appears to be consistent with the decrease in reaction rate that is observed when this nitrogen is replaced by a carbon.

Similarly, the presence of the exocyclic 2-amino group has been found to be essential to the ATase-depleting ability of compounds (1-3). Compounds without this 2-amino group, such as O^6 -benzylhypoxanthine (4) show diminished ATase depleting-activity.⁴⁵



(4)

The nature of the group attached through the exocyclic oxygen at the 6-position is also directly related to the level of activity of an ATase inactivator. It has been shown that a benzyl or allyl group attached at this position is necessary to produce activity greatly in excess of that of O^6 -methylguanine. The ATase-depleting activity of such O^6 -derivatives follows the order of reactivity typical of bimolecular substitution (S_N2) reactions: benzyl > allyl > methyl.^{45,46} Reaction between the cysteine thiol group at the active site of ATase and the benzyl (or allyl) group of the inactivator is accelerated due to the energies of their transition states being reduced by p orbital overlap. In the transition state of such a S_N2 reaction, the carbon atom at which

substitution occurs is sp^2 hybridized resulting in the incoming nucleophile (thiol group) and the leaving group (guanine) being partially bonded to opposite lobes of the carbon p orbital (Fig. 1.13). The overlap of this p orbital with that of p orbitals of double bonds in the allyl or benzyl group provides additional bonding in the transition state therefore lowering the energy and accelerating reaction.

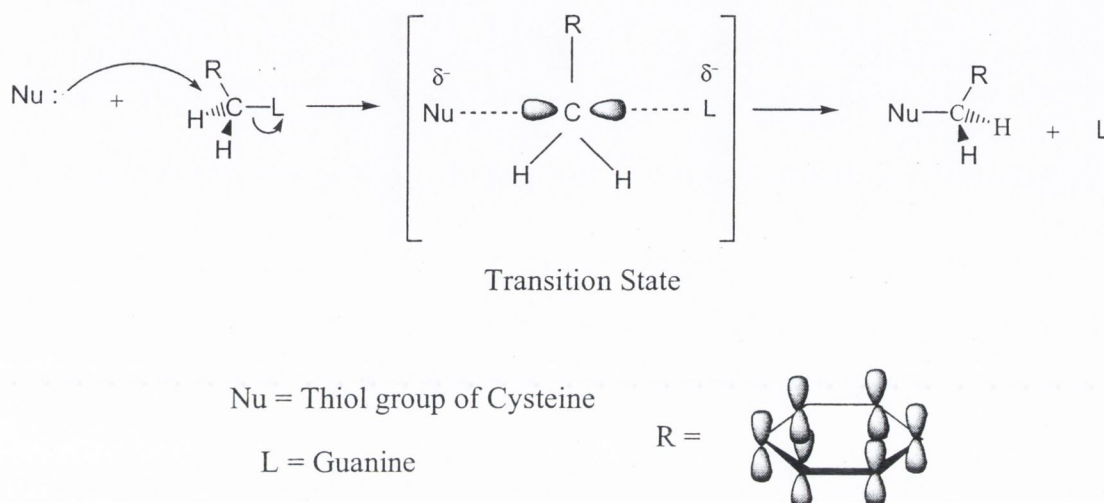


Fig. 1.13

Although this results in better ATase inactivators, due to participation in more energetically viable reactions with the active site, other factors are at least as important as the electronic ones.

Preliminary quantitative structure-activity relationship (QSAR) studies carried out by Rozas *et al.*^{*} indicate a number of features that are associated with good ATase inactivators. The study has been carried out to determine the properties of O^6 -substituents that influence the ATase-depleting ability of an inactivator. A QSAR model was developed from a set of O^6 -alkylguanine inactivators using electronic, lipophilic, steric and theoretical parameters. This model was found to be in agreement with experimental I_{50} results when applied to a test set of inactivators. The results obtained seem to indicate that the nature of the charges on the 6-oxygen atom, and the

^{*} Dr. Isabel Rozas, Trinity College Dublin, unpublished results.

CH₂ group linking the guanine with the aromatic moiety are essential for activity. The more negative the charge on the oxygen atom, the better the inhibitory activity. Conversely, the more positive the charge on the carbon atom of the CH₂ group, the better the activity. The results also indicated that the size and shape of the aromatic moiety influence activity. The best inactivators were found to be those with either a *meta* or *para* substituent, or no substituent at all. The results also suggest that hydrophilicity plays an important role in the inhibitory activity. The aromatic moieties in the O⁶-position with the greatest number of oxygen, nitrogen and hydrogen atoms on their surface have the greatest capacity to hydrogen-bond and therefore show the best biological activity. Not only do these results indicate the characteristics of the best inactivators but they give us a greater insight into the reaction of the ATase active site with the inactivator. The most effective ATase inactivators are those that react best with the ATase active site.

The indifference of the *E. coli* ATase to O⁶-benzylguanine (**2**) but not to O⁶-methylguanine (**1**), has been shown by X-ray crystallography structure-activity studies to be the result of the steric exclusion of (**2**) from its binding pocket.^{41,44} Such steric exclusions, and thus resistance to (**2**), have also been reported for modified human ATase, in which a number of point mutations in the protein (Fig. 1.4) result in the alteration of amino acid residues located in the binding pocket.^{47,48}

The characterization of human ATase through X-ray crystallography studies has provided useful information for structure-based inactivator design. However, some computational approaches based on the coordinates of the crystal structure for ATase yield results that often correlate poorly with the biological I₅₀ results.⁴⁹ Other techniques such as the covalent capture of the ATase-DNA complex, reported by Noll *et al.*⁵⁰ using oligodeoxyribonucleotides containing the novel nucleoside N¹, O⁶-

ethanoxanthosine, will hopefully aid a more rational approach to the design of ATase inactivators.

1.7 *O*⁶-(4-Bromothienyl)guanine

Synthesized in the Chemistry Department, Trinity College Dublin, and biologically tested in the Paterson Institute, Manchester, *O*⁶-(4-bromothienyl)guanine (**3**) or PaTrin-2 (Pa from Paterson, Trin from Trinity) was found to be the most effective of over 150 compounds of its type. PaTrin-2, with an *I*₅₀ of 0.0034 μM, is about to enter Phase II clinical trials. A successful Phase I trial has been carried out in the Christie Hospital, Manchester and University College Hospital, London. These new trials will assess the potentiating effect of PaTrin-2 when combined with temozolomide in melanoma and in colorectal tumours. Another trial will involve biopsiable tumours where the levels of ATase can be monitored.

PaTrin-2 has been shown to increase the therapeutic effectiveness of temozolomide (Fig. 1.6) in a mouse melanoma model.⁴⁰ This triazene is widely used in the clinical treatment of metastatic melanoma, but only 15% of patients respond, with 1% to 2% surviving long-term. Studies have shown that the administration of PaTrin-2 prior to temozolomide is more effective than compressed scheduling (more frequent administration) of temozolomide alone. Frequent dosage of temozolomide is extremely toxic, so less frequent dosage when used in conjunction with PaTrin-2 is highly preferable. Toxicity resulting from this combined treatment is no greater than for temozolomide used alone.⁹

Current PaTrin-2 research involves the design, synthesis and assaying of various analogues of this potent ATase inactivator, the results of which provide information on the types of groups that may be attached to the molecule without significantly

lowering its ATase-inactivating ability. Previous results with PaTrin-2 and other ATase inactivators indicate that only the O^6 , C-8 and N-9 positions in the purine ring core are suitable for structural variation.^{40,45,51,52} Structural modification to substituents at the O^6 or C-8 position could possibly alter the electronic properties of the purine ring system and enhance the leaving ability of the O^6 -alkyl group, producing an inactivator superior to PaTrin-2. However, the introduction of substituents to alter the pharmacokinetics of PaTrin-2 is as important a task.

PaTrin-2, the most potent ATase inactivator, has major disadvantages. It exhibits limited selectivity for neoplastic tissue and, since all cells contain ATase, its use leads to reduced repair capacity in normal tissue and loss of protection against the side effects of chemotherapy. PaTrin-2 is not very water-soluble and undergoes rapid plasma clearance. However, PaTrin-2 is not alone, since these problems are characteristic of all reported ATase inactivators, and indeed of many drugs.

Much effort has been devoted to the introduction of substituents at the N-9 position of these ATase pseudosubstrates in order to alter the pharmacokinetics by changing their water-solubility, and to increase their selectivity towards tumour cells in preference to normal cells.

The approach we have decided to take in order to increase the water-solubility and selectivity of PaTrin-2 is that of bioreversible derivatization or prodrug synthesis.

1.8 Prodrugs

The prodrug approach is commonly used to improve upon the properties of biologically active substances so as to increase their effective therapeutic use. This approach involves the bioreversible derivatization of the parent drug by a group (**R**)

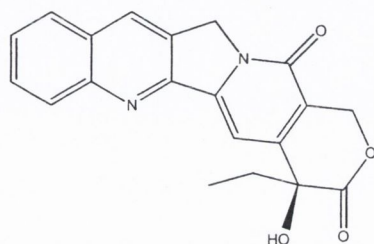
that will impart certain desirable properties to the prodrug as a whole. The most common of these desired properties include:

- Increased water-solubility
- Increased selectivity
- Higher lipophilicity
- Slower plasma clearance, through slow release of active agent
- Decreased toxicity

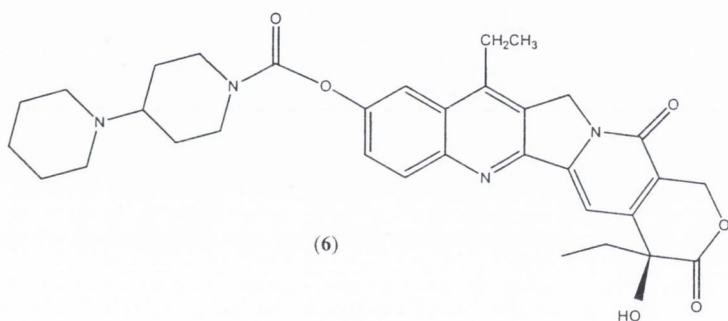
The biological reversal of this derivatization and the activation of the drug *in vivo* is dependent on the nature of **R**. Whether hydrolysis is enzymatic or chemical, if it occurs under physiological conditions (pH=7.4, T=37 °C) or not, are all controllable factors. By changing the nature of **R**, the rate at which hydrolysis occurs can be manipulated. This has important implications for the frequency of drug administration and toxicity. For a drug that has a short biological half-life, and is quickly degraded following administration, the prodrug approach may be used to manipulate the rate of release of the drug through slow prodrug hydrolysis. This approach decreases toxicity and the slow time release allows the drug to be active over a longer period of time.

The usefulness of the prodrug approach is best illustrated by example. As already mentioned, some of the commonly used chemotherapeutic agents are prodrugs that require *in vivo* activation. 20(S)-Camptothecin (**5**), an alkaloid, was shown to have significant inhibitory activity towards leukaemia, acting as a specific topoisomerase-I inhibitor. In clinical trials of its water-soluble sodium salt, it demonstrated severe toxicity to bone marrow and bladder.⁵³ Clearly it was a candidate for the prodrug approach. A stable carbamate linkage demonstrated to be selectively hydrolyzed in some tumour cells was chosen for modifying the drug, and a series of carbamate prodrugs prepared. Of the (C-ethylated) prodrugs prepared and tested, CPT-11,

commercially known as Irinotecan hydrochloride trihydrate or Campto[®] (6) was found to be the best.⁵³ CPT-11, the prodrug, is rapidly deesterified in the body to give the active metabolite SN-38 (7).⁵⁴ Campto[®] is used effectively worldwide to treat metastatic colorectal cancer and recently Okamoto *et al.*⁵⁵ have reported the involvement of ATase in resistance to Campto[®] and its active metabolite SN-38 (7).

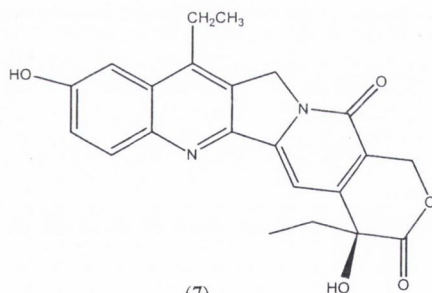


(5)



(6)

.HCl . 3 H₂O



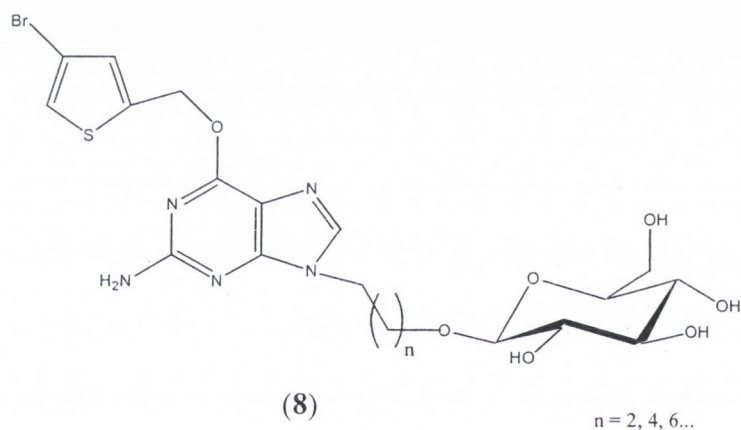
(7)

1.8.1 PaTrin-2 - prodrug approach

With previously published results indicating that several positions in the purine core are suitable for structural modification,^{40,45,51,52} the N-9 position of PaTrin-2 was chosen as a point for bioreversible derivatization and development of an effective

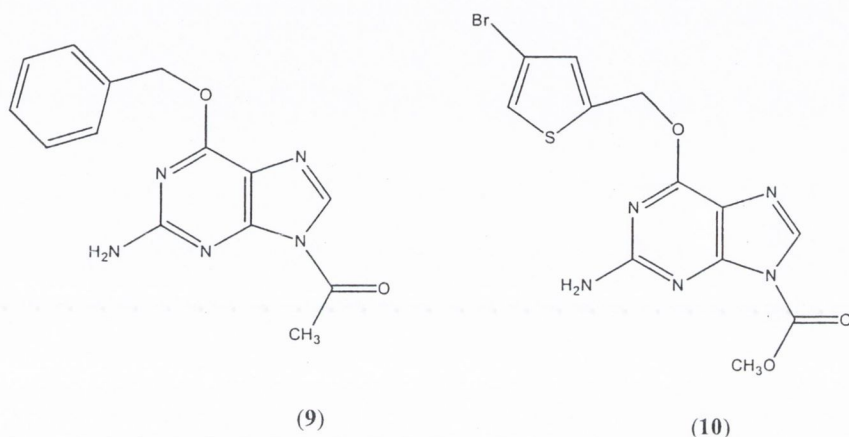
prodrug. It is known that alkylation of N-9 with certain substituents only marginally affects the ATase-depleting activity of PaTrin-2. Thus, while highly polar groups lead to a dramatic decrease in ATase depleting-activity, small or bulky lipophilic groups are well tolerated by the active site.⁵¹

Several reports of the introduction of alkyl substituents at the N-9 position of guanine derivatives in an effort to increase their tumour targeting ability are found in the literature.^{51,49} Reinhard *et al.*^{49,56} recently reported the alkylation of the N-9 position of several ATase depletors, including *O*⁶-(4-bromophenyl)guanine, with alkyl- β -D-glucosides (**8**). With enhanced expression of the glucose co-transport protein in tumour cells, they hope that glucosidation will provide a general strategy for improving targeting, as well as drug solubility. These targeted receptors are membrane-bound and sodium-dependent.



While there are many references to the N-9 alkylation of guanine derivatives in the literature, little is mentioned of N-9 acylation. Chae *et al.*⁵¹ reported the synthesis of 9-acetyl-*O*⁶-benzylguanine (**9**), which, when assayed, had an I_{50} almost comparable to that of *O*⁶-benzylguanine. They attributed this to the ready hydrolysis of (**9**) in aqueous medium liberating *O*⁶-benzylguanine, and so accounting for the higher than expected activity of (**9**). We have found that a similar PaTrin-2 derivative, *O*⁶-(4-

bromophenyl)-9-(methoxycarbonyl)guanine (**10**) has an I_{50} of 0.0048 μM , a value comparable to that of O^6 -(4-bromophenyl)guanine. It is stable under physiological conditions ($\text{pH}=7.4$, $T=37^\circ\text{C}$) for several hours, and undergoes decomposition to release free PaTrin-2 at pH 8.3. While 9-acetyl- O^6 -benzylguanine (**9**) is expected to be more susceptible to hydrolysis than the PaTrin-2 derivative (**10**), hydrolysis of this compound may not occur as readily as reported by Chae *et al.*⁵¹.

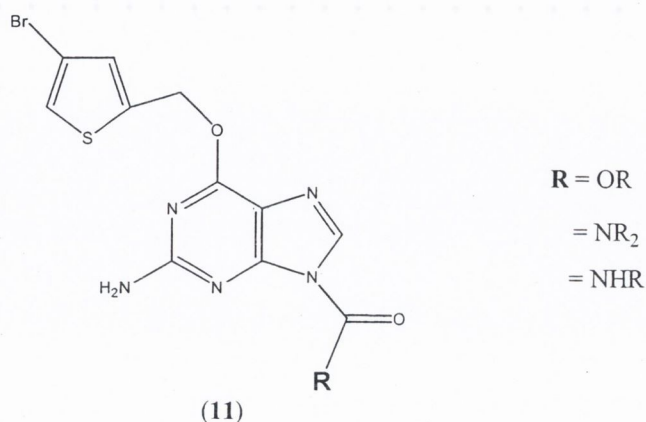


The properties of this simple methoxycarbonyl derivative have provided the lead for the design of a series of prodrugs whose chemical synthesis and biological assaying shall be discussed in the following chapters.

1.9 Aims of project

By changing the nature of group “**R**” coupled to a PaTrin-2 moiety *via* a C=O linker (**11**), we will discuss in the following chapters the synthesis of a series of prodrugs which we hope will confer upon this ATase inactivator some important pharmacological properties. The prodrugs we propose to investigate may be divided into 3 categories; where **R**=OR (Chapter 2), **R**=NR₂ (Chapter 3) and **R**=NHR (Chapter 4). The purpose of varying **R**, as already mentioned (Section 1.3.2), is that

by changing the nature of **R**, it is possible to manipulate the rate of prodrug hydrolysis and the liberation of the active drug.



Two of the major therapeutic disadvantages to be overcome by the prodrug approach are the poor solubility and the lack of selectivity for neoplastic tissue above normal tissue. In the following chapters we will demonstrate that by including a basic centre in **R**, such as a tertiary amino group, it is possible to facilitate the synthesis of water-soluble salts with considerable advantages for formulation.

To increase the targeting ability of our ATase depletor, using the prodrug approach outlined above, we plan to exploit some of the properties that differentiate tumour cells from normal cells. One property we hope to exploit is the over-expression of steroid receptors in steroid-dependent breast and testicular cancers.* Breast cancers have been shown to have enhanced expression of oestrogen receptors compared to normal tissues.⁵⁸ A similar situation is found in testicular cancer with the over-expression of testosterone receptors. Several chemotherapeutic agents such as the DNA-crosslinking nitrosourea *N*-(2-chloroethyl)-*N*-nitrosocarbamoyl(CNC)-L-alanine,⁵⁹⁻⁶¹ and the topoisomerase-II inhibitor ellipticine,^{62,63} have already been

* Oestrogen receptors have also been found in malignant gastrointestinal tumours.⁵⁷

successfully linked with oestradiol, in attempts to produce more selective chemotherapeutic agents. By incorporating either oestradiol or testosterone residues in **R** (Chapter 2) we hope in the same way to produce a more selective agent; the steroidal moiety encourages delivery to the steroid-dependent tumour where the prodrug will undergo hydrolysis releasing PaTrin-2.

To provide us with valuable biological data we have aimed to synthesize a variety of N-9 derivatives within each of the three categories proposed. The purpose of this, as already outlined, is to examine not only how the pharmacokinetics can vary between the three categories, but how by changing the nature of **R** (*i.e.* OR, NR₂, NHR) significant pharmacological differences may be observed within each individual category. To supply us with a wide range of prodrugs we set out to devise and execute within each of the categories general routes to the synthesis of the desired N-9 derivatives. However, as will become apparent over the following chapters, reaction with the N-9 position of *O*⁶-(4-bromophenyl)guanine, differs significantly from reaction with simple secondary amines. The synthetic approaches required have provided us with some useful chemical insights into reaction mechanism.

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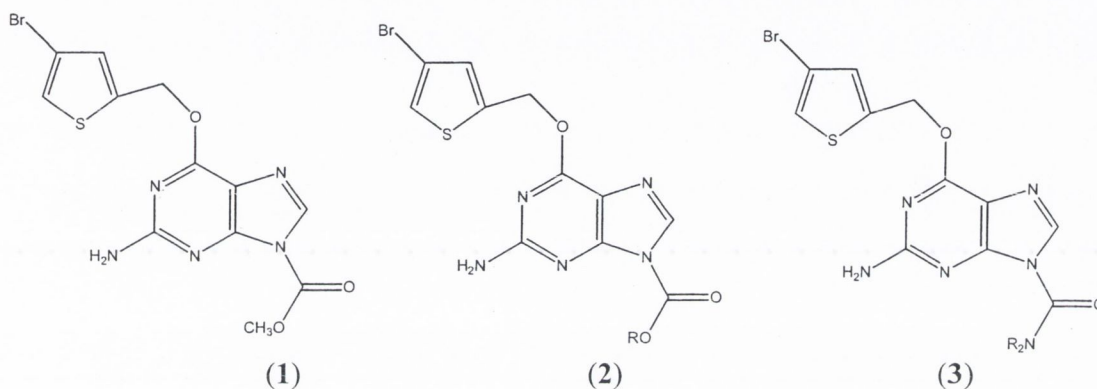
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CHAPTER 2

Chapter 2: Synthesis of 9-(alkoxycarbonyl)-*O*⁶-(4-bromophenyl)guanines

2.1 Introduction

The observed stability of *O*⁶-(4-bromophenyl)-9-(methoxycarbonyl)guanine (**1**) under physiological conditions (pH=7.4, T=37°C) for several hours, provides an encouraging impetus to the investigation of the N-9 alkoxycarbonyl derivatives (**2**) whose synthesis will be discussed in the following sections of this chapter.



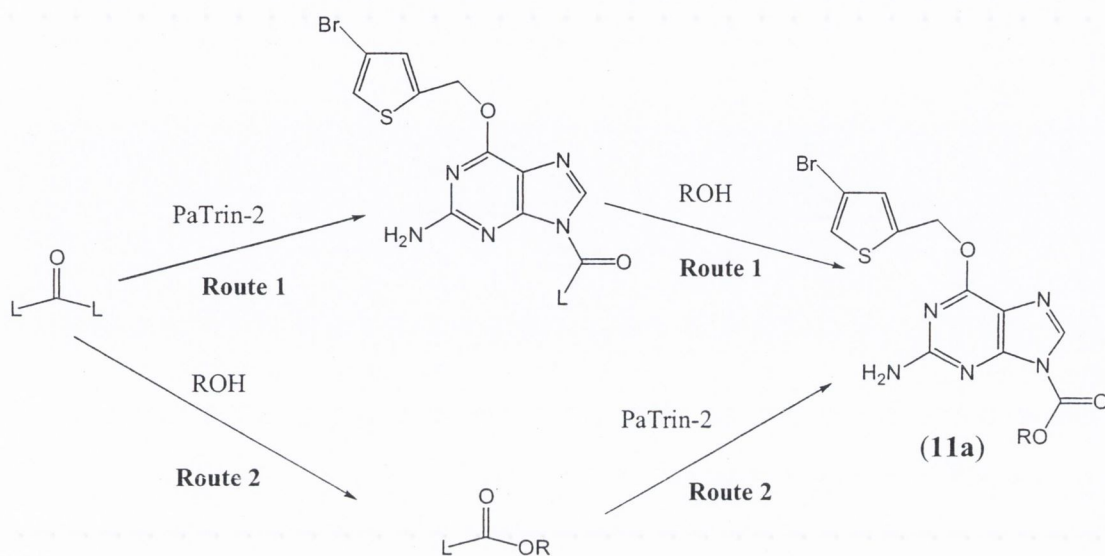
The development of effective and reliable methods for the alkoxycarbonylation of amines is of considerable interest in medicinal chemistry.¹⁻⁴ Accounts of alkoxycarbonylation of parent drugs as a form of bioreversible derivatization *in vivo* are plentiful in the literature.⁵⁻⁸

Although the carbamates (**2**) are quite susceptible to hydrolysis, predictably more so than the corresponding ureas (**3**), the hydrolysis results from (**1**), as well as numerous literature reports, made this series of analogues worth assessing for their suitability as prodrugs.

2.1.1 Alkoxycarbonylation of PaTrin-2

Aiming to prepare a large variety of N-9 alkoxycarbonyl derivatives for pharmacological evaluation, we set out to develop a general and reliable route to the synthesis of these potential prodrugs. Many of the reported methods of the

alkoxycarbonylation of amines involved the use of leaving groups.^{1,2} Based on this we outlined two synthetic approaches (Scheme 2.1).



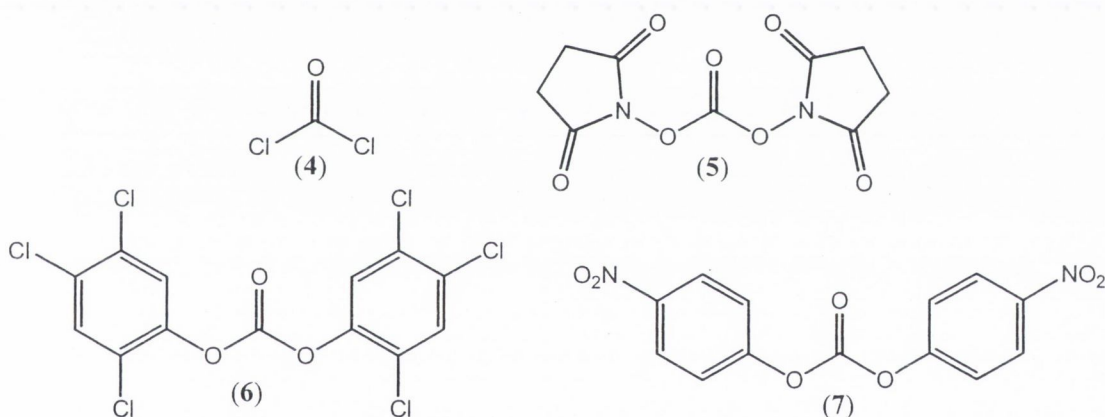
Scheme 2.1

Route 1 involved the reaction of an activated carbonic acid derivative $CO(L)_2$ (where L = a suitable leaving group) with PaTrin-2, followed by treatment with an alcohol ROH . In **Route 2** the order of the steps was reversed, reacting the activated carbonic acid derivative first with the alcohol and then with PaTrin-2. Initially there appears to be little novelty associated with the synthesis of carbamate esters *via* the preparative routes outlined in Scheme 2.1. However, in recent years several alkoxycarbonylation methods have appeared in the literature, reiterating that the scope of existing methodologies is often limited, particularly by the steric and electronic effects of the reagents involved.¹ Such is true in our investigation of the alkoxycarbonylation of PaTrin-2. Important to the stability of such alkoxycarbonyl derivatives, as in the whole area of carbonic acid chemistry, are the electronic contributions of functional groups on either side of the carbonyl group.⁹ As will be illustrated in the following sections, the electronic and steric characteristics of the purine ring system explain the

occasional seemingly poor reactivity of PaTrin-2, and also its ability to behave as a competitive leaving group in these reactions.

2.1.2 Activated carbonic acid derivatives

The synthetic approaches, **Route 1** and **Route 2** (Scheme 2.1) involve the use of an activated carbonic acid derivative $\text{CO}(\text{L})_2$. The possibilities for L (the leaving or activating group), and the range of activated derivatives available are large. Since it would be impossible to investigate every reagent thoroughly, we chose to compare several activated carbonic acid derivatives that differed in reactivity.¹⁰



The most reactive of these reagents is phosgene (4), which we generated from its precursor bis(trichloromethyl) carbonate¹¹ (see below, Scheme 2.5, pg 49). The carbonyl is highly activated by the electron-withdrawing chloride groups. Similarly, the active nature of disuccinimidyl carbonate (5)¹², bis(2,4,5-trichlorophenyl) carbonate (6) and bis(4-nitrophenyl) carbonate (7), which vary marginally in their reactivities, is determined by the difference in the electron-withdrawing abilities of the activating groups on either side of their carbonyl group.

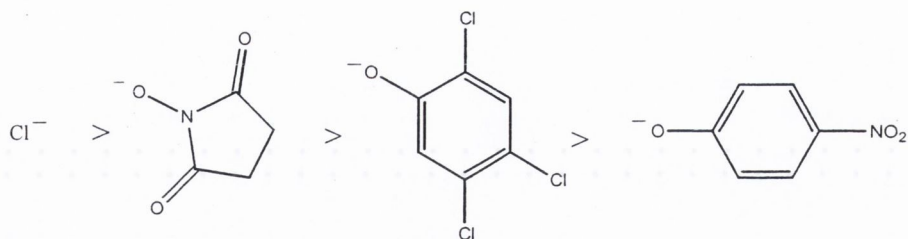


Fig. 2.1

Chloride is the best leaving group, making phosgene (**4**) the most activated carbonic acid derivative in this series (Fig. 2.1). The usefulness of each of these leaving groups in effectively facilitating the reaction sequences proposed for **Route 1** and **Route 2** will be investigated in this chapter.

2.2 Summary of Chapter

The following sections of this chapter will discuss the development of a general approach to the synthesis of N-9 alkoxy carbonyl derivatives. We will examine the effectiveness of **Route 1** and **Route 2** in facilitating the synthesis of this class of prodrugs. We will begin by discussing our investigation of **Route 1** (Section 2.3). However, it will be demonstrated that synthesis *via* this pathway is limited.

Route 2 is shown to provide a more versatile and practical route (Section 2.4). However, although it is a successful route, we will report the dependence of **Route 2** on the type of activated carbonic acid derivative involved. A comparison of the reactions of bis(4-nitrophenyl) carbonate (**7**) and bis(2,4,5-trichlorophenyl) carbonate (**6**) shows (**7**) to be, by far, the more versatile reagent (Section 2.7).

Although, as we report, **Route 2** using bis(4-nitrophenyl) carbonate (**7**) generally provides a reliable route to the synthesis of our potential prodrugs, the preparation of a few N-9 alkoxy carbonyl derivatives was not possible. Therefore, it was worthwhile to investigate the synthesis of chloroformates and their reaction with PaTrin-2 (Section 2.9). As well as making possible the synthesis of further N-9 alkoxy carbonyl

derivatives, we will show that the reactions involving chloroformates provide a useful comparison to those involving activated esters* derived from both bis(2,4,5-trichlorophenyl) carbonate (6) and bis(4-nitrophenyl) carbonate (7).

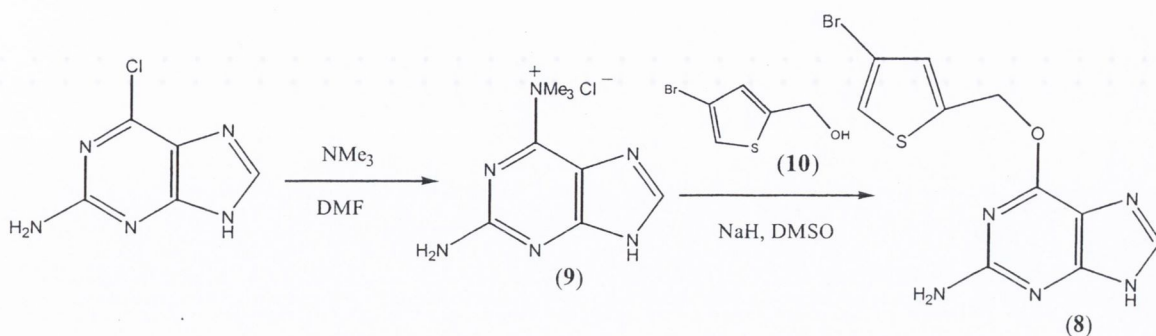
This chapter will illustrate the effectiveness of **Route 2** for the synthesis of prodrugs of PaTrin-2. However, we hope to show that **Route 2** is limited not only by the type of carbonic acid derivative employed, but also by the electronic and steric effects of the purine ring system. The comparison of pathways and reagents provides valuable information on the limitations and practicalities of the alkoxycarbonylation of PaTrin-2. The final section of this chapter (Section 2.11) will show the further application of our findings as a means of synthesizing prodrugs with targeting abilities.

2.3 Investigation of Route 1

2.3.1 Synthesis of O^6 -(4-bromophenyl)guanine¹³

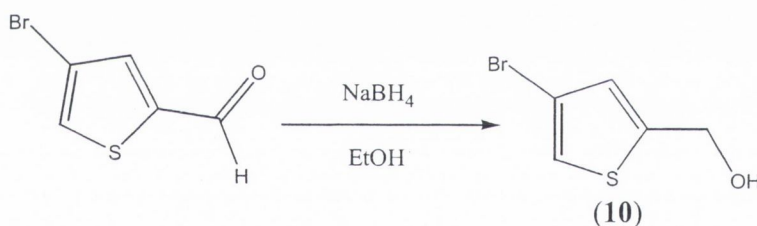
Stage one of **Route 1** involved the reaction of PaTrin-2 (8) with an activated carbonic acid derivative. PaTrin-2 (8), O^6 -(4-bromophenyl)guanine, was synthesized by an improved and milder method used by McElhinney *et al.*¹⁴ for the synthesis of O^6 -substituted guanines. The previous preparation involved harsh conditions, leading to low yields for compounds with sensitive substituents.^{15,16,17} The reaction developed here involved the conversion of 2-amino-6-chloropurine into the trimethylammonium salt (9).¹⁸ Subsequently, the trimethylammonio group was displaced by the 4-bromophenyloxy anion to give O^6 -(4-bromophenyl)guanine (8) in good yield. The reaction occurred at room temperature (Scheme 2.2), unlike the previous method that required temperatures of 130-180 °C.

* Although carbonates, these compounds are conventionally referred to as "activated esters".



Scheme 2.2

4-Bromophenyl alcohol (**10**) was prepared by the reduction of 4-bromothiophene-2-carboxaldehyde by sodium borohydride (Scheme 2.3).¹⁹



Scheme 2.3

2.3.2 ¹H NMR analysis of *O*⁶-(4-bromophenyl)guanine

The initial characterization of each of the N-9 alkoxy carbonyl derivatives, whose synthesis we will discuss in this chapter, was carried out by the analysis of their ¹H NMR spectra.♦ This was achieved easily by comparing the ¹H NMR spectrum of each derivative with that of *O*⁶-(4-bromophenyl)guanine (**8**). The ¹H NMR spectrum of *O*⁶-(4-bromophenyl)guanine (**8**) is given below (Fig. 2.2) and the assignments of the signals are given in Table 2.1.

♦ Chemical shifts of all ¹H and ¹³C NMR spectra were measured using solutions in deuterated DMSO (unless otherwise stated), and at 400 and 100 MHz respectively.

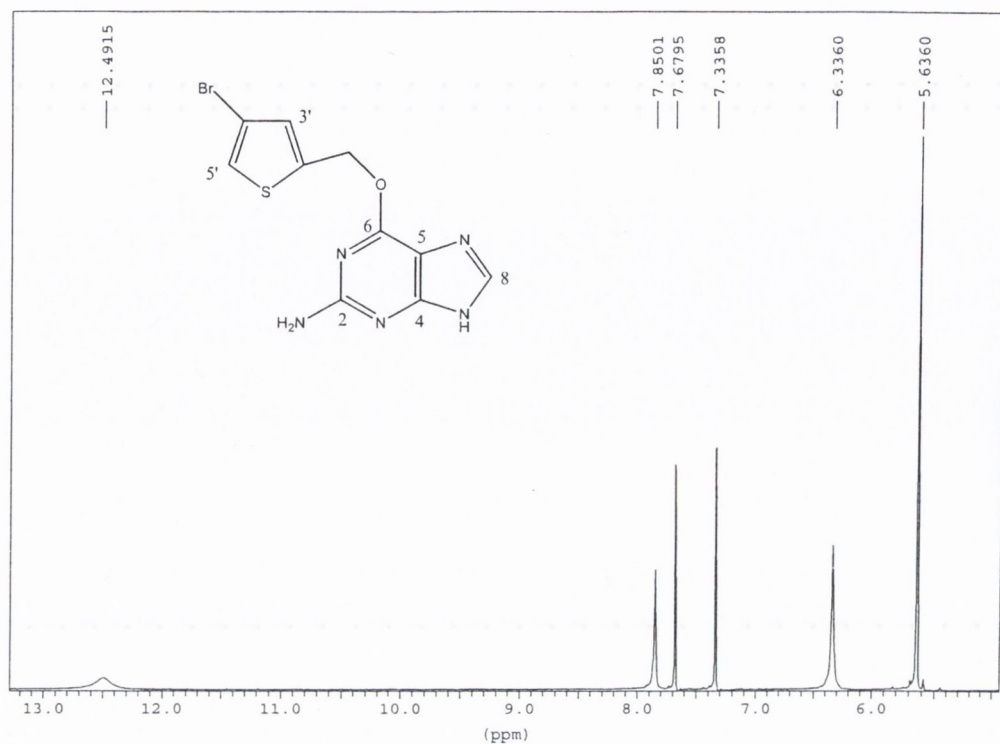


Fig. 2.2

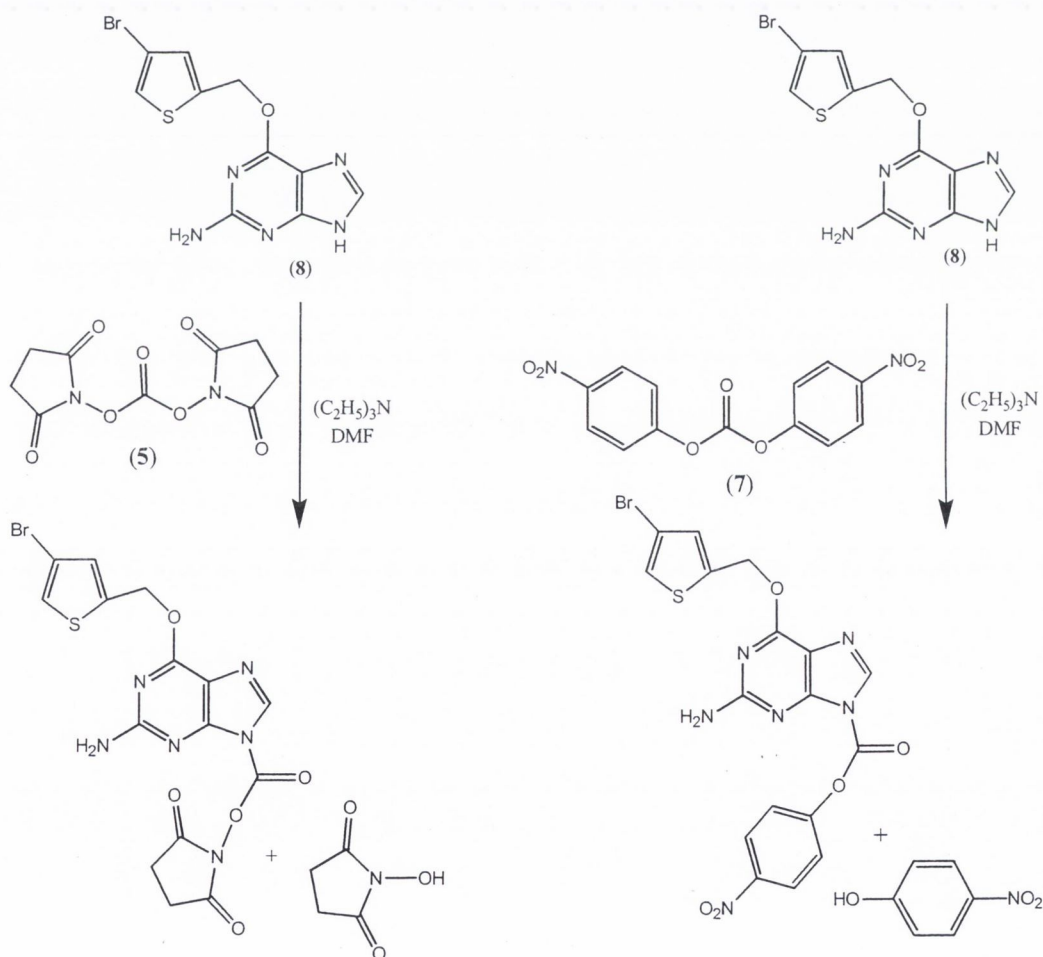
Hydrogen	NMR δ (ppm from TMS)
CH ₂	5.64
NH ₂	6.34
H-3'	7.34
H-5'	7.68
H-8	7.85
NH	12.49

Table 2.1

Full analysis of the ¹³C NMR spectrum of *O*⁶-(4-bromophenyl)guanine (**8**) can be found in the Experimental (Section 2.13).

2.3.3 Reaction of PaTrin-2 with activated carbonic acid derivatives

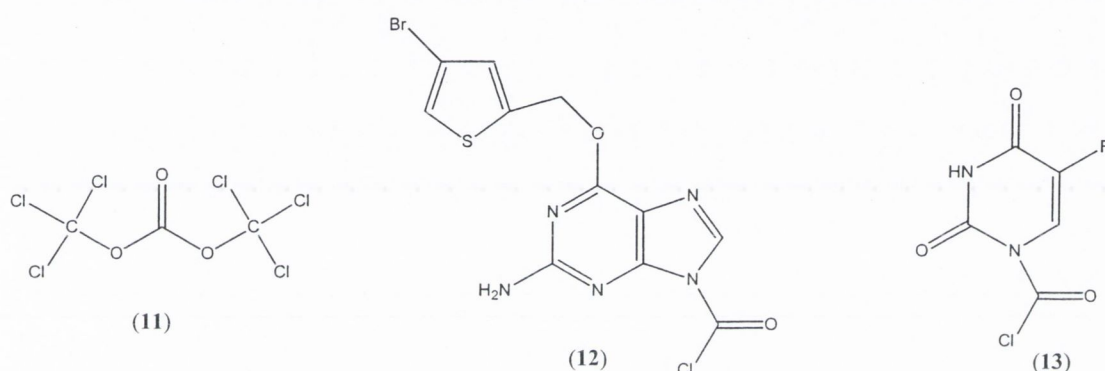
Stage one of **Route 1** involved the reaction of PaTrin-2 with an activated carbonic acid derivative (Section 2.1.2). The pK_a values of PaTrin-2 are not known, however the amino group in the 9-position of guanine is reported to have a pK_a of 12.3 and may be similar to PaTrin-2. The reaction of PaTrin-2 with both disuccinimidyl carbonate (**5**) and bis(4-nitrophenyl) carbonate (**7**), in the presence of an equivalent of triethylamine^{*}, proceeded well (Scheme 2.4), but resulted in inseparable mixtures. Attempts made to isolate either of the N-9 alkoxy carbonyl derivatives resulted in their gradual decomposition, due to their susceptibility to hydrolysis.



Scheme 2.4

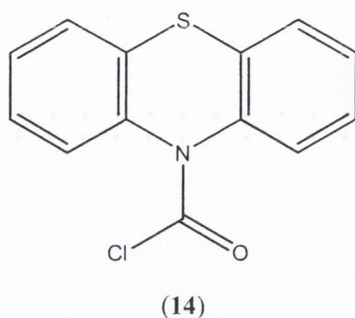
^{*} The use of an organic base such as $(C_2H_5)_3N$ avoids the aqueous work-up that was found to be required for PaTrin-2 reactions involving an inorganic base.

Using bis(trichloromethyl) carbonate (**11**) as a source of phosgene (**4**) (see below, Scheme 2.5, pg 49), and pyridine as a base, we attempted to prepare *O*⁶-(4-bromothienyl)-9-(chloroformyl)guanine (**12**). However, this derivative proved too unstable to isolate. This result mirrors the findings of Ozaki *et al.*⁸ who reported the synthesis of a 5-fluorouracil derivative, 1-chloroformyl-5-fluorouracil (**13**), using phosgene. This was also too unstable to isolate, but it proved stable enough at low temperatures in pyridine to be used in a “one-pot” synthesis of urea derivatives.



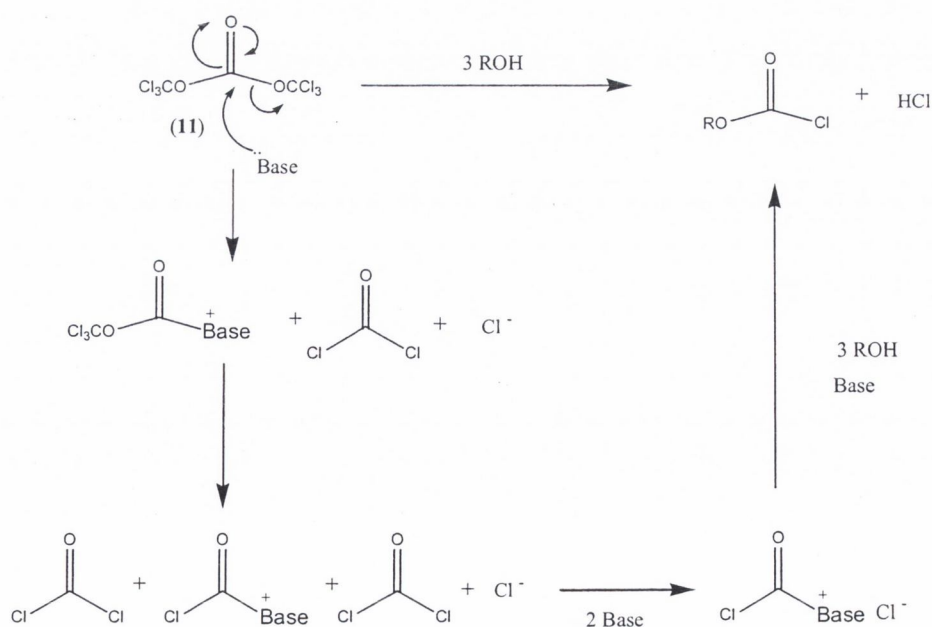
We carried out several low temperature reactions (0 °C to –40 °C) involving PaTrin-2, trying both DMF and pyridine as suitable solvent systems for the synthesis of (**12**), with methanol being subsequently added in an attempt to displace the chloride. The product should have been *O*⁶-(4-bromothienyl)-9-(methoxycarbonyl)guanine (**1**), which had been characterized already. In all reaction conditions explored, (**12**) proved too unstable to observe or to use for subsequent “one-pot” synthesis.

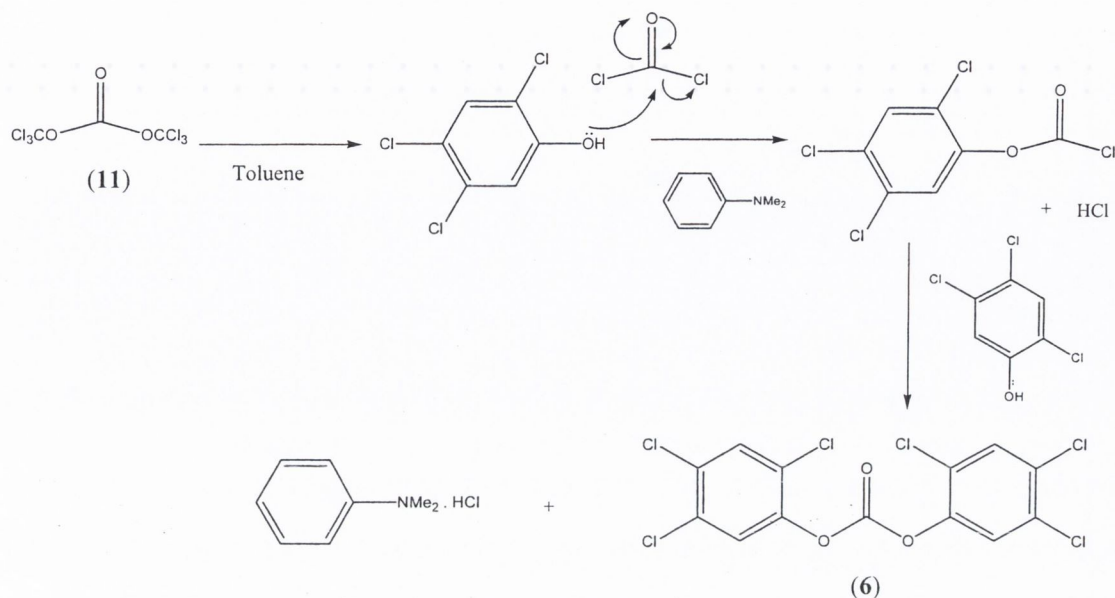
Stable chloroformyl derivatives have been synthesized successfully from phosgene. In 1891 Paschkowetzky²⁰ reported the synthesis of the stable crystalline solid 10-chloroformylphenothiazine (**14**), which has been used successfully in the preparation of its corresponding COOR derivatives.²¹ The difference in the stabilities of (**12**) and (**13**) compared to the 10-chloroformylphenothiazine (**14**) is associated with the unique electronic effects of the purine and pyrimidine ring systems.



2.3.4 Synthesis of *O*⁶-(4-bromophenyl)-9-(2,4,5-trichlorophenoxy carbonyl) guanine

Unlike disuccinimidyl carbonate (**5**) and bis(4-nitrophenyl) carbonate (**7**), bis(2,4,5-trichlorophenyl) carbonate (**6**) was not available commercially. This symmetrical carbonate was synthesized in 80% yield from 2,4,5-trichlorophenol and bis(trichloromethyl) carbonate (**11**) using *N,N*-dimethylaniline as a base, according to a method reported by McCormick *et al.*²² (Scheme 2.6). Bis(trichloromethyl) carbonate (BTC) (**11**) liberates three molecules of phosgene (**4**) by the mechanism proposed by Eckert (Scheme 2.5, below).^{11,23}





Scheme 2.6

Bis(2,4,5-trichlorophenyl) carbonate (6) was characterized by infrared spectroscopy and ^1H NMR. Its carbonyl stretching frequency is observed at 1774 cm^{-1} in the infrared spectrum. Compound (6) is characterized by two signals at 8.06 ppm and 8.14 ppm in the ^1H NMR spectrum (Fig. 2.3). Signals associated with traces of 2,4,5-trichlorophenol are also observed at 7.15, 7.69 and 10.97 ppm.

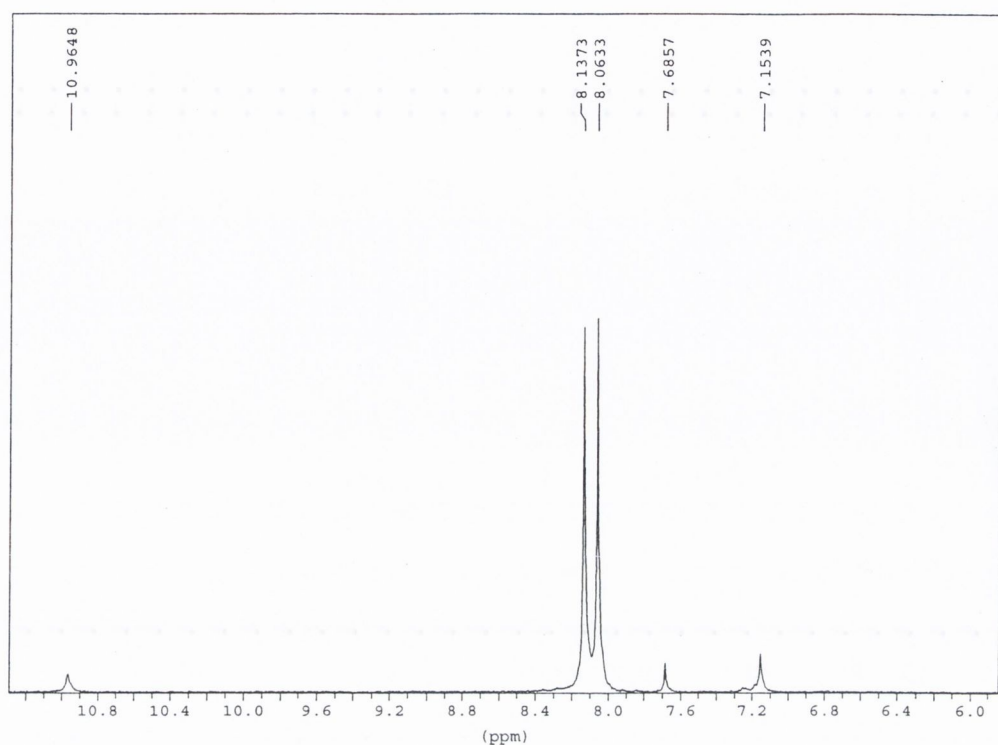
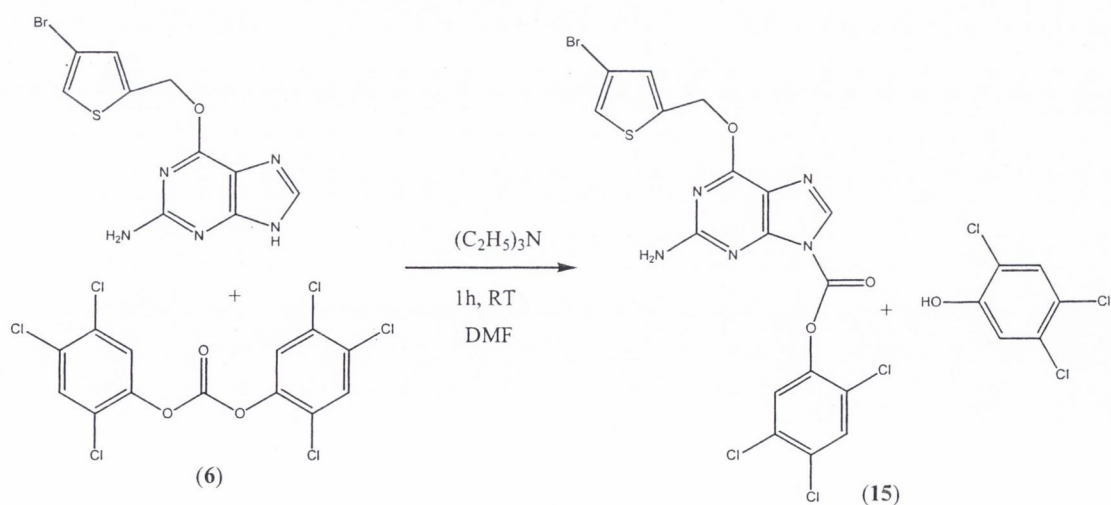


Fig. 2.3

The reaction of bis(2,4,5-trichlorophenyl) carbonate (**6**) with PaTrin-2 was carried out for 1 hour at room temperature in DMF, using an equimolar amount of triethylamine (Scheme 2.7). Reaction resulted in 80% yield of *O*⁶-(4-bromophenyl)-9-(2,4,5-trichlorophenoxy)carbonylguanine (**15**).



Scheme 2.7

The advantage of this reaction over those briefly outlined in Section 2.3.3 is that (15) can be isolated due to its extreme insolubility in common solvents.

2.3.5 Characterization of *O*⁶-(4-bromophenyl)-9-(2,4,5-trichlorophenoxy)carbonyl guanine

The N-9 derivative *O*⁶-(4-bromophenyl)-9-(2,4,5-trichlorophenoxy)carbonyl guanine (15) was characterized by analysis of its ¹H NMR spectrum (Fig. 2.4). The assignments for the signals in this spectrum are given in Table 2.2. Evidence for the formation of a N-9 derivative can be found by observing the positions of the signals associated with the NH₂ and H-8 protons. Comparing the ¹H NMR spectrum shown in Fig. 2.4 with that of PaTrin-2 shown in Fig. 2.2, we find that the signals associated with the NH₂ and the H-8 have shifted downfield to 7.04 ppm and 8.47 ppm respectively. The downfield shift of the signals associated with the NH₂ and the H-8 occurs upon the formation of all N-9 derivatives.

The N-9 alkoxycarbonyl derivatives that will be discussed in this chapter were all characterized in a similar manner. Full ¹H and ¹³C NMR spectral assignments for these compounds are given in the Experimental (Section 2.13).[♦]

[♦] A selection of NMR spectra from the three categories of prodrug can be found in the Appendix.

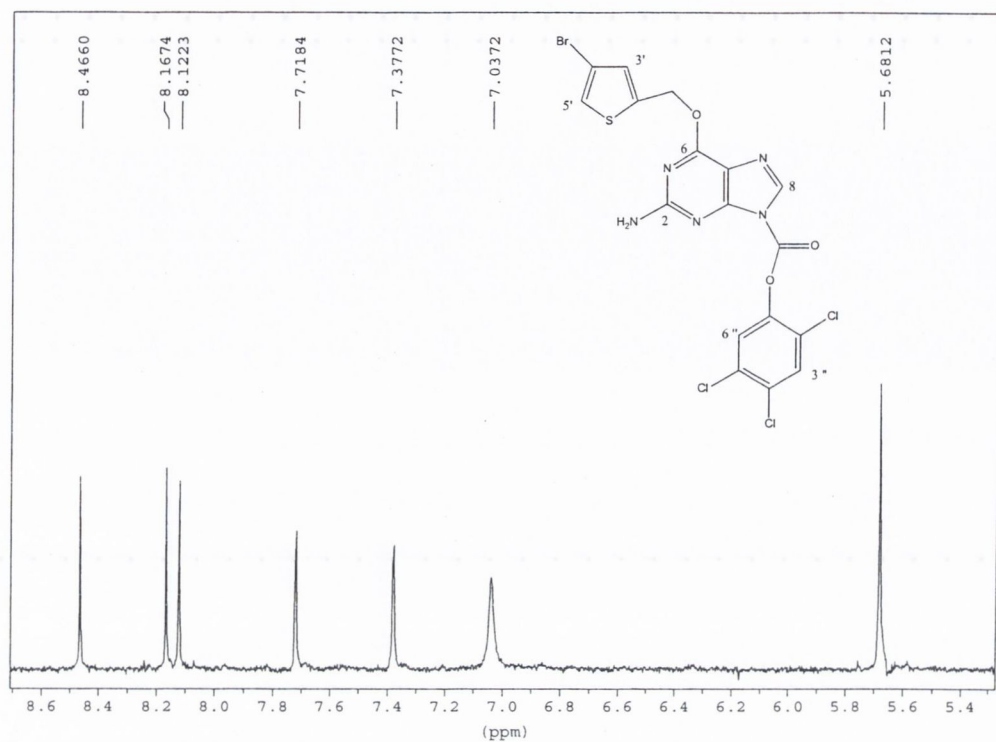


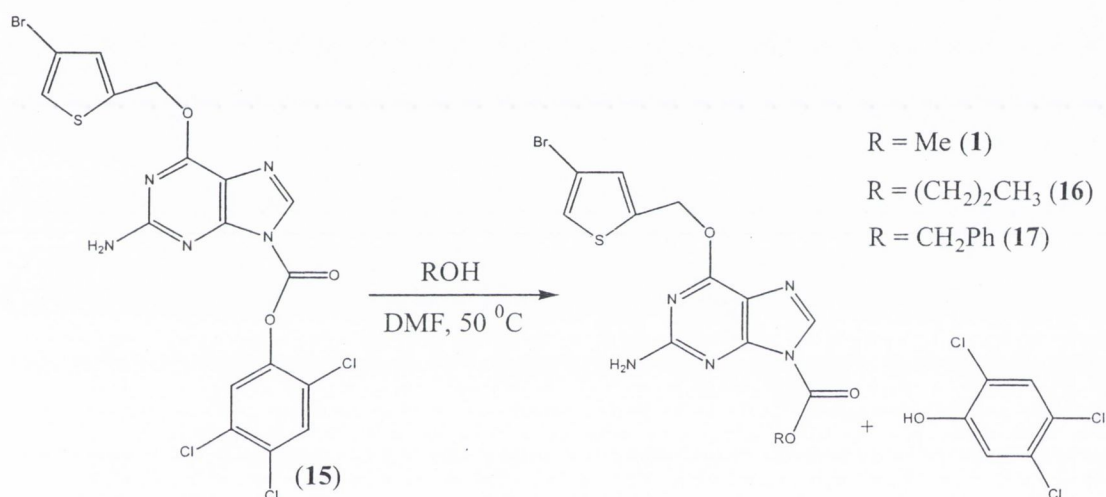
Fig. 2.4

Hydrogen	NMR δ (ppm from TMS)
CH ₂	5.68
NH ₂	7.04
H-3'	7.38
H-5'	7.72
H-3''	8.12
H-6''	8.17
H-8	8.47

Table 2.2

2.3.6 Use of *O*⁶-(4-bromophenyl)-9-(2,4,5-trichlorophenoxy)guanine

To see how readily the 2,4,5-trichlorophenoxide anion of *O*⁶-(4-bromophenyl)-9-(2,4,5-trichlorophenoxy)guanine (**15**) could be displaced, we treated it with an excess of methanol. Due to the insolubility of (**15**), the reaction was carried out in DMF at 50 °C (Scheme 2.8). Excess methanol was added, and after 2 hours, the solvent was removed, and ethanol was added to the remaining residue to precipitate the product. The product, *O*⁶-(4-bromophenyl)-9-(methoxycarbonyl)guanine (**1**)^{*}, was obtained in approximately 70% yield.



Scheme 2.8

The synthesis shown in Scheme 2.8 provides a high yielding route to compound (**1**), which had been characterized previously. Likewise, using an excess of either propanol or benzyl alcohol, *O*⁶-(4-bromophenyl)-9-(1-propoxycarbonyl)guanine (**16**) and 9-(benzyloxycarbonyl)-*O*⁶-(4-bromophenyl)guanine (**17**) were prepared (Scheme 2.8). However, the preparation of (**17**) required the presence of an equimolar amount of triethylamine. With the added base, the reaction proceeded well, but a 10 to 1 ratio of alcohol to (**15**) was required. Attempts to reduce the ratio of benzyl alcohol to (**15**)

^{*} This compound was previously prepared by Dr. D.J. Donnelly by a reaction involving PaTrin-2 and methyl chloroformate.

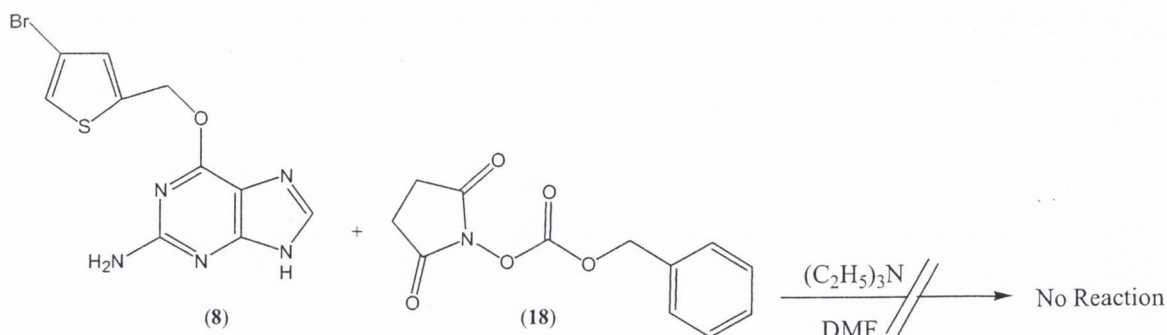
failed, resulting in dissociation to PaTrin-2 (**8**), and the formation of 2,4,5-trichlorophenol.

This appeared to be an inefficient method for the preparation of N-9 alkoxy carbonyl derivatives. This was even more apparent when no products were obtained when *O*⁶-(4-bromophenyl)-9-(2,4,5-trichlorophenoxy carbonyl)guanine (**15**) was treated with secondary alcohols under similar conditions. The procedure was also found to fail when primary alcohols containing a tertiary amino group were used.

With the scope for synthesis of our N-9 alkoxy carbonyl derivatives *via* **Route 1** severely limited, we decided that **Route 2** could potentially provide a more efficient and predictable synthetic pathway.

2.4 Investigation of Route 2

The first stage in **Route 2** (Scheme 2.1) involved the synthesis of an activated ester that could then react with PaTrin-2 to yield the desired N-9 alkoxy carbonyl derivative. The activated ester benzyl succinimidyl carbonate (**18**) was commercially available, so the second stage of **Route 2**, reaction with PaTrin-2 (**8**), was attempted (Scheme 2.9).

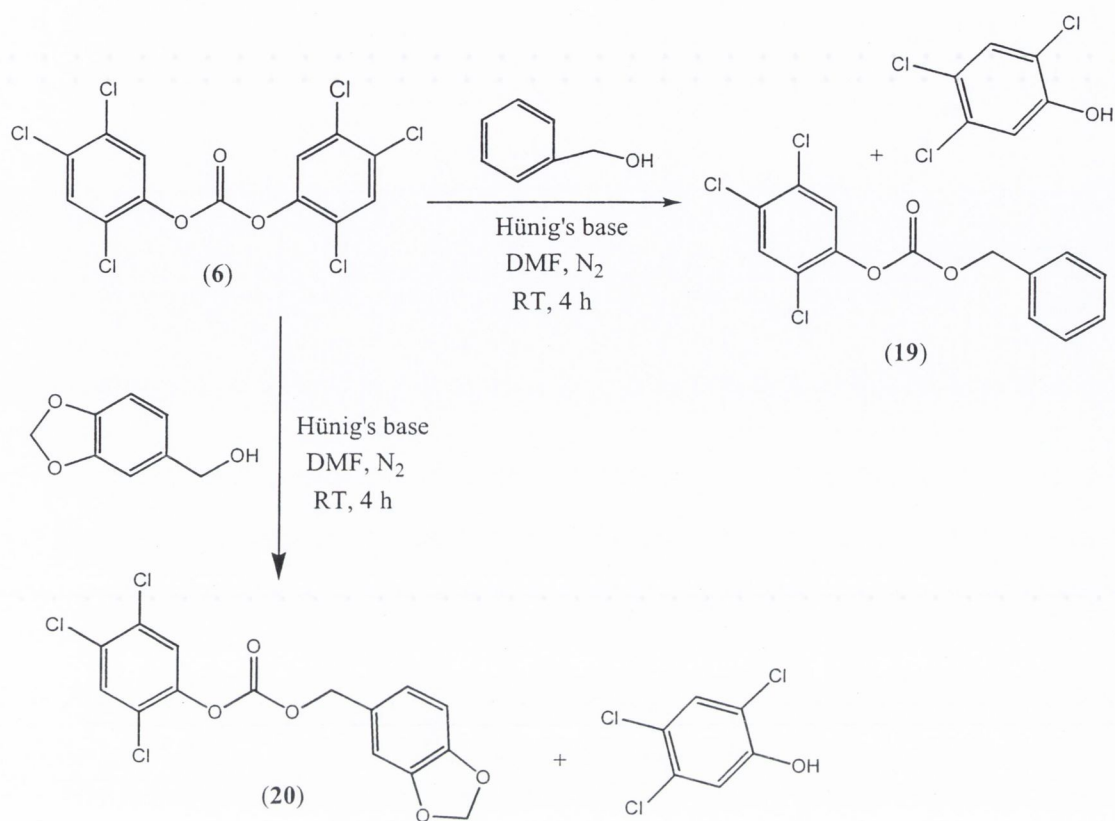


Scheme 2.9

The reaction of (18) with PaTrin-2 was initially attempted at room temperature using an equivalent of triethylamine as base. However reaction over 18 hours resulted only in the recovery of starting materials. Increases in reaction temperature, and use of DMAP instead of triethylamine further provided no signs of reaction. In fact (18) appeared surprisingly stable, with no signs of its decomposition. It was recovered in all reactions carried out.

2.4.1 Synthesis of 2,4,5-trichlorophenyl esters

Finding benzyl succinimidyl carbonate (18) to be unreactive, we decided to synthesize benzyl(2,4,5-trichlorophenyl) carbonate (19)²⁴ which we hoped would be a more activated ester. Many reports of the preparation of activated esters like (19) involve the treatment of alcohols with chloroformates.^{24,25} However, with a limited number of chloroformates available, and with the synthesis of these unstable reagents requiring the use of phosgene, the use of symmetrical carbonates like bis(2,4,5-trichlorophenyl) carbonate (6) to prepare mixed carbonates like (19) promised to be the favoured route. The 2,4,5-trichlorophenyl ester (19) was synthesized in good yield applying a method described by Dubowchik *et al.*²⁶ for the preparation of an activated ester. The reaction was carried out at room temperature for 4 hours using a molar equivalent of Hünig's base (*i*-Pr₂EtN) (Scheme 2.10).



Scheme 2.10

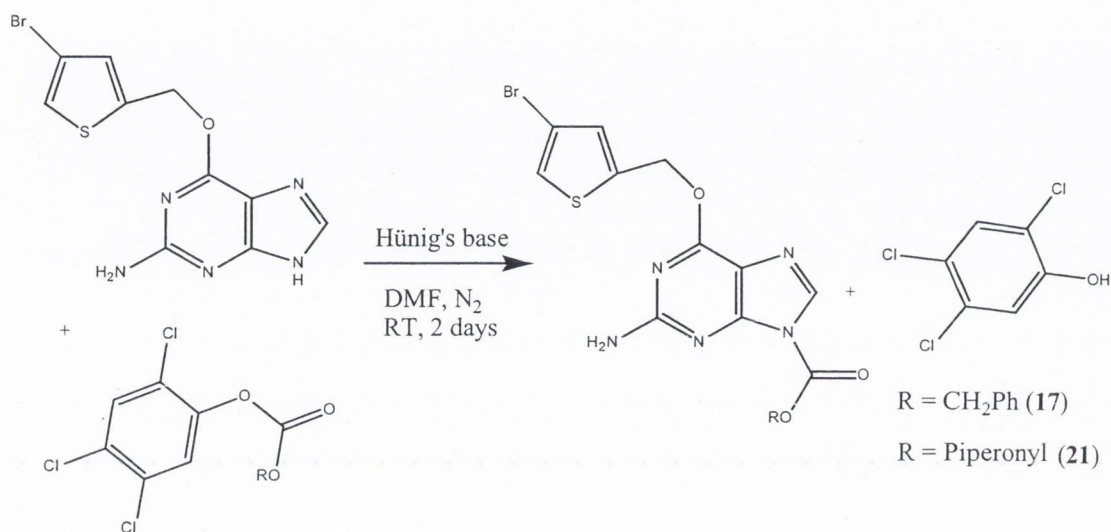
At the end of the reaction, when DMF was removed and light petroleum was added to the residue, (19) precipitated. Piperonyl(2,4,5-trichlorophenyl) carbonate (20) was also successfully synthesized in this way (Scheme 2.10).

Prior to the use of Hünig's base, as in the procedure by Dubowchik *et al.*²⁶, similar reactions were carried out using triethylamine or pyridine as bases. These reactions were unsuccessful in the synthesis of (19) or (20).

2.4.2 Reaction of PaTrin-2 with 2,4,5-trichlorophenyl esters

The subsequent reactions of the 2,4,5-trichlorophenyl esters (19) and (20) with PaTrin-2 were carried out in DMF using Hünig's base (Scheme 2.11). These reactions were slow, and required 2 days at room temperature for completion. The synthesis

resulted in almost quantitative yields of 9-benzyloxycarbonyl-*O*⁶-(4-bromophenyl)guanine (**17**) (prepared previously, see Section 2.3.6) and *O*⁶-(4-bromophenyl)-9-(piperonyloxycarbonyl)guanine (**21**).



Scheme 2.11

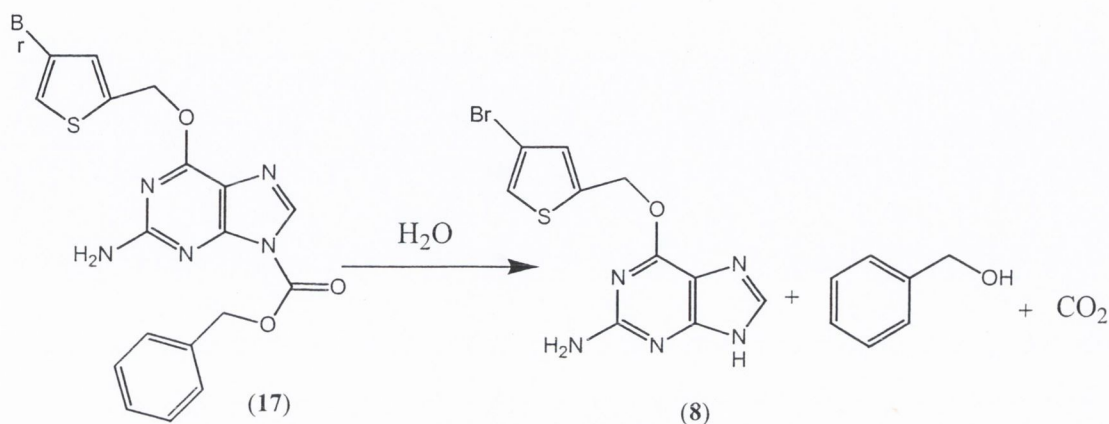
It was also established that a “one-pot” synthesis was as efficient and high-yielding a route to (**17**) and (**21**). “One-pot” synthesis eliminated the need to isolate the intermediates (**19**) and (**20**).

Thus, using **Route 2** (Scheme 2.1) and the 2,4,5-trichlorophenoxide anion as a leaving group, two examples of N-9 alkoxyguanine derivatives, 9-benzyloxycarbonyl-*O*⁶-(4-bromophenyl)guanine (**17**) and *O*⁶-(4-bromophenyl)-9-(piperonyloxycarbonyl)guanine (**21**) were synthesized in good yield from equimolar amounts of the corresponding primary alcohols. As well as having provided us with two simple prodrugs for pharmacological evaluation, we appeared to be equipped with an efficient synthetic route to the preparation of more N-9 derivatives.

2.5 Investigation of other alcohols

The N-9 alkoxyguanine derivatives (**17**) and (**21**) were synthesized from primary alcohols. We also thought that carbamates derived from secondary alcohols might

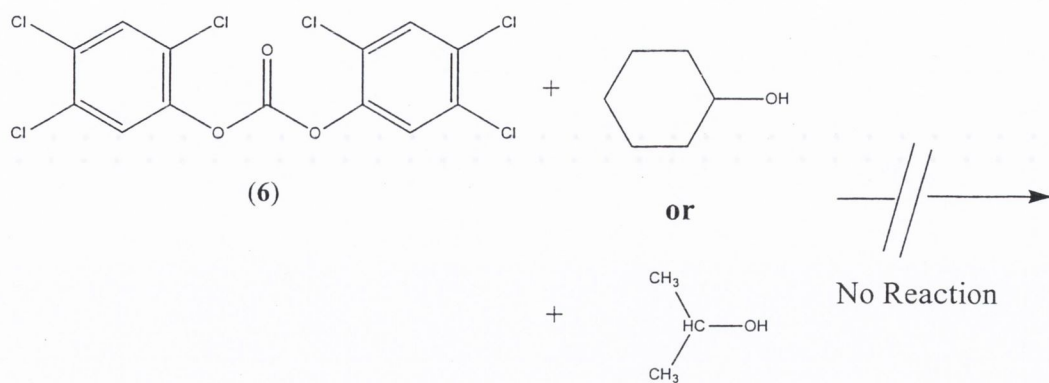
prove interesting as prodrugs. They should be hydrolysed more slowly than the carbamates obtained from primary alcohols. All these carbamates should be hydrolysed by the reaction shown for the benzyl derivative (17) in Scheme 2.12.



Scheme 2.12

2.5.1 Synthesis of 2,4,5-trichlorophenyl esters from secondary alcohols

Using the method for the synthesis of 2,4,5-trichlorophenyl esters as outlined in Scheme 2.8, we attempted the reaction of bis(2,4,5-trichlorophenyl) carbonate (6) with both cyclohexanol and isopropanol (Scheme 2.13). Treatment of (6) with each of these secondary alcohols, in the presence of a molar equivalent of Hünig's base, over 4 hours at room temperature resulted only in the recovery of unreacted bis(2,4,5-trichlorophenyl) carbonate (6). A secondary alcohol would be expected to be less reactive than a primary alcohol, so these reactions were repeated, but for longer periods of time. However, no reaction occurred after 2 days at room temperature or at elevated temperatures (up to $80^{\circ}C$). Use of an excess of Hünig's base or the slightly more basic triethylamine also afforded no product.



Scheme 2.13

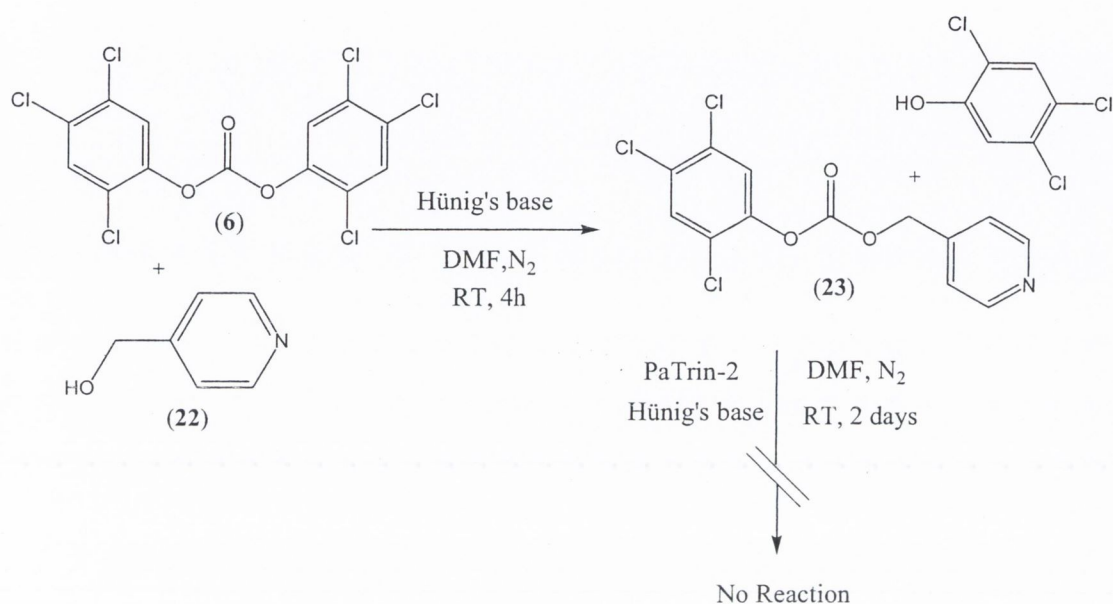
The scope for the synthesis of 2,4,5-trichlorophenyl esters appeared to be limited to reactions involving primary alcohols. As a result, we decided it was important to investigate further the limitations of reaction with bis(2,4,5-trichlorophenyl) carbonate (6).

2.5.2 Synthesis of 2,4,5-trichlorophenyl esters from alcohols containing a tertiary amino group

One of the more important aims in the synthesis of N-9 alkoxy carbonyl derivatives, as potential prodrugs, was the preparation of derivatives in which the N-9 substituent incorporates a tertiary amino group. Such a derivative would facilitate the preparation of salts to improve solubility and therefore the formulation and delivery of a prodrug. Consequently, it was essential to examine the usefulness of bis(2,4,5-trichlorophenyl) carbonate (6) in the synthesis of an activated ester from a primary alcohol containing a tertiary amino group.

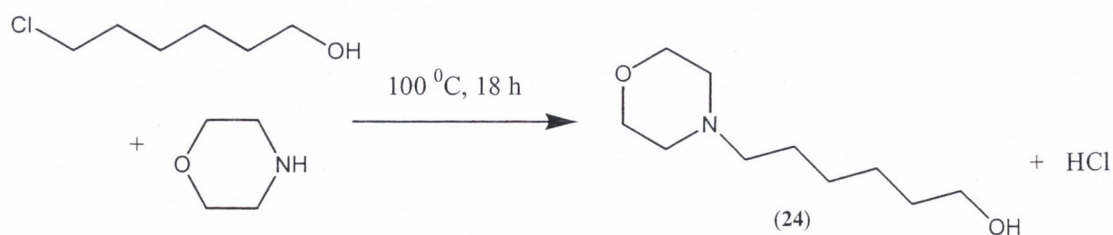
As it was similar in structure to benzyl alcohol, we chose to examine the reaction of 4-picolyl alcohol (22) with bis(2,4,5-trichlorophenyl) carbonate (6). The reaction was carried out for 4 hours at room temperature using an equimolar amount of Hünig's base (Scheme 2.14). The reaction was successful, resulting in a high yield of the

2,4,5-trichlorophenyl ester (**23**). However, the reaction of PaTrin-2 with (**23**), attempted under a variety of conditions, was unrewarding. Only PaTrin-2 was recovered.



Scheme 2.14

Similar behaviour was found with 3-picolyl alcohol which gave the corresponding 2,4,5-trichlorophenyl ester, but this ester also failed to react with PaTrin-2. Another alcohol, 6-morpholinohexan-1-ol (**24**)^{*}, synthesized by the reaction of 6-chlorohexan-1-ol with morpholine (Scheme 2.15)²⁷, did not even yield the intermediate ester with bis(2,4,5-trichlorophenyl) carbonate (**6**) under the standard (Scheme 2.10) or under other conditions.



Scheme 2.15

^{*} A sample of 6-morpholinohexan-1-ol (**24**) was kindly provided by Dr. D. Scully.

Using bis(2,4,5-trichlorophenyl) carbonate (**6**), it appeared the development of a reliable route to the preparation of a variety of N-9 alkoxy carbonyl derivatives would not be possible. To develop an efficient route, and to rationalise the reactions involving bis(2,4,5-trichlorophenyl) carbonate (**6**), we decided to examine the reactions of bis(4-nitrophenyl) carbonate (**7**) with the same alcohols.

2.6 Bis(4-nitrophenyl) carbonate

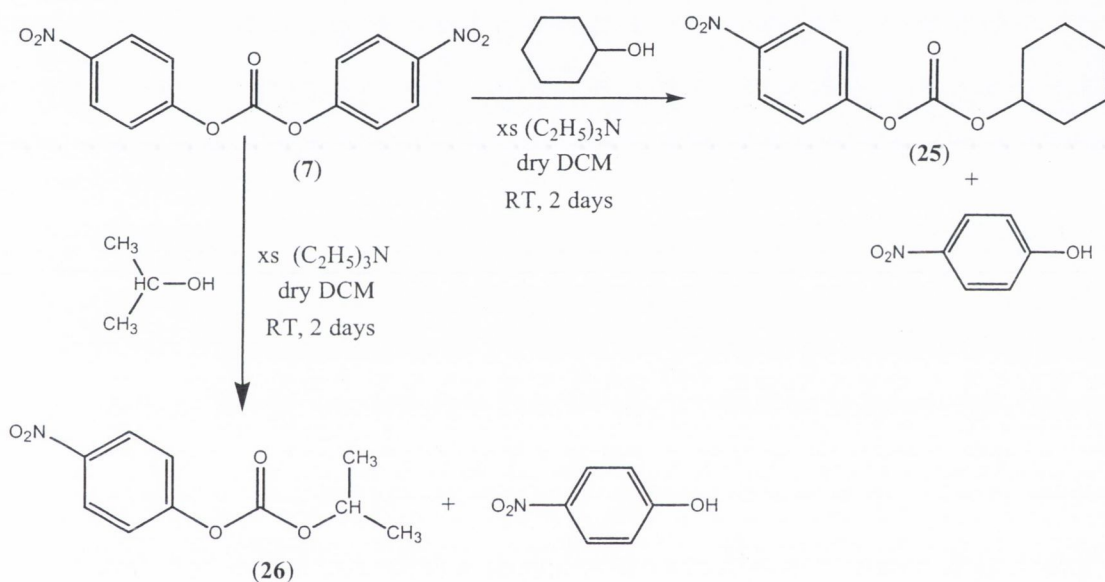
Activated esters with the 4-nitrophenoxy group as the activating group are numerous in the literature.^{24,25,28-31} The popularity of such activated esters stems from the lower reactivity that the 4-nitrophenoxy group confers upon such carbonates relative to the corresponding chloroformates.^{28,29} The 4-nitrophenoxy anion is not as good a leaving group as the chloride anion.

Many literature reports propose the preparation of 4-nitrophenyl esters either by the reaction of an alcohol with 4-nitrophenyl chloroformate,^{24,31} or the reaction of a chloroformate with 4-nitrophenol.^{24,28} The reaction of 4-nitrophenyl chloroformate with alcohols, such as benzyl alcohol, has been shown to result in low yields of activated esters.²⁴ We also found similar difficulties with the use of 4-nitrophenyl chloroformate and we abandoned its use. There are limited accounts in the literature of the use bis(4-nitrophenyl) carbonate (**7**) in the preparation of activated esters, but the available reports suggest synthetic advantages with its use.³²

Reaction of bis(4-nitrophenyl) carbonate (**7**) with benzyl alcohol was successfully repeated using an equivalent of Hünig's base in a reaction at room temperature for 4 hours. The subsequent reaction of PaTrin-2 with the mixed 4-nitrophenyl ester obtained proved to be as efficient as with benzyl(2,4,5-trichlorophenyl) carbonate (**19**) (Scheme 2.11).

2.6.1 Synthesis of 4-nitrophenyl esters from secondary alcohols

Bis(2,4,5-trichlorophenyl) carbonate (6) had failed to react with secondary alcohols. Therefore we attempted the reaction of bis(4-nitrophenyl) carbonate (7) with cyclohexanol. No reaction was observed using either one equivalent of Hünig's base or of triethylamine. However, use of an excess of triethylamine (80% excess)* in a reaction carried out at room temperature in dry DCM for 2 days proved successful (Scheme 2.16). This reaction worked equally well with isopropanol.

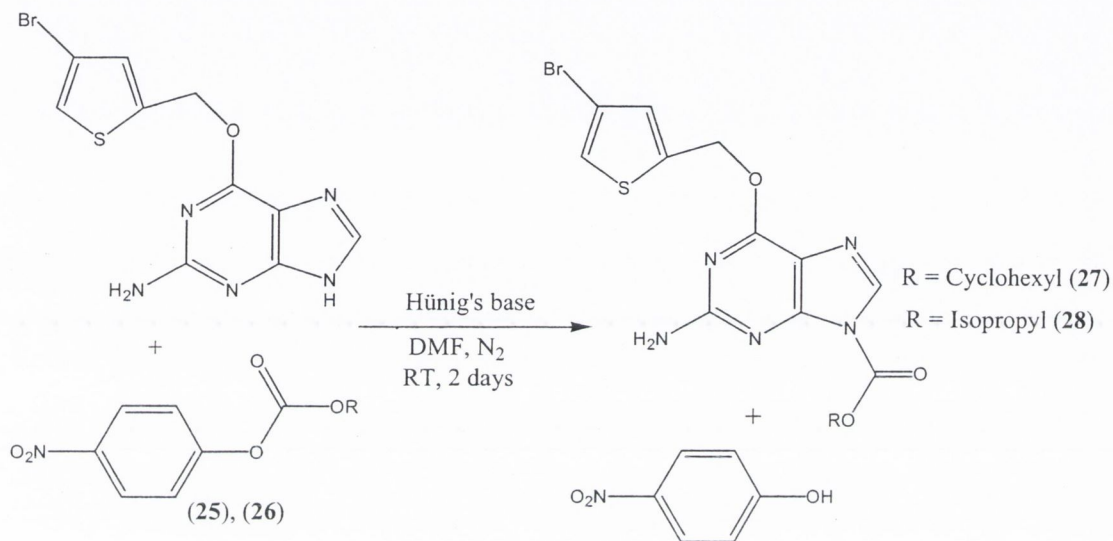


Scheme 2.16

The 4-nitrophenyl esters (25) and (26) were found to be very susceptible to hydrolysis and obtained only in approximately 50% yield. This was due to the aqueous work-up required to extract the free 4-nitrophenol. Total purification of (25) and (26) was difficult, with their gradual decomposition over time to liberate 4-nitrophenol. This instability required that the subsequent reaction with PaTrin-2 be carried out immediately. Perhaps due to the large excess of base required in stage one, one-pot synthesis (Section 2.4.2) was not successful.

* Use of excess $(\text{C}_2\text{H}_5)_3\text{N}$ was suggested by the findings of Úna Hanafin from a related experiment.

The reaction of the 4-nitrophenyl esters (**25**) and (**26**) with PaTrin-2 were carried out in DMF, in the presence of an equivalent of Hünig's base at room temperature for 2 days (Scheme 2.17). Kim *et al.*²⁸ had reported problems related to the use of the 4-nitrophenyl ester (**26**) in DMF, suggesting that DMF might itself be reacting with (**26**).

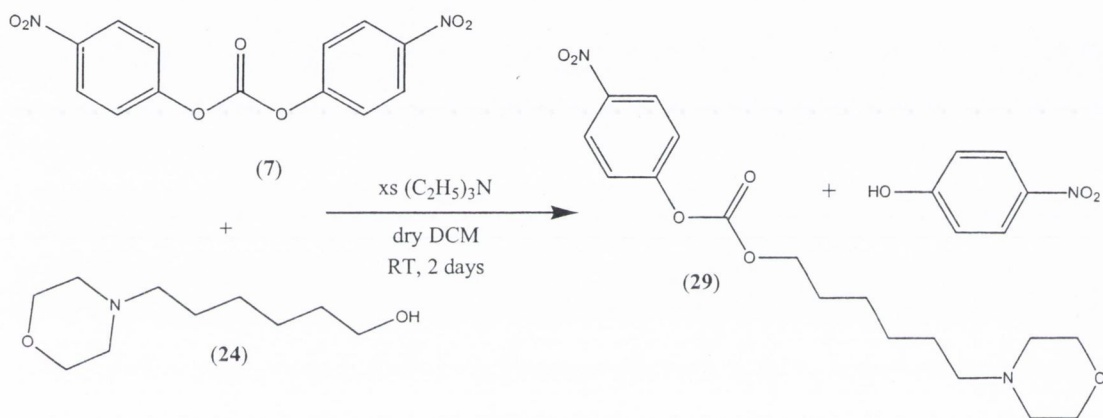


Scheme 2.17

Initially both reactions appeared to be low yielding. However, NMR analysis of the crude product prior to work-up showed high yields of *O*⁶-(4-bromophenyl)-9-(cyclohexyloxycarbonyl)guanine (**27**) and *O*⁶-(4-bromophenyl)-9-(isopropoxycarbonyl)guanine (**28**). The reactions were, in fact, very successful, with no notable complications due to DMF. The low yields observed in the initial experiments again resulted from the work-up procedures involved in the separation of these derivatives from the liberated 4-nitrophenol, and unreacted PaTrin-2 was an additional complication. Hydrolysis of these N-9 alkoxycarbonyl derivatives must occur very easily. Flash chromatography or aqueous work-ups resulted only in the recovery of small percentages of (**27**) and (**28**). However, we found that both 4-nitrophenol and PaTrin-2 were extremely soluble in hot acetonitrile, while (**27**) and (**28**) were sufficiently insoluble that they could be separated in better yields.

2.6.2 Synthesis of *O*⁶-(4-bromophenyl)-9-(6-morpholinohexyloxycarbonyl)guanine

As mentioned above, the development of an efficient route to the synthesis of N-9 alkoxycarbonyl derivatives must include reactions with alcohols containing a tertiary amino group. 6-Morpholinohexan-1-ol (**24**) had failed to react with bis(2,4,5-trichlorophenyl) carbonate (**6**) (Section 2.5.2), therefore we examined its reaction with bis(4-nitrophenyl) carbonate (**7**). The preparation of the corresponding 4-nitrophenyl ester (**29**) required 2 days at room temperature and an excess of triethylamine (80% excess) (Scheme 2.18).

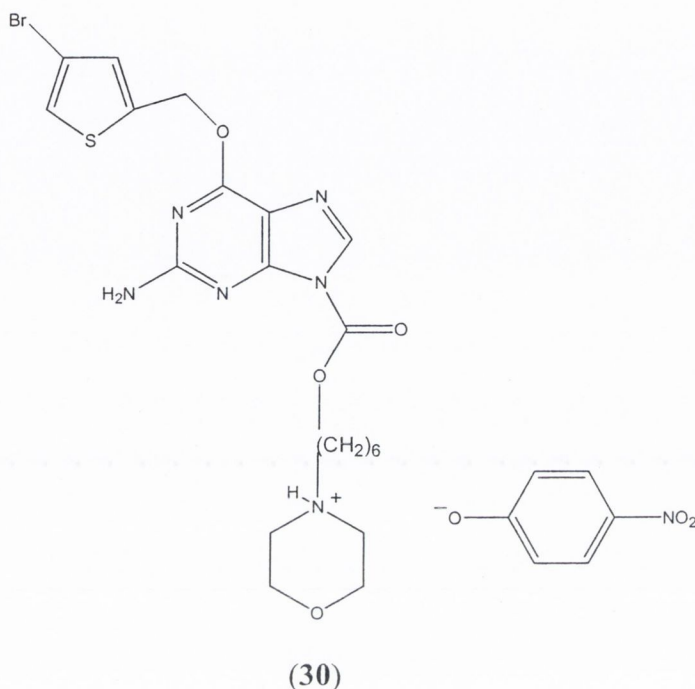


Scheme 2.18

No reaction was observed in the presence of an equimolar amount of base, or when the reaction was carried out in the absence of base. The 4-nitrophenyl ester (**29**) was isolated in approximately 50% yield following the repeated washing of the DCM reaction solution with a 10% NaHCO₃ solution, which was effective in removing the liberated 4-nitrophenol.

Analogous to the synthetic route shown in Scheme 2.17, the 4-nitrophenyl ester (**29**) was reacted with PaTrin-2 using Hünig's base, and the reaction was monitored by TLC. However, after 2 days at room temperature no new compound appeared to have been produced. Instead TLC indicated only what appeared to be the presence of PaTrin-2 on the baseline. The reaction was subsequently stopped and the DMF

removed. NMR analysis of the crude residue showed that complete reaction had taken place, with no trace of unreacted PaTrin-2. NMR and TLC results showed that the liberated 4-nitrophenol had formed a salt with the PaTrin-2 derivative (30).



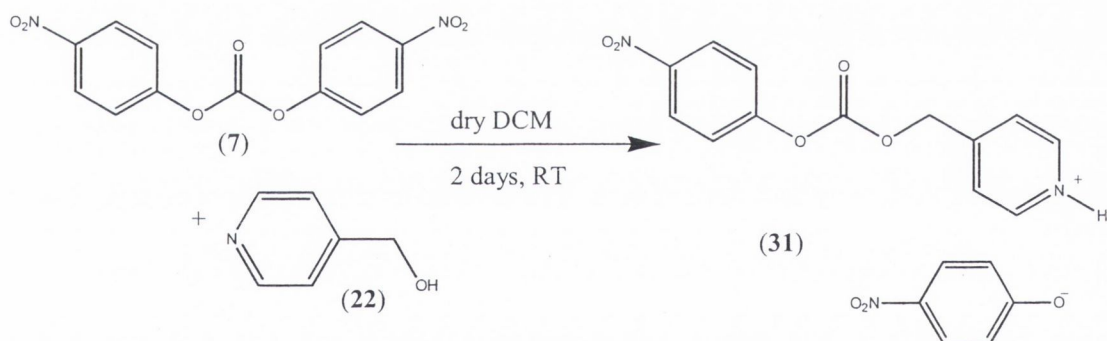
Although the eventual objective of synthesizing such basic N-9 alkoxy carbonyl derivatives was the preparation of salts, such a 4-nitrophenol salt was not desirable. We attempted to break down the salt using an aqueous NaHCO_3 solution. This worked, gradually removing 4-nitrophenol, but the number of washes required for completion resulted in extensive hydrolysis of the N-9 derivative to give mostly PaTrin-2 and only a small quantity of the desired product.

Effort was directed towards the conversion of the 4-nitrophenol salt to a hydrochloride using an anion exchange resin (Amberlite, IRA 400 (Cl)). However this too resulted in the decomposition of product. Eventually a solution of (30) in DCM was washed quickly with a 0.5 M NaOH solution, dried over MgSO_4 , and the DCM removed to afford *O*⁶-(4-bromophenyl)-9-(6-morpholinohexyloxycarbonyl)guanine in

76% yield. The immediate reaction of strong alkali with 4-nitrophenol minimised any loss of product through increased sensitivity.

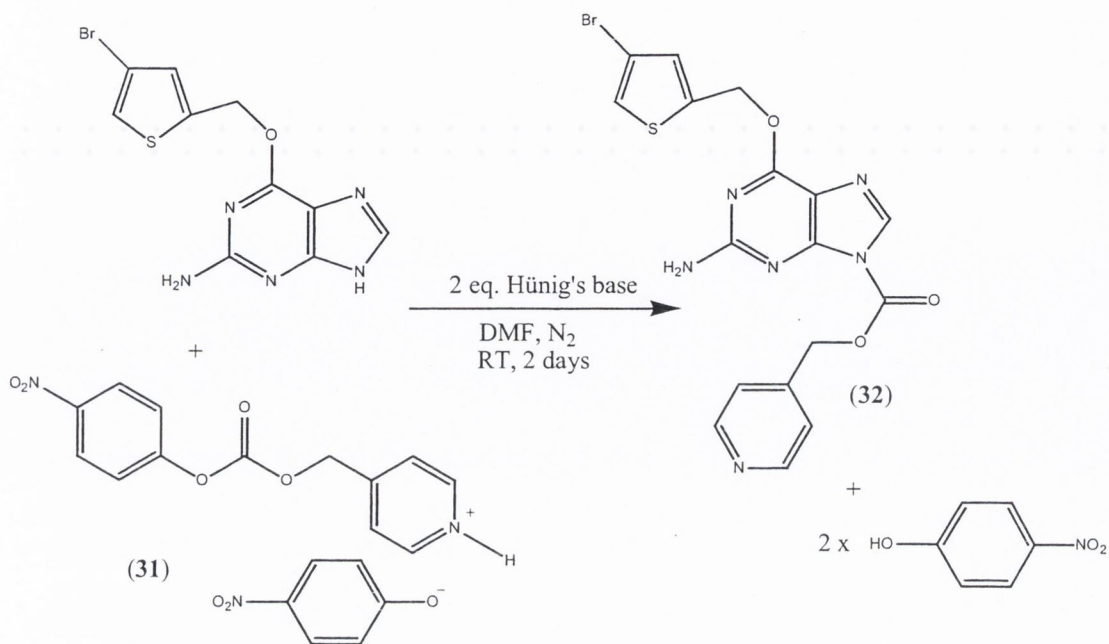
2.6.3 Synthesis of *O*⁶-(4-bromophenyl)-9-(4-picolylloxycarbonyl)guanine

Bis(4-nitrophenyl) carbonate (**7**) was proving a more versatile reagent than bis(2,4,5-trichlorophenyl) carbonate (**6**), as we continued with our comparison of these two reagents. 4-Picolyl(2,4,5-trichlorophenyl) carbonate (**23**) had been successfully synthesized from the reaction of (**6**) with 4-picolyl alcohol (**22**), but it had failed to react with PaTrin-2 (Section 2.5.2). Using a method described by Veber *et al.*³³ we synthesized the 4-nitrophenol salt of the 4-nitrophenyl ester (**31**) (Scheme 2.19). As reported, complete reaction required 2 days at room temperature. The salt was precipitated from ethyl acetate, in good yield, by the addition of hexane.



Scheme 2.19

Subsequent reaction with PaTrin-2 using two equivalents of Hünig's base resulted in a good yield of *O*⁶-(4-bromophenyl)-9-(4-picolylloxycarbonyl)guanine (**32**) (Scheme 2.20). The reaction was also shown to be successful when the free base derived from (**31**) was used. However, unlike 4-picolyl alcohol, 3-picolyl alcohol failed to react with bis(4-nitrophenyl) carbonate (**7**) under a variety of conditions.



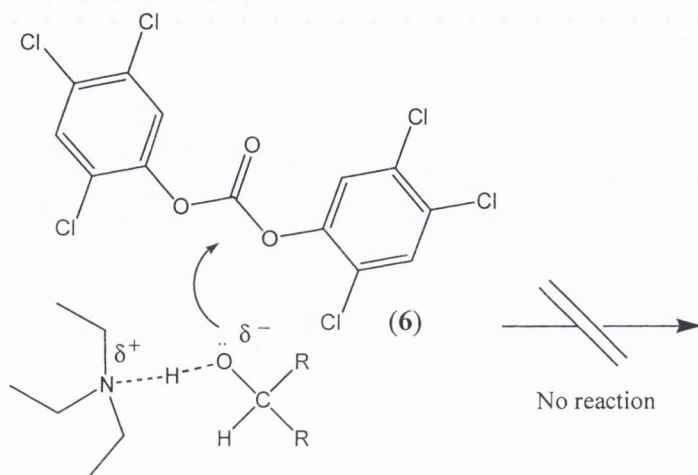
Scheme 2.20

2.7 Bis(4-nitrophenyl) carbonate versus bis(2,4,5-trichlorophenyl) carbonate

Using bis(4-nitrophenyl) carbonate (**7**), **Route 2** (Scheme 2.1) was shown to be an effective route to the synthesis of N-9 alkoxy carbonyl derivatives using primary, secondary and basic primary alcohols. Although successful in its reactions with primary alcohols, bis(2,4,5-trichlorophenyl) carbonate (**6**) had proved suitable only for reactions involving primary alcohols. The 2,4,5-trichlorophenoxide anion is a better leaving group than the 4-nitrophenoxide anion, but only marginally so, and not sufficient to account for the differences observed in reactions of (**6**) and (**7**).

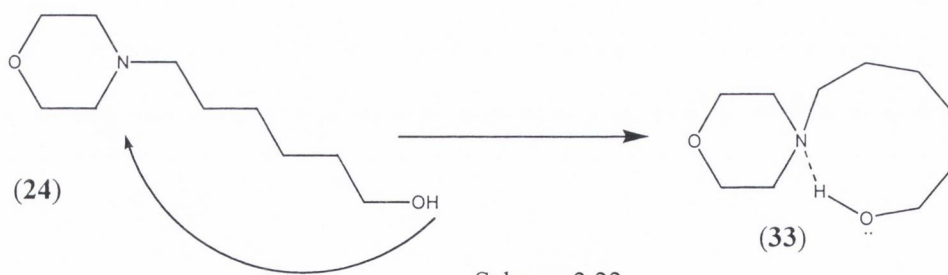
The reaction of bis(4-nitrophenyl) carbonate (**7**) with secondary alcohols, although slow and requiring an excess of base, succeeds where bis(2,4,5-trichlorophenyl) carbonate (**6**) fails, possibly due to steric hindrance in the latter. If the preferred conformation of (**6**) is that shown in Scheme 2.21, the 2-chlorine would interfere with attack on the carbonyl group by the already hindered secondary alcohol. This must occur from above the O-CO-O plane, but steric factors still would be involved. With

recovery of (6) in all reactions carried out (Section 2.5.1), steric hindrance (as illustrated by the red substituents in Scheme 2.21) seems likely.



Scheme 2.21

Although bis(2,4,5-trichlorophenyl) carbonate (6) reacted efficiently with simple primary alcohols, it failed to react with 6-morpholinohexan-1-ol (24). This perhaps can be rationalised again by steric hindrance. Due to hydrogen bonding involving the hydroxyl group and the tertiary amino group in (24) (Scheme 2.22), the hydroxy oxygen in (33) may be hindered in its attack on the carbonyl of bis(2,4,5-trichlorophenyl) carbonate (6).



Scheme 2.22

However, attack of (33) is still possible on the less hindered carbonyl of bis(4-nitrophenyl) carbonate (7). The oxygen atom in (33) is activated by a hydrogen bonding interaction with the tertiary amino group that acts as a base.

This explanation for the differences in the reactions of bis(2,4,5-trichlorophenyl) carbonate (6) and bis(4-nitrophenyl) carbonate (7) is due to the different steric environments of their carbonyl groups. However, such reasoning failed to rationalise the reactions involving 4-picolyl alcohol (22).

4-Picolyl(2,4,5-trichlorophenyl) carbonate (23) was successfully synthesized but failed to react with PaTrin-2 under our normal conditions. However, when we used ultra-dry DMF, we were able to isolate *O*⁶-(4-bromophenyl)-9-(4-picolylloxycarbonyl)guanine (32) in a respectable yield. This suggests that the carbonate (23) is very susceptible to hydrolysis by traces of water in the solvent as postulated in Fig. 2.5. The corresponding 4-nitrophenyl derivative (31) did not require ultra-dry DMF, and so the latter reagent was preferred.

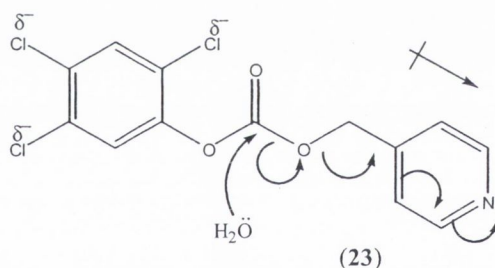


Fig. 2.5

It was not possible to completely rationalise the differences in reactions involving bis(2,4,5-trichlorophenyl) carbonate (6) and bis(4-nitrophenyl) carbonate (7). However, it was clear that, whether for steric or electronic reasons, bis(4-nitrophenyl) carbonate (7) was the more versatile reagent.

2.8 Synthesis of further alkoxycarbonyl derivatives

In an investigation of N-9 alkyl derivatives for use as targeting agents, it was found that lipophilic substituents, even bulky ones, were well tolerated by the ATase active site and had little diminishing effect on the activity of the inactivator.³⁴ Molecular

modelling studies³⁵ showed that the orientation of the purine ring system in the ATase binding pocket directed N-9 alkyl substituents along a hydrophobic cleft. It was calculated that the best N-9 substituted inactivators would be those with long flexible chains (*i.e.* eight to twelve CH₂ groups). The interaction of amino acid residues in the cleft with the alkyl chain was proposed to be responsible for increasing the stabilization of the guanyl moiety in the binding pocket, resulting in a better I₅₀ value. However, the biological I₅₀ results were found not to correlate entirely with the computational result. What such reports do underline is that a N-9 substituent can play a potentiating role in the activity of ATase inhibitors. The need to synthesize more N-9 alkoxy carbonyl derivatives was therefore apparent.

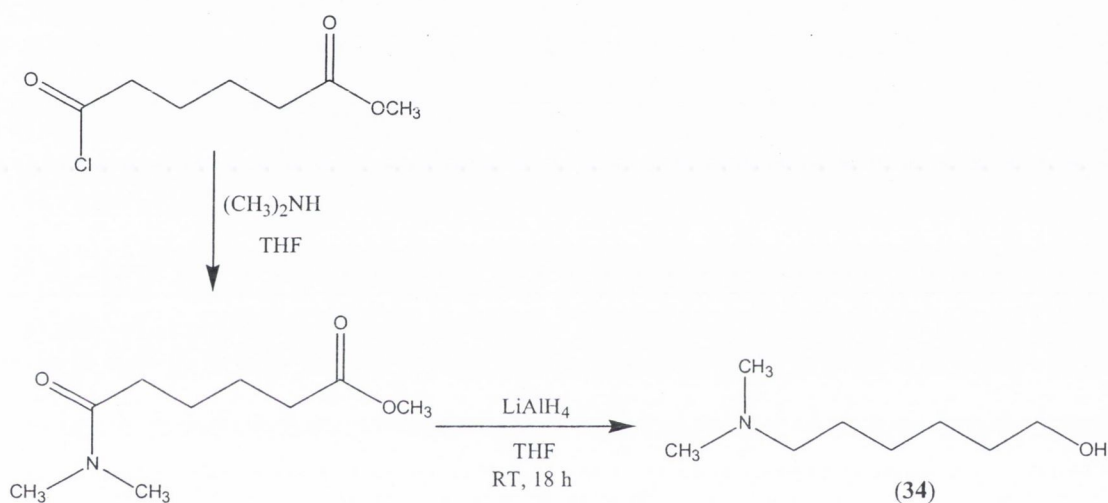
2.8.1 Further use of bis(4-nitrophenyl) carbonate

Using bis(4-nitrophenyl) carbonate and following **Route 2** (Scheme 2.1) we had successfully synthesized six examples of N-9 alkoxy carbonyl derivatives from primary and secondary alcohols, as well as of primary alcohols substituted with tertiary amino groups, thus defining an efficient route to the alkoxy carbonylation of the N-9 position in the purine ring system. The reliability of this synthetic method was further demonstrated by the synthesis of several more alkoxy carbonyl derivatives for biological evaluation.

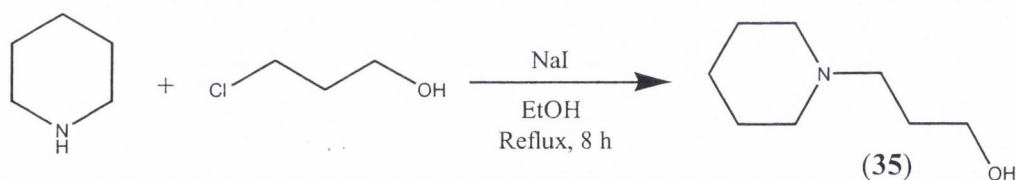
To allow for the preparation of salts, the synthesis of more N-9 alkoxy carbonyl derivatives containing a basic centre was of great importance to us. We chose to examine several alcohols that differed in chain length. 6-Dimethylaminohexan-1-ol (**34**), 3-piperidinopropan-1-ol (**35**) and *N*-methylpiperidin-4-ol (**36**) fulfilled such requirements. A PaTrin-2 derivative with a N-9 side chain derived from (**34**) would provide a useful comparison with *O*⁶-(4-bromophenyl)-9-(6-

morpholinohexyloxycarbonyl)guanine (**30**). It differs only from (**30**) in having a more flexible tertiary amino group.

Alcohol (**34**) was synthesized* in high yield by the reduction of the corresponding amido-ester using LiAlH_4 (Scheme 2.23).³⁶ The amido-ester, 5-methoxycarbonyl-*N,N*-dimethylpentanamide was afforded by treating 6-methoxycarbonylpentanoyl chloride with dimethylamine (Scheme 2.23).³⁷



3-Piperidinopropan-1-ol (**35**) was prepared* by the reaction of 3-chloropropan-1-ol with piperidine (Scheme 2.24).³⁸



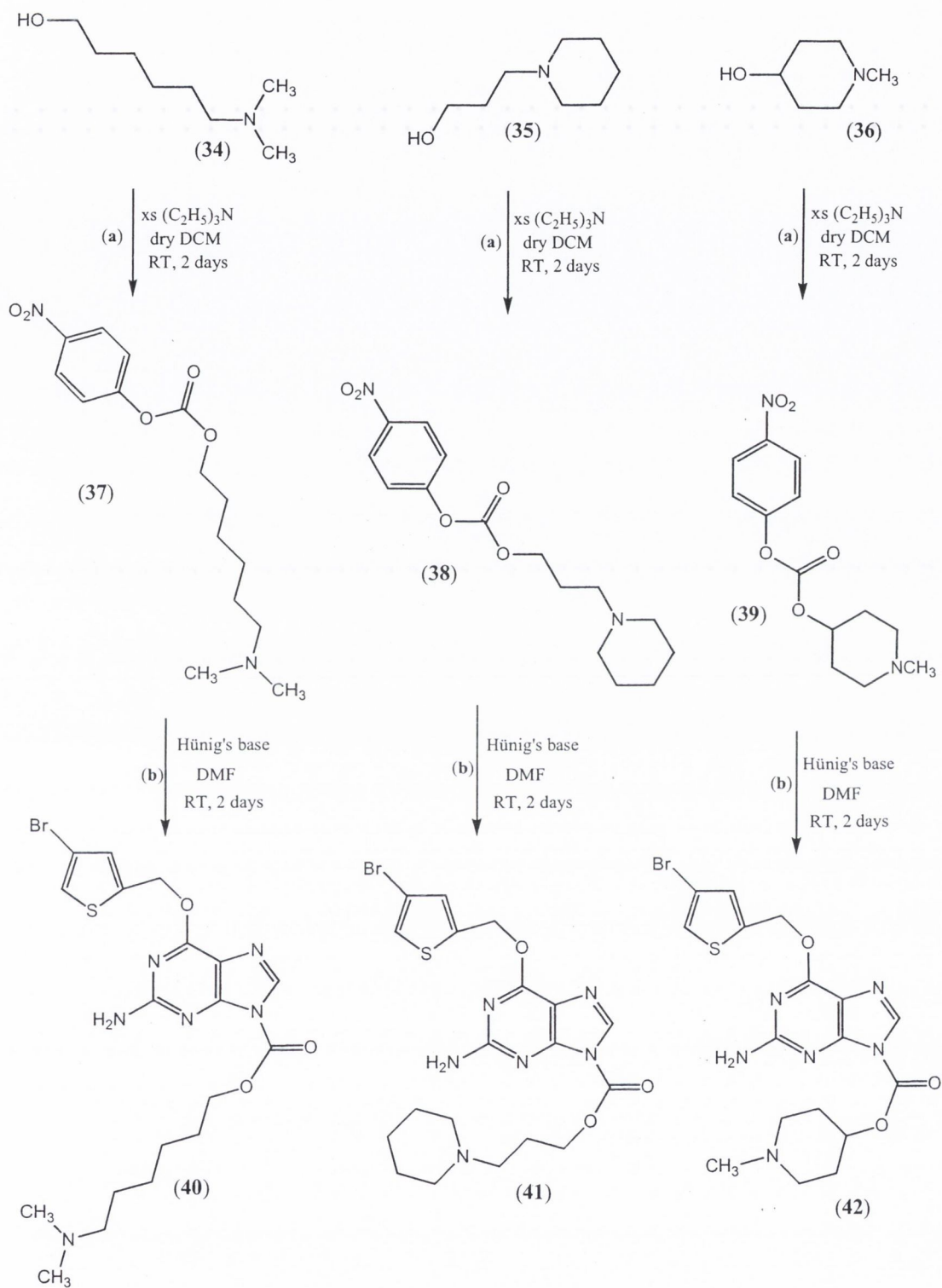
We successfully prepared the 4-nitrophenyl esters (**37**), (**38**) and (**39**) by the reaction of bis(4-nitrophenyl) carbonate (**7**) with the alcohols (**34**), (**35**) and (**36**) respectively.

The 4-nitrophenyl esters were prepared in a similar way to 6-morpholinohexyl 4-

* Alcohols (**34**) and (**35**) were kindly prepared by Dr. D.J. Donnelly.

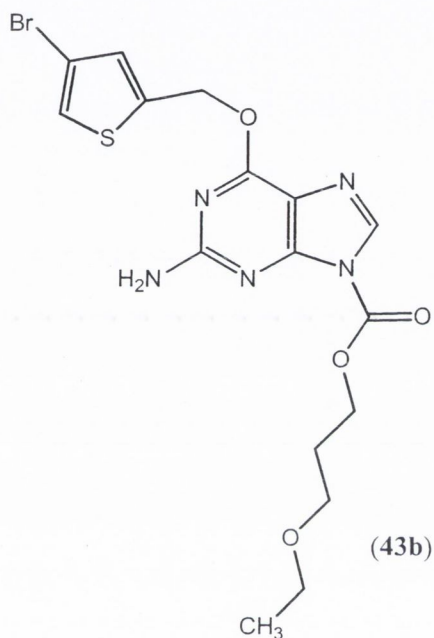
nitrophenyl carbonate (**29**) (Section 2.6.2), and an excess of triethylamine was used in reactions carried out for 2 days (Scheme 2.25a).

The optimum conditions for the subsequent reaction with PaTrin-2 were found to involve the esters as free bases, and not as their 4-nitrophenol salts (see above, Section 2.6.3, pg 67). As with previous reactions of PaTrin-2 with 4-nitrophenyl esters, Hünig's base was found to be the best base, resulting after 2 days at room temperature in good yields of O^6 -(4-bromophenyl)-9-(6-dimethylaminohexyloxycarbonyl)guanine (**40**), O^6 -(4-bromophenyl)-9-(3-piperidinopropoxycarbonyl)guanine (**41**) and O^6 -(4-bromophenyl)-9-(*N*-methyl-4-piperidyloxycarbonyl)guanine (**42**) (Scheme 2.25b). These syntheses resulted in the N-9 derivatives (**40**) and (**41**) being obtained as 4-nitrophenol salts. Washing DCM solutions of these salts with a 0.5 M NaOH solution removed most of the 4-nitrophenol. Residual traces of 4-nitrophenol were removed by ethyl acetate in which (**40**), (**41**) and (**42**) were insoluble, while 4-nitrophenol was fully soluble.



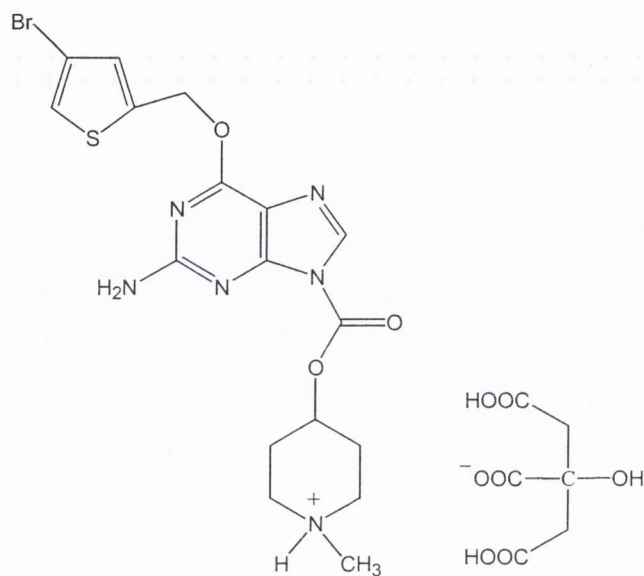
Scheme 2.25

The intermediate 3-ethoxypropyl 4-nitrophenyl carbonate (**43a**) was also prepared using the route shown in Scheme 2.25a and was subsequently used in the preparation of *O*⁶-(4-bromophenyl)-9-(3-ethoxypropoxycarbonyl) guanine (**43b**) following the route shown in Scheme 2.25b. The pharmacological evaluation of (**43b**) will show the influence, if any, of the terminal alkoxy group.



2.8.2 Preparation of citric acid salts

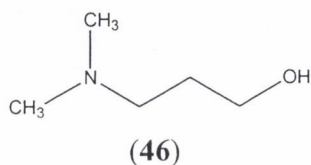
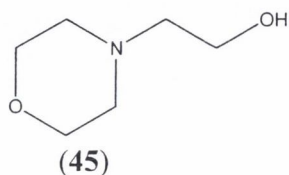
To demonstrate the ease by which a salt could be formed from a PaTrin-2 derivative with an amino group in the 9-substituent, *O*⁶-(4-bromophenyl)-9-(*N*-methyl-4-piperidyloxycarbonyl)guanine (**42**) was dissolved in the minimum amount of methanol. To this solution was added a molar equivalent of citric acid and the mixture set aside for 10 minutes. Longer reaction times lead to the gradual hydrolysis of the alkoxy carbonyl derivative to regenerate PaTrin-2 (**8**). The citric acid salt (**44**), a white crystalline solid, was obtained in quantitative yield and was found to be water-soluble.



(44)

2.8.3 Problems with the use of bis(4-nitrophenyl) carbonate

Using bis(4-nitrophenyl) carbonate (7), **Route 2** (Scheme 2.1) had so far appeared to be a reliable and consistently efficient route to the synthesis of N-9 alkoxy carbonyl derivatives. However, employing the method used in the synthesis of derivatives (40) (41) and (42), the preparation of two 4-nitrophenyl esters under the standard conditions (Scheme 2.25) were not possible. Bis(4-nitrophenyl) carbonate (7) failed to react with either 2-morpholinoethanol (45) or 3-dimethylaminopropan-1-ol (46). Although we had previously been unable to prepare a 4-nitrophenyl ester from 3-picolyl alcohol, this appeared to be a different problem.



The alcohol (45) had been expected to behave in similar fashion to its longer chain homologue 6-morpholinohexan-1-ol (24). Likewise, (46) should be comparable in its reaction to 3-piperidinopropan-1-ol (35), having the same number of spacer carbons between the hydroxyl group and the tertiary amino group. The N-9 alkoxy carbonyl derivatives prepared from (45) and (46) would have provided important pharmacological comparisons to previously synthesized potential prodrugs; *O*⁶-(4-bromophenyl)-9-(6-morpholinohexyloxycarbonyl)guanine (30), *O*⁶-(4-bromophenyl)-9-(6-dimethylaminohexyloxycarbonyl)guanine (40) and *O*⁶-(4-bromophenyl)-9-(3-piperidinopropoxycarbonyl)guanine (41).

With this in mind, we decided that the investigation of an alternative route to their synthesis should be undertaken. This would also provide useful comparisons with the routes described above.

2.9 Chloroformates

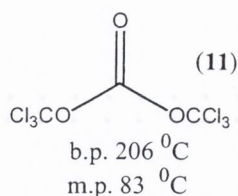
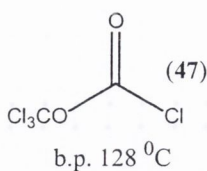
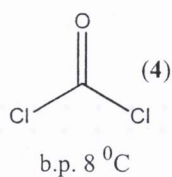
The chloride anion is an excellent leaving group, making chloroformates extremely reactive, but also very unstable. Although they are effective acylating agents, there are many reports of their reactivity resulting in low yields because of their reaction at secondary locations in the molecule being acylated.^{28,31} Such difficulties are illustrated in the literature by an overwhelming preference for acylating agents containing other active groups, such as the 4-nitrophenoxy group, which is less reactive.²⁸ Another disadvantage of chloroformates lies in the operational complexity required in their synthesis, which involves the use of highly toxic phosgene (4). Safer phosgene sources, such as the liquid trichloromethyl chloroformate (47) and the solid bis(trichloromethyl) carbonate (11), are available, but there is an understandable stigma attached to the generation of phosgene by any means.

However, many chloroformates are commercially available. *O*⁶-(4-bromophenyl)-9-(methoxycarbonyl)guanine (**1**), the lead compound for our prodrug synthesis, was originally prepared by the reaction of methyl chloroformate with PaTrin-2. This was a successful and high yielding reaction. Further preparation of N-9 alkoxycarbonyl derivatives by this route was abandoned due to the limited number of chloroformates being available on the market. Furthermore, there were difficulties encountered by others in this laboratory in the synthesis of chloroformates.

Our requirement to synthesize additional N-9 derivatives, which we had been unable to prepare by the standard routes above, suggested that we should investigate the preparation and reactions of chloroformates.

2.9.1 Chloroformate synthesis

A thorough investigation of the use of bis(trichloromethyl) carbonate (BTC) (**11**) as an important building block in organic synthesis has only occurred in recent years.²³ First prepared in 1880, its usefulness to organic chemistry appears to have been tarnished by its similarity to phosgene (**4**). As proposed by the Eckert mechanism (Scheme 2.5) BTC, also referred to as “triphosgene”, behaves as three molecules of phosgene.¹¹ A crystalline stable solid (m.p. = 80 °C, b.p. = 206 °C), BTC has been reputed to be much safer to use than phosgene and liquid trichloromethyl chloroformate (“diphosgene”).^{11,23} Trichloromethyl chloroformate (**47**) has been more commonly used as a phosgene substitute in all common phosgene reactions,³⁹⁻⁴² but as a liquid, its transport and storage still pose considerable dangers. Although BTC is a stable crystalline solid, recent surveys suggest that it is of the same toxicity as phosgene and although easier to handle, it should be used with the appropriate precautions.²³



Since the use of BTC in organic synthesis has only been reported more widely in recent years, it was the literature reports of the preparation of alkyl chloroformates using phosgene that came first to our attention. Standard procedures for the preparation of chloroformates according to Kenyon *et al.*,⁴³ as well as Nakanishi *et al.*,^{44,45} involved the simple reaction of an alcohol in ether containing one equivalent of quinoline, with an ice-cold solution of an equimolar quantity of phosgene in ether. However, repeating this procedure using a third of a mole of BTC to one mole of alcohol produced unsatisfactorily low yields of chloroformate. Due to the instability of the prepared chloroformate, subsequent reactions of this with the second nucleophile are usually carried out *in situ*. To successfully achieve this, an almost quantitative yield of chloroformate is required. It was apparent that the procedures described using phosgene would not work using BTC.

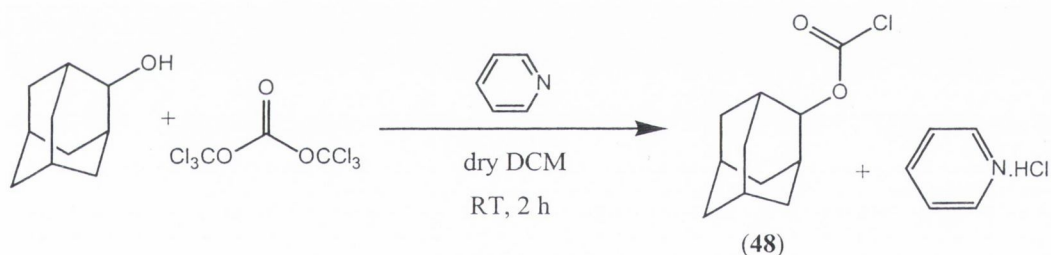
An investigation by Konakahara *et al.*⁴⁶ had found that chloroformates could be synthesized in high yield using BTC with an equivalent of Hünig's base. The synthesis involved the dropwise addition of Hünig's base in DCM to a solution of BTC and alcohol in DCM at -30 °C. Following the addition, stirring was continued at 0 °C for 3 hours, and then at 20 °C for 6 hours. Finally the mixture was refluxed for 2 hours.

However, we found that this rather involved procedure produced an unsatisfactorily low yield of chloroformate from simple alcohols. Other similar routes for the

synthesis of chloroformates were no better.⁴⁷ A more direct procedure, reproducible in high yield, was eventually found and is reported in the following section.

2.9.2 Synthesis of 9-(2-adamantyloxycarbonyl)-O⁶-(4-bromophenyl)guanine

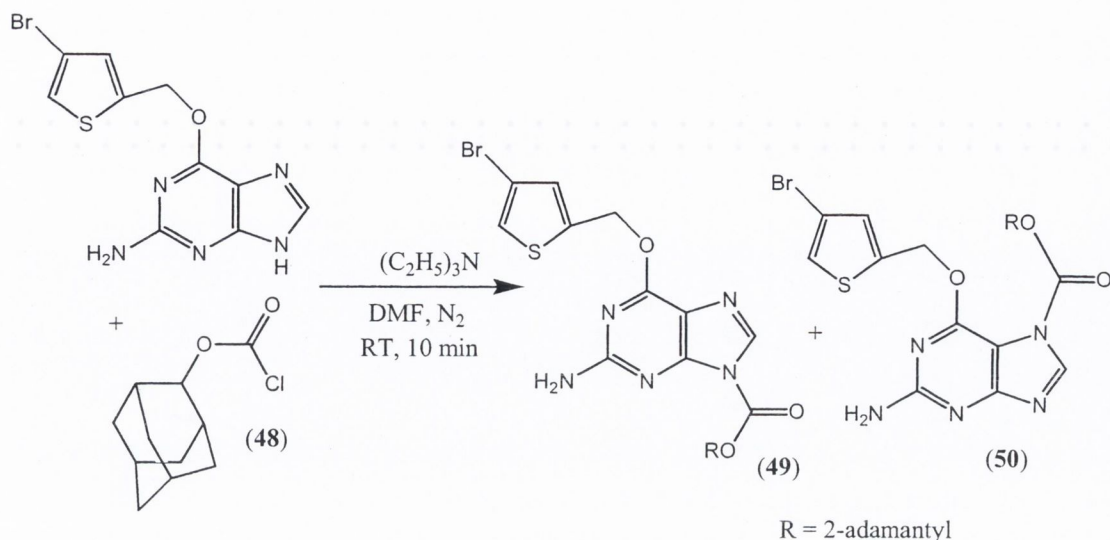
Horwell *et al.*⁴⁸ reported the synthesis of 2-adamantyl chloroformate (**48**) in quantitative yield. Repeating the procedure, a cooled solution of BTC and pyridine in dry DCM was added to an ice-cold solution of 2-adamantanol in dry DCM (Scheme 2.26). The reaction mixture was warmed to room temperature and after 2 hours, the DCM was removed and ethyl acetate added to the residue to precipitate the pyridine hydrochloride. This precipitate was collected and the solvent removed from the filtrate to give 2-adamantyl chloroformate (**48**) in 90% yield.



Scheme 2.26

The reaction was followed by infrared spectroscopy. It was possible to monitor the reaction by the disappearance of the BTC carbonyl stretching frequency at 1832 cm⁻¹ in the infrared spectrum, and the appearance of the chloroformate carbonyl at 1778 cm⁻¹. The carbonyl stretching frequencies of chloroformates are normally found between 1770 cm⁻¹ and 1780 cm⁻¹.⁴⁹

Having found it possible to synthesize this chloroformate in high yield we decided to investigate its subsequent reaction with PaTrin-2. An equivalent of 2-adamantyl chloroformate (**48**) was added to a solution of PaTrin-2 and an equimolar amount of triethylamine in DMF (Scheme 2.27).



Scheme 2.27

The reaction was monitored by TLC and was complete after 10 minutes. The solvent was removed to leave a white solid, the ^1H NMR of which showed a 50:50 mixture of the desired N-9 alkoxy carbonyl derivative (49) and the corresponding N-7 isomer (50). The N-9 isomer was isolated by flash chromatography. Partial hydrolysis to generate PaTrin-2 occurred during the process.

2.9.3 N-9 and N-7 isomers

The alkylation of O^6 -substituted guanines always results in N-9/N-7 isomeric mixtures,^{50,51} normally in the ratio of about 4:1.⁵⁰ These regio-isomers have been characterized by 2-D heteronuclear ^1H - ^{13}C correlated NMR spectroscopy.^{52,53} It is possible to distinguish between the N-9 and the N-7 isomer in their ^1H NMR spectra by the difference in the shifts of the H-8 and NH_2 signals. In the spectra of 9-(2-adamantyloxycarbonyl)- O^6 -(4-bromophenyl)guanine (49) (Fig. 2.6) and its corresponding N-7 isomer (50) (Fig. 2.7), the signal of H-8 for the N-9 isomer is shifted upfield relative to the corresponding H-8 signal for the N-7 isomer.

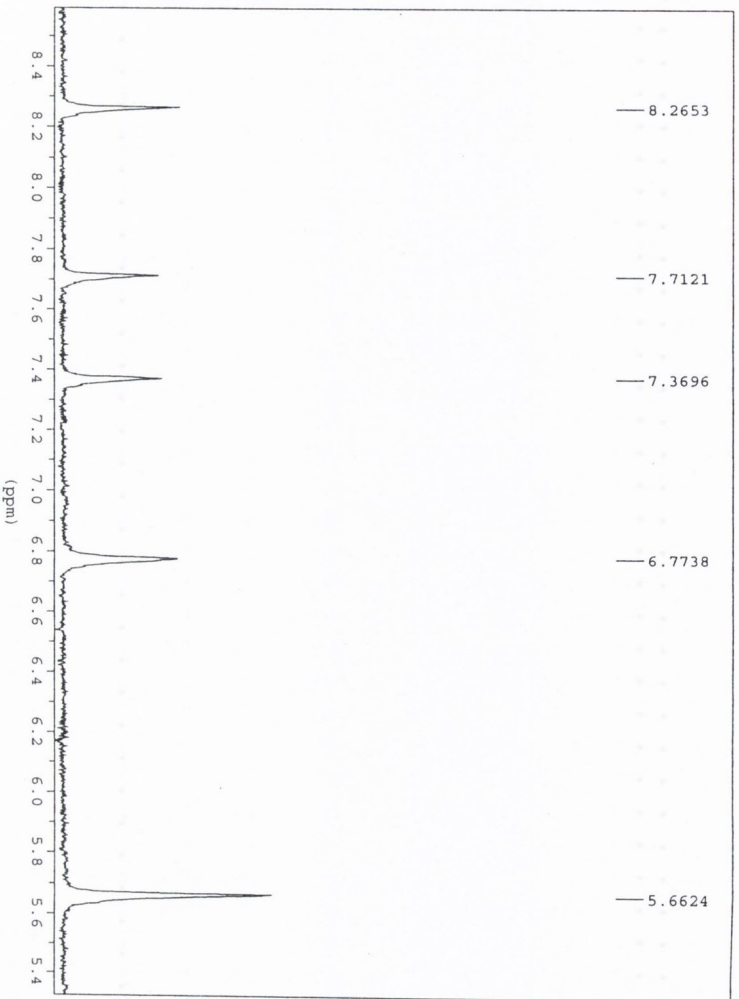


Fig. 2.6

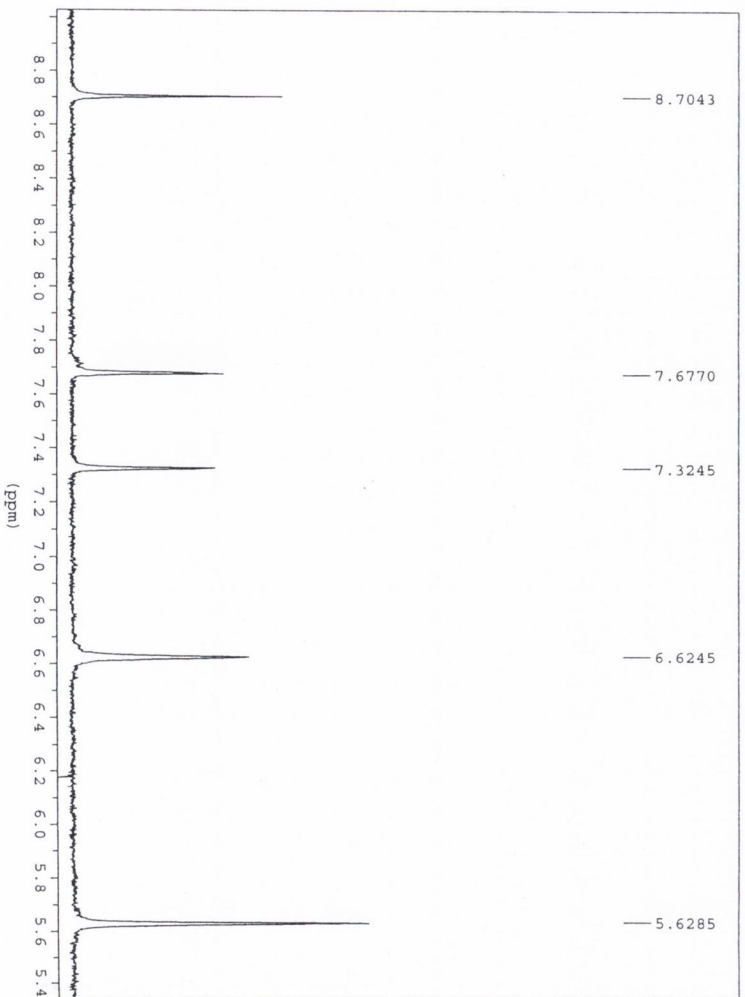


Fig. 2.7

The corresponding NH₂ signal is shifted downfield for the N-9 isomer relative to the corresponding NH₂ signal for the N-7 isomer. In a mixture, the ratio of N-9 and N-7 isomers can be determined by the integration of their respective H-8 signals.

The biological activities of ATase inactivators like PaTrin-2 (**8**) are only slightly reduced by alkyl substituents in the N-9 position.^{34,54} However, the corresponding N-7 derivatives greatly reduce their inactivating ability.⁵⁴ The cause of the reduced activity is thought to be due to the steric hindrance caused by the N-7 substituent when the molecule becomes involved with the ATase active site.⁵⁴ It also is suggested that the N-7 in *O*⁶-substituted guanines might be hydrogen bonded to a serine residue in the protein during the reaction with the ATase active site.⁵⁵

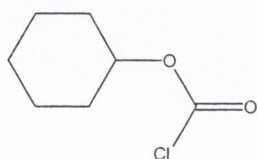
Since steric hindrance is also thought to be responsible for the smaller amount of the N-7 isomer formed in alkylation reactions, it was unusual that the reaction shown in Scheme 2.27 had resulted in a 50:50 ratio of isomers. This suggests that the N-7 position is capable of accommodating a bulky group.

Though we already had synthesized a large number of N-9 alkoxycarbonyl derivatives, it was very unusual that this was the first example of acylation at the N-7 position. There are numerous accounts in the literature of attempts to separate efficiently the desired N-9 alkyl from the N-7 isomers,⁵⁶ and there are also many reports of the development of more selective routes to N-9 alkylation.^{50,51} By using 4-nitrophenoxide as a leaving group we possibly had developed a route involving regioselective acylation.

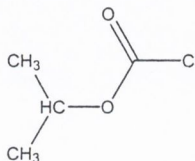
2.9.4 4-Nitrophenoxide versus chloride as a leaving group

The formation of an N-7 alkoxycarbonyl derivative in reactions of PaTrin-2 with 4-nitrophenyl esters had not been observed. In order to make a direct comparison

between 4-nitrophenoxide and chloride as leaving groups, it was necessary to compare reactions that were identical, but for the nature of the leaving group. We decided to synthesize cyclohexyl chloroformate (**51**) and isopropyl chloroformate (**52**) in order to compare their reactions with PaTrin-2, to those of the corresponding 4-nitrophenyl esters (**25**) and (**26**).



(51)



(52)

The chloroformates (**51**) and (**52**) were prepared in the same way as 2-adamantyl chloroformate (Scheme 2.26).[♦] Due to the volatile nature of (**52**)⁵⁷ it was used as a solution in ethyl acetate in its subsequent reaction with PaTrin-2. As with the reaction shown in Scheme 2.27, (**51**) and (**52**) both reacted quickly with PaTrin-2 in the presence of an equimolar amount of triethylamine. Following the removal of DMF, ¹H NMR analysis of the crude products showed the presence of traces (<10%) of the corresponding N-7 alkoxy carbonyl derivatives. ¹H NMR analysis of the crude products following an identical reaction of PaTrin-2 with the corresponding 4-nitrophenyl esters (**25**) and (**26**) showed no trace of the N-7 isomer.

2.9.5 Use of the 4-nitrophenoxy group in regioselective synthesis

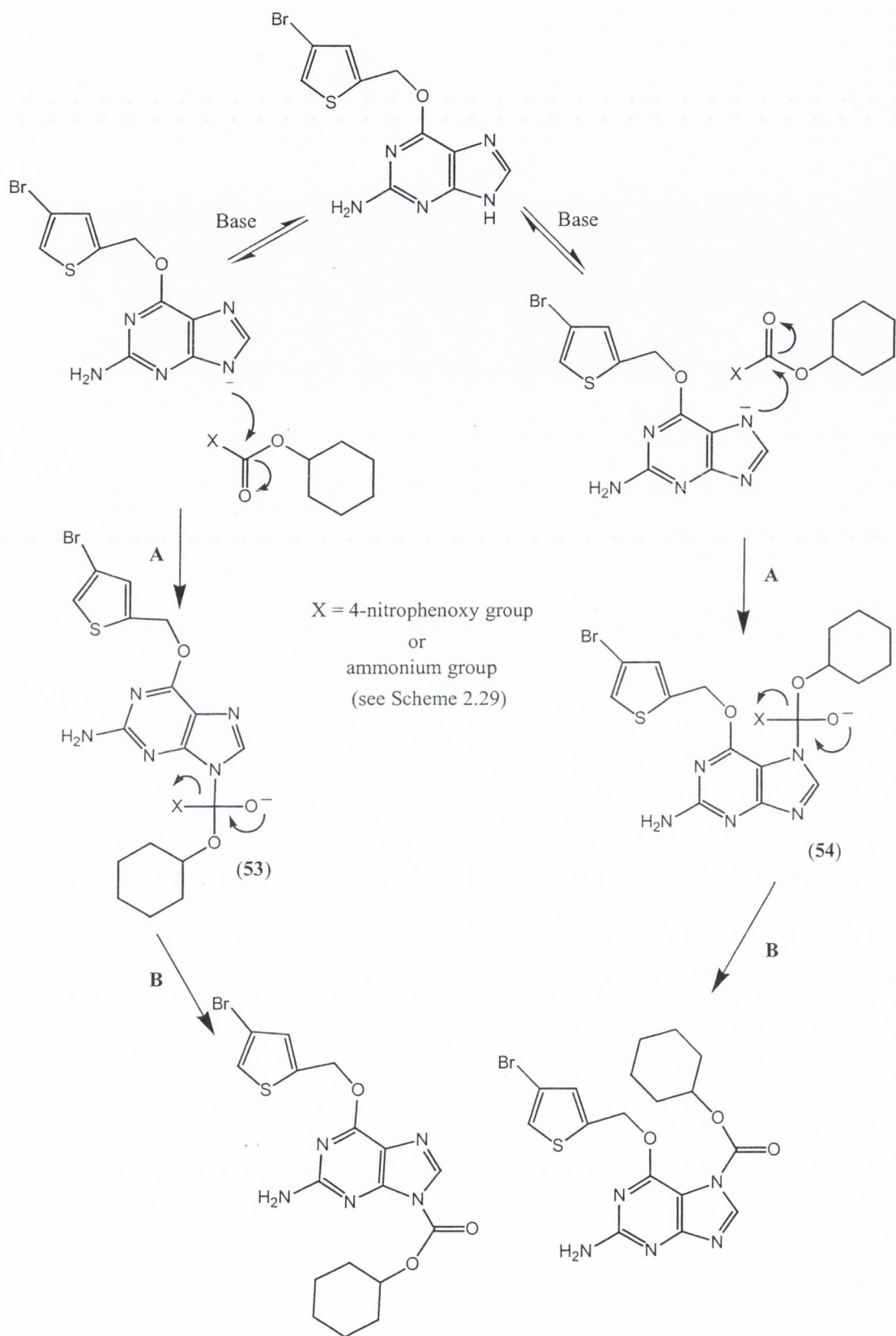
The ratio of N-9 to N-7 alkylated products obtained in a reaction has been reported to be dependent on several factors.⁵⁰ The nature of the substituent in the O⁶-position, the reaction temperature and the choice of base can all affect the ratio of isomers. By

[♦] Saunders *et al.*⁵⁷ reported the preparation of cyclohexyl and isopropyl chloroformate by a procedure involving phosgene.

varying these parameters, the regioselective synthesis of N-9 alkylguanines has been demonstrated. However, even in these reported methods some N-7 isomer was found, if only in minor amounts.⁵⁰

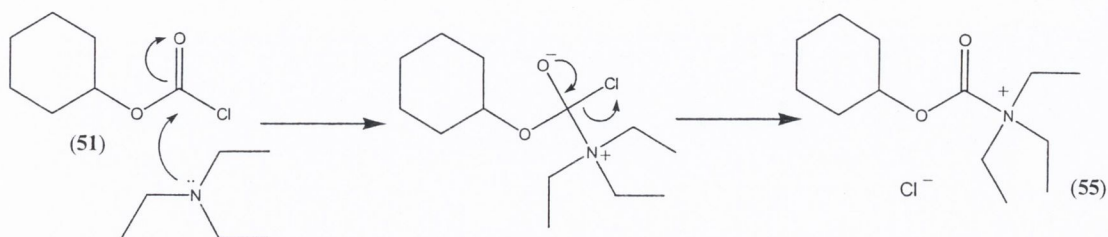
By substituting a 4-nitrophenoxy group for a chlorine we had demonstrated that alkoxycarbonylation exclusively at the N-9 position was possible. To further exploit such selective N-9 synthesis it was important to understand the factors involved in directing reaction towards the N-9 position in preference to that of the N-7. There are several plausible reasons for the apparent preference for the N-9 position and we attempted to outline the factors that had the greatest influence.

The most notable difference between the two active reagents lies in their reactivity. Comparing cyclohexyl chloroformate (**51**) with cyclohexyl(4-nitrophenyl) carbonate (**25**), we can suggest kinetic versus thermodynamic control for the difference in their reaction with PaTrin-2. A steric reason may also be linked with these. The reaction of PaTrin-2 with both (**51**) and (**25**) proceeds *via* tetrahedral intermediates (**53**) or (**54**) respectively (Scheme 2.28).



Scheme 2.28

Evidence suggests that the chloroformate (**51**) is so reactive that it reacts with triethylamine to give (**55**) (Scheme 2.29). Therefore in the intermediates (**53**) and (**54**), shown in Scheme 2.28, X is likely to be a triethylamino group.



Scheme 2.29

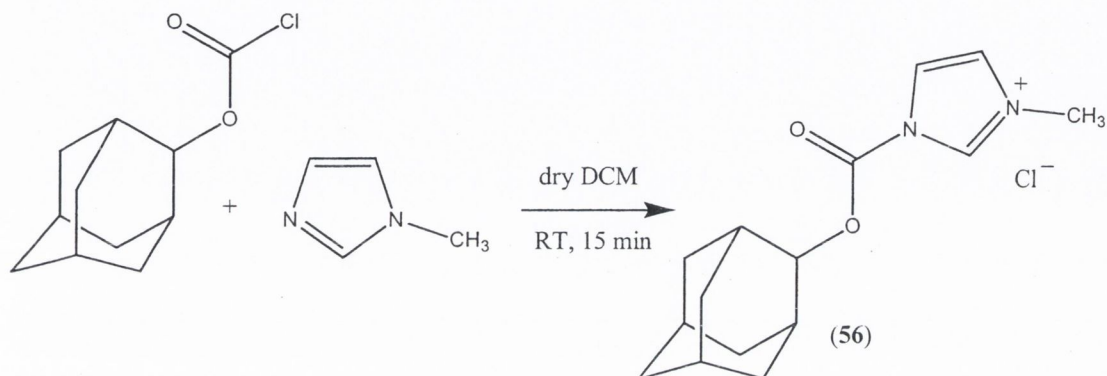
The reactive species (**55**) is quickly formed (Scheme 2.29). Subsequently, step **A** (Scheme 2.28) will be fast, and the reactive species (**55**) does not distinguish between the N-7 and N-9 anionic positions. This results in step **B**, the slower step, being the rate-controlling step. With the less reactive 4-nitrophenyl ester (**25**) the reaction occurs more slowly to generate the more thermodynamically stable intermediate (**53**). In this reaction step **A** is slower and therefore the rate-controlling step.

The failure of the 4-nitrophenyl ester (**25**) to react with the N-7 position could also be the result of steric crowding in the transition state leading to the intermediate (**54**). The intermediate (**54**), where X is the 4-nitrophenoxy group may form, but due to the steric crowding it may be thermodynamically unstable and decompose by a reaction in which PaTrin-2 is the leaving group.

To further investigate the steric and kinetic effects involved in this reaction, we decided to examine the reaction of an activated ester, similar in size to a 4-nitrophenyl ester, but of increased reactivity.

Watkins *et al.*,⁵⁸ amongst others,⁵⁹ have reported the reaction of less reactive chloroformates with *N*-methylimidazole to give acylimidazolium salts, which have proved extremely potent acylating agents. Reacting 2-adamantyl chloroformate (**48**)

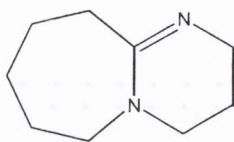
with *N*-methylimidazole we obtained the 2-adamantylmethylimidazolium salt (**56**) in quantitative yield (Scheme 2.30). If the reaction of 4-nitrophenyl esters exclusively at the N-9 position was solely due to bulk, then the reaction of (**56**) with PaTrin-2, as in Scheme 2.27, should also give only N-9.



Scheme 2.30

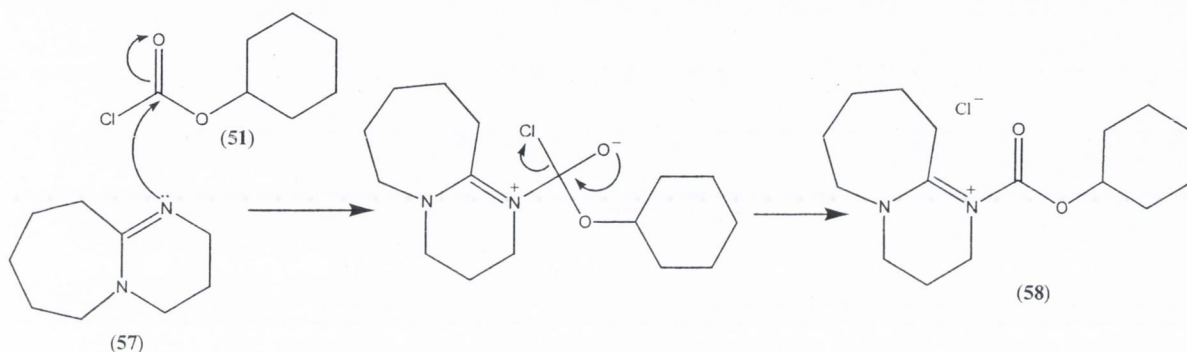
Instead the reaction of the 2-adamantylmethylimidazolium salt (**56**) with PaTrin-2 gave both the N-9 and the N-7 isomer, although in a 70:30 ratio, as opposed to the 50:50 ratio when chloride was the leaving group. This negative result indicated that steric bulk of the tetrahedral intermediate may not be the explanation of selective N-9 alkoxycarbonylation. It may be due to kinetic control of the reaction.

There was one final reaction that supported this explanation. The reactions of all chloroformates with PaTrin-2 carried out in the presence of equimolar amounts of triethylamine or Hünig's base gave a mixture of N-9 and N-7 isomers. However, when these reactions were carried out under identical conditions, but using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (**57**) as a base, reaction occurred exclusively at the N-9 position.



(57)

This result can be explained by considering the reaction of the PaTrin-2 anion with the reactive species (58). Species (58) is formed by the reaction of DBU with cyclohexyl chloroformate (51) (Scheme 2.31).



Scheme 2.31

Species (58) is expected to be less reactive than the reactive species (55) derived from triethylamine (Scheme 2.29). The greater stability arises from the distribution of the positive charge between resonance structures (Fig. 2.8).

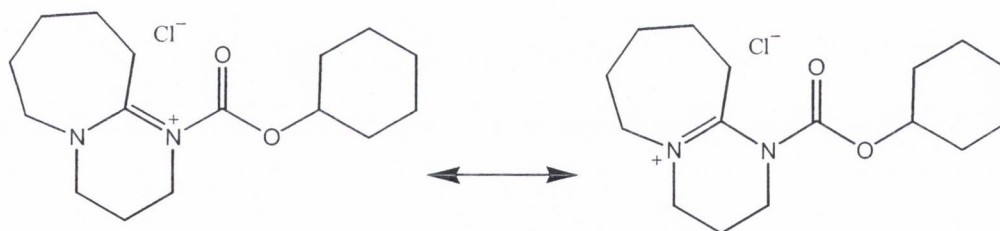


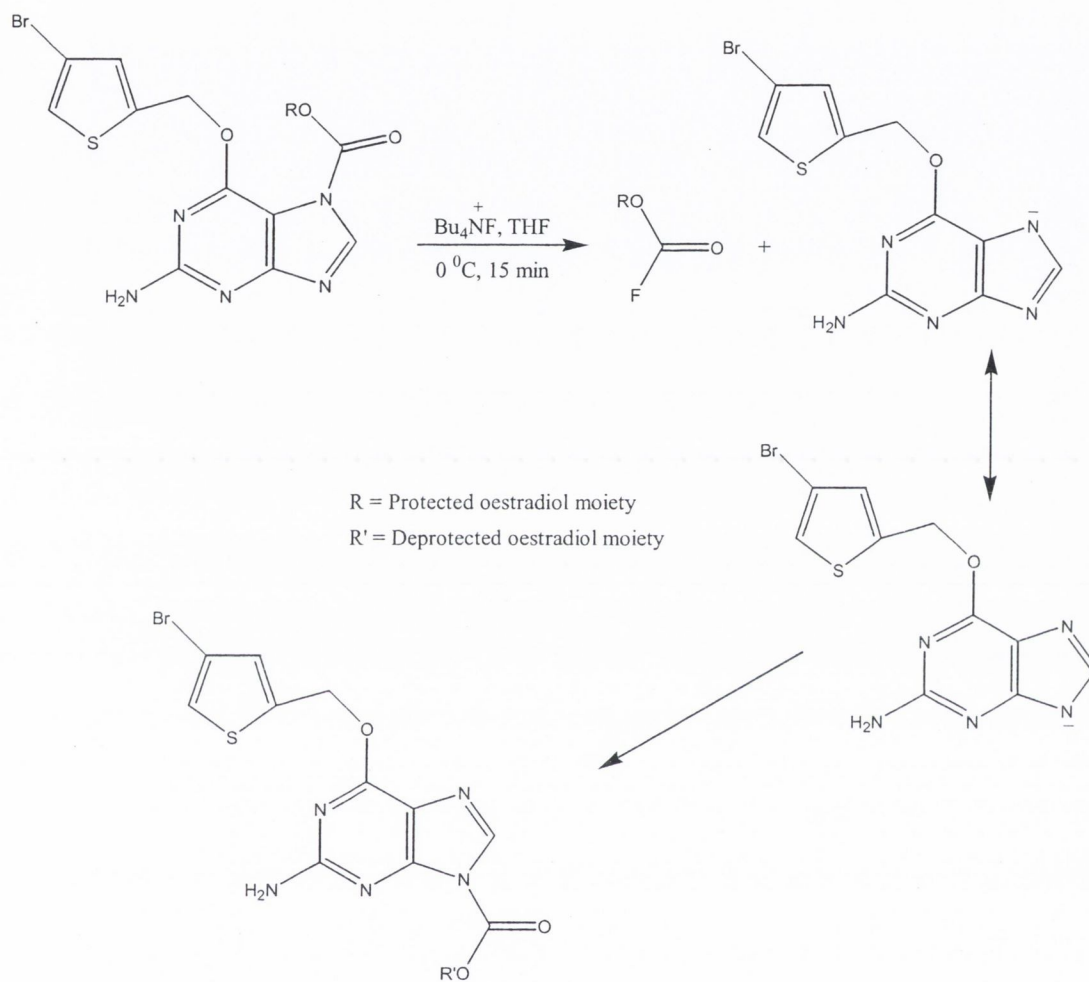
Fig. 2.8

We propose that (58) is similar in its reactivity to cyclohexyl(4-nitrophenyl) carbonate (25) and reacts with the PaTrin-2 anion to give the more thermodynamically stable N-9 product. Although a steric reason could be suggested for the failure of (58) to react with the N-7 position, the evidence suggests that these reactions are more strongly influenced by kinetic factors.

The results discussed above suggest that 4-nitrophenyl esters, being less reactive than chloroformates, allow for the synthesis of the more thermodynamically stable isomer. We have proposed that this is the N-9 isomer. This is supported by the results of a reaction that suggest the rearrangement of an N-7 isomer to give the more stable N-9 isomer.

In Section 2.11.4 we will discuss the preparation of *O*⁶-(4-bromophenyl)-9-[3-hydroxy-oestra-1,3,5(10)-triene-17 β -yl-oxycarbonyl]guanine, a PaTrin-2 derivative derived from oestradiol. However, it is instructive at this point to discuss an observation made during the course of its synthesis. Oestradiol contains both a phenolic and an alcoholic hydroxyl group. It was necessary to protect the phenolic hydroxyl group before reaction could be attempted with the alcoholic hydroxyl group. This was achieved using a *t*-butyldimethylsilyl moiety.⁶⁰ The reaction of PaTrin-2 with a chloroformate derived from this protected oestradiol moiety resulted in substantial amounts of both the N-9 and N-7 isomer. The isomers were separated by flash chromatography. Tetrabutylammonium fluoride was used to remove the protecting group from the PaTrin-2 derivatives.⁶¹ A pure sample of the N-7 alkoxycarbonyl derivative in THF at 0 °C was treated with one equivalent of a 1M solution of tetrabutylammonium fluoride in THF. The reaction was worked up and ¹H NMR analysis of the crude reaction mixture showed that the protecting group had been removed, but some hydrolysis of the derivative had occurred as traces of PaTrin-2 were also observed. More interestingly, none of the signals associated with the N-7 isomer remained. Instead only the signals associated with the N-9 isomer were observed. This rearrangement could be explained by a reaction similar to that shown in Scheme 2.32, however similar rearrangements were not observed when other N-7 alkoxycarbonyl derivatives were treated in the same way. Although the

exact mechanism for this reaction may be unknown, the result of this reaction does suggest that rearrangement occurred to give the more thermodynamically stable N-9 isomer.



Scheme 2.32

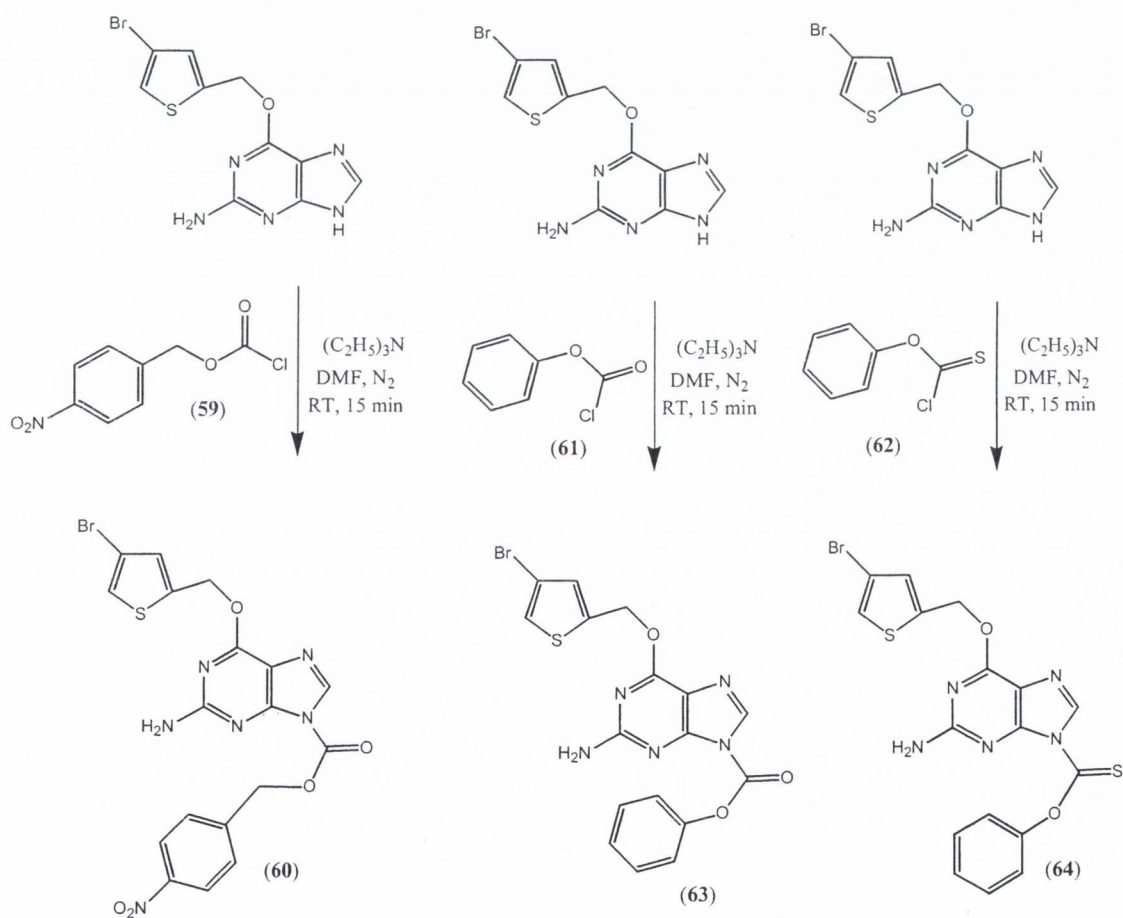
2.10 Further use of chloroformates

The reaction of PaTrin-2 with a chloroformate always results in an isomeric mixture.^{50,51} However, these reactions have the advantage of proceeding faster than those involving 4-nitrophenyl esters. In cases where the N-7 isomer is obtained only in trace amounts, the corresponding N-9 isomer can be purified in high yield.

The reaction of PaTrin-2 with the commercially available 4-nitrobenzyl chloroformate (**59**) afforded the N-9 derivative *O*⁶-(4-bromophenyl)-9-(4-nitrobenzyloxycarbonyl)-

guanine (**60**) in high yield. As with previous reactions involving chloroformates, the reaction was carried out in the presence of an equimolar amount of triethylamine and was complete within 15 minutes (Scheme 2.33). The potential targeting ability of this N-9 derivative will be discussed below in the final sections of this chapter.

The reaction of PaTrin-2 with phenyl chloroformate (**61**) and phenyl chlorothionoformate (**62**) also afforded in high yield their respective N-9 derivatives, *O*⁶-(4-bromophenyl)-9-(phoxycarbonyl)guanine (**63**) and *O*⁶-(4-bromophenyl)-9-(phoxythiocarbonyl)guanine (**64**) (Scheme 2.33). The N-9 derivative (**64**) will provide a useful pharmacological comparison with compound (**63**). Traces of the N-7 derivative were observed in each case.

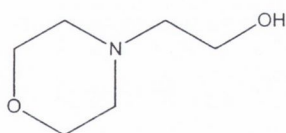


Scheme 2.33

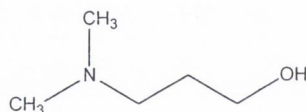
The above reactions resulted in high isolated yields of the N-9 derivatives (**60**), (**63**) and (**64**) due to a successful work-up procedure. Each of these N-9 isomers were less soluble in hot ethanol than the corresponding N-7 isomers, and so could be separated in a pure state.

2.10.1 The preparation of chloroformates from alcohols containing a tertiary amino group

The use of bis(4-nitrophenyl) carbonate (**7**) to give the 4-nitrophenyl ester intermediates and subsequently the N-9 derivatives of PaTrin-2 was successful in many cases. As pointed out previously, the process failed with two alcohols, 2-morpholinoethanol (**45**) and 3-dimethylaminopropan-1-ol (**46**). We decided to see whether the chloroformates derived from these alcohols would afford the PaTrin-2 derivatives. In addition, the reaction of chloroformates with PaTrin-2 would provide a useful comparison with the reactions involving 4-nitrophenyl esters.



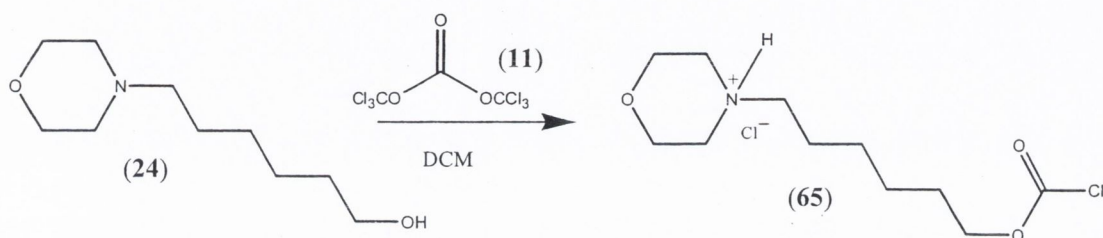
(45)



(46)

There are many procedures in the literature for the preparation of chloroformates from alcohols containing a tertiary amino group.^{62,63} However, all these syntheses involve the use of phosgene. No references to the preparation of such chloroformates using the phosgene source bis(trichloromethyl) carbonate (BTC) (**11**) were found. However, we had already established a route to the synthesis of simple chloroformates following a method reported by Horwell *et al.*⁴⁸ (Section 2.9.2). We decided to adapt this method and to use 6-morpholinohexan-1-ol (**24**) as a model.

Using a 10% excess of BTC (11), with no added base, the hydrochloride of 6-morpholinohex-1-yl chloroformate was prepared (65) (Scheme 2.34). The optimum conditions for this reaction involved the dropwise addition of an ice-cold solution of the alcohol in dry DCM to a solution of BTC (11) in DCM at less than -5°C .



Scheme 2.34

Following the addition of the alcohol, the reaction was set aside at room temperature for a further 20 minutes. Dry conditions were essential to the success of this reaction.

The subsequent reaction of the chloroformate (65) with PaTrin-2 was found to be most efficient when the chloroformate was used without purification. Attempts to isolate the chloroformate resulted in its decomposition. Therefore, following the 20 minutes at room temperature, and detection of the chloroformate by infrared spectroscopy, the DCM was removed from the reaction mixture at room temperature, under reduced pressure.

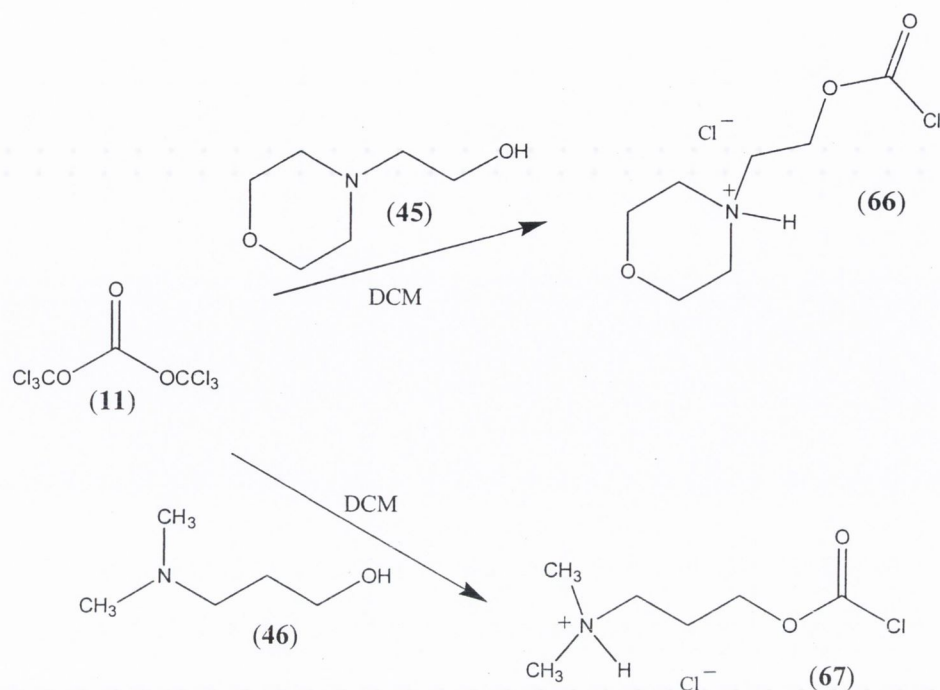
The reaction of (65) with PaTrin-2 proceeded well, requiring two equivalents of triethylamine. The N-9 derivative O^6 -(4-bromophenyl)-9-(6-morpholinohexyloxycarbonyl)guanine (30) was obtained in high yield, identical with the sample prepared earlier (Section 2.6.2, pg 66). However, trace amounts of the N-7 isomer made the purification of the N-9 isomer difficult. Similar difficulties in purifying the N-9 derivative were found when the above procedures were repeated but using 6-dimethylaminohexan-1-ol (34). The product, O^6 -(4-bromophenyl)-9-(6-dimethylaminohexyloxycarbonyl)guanine (40), which had previously been prepared

using bis(4-nitrophenyl)carbonate (**7**), decomposed completely when separation was attempted by flash chromatography. The N-9 derivative (**30**) also decomposed on attempting purification by chromatography.

The synthesis and purification of N-9 alkoxycarbonyl derivatives containing a tertiary amino group are thus more readily achieved using bis(4-nitrophenyl) carbonate (**7**) as starting material. However, it was still important to investigate the reactions of 2-morpholinoethanol (**45**) and 3-dimethylaminopropan-1-ol (**46**) with BTC (**11**), and the subsequent reaction of the corresponding chloroformates with PaTrin-2.

2.10.2 Failure of chloroformates to react with PaTrin-2

The procedure outlined above for the synthesis of chloroformates was repeated for both 2-morpholinoethanol (**45**) and 3-dimethylaminopropan-1-ol (**46**) (Scheme 2.35). The corresponding chloroformates (**66**) and (**67**) were synthesized as hydrochlorides, and characterized by infrared spectroscopy. The infrared carbonyl stretching frequencies of (**66**) and (**67**) were observed to be 1756 cm^{-1} and 1758 cm^{-1} respectively. However, no products were obtained in the subsequent reactions of both (**66**) and (**67**) with PaTrin-2.



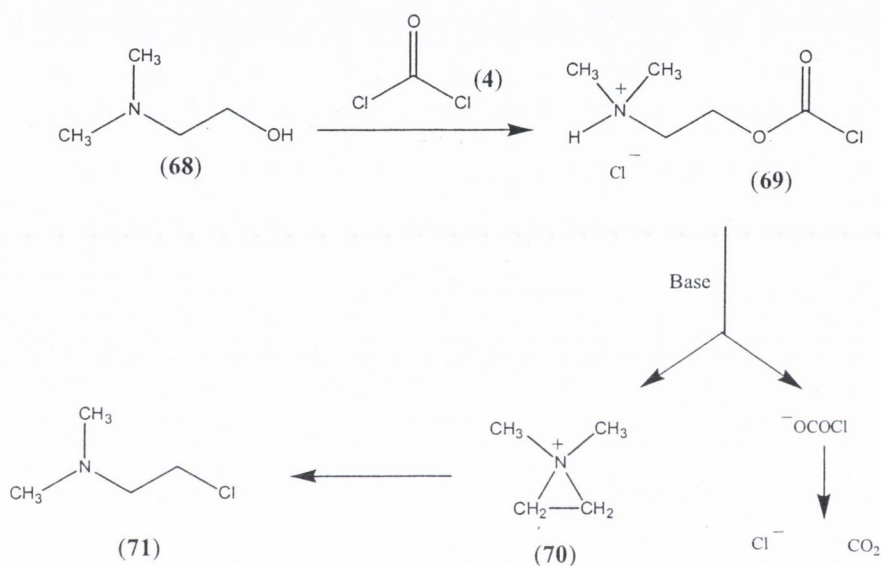
Scheme 2.35

Infrared spectroscopy had indicated the synthesis of both chloroformates. With such reactive species present, it was unusual not to observe some reaction with PaTrin-2. The reaction of 6-morpholinohex-1-yl chloroformate (65) with PaTrin-2 had been high yielding. This would suggest that the chloroformate was synthesized in almost quantitative yield. Therefore it was evident that the procedure for its synthesis was efficient. We varied the reaction conditions for the synthesis of (66) and (67), but to no avail. In all reactions the presence of these chloroformates was detected, but no subsequent reaction with PaTrin-2 was observed.

2.10.3 Problems with chloroformates containing a tertiary amino group

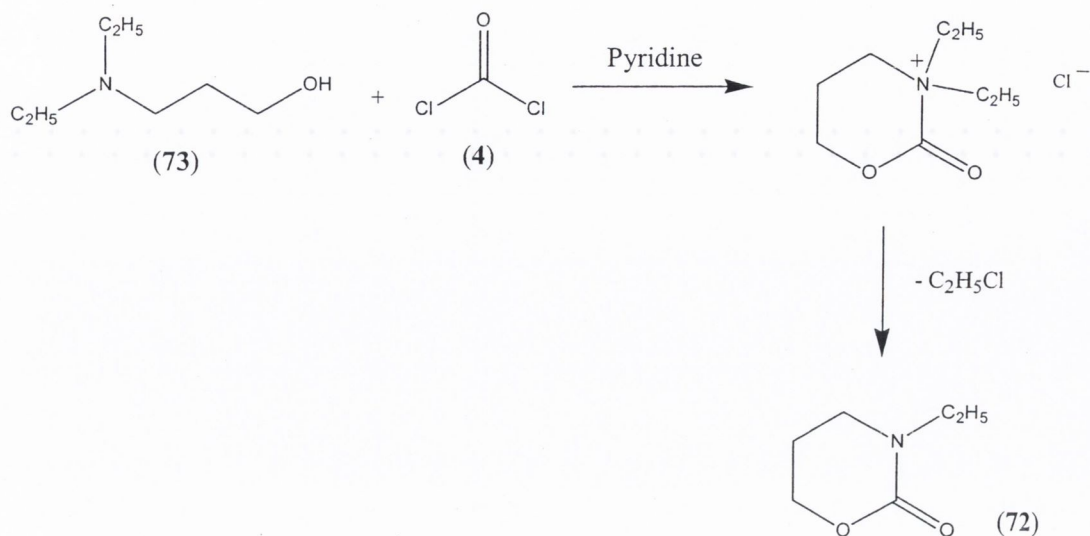
In the literature, problems encountered in the synthesis of chloroformates derived from alcohols that contain a tertiary amino group are reported.^{62,63} Murdock⁶² suggested that these chloroformates could undergo one of two types of intramolecular reaction. He reported the reaction of 2-dimethylaminoethanol (68) with phosgene (4)

to give the corresponding chloroformate (69), which was characterized by infrared spectroscopy (Scheme 2.36). Upon the addition of base, the amino group became free to participate in an intramolecular reaction to form an ethylenimmonium intermediate (70). This was attacked by a chloride anion, resulting in the major product of this reaction being 2-dimethylaminoethyl chloride (71).



Scheme 2.36

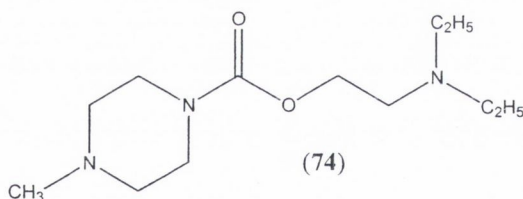
The second intramolecular reaction proposed by Murdock⁶² resulted in the formation of an oxazolidinone. He suggested that this was the favoured reaction of chloroformates derived from branched dialkylamino alcohols. He also reported the synthesis of an oxazinone (72) in low yield from 3-diethylaminopropan-1-ol (73) (Scheme 2.37).



Scheme 2.37

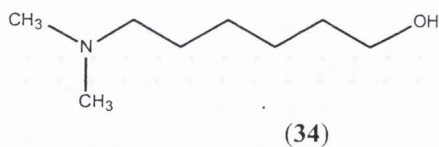
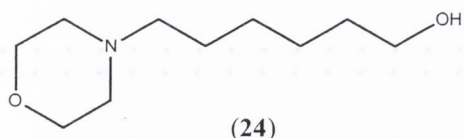
He did not observe a similar reaction with 3-dimethylaminopropan-1-ol (46), whose chloroformate (67) we had prepared above (Section 2.10.2).

Angier *et al.*⁶³ examined the reactions of a large number of basic alcohols whilst investigating the synthesis of analogues of the potent antiviral agent 2-diethylaminoethyl 4-methylpiperazine-1-carboxylate (74).

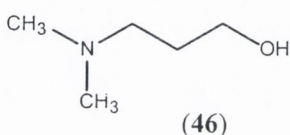
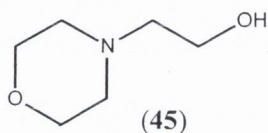


They reported that procedures involving chloroformates were only successful if the amino and the hydroxyl group in the alcohol were separated by three or more atoms. However, they found that this limitation did not exist if the amino group was part of an aromatic or alicyclic ring system.

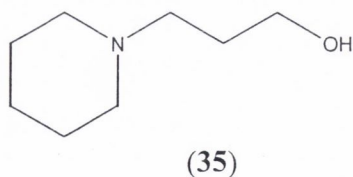
We compared these findings with our own. No problems had been encountered in the reaction of PaTrin-2 with the chloroformates derived from either 6-morpholinohexan-1-ol (24) or 6-dimethylaminohexan-1-ol (34) (Section 2.10.1).



These alcohols have six atoms between the amino and the hydroxyl group, resulting in the synthesis of stable chloroformates. Our problems had been encountered with 2-morpholinoethanol (**45**) and 3-dimethylaminopropan-1-ol (**46**).



If Angier *et al.*⁶³ were correct both compounds should form effective chloroformates. The alcohol (**45**), although having only two atoms between the amino and the hydroxyl group should produce a stable chloroformate as the amino group is in a rigid position within an alicyclic ring. Similarly, (**46**) which has three atoms between the amino and the hydroxyl group would be expected to form a stable chloroformate. It had been possible to prepare *O*⁶-(4-bromophenyl)-9-(3-piperidinopropoxycarbonyl)-guanine (**41**) from 3-piperidinopropan-1-ol (**35**).

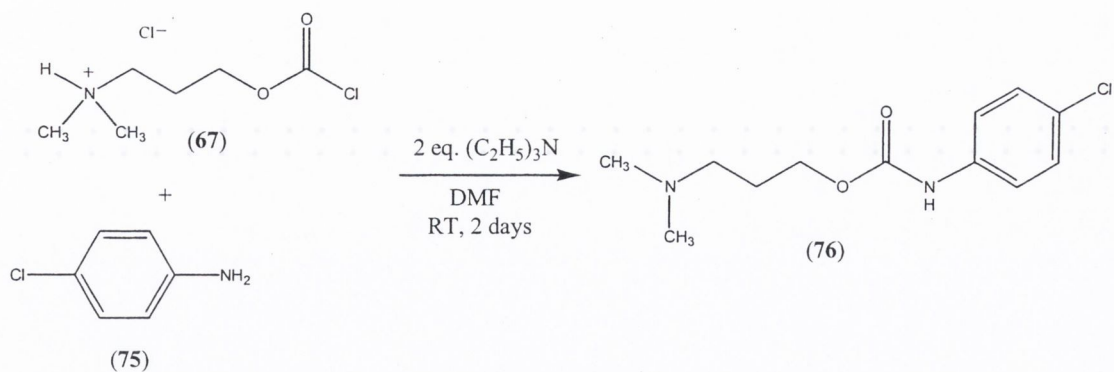


The reaction with PaTrin-2 was successful when carried out with either the corresponding 4-nitrophenyl ester (as shown in Scheme 2.25) or chloroformate. Alcohol (**35**) only differs from (**46**) because its amino group is part of a heterocyclic ring.

The failure of the 3-dimethylaminoprop-1-yl chloroformate (**67**) to react with PaTrin-2 was even more surprising, as Angier *et al.*⁶³ reported the successful use of 3-dimethylaminopropan-1-ol (**46**). However, they had synthesized their antiviral analogue by a transesterification reaction involving ethyl 4-methylpiperazine-1-carboxylate and (**46**), and not by using a procedure involving the corresponding chloroformate.

The evidence from the literature suggested that 2-morpholinoethyl chloroformate (**66**) and 3-dimethylaminoprop-1-yl chloroformate (**67**) should not undergo intramolecular reactions. These chloroformates were predicted to be stable enough to undergo subsequent reaction with a nucleophile. Therefore it was reasonable to suggest that the problem was associated with PaTrin-2.

Based on this theory, we observed the reaction of the hydrochloride of 3-dimethylaminoprop-1-yl chloroformate (**67**) with an alternative nucleophile. The primary amine, 4-chloroaniline (**75**) was chosen to test the reactivity of the chloroformate (**67**). The procedure was repeated under identical conditions to those involving PaTrin-2. The amine (**75**) was treated with the chloroformate (**67**), using two equivalents of triethylamine, to give the desired product (**76**) in 50% yield (Scheme 2.38). The optimum reaction time was found to be 2 days. However, the carbamate (**76**) slowly decomposed on standing.

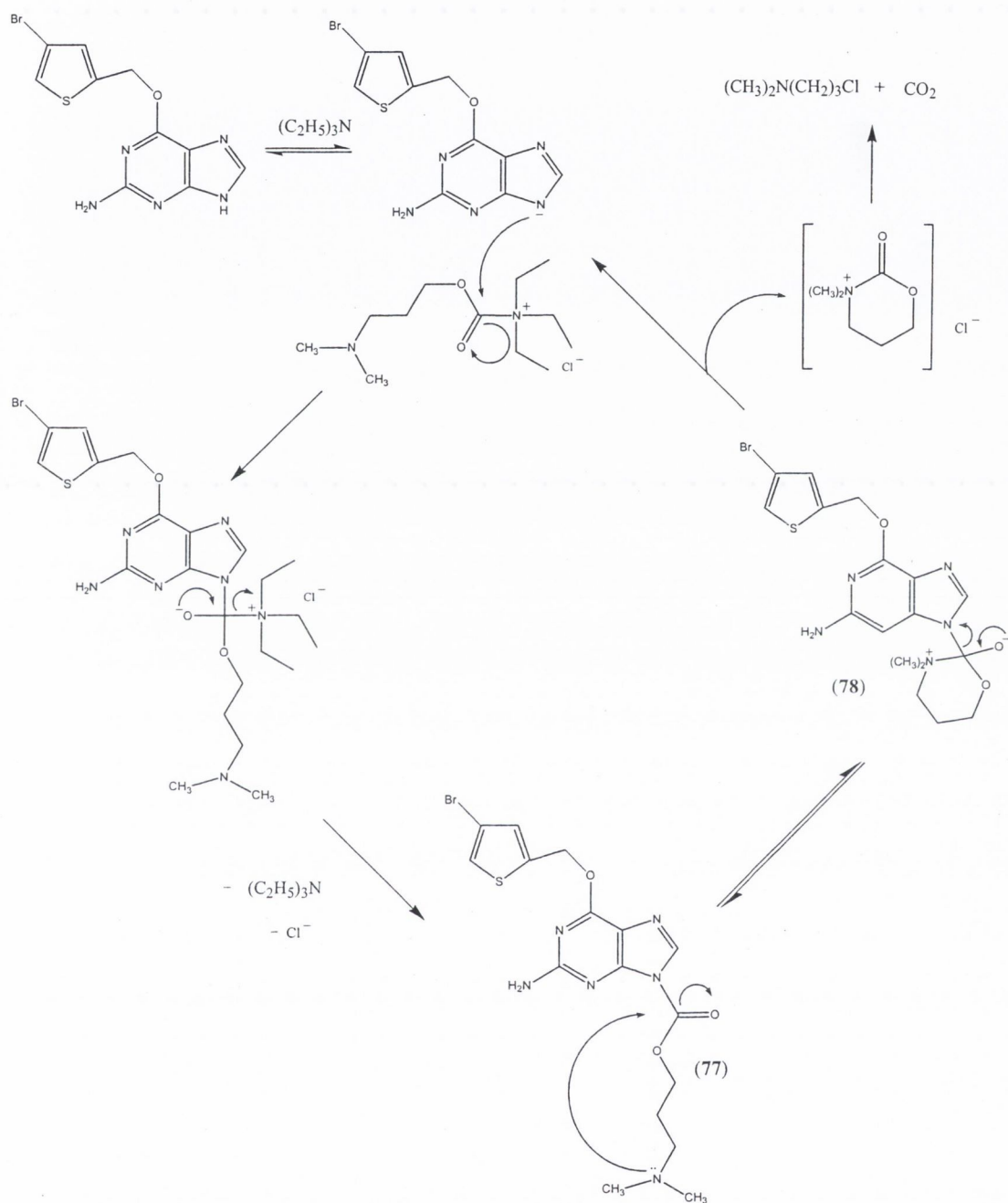


Scheme 2.38

Nevertheless, the success of this reaction demonstrated that the chloroformate (67) had been prepared in a reasonable yield by the synthetic procedure shown in Scheme 2.35. Therefore, since the chloroformate (67) was capable of reacting with an alternative nucleophile, why had it failed to give a product with PaTrin-2? Does the instability of (76) point to the answer?

We believe that O^6 -(4-bromophenyl)-9-(3-dimethylaminopropoxycarbonyl)guanine (77) is formed, but that it readily decomposes. The decomposition reaction is driven by the tendency of the PaTrin-2 anion to act as a leaving group (Scheme 2.39). The intermediate (78), which involves a six-membered ring, can either return to (77) or decompose to PaTrin-2, and eventually 3-dimethylaminopropyl chloride and carbon dioxide. We attempted to synthesize (77) as a salt, hoping that this would result in a more stable N-9 derivative. However, these attempts failed.

This was not the first time we had observed PaTrin-2 to behave as a leaving group. In the course of our investigation of basic alcohols we had observed a similar reaction involving an N-9 derivative derived from 1-dimethylaminopropan-2-ol.

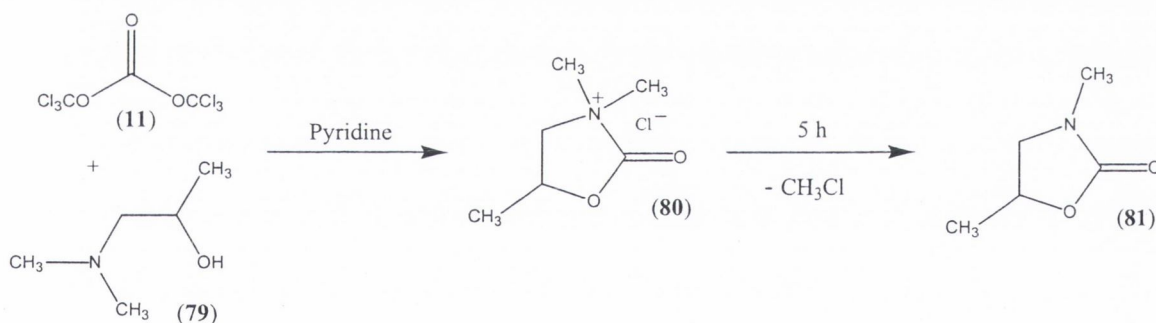


Scheme 2.39

2.10.4 *O*⁶-(4-Bromophenyl)-9-(2-dimethylaminoisopropoxycarbonyl)guanine

It has been suggested that the intramolecular reaction favoured by chloroformates derived from branched dialkylamino alcohols results in the formation of oxazolidinones (see Scheme 2.40).⁶² This reaction proceeds *via* an *N*-acylammonium salt, which is a powerful acylating agent. We decided to examine the reaction of an *N*-acyl ammonium salt with PaTrin-2 in the hope of preparing an example of a branched *N*-9 derivative for pharmacological analysis.

Murdock⁶² thoroughly investigated the reaction of 1-dimethylaminopropan-2-ol (**79**) with phosgene to give the corresponding *N*-acylammonium salt (**80**). This potent acylating agent was found to be short lived, decomposing within a few hours to give 3,5-dimethyl-2-oxazolidinone (**81**). We repeated the procedure for the preparation of (**80**) but using bis(trichloromethyl) carbonate (BTC) (**11**) (Scheme 2.40).



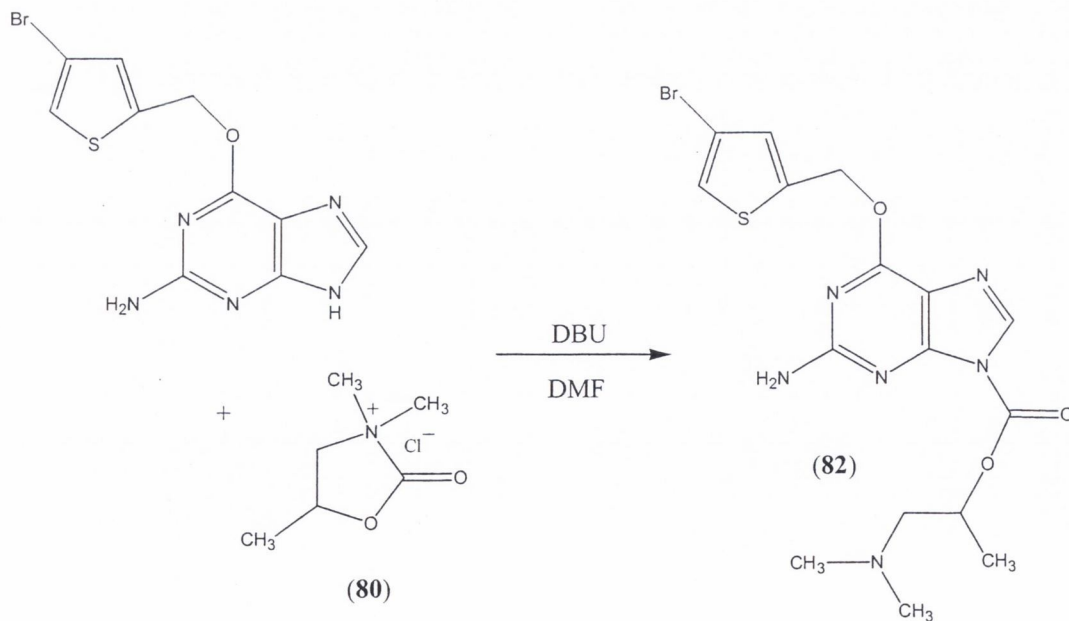
Scheme 2.40

An ice-cold solution of the alcohol (**79**) and pyridine in dry DCM were added dropwise to a solution of BTC (**11**) in DCM at -40°C . The reaction was then set aside at room temperature for 2 hours. The precipitate was collected and washed with DCM to remove the pyridine hydrochloride in which this hydrochloride was soluble. The remaining precipitate (**80**) was found to be extremely hygroscopic and so was kept under vacuum until its subsequent use within several hours. The compound (**80**) was not obtained in reactions carried out on less than a 5 mmol scale due to the

hygroscopic nature of pyridine hydrochloride. The presence of water caused the hydrolysis of the product. The *N*-acylammonium salt (**80**) was characterized by infrared spectroscopy. The carbonyl stretching frequency in the infrared spectrum was observed at 1845 cm^{-1} . When the *N*-acylammonium salt (**80**) decomposed, this peak disappeared.

The literature reports (**80**) to be an excellent acylating agent.⁶² The reaction of (**80**) with aniline is complete within a couple of minutes, giving the corresponding carbamate in high yield.⁶²

The reaction of PaTrin-2 with the *N*-acyl ammonium salt (**80**) also proceeded quickly (Scheme 2.41). However, the reaction was not high yielding. From ^1H NMR analysis of the reaction mixture following the removal of solvent, the reaction was observed to give 40% yield of the *N*-9 alkoxy carbonyl derivative (**82**) with DBU as a base. Using sodium hydride the reaction resulted in a 25% yield. No reaction was observed using triethylamine.



Scheme 2.41

The optimum conditions for the preparation of (82) involved the addition of (80) to an ice-cold solution of PaTrin-2 and base in DMF. The reaction was set aside at room temperature for 15 minutes. Following the removal of the solvent, the N-9 alkoxy carbonyl derivative present in the reaction mixture was characterized by ^1H NMR (Fig. 2.9). The product was distinguished from the unreacted PaTrin-2 in the mixture by the positions in the ^1H NMR spectrum of their H-8 and NH_2 signals. The signal of H-8 for the N-9 derivative was shifted downfield to 8.34 ppm (H-8 signal for PaTrin-2; 7.85 ppm). Similarly the NH_2 signal of the N-9 derivative also appeared further downfield at 6.91 ppm (NH_2 signal for PaTrin-2; 6.34 ppm).

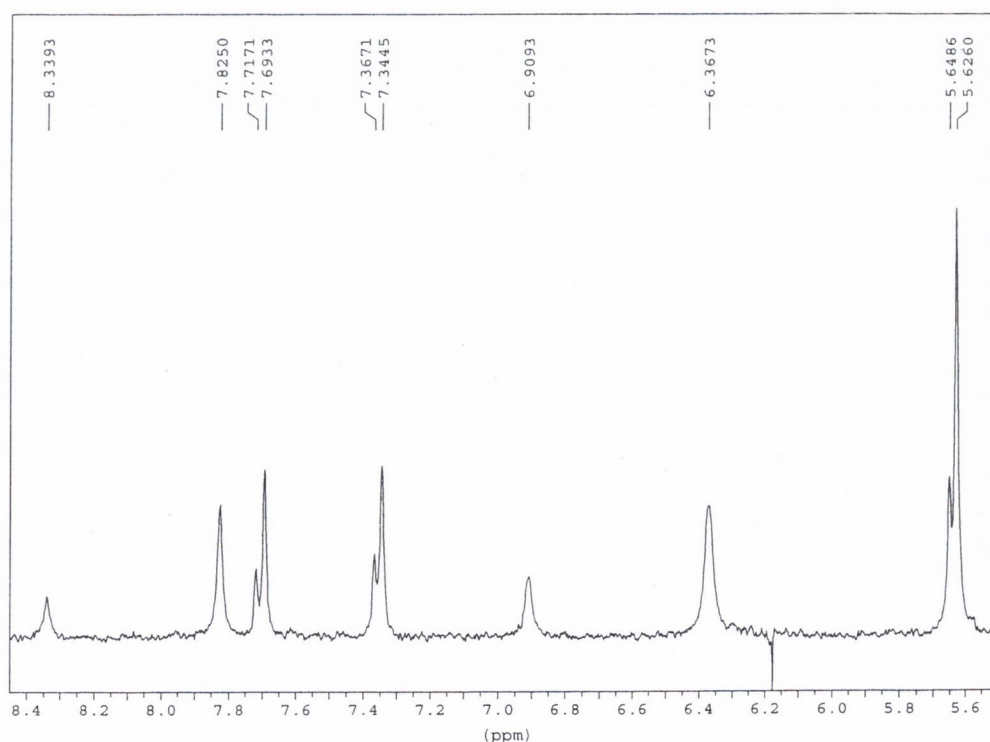
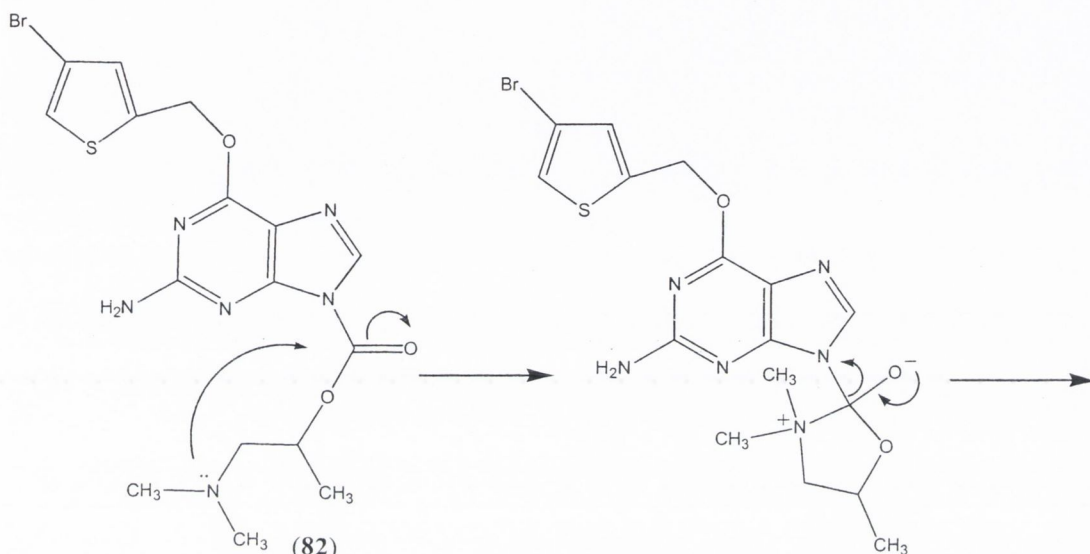


Fig. 2.9

Although the N-9 alkoxy carbonyl derivative was present its separation from the reaction mixture was not possible. All separation techniques attempted resulted in the total decomposition of (82). We proposed that it decomposed by a route similar to that

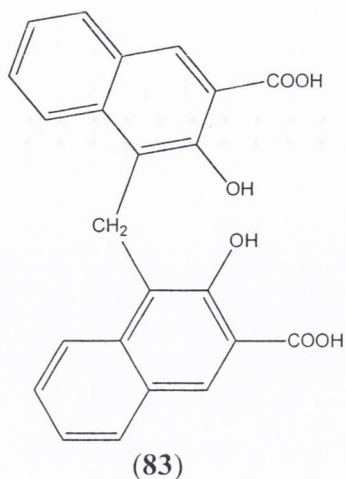
shown in Scheme 2.39, where the lone pair of electrons on the nitrogen attacks the carbonyl group resulting in the PaTrin-2 anion becoming a leaving group (Scheme 2.42).



Scheme 2.42

Unstable basic chloroformates are stabilised as their hydrochlorides (Sections 2.10.2 and 2.10.3). Therefore, in an effort to prevent the intramolecular reaction shown in Scheme 2.42, we attempted to synthesize the hydrochloride of (82). The reaction of the *N*-acyl ammonium salt (80) with PaTrin-2 was carried out in the absence of base. The reaction proceeded well and the N-9 derivative (82) was again produced in approximately 40% yield. However, attempts to separate the hydrochloride of (82) also failed.

The use of pamoic acid (4,4'-methylenebis(3-hydroxy-2-naphthoic acid)) (83) has been reported to afford insoluble crystalline salts, which facilitate the separation of compounds from otherwise inseparable mixtures.⁶⁴



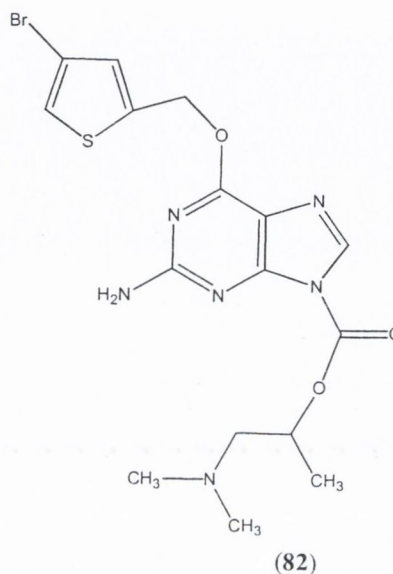
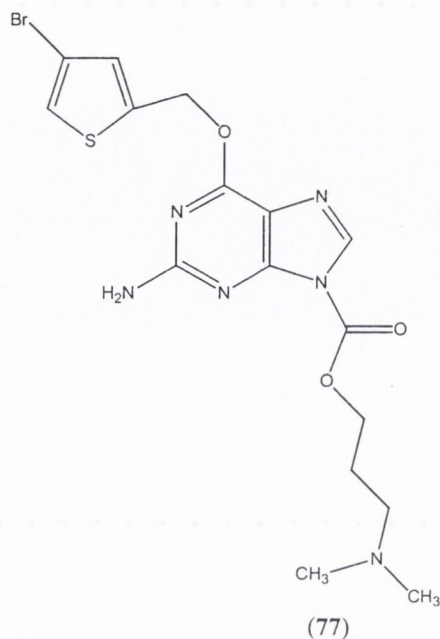
An appropriate quantity of pamoic acid was added to a solution of the reaction mixture in DMF and allowed to stir at room temperature for 20 minutes. Following the removal of the solvent, the ^1H NMR spectrum showed the presence of a pamoate salt. However, the NMR of this mixture showed none of the signals we associate with the N-9 substituted PaTrin-2. Hydrolysis of the N-9 side chain had occurred. Subsequent attempts to use citric acid for the same purpose also resulted in the cleavage of the N-9 chain from the PaTrin-2 moiety.

Although we had no evidence for the formation of O^6 -(4-bromophenyl)-9-(3-dimethylaminopropoxycarbonyl)guanine (77) discussed in Section 2.10.3, it is possible that failure to isolate either it or (82) is the result of intramolecular decomposition with the PaTrin-2 anion behaving as a leaving group.

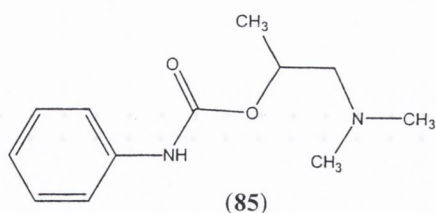
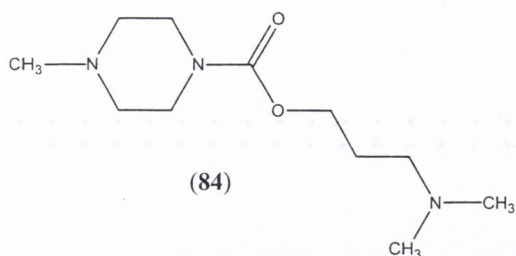
2.10.5 PaTrin-2 as a leaving group – a summary

Angier *et al.*⁶³ reported the failure of certain chloroformates derived from alcohols containing a tertiary amino group to undergo a subsequent reaction with a second nucleophile (Section 2.10.3). Similarly, Murdock⁶² reported the types of intramolecular reactions these chloroformates could undergo. However, the findings of both these reports do not explain our failure to prepare O^6 -(4-bromophenyl)-9-(3-

dimethylaminopropoxycarbonyl)guanine (77) or to isolate *O*⁶-(4-bromophenyl)-9-(2-dimethylaminoisopropoxycarbonyl)guanine (82).



We proposed that both compounds are formed, but that the failure to obtain samples of either of these N-9 alkoxy carbonyl derivatives is due to decomposition resulting from an attack by the tertiary amino group on the carbonyl group (as illustrated by Scheme 2.39 and Scheme 2.42 respectively). However, Angier *et al.*⁶³ has reported the synthesis of 3-dimethylaminopropyl 4-methylpiperazine-1-carboxylate (84), containing a side chain derived from 3-dimethylaminopropan-1-ol (46), and Murdock⁶⁴ reported the synthesis of a carbamate (85) from the reaction of aniline with a *N*-acyl ammonium salt derived from 1-dimethylaminopropan-2-ol (79). Both appear to be stable.

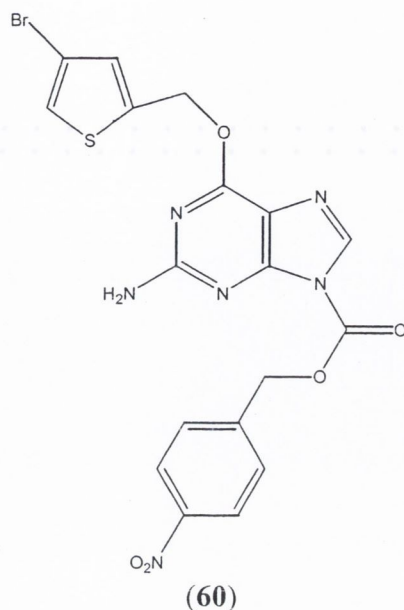


Comparing the N-9 derivatives (77) and (82) with the above compounds, it is strange that (84) and (85) are stable compounds, but that the N-9 derivatives of PaTrin-2 are not. The explanation of the instability of the N-9 derivatives (77) and (82) must be associated with PaTrin-2. It is possible that (84) and (85) do experience intramolecular attack by their amino group on their carbonyl group, but that it is a reversible reaction. However, when the corresponding reaction occurs in the N-9 derivatives (77) and (82), the PaTrin-2 anion leaves and the reaction is irreversible.

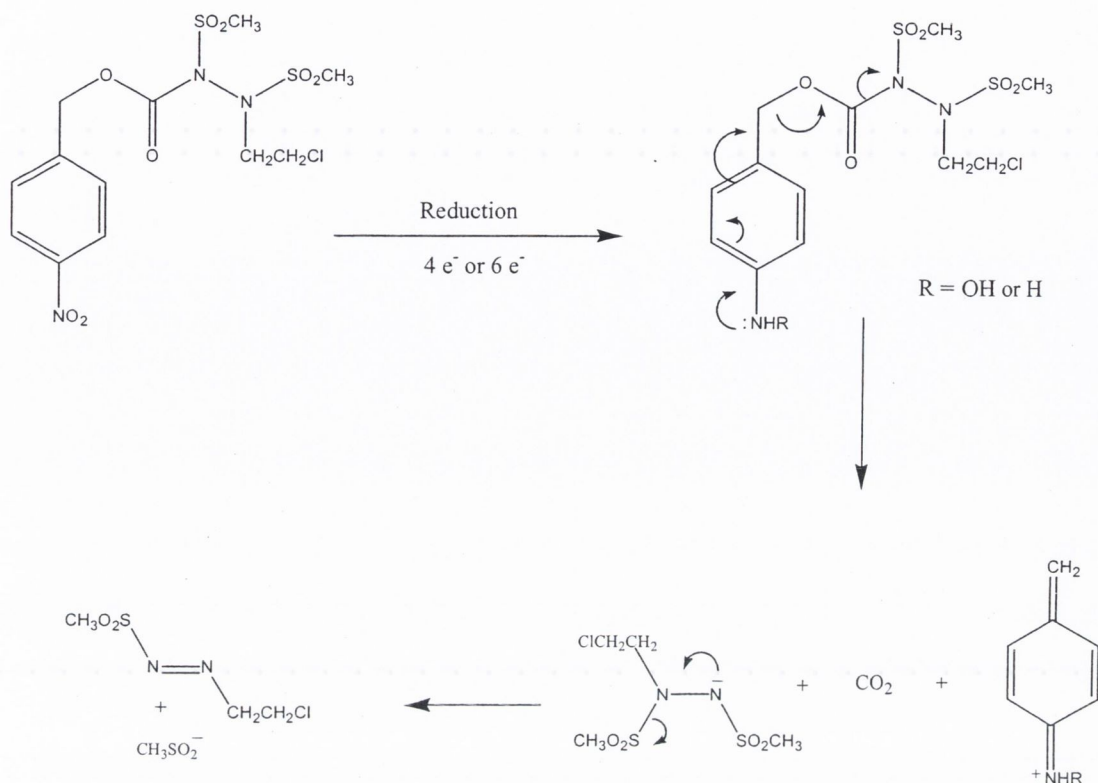
It is evident from the reactions discussed above, that reaction with PaTrin-2 is very different to similar reactions involving other compounds. It is likely that these differences result from the unique electronic properties of the purine ring system, with its size and shape also being factors that help to explain the reactions of PaTrin-2.

2.11 N-9 Alkoxycarbonyl groups as targeting agents

In Chapter 1 (Section 1.9, pg 33) we discussed the synthesis of prodrugs with targeting ability. To increase the targeting ability of our ATase inactivator we discussed exploiting some of the properties that differentiate tumour cells from normal cells. In Section 2.10 we detailed the synthesis of *O*⁶-(4-bromophenyl)-9-(4-nitrobenzyloxycarbonyl)guanine (60). Even this simple N-9 derivative could potentially have targeting ability.



Tumour tissue is generally somewhat more acidic than normal tissues. The lower pH results from the fact that solid tumours contain regions of hypoxia.^{65,66} These oxygen-deficient cells have a greater reliance on anaerobic respiration, which changes glucose to lactic acid in abundance, so contributing to the increased acidity. It has been suggested that tumour hypoxia could be exploited.⁶⁵⁻⁶⁷ There are many reports of the design of hypoxia-selective cytotoxic agents that may be activated to generate the cytotoxic agent by reduction at the reduced oxygen levels in these tumours.⁶⁵⁻⁶⁷ This mechanism is commonly referred to as bioreductive activation. Much effort has been devoted to the design and synthesis of nitro-containing agents as hypoxia-selective cytotoxic agents. Shyam *et al.*⁶⁵ recently reported the synthesis of several classes of prodrugs of the chemotherapeutic agent 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine. One of these, a nitrobenzyl-containing derivative, was shown to be quickly activated under hypoxic conditions to generate the active alkylating agent (Scheme 2.43).



The induced lability of the carbamate moiety is considered to be caused by the reduction of the electron-withdrawing nitro group to an electron-releasing hydroxylamino or amino group. This results in the lone pair assisted expulsion of carbon dioxide and the active agent.⁶⁵

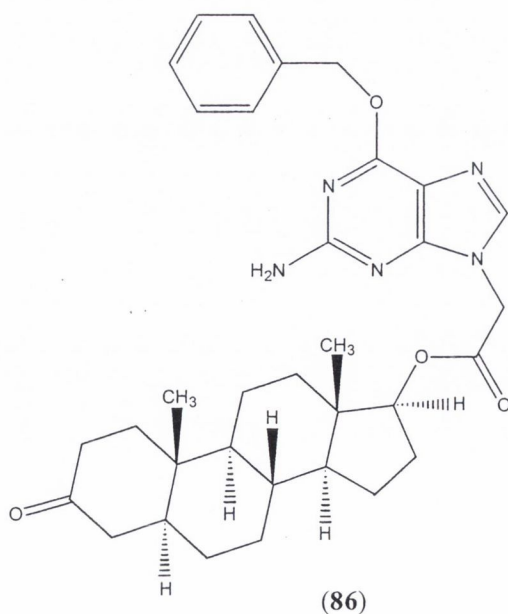
The N-9 derivative *O*⁶-(4-bromophenyl)-9-(4-nitrobenzyloxycarbonyl)guanine (**60**) will also be pharmacologically evaluated to observe whether it can release PaTrin-2 in a similar fashion. Although, the lability of the carbamate moiety of (**60**) could be induced by such a reductive mechanism, it is possible that some of the other potential prodrugs we have synthesized could also be hydrolyzed selectively at the lower pH in hypoxic tumours. This could also provide a useful targeting strategy.

In the final sections of this chapter we will discuss the use of steroid hormones as carrier molecules for PaTrin-2. There are numerous reports in the literature that suggest that linking antineoplastic agents with steroid hormones provides a useful

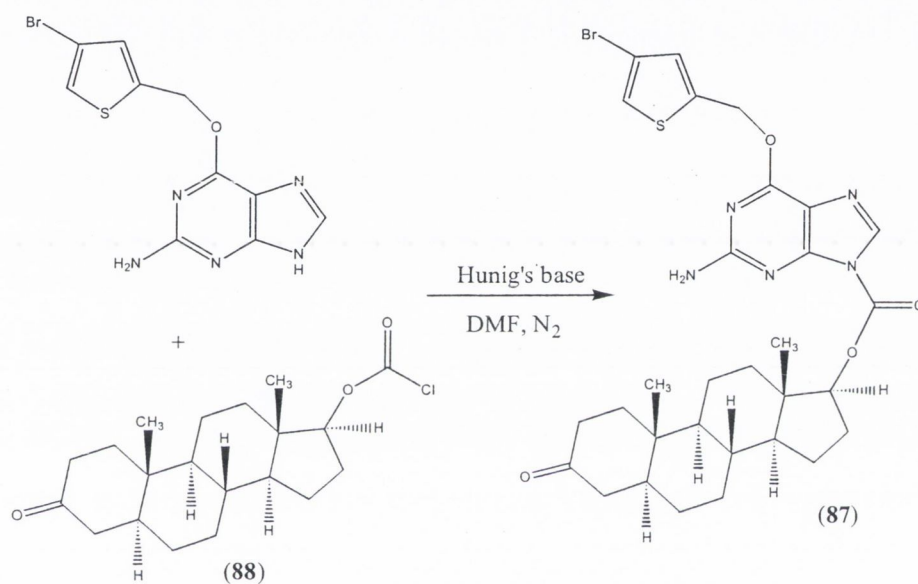
way to target steroid-dependent tumours.⁶⁸⁻⁷² In the subsequent sections we will discuss the synthesis of N-9 alkoxy carbonyl derivatives from steroid residues. The synthetic procedure we will follow will be based on the general route developed above.

2.11.1 Targeting steroid dependent tumours

Many human tumours have been found to contain hormone receptors. In the introduction we discussed the over-expression of steroid receptors in steroid dependent cancers like breast and testicular cancers.⁷³ Many attempts have been made to target oestrogen receptors in breast cancers. Several chemotherapeutic agents like the topoisomerase-II inhibitor ellipticine have already been successfully linked with oestradiol, and their selectivity investigated.^{68,69} Chae *et al.*³⁴ have also reported the N-9 substitution of the ATase depletor *O*⁶-benzylguanine with a dihydrotestosterone residue (**86**) *via* a N-9 carboxymethyl linkage. They hoped that such a derivative would selectively inactivate ATase in steroid responsive tumours.



Similarly, several N-9 substituted O^6 -(4-bromophenyl)guanine steroid derivatives have been prepared in our laboratory and tested as ATase inactivators. Amongst these inactivators, there was an example of an N-9 alkoxy carbonyl derivative containing a dihydrotestosterone residue. O^6 -(4-Bromophenyl)-9-(3-oxo-5 α -androst-17 β -yl-oxycarbonyl)guanine (**87**) has been synthesized by Dr. Dorothy J. Donnelly, by reacting 5 α -androst-3-one 17 β -chloroformate (**88**) and PaTrin-2 (Scheme 2.44).



Scheme 2.44

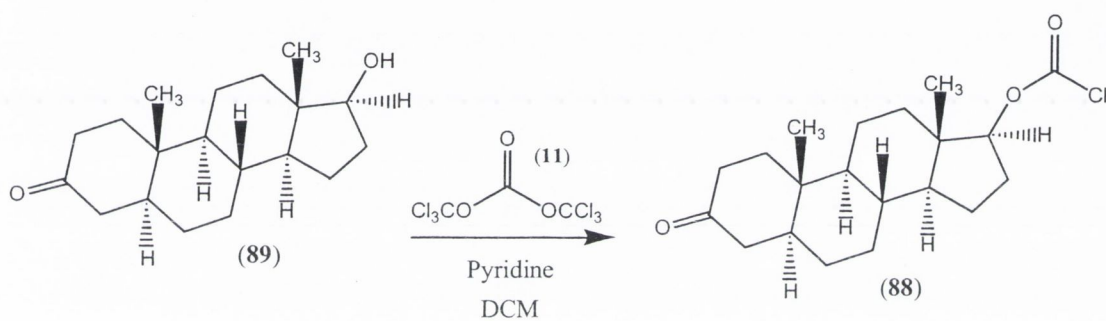
However, this N-9 derivative (**87**) was obtained in extremely low yield (<10%) in a reaction that proved difficult to repeat. It was tested for its ATase-depleting ability, and found to have a high level of activity (I_{50} 0.025 μ M). To provide more material for pharmacological studies on the targeting properties of (**87**), our aim was to improve the synthetic procedure.

Having already investigated reactions involving chloroformates (Section 2.8), it was apparent that the difficulty experienced in the synthesis of (**87**) was associated with the quality of 5 α -androst-3-one 17 β -chloroformate (**88**).[♦] The procedure reported by Konakahara *et al.*⁴⁶ was followed for the synthesis of the chloroformate (**88**). We

[♦] Nakanishi *et al.*⁴⁵ reported the synthesis of the chloroformate (**88**) by a procedure involving phosgene.

had already examined this procedure for the synthesis of chloroformates using BTC (11), but had found it to be complicated and low yielding (Section 2.9.1). The method described by Horwell *et al.*⁴⁸ was found to be a higher yielding and a more direct route to the synthesis of chloroformates (Section 2.9.2).

We repeated the procedure described by Horwell *et al.*⁴⁸ and the chloroformate (88) was prepared in high yield. The reaction involved the dropwise addition of an ice-cold solution of BTC (11) and pyridine in dry DCM to an ice-cold solution of dihydrotestosterone (89) in DCM (Scheme 2.45).



Scheme 2.45

The reaction was set aside for 2 hours at room temperature. The solvent was removed, and ethyl acetate was added to precipitate the pyridine hydrochloride. This precipitate was collected, and the solvent removed from the filtrate to leave the product (88). 5α-Androstan-3-one 17β-chloroformate (88) was characterized by infrared spectroscopy. The carbonyl stretching frequency of the chloroformate group was observed at 1774 cm^{-1} .

The reaction of 5α-androstan-3-one 17β-chloroformate (88) with PaTrin-2 was carried out using an equimolar amount of triethylamine as base, in a reaction otherwise analogous to that shown in Scheme 2.44. The reaction was complete within 15 minutes. Analysis of the ^1H NMR spectrum of the reaction mixture showed approximately 60% of the N-9 derivative (87) and 20% of the N-7 isomer, as well as

approximately 20% of unreacted PaTrin-2. The N-9 isomer (**87**) was isolated by flash chromatography in approximately 25% yield. A small amount of the N-7 isomer was also obtained. The purification procedures led to considerable decomposition, and might be improved upon. Difficulty experienced with the isolation of the N-9 isomer was also attributed to the similar R_F values of the two isomers.

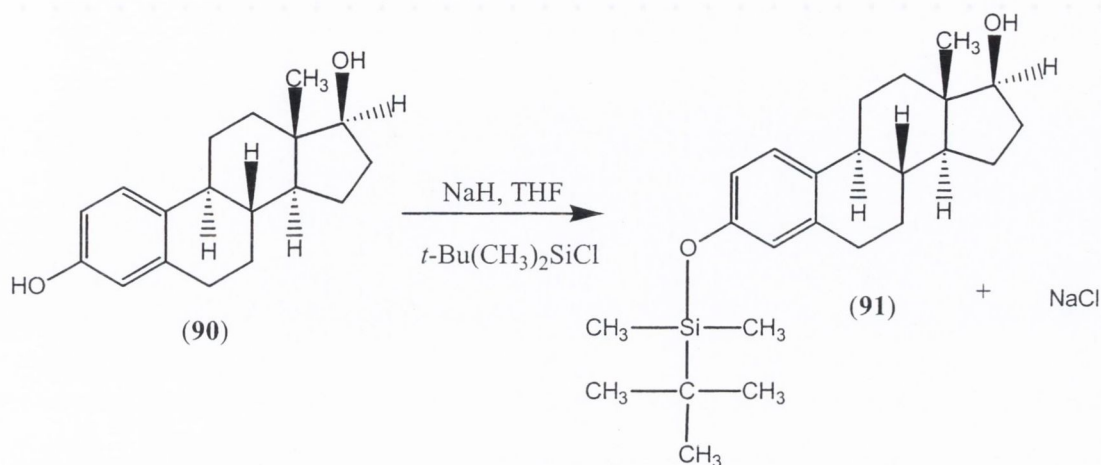
Previously, we had shown that by using DBU as a base the formation of the N-7 isomer could be prevented (Section 2.9.5) and we could obtain pure N-9 derivatives by this route. Although N-7 alkyl derivatives have little or no ATase-inactivating ability (Section 2.9.3), we anticipated that N-7 alkoxycarbonyl derivatives with targeting ability once delivered to their target would undergo hydrolysis to release PaTrin-2. We thought it would be worthwhile to synthesize both the N-9 and N-7 isomer to compare their respective rates of hydrolysis.

We had significantly improved upon the original method proposed for the synthesis of O^6 -(4-bromophenyl)-9-(3-oxo-5 α -androstan-17 β -yl-oxycarbonyl)guanine (**87**). We decided to repeat this procedure for the synthesis of the corresponding oestradiol derivative.

2.11.2 The selective protection of a phenolic hydroxyl group

β -Oestradiol (**90**) contains both phenolic and alcoholic hydroxyl groups. Reports suggest that for oestradiol to bind to the appropriate receptors a free phenolic 3-OH is required.^{72,74} As we found both hydroxyl groups to be reactive, it was necessary to protect the 3-OH before the reaction with the 17-OH could be attempted. One of the most commonly used hydroxyl protecting groups is the *t*-butyldimethylsilyl moiety.^{60,75,76} Of several reported methods for its introduction to protect a phenolic hydroxyl group, we found that a method described by Top *et al.*⁶⁰ worked best.

Following this procedure, 3-(dimethyl-*t*-butylsiloxy)-17 β -hydroxyoestra-1,3,5(10)-triene (**91**) was prepared in 70% yield (Scheme 2.46).

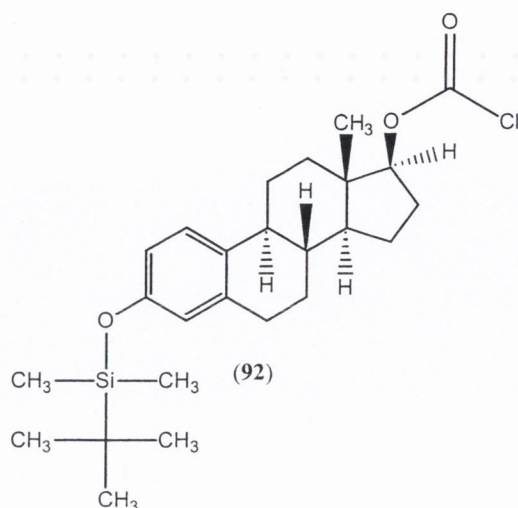


Scheme 2.46

The reaction involved the slow addition of a solution of β -oestradiol in dry THF to a suspension of NaH in dry THF. This mixture was stirred for 30 minutes before the addition of *t*-butyldimethylsilyl chloride. The reaction was allowed to stand for 3 hours. Decomposition of the excess hydride was carried out with ice-water, and the product was extracted into DCM. The product, 3-(dimethyl-*t*-butylsiloxy)-17 β -hydroxyoestra-1,3,5(10)-triene (**91**), was recrystallized from light petroleum.

2.11.3 Chloroformate synthesis

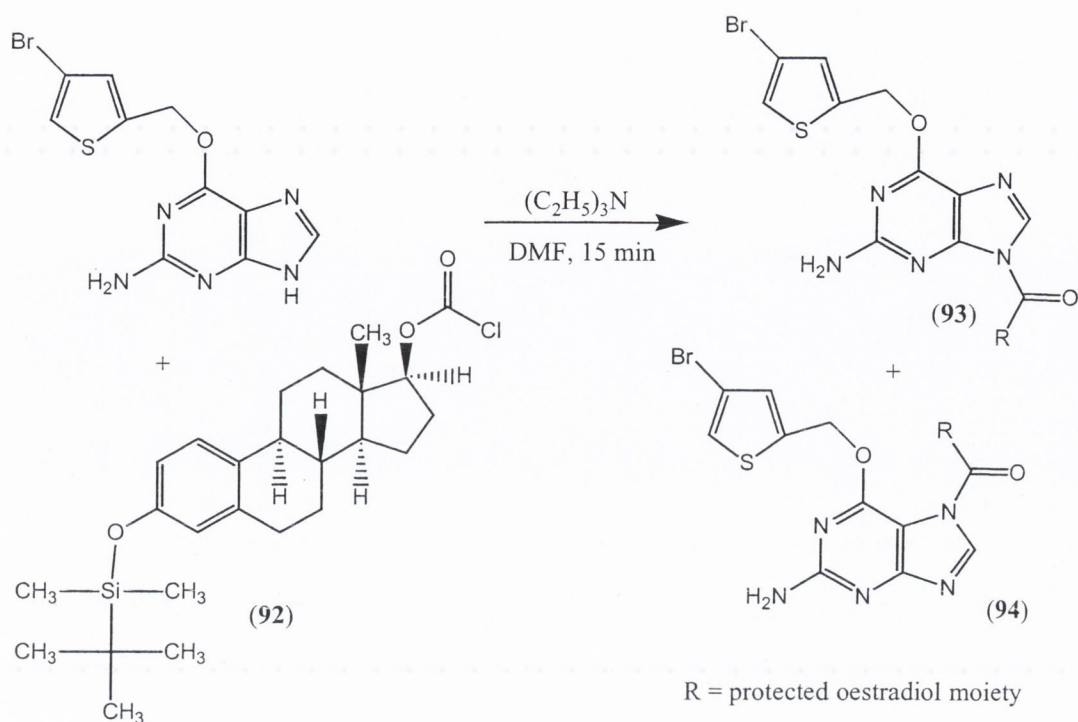
With the 3-OH protected, we proceeded to react the 17-OH of 3-(dimethyl-*t*-butylsiloxy)-17 β -hydroxyoestra-1,3,5(10)-triene (**91**) with the phosgene source, BTC (**11**). 3-(Dimethyl-*t*-butylsiloxy)-oestra-1,3,5(10)-triene-17 β -yl chloroformate (**92**) was prepared by the addition of an ice-cold solution of the protected oestradiol in dry DCM, to an ice-cold solution of BTC and pyridine in dry DCM. This reaction was analogous to that shown in Scheme 2.45.



The reaction was complete after 2 hours at room temperature. The chloroformate (92) was characterized by infrared spectroscopy, with the carbonyl stretching frequency observed at 1774 cm^{-1} . The chloroformates prepared previously had been separated from pyridine hydrochloride. However, the purification of 3-(dimethyl-*t*-butylsiloxy)-oestra-1,3,5(10)-triene-17 β -yl chloroformate (92) was not possible, as the two compounds had similar solubility in the solvent mixture. Instead the subsequent reaction with PaTrin-2 was carried out using the crude reaction mixture.

2.11.4 Synthesis of *O*⁶-(4-bromophenyl)-9-[3-hydroxy-oestra-1,3,5(10)-triene-17 β -yl-oxycarbonyl]guanine

The reaction of PaTrin-2 with 3-(dimethyl-*t*-butylsiloxy)-oestra-1,3,5(10)-triene-17 β -yl chloroformate (92) was carried out in the presence of an equimolar amount of triethylamine (Scheme 2.47) and was complete within 15 minutes. The reaction resulted in equal amounts of the N-9 (93) and the N-7 (94) isomers. Approximately 40% of unreacted PaTrin-2 was also observed based on an analysis of the ^1H NMR spectrum of the crude reaction mixture.



Scheme 2.47

Flash chromatography was used to separate the components of the reaction mixture. However, this procedure resulted in the partial decomposition of (93) and (94) to give PaTrin-2. It was also observed that the N-9 isomer (93) was more easily hydrolysed during the separation than the N-7 isomer. The R_F values of the N-9 isomer and the protected oestradiol (91), generated from the decomposition of the unreacted chloroformate (92), were similar thus preventing complete chromatographic separation. However, it was found that the N-9 isomer (93) could be successfully purified by its insolubility in hot ethyl acetate.

Some desilylation of the N-9 isomer (93) occurred during chromatography. However a method for the deprotection of both the remaining N-9 isomer, as well as the N-7 isomer was required.

There are several methods in the literature for the deprotection of phenolic silyl ethers.⁷⁷⁻⁸² Tetrabutylammonium fluoride is regarded as one of the most useful

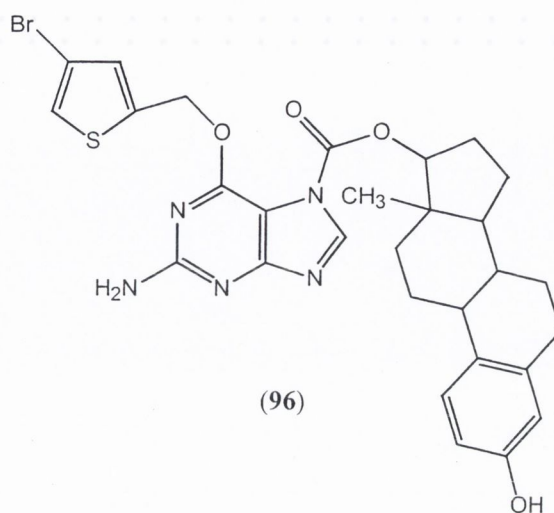
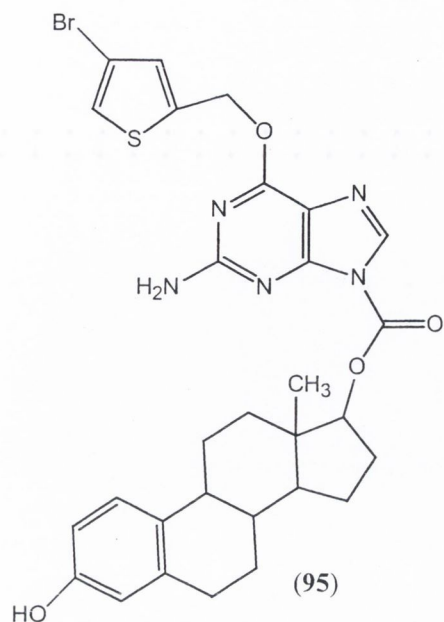
reagents for the removal of the silyl group.⁶¹ Successful removal of the silyl group from both the N-9 and N-7 isomers would require mild conditions. It was feared that harsh conditions would result in the hydrolysis of these alkoxycarbonyl derivatives to generate PaTrin-2. A method reported by Collington *et al.*⁶¹ met this requirement.

The reaction involved the addition of one equivalent of a 1M solution of tetrabutylammonium fluoride in THF to a solution of the silyl ether in THF at 0 °C. After 15 minutes an excess of aqueous NH₄Cl was added, and the desilylated product was extracted into ether.

We repeated this procedure with the N-7 alkoxycarbonyl derivative (**94**). Although the silyl group was removed in the reaction, partial hydrolysis occurred giving a mixture of the desilylated alkoxycarbonyl derivative^{*} and PaTrin-2. This mixture could not be separated. Although the report by Collington *et al.*⁶¹ suggested flash chromatography following desilylation, we had insufficient material to carry on from here.

An alternative approach was eventually found. Following the reaction of 3-(dimethyl-*t*-butylsiloxy)-oestra-1,3,5(10)-triene-17 β -yl chloroformate (**92**) with PaTrin-2 (as shown in Scheme 2.47), the crude reaction mixture was treated, as above, with an equivalent of tetrabutylammonium fluoride. The resulting mixture was then successfully separated by flash chromatography. Both the desilylated *O*⁶-(4-bromophenyl)-9-[3-hydroxy-oestra-1,3,5(10)-triene-17 β -yl-oxycarbonyl]guanine (**95**) and the corresponding N-7 isomer (**96**) were recovered in reasonable yield by this route.

^{*} As discussed in Section 2.9.5, rearrangement occurred to give the N-9 derivative (see Scheme 2.32).



2.11.5 Summary of steroid reactions

We had successfully linked PaTrin-2 to both a dihydrotestosterone and an oestradiol residue. However, the need to separate the isomers by flash chromatography resulted in substantial decomposition. In addition, the reaction of PaTrin-2 with 3-(dimethyl-*t*-butylsiloxy)-oestra-1,3,5(10)-triene-17 β -yl chloroformate (**92**) was not as high-yielding as the reaction involving 5 α -androstan-3-one 17 β -chloroformate (**88**). Under the optimum conditions only 60% of PaTrin-2 usually reacted and moreover equal amounts of both the N-9 and N-7 isomers were normally formed.

We considered alternative routes to the synthesis of these steroid derivatives. We investigated the reactions of both bis(2,4,5-trichlorophenyl) carbonate (**6**) and bis(4-nitrophenyl) carbonate (**7**) with each of the steroids. The corresponding esters were synthesized, but never in more than 20% yield and their subsequent reactions with PaTrin-2 were equally low yielding. Although these reactions resulted in the formation of only the N-9 isomer, syntheses still resulted in mixtures, which required separation by flash chromatography. In the light of these reactions, the chloroformate

route appears to be the most satisfactory route to the synthesis of these alkoxycarbonyl derivatives.

The N-9 and N-7 isomers derived from both dihydrotestosterone and oestradiol will provide very interesting pharmacological results.

2.12 N-9 Alkoxycarbonylation - a summary

This chapter has discussed fully a comprehensive search for the most reliable route to the synthesis of N-9 alkoxycarbonyl derivatives. Of the two approaches originally proposed for the synthesis of this series of prodrugs (Section 2.1.2), **Route 2** was found to be the more efficient pathway. This route involved the reaction of an activated carbonic acid derivative with an alcohol, which gave an intermediate that could react with PaTrin-2. However, the success of **Route 2** was found to be dependent on the type of activated derivative involved. We investigated the usefulness of both bis(2,4,5-trichlorophenyl) carbonate (**6**) and bis(4-nitrophenyl) carbonate (**7**) in these reactions, and have demonstrated the latter to be the more versatile reagent.

Using bis(4-nitrophenyl) carbonate (**7**) we synthesized a variety of prodrugs derived from primary and secondary alcohols, and also from alcohols containing a tertiary amino group. These N-9 derivatives will provide valuable pharmacological results that will determine the type of derivative that has greatest potential as a prodrug.

We also examined the reactions of chloroformates with PaTrin-2. These reactions were found to be rapid, but resulted in both the N-9 and N-7 isomers. Such isomeric mixtures were difficult to separate, and further complicated if the N-9 chain contained a tertiary amino group. In contrast, use of bis(4-nitrophenyl) carbonate provided a regioselective route to the synthesis of N-9 derivatives.

As well as to synthesize a variety of N-9 derivatives as potential prodrugs, the aim of the research discussed in this chapter was to establish a reliable route to the synthesis of any N-9 alkoxycarbonyl derivatives in general. We have shown bis(4-nitrophenyl) carbonate to be the reagent of choice in the synthesis of N-9 alkoxycarbonyl derivatives. However, as the above section on steroids has illustrated, chloroformates can also be of use, but only when it is possible to separate the resulting isomeric mixtures. Our investigation has provided useful results and outlined an approach that should allow for the facile synthesis of subsequent derivatives.

2.13 Experimental

Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker 400 MHz spectrometer. Chemical shifts of ^1H and ^{13}C NMR spectra were measured using solutions in deuterated DMSO (unless otherwise stated), and at 400 and 100 MHz respectively. Chemical shifts are reported in parts per million (ppm) and coupling constants (J) are quoted in Hertz (Hz). Signal assignments were made with the aid of 2D heteronuclear (^1H - ^{13}C) and homonuclear (^1H - ^1H) correlated spectra.

Infrared (IR) spectra were recorded as nujol mulls for solids or as liquid films for oils using a Mattson Genesis II FTIR spectrophotometer. The wavenumber (ν) is reported in reciprocal centimetres (cm^{-1}).

Ultraviolet (UV) spectra were recorded using a Unicam UV4 spectrometer and solutions in methanol. The wavelength (λ) is reported in nanometers (nm). Due to the poor solubility of all compounds in methanol we did not calculate the molar extinction coefficient (ϵ).

Accurate mass was measured by electrospray ionisation mass spectrometry using a Micromass LCT TOF mass spectrometer and solutions in methanol.

Melting points were measured using a Stuart Scientific SMP1 melting point apparatus.

Thin layer chromatography was carried out using either Merck Kieselgel 60 F₂₅₄ silica gel plates or Merck 60 F₂₅₄ aluminium oxide neutral plates. Visualisation was by means of ultraviolet light or by development in a 10% solution of phosphomolybdic acid in ethanol (followed by gentle charring). Flash chromatography was carried out using Merck Kieselgel 60 silica gel (230 – 400 mesh).

Dry DMF was 99.8% anhydrous and supplied by Aldrich.

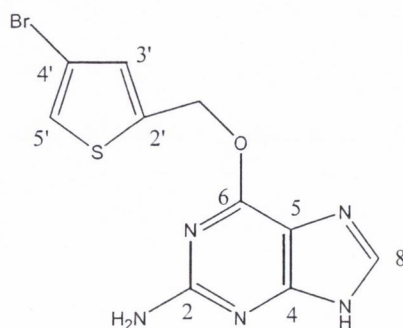
DCM was dried by treatment of the distilled solvent with calcium hydride.

Dry THF was prepared by treatment with calcium chloride, followed by treatment with sodium wire and benzophenone.

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

(Full analysis of 2,4,5-trichlorophenyl and 4-nitrophenyl intermediates was not always possible due to the unstable nature of these compounds)

Numbering system followed for *O*⁶-(4-bromophenyl)guanine:



2-Amino-N,N,N-trimethyl-9H-purine-6-yl-ammonium chloride (9)^{13,18} – An ice-cold solution of trimethylamine (30 ml) in DMF (30 ml) was added to an ice-cold solution of 2-amino-6-chloropurine (3.39 g, 20 mmol) in DMF (132 ml) with continuous stirring. The crystalline solid that precipitated was collected, washed first with DMF and then with ether, and dissolved in water (10.4 ml) at 40 °C. Upon the subsequent addition of acetone (200 ml), a solid precipitated and was collected. Washing first with a mixture of acetone and water (20:1), then with acetone, and drying *in vacuo* over P₂O₅ afforded the trimethylammonium salt (9) (3.2 g, 70%).

4-Bromophenyl alcohol (10)¹⁹ – NaBH₄ (380 mg, 10 mmol) in ethanol (12 ml) was added dropwise over 15 min to an ice-cold solution of 4-bromothiophene-2-carboxaldehyde (3.82 g, 20 mmol) in ethanol (24 ml). The reaction mixture was stirred at room temperature for 2 h. The solution was cooled and acetic acid added until there was no further effervescence. The solvent was removed under reduced pressure to afford a white paste. The paste was dissolved in ether, washed in turn with water, aqueous NaHCO₃, water, and brine, and dried over MgSO₄. The ether was removed to give 4-bromophenyl alcohol (10) (3.4g, 88%) as a colourless oil.

$\delta^1\text{H}$ (400MHz, DMSO): 4.61 (2H, d, $J=7.0$, CH₂), 5.61 (1H, t, $J=8.0$, OH), 6.96 (1H, s, 3-CH), 7.53 (1H, s, 5-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 58.0 (CH₂), 107.8 (4-C), 122.3 (5-CH), 125.9 (3-CH), 148.5 (2-C).

O⁶-(4-Bromophenyl)guanine (PaTrin-2) (8)¹³ – NaH (60% in oil; 800 mg, 20 mmol) was added to a solution of 4-bromophenyl alcohol (10) (5.79 g, 30 mmol) in DMSO (5 ml). The mixture was stirred at room temperature for 30 min. The

trimethylammonium salt (**9**) (2.29 g, 10 mmol) was added, and stirring was continued for 2 h. The mixture was treated with a solution of acetic acid (1.63 ml) in ether (300 ml), and stirring was continued for a further 30 min. The white solid that precipitated was collected and washed with ether, and then with water to give *O*⁶-(4-bromophenyl)guanine (**8**), which was then dried *in vacuo* over P₂O₅ (2.28 g, 70%).

m.p. 212 °C

λ_{max} : 284nm

$\delta^1\text{H}$ (400MHz, DMSO): 5.63 (2H, s, 6-OCH₂), 6.31 (2H, s, NH₂), 7.31 (1H, s, 3'-CH), 7.68 (1H, s, 5'-CH), 7.85 (1H, s, 8-CH), 12.45 (1H, s, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 60.5 (6-OCH₂), 108.0 (4'-C), 113.0 (5-C), 125.1 (5'-CH), 130.7 (3'-CH), 138.0 (8-CH), 140.7 (2'-C), 155.4 (4-C), 159.1 (2-C), 159.4 (6-C).

Bis(2,4,5-trichlorophenyl) carbonate (**6**)²² – Bis(trichloromethyl) carbonate (989 mg, 6.6 mmol) in toluene (15 ml), was added to a solution of 2,4,5-trichlorophenol (3.95 g, 20 mmol) and *N,N*-dimethylaniline (2.54 ml, 20 mmol) in toluene (25 ml) under nitrogen. The reaction was stirred at 80 °C for 2 h. Ice (20 g) was then added. When this had melted the precipitate was collected and washed with water, and then with toluene. The solid was recrystallised from ethyl acetate by hot charcoal filtration to afford (**6**) (5.9 g, 70%).

ν_{max} : 1639, 1774(C=O) cm⁻¹ (lit.²² 1768 cm⁻¹, KBr)

m.p. 171 °C (lit.⁸³ 166-167 °C, EtOAc)

$\delta^1\text{H}$ (400MHz, DMSO): 8.06 (2H, s, arom. 6, 6'-CH), 8.14 (2H, s, arom. 3, 3'-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 117.4 (6, 6'-CH), 120 (2, 2'-C), 120.8 (4, 4', 5, 5'-C), 130.0 (C=O), 130.5 (3, 3'-CH), 153.0 (1, 1'-C).

***O*⁶-(4-Bromophenyl)-9-(2,4,5-trichlorophenoxycarbonyl)guanine (15)** – Bis(2,4,5-trichlorophenyl) carbonate (42 mg, 0.1 mmol) was added to a stirred solution of PaTrin-2 (33 mg, 0.1 mmol) and triethylamine (14 μ l, 0.1 mmol) in DMF (0.5 ml). After 2 h the solvent was evaporated and ethanol was added to the residue. The white precipitate of (15) (50.5 mg, 92%) was collected and dried.

ν_{max} : 1581, 1647, 1790(C=O) cm^{-1}

m.p. 182 $^{\circ}\text{C}$

λ_{max} : 282, 286nm

$\delta^1\text{H}$ (400MHz, DMSO): 5.68 (2H, s, 6-OCH₂), 7.04 (2H, s, NH₂), 7.38 (1H, s, 3'-CH), 7.72 (1H, s, 5'-CH), 8.12 (1H, s, arom. 6''-CH), 8.17 (1H, s, arom. 3''-CH), 8.47 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 61.1 (6-OCH₂), 108.1 (4'-C), 113.9 (5-C), 125.4 (5'-CH), 125.9 (6''-CH), 130.7 (1''-C), 130.8 (2''-C), 131.1 (3'-CH), 131.4 (3''-CH), 137.8 (8-CH), 140.1 (2'-C), 144.8 (5''-C), 144.9 (4''-C), 153.9 (C=O), 159.9 (4-C), 160.9 (2-C), 161.0 (6-C).

Found: **C**, 37.06; **H**, 1.66; **N**, 12.68 %. C₁₇H₉BrN₅O₃SCl₃ requires **C**, 37.15; **H**, 1.65; **N**, 12.74 %

(This compound proved too insoluble to allow for the measurement of accurate mass)

***O*⁶-(4-Bromophenyl)-9-(methoxycarbonyl)guanine (1)^{*}** – Methanol (1 ml) was added to a solution of *O*⁶-(4-bromophenyl)-9-(2,4,5-trichlorophenoxycarbonyl)guanine (15) (27.5 mg, 0.05 mmol) in DMF (0.5 ml) at 50 $^{\circ}\text{C}$. The reaction was set aside for 2 h. The solvent was evaporated, and ethanol was added to precipitate a white solid. The solid was collected and dried to afford (1) (17 mg, 90%).

^{*} Compound identical to that prepared by Dr. D.J. Donnelly (from methyl chloroformate).

$\nu_{\max}$: 1580, 1626, 1779(C=O) cm^{-1}

$\lambda_{\max}$: 270nm

m.p. 167 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 3.99 (3H, s, OCH_3), 5.65 (2H, s, 6- OCH_2), 6.88 (2H, s, NH_2), 7.36 (1H, s, 3'-CH), 7.70 (1H, s, 5'-CH), 8.22 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 54.6 (OCH_3), 60.9 (6- OCH_2), 108.1 (4'-C), 113.9 (5-C), 125.3 (5'-CH), 131.1 (3'-CH), 137.9 (8-CH), 140.1 (2'-C), 148.4 (C=O), 153.6 (4-C), 159.7 (2-C), 160.7 (6-C).

Found: C, 36.49; H, 2.51; N, 17.24 %. $\text{C}_{12}\text{H}_{10}\text{BrN}_5\text{O}_3\text{S}\cdot 0.75 \text{H}_2\text{O}$ requires C, 36.24; H, 2.91; N, 17.61 %

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{12}\text{H}_{11}\text{BrN}_5\text{O}_3\text{S}$ 383.9766, Found 383.9739.

O⁶-(4-Bromophenyl)-9-(propoxycarbonyl)guanine (16) – This compound (16.5 mg, 80%) was prepared following the above procedure, but using propanol instead of methanol.

$\nu_{\max}$: 1578, 1623, 1771(C=O) cm^{-1}

$\lambda_{\max}$: 273nm

m.p. 156 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 0.98 (3H, t, $J=7.3$, 3''- CH_3), 1.74 (2H, m, 2''- CH_2), 4.32 (2H, t, $J=6.5$, 1''- CH_2), 5.64 (2H, s, 6- OCH_2), 6.89 (2H, s, NH_2), 7.37 (1H, s, 3'-CH), 7.72 (1H, s, 5'-CH), 8.23 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 10.2 (3''- CH_3), 21.4 (2''- CH_2), 60.9 (6- OCH_2), 69.1 (1''- CH_2), 108.1 (4'-C), 113.9 (5-C), 125.4 (5'-CH), 131.1 (3'-CH), 137.9 (8-CH), 140.2 (2'-C), 147.6 (C=O), 153.8 (4-C), 159.6 (2-C), 160.7 (6-C).

Found: **C**, 40.57; **H**, 3.36; **N**, 16.69 %. $C_{14}H_{14}BrN_5O_3S$ requires **C**, 40.79; **H**, 3.42; **N**, 16.99 %

MS (ES) $[M+H]^+$ Calcd m/z for $C_{14}H_{15}BrN_5O_3S$ 412.0079, Found 412.0109.

Benzyl(2,4,5-trichlorophenyl) carbonate (19) – Benzyl alcohol (105 μ l, 1 mmol), followed by Hünig's base (170 μ l, 1 mmol), were added to a solution of bis(2,4,5-trichlorophenyl) carbonate (420 mg, 1 mmol) in dry DMF (0.5 ml). Stirring was continued at room temperature for 4 h. The solvent was removed under reduced pressure, and light petroleum (b.p. 40 - 60 $^{\circ}$ C) was added to the residue. A white solid precipitated, and was collected, washed with light petroleum and dried to afford **(19)** (166 mg, 50%).

$\nu_{\max}$: 1590, 1662, 1753(C=O) cm^{-1}

m.p. 70 $^{\circ}$ C (lit.²⁴ 86-87 $^{\circ}$ C, prepared using benzyl chloroformate)

$\delta^1\text{H}$ (400MHz, DMSO): 5.33 (2H, s, OCH_2Ar), 7.43 (5H, m, arom. 2', 3', 4', 5', 6'-CH), 8.01 (1H, s, arom. 6-CH), 8.08 (1H, s, arom. 3-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 70.8 (CH_2), 125.7 (arom. 6-CH), 125.8 (arom. 2-C), 128.4 (arom. 3', 5'-CH), 128.6 (arom. 2', 6'-CH), 128.8 (arom. 4'-CH), 130.0 (arom. 4-C), 130.7 (arom. 5-C), 131.6 (arom. 3-CH), 134.5 (arom. 1'-C), 145.8 (C=O), 151.5 (arom. 1-C).

Piperonyl(2,4,5-trichlorophenyl) carbonate (20) – This compound (338 mg, 90%) was prepared following the above procedure, but using piperonyl alcohol (152 mg, 0.1 mmol) instead of benzyl alcohol.

$\nu_{\max}$: 1594, 1656, 1745(C=O) cm^{-1}

m.p. 136 $^{\circ}$ C

$\delta^1\text{H}$ (400MHz, DMSO): 5.22 (2H, s, OCH_2Ar), 6.05 (2H, s, OCH_2O), 6.95 (2H, s, arom. 5', 6'-CH), 7.03 (1H, s, arom. 2'-CH), 7.99 (1H, s, arom. 6-CH), 8.08 (1H, s, arom. 3-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 70.9 (OCH_2Ar), 101.3 (OCH_2O), 108.3 (arom. 5'-CH), 109.2 (arom. 2'-CH), 123.0 (arom. 6'-CH), 125.7 (arom. 6-CH), 128.1 (arom. 2-C), 130.0 (arom. 4-C), 130.6 (arom. 5-C), 131.1 (arom. 3-CH), 145.8 (arom. 1'-C), 147.4 (arom. 3', 4'-C), 147.7 ($\text{C}=\text{O}$), 151.4 (arom. 1-C).

9-(Benzyloxycarbonyl)-O⁶-(4-bromophenyl)guanine (17) – Method (a):

Benzyl(2,4,5-trichlorophenyl) carbonate (**19**) (33.2 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and Hünig's base (17 μl , 0.1 mmol) in dry DMF (0.5 ml) under nitrogen. After 2 days the solvent was evaporated and DCM added to the residue, followed by hexane, to precipitate a white solid (**17**) (35 mg, 76%).

Method (b): This compound (12 mg, 52%) was prepared following the above procedure for the preparation of O⁶-(4-bromophenyl)-9-(methoxycarbonyl)guanine (**1**), but in the presence of triethylamine (7 μl , 0.05 mmol) and using benzyl alcohol (53 μl , 0.5 mmol) instead of methanol.

ν_{max} : 1580, 1639, 1772($\text{C}=\text{O}$) cm^{-1}

λ_{max} : 280nm

m.p. 130 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 5.47 (2H, s, OCH_2Ar), 5.65 (2H, s, 6- OCH_2), 6.86 (2H, s, NH_2), 7.37 (1H, s, 3'-CH), 7.33-7.45 (4H, m, arom. 3'', 4'', 5''-CH), 7.54 (2H, d, $J=7.5$, arom. 2'', 6''-CH), 7.70 (1H, s, 5'-CH), 8.23 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 60.9 (6-OCH₂), 68.7 (OCH₂Ar), 107.7 (4'-C), 113.8 (5-C), 125.2 (5'-CH), 127.9 (arom. 6'', 2''-CH), 128.3 (arom. 4''-CH), 128.5 (arom. 3'', 5'-CH), 130.5 (3'-CH), 134.9 (arom. 1''-C), 138.0 (8-CH), 140.2 (2'-C), 146.8 (C=O), 154.3 (4-C), 160.2 (2-C), 160.9 (6-C).

Found: C, 44.55; H, 2.88; N, 15.46 %. C₁₈H₁₄BrN₅O₃S requires C, 46.99; H, 3.07; N, 15.22 %

MS (ES) [M+H]⁺ Calcd m/z for C₁₈H₁₅BrN₅O₃S 460.0079, Found 460.0124.

O⁶-(4-Bromophenyl)-9-(piperonyloxycarbonyl)guanine (21) – This compound (22 mg, 44%) was prepared following the above procedure [Method (a)], but using (20) (38 mg, 0.1 mmol) instead of (19).

ν_{max} : 1583, 1632, 1783(C=O) cm⁻¹

λ_{max} : 284nm

m.p. 140 °C

$\delta^1\text{H}$ (400MHz, DMSO): 5.35 (2H, s, OCH₂Ar), 5.64 (2H, s, 6-OCH₂), 6.03 (2H, s, OCH₂O), 6.85 (2H, s, NH₂), 6.93 (1H, d, $J=7.5$, arom. 5''-CH), 7.03 (1H, d, $J=7.5$, arom. 6''-CH), 7.12 (1H, s, arom. 2''-CH), 7.35 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.21 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 60.9 (6-OCH₂), 68.8 (OCH₂Ar), 101.1 (OCH₂O), 108.1 (4'-C), 108.2 (arom. 5''-CH), 108.9 (arom. 2''-CH), 113.9 (5-C), 122.4 (arom. 6''-CH), 125.1 (5'-CH), 128.5 (arom. 1''-C), 130.9 (3'-CH), 138.0 (8-CH), 140.2 (2'-C), 147.3 (arom. 3'', 4''-C), 147.4 (C=O), 153.8 (4-C), 159.7 (2-C), 160.7 (6-C).

Found: C, 44.47; H, 2.77; N, 14.07 %. C₁₉H₁₄BrN₅O₅S·0.25 H₂O requires C, 44.85; H, 2.87; N, 13.77 %.

MS (ES) [M+H]⁺ Calcd m/z for C₁₉H₁₅BrN₅O₅S 503.9977, Found 503.9962.

6-Morpholinohexan-1-ol (24)^{27*} – A mixture of 6-chlorohexan-1-ol (6.7 ml, 50 mmol) and morpholine (13 ml, 150 mmol) was heated at 100 °C for 15 h. After cooling, DCM was added to the mixture and the solution was washed with water, then brine, and dried over MgSO₄. DCM was removed to afford (**24**) (8.19 g, 87%).

$\delta^1\text{H}$ (400MHz, DMSO): 1.27-1.43 (8H, m, 2, 3, 4, 5-CH₂), 2.22 (2H, t, $J=7.3$, 6-NCH₂), 2.31 (4H, br s, 3', 5'-CH₂), 3.36 (2H, m, 1-OCH₂), 3.55 (4H, m, 2', 6'-CH₂), 4.30 (1H, t, $J=5.0$, OH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 25.5 (3-CH₂), 26.0 (4-CH₂), 26.8 (5-CH₂), 32.5 (2-CH₂), 53.4 (3', 5'-CH₂), 58.3 (6-NCH₂), 60.7 (1-OCH₂), 66.2 (2', 6'-CH₂).

Cyclohexyl(4-nitrophenyl) carbonate (25)^{*} – Cyclohexanol (105 μl , 1 mmol) was added to a solution of bis(4-nitrophenyl) carbonate (304 mg, 1 mmol) in DCM (3 ml). Triethylamine (250 μl , 1.8 mmol) was subsequently added. After 2 days at room temperature, the reaction mixture in DCM was washed three times with a 10% NaHCO₃ solution and once with brine, before being dried over MgSO₄. The solvent was evaporated to afford (**25**) (133 mg, 50%) as an oil which later solidified.

ν_{max} : 1527, 1595, 1610, 1755(C=O) cm⁻¹

m.p. 62 °C

$\delta^1\text{H}$ (400MHz, DMSO): 1.25 (4H, m, 4'-CH₂, 3'_{ax}-CH, 5'_{ax}-CH), 1.52 (2H, m, 2'_{ax}, 6'_{ax}-CH), 1.69 (2H, m, 3'_{eq}, 5'_{eq}-CH), 1.93 (2H, m, 2'_{eq}, 6'_{eq}-CH), 4.70 (1H, m, 1'-CH), 7.54 (2H, d, $J=9.0$, arom. 2, 6-CH), 8.29 (2H, d, $J=9.0$, arom. 3, 5-CH).

* A sample of alcohol (**24**) was provided by Dr. D. Scully.

* Iimori *et al.*⁸⁴ reported the synthesis of (**25**) by a procedure involving 4-nitrophenyl chloroformate, however, no experimental details were given.

$\delta^{13}\text{C}$ (100MHz, DMSO): 22.9 (3', 5'-CH₂), 24.6 (4'-CH₂), 30.7 (2', 6'-CH₂), 77.9 (1'-CH), 122.6 (arom. 2, 6-CH), 125.4 (arom. 3, 5-CH), 145.1 (arom. 1-C), 151.4 (C=O), 155.3 (arom. 4-C).

Isopropyl(4-nitrophenyl) carbonate (26) – This compound (146 mg, 65%) was prepared following the above procedure, but using isopropanol (70 μl , 1 mmol) instead of cyclohexanol.

m.p. 54 $^{\circ}\text{C}$ (lit.⁸⁵ 55-57 $^{\circ}\text{C}$)

ν_{max} : 1593, 1614, 1751(C=O) cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 1.33 (6H, d, $J=6.0$, (CH₃)₂), 4.89 (1H, sept, $J=6.0$, OCH), 7.55 (2H, d, $J=9.0$, arom. 2', 6'-CH), 8.31 (2H, d, $J=9.0$, arom. 3', 5'-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 21.3 ((CH₃)₂), 73.7 (CH), 122.5 (arom. 2', 6'-CH), 125.3 (arom. 3', 5'-CH), 145.0 (arom. 1'-C), 151.4 (C=O), 155.3 (arom. 4'-C).

O⁶-(4-Bromophenyl)-9-(cyclohexyloxycarbonyl)guanine (27) – **Method (a):**

Cyclohexyl(4-nitrophenyl) carbonate (**25**) (27 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and Hünig's base (17 μl , 0.1 mmol) in dry DMF (0.5 ml). After 2 days the solvent was evaporated, acetonitrile added to the residue, and (**27**) (23 mg, 50%) as a white solid separated and was collected and dried.

Method (b): Cyclohexyl chloroformate (**51**) (16.3 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1mmol) and triethylamine (14 μl , 0.1 mmol) in DMF (0.5 ml) under nitrogen. The reaction was stirred at room temperature for 15 min. The solvent was removed under reduced pressure to give a mixture of the N-9 isomer (**27**) and the N-7 isomer (< 10%). These were not separated, but the N-7 isomer was identified by the ^1H NMR signals at δ_{H} 4.96 (NH₂) and 8.67 (8-CH).

ν_{max} : 1580, 1640, 1774(C=O) cm^{-1}

λ_{max} : 280nm

m.p. 182 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 1.31 (3H, m, $3_{\text{ax}}''$, $4_{\text{ax}}''$, $5_{\text{ax}}''\text{-CH}$), 1.40 (1H, m, 4_{eq}-CH), 1.64 (2H, m, $2_{\text{ax}}''$, $6_{\text{ax}}''\text{-CH}$), 1.76 (2H, m, $3_{\text{eq}}''$, $5_{\text{eq}}''\text{-CH}$), 1.92 (2H, m, $2_{\text{eq}}''$, $6_{\text{eq}}''\text{-CH}$), 4.94 (1H, br s, $1''\text{-CH}$), 5.63 (2H, s, 6-OCH₂), 6.81 (2H, s, NH₂), 7.35 (1H, s, $3'\text{-CH}$), 7.70 (1H, s, $5'\text{-CH}$), 8.21 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 22.7 ($3''$, $5''\text{-CH}_2$), 24.8 ($4''\text{-CH}_2$), 30.6 ($2''$, $6''\text{-CH}_2$), 60.9 (6-OCH₂), 76.4 ($1''\text{-CH}$), 108.1 ($4'\text{-C}$), 114.0 (5-C), 125.3 ($5'\text{-CH}$), 131.0 ($3'\text{-CH}$), 137.9 (8-CH), 140.2 ($2'\text{-C}$), 146.8 (C=O), 153.9 (4-C), 159.7 (2-C), 160.7 (6-C).

Found: C, 44.13; H, 3.90; N, 15.90 %. C₁₇H₁₈BrN₅O₃S·0.5H₂O requires C, 44.26; H, 4.15; N, 15.18 %

MS (ES) [M+H]⁺ Calcd m/z for C₁₇H₁₉BrN₅O₃S 452.0392, Found 452.0410.

O⁶-(4-Bromophenyl)-9-(isopropoxycarbonyl)guanine (28) – Method (a): This compound (17 mg, 42%) was prepared following the above procedure [Method (a)], but using (26) (23 mg, 0.1 mmol) instead of (25).

Method (b): This compound was prepared following the above procedure [Method (b)], but using an ethyl acetate solution of isopropyl chloroformate (52) (500 μl , 0.1 mmol) instead of cyclohexyl chloroformate (51). Synthesis resulted in a mixture of the N-9 isomer (28) and the N-7 isomer (< 10%). These were not separated, but the N-7 isomer was identified by the ¹H NMR signals at δ_{H} 6.62 (NH₂) and 8.65 (8-CH).

ν_{max} : 1612, 1770(C=O) cm^{-1}

λ_{max} : 271nm

m.p. 184 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 1.38 (6H, d, $J=6.0$, $(\text{CH}_3)_2$), 5.11 (1H, sept., $J=6.0$, OCH), 5.65 (2H, s, 6-OCH₂), 6.81 (2H, s, NH₂), 7.35 (1H, s, 3'-CH), 7.69 (1H, s, 5'-CH), 8.19 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 21.4 ($(\text{CH}_3)_2$), 60.9 (6-OCH₂), 72.4 (CH), 108.1 (4'-C), 114.0 (5-C), 125.3 (5'-CH), 131.0 (3'-CH), 137.9 (8-CH), 140.2 (2'-C), 146.9 (C=O), 153.9 (4-C), 159.7 (2-C), 160.7 (6-C).

Found: C, 40.01; H, 3.25; N, 16.84 %. C₁₄H₁₄BrN₅O₃S·0.5 H₂O requires C, 39.93; H, 3.59; N, 16.64 %

MS (ES) [M+H]⁺ Calcd m/z for C₁₄H₁₅BrN₅O₃S 412.0079, Found 412.0095.

6-Morpholinohexyl 4-nitrophenyl carbonate (29) – 6-Morpholinohexan-1-ol (187 mg, 1 mmol) was added to a solution of bis(4-nitrophenyl) carbonate (304 mg, 1 mmol) in DCM (3 ml) followed by triethylamine (250 μl , 1.8 mmol). The reaction mixture was stirred at room temperature for 2 days, and then washed three times with a 10% NaHCO₃ solution and once with brine. The DCM solution was dried over MgSO₄ and evaporated to afford (29) (243 mg, 69%), an oil.

ν_{max} : 1525, 1595, 1610, 1764(C=O) cm⁻¹

$\delta^1\text{H}$ (400MHz, DMSO): 1.25 – 1.44 (6H, m, 3', 4', 5'-CH₂), 1.66 (2H, m, 2'-CH₂), 2.23 (2H, t, $J=7.3$, 6'-NCH₂), 2.32 (4H, br s, 3'', 5''-CH₂), 3.55 (4H, br s, 2'', 6''-CH₂), 4.25 (2H, t, $J=6.5$, 1'-OCH₂), 7.55 (2H, d, $J=9.5$, arom. 2, 6-CH), 8.30 (2H, d, $J=9.5$, arom. 3, 5-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 25.1 (3'-CH₂), 25.8 (4'-CH₂), 26.5 (5'-CH₂), 27.9 (2'-CH₂), 53.4 (3'', 5''-CH₂), 58.2 (6'-NCH₂), 66.2 (2'', 6''-CH₂), 69.2 (1'-OCH₂), 122.5 (arom. 2, 6-CH), 125.3 (arom. 3, 5-CH) 145.1 (arom. 1-C), 152.0 (C=O), 155.4 (arom. 4-C).

O⁶-(4-Bromophenyl)-9-(6-morpholinohexyloxycarbonyl)guanine (30) – Method (a):

6-Morpholinohexyl 4-nitrophenyl carbonate (**29**) (35 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and Hünig's base (17 μ l, 0.1 mmol) in dry DMF (0.5 ml). After 2 days the solvent was removed under reduced pressure. TLC (hexane:EtOH, 4:1) showed the presence of a 4-nitrophenol salt. The residue was dissolved in DCM, and the solution washed three times with 0.5 M NaOH, dried over MgSO₄ and evaporated to afford (**30**) (41 mg, 76%).

Method (b): A crude sample of 6-morpholinohex-1-yl chloroformate (0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and triethylamine (28 μ l, 0.2 mmol) in dry DMF (0.5 ml). After 1 h the solvent was removed to give a mixture containing the N-9 isomer (**30**) and the N-7 isomer (< 5%). These were not separated, but the N-7 isomer was identified by the ¹H NMR signals at δ_{H} 6.61 (NH₂) and 8.63 (8-CH).

ν_{max} : 1582, 1635, 1765(C=O) cm⁻¹

λ_{max} : 280nm

m.p. 180 °C

$\delta^1\text{H}$ (400MHz, DMSO): 1.33 (2H, m, 4''-CH₂), 1.43 (4H, m, 3'', 5''-CH₂), 1.73 (2H, m, 2''-CH₂), 2.23 (2H, m, 6''-NCH₂), 2.30 (4H, br s, 3''', 5'''-CH₂), 3.53(4H, br s, 2''', 6'''-CH₂), 4.36 (2H, m, 1''-OCH₂), 5.65 (2H, s, 6-OCH₂), 6.85 (2H, s, NH₂), 7.36 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.22 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 25.1 (3''-CH₂), 25.8 (4''-CH₂), 26.5 (5''-CH₂), 27.9 (2''-CH₂), 53.4 (3''', 5'''-CH₂), 58.2 (6''-NCH₂), 60.9 (6-OCH₂), 66.2 (2''', 6'''-CH₂), 67.7 (1''-OCH₂), 108.09 (4'-C), 113.9 (5-C), 125.3 (5'-CH), 131.0 (3'-CH), 137.9 (8-CH), 140.2 (2'-C), 147.6 (C=O), 153.8 (4-C), 159.7 (2-C), 160.7 (6-C).

Found: C, 46.53; H, 5.08; N, 14.40 %. $C_{21}H_{27}BrN_6O_4S \cdot 0.5 H_2O$ requires C, 45.94; H, 4.84; N, 14.96 %

MS (ES) $[M+H]^+$ Calcd m/z for $C_{21}H_{28}BrN_6O_4S$ 539.1076, Found 539.1130.

4-Nitrophenol salt of 4-picolyl 4-nitrophenyl carbonate (31)³³ – Bis(4-nitrophenyl) carbonate (304 mg, 1 mmol) was added to a solution of 4-picolyl alcohol (109 mg, 1 mmol) in DCM (5 ml). After 2 days the solvent was evaporated and ethyl acetate added to the residue and upon the addition of hexane a pale yellow solid precipitated. The solid was collected and dried to afford the 4-nitrophenol salt (**31**) (252 mg, 61%).

$\nu_{\max}$: 1516, 1587, 1605, 1764(C=O) cm^{-1}

m.p. 110 °C (lit.³³ 112-113 °C)

δ^1H (400MHz, DMSO): 5.37 (2H, s, CH_2Ar), 6.92 (2H, d, $J=9.0$, 4-nitrophenol 2'', 6''-CH), 7.45 (2H, d, $J=6.0$, picolyl 3', 5'-CH), 7.61 (2H, d, $J=9.0$, arom. 2, 6-CH), 8.11 (2H, d, $J=9.5$, 4-nitrophenol 3'', 5''-CH), 8.33 (2H, d, $J=9.0$, arom. 3, 5-CH), 8.6 (2H, d, $J=6.0$, picolyl 2', 6'-CH), 11.02 (1H, br s, 4-nitrophenol-OH).

$\delta^{13}C$ (100MHz, DMSO): 68.3 (CH_2Ar), 115.8 (4-nitrophenol 2'', 6''-CH), 122.0 (picolyl 3', 5'-CH), 122.7 (arom. 2, 6-CH), 125.5 (arom. 3, 5-CH), 126.2 (4-nitrophenol 3'', 5''-CH), 139.6 (4-nitrophenol 1''-C), 143.8 (arom. 1-C), 145.3 (picolyl 4'-C), 150.0 (picolyl 2', 6'-CH), 151.9 (C=O), 155.2 (arom. 4-C), 163.9 (4-nitrophenol 4''-C).

* The free base of the 4-nitrophenol salt (**31**) may be prepared by washing the salt with a 0.5 M NaOH solution.

O⁶-(4-Bromophenyl)-9-(4-picolylloxycarbonyl)guanine (32)[•] – The 4-nitrophenol salt of 4-picolyl 4-nitrophenyl carbonate (**31**) (41 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and Hünig's base (34 μ l, 0.2 mmol) in dry DMF (0.5 ml). After 2 days the solvent was removed under reduced pressure. Ethyl acetate was added to the residue, then hexane, which precipitated a white solid (**32**) (18 mg, 39%).

ν_{max} : 1578, 1610, 1776(C=O) cm^{-1}

λ_{max} : 280nm

m.p. 90 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 5.53 (2H, s, OCH_2Ar), 5.66 (2H, s, 6- OCH_2), 6.89 (2H, s, NH_2), 7.36 (1H, s, 3'-CH), 7.57 (2H, d, $J=6.0$, 2 arom. 3'', 5''-CH), 7.70 (1H, s, 5'-CH), 8.32 (1H, s, 8-CH), 8.61 (2H, d, $J=6.0$, 2 arom. 2'', 6''-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 60.9 (6- OCH_2), 66.8 (OCH_2Ar), 108.1 (4'-C), 113.8 (5-C), 121.5 (arom. 3'', 5''-CH), 125.2 (5'-CH), 131.0 (3'-CH), 137.9 (8-CH), 140.2 (2'-C), 144.0 (arom. 1''-C), 147.6 (C=O), 149.7 (arom. 2'', 6''-CH), 153.6 (4-C), 159.6 (2-C), 161.0 (6-C).

Found: C, 44.15; H, 2.85; N, 19.98 %. $\text{C}_{17}\text{H}_{13}\text{BrN}_6\text{O}_3\text{S}$ requires C, 44.26; H, 2.84; N, 18.22 %

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{17}\text{H}_{14}\text{BrN}_6\text{O}_3\text{S}$ 461.0031, Found 461.0002.

[•] Compound (**32**) may also be prepared by using the free base of (**31**) and a single equivalent of Hünig's base.

6-Dimethylaminohexan-1-ol (34)^{36,37*} – Dimethylamine in THF (2 M; 55 ml, 110 mmol) was added dropwise over 20 min to an ice-cold solution of 6-methoxycarbonylpentanoyl chloride (8.9 g, 50 mmol) in dry THF (10 ml). Following 30 min at 0 °C, the reaction mixture was stirred at room temperature for 15 min and then refluxed for 2 h. The dimethylamine hydrochloride was collected and washed with THF. The solvent was removed from the filtrate and the residue taken up in DCM, and the solution was washed with water, then brine and dried over MgSO₄. The solvent was evaporated to afford the amido-ester 5-methoxycarbonyl-*N,N*-dimethylpentanamide (7.2 g, 77%).

A solution of the amido-ester (6.6 g, 35 mmol) in dry THF (65 ml) was added slowly to a suspension of LiAlH₄ (2.0 g, 53 mmol) in dry THF (55 ml). Following the addition, the mixture was refluxed for 18 h. The reaction mixture was decomposed by the successive addition of water (2 ml), NaOH solution (15%; 2 ml), and then water (6 ml) again. A white solid precipitated, was collected and washed with THF. The solvent was removed from the combined filtrates to give **(34)** (4.26 g, 84%), b.p. 102 °C/4.5 mm Hg. (lit.³⁷ b.p. 114-116 °C/12 mm Hg)

$\delta^1\text{H}$ (400MHz, CDCl₃): 1.30-1.59 (8H, m, 2, 3, 4, 5-CH₂), 2.20 (6H, s, N(CH₃)₂), 2.23 (2H, t, *J*=7.5, NCH₂), 2.72 (1H, br s, OH), 3.59 (2H, t, *J*=6.5, CH₂OH).

$\delta^{13}\text{C}$ (100MHz, CDCl₃): 25.2 (3-CH₂), 26.7 (4-CH₂), 27.1 (5-CH₂), 32.3 (2-CH₂), 44.9 (N(CH₃)₂), 59.3 (6-NCH₂), 62.1 (1-OCH₂).

3-Piperidinopropan-1-ol (35)^{38*} – A solution of piperidine (9.9 ml, 0.1 mol) and NaI (350 mg, 2.3 mmol) in ethanol (10 ml) was treated with 3-chloropropan-1-ol (4.2 ml, 50 mmol) and refluxed for 8 h. The mixture was cooled to room temperature and a

* Alcohols **(34)** and **(35)** were prepared by Dr. D.J. Donnelly.

solution of sodium ethoxide added. NaCl precipitated, was collected and washed with ether. Solvent and excess piperidine were evaporated from the combined filtrates. The residue was then diluted to three times its volume with ether, dried, evaporated and distilled to give (35) (5.58 g, 78%), a clear oil, b.p. 122 - 124 °C. (lit.³⁸ b.p. 114 °C/19 mm Hg)

$\delta^1\text{H}$ (400MHz, DMSO): 1.37 – 1.59 (8H, m, 2, 3', 4', 5'-CH₂), 2.26 (6H, m, 3, 2', 6'-CH₂), 3.41 (2H, t, $J=6.3$, 1-CH₂OH), 4.48 (1H, br s, OH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 24.2 (4'-CH₂), 25.7 (3', 5'-CH₂), 29.6 (2-CH₂), 54.2 (2', 6'-CH₂), 56.1 (3-NCH₂), 59.8 (1-OCH₂).

6-Dimethylaminohexyl 4-nitrophenyl carbonate (37) – 6-Dimethylaminohexan-1-ol (34) (145 mg, 1 mmol) was added to a solution of bis(4-nitrophenyl) carbonate (304 mg, 1 mmol) in DCM (3 ml) followed by triethylamine (250 μl , 1.8 mmol). After 2 days the reaction mixture was washed three times with a 10% NaHCO₃ solution and once with brine. The DCM solution was dried over MgSO₄ and evaporated to afford (37) (202 mg, 65%), a yellow oil.

ν_{max} : 1527, 1595, 1610, 1767(C=O), 2767 cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 1.33 (6H, m, 3', 4', 5'-CH₂), 1.68 (2H, m, 2'-CH₂), 2.11 (6H, s, N(CH₃)₂), 2.17 (2H, br s, 6'-NCH₂), 4.24 (2H, t, $J=6.5$, 1'-OCH₂), 7.55 (2H, d, $J=9.0$, arom. 2, 6-CH), 8.31 (2H, d, $J=9.0$, arom. 3, 5-CH).

3-Piperidinopropyl 4-nitrophenyl carbonate (38) – This compound (185 mg, 60%) was prepared following the above procedure, but using (35) (143 mg, 1 mmol) instead of (34).

ν_{max} : 1525, 1595, 1610, 1767(C=O) cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 1.38 (2H, m, 4''-CH₂), 1.46 (4H, m, 3'', 5''-CH₂), 1.81 (2H, m, 2'-CH₂), 2.28 (6H, m, 3', 2'', 6''-CH₂), 4.27 (2H, t, $J=6.8$, 1'-OCH₂), 7.55 (2H, d, $J=9.0$, arom. 2, 6-CH), 8.31 (2H, d, $J=9.0$, arom. 3, 5-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 24.0 (4''-CH₂), 25.4 (3'', 5''-CH₂), 53.9 (2'', 6''-CH₂), 54.6 (2'-CH₂), 66.0 (3'-NCH₂), 67.8 (1'-OCH₂), 122.5 (arom. 2, 6-CH), 125.3 (arom. 3, 5-CH), 145.1 (arom. 1-C), 152.0 (C=O), 155.4 (arom. 4-C).

N-Methyl-4-piperidiny 4-nitrophenyl carbonate (39) – This compound (126 mg, 45%) was prepared following the above procedure, but using *N*-methylpiperidin-4-ol (115 mg, 1 mmol) instead of (34).

ν_{max} : 1580, 1738(C=O) cm⁻¹

$\delta^1\text{H}$ (400MHz, DMSO): 1.72 (2H, br s, 3'_{ax}, 5'_{ax}-CH), 1.94 (2H, br s, 3'_{eq}, 5'_{eq}-CH), 2.18 (3H, s, NCH₃), 2.20 (2H, br s, 2'_{ax}, 6'_{ax}-CH), 2.51 (2H, br s, 2'_{eq}, 6'_{eq}-CH), 4.70 (1H, br s, 4'-CH), 7.56 (2H, d, $J=9.0$, arom. 2, 6-CH), 8.31 (2H, d, $J=9.0$, arom. 3, 5-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 30.1 (3', 5'-CH₂), 45.7 (N-CH₃), 52.0 (2', 6'-CH₂), 75.4 (4'-CH), 122.6 (arom. 2, 6-CH), 125.4 (arom. 3, 5-CH), 145.1 (arom. 1-C), 151.4 (C=O), 155.3 (arom. 4-C).

3-Ethoxypropyl 4-nitrophenyl carbonate (43a) – This compound (145 mg, 54%) was prepared following the above procedure, but using 3-ethoxypropan-1-ol (115 μl , 1 mmol) instead of (34).

ν_{max} : 1595, 1616, 1767(C=O) cm⁻¹

m.p. 60 °C

$\delta^1\text{H}$ (400MHz, DMSO): 1.10 (3H, t, $J=7.0$, CH_3), 1.92 (2H, m, $2'\text{-CH}_2$), 3.41 (4H, m, $3'\text{-CH}_2$, $1''\text{-CH}_2$), 4.30 (2H, t, $J=6.3$, $1'\text{-OCH}_2$), 7.56 (2H, d, $J=9.0$, arom. 2, 6-CH), 8.31 (2H, d, $J=9.0$, arom. 3, 5-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 15.0 (CH_3), 28.4 ($2'\text{-CH}_2$), 65.4 ($1''\text{-CH}_2$), 66.0 ($3'\text{-CH}_2$), 66.7 ($1'\text{-OCH}_2$), 122.6 (arom. 2, 6-CH), 125.4 (arom. 3, 5-CH), 145.1 (arom. 1-C), 152.0 (C=O), 155.3 (arom. 4-C).

O⁶-(4-Bromophenyl)-9-(6-dimethylaminohexyloxycarbonyl)guanine (40) – 6-Dimethylaminohexyl 4-nitrophenyl carbonate (**37**) (31 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and Hünig's base (17 μl , 0.1 mmol) in dry DMF (0.5 ml). After 2 days the solvent was evaporated and TLC (hexane:EtOH, 4:1) showed the presence in the residue of a 4-nitrophenol salt. The residue was dissolved in DCM, the solution washed three times with 0.5 M NaOH, and dried over MgSO_4 . The DCM was removed under reduced pressure to afford (**40**) (27 mg, 55%) as a white solid.

ν_{max} : 1585, 1630, 1745(C=O) cm^{-1}

λ_{max} : 293nm

m.p. 198 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 1.32 (6H, m, $3''$, $4''$, $5''\text{-CH}_2$), 1.71 (2H, m, $2''\text{-CH}_2$), 2.09 (6H, s, $\text{N}(\text{CH}_3)_2$), 2.15 (2H, t, $J=7.0$, $6''\text{-CH}_2$), 4.36 (2H, t, $J=6.5$, $1''\text{-CH}_2$), 5.65 (2H, s, 6-OCH_2), 6.83 (2H, s, NH_2), 7.36 (1H, s, $3'\text{-CH}$), 7.70 (1H, s, $5'\text{-CH}$), 8.21 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 25.1 ($3''\text{-CH}_2$), 26.5 ($4''\text{-CH}_2$), 26.9 ($5''\text{-CH}_2$), 27.9 ($2''\text{-CH}_2$), 45.2 ($\text{N}(\text{CH}_3)_2$), 59.1 ($6''\text{-CH}_2$), 60.9 (6-OCH_2), 67.7 ($1''\text{-CH}_2$), 108.1 ($4'\text{-C}$), 113.9 (5-

C), 125.3 (5'-CH), 131.0 (3'-CH), 137.9 (8-CH), 140.2 (2'-C), 147.6 (C=O), 153.8 (4-C), 159.7 (2-C), 160.7 (6-C).

Found: C, 45.08; H, 5.12; N, 16.87 %. $C_{19}H_{25}BrN_6O_3S \cdot 0.5 H_2O$ requires C, 45.06; H, 5.08; N, 16.60 %

MS (ES) $[M+H]^+$ Calcd m/z for $C_{19}H_{26}BrN_6O_3S$ 497.0970, Found 497.0983.

O⁶-(4-Bromophenyl)-9-(3-piperidinopropoxycarbonyl)guanine (41) – This compound (20 mg, 40%) was prepared following the above procedure, but using (38) (31 mg, 0.1 mmol) instead of (37).

$\nu_{\max}$: 1581, 1639, 1770(C=O) cm^{-1}

$\lambda_{\max}$: 290nm

m.p. 128 °C

δ^1H (400MHz, DMSO): 1.34 (2H, br s, 4'''-CH₂), 1.42 (4H, br s, 3''', 5'''-CH₂), 1.86 (2H, m, 2''-CH₂), 2.31 (4H, br s, 2''', 6'''-CH₂), 2.37 (2H, t, $J=6.8$, 3''-CH₂), 4.40 (2H, t, $J=6.5$, 1''-CH₂), 5.65 (2H, s, 6-OCH₂), 6.86 (2H, s, NH₂), 7.36 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.23 (8-CH).

$\delta^{13}C$ (100MHz, DMSO): 24.1 (4'''-CH₂), 25.3 (2''-CH₂), 25.6 (3''', 5'''-CH₂), 54.0 (2''', 6'''-CH₂), 54.9 (3''-CH₂), 60.9 (6-OCH₂), 66.5 (1''-CH₂), 108.1 (4'-C), 113.9 (5-C), 125.4 (5'-CH), 131.1 (3'-CH), 137.9 (8-CH), 140.2 (2'-C), 147.5 (C=O), 153.8 (4-C), 159.6 (2-C), 160.7 (6-C).

Found: C, 46.01; H, 4.66; N, 16.92 %. $C_{19}H_{23}BrN_6O_3S$ requires C, 46.06; H, 4.68; N, 16.96 %

(This compound proved too unstable to allow for the measurement of accurate mass)

O⁶-(4-Bromophenyl)-9-(N-methyl-4-piperidylloxycarbonyl)guanine (42) – This compound was prepared following the above procedure, but using (39) (28 mg, 0.1 mmol) instead of (37). Following the removal of DMF, acetonitrile was added to the residue, and the white solid, which separated, was the free base (42) (25 mg, 54%).

$\nu_{\max}$: 1583, 1620, 1765(C=O) cm⁻¹

$\lambda_{\max}$: 282nm

m.p. 180 °C

$\delta^1\text{H}$ (400MHz, DMSO): 1.80 (2H, br s, 3_{ax}'', 5_{ax}''-CH), 1.95 (2H, br s, 3_{eq}'', 5_{eq}''-CH), 2.20 (3H, s, NCH₃), 2.24 (2H, br s, 2_{ax}'', 6_{ax}''-CH), 2.61 (2H, br s, 2_{eq}'', 6_{eq}''-CH), 4.95 (1H, br s, 4''-CH), 5.65 (2H, s, 6-OCH₂), 6.83 (2H, s, NH₂), 7.36 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.22 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 29.5 (3'', 5''-CH₂), 45.2 (NCH₃), 51.3 (2'', 6''-CH₂), 60.3 (6-OCH₂), 73.3 (4''-CH), 107.5 (4'-C), 113.4 (5-C), 124.7 (5'-CH), 130.5 (3'-CH), 137.3 (8-CH), 139.6 (2'-C), 146.1 (C=O), 153.3 (4-C), 159.1 (2-C), 160.1 (6-C).

Found: C, 43.55; H, 4.00; N, 17.68 %. C₁₇H₁₉BrN₆O₃S requires C, 43.69; H, 4.10; N, 17.98 %

MS (ES) [M+H]⁺ Calcd m/z for C₁₇H₂₀BrN₆O₃S 467.0501, Found 467.0525.

O⁶-(4-Bromophenyl)-9-(3-ethoxypropoxycarbonyl)guanine (43b) – This compound was prepared following the above procedure, but using 3-ethoxypropyl 4-nitrophenyl carbonate (27 mg, 0.1 mmol) instead of (37). After the removal of DMF, ethyl acetate was added to the residue followed by hexane to precipitate (43b) (22 mg, 49%) as a white solid.

$\nu_{\max}$: 1578, 1632, 1776(C=O) cm⁻¹

$\lambda_{\max}$: 287nm

m.p. 118 °C

$\delta^1\text{H}$ (400MHz, DMSO): 1.06 (3H, t, $J=7.0$, CH_3), 1.97 (2H, m, $2''\text{-CH}_2$), 3.10 (2H, q, $J=7.0$, $1'''\text{-CH}_2$), 3.45 (2H, t, $J=6.0$, $3''\text{-CH}_2$), 4.43 (2H, t, $J=6.5$, $1''\text{-CH}_2$), 5.65 (2H, s, 6-OCH_2), 6.84 (2H, s, NH_2), 7.36 (1H, s, $3'\text{-CH}$), 7.70 (1H, s, $5'\text{-CH}$), 8.22 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 15.0 (CH_3), 28.4 ($2''\text{-CH}_2$), 60.9 (6-OCH_2), 65.3 ($1''\text{-CH}_2$), 66.2 ($3''$, $1'''\text{-CH}_2$), 108.1 ($4'\text{-C}$), 113.9 (5-C), 125.3 ($5'\text{-CH}$), 131.0 ($3'\text{-CH}$), 137.9 (8-CH), 140.2 ($2'\text{-C}$), 147.6 (C=O), 153.8 (4-C), 159.7 (2-C), 160.7 (6-C).

Found: C, 41.50; H, 3.87; N, 15.29 %. $\text{C}_{16}\text{H}_{18}\text{BrN}_5\text{O}_4\text{S}\cdot 0.2 \text{H}_2\text{O}$ requires C, 41.81; H, 4.03; N, 15.24 %

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{16}\text{H}_{19}\text{BrN}_5\text{O}_4\text{S}$ 456.0341, Found 456.0374.

O⁶-(4-Bromophenyl)-9-(N-methyl-4-piperidylloxycarbonyl)guanine citrate (44) -

Citric acid (10 mg, 0.05 mmol) was added to a solution of *O⁶-(4-bromophenyl)-9-(N-methyl-4-piperidylloxycarbonyl)guanine (42)* (23.4 mg, 0.05 mmol) in methanol (2 ml). After 10 min the solvent was removed under reduced pressure. Ether was added to the residue and the citrate precipitated. The salt was collected and dried to afford **(44)** (31 mg, 95%).

ν_{max} : 1580, 1626, 1703, 1770(C=O) cm^{-1}

λ_{max} : 282nm

m.p. decomp. > 70 °C

$\delta^1\text{H}$ (400MHz, DMSO): 1.92 (4H, br s, $3''$, $5''\text{-CH}_2$), 2.60 (4H, br s, citric acid- CH_2), 2.68 (3H, s, NCH_3), 3.04 (2H, br s, $2_{\text{ax}}''$, $6_{\text{ax}}''\text{-CH}$), 3.12 (2H, br s, $2_{\text{eq}}''$, $6_{\text{eq}}''\text{-CH}$), 5.15 (1H, s, $4''\text{-CH}$), 5.66 (2H, s, 6-OCH_2), 6.85 (2H, s, NH_2), 7.36 (1H, s, $3'\text{-CH}$), 7.71 (1H, s, $5'\text{-CH}$), 8.35 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 27.7 (3'', 5''-CH₂), 43.4 (NCH₃), 43.9 (citric acid-CH₂), 50.0 (2'', 6''-CH₂), 60.9 (6-OCH₂), 70.2 (4''-CH), 71.4 (citric acid-C), 108.1 (4'-C), 113.9 (5-C), 125.4 (5'-CH), 131.1 (3'-CH), 138.2 (8-CH), 140.2 (2'-C), 146.5 (C=O), 153.9 (4-C), 159.7 (2-C), 160.7 (6-C), 171.2 (citric acid-COOH), 176.4 (citric acid-COO⁻).

Found: **C**, 40.16; **H**, 4.04; **N**, 12.22 %. C₂₃H₂₇BrN₆O₁₀S·1.5 H₂O requires **C**, 40.27; **H**, 4.19; **N**, 12.25 %

2-Adamantyl chloroformate (48)⁴⁸ – A solution of bis(trichloromethyl) carbonate (326 mg, 1.1 mmol) and pyridine (243 μl , 3 mmol) in dry DCM (5 ml) was added to an ice-cold solution of 2-adamantanol (456 mg, 3 mmol) in dry DCM (7 ml). The reaction was stirred at room temperature for 2 h. The solvent was removed under reduced pressure. Ethyl acetate (15 ml) was added to the residue, and after 10 min the precipitate, pyridine hydrochloride, was collected. The solvent was removed from the filtrate to leave the product (580 mg, 90%), an oil which later solidified.

ν_{max} : 1774(C=O) cm⁻¹ (lit.⁴⁸ 1778 cm⁻¹)

9-(2-Adamantyloxycarbonyl)-O⁶-(4-bromophenyl)guanine (49) – **Method (a)**: 2-Adamantyl chloroformate (**48**) (43 mg, 0.2 mmol) was added to a solution of PaTrin-2 (66 mg, 0.2 mmol) and triethylamine (28 μl , 0.2 mmol) in DMF (1 ml) under nitrogen. The reaction was stirred at room temperature for 15 min. The solvent was removed under reduced pressure. The residue was a mixture of the N-9 and N-7 isomers (**49**) and (**50**)[†]. The N-9 isomer (**49**) (40 mg, 40%) was isolated by flash chromatography [hexane:EtOH; 4:1].

Method (b): The 2-adamantylmethyylimidazolium salt (**56**) (30 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and triethylamine (14 μl , 0.1

mmol) in DMF (1 ml). After 1 h the solvent was removed to give a mixture containing the N-9 and N-7 isomers (**49**) and (**50**). These were not separated, but were characterized by ^1H NMR.

ν_{max} : 1620, 1768(C=O) cm^{-1}

λ_{max} : 271nm

m.p. 220 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 1.57 (2H, m, $7_{\text{ax}}''$, $9_{\text{ax}}''\text{-CH}$), 1.75 (8H, m, $5''$, $8''\text{-CH}$, $4''$, $6''$, $10''\text{-CH}_2$), 2.10 (4H, m, $1''$, $3''\text{-CH}$, $7_{\text{eq}}''$, $9_{\text{eq}}''\text{-CH}$), 5.11 (1H, s, $2''\text{-CH}$), 5.66 (2H, s, 6-OCH_2), 6.77 (2H, s, NH_2), 7.37 (1H, s, $3'\text{-CH}$), 7.71 (1H, s, $5'\text{-CH}$), 8.27 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 26.3 ($5''\text{-CH}_2$), 26.6 ($8''\text{-CH}_2$), 31.1 ($1''$, $3''\text{-CH}_2$), 31.2 ($7''$, $9''\text{-CH}_2$), 35.6 ($4''$, $6''\text{-CH}_2$), 36.8 ($10''\text{-CH}_2$), 60.9 (6-OCH_2), 80.8 ($2''\text{-CH}$), 108.1 ($4'\text{-C}$), 114.0 (5-C), 125.3 ($5'\text{-CH}$), 131.0 ($3'\text{-CH}$), 137.8 (8-CH), 140.2 ($2'\text{-C}$), 146.8 (C=O), 153.8 (4-C), 159.7 (2-C), 160.7 (6-C).

Found: C, 49.22; H, 4.30; N, 13.40 %. $\text{C}_{21}\text{H}_{22}\text{BrN}_5\text{O}_3\text{S}\cdot 0.25 \text{H}_2\text{O}$ requires C, 49.60; H, 4.46; N, 13.78 %.

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{21}\text{H}_{23}\text{BrN}_5\text{O}_3\text{S}$ 504.0705, Found 504.0697.

♦ **7-(2-Adamantyloxycarbonyl)-O⁶-(4-bromophenyl)guanine (50)** – Approximately 2 mg of the N-7 isomer was isolated by flash chromatography [hexane:EtOH; 4:1] and the isomer was characterized by ^1H NMR.

$\delta^1\text{H}$ (400MHz, DMSO): 1.54 (2H, m, $7_{\text{ax}}''$, $9_{\text{ax}}''\text{-CH}$), 1.74 (8H, m, $5''$, $8''\text{-CH}$, $4''$, $6''$, $10''\text{-CH}_2$), 2.06 (4H, m, $1''$, $3''\text{-CH}$, $7_{\text{eq}}''$, $9_{\text{eq}}''\text{-CH}$), 5.07 (1H, s, $2''\text{-CH}$), 5.63 (2H, s, 6-OCH_2), 6.63 (2H, s, NH_2), 7.33 (1H, s, $3'\text{-CH}$), 7.68 (1H, s, $5'\text{-CH}$), 8.70 (1H, s, 8-CH).

Cyclohexyl chloroformate (**51**) – This compound (445 mg, 91%) was prepared following the above procedure for the preparation of 2-adamantyl chloroformate (**48**), but using cyclohexanol (318 μ l, 3 mmol) instead of 2-adamantanol.

$\nu_{\max}$: 1778(C=O) cm^{-1}

Isopropyl chloroformate (**52**) – This compound was prepared following the above procedure for the preparation of 2-adamantyl chloroformate (**48**), but using isopropanol (230 μ l, 3 mmol) instead of 2-adamantanol. Due to the volatility of (**52**) it was subsequently used as a solution in ethyl acetate.

$\nu_{\max}$: 1776(C=O) cm^{-1}

2-Adamantylmethylimidazolium salt (**56**) – *N*-Methyl imidazole (16 μ l, 0.2 mmol) was added to a solution of 2-adamantyl chloroformate (**48**) (43 mg, 0.2 mmol) in dry DCM (2 ml) at 0 $^{\circ}\text{C}$. The reaction was set aside at room temperature for 15 min. The salt (**56**) (42 mg, 71%) separated upon the addition of hexane.

$\nu_{\max}$: 1784(C=O) cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 1.59 (2H, m, $7_{\text{ax}}''$, $9_{\text{ax}}''$ -CH), 1.76 (8H, m, $5''$, $8''$ -CH, $4''$, $6''$, $10''$ -CH₂), 2.13 (4H, m, $1''$, $3''$ -CH, $7_{\text{eq}}''$, $9_{\text{eq}}''$ -CH), 3.96 (3H, s, CH₃), 5.21 (1H, s, $2''$ -CH), 7.89 (1H, s, arom. 5-CH), 8.18 (1H, s, arom. 4-CH), 9.91 (1H, s, arom. 2-CH).

O⁶-(4-Bromophenyl)-9-(4-nitrobenzyloxycarbonyl)guanine (**60**) – 4-Nitrobenzyl chloroformate (22 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and triethylamine (14 μ l, 0.1 mmol) in DMF (0.5 ml) under nitrogen, and stirring continued for 15 min. The solvent was evaporated and ethyl acetate added to the residue. The derivative (**60**) (32 mg, 63%) separated upon the addition of hexane.

$\nu_{\max}$: 1580, 1626, 1752(C=O) cm^{-1}

$\lambda_{\max}$: 293nm

m.p. 198 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 5.64 (2H, s, OCH_2Ar), 5.67 (2H, s, 6- OCH_2), 6.93 (2H, s, NH_2), 7.37 (1H, s, 3'-CH), 7.72 (1H, s, 5'-CH), 7.89 (2H, d, $J=8.5$, arom. 2'', 6''-CH), 8.32 (1H, s, 8-CH), 8.33 (2H, d, $J=8.5$, arom. 3'', 5''-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 61.0 (6- OCH_2), 67.5 (OCH_2Ar), 108.1 (4'-C), 114.0 (5-C), 123.7 (arom. 3'', 5''-CH), 125.3 (5'-CH), 128.2 (arom. 2'', 6''-CH), 131.1 (3'-CH), 137.9 (8-CH), 140.2 (2'-C), 142.7 (1''-C), 147.2 (4''-C), 147.7 (C=O), 153.7 (4-C), 159.8 (2-C), 160.7 (6-C).

Found: C, 42.58; H, 2.49; N, 16.98 %. $\text{C}_{18}\text{H}_{13}\text{BrN}_6\text{O}_5\text{S}$ requires C, 42.78; H, 2.59; N, 16.63 %.

(This compound proved too unstable to allow for the measurement of accurate mass)

O⁶-(4-Bromophenyl)-9-(phenoxycarbonyl)guanine (63) – This compound was prepared following the above procedure, but using phenyl chloroformate (13 μl , 0.1 mmol) instead of 4-nitrobenzyl chloroformate. After the removal of the DMF, ethanol was added to precipitate (63) (40 mg, 90%) as a white solid.

$\nu_{\max}$: 1579, 1643, 1783(C=O) cm^{-1}

$\lambda_{\max}$: 276nm

m.p. 202 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 5.68 (2H, s, 6- OCH_2), 6.93 (2H, s, NH_2), 7.37 – 7.44 (4H, m, 3'-CH, arom. 2'', 4'', 6''-CH), 7.50 (2H, m, arom. 3'', 5''-CH), 7.71 (1H, s, 5'-CH), 8.42 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 61.0 (6-OCH₂), 108.1 (4'-C), 114.0 (5-C), 121.7 (arom. 2'', 6''-CH), 125.3 (5'-CH), 126.8 (4''-CH), 129.7 (arom. 3'', 5''-CH), 131.0 (3'-CH), 138.2 (8-CH), 140.2 (2'-C), 146.3 (arom. 1''-C), 149.9 (C=O), 153.9 (4-C), 159.8 (2-C), 160.8 (6-C).

Found: C, 45.45; H, 2.61; N, 15.72 %. C₁₇H₁₂BrN₅O₃S requires C, 45.75; H, 2.71; N, 15.69 %.

MS (ES) [M+H]⁺ Calcd m/z for C₁₇H₁₃BrN₅O₃S 445.9922, Found 445.9950.

O⁶-(4-Bromophenyl)-9-[phenoxy(thiocarbonyl)]guanine (64) – This compound was prepared following the above procedure, but using phenyl chlorothionoformate (14 μ l, 0.1 mmol) instead of 4-nitrobenzyl chloroformate. After the removal of the DMF, ethanol was added to precipitate (**64**) (42 mg, 90%) as a white solid.

ν_{max} : 1581, 1622 cm⁻¹

λ_{max} : 276nm

m.p. decomp. > 60 °C

$\delta^1\text{H}$ (400MHz, DMSO): 5.68 (2H, s, 6-OCH₂), 6.94 (2H, s, NH₂), 7.34 – 7.40 (4H, m, 3'-CH, arom. 2'', 4'', 6''-CH), 7.53 (2H, m, arom. 3'', 5''-CH), 7.72 (1H, s, 5'-CH), 8.61 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 61.0 (6-OCH₂), 108.02 (4'-C), 114.2 (5-C), 122.1 (arom. 2'', 6''-CH), 125.3 (5'-CH), 127.0 (4''-CH), 129.8 (arom. 3'', 5''-CH), 131.0 (3'-CH), 139.3 (8-CH), 140.0 (2'-C), 152.7 (1''-C), 153.5 (4-C), 159.8 (2-C), 160.8 (6-C), 183.8 (C=S).

Found: C, 43.40; H, 2.54; N, 15.08 %. C₁₇H₁₂BrN₅O₂S₂·0.25 H₂O requires C, 43.73; H, 2.70; N, 15.01 %.

MS (ES) [M+H]⁺ Calcd m/z for C₁₇H₁₃BrN₅O₂S₂ 461.9694, Found 461.9654.

Morpholinohex-1-yl chloroformate (65) – A solution of 6-morpholinohexan-1-ol (18.7 mg, 0.1 mmol) in dry DCM (0.5 ml) was added dropwise to a solution of bis(trichloromethyl) carbonate (11 mg, 0.034 mmol) in dry DCM (0.5 ml) at -5°C . After 20 min at room temperature the solvent was removed to leave a mixture containing **(65)**.

ν_{max} : 1776 cm^{-1}

2-Morpholinoethyl chloroformate (66) – This compound was prepared following the above procedure, but using 2-morpholinoethanol (12 μl , 0.1 mmol) instead of 6-morpholinohexan-1-ol.

ν_{max} : 1756 cm^{-1}

3-Dimethylaminopropyl chloroformate (67) – This compound was prepared following the above procedure, but using 3-dimethylaminopropan-1-ol (12 μl , 0.1 mmol) instead of 6-morpholinohexan-1-ol.

ν_{max} : 1758 cm^{-1}

3-(N,N-Dimethylamino)propyl N-(4-chlorophenyl) carbamate (76) – 4-Chloroaniline (13 mg, 0.1 mmol) and triethylamine (28 μl , 0.2 mmol) were added to a solution of a crude sample of 3-dimethylaminopropyl chloroformate **(67)** (0.1 mmol) in dry DMF (1 ml). The reaction was set aside for 2 days. The solvent was removed under reduced pressure and DCM added to the residue. The compound **(76)** separated upon the addition of hexane.

ν_{max} : 1745 cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 2.03 (2H, m, 2- CH_2), 2.73 (6H, s, $\text{N}(\text{CH}_3)_2$), 3.09 (2H, m, 3- NCH_2), 4.15 (2H, t, $J=6.3$, 1- OCH_2), 7.26 (2H, d, $J=8.5$, arom. 2', 6'- CH), 7.46 (2H, d, $J=8.5$, arom. 3', 5'- CH).

3,3,5-Trimethyl-2-oxooxazolidinium chloride (80)⁶² – A solution of 1-dimethylaminopropan-2-ol (1.03 g, 10 mmol) and pyridine (809 μl , 10 mmol) in dry DCM (10 ml) was added dropwise to a solution of bis(trichloromethyl) carbonate (1 g, 3.4 mmol) in dry DCM (10 ml) at -40°C . The reaction was warmed to room temperature and stirred for 2 h. The precipitate was collected. It was washed with DCM to remove pyridine hydrochloride. The remaining solid (**80**) was kept in an evacuated container until required for use.

ν_{max} : 1845 cm^{-1} (lit.⁶² 1845 cm^{-1})

O⁶-(4-Bromophenyl)-9-(2-dimethylaminoisopropoxycarbonyl)guanine (82) – 3,3,5-Trimethyl-2-oxooxazolidinium chloride (**80**) (17 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and DBU (15 μl , 0.1 mmol) in dry DMF (0.5 ml) at -5°C . After 15 min at room temperature the solvent was removed to give a mixture containing (**82**). Compound (**82**) could not be isolated but was characterized by the ^1H NMR signals at δ_{H} 6.91 (NH_2) and 8.34 (8- CH) in a spectrum of the crude material.

5 α -Androstan-3-one 17 β -chloroformate (88)⁴⁵ – A solution of bis(trichloromethyl) carbonate (66 mg, 0.22 mmol) and pyridine (48.6 μl , 0.6 mmol) in dry DCM (1 ml), was added to an ice-cold solution of dihydrotestosterone (174 mg, 0.6 mmol) in dry DCM (1.5 ml). The reaction was stirred at room temperature for 2 h. The solvent was

removed under reduced pressure. Ethyl acetate was added, and the pyridine hydrochloride, which precipitated was collected. The solvent was removed from the filtrate to afford (**88**) (180 mg, 85%).

ν_{max} : (C=O) 1701, 1774 cm^{-1} (lit.⁴⁵ 1766 cm^{-1} , KBr)

O⁶-(4-Bromophenyl)-9-(3-oxo-5 α -androstan-17 β -yl-oxycarbonyl)guanine (87**)^{*}** -

5 α -androstan-3-one 17 β -chloroformate (**88**) (176 mg, 0.5 mmol) was added to a solution of PaTrin-2 (163 mg, 0.5 mmol) and triethylamine (70 μl , 0.5 mmol) in DMF (2.5 ml) under nitrogen. After 15 min the solvent was evaporated and TLC (5% EtOH in DCM) showed the presence of a mixture of the N-9 and N-7 isomers. The isomers were separated by flash chromatography, to afford pure (**87**) (80 mg, 25%).

ν_{max} : (C=O) 1704 cm^{-1} , 1768 cm^{-1}

λ_{max} : 275nm

m.p. 110 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 0.93 (3H, s, 18''-CH₃), 1.01 (3H, s, 19''-CH₃), 1.16 – 2.33 (24H, m, steroid backbone), 4.73 (1H, t, $J=10.9$, 17''-CH), 5.64 (2H, s, 6-OCH₂), 6.79 (2H, s, NH₂), 7.35 (1H, s, 3'-CH), 7.72 (1H, s, 5'-CH), 8.19 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 11.1 (18-CH₃), 12.1 (19-CH₃), 20.5 (CH₂), 23.1 (CH₂), 26.9 (CH₂), 28.2 (CH₂), 30.8 (CH₂), 34.6 (CH), 35.3 (C), 36.2 (CH₂), 36.6 (CH₂) 37.7 (CH₂), 37.9 (CH₂), 42.6 (C), 44.2 (CH₂), 45.9 (CH), 49.6 (CH), 52.9 (CH), 60.9 (6-OCH₂), 86.2 (17''-CH), 108.1 (4'-C), 113.9 (5-C), 125.4 (5'-CH), 131.1 (3'-CH), 137.7 (8-CH), 140.2 (2'-C), 147.3 (C=O), 153.8 (4-C), 159.6 (2-C), 160.6 (6-C), 210.5 (3''-C=O).

^{*} Compound identical to that prepared by Dr. D.J. Donnelly.

Found: **C**, 56.37; **H**, 5.70; **N**, 10.46 %. $C_{30}H_{36}BrN_5O_4S$ requires **C**, 56.07; **H**, 5.65; **N**, 10.90 %.

MS (ES) $[M+H]^+$ Calcd m/z for $C_{30}H_{37}BrN_5O_4S$ 642.1750, Found 462.1798.

3-(*t*-Butyldimethylsiloxy)-17 β -hydroxyoestra-1,3,5(10)-triene (91)⁶⁰ – A suspension of NaH (60% in oil; 50mg, 1.25mmol) in dry THF (2ml) was added to β -oestradiol (272mg, 1mmol) in dry THF (2ml). This mixture was stirred for 0.5 h and then *t*-BuMe₂SiCl (176mg, 1.17mmol) was added. The reaction was stirred at room temperature for 3 h. Hydrolysis was carried out by adding a small amount of ice water (10ml). The product was taken up in DCM (10 ml) and dried over MgSO₄. After evaporation, the residue was recrystallised from light petroleum (b.p. 40 - 60 °C), to give **(91)** (270 mg, 70%).

m.p. 160 °C (lit.⁶⁰ 158 °C)

δ^1H (400MHz, DMSO): 0.16 (6H, s, 2 x CH₃), 0.67 (3H, s, CH₃), 0.95 (9H, s, 3 x CH₃), 1.25 – 2.73 (15H, m, steroid backbone) 3.57 (1H, t, $J=10.9$, 17-CH), 4.46 (1H, s, 17-OH), 6.50 (1H, s, arom. 4-CH), 6.61 (1H, d $J=8.2$, arom. 2-CH), 7.10 (1H, d, $J=8.5$, arom. 1-CH).

3-(Dimethyl-*t*-butylsiloxy)-oestra-1,3,5(10)-triene-17 β -yl chloroformate (92) – This compound was prepared following the above procedure used in the preparation of **(88)**, but using **(91)** (270 mg, 0.7 mmol) instead of dihydrotestosterone. The pyridine hydrochloride could not be separated from the chloroformate in this case, and therefore the mixture was used in subsequent reactions.

ν_{max} : 1791(C=O) cm⁻¹

O⁶-(4-Bromophenyl)-9-[3-hydroxyoestra-1,3,5(10)-trien-17 β -yl-oxycarbonyl]

guanine (95) – The mixture containing 3-(dimethyl-*t*-butylsiloxy)-oestra-1,3,5(10)-triene-17 β -yl chloroformate (**92**) (0.7 mmol) was added to a solution of PaTrin-2 (228 mg, 0.7 mmol) and triethylamine (98 μ l, 0.7 mmol) in DMF (4 ml) under nitrogen. After 15 min, the solvent was evaporated and TLC (5% EtOH in DCM) showed the presence of both the N-9 (**93**) and N-7 (**94**) isomers[†]. The reaction mixture in dry THF (2 ml) was added to an ice-cold solution of tetrabutylammonium fluoride in THF (1 M; 700 μ l). After 15 min at 0 °C, an excess of NH₄Cl solution was added, and the product extracted into ether. Drying (MgSO₄) and evaporation gave a mixture that was separated by flash chromatography (5% EtOH in DCM) to afford pure samples of both the N-9 isomer (**95**) (44 mg, 10%) and the N-7 isomer (**96**) (66 mg, 15%).

$\nu_{\max}$: 1579, 1608, 1645, 1776(C=O) cm⁻¹

$\lambda_{\max}$: 277nm

m.p. 180 °C

$\delta^1\text{H}$ (400MHz, DMSO): 0.95 (3H, s, CH₃), 1.15 – 2.67 (15H, m, steroid backbone), 4.83 (1H, t, *J*=8.0, 17''-CH), 5.65 (2H, s, 6-OCH₂), 6.45 (1H, s, arom. 4''-CH), 6.51 (1H, d, *J*=8.1, arom. 2''-CH), 6.79 (2H, s, NH₂), 7.03 (1H, d, *J*=8.1, arom. 1''-CH), 7.35 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.21 (1H, s, 8-CH), 8.96 (1H, s, 3''-OH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 12.1 (CH₃), 22.8 (CH₂), 25.9 (CH₂), 26.8 (CH₂), 27.0 (CH₂), 29.1 (CH₂), 36.2 (CH₂), 38.3 (CH), 42.9 (C), 43.2 (CH), 48.7 (CH), 60.9 (6-OCH₂), 86.1 (17''-CH), 108.1 (4'-C), 112.8 (5'-C), 113.9 (arom. 2''-CH), 114.9 (arom. 4''-CH), 125.4 (5'-CH), 126.1 (arom. 1''-CH), 130.1 (10''-C), 131.1 (3'-CH), 137.1 (5''-C), 137.8 (8-CH), 140.2 (2'-C), 147.3 (C=O), 155.0 (3''-C), 155.0 (4-C), 159.7 (2-C), 160.6 (6-C).

Found: **C**, 50.76; **H**, 4.39; **N**, 9.80 %. $C_{29}H_{30}BrN_5O_4S \cdot 3.5 H_2O$ requires **C**, 50.65; **H**, 5.42; **N**, 10.19 %.

MS (ES) $[M+H]^+$ Calcd m/z for $C_{29}H_{31}BrN_5O_4S$ 624.1280, Found 624.1315.

O⁶-(4-Bromophenyl)-7-[3-hydroxyoestra-1,3,5(10)-trien-17 β -yl-oxycarbonyl]

guanine (96)

$\nu_{\max}$: 1574, 1612, 1774(C=O) cm^{-1}

$\lambda_{\max}$: 288, 302nm

m.p. 132 °C

δ^1H (400MHz, DMSO): 0.82 (3H, s, CH_3), 1.15 – 2.67 (15H, m, steroid backbone), 4.85 (1H, t, $J=8.0$, 17''-CH), 5.65 (2H, s, 6-OCH₂), 6.45 (1H, s, arom. 4''-CH), 6.51 (1H, d, $J=8.0$, arom. 2''-CH), 6.66 (2H, s, NH₂), 7.05 (1H, d, $J=8.5$, arom. 1''-CH), 7.36 (1H, s, 3'-CH), 7.70 (1H, s, 5'-CH), 8.68 (1H, s, 8-CH), 9.02 (1H, s, 3''-OH).

$\delta^{13}C$ (100MHz, DMSO): 11.9 (CH_3), 22.7 (CH_2), 25.8 (CH_2), 26.6 (CH_2), 26.8 (CH_2), 29.1 (CH_2), 36.2 (CH_2), 38.3 (CH), 43.0 (C), 43.1 (CH), 48.8 (CH), 61.2 (6-OCH₂), 86.8 (17''-CH), 102.5 (4'-C), 108.0 (5'-C), 112.8 (arom. 2''-CH), 114.9 (arom. 4''-CH), 125.4 (5'-CH), 126.1 (arom. 1''-CH), 130.1 (10''-C), 131.1 (3'-CH), 137.1 (5''-C), 140.3 (2'-C), 147.1 (8-CH), 147.6 (C=O), 155.0 (3''-C), 155.9 (4-C), 160.4 (2-C), 165.9 (6-C).

Found: **C**, 56.25; **H**, 5.16; **N**, 10.29 %. $C_{29}H_{30}BrN_5O_4S$ requires **C**, 55.77; **H**, 4.84; **N**, 11.21 %.

MS (ES) $[M+H]^+$ Calcd m/z for $C_{29}H_{31}BrN_5O_4S$ 624.1280, Found 624.1234.

*In another experiment, the silylated N-9 and N-7 derivatives (**93**) and (**94**) were separated by flash chromatography [hexane:EtOH; 4:1]. This resulted in several

fractions including the pure N-9 and N-7 silylated derivatives (**93**) and (**94**). These compounds were characterized by ^1H NMR.

O⁶-(4-Bromophenyl)-9-[3-(dimethyl-*t*-butylsiloxy)-oestra-1,3,5(10)-trien-17 β -yl-oxycarbonyl] guanine (93**):** $\delta^1\text{H}$ (400MHz, DMSO): 0.16 (6H, s, (CH₃)₂), 0.68 (3H, s, CH₃), 0.95 (9H, s, (CH₃)₃), 1.11 – 2.68 (15H, m, steroid backbone), 4.86 (1H, m, 17''-CH), 5.65 (2H, s, 6-OCH₂), 6.45 (1H, s, arom. 4''-CH), 6.53 (1H, d, *J*=8.0, arom. 2''-CH), 6.80 (2H, s, NH₂), 7.05 (1H, d, *J*=8.0, arom. 1''-CH), 7.36 (1H, s, 3'-CH), 7.70 (1H, s, 5'-CH), 8.22 (1H, s, 8-CH).

O⁶-(4-Bromophenyl)-7-[3-(dimethyl-*t*-butylsiloxy)-oestra-1,3,5(10)-trien-17 β -yl-oxycarbonyl] guanine (94**):** $\delta^1\text{H}$ (400MHz, DMSO): 0.17 (6H, s, (CH₃)₂), 0.84 (3H, s, CH₃), 0.95 (9H, s, (CH₃)₃), 1.17 – 2.67 (15H, m, steroid backbone), 4.86 (1H, m, 17''-CH), 5.65 (2H, s, 6-OCH₂), 6.53 (1H, s, arom. 4''-CH), 6.61 (2H, m, arom. 2''-CH, NH₂), 7.14 (1H, d, *J*=8.0, arom. 1''-CH), 7.35 (1H, s, 3'-CH), 7.69 (1H, s, 5'-CH), 8.67 (1H, s, 8-CH).

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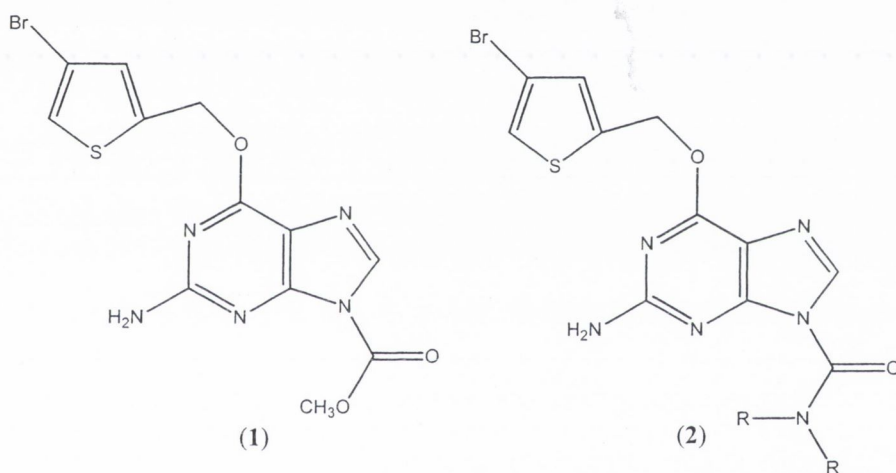
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CHAPTER 3

Chapter 3: Synthesis of *O*⁶-(4-bromophenyl)-9-(dialkylcarbamoyl)guanines

3.1 Introduction

In Chapter 2 we discussed the synthesis of a series of N-9 alkoxy carbonyl derivatives as potential prodrugs. The impetus for this research came from the observed stability of *O*⁶-(4-bromophenyl)-9-(methoxycarbonyl)guanine (1) under physiological conditions (pH=7.4, T=37 °C). In this chapter we will discuss the synthesis of PaTrin-2 N-9 dialkylcarbamoyl derivatives (2). Prior to this investigation, an example of such a N-9 carbamoyl derivative had not been prepared. Therefore, we had no indication of the stability of this category of PaTrin-2 prodrug.



The lability of both N-9 carbamoyl and alkoxy carbonyl derivatives is dependent on the reactivity of the carbonyl group. This reactivity is associated with the electronic effects of the groups attached.¹ Electron-donating groups reduce the reactivity. Electron-withdrawing groups make the carbonyl more positive, and therefore more reactive. Although oxygen and nitrogen, as in (1) and (2), exert an electron-withdrawing inductive effect on the carbonyl carbon atom, their unshared pairs of electrons interact to form a π -orbital with the carbonyl carbon atom, decreasing its positive character and therefore its reactivity. Comparing the general stability of N-9 alkoxy carbonyl derivatives with N-9 carbamoyl derivatives, the latter are predicted to

be more stable. Though the unique electronic contribution of the purine ring system cannot be ignored, the inductive effect of the alkyl group(s) attached to the nitrogen atom should increase the electron availability of the nitrogen atom (Fig. 3.1), and should therefore increase the stability of the carbonyl group.

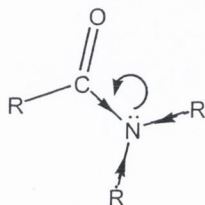


Fig. 3.1

The stability of a carbonyl to hydrolysis, due to the electronic effects of a dialkylamino group, can be exploited successfully in the design of a prodrug. Buur *et al.*^{2,3} reported the pharmacological evaluation of both alkoxycarbonyl and carbamoyl derivatives of 5-fluorouracil to investigate their suitability as prodrugs (Fig. 3.2). Although both categories were found to provide promising prodrug candidates that possessed increased lipophilicity, the dialkylcarbamoyl derivatives ($R = NR_2$) were found to offer greater stability under physiological conditions.

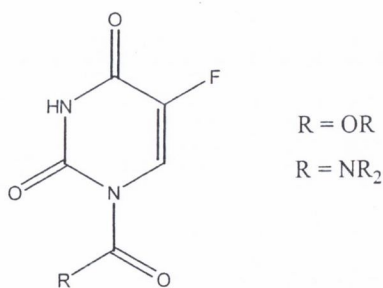


Fig. 3.2

The following sections of this chapter will discuss the synthesis of N-9 dialkylcarbamoyl derivatives of PaTrin-2 as potential prodrugs. It was important to prepare a variety of derivatives to provide pharmacological comparisons. Essential to

this research was the need to prepare derivatives containing a basic tertiary amino group to facilitate the synthesis of water-soluble salts.

3.2 Routes and reagents – a summary

In the previous chapter we outlined a general approach to the synthesis of N-9 alkoxycarbonyl derivatives. The findings of our research paved the way to the successful synthesis of a variety of prodrug candidates. Although details of the chemistry involved in the preparation of N-9 carbamoyl derivatives are significantly different from that discussed in Chapter 2, the basic chemistry is similar and has been outlined.

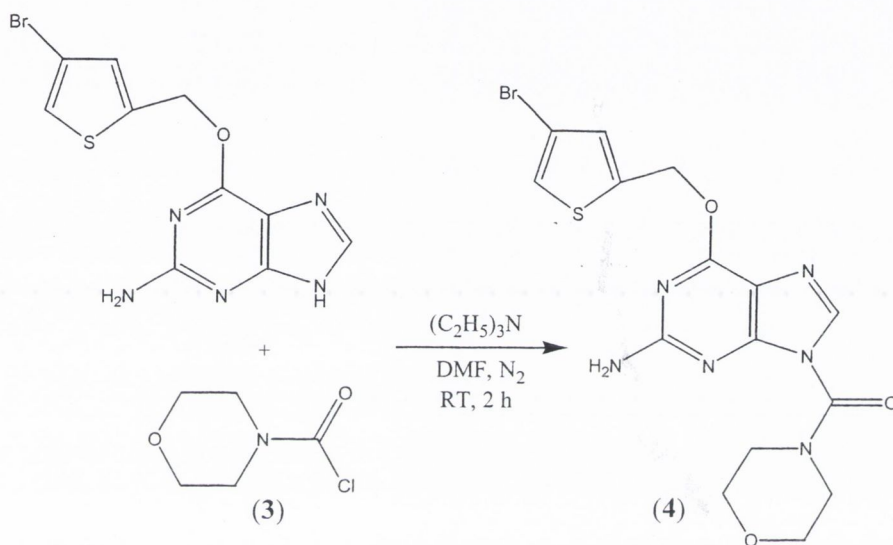
As previously reported, reactions of this type can be brought about by the treatment of PaTrin-2 with an activated derivative (*i.e.* **Route 2**). In the following sections we will discuss the synthesis of N-9 carbamoyl derivatives using this approach. Our investigation of the preparation of N-9 alkoxycarbonyl derivatives focussed on the advantages of using moderately activated derivatives such as those containing a 4-nitrophenoxy group. However, as will be discussed later in this chapter, the stability conferred upon the carbonyl group by the NR₂ group can make such derivatives too stable to undergo the subsequent reaction with PaTrin-2.

The more frequently reported route to the synthesis of related tetrasubstituted ureas involves the use of carbamoyl chlorides. As a result, the sections below will focus mainly on the reaction of carbamoyl chlorides with PaTrin-2, and their preparation from the phosgene source bis(trichloromethyl) carbonate.

3.3 The reaction of PaTrin-2 with carbamoyl chlorides

3.3.1 Synthesis of *O*⁶-(4-bromophenyl)-9-(morpholinocarbonyl)guanine

The reaction of PaTrin-2 with the commercially available morpholine-4-carbonyl chloride (**3**) was carried out at room temperature for 2 hours in the presence of an equimolar amount of triethylamine (Scheme 3.1).



Scheme 3.1

Following the removal of the solvent, ¹H NMR analysis of the crude residue indicated the synthesis of *O*⁶-(4-bromophenyl)-9-(morpholinocarbonyl)guanine (**4**) in almost quantitative yield. Trace amounts of the corresponding N-7 isomer were also observed. We found that the N-9 derivative (**4**) was less soluble in hot ethanol than the N-7 isomer. This allowed us to obtain a high isolated yield of (**4**).

3.3.2 ¹H NMR analysis of *O*⁶-(4-bromophenyl)-9-(morpholinocarbonyl)guanine

N-9 Carbamoyl derivatives, like *O*⁶-(4-bromophenyl)-9-(morpholinocarbonyl)guanine (**4**), are characterized easily by ¹H NMR. As with the characterization of N-9 alkoxycarbonyl derivatives in the previous chapter, the formation of an N-9 derivative is indicated in the downfield region of the ¹H NMR spectrum. The ¹H NMR spectrum

of *O*⁶-(4-bromophenyl)-9-(morpholinocarbonyl)guanine (**4**) is shown below (Fig. 3.3).

The signal assignments for the N-9 derivative (**4**) are given in Table 3.1.

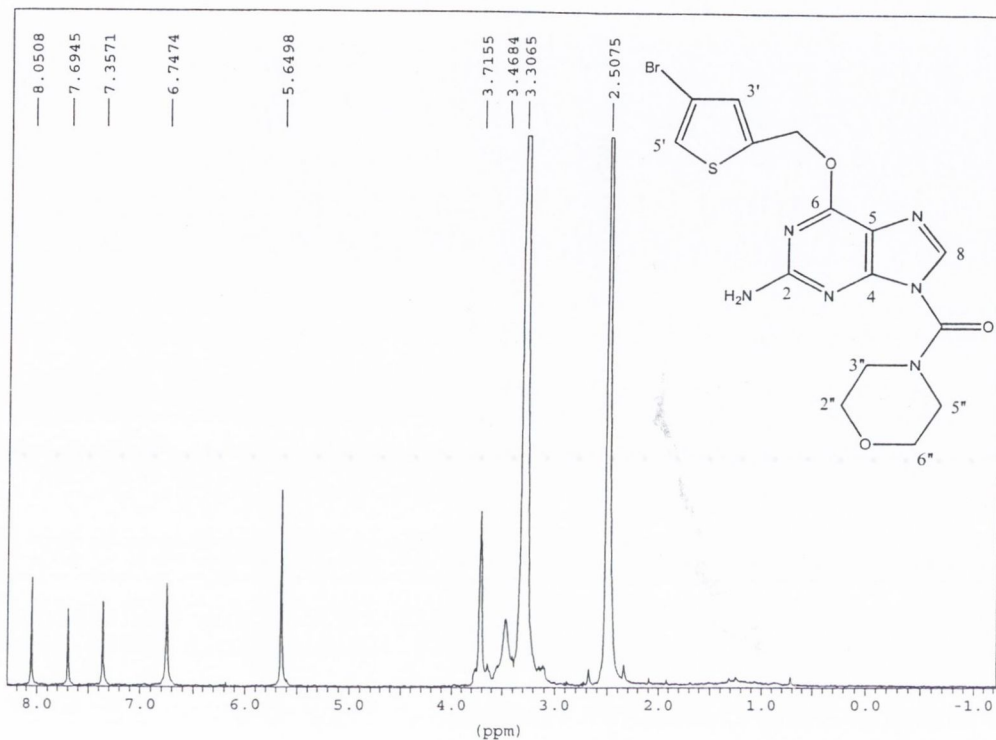


Fig. 3.3

Hydrogen	NMR δ (ppm from TMS)
3''/5''-CH ₂	3.47
2''/6''-CH ₂	3.72
CH ₂	5.65
NH ₂	6.75
H-3'	7.1
H-5'	7.7
H-8	8.05

Table 3.1

As illustrated in Chapter 2 (Section 2.3.1, pg 46), the signals associated with the H-8 and the NH₂ of PaTrin-2 are found at approximately 7.85 ppm and 6.34 ppm respectively. In the ¹H NMR spectrum (Fig. 3.3) of *O*⁶-(4-bromophenyl)-9-(morpholinocarbonyl)guanine (**4**) the H-8 and the NH₂ signals are shifted downfield to 8.05 ppm and 6.75 ppm respectively. The shifts of these signals are typical in the ¹H NMR spectra of all the N-9 carbamoyl derivatives whose synthesis we will discuss in the following sections of this chapter. Features of these and other spectra will be discussed in Section 3.5. Full ¹H and ¹³C NMR spectral assignments of these derivatives will be given in the experimental section of this chapter.*

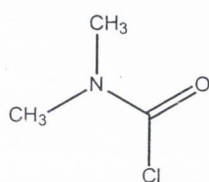
3.3.3 Attempted synthesis of *O*⁶-(4-bromophenyl)-9-(*N,N*-dimethylcarbamoyl)-guanine

Following the successful reaction of PaTrin-2 with morpholine-4-carbonyl chloride (**3**) we aimed to prepare several more examples of N-9 carbamoyl derivatives by the same procedure. Buur *et al.*² reported the pharmacological evaluation of a highly stable *N,N*-dimethylcarbamoyl derivative of 5-fluorouracil (see Fig. 3.2). They proposed that its stability to hydrolysis was associated with the increased steric hindrance caused by the two methyl groups. While this may not be the only reason for the stability of this compound (see Section 3.1), it was thought to be worthwhile to prepare the corresponding PaTrin-2 derivative.

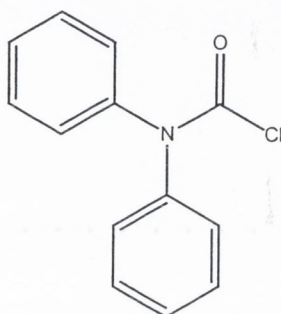
In a reaction analogous to that shown in Scheme 3.1, the commercially available *N,N*-dimethylcarbamoyl chloride (**5**) was added to a solution of PaTrin-2 and an equimolar amount of triethylamine in DMF. However, no reaction was observed after 2 hours at room temperature. The reaction was repeated, but the formation of products was not

* A selection of ¹H and ¹³C NMR from the three categories of prodrug can be found in the Appendix.

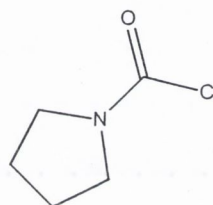
observed after three days or in reactions carried out at elevated temperatures. Both Hünig's base and pyridine were examined as alternative bases to triethylamine, but to no avail. Further investigations also revealed that neither *N,N*-diphenylcarbamoyl chloride (6) or pyrrolidine-1-carbonyl chloride (7) reacted with PaTrin-2 under the above conditions. Moreover, each of these carbamoyl chlorides was shown to react with 4-chloroaniline under equivalent conditions.



(5)



(6)



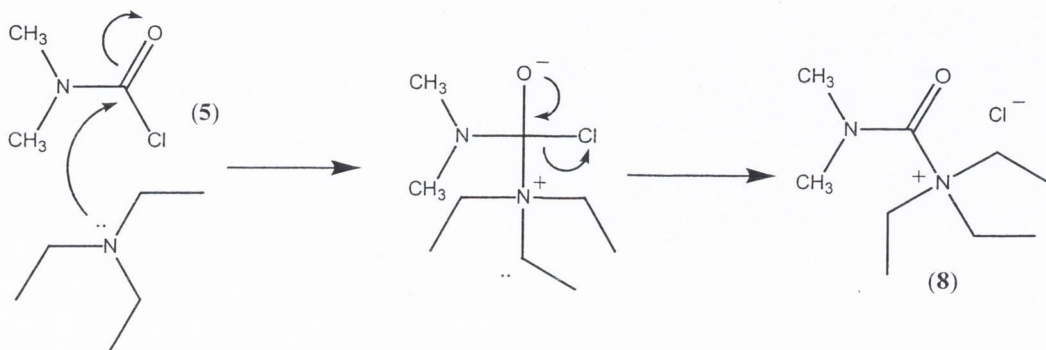
(7)

3.3.4 Steric hindrance

The reaction of morpholine-4-carbonyl chloride (3) with PaTrin-2 had proceeded without difficulty to give the corresponding N-9 derivative in a high yield. However, the carbamoyl chlorides (5), (6) and (7) failed to react with PaTrin-2 in the presence of triethylamine.

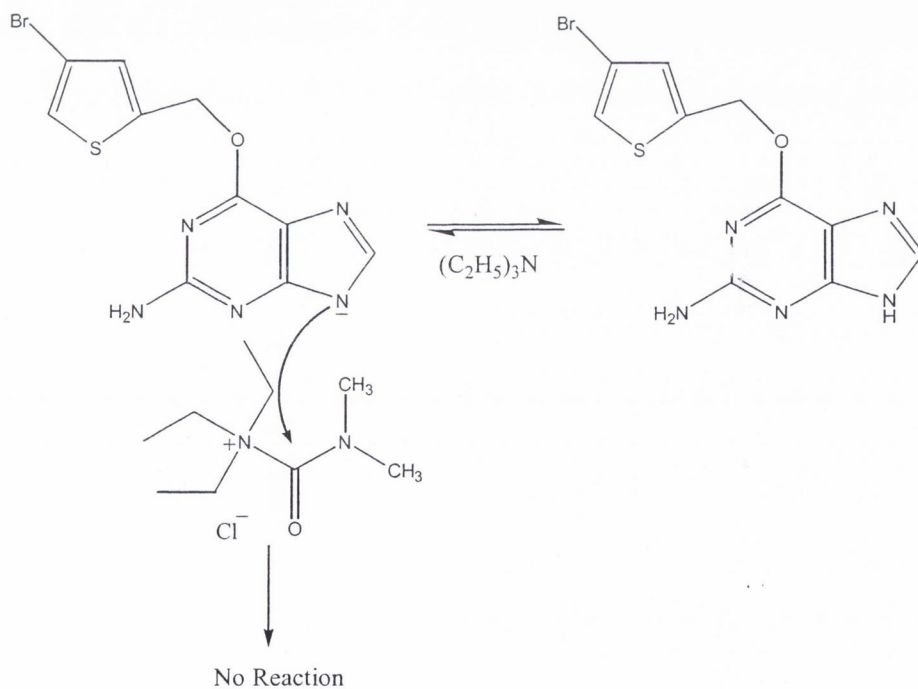
A structural comparison of morpholine-4-carbonyl chloride (3) and *N,N*-dimethylcarbamoyl chloride (5) suggests that the difference in their reactions with PaTrin-2 may be due to steric factors. As discussed above, Buur *et al.*² proposed that the decreased susceptibility of 1-(*N,N*-dimethylcarbamoyl)-5-fluorouracil to hydrolysis was associated with the steric hindrance caused by the two methyl groups. Like the chloroformates discussed in Chapter 2, carbamoyl chlorides are very reactive. Upon the addition of a carbamoyl chloride to a solution containing PaTrin-2 and base, it is likely that the carbamoyl chloride reacts quickly with the base to form a

reactive species that is then attacked by the PaTrin-2 anion. The reactive species (8) would result from the reaction of *N,N*-dimethylcarbamoyl chloride (5) with triethylamine (Scheme 3.2).



Scheme 3.2

An attack by a PaTrin-2 anion on the carbonyl carbon atom of the reactive species (8) would be sterically hindered (Scheme 3.3).



Scheme 3.3

This would also explain the failure to obtain products from the reaction of PaTrin-2 with *N,N*-diphenylcarbamoyl chloride (6) and pyrrolidine-1-carbonyl chloride (7).

The success or failure of the reaction of these carbamoyl chlorides with PaTrin-2 appeared to be dependent on the base. Both triethylamine and Hünig's base contributed to steric hindrance in the reactive species. Therefore the reactions involving these bases failed. Although the corresponding reactive species derived from pyridine would not hinder an attack by the PaTrin-2 anion on the carbonyl carbon, pyridine itself is not strong enough to deprotonate PaTrin-2. Therefore the reactions involving pyridine failed.

Our explanation for the failure of these carbamoyl chlorides to react with PaTrin-2 seemed increasingly likely when we found that each of these reactions proceeded using DBU as a base. DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) (**9**) is of similar basic strength to both triethylamine and Hünig's base (see Table 3.2).⁴

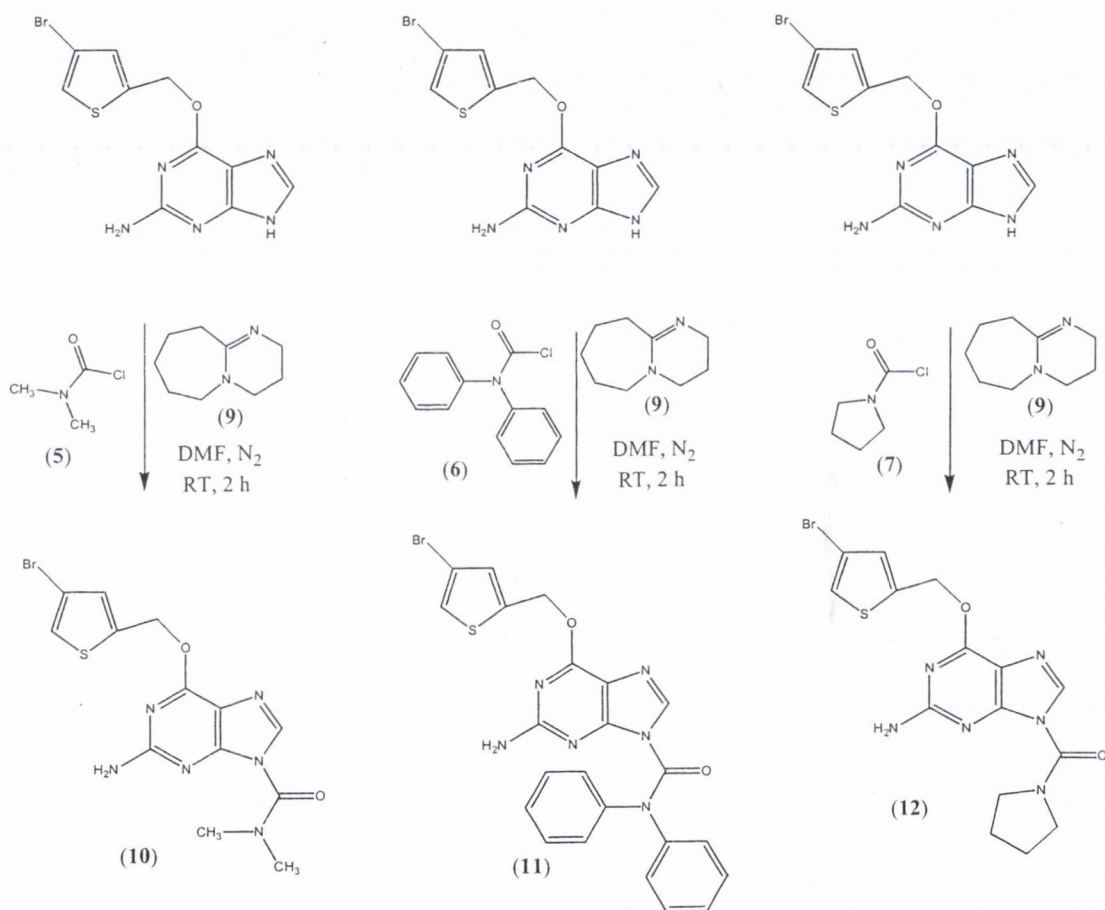
Base	pK _a (of conjugate acid)
Pyridine	5.29
Triethylamine	10.87
Hünig's Base	10.50
DBU	11.50

Table 3.2⁴

However, the reaction of the carbamoyl chlorides *N,N*-dimethylcarbamoyl chloride (**5**), *N,N*-diphenylcarbamoyl chloride (**6**) and pyrrolidine-1-carbonyl chloride (**7**) with DBU (**9**) would afford a reactive species with a sterically more accessible carbonyl carbon atom.

The reactions of the carbamoyl chlorides (**5**), (**6**) and (**7**) with PaTrin-2 were carried out at room temperature in the presence of an equimolar amount of DBU (**9**) (Scheme

3.4). After 2 hours, the solvent was removed, and in each case, ^1H NMR analysis of the crude reaction mixture showed a quantitative yield of the corresponding N-9 derivative. The derivatives O^6 -(4-bromophenyl)-9-(*N,N*-dimethylcarbamoyl)guanine (10), O^6 -(4-bromophenyl)-9-(*N,N*-diphenylcarbamoyl)guanine (11), O^6 -(4-bromophenyl)-9-(1-pyrrolidinylcarbonyl)guanine (12) were each isolated in high yield by precipitation from ethanol. Apart from water, ethanol was the only solvent in which DBU hydrochloride was found to be soluble.



Scheme 3.4

The reaction of PaTrin-2 with morpholine-4-carbonyl chloride (3) was shown to proceed as efficiently in the presence of DBU, as it had done in the presence of triethylamine.

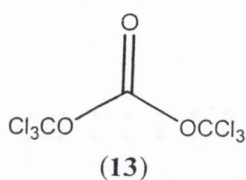
The success of each of the reactions in Scheme 3.4 has been shown to depend on the type of base used. This success is also dependent on the role of the base in the reaction. Ozaki *et al.*⁵ reported the successful reaction of *N,N*-dimethylcarbamoyl chloride (**5**) with 5-fluorouracil, using sodium hydride as a base. We repeated this reaction with PaTrin-2. The procedure resulted in a high yield of *O*⁶-(4-bromophenyl)-9-(*N,N*-dimethylcarbamoyl)guanine (**10**). However, the role of this strong inorganic base is solely to bring about the irreversible deprotonation of PaTrin-2. The PaTrin-2 anion reacts directly with the carbamoyl chloride to give the corresponding N-9 derivative in high yield. No intermediate reactive species is formed.

The reactions discussed above provided us with several derivatives for evaluation as potential prodrugs. The reactions also helped us to understand the role of the base. This would assist us in the preparation of subsequent N-9 derivatives.

3.4 PaTrin-2 derivatives containing a tertiary amino group

In the following sections we will report the synthesis of PaTrin-2 N-9 carbamoyl derivatives containing a tertiary amino group. As previously discussed, these derivatives allow for the preparation of salts to improve the solubility and therefore the formulation and delivery of a prodrug.

Many of the carbamoyl chlorides of the secondary amines we proposed to link to PaTrin-2 were not commercially available. Therefore we needed to investigate a route to their preparation from the phosgene precursor bis(trichloromethyl) carbonate BTC (**13**).

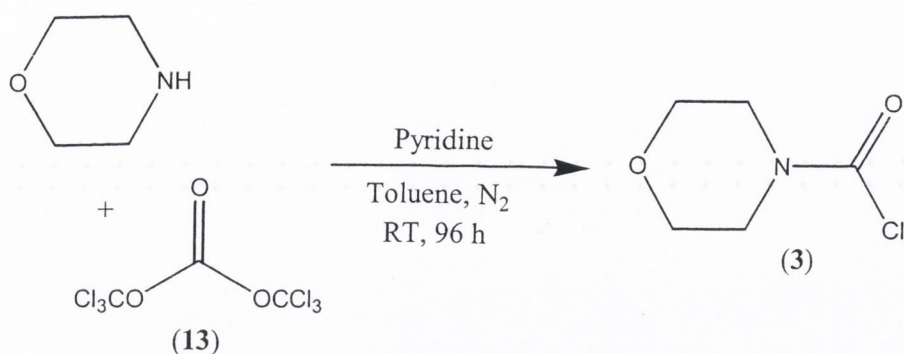


We decided to use morpholine as a model. This secondary amine is uncomplicated by the presence of a tertiary amino group, and we had already examined the reaction of morpholine-4-carbonyl chloride (3) with PaTrin-2.

3.4.1 Morpholine-4-carbonyl chloride – a model

Boon⁶ reported the synthesis of morpholine-4-carbonyl chloride using phosgene. The procedure involved the addition of the morpholine in toluene to a solution of excess phosgene in toluene at -10°C . We repeated this procedure, but substituting BTC (13) for phosgene. We did not observe the infrared carbonyl stretching frequency at 1732 cm^{-1} , which we found with the commercial sample of morpholine-4-carbonyl chloride (3), but upon the addition of base we did detect the presence of the carbamoyl chloride. However, the subsequent reaction of PaTrin-2 with this crude sample of carbamoyl chloride afforded *O*⁶-(4-bromophenyl)-9-(morpholinocarbonyl)-guanine (4) in less than 10% yield. This poor result mirrored previous efforts to substitute BTC for phosgene in procedures describing phosgene reactions.

A procedure reported by Pattenden *et al.*⁷ described the preparation of a carbamoyl chloride in a quantitative yield using BTC (13). The procedure involved the dropwise addition of the amine in benzene to a solution of BTC and pyridine in benzene. The resulting suspension was stirred under nitrogen for 96 hours. The pyridine hydrochloride was collected, and the solvent removed from the filtrate to leave the carbamoyl chloride. We repeated this procedure, but using toluene as a solvent (Scheme 3.5).



Scheme 3.5

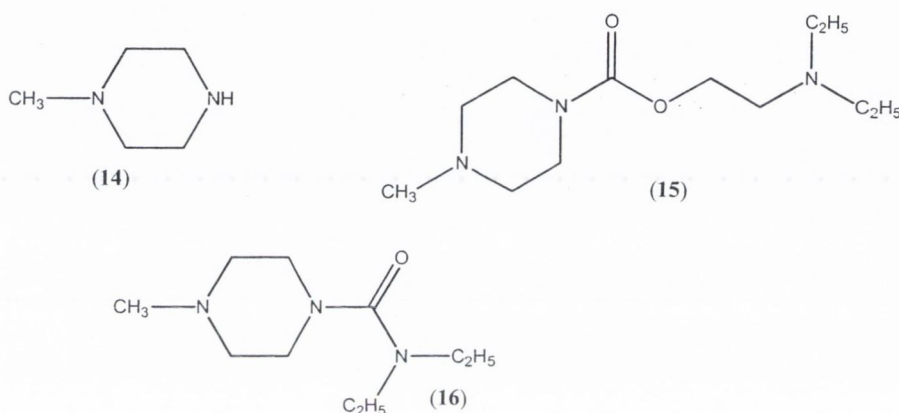
Although, the procedure reported by Pattenden *et al.*⁷ did not describe the purification of their carbamoyl chloride we decided to distil our sample. The reaction of PaTrin-2 with the distilled sample of morpholine-4-carbonyl chloride (3) gave *O*⁶-(4-bromophenyl)-9-(morpholinocarbonyl)guanine (4) in 40% yield. We subsequently found that refluxing the reaction mixture for several hours, prior to distillation, resulted in a sample of morpholine-4-carbonyl chloride (3) which reacted with PaTrin-2 in the presence of an equimolar amount of triethylamine to give an almost quantitative yield of (4). This mirrored the reaction involving the commercial sample of morpholine-4-carbonyl chloride (3). These results suggest that the compound prepared by the route shown in Scheme 3.5 is an unreactive precursor to morpholine-4-carbonyl chloride (3) and may be converted to the more reactive (3) upon heating. Such precursors resulting from reactions involving bis(trichloromethyl) carbonate (13) are discussed in more detail in Chapter 4.

We had established a high-yielding route to the synthesis of a carbamoyl chloride. Using this procedure we attempted the preparation of a carbamoyl chloride from a secondary amine containing a tertiary amino group.

3.4.2 Preparation of 4-methylpiperazine-1-carbonyl chloride hydrochloride

The literature reports the biological activity of several compounds derived from *N*-methylpiperazine (14). For example, the carbamate 2-diethylaminoethyl 4-methylpiperazine-1-carboxylate (15) exhibits antiviral activity against influenza,⁸ and the urea diethylcarbamazine (16) is a potent microfilaricidal agent.⁹

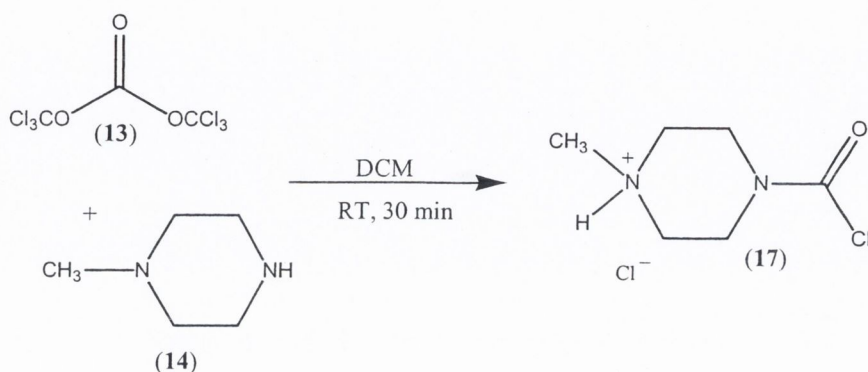
We decided that a PaTrin-2 derivative derived from *N*-methylpiperazine (14) might provide interesting pharmacological results.



We attempted to prepare 4-methylpiperazine-1-carbonyl chloride by repeating the procedure outlined by Pattenden *et al.*⁷ for the preparation of morpholine-4-carbonyl chloride (3). The infrared spectrum of the resulting product showed a stretching frequency at 1731 cm⁻¹. We believed this to be characteristic of the carbonyl group of the carbamoyl chloride. However, the subsequent reaction of this product with PaTrin-2 failed. We attempted the preparation of 4-methylpiperazine-1-carbonyl chloride several times using different bases and conditions, but the materials obtained in no case reacted with PaTrin-2.

Fujii *et al.*¹⁰ reported the preparation of 4-methylpiperazine-1-carbonyl chloride hydrochloride (17) using phosgene itself in the absence of base. We decided to repeat this procedure, but using BTC (13). The reaction involved the dropwise addition of an ice-cold solution of *N*-methylpiperazine (14) in dry DCM to an ice-cold solution of

BTC (13) in dry DCM (Scheme 3.6). The reaction was allowed to warm to room temperature, and was set aside for 30 minutes. The precipitate, 4-methylpiperazine-1-carbonyl chloride hydrochloride (17), was collected and dried.



Scheme 3.6

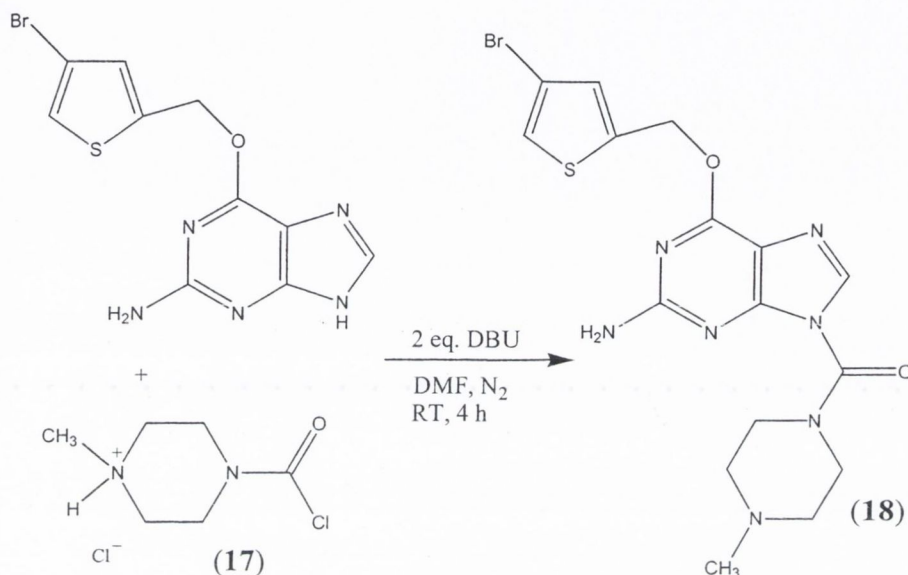
The carbamoyl chloride was characterized by an infrared stretching frequency at 1752 cm^{-1} . This frequency is different from that recorded for the sample prepared using Pattenden's procedure⁷ which we believe was the carbamoyl chloride itself, as the morpholine analogue absorbs at the same frequency. In the hydrochloride (17) a transannular or intermolecular interaction may increase the stretching frequency of the carbamoyl carbonyl group.

We had previously been unable to prepare high yields of carbamoyl chlorides (or chloroformates) by substituting BTC for phosgene in procedures describing reactions involving phosgene. The presence of the tertiary amino group in *N*-methylpiperazine may effectively substitute for an external base.

3.4.3 *O*⁶-(4-Bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine

4-Methylpiperazine-1-carbonyl chloride hydrochloride (17) had precipitated from the reaction mixture. We believed we could use this pure sample of the carbamoyl chloride hydrochloride (17) in the reaction with PaTrin-2. However, it was found that the reaction of PaTrin-2 with the crude reaction mixture, following the removal of the

reaction solvent, proceeded better than reactions involving the “isolated” carbamoyl chloride. The reaction of PaTrin-2 with 4-methylpiperazine-1-carbonyl chloride hydrochloride (**17**) was carried out at room temperature in the presence of two equivalents of DBU (Scheme 3.7).



Scheme 3.7

After 4 hours, the solvent was removed, and ¹H NMR analysis of the crude reaction mixture showed approximately 50% yield of *O*⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**). Ethanol was added to the residue, and the base (**18**) precipitated and was collected.

The above reaction was found to result in a lower yield of (**18**) when two equivalents of triethylamine were used instead of DBU and some of the corresponding N-7 isomer was detected. Also, we found that using a single equivalent of DBU did not result in the hydrochloride of (**18**).

During the course of our investigation of the synthesis of *O*⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**) from *N*-methylpiperazine (**14**), 4-methylpiperazine-1-carbonyl chloride hydrochloride (**17**) became commercially available. We repeated the reaction of PaTrin-2 with a sample of this material in a

collapse and coalesce, eventually showing one sharp peak. At this temperature, the rate of rotation has speeded up and is faster than the NMR timescale.¹¹

*O*⁶-(4-Bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**) also illustrates the phenomenon (Fig. 3.5). In the ¹H NMR of (**18**) (Fig. 3.6), the proton signals due to the 2''- and 6''-CH₂ cannot be distinguished, but appear as a broad singlet at 3.44 ppm. In the ¹³C NMR spectrum (Fig. 3.7), the signals associated with the 2''- and 6''-CH₂ are too broad to detect, though the signal due to the 3''- and 5''-CH₂ are seen at 53.9 ppm.

In experiments at 70 °C, the ¹H NMR spectrum (Fig. 3.8) shows a sharpening of the 2''- and 6''-CH₂ proton signal at 3.47 ppm, and in the ¹³C NMR spectrum (Fig. 3.9) an additional peak at 45.8 ppm appears. A HMQC (Heteronuclear Multiple Quantum Coherence) experiment showed that this carbon signal was coupled to the signal at 3.47 ppm in the proton spectrum, associated with the 2''- and 6''-CH₂.

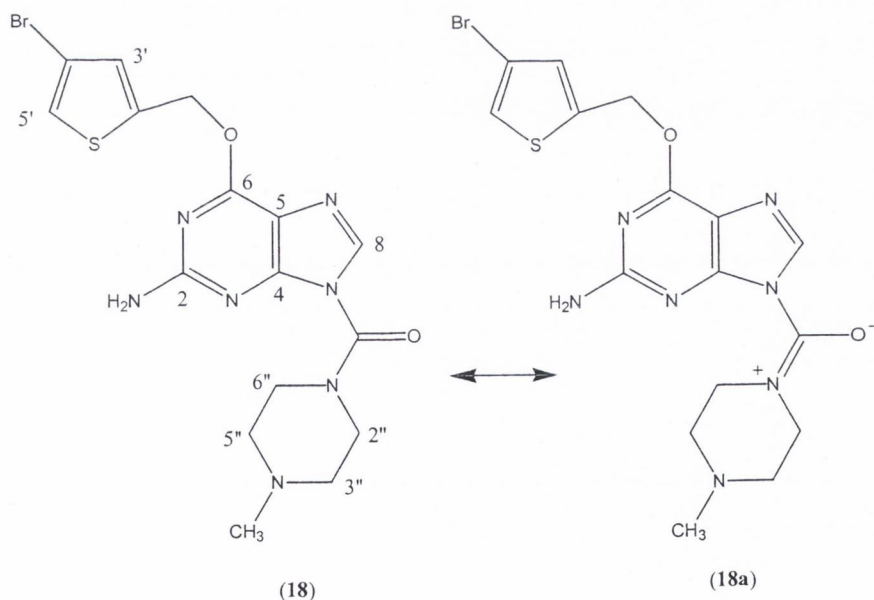


Fig. 3.5

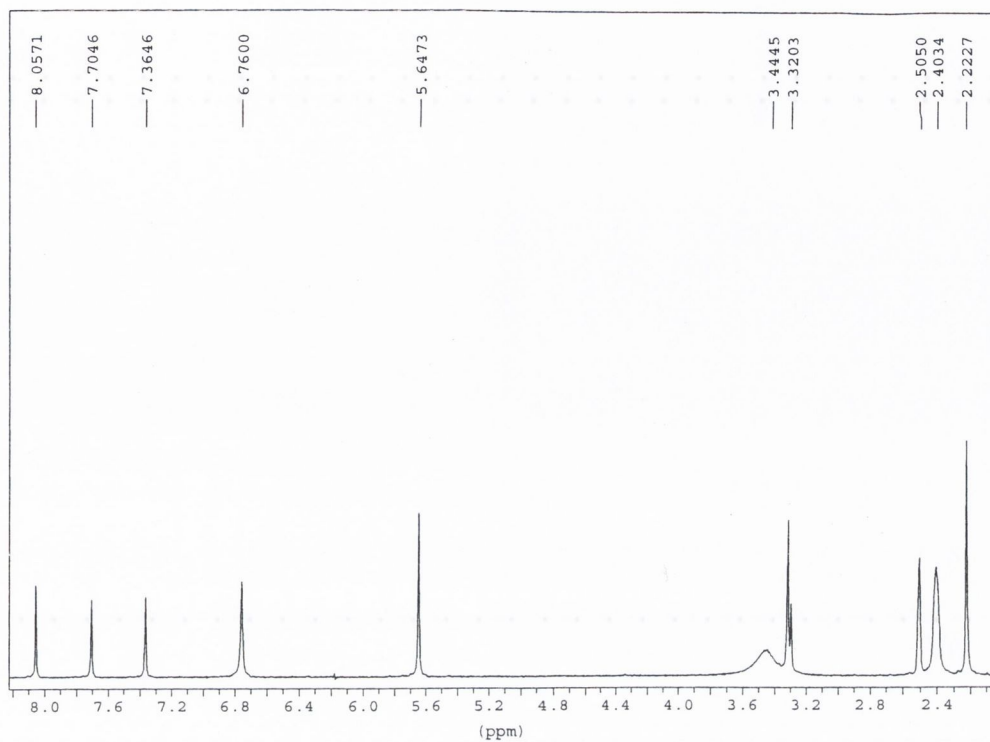


Fig. 3.6

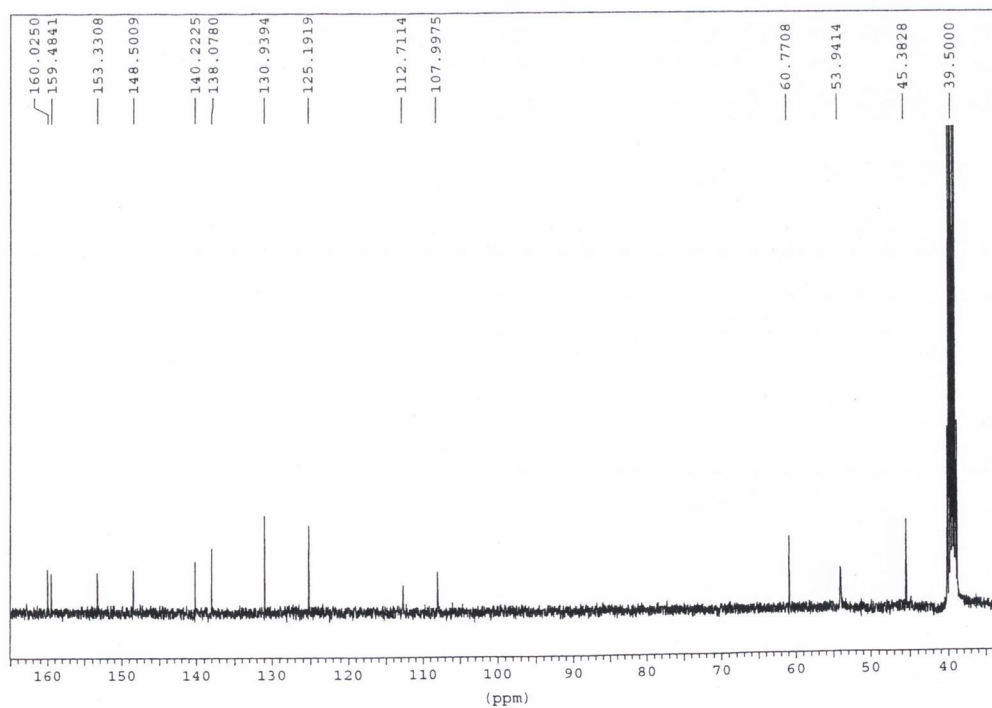


Fig. 3.7

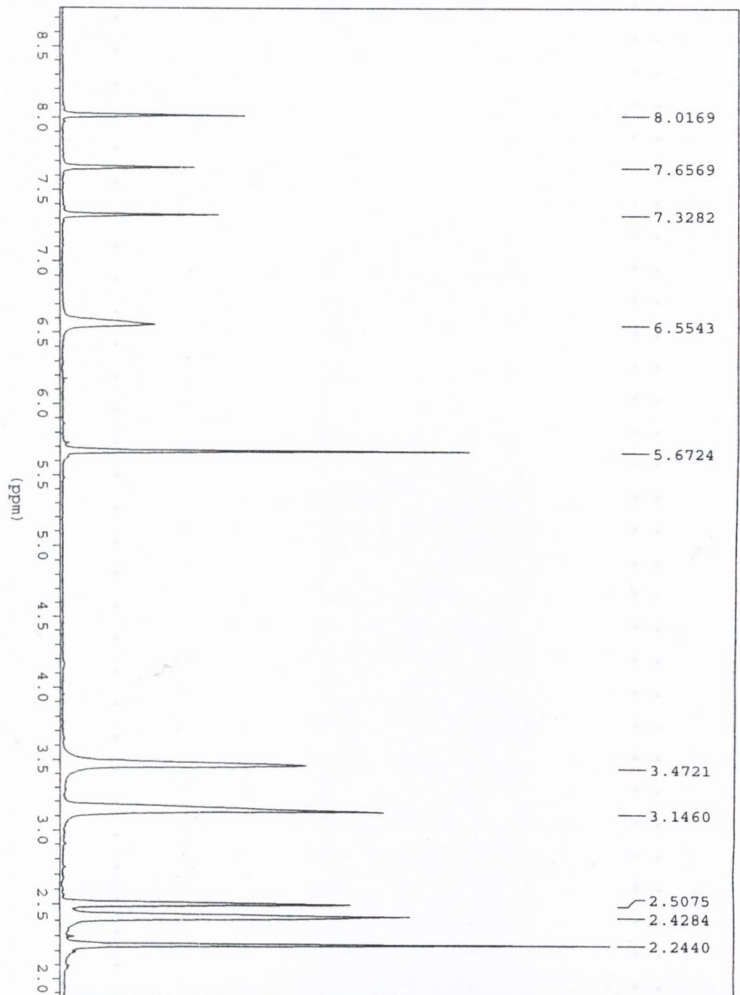


Fig. 3.8

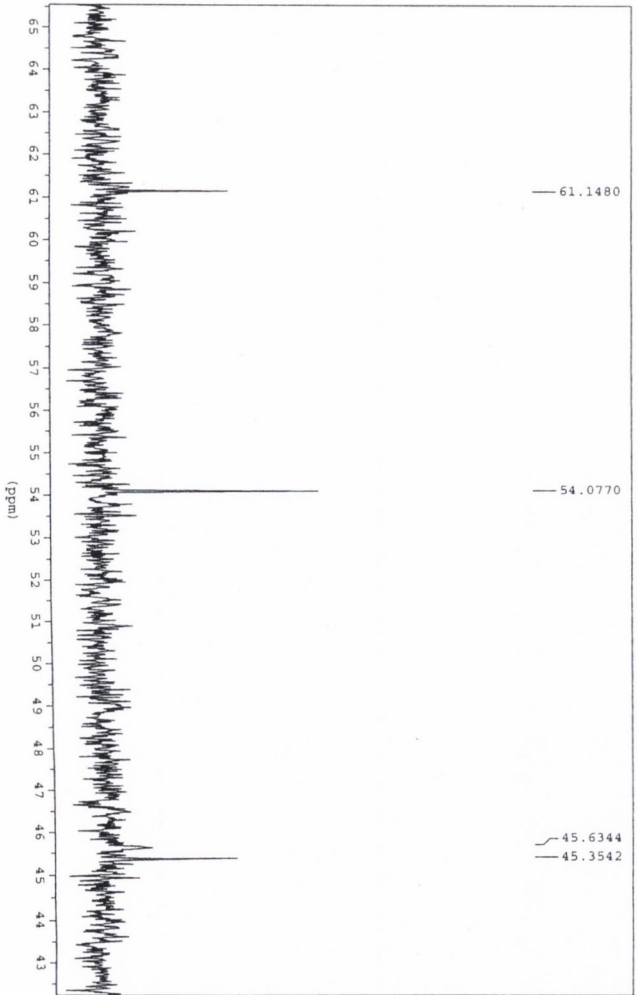


Fig. 3.9

The phenomenon discussed above is characteristic of all the PaTrin-2 N-9 carbamoyl derivatives reported in this chapter. The degree of C-N double bond character, and hence the rate of rotation about the bond, varies from compound to compound. Therefore the effect is not observed to the same extent in the ^1H and ^{13}C NMR spectra of every PaTrin-2 N-9 carbamoyl derivative. In Section 3.3.2 we used the ^1H NMR spectrum of *O*⁶-(4-bromophenyl)-9-(morpholinocarbonyl)guanine (**4**) (Fig. 3.3) to show the proton shifts of a typical PaTrin-2 N-9 carbamoyl derivative. As in the ^1H NMR spectrum of *O*⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**), the signal associated with the 3''- and the 5''-CH₂ at 3.47 ppm is very broad. However, the ^{13}C NMR spectrum of (**4**) is unlike that of (**18**), and the signal associated with the 3''- and the 5''-CH₂, although not very sharp, is observed.

*O*⁶-(4-Bromophenyl)-9-(*N,N*-dimethylcarbamoyl)guanine (**10**), whose synthesis was reported in Section 3.3.4, should be similar to *N,N*-dimethylformamide and two sharp signals should be observed for the *N*-methyl groups in its ^1H and ^{13}C NMR spectra. However, only one broad peak is observed at 2.99 ppm in the ^1H NMR spectrum (Fig. 3.10) and in the ^{13}C NMR spectrum the signals associated with these methyl groups are too broad to be detected. Nothing indicative of this phenomenon is observed in the NMR spectra of *O*⁶-(4-bromophenyl)-9-(*N,N*-diphenylcarbamoyl)guanine (**11**), though it might be difficult to find in the spectra.

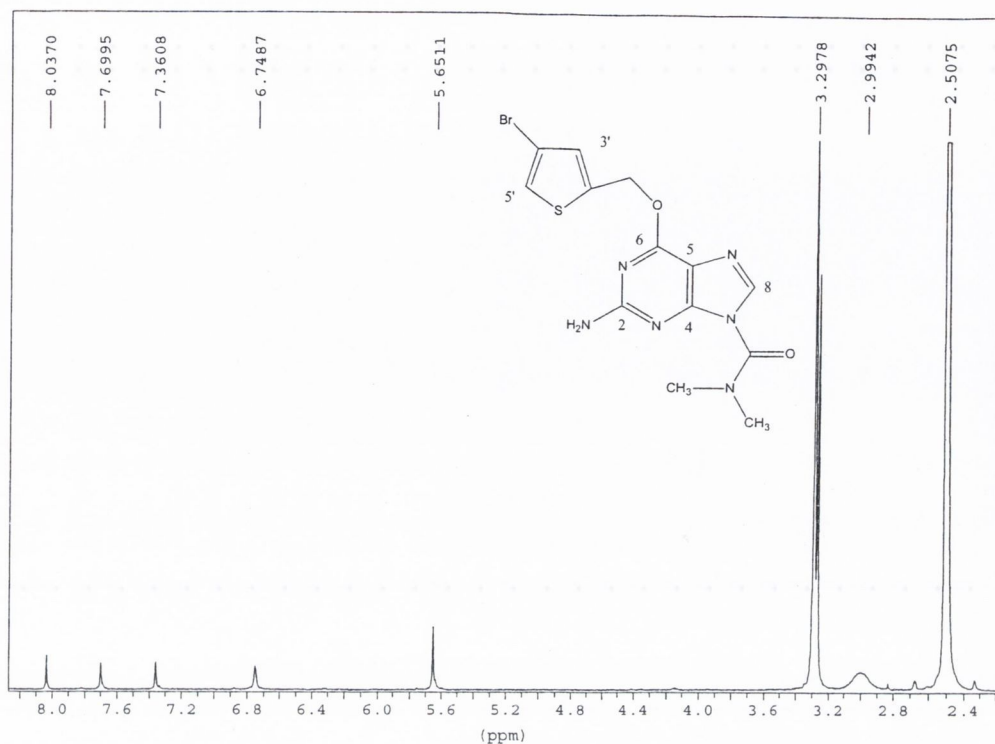


Fig. 3.10

The preparation of *O*⁶-(4-bromophenyl)-9-(1-pyrrolidinylcarbonyl)guanine (**12**) was also reported in Section 3.3.4. In this compound the rate of rotation about the bond is slower than the NMR timescale. This result is to be expected, as there should be more double bond character in the C-N bond because it is exocyclic to a five-membered ring. The increased stability of such a bond over a similar bond exocyclic to a six-membered ring is well known, and is due to the fewer eclipsed interactions in the five-membered ring.^{12,13} In the ¹³C NMR spectrum (Fig. 3.11) four signals are observed, each associated with one of the four CH₂ groups in the pyrrolidine moiety. The proton spectrum is not as clear-cut, but this can be attributed to the multiple H-H couplings.

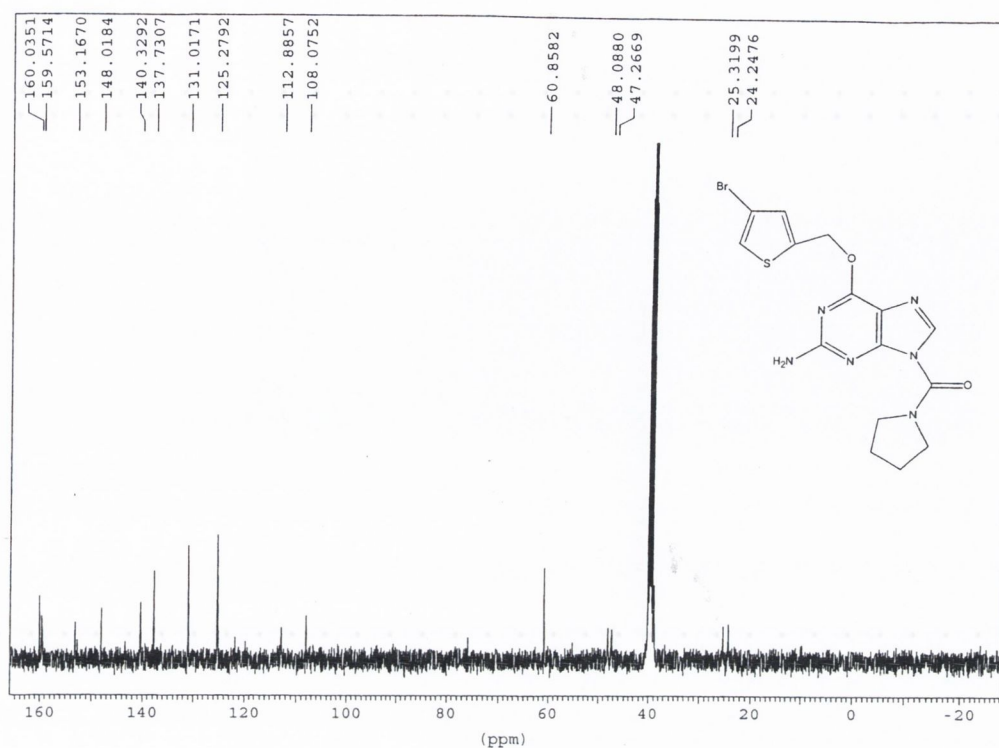


Fig. 3.11

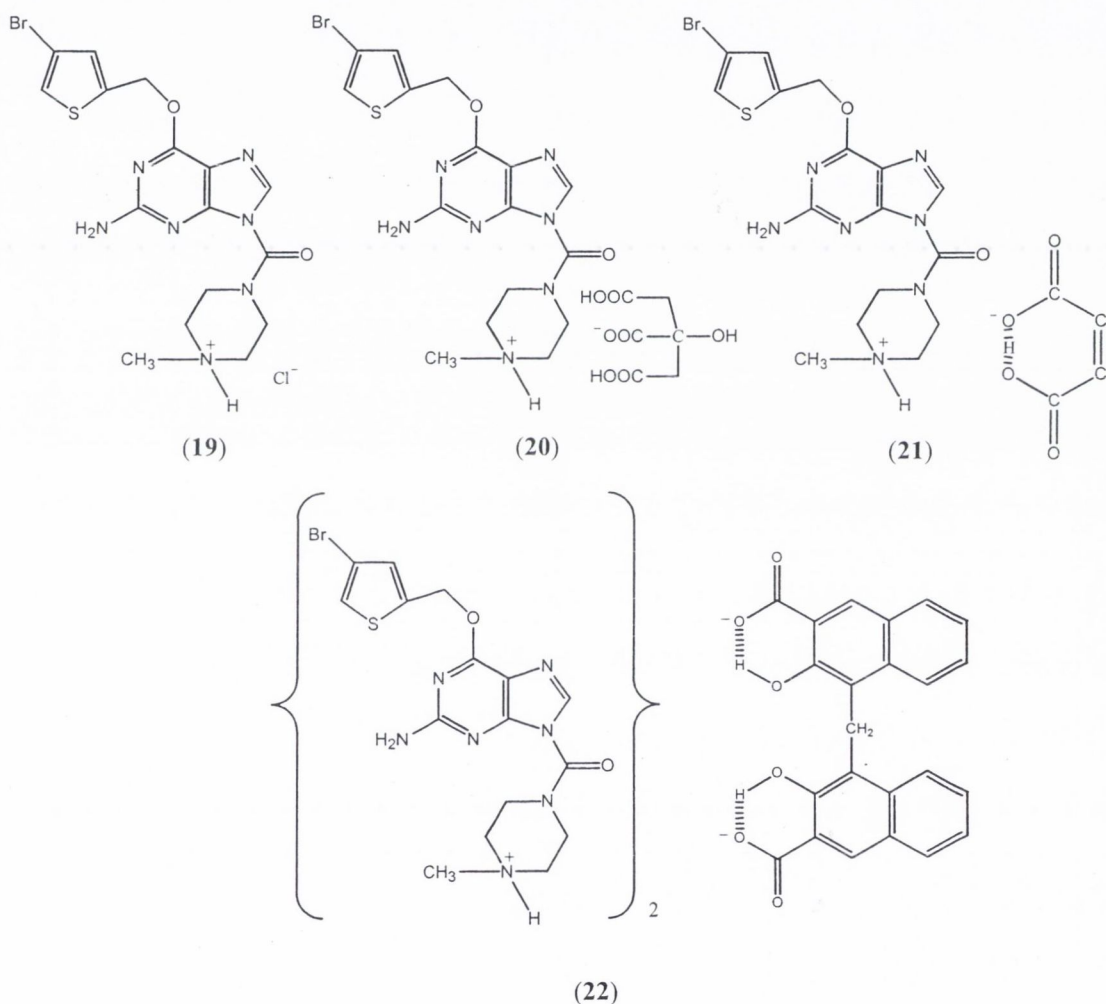
The variable temperature NMR experiments had allowed us to characterize fully *O*⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**) in the ¹H and ¹³C NMR spectra. In addition, an interesting observation was made. The sample of the N-9 derivative (**18**) had shown surprising stability in the DMSO solution. It had taken one week to acquire all the NMR data, and the sample had been exposed to elevated temperatures. However, this potential prodrug showed no signs of having undergone any hydrolysis.

Due to poor solubility in other deuterated solutions, NMR analysis of PaTrin-2 derivatives is always obtained in solutions in DMSO. However, the traces of water in the DMSO solvent can promote the hydrolysis of our N-9 derivatives in solution. Although this instability has led to difficulty in acquiring NMR data, the rate at which each potential prodrug is observed to undergo hydrolysis in the deuterated solution has given us an indication of its stability. These N-9 derivatives have been observed

to have half-lives of from several hours to several days. O^6 -(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**) was the first derivative found to be fully stable in the DMSO solution for a week, and under extreme conditions. The pharmacological results would yield more information on the stability of this potential prodrug under physiological conditions.

3.6 Salts of *O*⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine

*O*⁶-(4-Bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (18) provided us with an example of a stable prodrug which contained a tertiary amino group. We decided to prepare several salts of this N-9 derivative for pharmacological evaluation. We chose to synthesize a hydrochloride (19), a citrate (20), a maleate (21) and a pamoate (22).

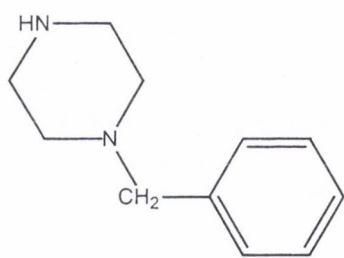


The salts (19), (20) and (21) were prepared by the addition of a solution of the respective acid in methanol to a solution of an equimolar amount of the base *O*⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (18) in methanol. The pamoate (22) was prepared by the addition of a methanol solution containing pamoic

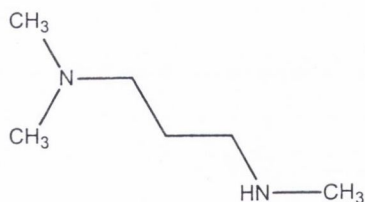
acid [4,4'-methylenebis(3-hydroxy-2-naphthoic acid)] and a molar equivalent of ammonia, to a solution containing two molar equivalents of *O*⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**) in methanol. Each of the reactions was set aside for approximately 10 minutes. The solvent was evaporated and ether was added to each of the residues and the salts precipitated. The salts (**19**), (**20**), (**21**) and (**22**) were isolated in an almost quantitative yield. We found the hydrochloride (**19**), the citrate (**20**) and the maleate (**21**) to be water-soluble. The pamoate (**22**) was less soluble and in the concentration that we used gentle heating was required to dissolve it.

3.7 Further N-9 carbamoyl derivatives containing a tertiary amino group

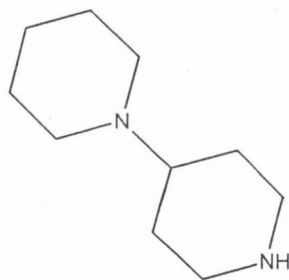
We decided to investigate the synthesis of three other PaTrin-2 N-9 carbamoyl derivatives derived from secondary-tertiary diamines. We chose to examine the reaction of PaTrin-2 with carbamoyl chlorides derived from *N*-benzylpiperazine (**23**), *N,N,N'*-trimethyl-1,3-propanediamine (**24**) and 4-piperidinopiperidine (**25**).



(23)



(24)

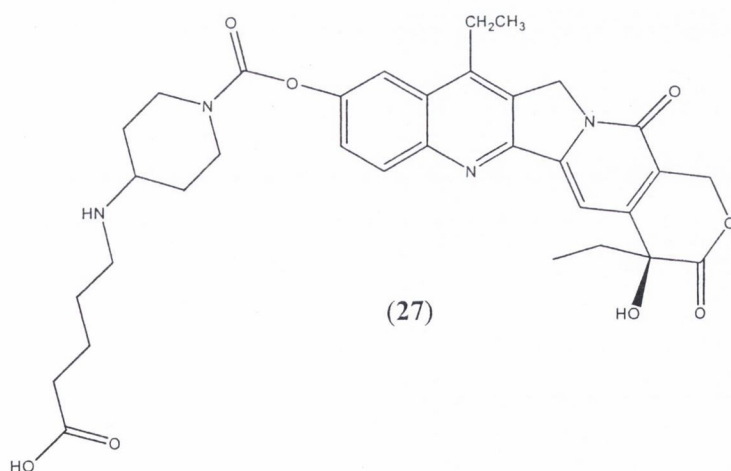
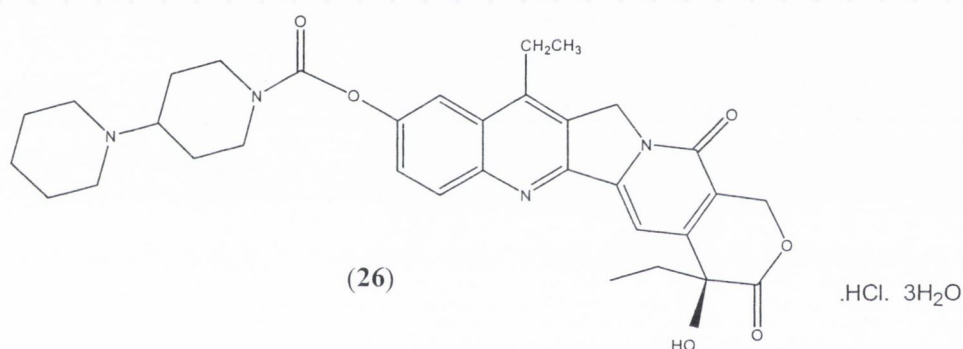


(25)

The PaTrin-2 derivative derived from *N*-benzylpiperazine (**23**) would provide a direct comparison to *O*⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**). By observing the reaction of PaTrin-2 with a carbamoyl chloride derived from *N,N,N'*-trimethyl-1,3-propanediamine (**24**) we hoped to make a comparison between

this reaction and the failed reaction we had examined previously involving PaTrin-2 and the chloroformate derived from 3-dimethylaminopropan-1-ol (Chapter 2, Section 2.10.3, pg 96).

In Chapter 1 (Section 1.8, pg 29) we discussed the prodrug Irinotecan hydrochloride trihydrate (26), an antineoplastic agent. Irinotecan (26) was found to be the most active of a series of prepared prodrugs of the topoisomerase-I inhibitor Camptothecin.¹⁴ Irinotecan (26) consists of a modified Camptothecin residue linked to 4-piperidinopiperidine (25) *via* a carbamate linkage. This prodrug was found to have an interesting metabolite (27), which is the product of a reaction involving the ring-opening oxidation of the terminal piperidine ring of Irinotecan.¹⁵

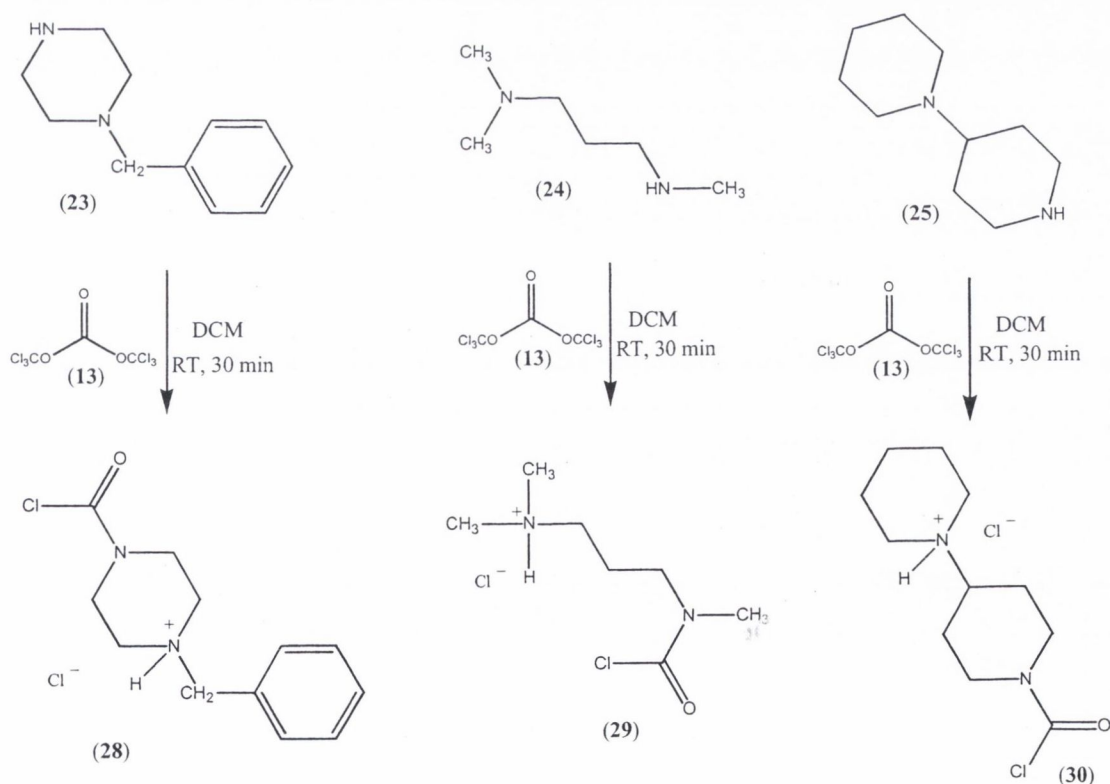


Irinotecan (26) was of interest to us because of its advantage over other prodrugs tested, and the fact that it gives rise to the metabolite (27). We thought that a PaTrin-2

N-9 carbamoyl derivative derived from a carbamoyl chloride of 4-piperidinopiperidine (**25**) might also provide interesting pharmacological results.

3.7.1 Preparation of carbamoyl chloride hydrochlorides

In Section 3.4.2 we reported the preparation of 4-methylpiperazine-1-carbonyl chloride hydrochloride. Following the same procedure we prepared the carbamoyl chloride hydrochlorides derived from *N*-benzylpiperazine (**23**), *N,N,N'*-trimethyl-1,3-propanediamine (**24**) and 4-piperidinopiperidine (**25**). The procedure involved the dropwise addition of a solution of the amine in dry DCM to an ice-cold solution of BTC (**13**) in dry DCM (Scheme 3.8). Each reaction was warmed to room temperature, and allowed to stand for 30 minutes. The solvent was removed, leaving a crude mixture containing the respective carbamoyl chloride hydrochloride.

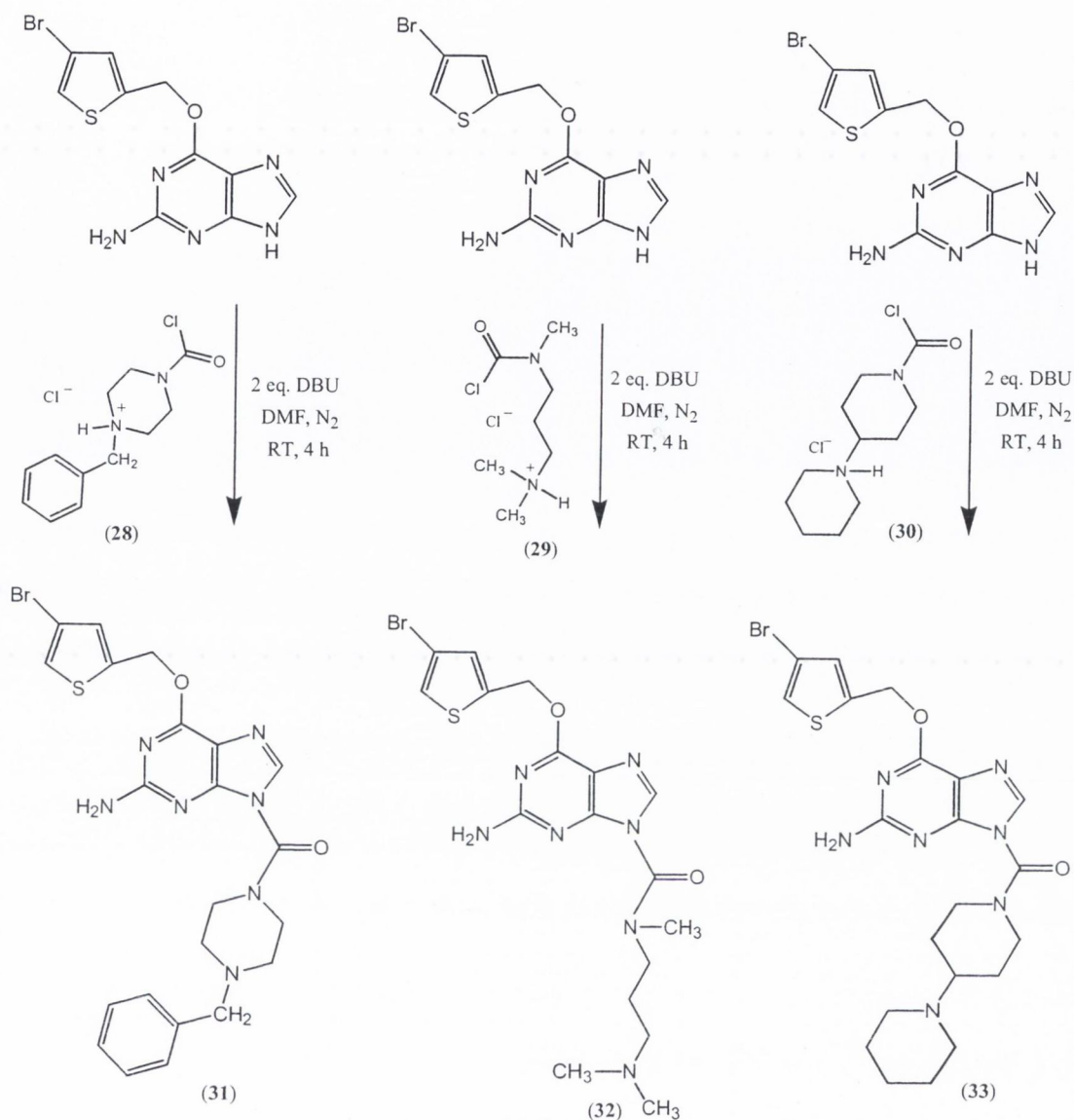


Scheme 3.8

Each of the carbamoyl chloride hydrochlorides was characterized by infrared spectroscopy. The carbonyl stretching frequency of 4-benzylpiperazine-1-carbonyl chloride hydrochloride (**28**) was observed at 1752 cm^{-1} , as was that of *N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl chloride hydrochloride (**29**). The carbonyl stretching frequency of 4-piperidinopiperidine-1-carbonyl chloride hydrochloride (**30**) was observed at 1735 cm^{-1} . This frequency is lower than that observed for (**28**) and (**29**), being similar to the frequency observed for typical carbamoyl chlorides. Although a hydrochloride, the structure of this compound does not permit the transannular interaction that we have proposed to be responsible for the higher frequencies observed for hydrochlorides like (**28**) (Section 3.4.2). The reaction involving PaTrin-2 was carried out using the crude samples of these carbamoyl chloride salts.

3.7.2 Reaction with PaTrin-2

The reaction of PaTrin-2 with each of the carbamoyl chloride hydrochlorides (**28**), (**29**) and (**30**) was carried out at room temperature, in DMF, for 4 hours, in the presence of two equivalents of DBU (Scheme 3.9). The solvent was removed, and each crude reaction mixture was analysed by ^1H NMR. The ^1H NMR spectrum of each residue showed a 50% yield of the corresponding N-9 derivative. Approximately 50% of the PaTrin-2 had not reacted. This suggested that although the carbamoyl chloride hydrochlorides (**28**), (**29**) and (**30**) were at least 50% pure, they perhaps were not 100% pure.



Scheme 3.9

We found that by reacting two equivalents of *N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl chloride hydrochloride (**(29)**) with PaTrin-2, under the above conditions, the ¹H NMR spectrum of the crude reaction mixture showed complete reaction. However, doubling the quantities of the other carbamoyl chloride hydrochlorides, (**(28)**) and (**(30)**), resulted only in the total recovery of PaTrin-2.

Although a substantial quantity of the N-9 derivatives (**(31)**), (**(32)**) and (**(33)**) was detected in the reaction mixtures difficulty was experienced with their isolation.

3.7.3 Isolation and characterization of N-9 carbamoyl derivatives

The insolubility of the N-9 derivative O^6 -(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**) in ethanol had allowed for its successful separation from the reaction mixture. However, we found it more difficult to isolate 9-(4-benzyl-1-piperazinylcarbonyl)- O^6 -(4-bromophenyl)guanine (**31**), O^6 -(4-bromophenyl)-9-[*N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl]guanine (**32**) and O^6 -(4-bromophenyl)-9-(4-piperidinopiperidinocarbonyl)guanine (**33**).

Attempts to isolate N-9 alkoxycarbonyl derivatives, containing a tertiary amino group, by flash chromatography had failed. Complete hydrolysis of these N-9 derivatives to give PaTrin-2 normally occurred, even when the silica was neutralised* prior to use and a non-hydrolytic solvent system was chosen. However, we did find it possible to isolate 9-(4-benzyl-1-piperazinylcarbonyl)- O^6 -(4-bromophenyl)guanine (**31**) by flash chromatography. Although partial decomposition occurred, the N-9 carbamoyl derivative (**31**) was successfully isolated in approximately 40% yield.

NMR analysis of 9-(4-benzyl-1-piperazinylcarbonyl)- O^6 -(4-bromophenyl)guanine (**31**) suggests it to be similar to O^6 -(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**) (Fig. 3.12). The signals associated with all the CH_2 groups of the piperazine residue in both the ^1H (Fig. 3.13) and ^{13}C (Fig. 3.14) are too broad to be observed. The only observable CH_2 groups are that of the benzyl group and the $O^6\text{-CH}_2$ group. In the ^1H NMR spectrum these methylene protons are observed at 3.54 ppm and 5.64 ppm respectively, and in the ^{13}C NMR spectrum the carbons are observed at 60.8 ppm and 61.6 ppm respectively.

* Silica was neutralised by washing with a solution of 1% $(\text{C}_2\text{H}_5)_3\text{N}$ in hexane prior to use.

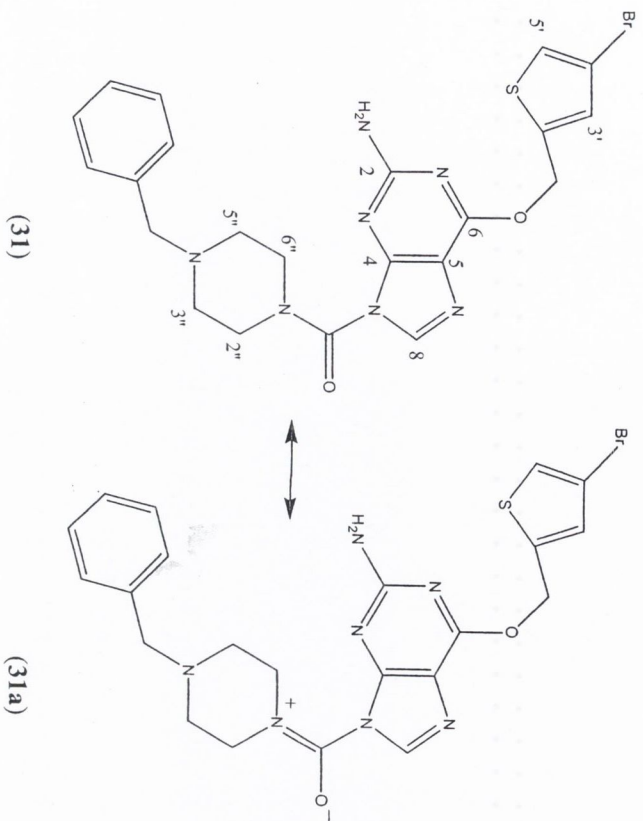


Fig. 3.12

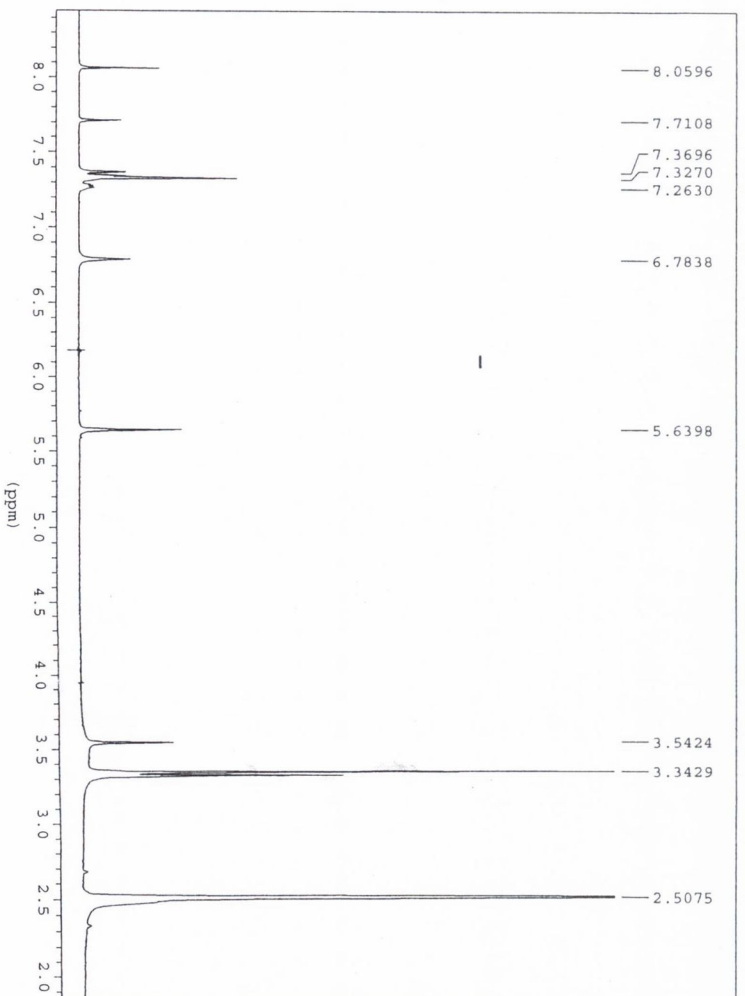


Fig. 3.13

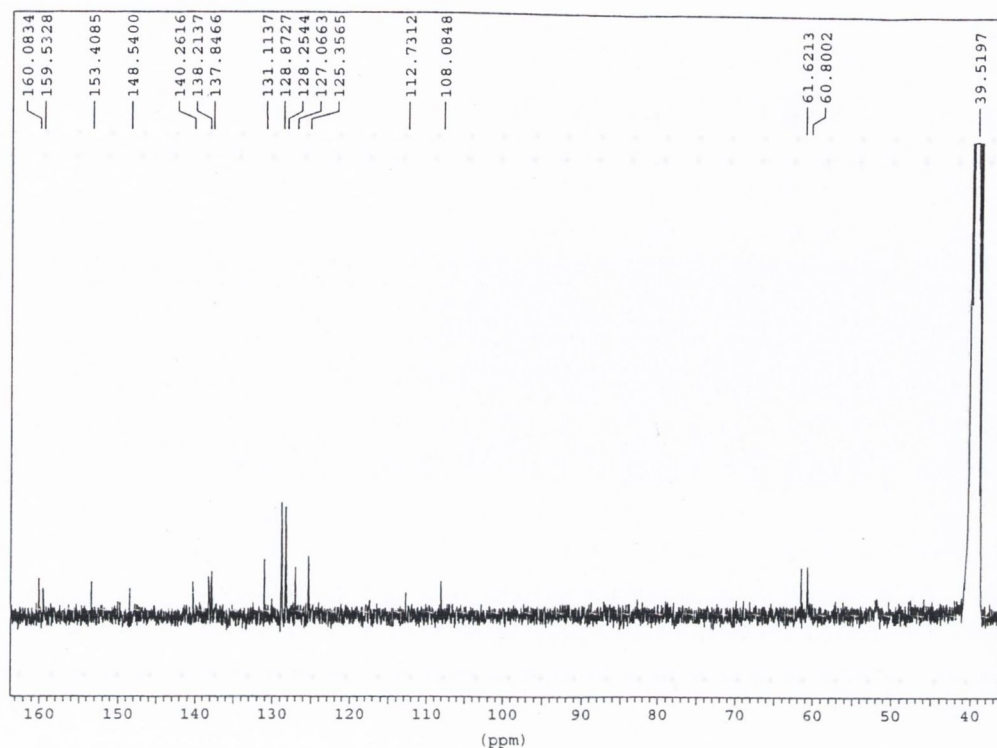


Fig. 3.14

Unlike O^6 -(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (**18**), the N-9 derivative (**31**) was not stable at elevated temperatures in variable temperature NMR experiments. As a result decomposition occurred prior to attaining the required information. However, the NMR data appear to suggest the presence of the missing CH_2 signals beneath the signal associated with water at 3.32 ppm in the ^1H NMR spectrum (Fig. 3.13).

It was not possible to isolate O^6 -(4-bromophenyl)-9-[*N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl]guanine (**32**) by flash chromatography. All attempts made resulted in its decomposition. Instead we found that it was possible to crystallise (**32**) slowly from a mixture of acetonitrile and ethyl acetate. A reasonably pure sample of (**32**) was obtained and was characterized by examining the downfield region in the ^1H NMR spectrum (Fig. 3.15). The NH_2 and the H-8 signals were observed downfield at 6.79 ppm and 8.07 ppm respectively.

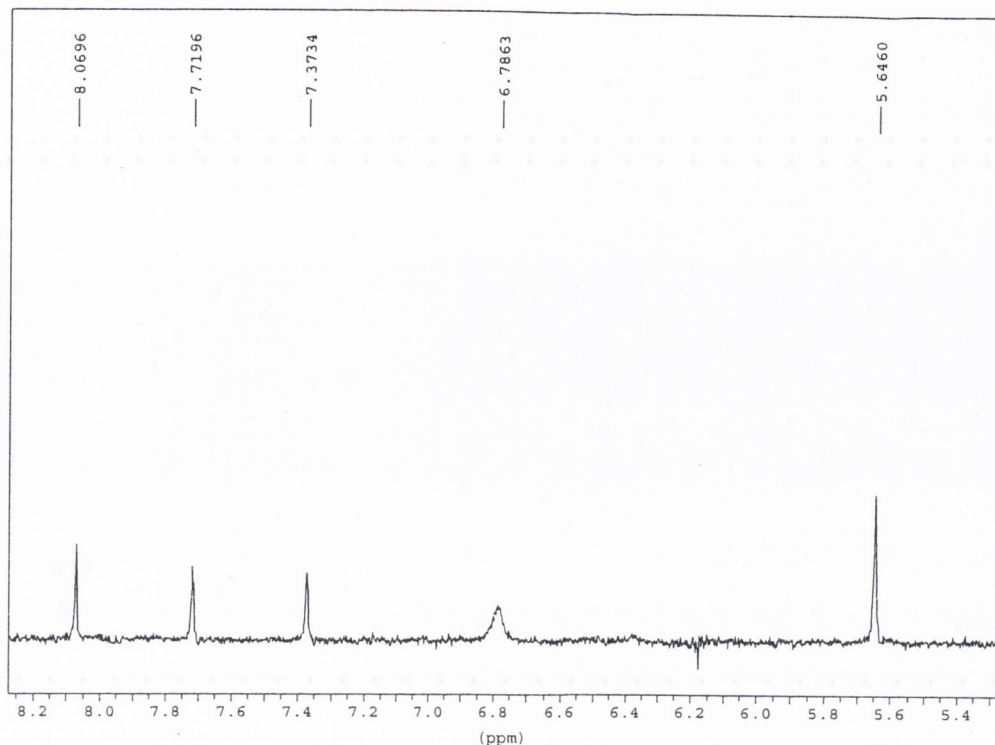
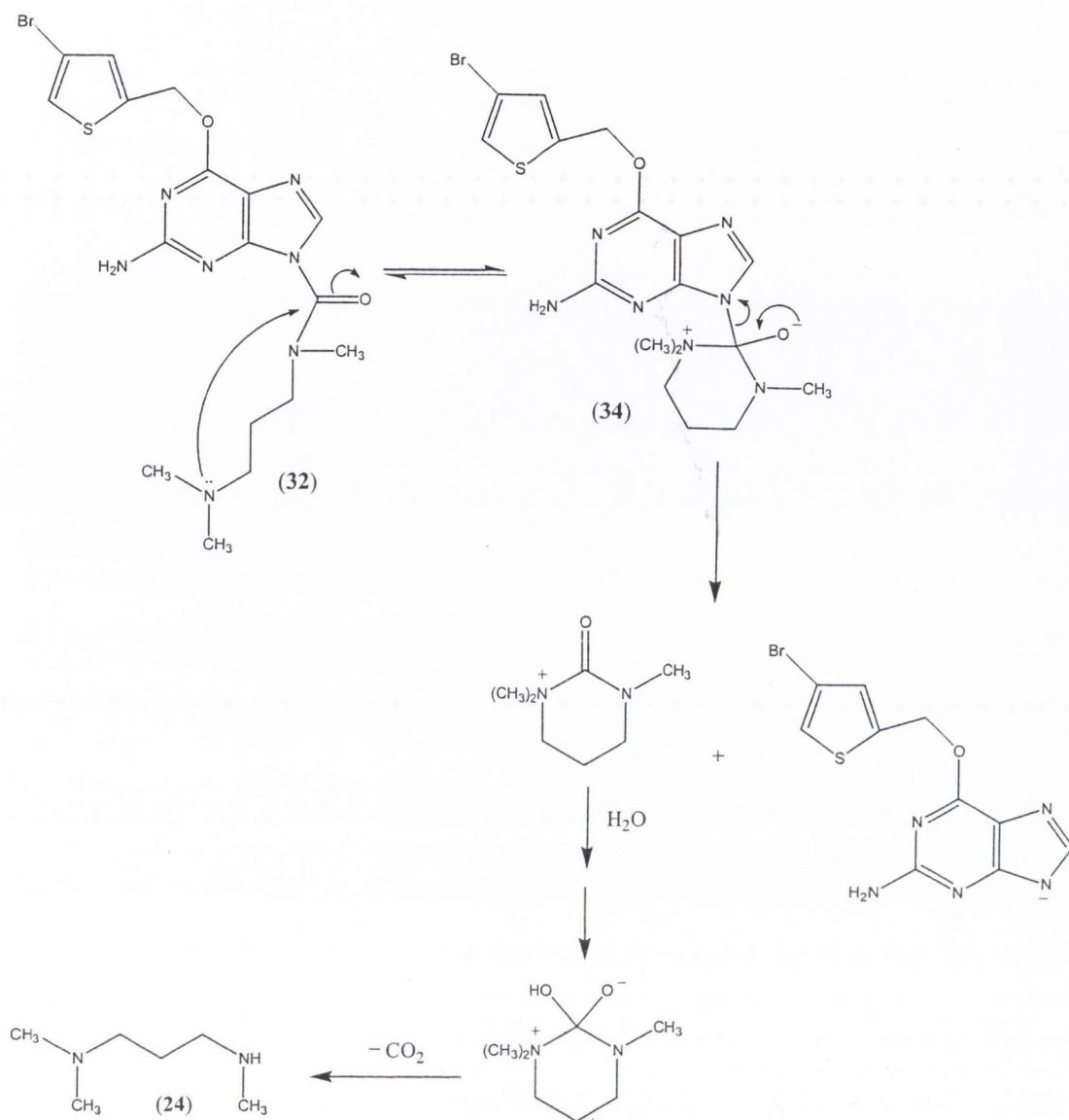


Fig. 3.15

Unfortunately, further characterization was not possible as the solid decomposed upon standing for several hours. In Chapter 2 (Sections 2.10.3 and 2.10.4) we proposed a decomposition mechanism for several N-9 alkoxycarbonyl derivatives whose isolation had not been possible. We proposed that the decomposition proceeded *via* an intramolecular reaction. The reaction involved an attack by the lone pair of electrons of the tertiary amino group on the carbonyl carbon atom. We proposed that the decomposition reaction was facilitated further by the ability of the PaTrin-2 anion to behave as a good leaving group.

Such a decomposition mechanism can also be proposed for *O*⁶-(4-bromophenyl)-9-[*N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl]guanine (**32**) (Scheme 3.10). As discussed above, an intramolecular reaction can give a six-membered cyclic intermediate (**34**) which can either return to (**32**) or decompose to give PaTrin-2 and *N,N,N'*-trimethyl-1,3-propanediamine (**24**).



Scheme 3.10

In an effort to prevent this intramolecular reaction we attempted to prepare the hydrochloride of (32) by carrying out the procedure for the preparation of (32), as shown in Scheme 3.9, was carried out in the absence of base. However, we were unable to produce a stable sample of the hydrochloride of (32).

Although, *O*⁶-(4-bromophenyl)-9-[*N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl]-guanine (32) had decomposed upon standing, we had found it possible to isolate both it and 9-(4-benzyl-1-piperazinylcarbonyl)-*O*⁶-(4-bromophenyl)guanine (31). However,

the isolation of O^6 -(4-bromophenyl)-9-(4-piperidinopiperidinocarbonyl)guanine (**33**) from its reaction mixture was not possible.

O^6 -(4-Bromophenyl)-9-(4-piperidinopiperidinocarbonyl)guanine (**33**) had been prepared in approximately 50% yield by the reaction of PaTrin-2 with 4-piperidinopiperidin-1-ylcarbonyl chloride hydrochloride (**30**). Compound (**33**) was not sufficiently stable to permit its isolation by chromatography. Due to the physical characteristics of the reaction mixture we were unable to isolate (**33**) by recrystallisation from a variety of solvents. We decided that it would be worthwhile to investigate the synthesis of (**33**) by an alternative route. By increasing the amount of (**33**) in the reaction mixture, or by changing the characteristics of the mixture, the isolation of (**33**) might be more feasible.

3.8 Alternative routes to the preparation of tetrasubstituted ureas

In Chapter 2 we investigated several methods for the preparation of N-9 alkoxycarbonyl derivatives. This allowed us to find the most efficient route to their synthesis. However, there are only a few established methods for the preparation of unsymmetrical tetrasubstituted ureas such as the PaTrin-2 N-9 dialkylcarbamoyl derivatives.¹⁶⁻¹⁸ The most frequently used method is that which we reported above, and involves the treatment of a carbamoyl chloride (synthesized from a phosgene source and a secondary amine) with a secondary amine.

The limited number of procedures available for the synthesis of tetrasubstituted ureas arises from the stability of the $CONR_2$ group to hydrolysis, or in the case of intermediates, attack by a nucleophile *i.e.* PaTrin-2 anion. As discussed in Section 3.1, the inductive effect of the alkyl groups attached to the nitrogen atom increases the electron availability on the nitrogen atom (Fig. 3.1). This in turn decreases the

positive character of the carbonyl carbon atom and therefore its reactivity. In a carbamoyl chloride (Fig. 3.16) the unshared pair of electrons on the chlorine atom also has a role in decreasing the positive character of the carbonyl carbon atom (mesomeric effect); however, the electron-withdrawing effect of the chlorine atom sufficiently increases the reactivity of the carbamoyl chloride toward nucleophilic attack.¹

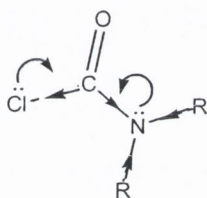
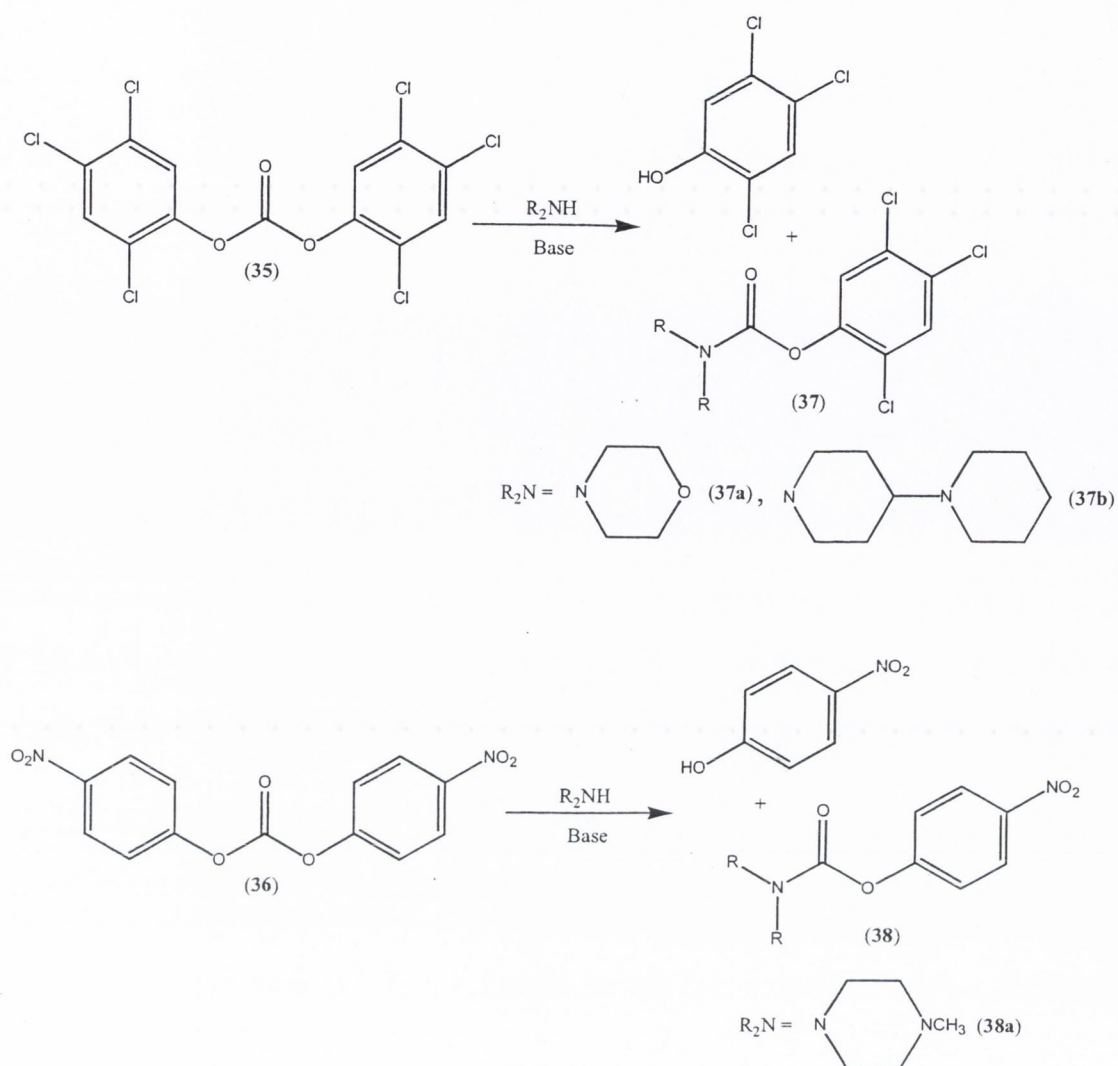


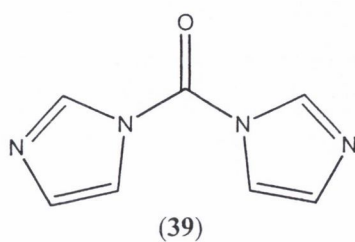
Fig. 3.16

Initially, we attempted to prepare our N-9 carbamoyl derivatives by reacting PaTrin-2 with carbamate esters. These esters were prepared by the reaction of a secondary amine with either bis(2,4,5-trichlorophenyl) carbonate (**35**) or bis(4-nitrophenyl) carbonate (**36**) in the presence of base (Scheme 3.11). However, carbamate esters like (**37**) and (**38**) proved to be too stable to undergo a reaction with a subsequent nucleophile.



Scheme 3.11

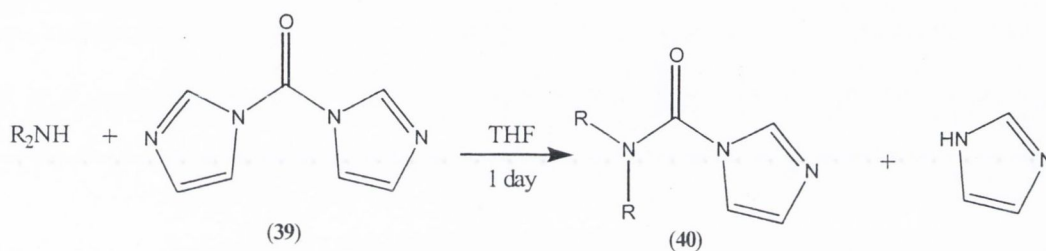
The literature reports the use of several activated carbonic acid derivatives other than carbamoyl chlorides.^{17,18} In the following sections we will discuss our attempt at using *N,N'*-carbonyldiimidazole (CDI) (**39**) to make possible the synthesis of *O*⁶-(4-bromophenyl)-9-(4-piperidinopiperidinocarbonyl)guanine (**33**).



3.9 Use of *N,N'*-carbonyldiimidazole

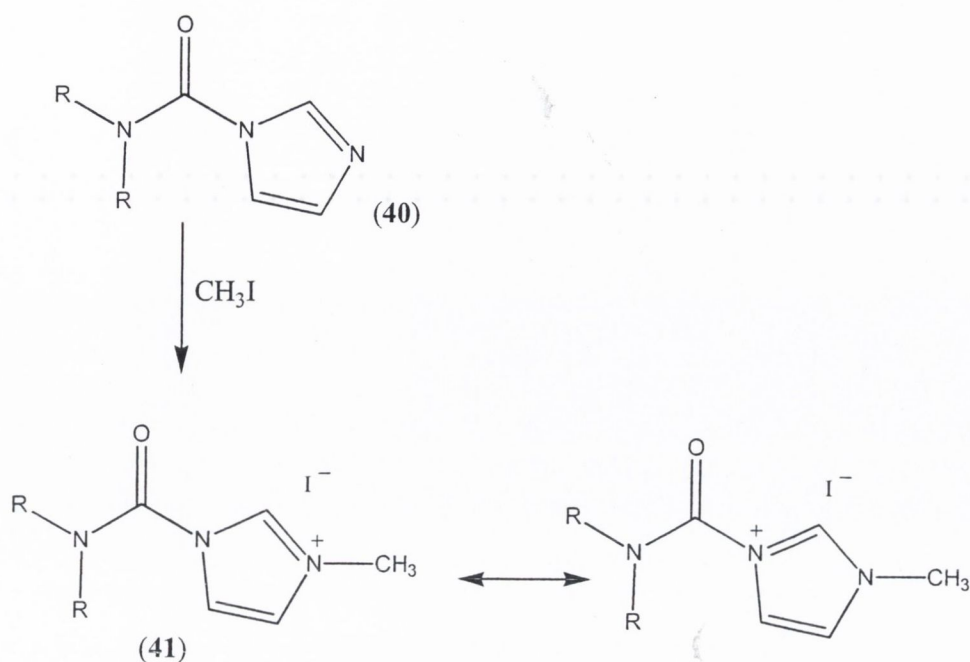
Batey *et al.*¹⁸ recently reported a new approach to the synthesis of unsymmetrical tetrasubstituted ureas, using cationic carbamoylimidazolium intermediates derived from CDI (39). Although CDI, which is commercially available, has been used in the preparation of disubstituted ureas,¹⁸ this was the first report of its use in the synthesis of tetrasubstituted ureas.

The reported procedure involves the reaction of a secondary amine with CDI (39) to generate a *N,N*-disubstituted carbamoylimidazole intermediate (40) (Scheme 3.12).



Scheme 3.12

Like the carbamate esters (37) and (38) discussed above, the intermediate (40) is too stable to undergo a reaction with a nucleophile. However, (40) can be activated by the *N*-alkylation of the imidazole moiety using methyl iodide to yield a resonance-stabilized carbamoylimidazolium salt (41) (Scheme 3.13).^{19,20}



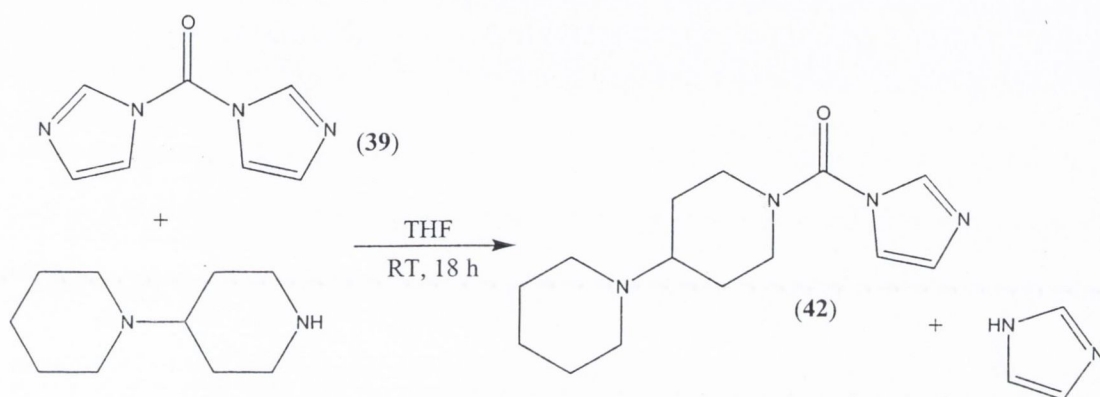
Scheme 3.13

The activation of acylimidazoles by the alkylation of the imidazole moiety is widely reported to increase their reactivity toward nucleophilic attack.¹⁸⁻²⁰ Similarly the subsequent treatment of (41) with a secondary amine affords tetrasubstituted ureas in high yield.¹⁸

The use of methyl iodide would not permit the preparation of the free base *O*⁶-(4-bromophenyl)-9-(4-piperidinopiperidinocarbonyl)guanine (33) as the tertiary amino group would compete with the imidazole moiety for methylation. However, we had planned to prepare examples of salts of our tertiary-amino-substituted prodrugs with alkylating agents as well as acids, therefore a reaction where the amino group is quaternised before reaction with PaTrin-2 to afford such a salt directly was highly favourable.

3.9.1 *O*⁶-(4-Bromophenyl)-9-[4-(*N*-methylpiperidinio)piperidinocarbonyl]guanine iodide

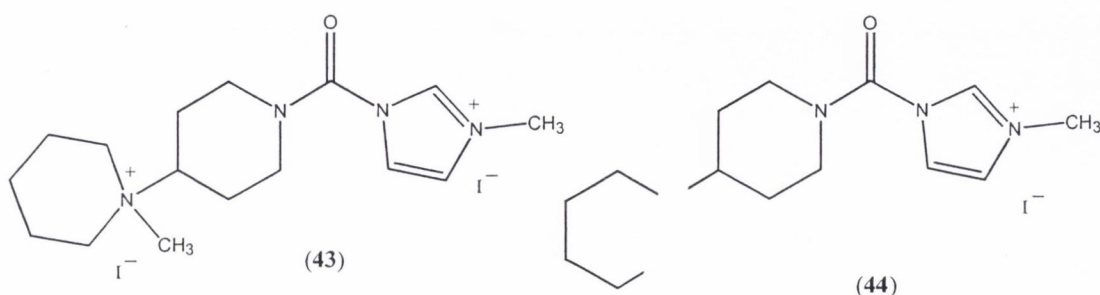
The reaction of 4-piperidinopiperidine with *N,N'*-carbonyldiimidazole (CDI) (39) was carried out in dry THF at room temperature for 18 hours (Scheme 3.14). This differed from the procedure reported by Batey *et al.*¹⁸ which required reaction at higher temperatures and for longer periods of time.



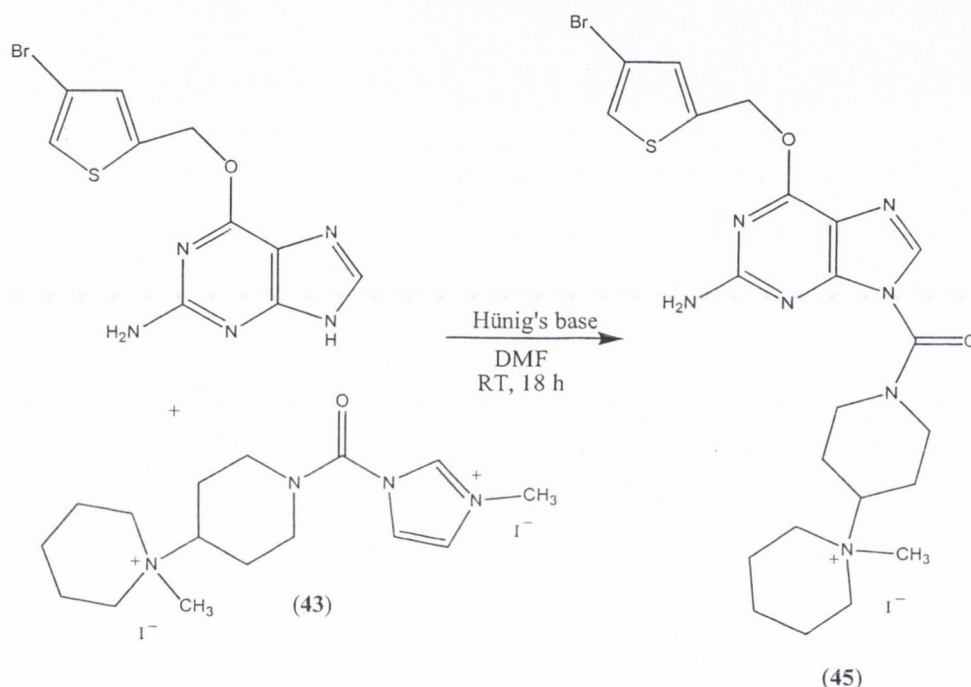
Scheme 3.14

The solvent was removed, and the residue was dissolved in DCM. This solution was washed three times with water and then once with brine to remove traces of free imidazole. The solution was dried over MgSO_4 , before being removed to afford 1-(4-piperidinopiperidinocarbonyl)imidazole (42). Compound (42), an oil, was characterized by ^1H NMR and infrared spectroscopy. The infrared carbonyl stretching frequency was observed at 1695 cm^{-1} .

Treatment of the carbamoylimidazole (42) with excess methyl iodide afforded the dimethylated carbamoylimidazolium salt (43), rather than the salt (44).



We did not investigate the use of one equivalent of methyl iodide, since in related experiments in other systems (Dr. D. J. Donnelly) this had not proved to be fruitful. The reaction was carried out in DMF at room temperature for 18 hours, and after removal of excess methyl iodide the DMF solution of (43) was treated directly with PaTrin-2 and Hünig's base (Scheme 3.15). After 18 hours at room temperature, evaporation of solvent and addition of ethanol caused precipitation of a white solid.



Scheme 3.15

The ^1H NMR spectrum of this solid showed the presence of a methyl group (2.96 ppm), helping to characterise the quaternary salt O^6 -(4-bromophenyl)-9-[4-(*N*-methylpiperidinio)piperidinocarbonyl]guanine iodide (45). Further evidence for this structure came from the observed solubility of (45) in water.

Following the procedure reported by Batey *et al.*¹⁸ we had successfully synthesized and isolated a tetrasubstituted urea type of PaTrin-2 derivative (9-CONR₂), whereas the procedure involving the corresponding carbamoyl chloride had not been successful (Section 3.7.3). Pharmacological evaluation of the quaternary salt O^6 -(4-

bromothienyl)-9-[4-(*N*-methylpiperidinio)piperidinocarbonyl]guanine iodide (45)

would provide useful results.

3.10 Chapter summary

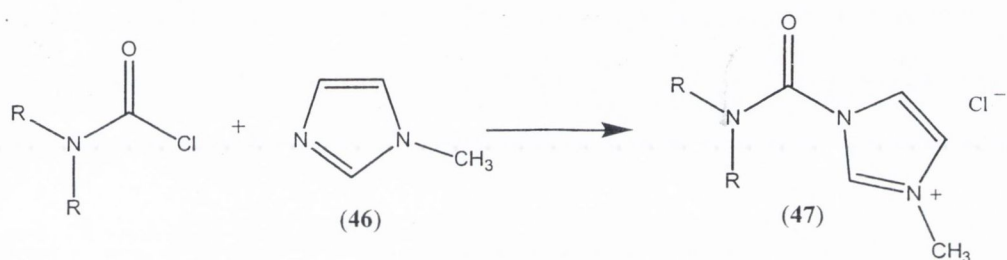
This chapter has focussed primarily on the preparation of N-9 derivatives by the reaction of PaTrin-2 with carbamoyl chlorides. Using this procedure, we have reported the successful synthesis of a series of potential prodrugs.

However, as discussed in Section 3.3.3, we have found that the reaction of PaTrin-2 with certain carbamoyl chlorides is dependent on the type of base used, and its role in the reaction. As in the previous chapter, we have shown that reaction at the N-9 position in PaTrin-2 is more complicated than with other secondary amines. The size and shape of PaTrin-2 have been implicated in making its anion less reactive to some reagents. In addition, the unique electronic effects of the purine ring system differentiate its behaviour from that of other secondary amines. As with the findings reported in Chapter 2, we have proposed again that the ability of the PaTrin-2 anion to behave as a good leaving group is the result of these unique electronic properties (Section 3.7.3).

Although we have mainly focussed on the use of carbamoyl chlorides for the synthesis of our potential prodrugs, we have demonstrated the usefulness of an alternative approach involving *N,N'*-carbonyldiimidazole (CDI) (39) (Section 3.9.1). This procedure incidentally resulted in the preparation of a quaternary salt of a N-9 carbamoyl derivative.

A direct comparison of the procedure involving the carbamoyl chloride with that involving the carbamoylimidazolium salt shows the latter to be a higher yielding reaction. However, this method is comparatively time-consuming, and if the amine

that is being linked to PaTrin-2 also contains a tertiary amino group, then synthesis results in a quaternary salt. The use of excess methyl iodide is responsible for methylation at sites other than the imidazole moiety. We did attempt an alternative approach that involved the reaction of a carbamoyl chloride with the commercially available *N*-methylimidazole (46) (Scheme 3.16). However, this did not go to completion, and difficulty was experienced when we attempted to isolate the resulting carbamoylimidazolium salt (47) from the reaction mixture. Direct reaction of this with PaTrin-2 gave a further inseparable mixture.

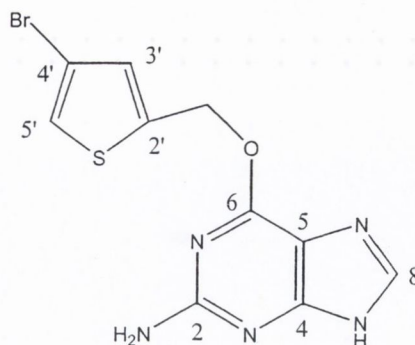


Scheme 3.16

Other reagents can be used in the preparation of tetrasubstituted ureas to provide alternative leaving groups to the chloride anion. However, as reported in the above sections of this chapter, we have satisfactorily prepared and employed carbamoyl chlorides for the synthesis of our potential prodrugs. In addition we have investigated a useful and direct route to the synthesis of quaternary salts. This type of water-soluble salt may be of use in improving drug formulation and delivery.

3.11 Experimental*

Numbering system followed for *O*⁶-(4-bromophenyl)guanine:



***O*⁶-(4-Bromophenyl)-9-(morpholinocarbonyl)guanine (4)** – Morpholine-4-carbonyl chloride (12 μ l, 0.1 mmol) was added to a solution of *O*⁶-(4-bromophenyl)guanine (33 mg, 0.1 mmol) and triethylamine (14 μ l, 0.1 mmol) in DMF (0.1 ml) under nitrogen. After 2 h, the solvent was evaporated and hot ethanol was added to the residue and a white solid (**4**) (38 mg, 86%) separated.

$\nu_{\max}$: 1579, 1614, 1693(C=O) cm^{-1}

$\lambda_{\max}$: 283nm

m.p. decomp. > 60 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 3.46 (4H, m, 3'', 5''-CH₂), 3.71 (4H, m, 2'', 6''-CH₂), 5.64 (2H, s, 6-OCH₂), 6.74 (2H, s, NH₂), 7.35 (1H, s, 3'-CH), 7.69 (1H, s, 5'-CH), 8.05 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 41.5 (3'', 5''-CH₂), 60.7 (6-OCH₂), 65.8 (2'', 6''-CH₂), 108.3 (4'-C), 112.9 (5-C), 125.3 (5'-CH), 131.0 (3'-CH), 138.3 (8-CH), 140.1 (2'-C), 148.4 (C=O), 153.1 (4-C), 159.7 (2-C), 159.9 (6-C).

Found: C, 40.06; H, 3.52; N, 17.84 %. C₁₅H₁₅BrN₆O₃S·H₂O requires C, 39.42; H, 3.75; N, 18.39 %

* For experimental details see Chapter 2, Section 2.13, pgs. 122-123.

MS (ES) $[M + H]^+$ Calcd m/z for $C_{15}H_{16}BrN_6O_3S$ 439.0188, Found 439.0172.

O⁶-(4-Bromophenyl)-9-(N,N-dimethylcarbamoyl)guanine (10) – This compound (28 mg, 71%) was prepared following the above procedure, but using *N,N*-dimethylcarbamoyl chloride (9 μ l, 0.1 mmol) instead of morpholine-4-carbonyl chloride and using DBU (15 μ l, 0.1 mmol) as a base instead of triethylamine.

$\nu_{\max}$: 1583, 1620, 1699(C=O) cm^{-1}

$\lambda_{\max}$: 283nm

m.p. decomp. > 40 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 3.01 (6H, br s, $\text{N}(\text{CH}_3)_2$), 5.65 (2H, s, 6-OCH₂), 6.75 (2H, s, NH₂), 7.36 (1H, s, 3'-CH), 7.70 (1H, s, 5'-CH), 8.04 (1H, s, 8-H).

$\delta^{13}\text{C}$ (100MHz, DMSO): 61.1 (6-OCH₂), 107.9 (4'-C), 112.8 (5-C), 125.0 (5'-CH), 131.0 (3'-CH), 137.8 (8-CH), 140.4 (2'-C), 149.9 (C=O), 153.2 (4-C), 159.4 (2-C), 160.0 (6-C).

Found: C, 39.65; H, 3.06; N, 20.03 %. $C_{13}H_{13}BrN_6O_2S \cdot 0.5 \text{ EtOH}$ requires C, 40.03; H, 3.84; N, 20.01 %

MS (ES) $[M + H]^+$ Calcd m/z for $C_{13}H_{14}BrN_6O_2S$ 397.0082, Found 397.0066.

O⁶-(4-Bromophenyl)-9-(N,N-diphenylcarbamoyl)guanine (11) – This compound (30 mg, 58%) was prepared following the above procedure, but using *N,N*-diphenylcarbamoyl chloride (23 mg, 0.1 mmol) instead of morpholine-4-carbonyl chloride and using DBU (15 μ l, 0.1 mmol) as a base instead of triethylamine.

$\nu_{\max}$: 1579, 1612, 1697(C=O) cm^{-1}

$\lambda_{\max}$: 282nm

m.p. 210 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 5.57 (2H, s, 6-OCH₂), 6.70 (2H, s, NH₂), 7.26 (2H, m, arom. 4'', 4'''-CH), 7.31 - 7.39 (9H, m, arom. 3', 2'', 2''', 3'', 3''', 5'', 5''', 6'', 6'''-CH), 7.69 (1H, s, 5'-CH), 7.99 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 60.9 (6-OCH₂), 108.1 (4'-C), 112.2 (5-C), 125.4 (5'-CH), 126.9 (arom. 2'', 2''', 6'', 6'''-CH), 127.4 (arom. 4'', 4'''-CH), 129.3 (arom. 3'', 3''', 5'', 5'''-CH), 131.1 (3'-CH), 137.6 (8-CH), 140.1 (2'-C), 141.9 (arom. 1'', 1'''-C), 148.7 (C=O), 153.5 (4-C), 159.2 (2-C), 160.0 (6-C).

Found: C, 53.26; H, 3.39; N, 15.82 %. C₂₃H₁₇BrN₆O₂S requires C, 52.98; H, 3.29; N, 16.12 %

MS (ES) [M + H]⁺ Calcd *m/z* for C₂₃H₁₈BrN₆O₂S 521.0395, Found 521.0381.

O⁶-(4-Bromophenyl)-9-(1-pyrrolidinylcarbonyl)guanine (12) – This compound (27 mg, 65%) was prepared following the above procedure, but using pyrrolidine-1-carbonyl chloride (13.4 μl , 0.1 mmol) instead of morpholine-4-carbonyl chloride and using DBU (15 μl , 0.1 mmol) as a base instead of triethylamine.

ν_{max} : 1610, 1695(C=O) cm⁻¹

λ_{max} : 283nm

m.p. 192 °C

$\delta^1\text{H}$ (400MHz, DMSO): 1.85 (4H, m, 3'', 4''-CH₂), 3.46 (4H, m, 2'', 5''-CH₂), 5.65 (2H, s, 6-OCH₂), 6.79 (2H, s, NH₂), 7.38 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.07 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 24.3 (3'', 4''-CH₂), 25.3 (3'', 4''-CH₂), 47.3 (2'', 5''-CH₂), 48.1 (2'', 5''-CH₂), 60.9 (6-OCH₂), 108.1 (4'-C), 112.9 (5-C), 125.3 (5'-CH), 131.0 (3'-CH), 137.7 (8-CH), 140.3 (2'-C), 148.0 (C=O), 153.2 (4-C), 159.6 (2-C), 160.0 (6-C).

Found: C, 42.34; H, 3.58; N, 19.81 %. $C_{15}H_{15}BrN_6O_2S$ requires C, 42.56; H, 3.57; N, 19.85

MS (ES) $[M + H]^+$ Calcd m/z for $C_{15}H_{16}BrN_6O_2S$ 423.0239, Found 423.0257.

4-Methylpiperazine-1-carbonyl chloride hydrochloride (17) – *N*-Methylpiperazine (10 mg, 0.1 mmol) in dry DCM (0.5 ml) was added to an ice-cold solution of bis(trichloromethyl) carbonate (11 mg, 0.037 mmol) in dry DCM (0.5 ml). The reaction was set aside at room temperature for 30 min. The solvent was evaporated and compound (17) was subsequently used without further purification.

$\nu_{\max}$: 1752(C=O) cm^{-1}

***O*⁶-(4-Bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (18)** – 4-Methylpiperazine-1-carbonyl chloride hydrochloride (17) (20 mg, 0.1 mmol) was added to a solution of *O*⁶-(4-bromophenyl)guanine (33 mg, 0.1 mmol) and DBU (30 μ l, 0.2 mmol) in DMF (1 ml) under nitrogen. After 4 h, the solvent was evaporated and ethanol was added to the residue to precipitate (18) (27 mg, 60%) as a white solid.

$\nu_{\max}$: 1608, 1691(C=O) cm^{-1}

$\lambda_{\max}$: 282nm

m.p. 198 °C

δ^1H (400MHz, DMSO): 2.23 (3H, s, NCH_3), 2.41 (4H, br s, 3'', 5''-CH₂), 3.47 (4H, br s, 2'', 6''-CH₂), 5.65 (2H, s, 6-OCH₂), 6.75 (2H, s, NH_2), 7.37 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.06 (1H, s, 8-CH).

$\delta^{13}C$ (100MHz, DMSO): 45.4 ($N-CH_3$), 45.2 (2'', 6''-CH₂), 53.9 (3'', 5''-CH₂), 60.8 (6-OCH₂), 108.0 (4'-C), 112.7 (5-C), 125.2 (5'-CH), 130.9 (3'-CH), 138.1 (8-CH), 140.2 (2'-C), 148.5 (C=O), 153.3 (4-C), 159.5 (2-C), 160.0 (6-C).

Found: **C**, 42.34; **H**, 4.06; **N**, 21.48%. $C_{16}H_{18}BrN_7O_2S$ requires **C**, 42.48; **H**, 4.01; **N**, 21.68 %

MS $[M + H]^+$ (ES) Calcd m/z for $C_{16}H_{19}BrN_7O_2S$ 452.0504, Found 452.0470.

O⁶-(4-Bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine hydrochloride (19)

Hydrochloric acid (1M; 100 μ l) was added to a solution of *O⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (18)* (45.2 mg, 0.1 mmol) in methanol (4 ml). The reaction was set aside for 10 min. The solvent was evaporated, and ether added to the residue and a white solid (**19**) (44 mg, 90%) separated.

$\nu_{\max}$: 1580, 1617, 1699(C=O) cm^{-1}

$\lambda_{\max}$: 282nm

m.p. 222 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 2.51 (4H, br s, 3'', 5''-CH₂), 2.84 (3H, s, NCH₃), 3.44 (4H, br s, 2'', 6''-CH₂), 5.65 (2H, s, 6-OCH₂), 6.91 (2H, s, NH₂), 7.38 (1H, s, 3'-CH), 7.73 (1H, s, 5'-CH), 8.06 (1H, s, 8-CH), 10.74 (1H, s, HCl).

$\delta^{13}\text{C}$ (100MHz, DMSO): 42.2 (NCH₃), 43.1 (2'', 6''-CH₂), 52.1 (3'', 5''-CH₂), 60.9 (6-OCH₂), 108.1 (4'-C), 112.9 (5-C), 125.3 (5'-CH), 131.0 (3'-CH), 138.5 (8-CH), 140.3 (2'-C), 148.7 (C=O), 153.2 (4-C), 159.7 (2-C), 160.0 (6-C).

Found: **C**, 39.11; **H**, 3.99; **N**, 19.81 %. $C_{16}H_{19}BrN_7O_2\text{SCl}$ requires **C**, 39.31; **H**, 3.92; **N**, 20.06 %

O⁶-(4-Bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine citrate (20) – This compound (61 mg, 95%) was prepared following the above procedure, but using citric acid (19.2 mg, 0.1 mmol) instead of hydrochloric acid.

$\nu_{\max}$: 1618, 1719(C=O) cm^{-1}

λ_{max} : 284, 366nm

m.p. decomp. > 100 °C

$\delta^1\text{H}$ (400MHz, DMSO): 2.39 (3H, s, NCH_3), 2.63 (6H, m, 3'', 5''- CH_2 , citric acid- CH_2), 3.52 (4H, br s, 2'', 6''- CH_2), 4.08 (1H, s, citric acid-OH), 5.65 (2H, s, 6-O CH_2), 6.77 (2H, s, NH_2), 7.37 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.05 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 43.1 (citric acid- CH_2), 44.7 (NCH_3), 53.6 (3'', 5''- CH_2), 60.8 (6-O CH_2), 71.9 (citric acid-C), 108.0 (4'-C), 112.7 (5-C), 125.2 (5'-CH), 131.0 (3'-CH), 138.2 (8-CH), 140.2 (2'-C), 148.5 ($\text{C}=\text{O}$), 153.3 (4-C), 159.5 (2-C), 160.0 (6-C), 171.1 (citric acid-COOH).

Found: C, 38.93; H, 4.09; N, 14.11 %. $\text{C}_{22}\text{H}_{26}\text{BrN}_7\text{O}_9\text{S}\cdot 1.75 \text{H}_2\text{O}$ requires C, 39.11; H, 4.40; N, 14.52 %

***O*⁶-(4-Bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine maleate (21) –**

This compound (51 mg, 90%) was prepared following the above procedure, but using maleic acid (11.6 mg, 0.1 mmol) instead of hydrochloric acid.

ν_{max} : 1616, 1580, 1699($\text{C}=\text{O}$) cm^{-1}

λ_{max} : 278nm

m.p. 55 °C

$\delta^1\text{H}$ (400MHz, DMSO): 2.78 (3H, s, NCH_3), 3.17 (4H, s, 3'', 5''- CH_2), 3.64 (4H, s, 2'', 6''- CH_2), 5.66 (2H, s, 6-O CH_2), 6.07 (2H, s, maleic acid-CH), 6.85 (2H, s, NH_2), 7.37 (1H, s, 3'-CH), 7.72 (1H, s, 5'-CH), 8.06 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 43.0 (NCH_3), 43.7 (2'', 6''- CH_2), 52.7 (3'', 5''- CH_2), 60.8 (6-O CH_2), 108.0 (4'-C), 112.8 (5-C), 125.2 (5'-CH), 130.9 (3'-CH), 135.2 (maleic acid-CH), 138.3 (8-CH), 140.2 (2'-C), 148.6 ($\text{C}=\text{O}$), 153.1 (4-C), 159.6 (2-C), 160.0 (6-C), 167.0 (maleic acid-COOH).

Found: C, 40.41; H, 3.77; N, 16.15 %. $C_{20}H_{22}BrN_7O_6S \cdot 1.5 H_2O$ requires C, 40.34; H, 4.23; N, 16.47 %

O⁶-(4-Bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine pamoate (22) – A solution of pamoic acid (19.4 mg, 0.05 mmol) and ammonia (16 μ l, 0.05 mmol) in methanol (1 ml) was added to a solution of *O⁶-(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (18)* (45.2 mg, 0.1 mmol) in methanol (4 ml). The reaction was set aside for 10 min. The solvent was evaporated, and ether added to the residue and a yellow solid (**22**) (58 mg, 90%) separated.

$\nu_{\max}$: 1579, 1618, 1700(C=O) cm^{-1}

$\lambda_{\max}$: 284nm

m.p. decomp. > 80 $^{\circ}C$

δ^1H (400MHz, DMSO): 2.34 (6H, s, 2 x NCH_3), 2.63 (8H, s, 2 x 3'', 5''-CH), 3.52 (8H, s, 2 x 2'', 6''-CH), 4.70 (2H, s, pamoic- CH_2), 5.65 (4H, s, 2 x 6-O CH_2), 6.77 (4H, s, 2 x NH_2), 7.02 (2H, t, $J=7.0$, pamoic 7-CH), 7.14 (2H, t, $J=8.0$, pamoic 6-CH), 7.37 (2H, s, 2 x 3'-CH), 7.67 (2H, d, $J=8.0$, pamoic 8-CH), 7.71 (2H, s, 2 x 5''-CH), 8.05 (2H, s, 2 x 8-CH), 8.18 (4H, m, pamoic 5-CH).

$\delta^{13}C$ (100MHz, DMSO): 19.9 (pamoic- CH_2), 44.5 (NCH_3), 48.5 (2'', 6''- CH_2), 53.5 (3'', 5''- CH_2), 60.9 (6-O CH_2), 108.1 (4'-C), 112.8 (5-C), 119.6 (pamoic 5-CH), 120.9 (pamoic 4-C), 121.5 (pamoic 7-CH), 123.7 (pamoic 6-CH), 125.3 (5'-CH), 126.3 (pamoic 8-CH), 129.2 (pamoic 1-CH), 129.3 (pamoic 9-C), 131.0 (3'-CH), 135.4 (pamoic 10-C), 138.3 (8-CH), 140.3 (2'-C), 148.6 (C=O), 153.3 (4-C), 156.1 (pamoic-COH), 159.6 (2-C), 160.1 (6-C), 171.6 (pamoic-COOH).

Found: C, 47.4; H, 3.93; N, 15.35%. $C_{55}H_{52}Br_2N_{14}O_{10}S_2$ requires C, 51.08; H, 4.02; N, 15.17 %

4-Benzylpiperazine-1-carbonyl chloride hydrochloride (28) – *N*-Benzylpiperazine (87 μ l, 0.5 mmol) in dry DCM (2 ml) was added to an ice-cold solution of bis(trichloromethyl) carbonate (55 mg, 0.185 mmol) in dry DCM (2 ml). The reaction was set aside at room temperature for 30 min. The solvent was removed under reduced pressure. Compound (28) was subsequently used without further purification.

ν_{max} : 1752(C=O) cm^{-1}

***N*-(3-Dimethylaminopropyl)-*N*-methylcarbamoyl chloride hydrochloride (29)** – This compound was prepared following the above procedure, but using *N,N,N'*-trimethyl-1,3-propanediamine (73 μ l, 0.5 mmol) instead of *N*-benzylpiperazine. This compound was used without further purification.

ν_{max} : 1752(C=O) cm^{-1}

4-Piperidinopiperidine-1-carbonyl chloride hydrochloride (30) – This compound was prepared following the above procedure, but using 4-piperidinopiperidine (84 mg, 0.5 mmol) instead of *N*-benzylpiperazine. This compound was used without further purification.

ν_{max} : 1735(C=O) cm^{-1}

9-(4-Benzyl-1-piperazinylcarbonyl)-*O*⁶-(4-bromophenyl)guanine (31) – 4-Benzylpiperazine-1-carbonyl chloride hydrochloride (28) (0.5 mmol) was added to a solution of *O*⁶-(4-bromophenyl)guanine (163 mg, 0.5 mmol) and DBU (150 μ l, 1 mmol) in DMF (3 ml) under nitrogen. After 4 h, the solvent was evaporated and compound (31) (106 mg, 40%) was isolated by flash chromatography [EtOAc:hexane; 1:1].

$\nu_{\max}$: 1689(C=O) cm^{-1}

$\lambda_{\max}$: 251, 281 nm

m.p. decomp. > 200 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400 MHz, DMSO): 3.54 (2H, s, NCH_2Ar), 3.63 (4H, br s, 3'', 5''- CH_2), 4.11 (4H, br s, 2'', 6''- CH_2), 5.64 (2H, s, 6-O CH_2), 6.78 (2H, s, NH_2), 7.26 (1H, m, arom. 4'''-CH), 7.33 (4H, m, arom. 2''', 3''', 5''', 6'''-CH), 7.37 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.06 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100 MHz, DMSO): 60.8 (6-O CH_2), 61.6 (NCH_2Ar), 108.1 (4'-C), 112.7 (5-C), 125.4 (5'-CH), 127.1 (arom. 4'''-CH), 128.3 (arom. 3''', 5'''-CH), 128.9 (arom. 2''', 6'''-CH), 131.1 (3'-CH), 137.9 (arom. 1'''-C), 138.2 (8-CH), 140.3 (2'-C), 148.5 (C=O), 153.4 (4-C), 159.5 (2-C), 160.1 (6-C).

Found: C, 46.60; H, 4.07; N, 16.96 %. $\text{C}_{22}\text{H}_{22}\text{BrN}_7\text{O}_2\text{S}\cdot 2\text{H}_2\text{O}$ requires C, 46.85; H, 4.65; N, 17.39 %

(This compound proved too unstable to allow for the measurement of accurate mass)

O⁶-(4-Bromophenyl)-9-[N-(3-dimethylaminopropyl)-N-methylcarbamoyl]guanine

(32) – This compound was prepared following the above procedure, but using two equivalents of *N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl chloride hydrochloride (29) (1 mmol) instead of benzylpiperazine-1-carbonyl chloride hydrochloride (28). A small amount of (32) was isolated by its slow crystallisation from a mixture of acetonitrile and ethyl acetate. The compound (32) was characterized by observing the downfield signals in the ^1H NMR spectrum.* Further characterization was not possible as the solid decomposed upon standing for several hours.

* Full characterization of the upfield region of this spectrum was not possible due to the presence of signals associated with several solvents that had been used in attempts to crystallize (32).

$\delta^1\text{H}$ (400MHz, DMSO): 5.65 (2H, s, 6- OCH_2), 6.79 (2H, s, NH_2), 7.37 (1H, s, 3'- CH), 7.72 (1H, s, 5'- CH), 8.07 (1H, s, 8- CH).

Morpholino 2,4,5-trichlorophenyl carbonate (37a) – Morpholine (54 μl , 0.6 mmol) and Hünig's base (102 μl , 0.6 mmol) were added to a solution of bis(2,4,5-trichlorophenyl) carbonate (252 mg, 0.6 mmol) in dry DMF. The reaction was set aside with stirring for 2 days. The solvent was removed and DCM was added to the residue. Compound (37a) (180 mg, 97%) separated upon the addition of hexane.

ν_{max} : 1691($\text{C}=\text{O}$) cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 3.44 (4H, br s, 3, 5- CH_2), 3.66 (4H, br s, 2, 6- CH_2), 7.81 (1H, s, arom. 6'- CH), 8.01 (1H, s, arom. 3'- CH).

4-Piperidinopiperidino 2,4,5-trichlorophenyl carbonate (37b) – 4-Piperidinopiperidine (17 mg, 0.1 mmol) was added to a solution of bis(2,4,5-trichlorophenyl) carbonate (42 mg, 0.1 mmol) in DMF at 80 $^{\circ}\text{C}$. The reaction was set aside at this temperature for 18 h. The solvent was removed and DCM was added to the residue. Compound (37b) (27 mg, 50%) separated upon the addition of hexane.

ν_{max} : 1714($\text{C}=\text{O}$) cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 1.39 (2H, m, 3 $_{\text{ax}}''$, 5 $_{\text{ax}}''$ - CH), 1.50 (6H, m, 3 $_{\text{eq}}''$, 5 $_{\text{eq}}''$, 3 $_{\text{ax}}'$, 5 $_{\text{ax}}'$, 4 $_{\text{ax}}'$, 4 $_{\text{eq}}'$ - CH), 1.77 (2H, m, 3 $_{\text{eq}}'$, 5 $_{\text{eq}}'$ - CH), 2.44 (5H, m, 2 $_{\text{ax}}''$, 6 $_{\text{ax}}''$, 2 $_{\text{eq}}''$, 6 $_{\text{eq}}''$, 4'- CH), 3.05 (2H, m, 2 $_{\text{ax}}'$, 6 $_{\text{ax}}'$ - CH), 3.99 (2H, m, 2 $_{\text{eq}}'$, 6 $_{\text{eq}}'$ - CH), 7.79 (1H, s, arom. 6'- CH), 7.98 (1H, s, arom. 3'- CH).

4-Methylpiperazin-1-yl 4-nitrophenyl carbonate (38a) – *N*-Methylpiperazine (44 μl , 0.4 mmol) was added to a solution of bis(4-nitrophenyl) carbonate (122 mg, 0.4

mmol) in dry DCM. After 4 h the reaction mixture was washed three times with a 10% NaHCO₃ solution and once with brine. The DCM solution was dried over MgSO₄ and evaporated to afford compound (38a) (48 mg, 45%), an oil, which later solidified.

$\delta^1\text{H}$ (400MHz, DMSO): 2.23 (3H, s, NCH₃), 2.39 (4H, br s, 3, 5-CH₂), 3.46 (4H, br s, 2, 6-CH₂), 7.43 (2H, d, $J=9.0$, arom. 2', 6'-CH), 8.26 (2H, d, $J=9.0$, arom. 3', 5'-CH).

1-(4-Piperidinopiperidinocarbonyl)imidazole (42) – *N,N'*-Carbonyldiimidazole (89 mg, 0.55 mmol) in dry THF (1 ml) was added to a solution of 4-piperidinopiperidine (84mg, 0.5 mmol) in dry THF (1 ml). The reaction was set aside with stirring for 18 h. The solvent was removed under reduced pressure and DCM was added to the residue. The solution was washed three times with water and once with brine, and dried over MgSO₄. The solvent was evaporated to afford (42) (73 mg, 56%) as an oil.

ν_{max} : 1695(C=O) cm⁻¹

$\delta^1\text{H}$ (400MHz, DMSO): 1.37 (2H, m, 3_{ax}'', 5_{ax}''-CH), 1.46 (6H, m, 3_{eq}'', 5_{eq}'', 3_{ax}', 5_{ax}', 4_{ax}', 4_{eq}'-CH), 1.75 (2H, m, 3_{eq}', 5_{eq}'-CH), 2.44 (5H, m, 2_{ax}'', 6_{ax}'', 2_{eq}'', 6_{eq}'', 4'-CH), 3.04 (2H, m, 2_{ax}', 6_{ax}'-CH), 3.89 (2H, m, 2_{eq}', 6_{eq}'-CH), 7.02 (1H, s, arom. 5-CH), 7.45 (1H, s, arom. 4-CH), 8.0 (1H, s, arom. 2-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 24.5 (4''-CH₂), 26.0 (3', 5'-CH₂), 27.4 (3'', 5''-CH₂), 45.4 (2', 6'-CH₂), 49.7 (2'', 6''-CH₂), 61.1 (4'-CH) 118.6 (arom. 5-CH), 128.7 (arom. 4-CH), 137.0 (arom. 2-CH), 150.1 (C=O).

O⁶-(4-Bromophenyl)-9-[4-(*N*-methylpiperidinio)piperidinocarbonyl]guanine iodide (45) – An excess of methyl iodide (25 μl) was added to a solution of 1-(4-piperidinopiperidinocarbonyl)imidazole (42) in DMF (0.5 ml). The reaction was set

aside at room temperature for 18 h. The excess methyl iodide was removed under reduced pressure. *O*⁶-(4-bromophenyl)guanine (33 mg, 0.1 mmol) and Hünig's base (17 μ l, 0.1 mmol) were added to the solution in DMF. After 18 h, the solvent was evaporated and ethanol added to the residue. A white solid separated, was collected and dried to afford the quaternary salt (**45**) (60 mg, 90%).

ν_{max} : 1576, 1616, 1693 (C=O) cm^{-1}

λ_{max} : 282nm

m.p. decomp. > 200 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 1.59 (2H, m, $3_{\text{ax}}'''$, $5_{\text{ax}}'''$ -CH), 1.59 (6H, m, $3_{\text{ax}}''$, $5_{\text{ax}}''$, $3_{\text{eq}}'''$, $5_{\text{eq}}'''$, $4_{\text{ax}}'''$, $4_{\text{eq}}'''$ -CH), 2.16 (2H, m, $3_{\text{eq}}''$, $5_{\text{eq}}''$ -CH), 2.96 (3H, s, CH_3), 3.09 (2H, m, $2_{\text{ax}}'''$, $6_{\text{ax}}'''$ -CH), 3.41 (4H, m, $2_{\text{eq}}'''$, $6_{\text{eq}}'''$, $2_{\text{ax}}''$, $6_{\text{ax}}''$ -CH), 3.90 (3H, m, $2_{\text{eq}}''$, $6_{\text{eq}}''$, $4''$ -CH), 5.66 (2H, s, 6-OCH₂), 6.80 (2H, s, NH₂), 7.37 (1H, s, $3'$ -CH), 7.72 (1H, s, $5'$ -CH), 8.06 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 19.2 ($4'''$ -CH₂), 20.7 ($3'''$, $5'''$ -CH₂), 24.4 ($3''$, $5''$ -CH₂), 43.6 (CH_3), 58.7 ($2'''$, $6'''$ -CH₂), 60.9 (6-OCH₂), 66.9 ($4''$ -CH), 108.1 ($4'$ -C), 112.9 (5 -C), 125.3 ($5'$ -CH), 131.1 ($3'$ -CH), 138.5 (8-CH), 140.3 ($2'$ -C), 148.4 (C=O), 153.4 (4 -C), 159.7 (2 -C), 160.1 (6 -C).

Found: **C**, 39.57; **H**, 4.44; **N**, 14.40 %. C₂₂H₂₉BrIN₇O₂S requires **C**, 39.89; **H**, 4.41; **N**, 14.80 %

MS (ES) Calcd m/z for C₂₂H₂₉BrN₇O₂S 534.1287, Found 534.1282.

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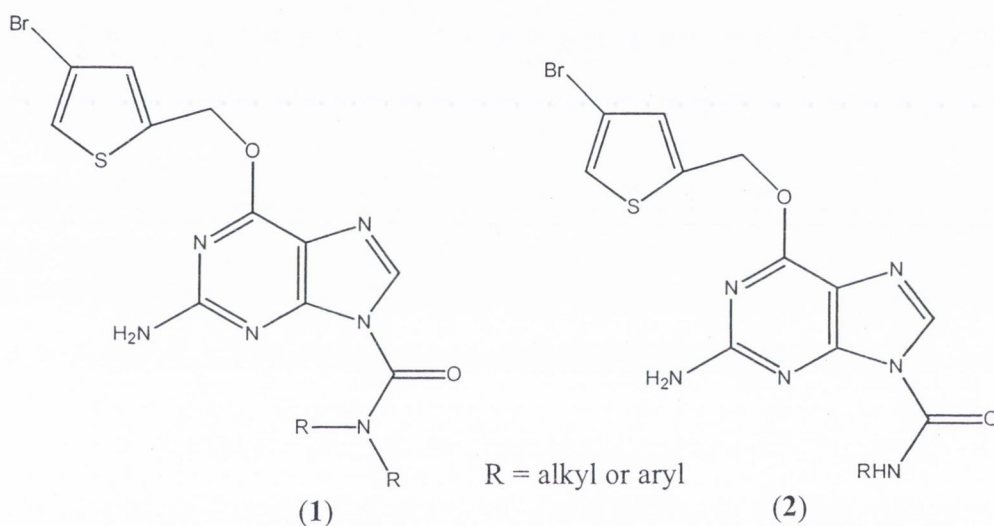
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CHAPTER 4

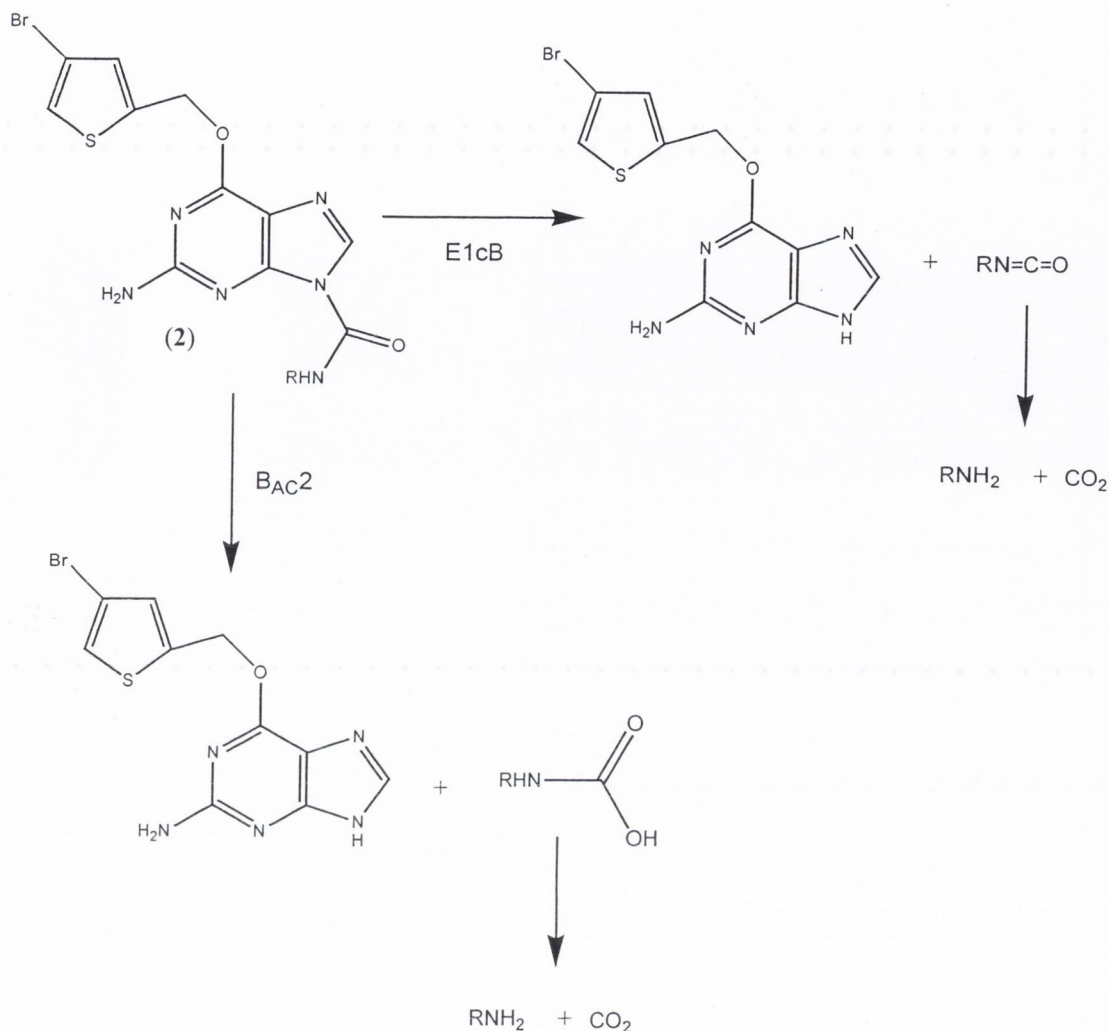
Chapter 4: Synthesis of O^6 -(4-bromophenyl)-9-(monoalkylcarbamoyl)guanines

4.1 Introduction

In Chapter 3 we reported the synthesis of a series of O^6 -(4-bromophenyl)-9-(dialkylcarbamoyl)guanines (**1**) that were prepared by the reaction of PaTrin-2 with activated derivatives derived from secondary amines. In this chapter we will discuss the preparation of N-9 carbamoyl derivatives (**2**) derived from primary amines. This latter type of prodrug (**2**) is predictably more susceptible to hydrolysis than the prodrug (**1**), as is evident from a consideration of the mechanism of hydrolysis.



Reports in the literature suggest that this may involve a pathway involving a reactive isocyanate intermediate (Scheme 4.1).²⁻⁵ Hegarty *et al.*³ have established that compounds with good leaving groups decompose by this mechanism, which is equivalent to the E1cB elimination mechanism. Although we have found the PaTrin-2 anion to be a good leaving group, we are presently unable to say whether such N-9 carbamoyl derivatives (**2**) would undergo hydrolysis by this mechanism or by the alternative B_{AC}2 pathway (Scheme 4.1) involving attack on the amide carbonyl by a hydroxide anion to give a tetrahedral intermediate. The B_{AC}2 pathway is the proposed pathway for the hydrolysis of the prodrugs discussed previously (Chapters 2 and 3).

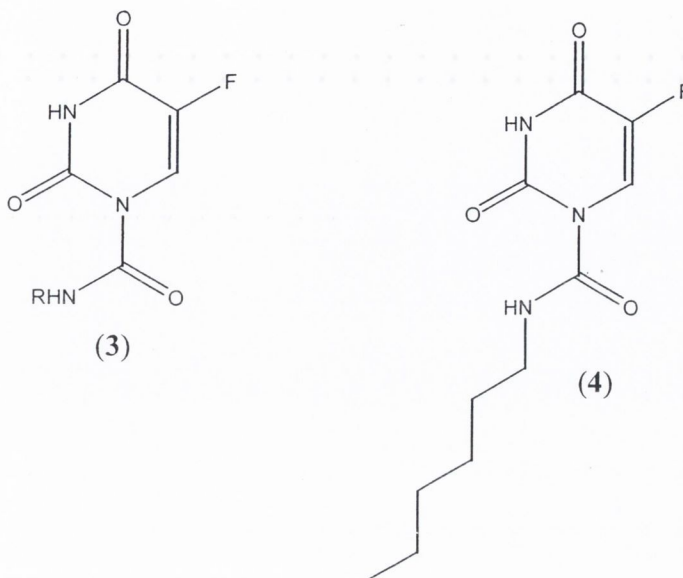


Scheme 4.1

Even though prodrugs of type (2) may be readily susceptible to hydrolysis, pharmacological evaluation may indicate this to be an advantage.

There are many reports discussing the preparation of prodrugs of the antitumour agent, 5-fluorouracil, some of which we have mentioned previously. Ozaki *et al.*⁶ reported the synthesis of 1-carbamoyl-5-fluorouracil derivatives (3). Their interest in this type of prodrug arose from its susceptibility to hydrolysis. They reported that several other prodrugs that would be expected to liberate 5-fluorouracil have little or no activity towards tumours. In these prodrugs, removal of the alkoxycarbonyl,

dialkylcarbamoyl and some other substituents by hydrolysis is too slow and the drugs do not undergo biodegradation.



Of all the structural analogues of this type that were prepared by Ozaki *et al.*⁶, 1-hexylcarbamoyl-5-fluorouracil (4) was the most effective derivative. The lipophilic hexyl group helped to deliver the prodrug to tumour tissues. The prodrug (4) was also found to be effective against a wider tumour spectrum than the parent drug. Amongst the potential prodrugs whose synthesis we will discuss in the following sections is a PaTrin-2 N-9 hexylcarbamoyl derivative analogous to (4).

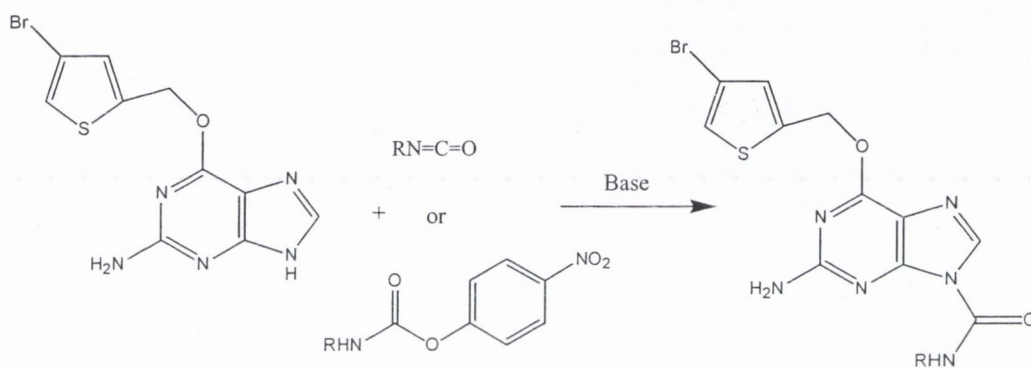
The hydrolysis pattern of prodrugs of PaTrin-2 may not be similar to prodrugs of 5-fluorouracil, providing a further incentive to investigate this category. Such derivatives, of type (2), will provide a useful comparison to the prodrugs from the two other categories whose synthesis we have already discussed.

4.2 Routes and reagents – a summary

In the following sections of this chapter we will report the synthesis of a series of N-9 monoalkylcarbamoyl derivatives. In Chapter 2 we investigated the most efficient route to acylation at the N-9 position of PaTrin-2 using an activated carbonic acid

derivative (**Route 2**). As previously discussed, there are many types of activated agent that may be employed.

This chapter will discuss the usefulness of two categories of reagent for the carbamoylation of PaTrin-2 (Scheme 4.2). The following sections will focus initially on the reaction of isocyanates with PaTrin-2, and their preparation from the phosgene source bis(trichloromethyl) carbonate. We will then consider an activated derivative containing a 4-nitrophenoxy group as an alternative reagent to an isocyanate.



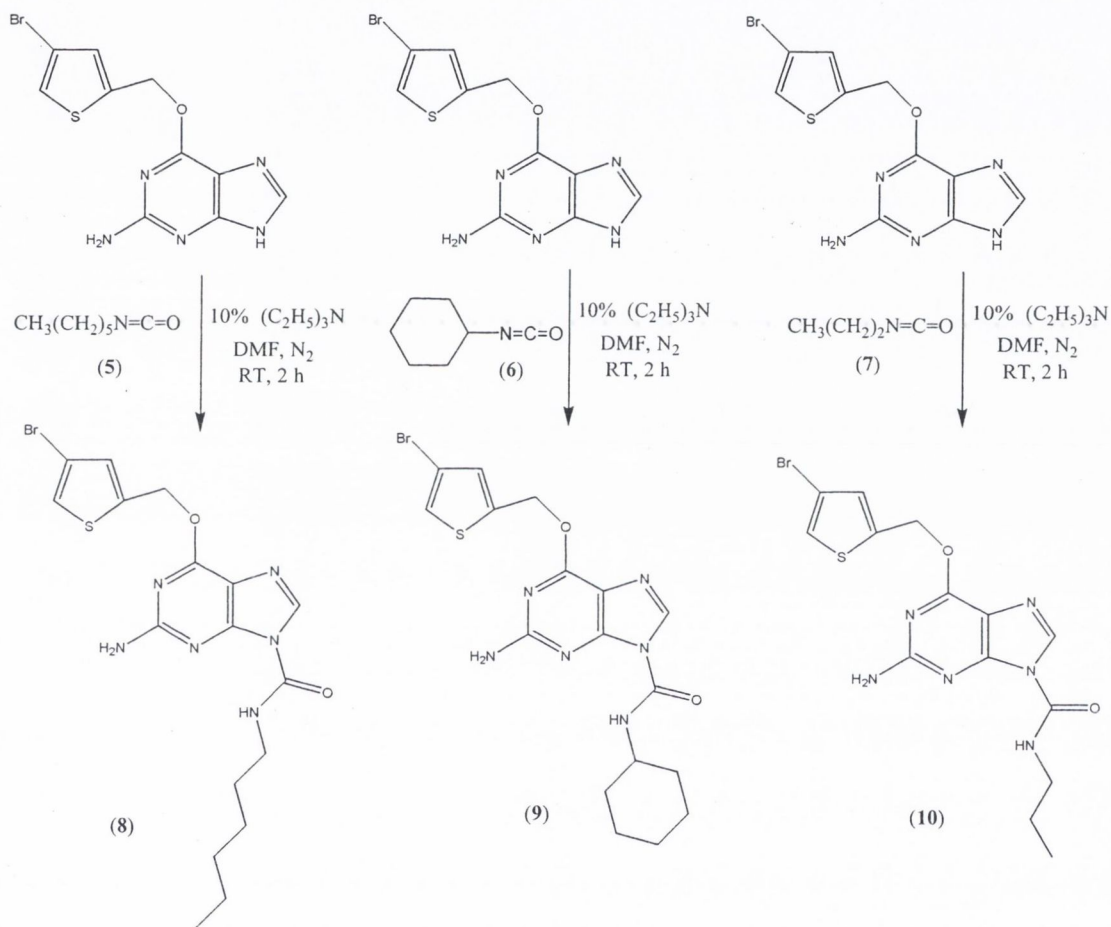
Scheme 4.2

As with the other categories of prodrugs we have discussed, it was again important to prepare derivatives that contain a tertiary amino group. As mentioned previously, this makes possible the preparation of water-soluble salts for improved drug formulation and therefore delivery.

Our discussion of the preparation of *O*⁶-(4-bromophenyl)-9-(monoalkylcarbamoyl)-guanines will again demonstrate the special properties of PaTrin-2 as a reactant. We will again show that the electronic effects of the purine ring system and the size and shape of the PaTrin-2 molecule are factors important in rationalising its reactions.

4.3 The reaction of PaTrin-2 with isocyanates

The reaction of PaTrin-2 with the commercially available hexyl, cyclohexyl and propyl isocyanate, (5), (6) and (7) respectively, was carried out in DMF in the presence of a catalytic amount of triethylamine (Scheme 4.3).



Scheme 4.3

The reactions were set aside for 2 hours. The solvent was removed, and analysis of the ^1H NMR spectrum of each crude residue showed an almost quantitative yield of the corresponding N-9 derivatives. DCM was added to each of the residues, followed by hexane to precipitate each of the N-9 carbamoyl derivatives. The derivatives *O*⁶-(4-bromophenyl)-9-(*N*-hexylcarbamoyl)guanine (8), *O*⁶-(4-bromophenyl)-9-(*N*-cyclohexylhexylcarbamoyl)guanine (9) and *O*⁶-(4-bromophenyl)-9-(*N*-

propylcarbamoyl)guanine (**10**) were all isolated in high yield. The reactions shown in Scheme 4.3 were found to proceed as efficiently in the presence of a catalytic amount of Hünig's base.

4.4 Characterization of *O*⁶-(4-bromophenyl)-9-(monoalkylcarbamoyl)guanines

As we have seen previously (Chapters 2 and 3), PaTrin-2 N-9 derivatives can be characterized easily by analysis of ¹H NMR spectra. The evidence for the formation of a N-9 derivative in a ¹H NMR spectrum lies in the positions of the signals associated with the NH₂ and H-8 protons. This is best illustrated by example.

Comparing the ¹H NMR spectrum of *O*⁶-(4-bromophenyl)-9-(*N*-propylcarbamoyl)guanine (**10**), shown in Fig. 4.1, with that of PaTrin-2 (Chapter 2, Section 2.3.1, pg 46) we find that the signals associated with the NH₂ and the H-8 have shifted downfield to 7.03 ppm and 8.24 ppm respectively. The signal observed furthest downfield at 8.89 ppm is associated with the NH in the N-9 substituent. Full assignment of the signals in this ¹H NMR spectrum can be found in Table 4.1.

The N-9 carbamoyl derivatives discussed in this chapter were all characterized in a similar manner. Full ¹H and ¹³C NMR spectral assignments for these compounds are given in the Experimental Section (4.10)*.

* A selection of NMR spectra from the three categories of prodrug can be found in the Appendix.

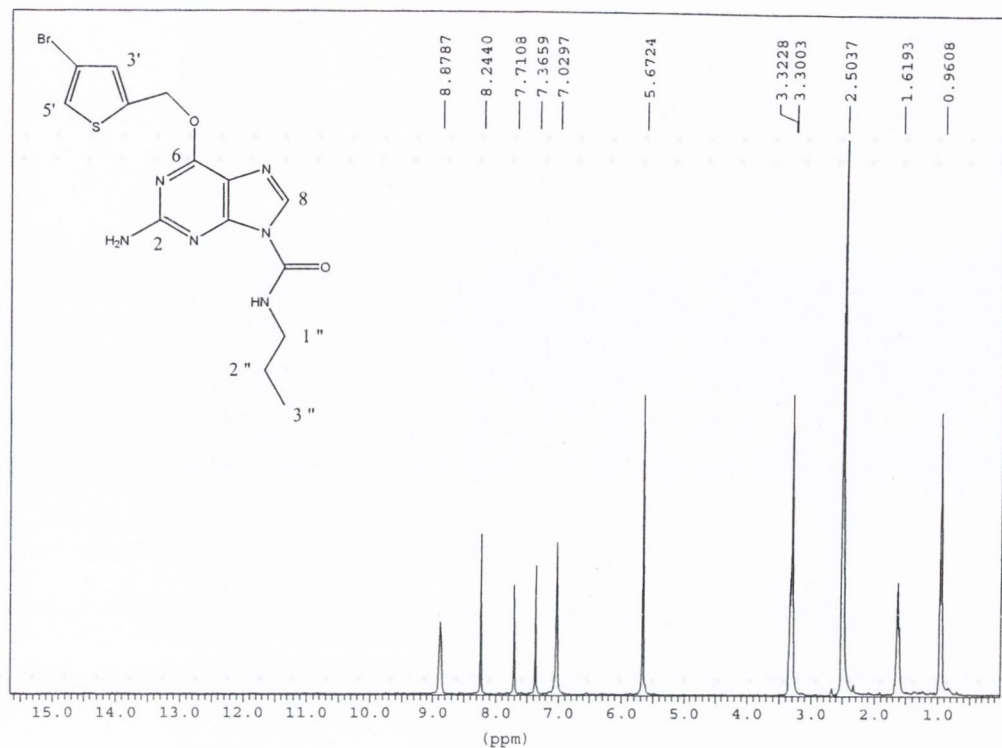


Fig. 4.1

Hydrogen	NMR δ (ppm from TMS)
3''-CH ₃	0.96
2''-CH ₂	1.62
1''-CH ₂	3.32
CH ₂	5.67
NH ₂	7.03
H-3'	7.37
H-5'	7.71
H-8	8.24
NH	8.89

Table 4.1

4.5 Isocyanate synthesis

For the general procedure for the synthesis of unsymmetrical ureas like our N-9 carbamoyl derivatives, only a limited number of isocyanates are available commercially. Therefore we needed to establish a route to the synthesis of isocyanates. This would allow for the preparation of any N-9 carbamoyl derivative, not only during the course of this research, but also if the biological results were to require the synthesis of further analogues of this type.

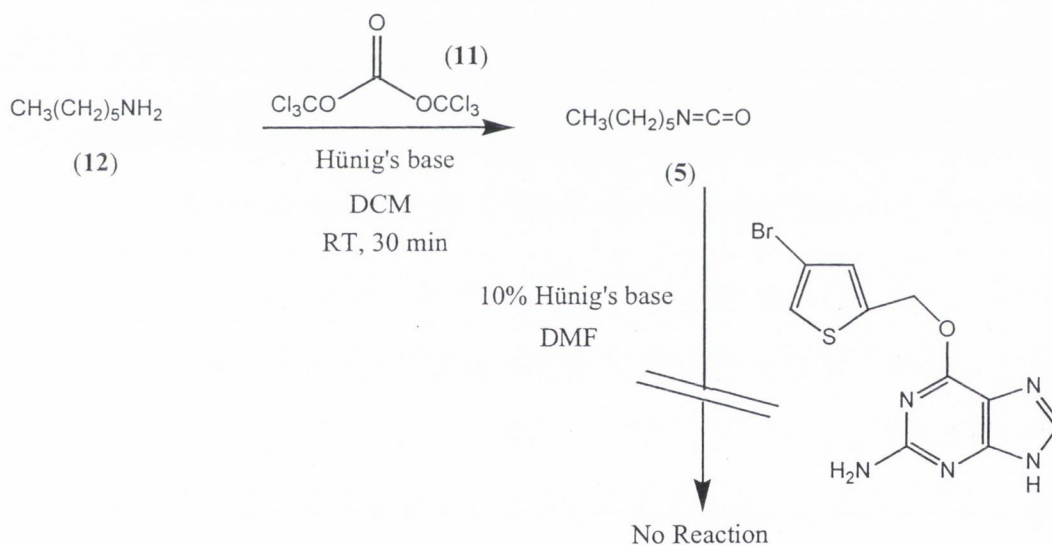
Isocyanates can be prepared by bubbling phosgene gas through a solution of an amine at an elevated temperature. An improved method for the preparation of isocyanates was introduced by Norwich *et al.*⁷ and involves the reaction of a primary amine with phosgene in the presence of base. Majer *et al.*⁸ report a variation of this procedure using bis(trichloromethyl) carbonate (BTC) (**11**) instead of phosgene. We had already found BTC to be useful in the preparation of chloroformates and carbamoyl chlorides therefore we decided to attempt the synthesis of an isocyanate following this procedure.

4.5.1 Synthesis of hexyl isocyanate

We chose hexyl isocyanate (**5**) as a suitable example, since we had already observed the reaction of PaTrin-2 with a commercial sample of this compound in the preparation of *O*⁶-(4-bromophenyl)-9-(*N*-hexylcarbamoyl)guanine (**8**) (Section 4.3).

Majer *et al.*⁸ reported a “one-pot” procedure for the synthesis of unsymmetrical disubstituted ureas. Synthesis involved the sequential addition of two different amino components to a solution of triphosgene in DCM. The reaction was carried out in the presence of Hünig’s base. We repeated this procedure.

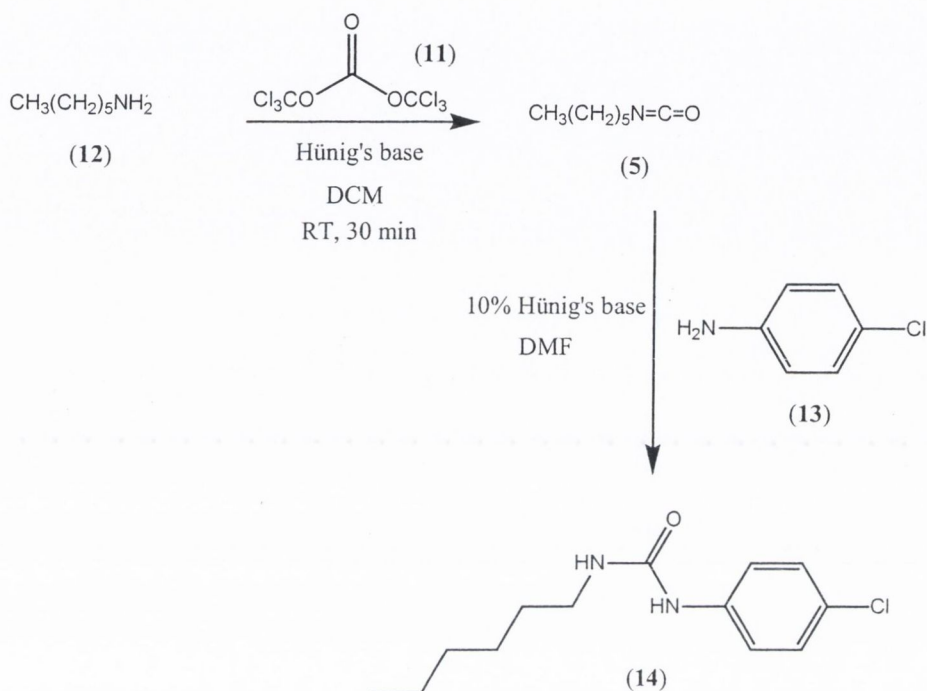
A solution of hexylamine (**12**) and Hünig's base in dry DCM was added dropwise to a solution of bis(trichloromethyl) carbonate (BTC) (**11**) in dry DCM (Scheme 4.4). The reaction was set aside for 30 minutes. The product was characterized by infrared spectroscopy, with the associated stretching frequency being found at 2269 cm^{-1} . This is consistent with the presence of $\text{N}=\text{C}=\text{O}$ (normal range $2250 - 2275\text{ cm}^{-1}$).⁹ The DCM was exchanged for DMF, and an equimolar amount of PaTrin-2 and a catalytic amount of Hünig's base were added. After 2 hours TLC did not indicate the presence of *O*⁶-(4-bromophenyl)-9-(*N*-hexylcarbamoyl)guanine (**8**), so the reaction was continued for a further 18 hours. The solvent was removed, but analysis of the ^1H NMR spectrum of the crude residue still showed no products.



Scheme 4.4

We attempted to prepare the isocyanate under alternative conditions. We used triethylamine as an alternative to Hünig's base, and also ran the reaction shown in Scheme 4.4 at lower temperatures. However, although all the products had characteristic infrared spectra, none would react with PaTrin-2.

Accordingly, we decided to observe the reaction, if any, with an alternative reagent. The reaction as shown in Scheme 4.4 was repeated but instead of adding an equimolar amount of PaTrin-2 we added an equivalent of 4-chloroaniline (**13**) (Scheme 4.5).



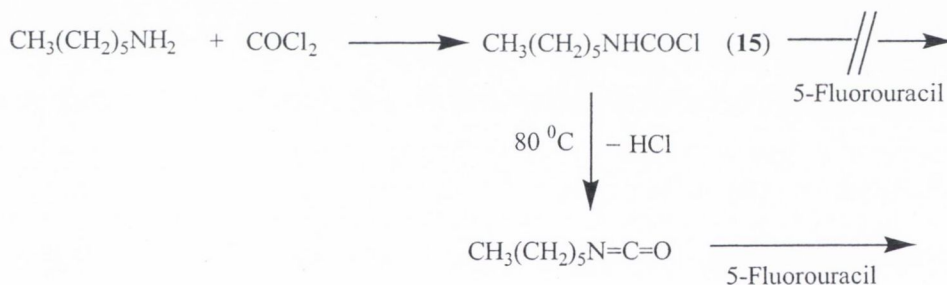
Scheme 4.5

After 2 hours, the solvent was removed, and analysis of the ^1H NMR spectrum of the crude residue showed the presence of the product **(14)** and none of the signals associated with 4-chloroaniline (**13**). The positive outcome of this reaction, together with the observed infrared spectrum, seemed to indicate the successful preparation of the isocyanate. However, it remained unclear why the material had failed to react with PaTrin-2. A closer look at the reaction mechanism involved in the synthesis of the isocyanate was required.

4.5.2 Problems with the synthesis of hexyl isocyanate

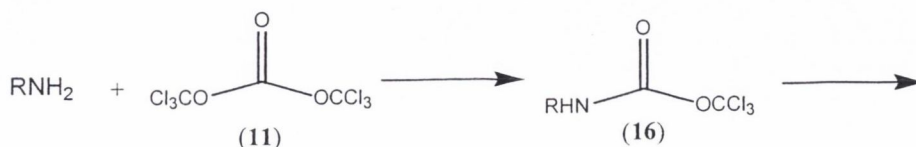
We had synthesized successfully a reactive species that behaved similarly to authentic hexyl isocyanate, but yet was somehow different. This difference had not affected its reaction with 4-chloroaniline, but had prevented it from reacting with PaTrin-2. Several reports in the literature suggested an explanation.

Ozaki *et al.*⁶ reported the synthesis of 1-hexylcarbamoyl-5-fluorouracil using a sample of hexyl isocyanate that had been prepared using phosgene. However, no product was obtained when the reaction was carried out at room temperature, even in the presence of base. They reported that the reaction was successful if carried out at 80 °C. The suggested explanation was that the reaction of hexylamine with phosgene resulted in the intermediate carbamoyl chloride (**15**) that was only converted to the reactive isocyanate at an elevated temperature (Scheme 4.6).



Scheme 4.6

Similarly, Cotarca *et al.*¹⁰ reported that the reaction of bis(trichloromethyl) carbonate (**11**) with primary amines (Scheme 4.7) initially resulted in the formation of a trichloromethyl carbamate (**16**) which then decomposed to an isocyanate.

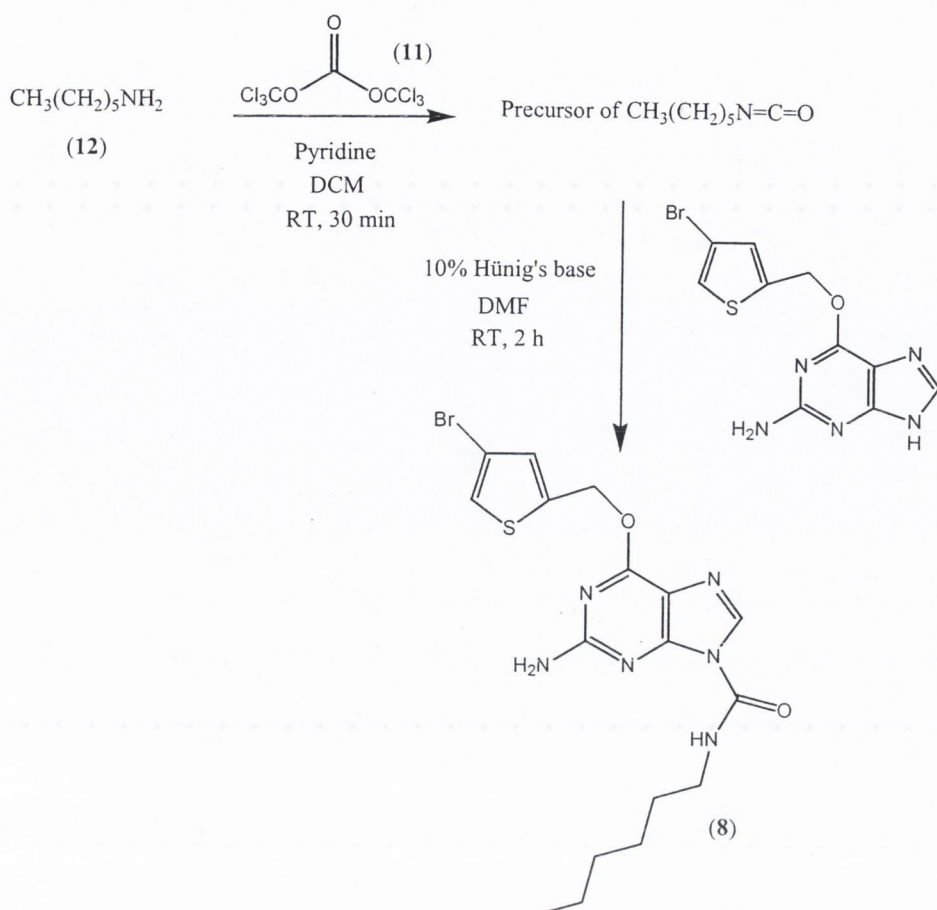


Scheme 4.7

The existence of the trichloromethyl carbamate (**16**) as the isocyanate precursor is suggested by kinetic and mechanistic studies.¹¹ The formation of (**16**) is also suggested by the Eckert mechanism¹² (Chapter 2, Section 2.3.4, Scheme 2.5, pg 49), which outlines the reaction of BTC (**11**) with nucleophiles. However, what is not evident from the report by Cotarca *et al.*¹⁰ is whether an energy barrier exists for the conversion of (**16**) into an isocyanate.

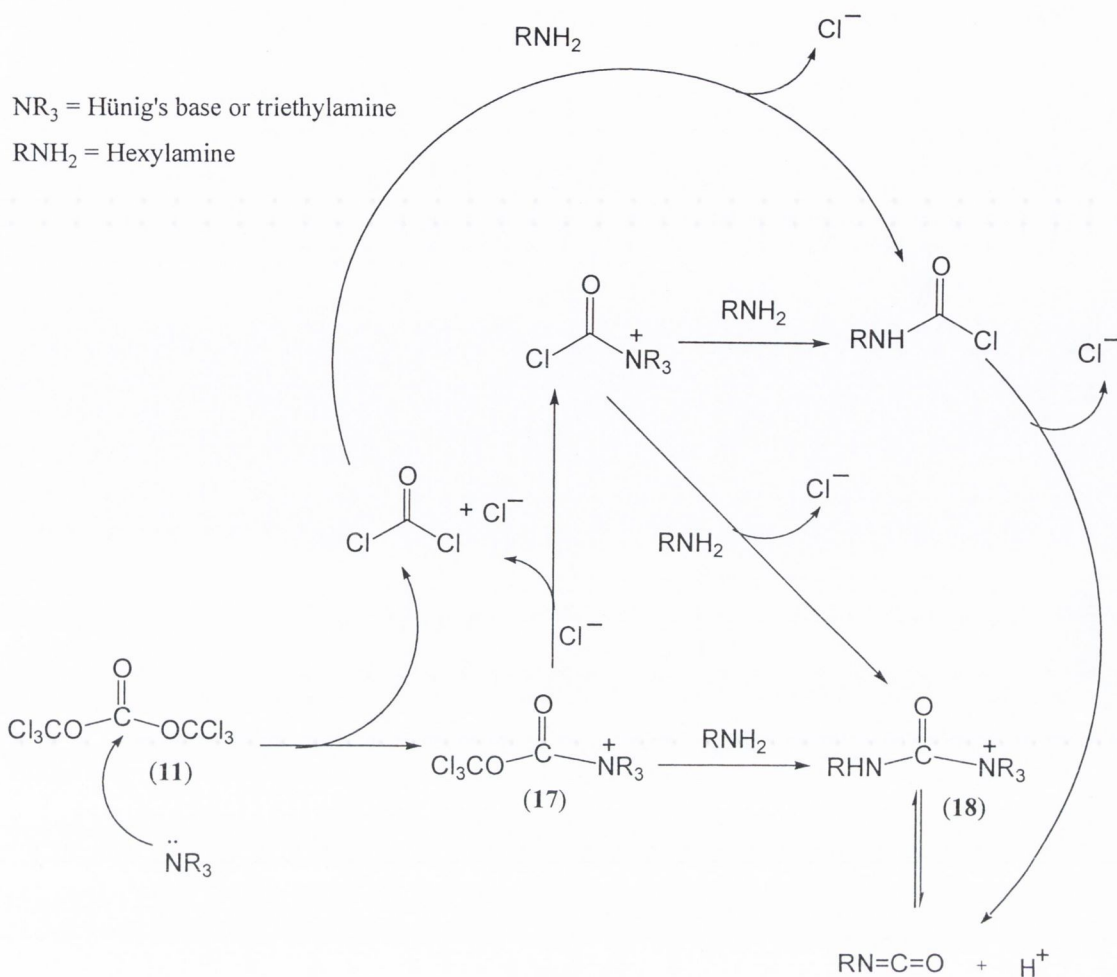
The literature reports suggested the possibility that our apparent samples of hexyl isocyanate were in fact of a precursor of the isocyanate. As reported by Ozaki *et al.*⁶, we found that elevated temperatures promoted reaction, although when the products of these reactions were treated with PaTrin-2, less than 5% yield of the corresponding N-9 derivative was obtained. A precursor was apparently involved, however the result of one further reaction suggested that (**16**) was perhaps not the precursor with which PaTrin-2 had failed to react.

Whether or not PaTrin-2 reacted with the precursor of hexyl isocyanate was found to be dependent on the type of base used in its preparation. We repeated the procedure reported by Majer *et al.*⁸, but instead of using Hünig's base in the preparation of the isocyanate, as shown in Scheme 4.4, we used a molar equivalent of pyridine. The reaction was otherwise identical to that shown in Scheme 4.4. A solution of hexylamine and pyridine in dry DCM was added dropwise to a solution of BTC (**11**) in dry DCM (Scheme 4.8). After 2 hours, the solvent was removed, and following reaction with PaTrin-2 as before, analysis of the ¹H NMR spectrum of the crude residue showed *O*⁶-(4-bromophenyl)-9-(*N*-hexylcarbamoyl)guanine (**8**) and very little unreacted PaTrin-2.



Scheme 4.8

We propose that, since the success of the above reaction was dependent on the type of base used in the synthesis of hexyl isocyanate, its precursor must incorporate the basic moiety in its structure. We considered the reaction mechanism that would result in such a precursor (Scheme 4.9).



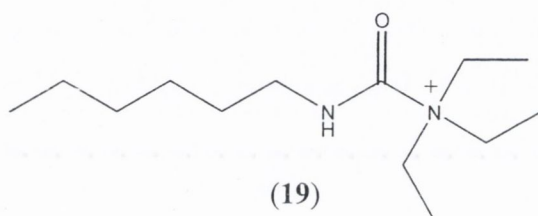
Scheme 4.9

Scheme 4.9 shows the Eckert mechanism¹² (in black). The nucleophile NR_3 reacts with BTC (11) generating one molecule of phosgene in the process. This reaction results in the formation of an intermediate (17), which is analogous to the trichloromethyl carbamate (16) shown in Scheme 4.7. According to the Eckert mechanism, (17) reacts with the chloride anion to generate another molecule of phosgene and regenerate a chloride anion. This pathway results in the formation of the isocyanate.

However, it would appear from our findings above that an alternative pathway (in red) might be possible. We propose that the reaction of (17) with the primary amine RNH_2 results in an intermediate (18) that is in equilibrium with the isocyanate. For this to

occur the trichloromethoxide anion would have to be a better leaving group than the trialkylammonium group in the tetrahedral intermediate derived from (17). As illustrated by Scheme 4.9, it is also possible that even if the Eckert pathway (in black) is followed, the presence of the hydrochlorides of either Hünig's base or triethylamine in the reaction mixture may also promote the equilibrium shown in red.

As discussed previously, the PaTrin-2 anion is hindered. Therefore it is reasonable to propose that the PaTrin-2 anion might be too hindered to attack the intermediate (18), for example if the $^+NR_3$ group is triethylammonium as in (19).

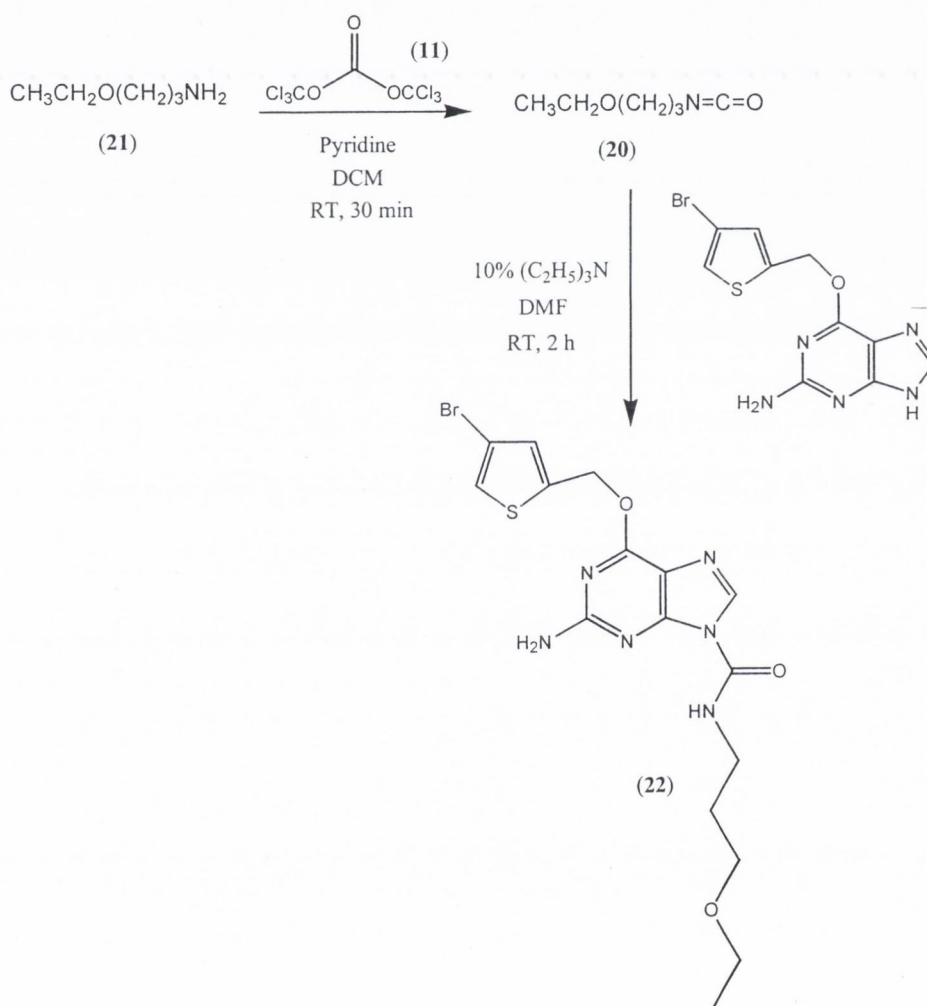


The fact that the reaction with PaTrin-2 proceeds when pyridine is used in the preparation of the isocyanate may be for one of two reasons. An intermediate (18) containing a pyridinium group would be more accessible to attack by a PaTrin-2 anion. Alternatively the reaction may occur by the Eckert pathway (shown in black) and lead conclusively to the formation of the isocyanate that then reacts directly with PaTrin-2.

The findings discussed above appear to be consistent with the formation of a precursor of hexyl isocyanate in our attempted preparation of this reagent. Although the evidence in the literature also suggests the existence of such precursors, we can now suggest that this precursor is the intermediate (18), with the observed infrared spectrum and reactivity towards the relatively unhindered 4-chloroaniline.

4.5.3 Synthesis of *O*⁶-(4-bromophenyl)-9-[*N*-(3-ethoxypropyl)carbamoyl]guanine

Following the procedure outlined above we also attempted to prepare the *N*-9 carbamoyl derivative *O*⁶-(4-bromophenyl)-9-[*N*-(3-ethoxypropyl)carbamoyl]guanine (**22**). 3-Ethoxypropyl isocyanate (**20**) [or its reactive precursor *i.e.* (**18**)] was synthesized by the dropwise addition of a solution of 3-ethoxypropylamine (**21**) and pyridine in dry DCM to a solution of BTC (**11**) in dry DCM (Scheme 4.10). As before, after 30 minutes, the DCM was then exchanged for DMF in an otherwise “one-pot” reaction. An equimolar amount of PaTrin-2 and catalytic triethylamine were added to the reaction mixture.



Scheme 4.10

After 2 hours the solvent was removed, and analysis of the ^1H NMR spectrum of the crude residue showed an almost quantitative yield of O^6 -(4-bromophenyl)-9-[*N*-(3-ethoxypropyl)carbamoyl]guanine (**22**). However, we found it difficult to isolate this product from the reaction mixture. Flash chromatography was unsuccessful and resulted only in the recovery of PaTrin-2.

We required O^6 -(4-bromophenyl)-9-[*N*-(3-ethoxypropyl)carbamoyl]guanine (**22**) for comparison with the potential prodrug O^6 -(4-bromophenyl)-9-(3-ethoxypropoxycarbonyl)guanine whose synthesis was discussed in Chapter 2. We eventually prepared (**22**) by an alternative synthetic route that will be described in Section 4.7.1.

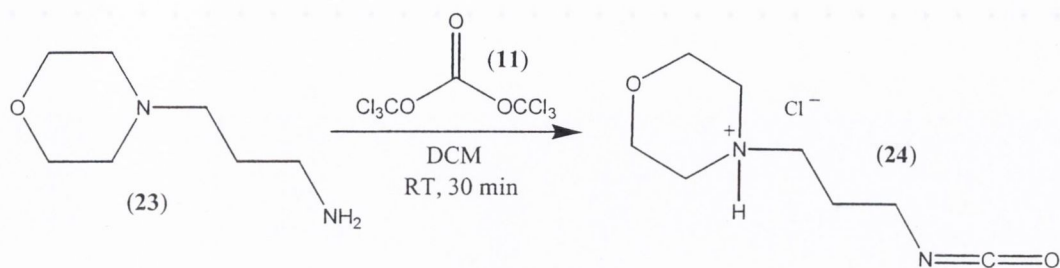
4.6 Synthesis of N-9 carbamoyl derivatives containing a tertiary amino group

In the previous section we reported a high-yielding procedure for the synthesis of N-9 carbamoyl derivatives of PaTrin-2 from isocyanates (or their equivalents). In the following sections we will discuss the corresponding reactions with primary amines containing a tertiary amino group. These N-9 carbamoyl derivatives will allow the preparation of water-soluble salts that will hopefully improve drug formulation and therefore delivery.

4.6.1 Synthesis of O^6 -(4-bromophenyl)-9-[*N*-(3-morpholinopropyl)carbamoyl]guanine

A solution of 4-(3-aminopropyl)morpholine (**23**) in dry DCM was added dropwise to an ice-cold solution of BTC (**11**) in dry DCM (Scheme 4.11). The reaction was carried out in the absence of added base and was set aside at room temperature for 30 minutes. The solvent was removed and the corresponding isocyanate hydrochloride

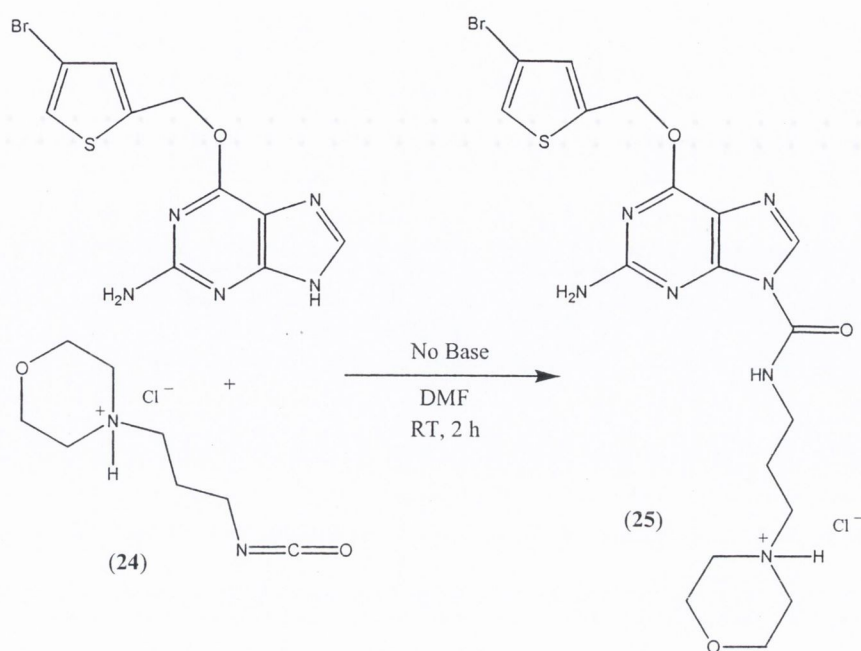
(24) was characterized by infrared spectroscopy. The stretching frequency associated with the isocyanate group was found at 2279 cm^{-1} .



Scheme 4.11

The isocyanate hydrochloride (24) was used directly in the subsequent reaction with PaTrin-2. A solution containing PaTrin-2 and triethylamine (two equivalents) in DMF was added to a solution of (24) in DMF. After 2 hours, the solvent was removed and ^1H NMR analysis of the crude residue showed the presence of *O*⁶-(4-bromophenyl)-9-[*N*-(3-morpholinopropyl)carbamoyl]guanine but in only approximately 40% yield. A similar yield was found when DBU (two equivalents) was used instead of triethylamine. In both cases it was not possible to isolate the N-9 derivative. All the separation techniques attempted resulted in the decomposition of the derivative.

A more successful route to the synthesis of *O*⁶-(4-bromophenyl)-9-[*N*-(3-morpholinopropyl)carbamoyl]guanine, as its hydrochloride (25), was found by carrying out the reaction with PaTrin-2 in the absence of base. The isocyanate hydrochloride (24) was prepared as above (Scheme 4.11), the DCM exchanged for DMF, and an equimolar amount of PaTrin-2 added to the solution (Scheme 4.12). After 2 hours a small amount of precipitate was observed. The solvent was removed and analysis of the ^1H NMR spectrum of the crude residue showed the corresponding N-9 derivative (25) in a quantitative yield.



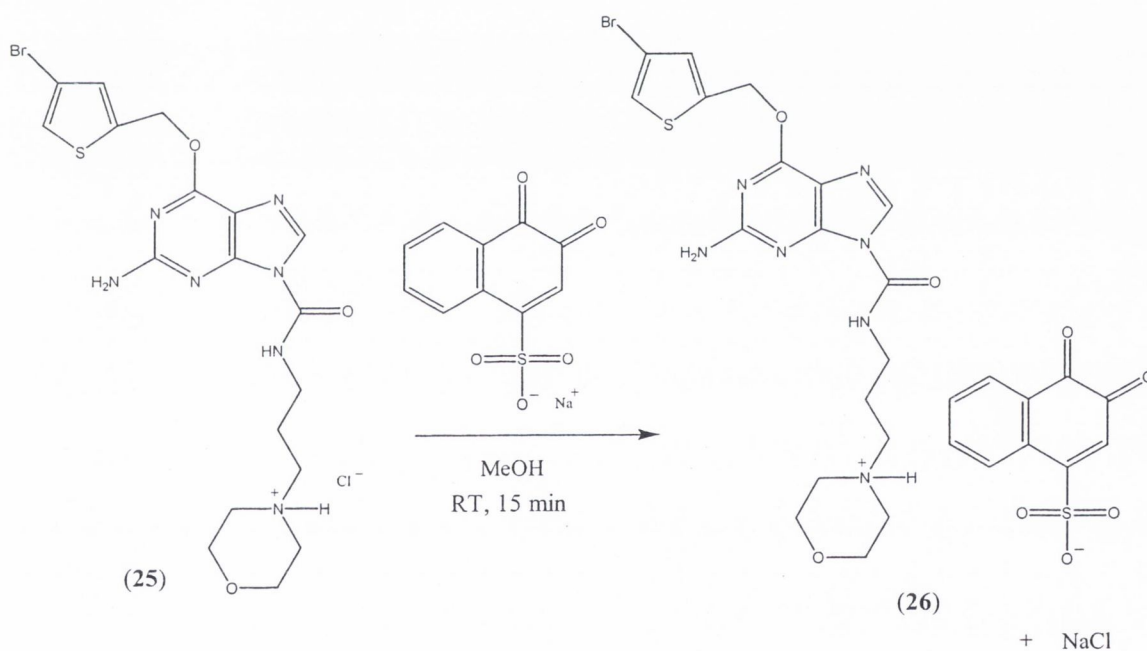
Scheme 4.12

Hot acetonitrile was added to the residue and a solid precipitated. The precipitate was collected and dried to afford, in low yield, *O*⁶-(4-bromophenyl)-9-[*N*-(3-morpholinopropyl)carbamoyl]guanine hydrochloride (**25**). It was found that (**25**) could be isolated in a higher yield (65%) by leaving the reaction in DMF for 18 hours and collecting the resulting precipitate. The hydrochloride (**25**) was found to be water-soluble.

The successful isolation of the hydrochloride (**25**) suggested that it was more stable than the free base, which we had previously failed to isolate. The instability of the free base may also explain the lower yield (40%) that resulted from these previous reactions. However, it is also possible that the reactive isocyanate hydrochloride (**24**) may react with the added base in these reactions (*i.e.* triethylamine or DBU) resulting in a reactive intermediate analogous to (**18**) (see Scheme 4.9 *i.e.* equilibrium reaction). As discussed in Section 4.5.2, the PaTrin-2 anion may be hindered in its attack on

such a reactive intermediate. This, and the instability of the free base itself, may explain the lower yields observed in these reactions.

Although we had not succeeded in preparing the free base of *O*⁶-(4-bromophenyl)-9-[*N*-(3-morpholinopropyl)carbamoyl]guanine we were able to demonstrate the preparation of another salt of this compound. A less readily water-soluble salt was prepared by the addition of a solution of the hydrochloride (25) in methanol to a solution of the sodium salt of 1,2-naphthoquinone-4-sulfonic acid in methanol (Scheme 4.13). The reaction was set aside for 15 minutes, the solvent removed and ether added to the residue. The precipitate was collected to afford the corresponding naphthoquinone-sulfonate salt (26) in an almost quantitative yield.



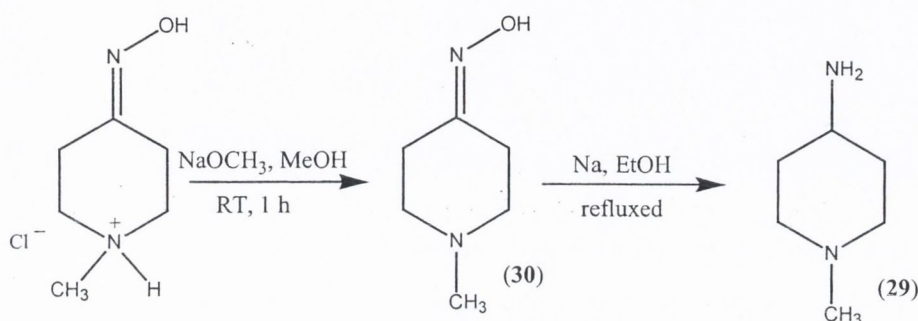
Scheme 4.13

4.6.2 Preparation of further derivatives containing a tertiary amino group

We had prepared successfully a derivative containing a tertiary amino group and we attempted to prepare several more *N*-9 carbamoyl derivatives that could be directly

compared with their alkoxy carbonyl homologues. However, as will be discussed below, the synthesis of these derivatives was not as straightforward as anticipated.

The primary amines 4-picolylamine (**27**) and 3-dimethylaminopropylamine (**28**) were commercially available. However, 4-amino-*N*-methylpiperidine (**29**) had to be prepared* by reduction of *N*-methyl-4-piperidone oxime (**30**) (Scheme 4.14).¹³

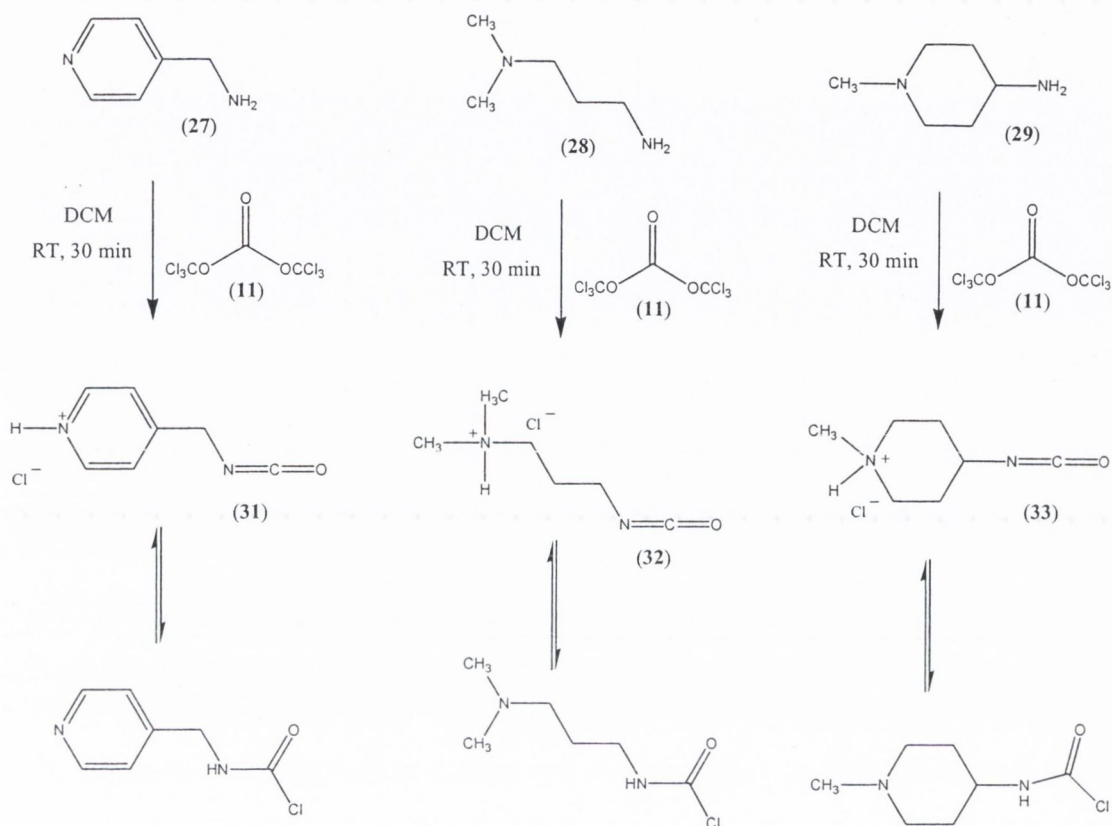


Scheme 4.14

As with the procedure discussed above in Section 3.1, the isocyanate hydrochlorides were prepared by the dropwise addition of a solution of the primary amine in dry DCM to an ice-cold solution of BTC (**11**) in dry DCM (Scheme 4.15). As before, the reactions were carried out in the absence of base and were set aside at room temperature for 30 minutes. The DCM was removed and the isocyanates (**31**), (**32**) and (**33**) were characterized by infrared spectroscopy. 4-Picolyl isocyanate (**31**) was characterized by an N=C=O stretching frequency at 2274 cm⁻¹. The N=C=O stretching frequencies of 3-dimethylaminopropyl isocyanate (**32**) and *N*-methyl-4-piperidyl isocyanate (**33**) were found at 2279 cm⁻¹ and 2272 cm⁻¹ respectively. The presence of a weak carbonyl stretching frequency between 1750 cm⁻¹ and 1777 cm⁻¹ in these spectra also suggested the presence of carbamoyl chlorides and therefore an equilibrium between the isocyanate hydrochloride and carbamoyl chloride may be occurring (Scheme 4.15). A peak in this range was not found in an earlier case

* 4-Amino-*N*-methylpiperidine (**29**) was prepared by Dr. D.J. Donnelly.

(Section 4.6.1). The isocyanates hydrochlorides (31), (32) and (33) were subsequently used without further purification.

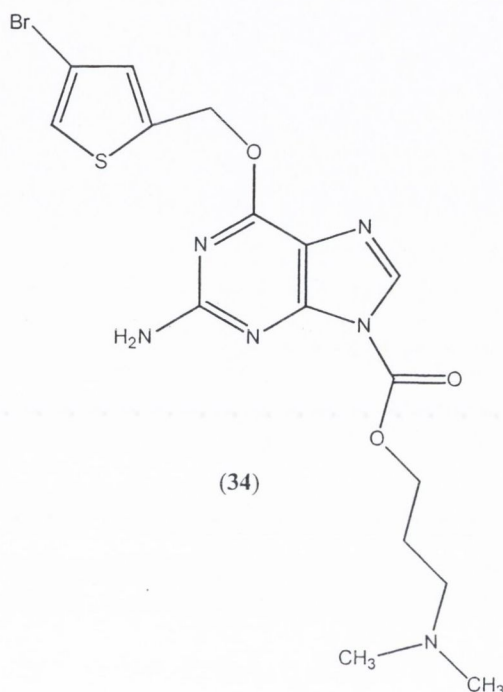


Scheme 4.15

The reaction of PaTrin-2 with each of the isocyanate hydrochlorides was attempted under conditions similar to those used in the preparation of *O*⁶-(4-bromophenyl)-9-[*N*-(3-morpholinopropyl)carbamoyl]guanine hydrochloride (25), as shown in Scheme 4.12. However, no products were obtained in the absence of added base. The procedures were repeated in the presence of either triethylamine or Hünig's base, but all experiments again resulted in the complete recovery of PaTrin-2.

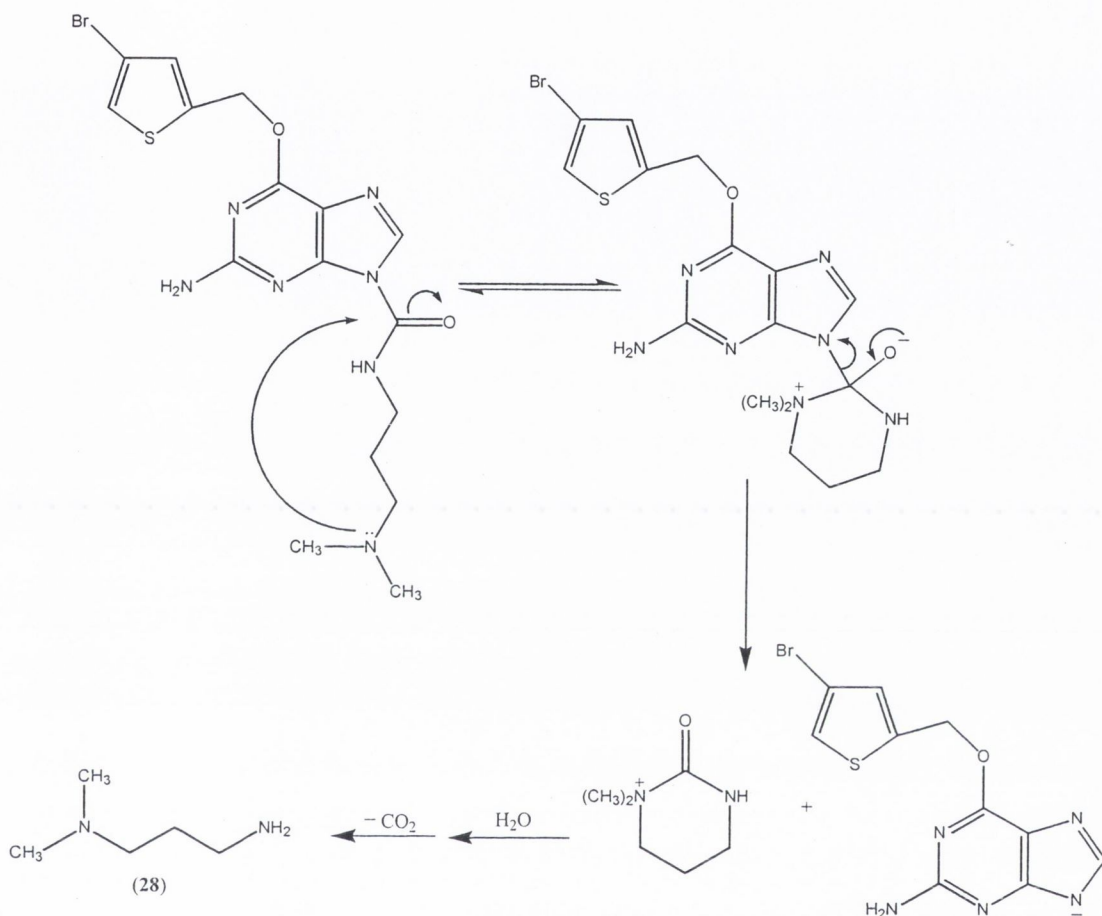
We attempted to understand why each of these isocyanates had failed to react with PaTrin-2. The findings above were compared with the results of the reactions involved in the preparation of the alkoxycarbonyl analogues. An explanation for the

failure of 3-dimethylaminopropyl isocyanate (**32**) to react with PaTrin-2 could be suggested since we had also been unable to prepare *O*⁶-(4-bromophenyl)-9-(3-dimethylaminopropoxycarbonyl)guanine (**34**) as discussed in Chapter 2, Section 2.10.3 (pg 96) (see Scheme 2.39, pg 102).



A similar explanation can be proposed for the reaction involving 3-dimethylaminopropyl isocyanate (**32**). The isocyanate derived from 4-(3-aminopropyl)morpholine (**23**) reacted successfully with PaTrin-2 (as shown in Scheme 4.12). Comparing this isocyanate with (**32**), we find that the only difference is in the nature of their amino groups. 3-Dimethylaminopropyl isocyanate (**32**) is flexible, and the amino group therefore more available for intramolecular interactions. If the isocyanate did react with PaTrin-2 to afford the corresponding derivative the lone pair of electrons on the nitrogen atom might interact with the carbonyl carbon atom (Scheme 4.16) and could initiate a decomposition reaction similar to that proposed in Chapter 2 (Scheme 2.39, pg 102). The PaTrin-2 derivative derived from

4-(3-aminopropyl)morpholine (**23**) is comparatively more stable. The cyclic morpholine residue makes the side chain less flexible and hence less available.

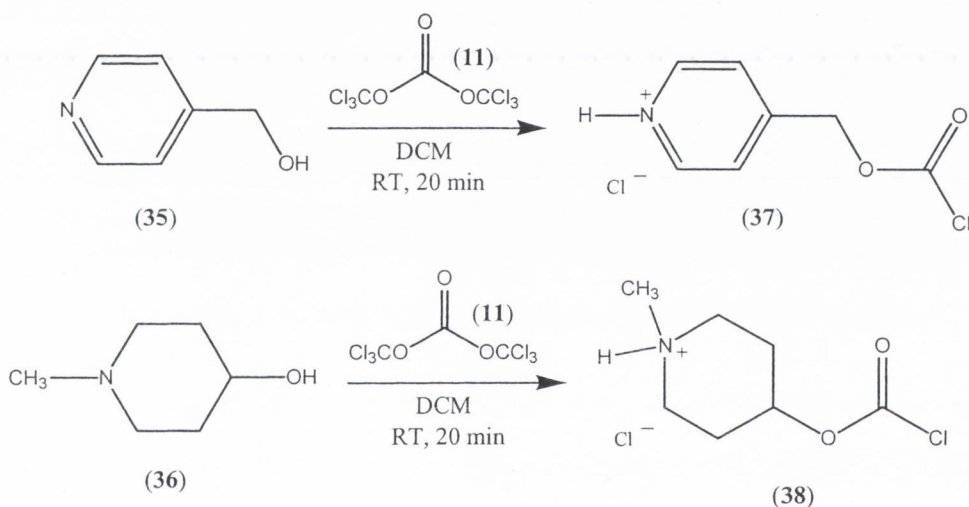


Scheme 4.16

This type of intramolecular reaction does not explain the failure of 4-picolyl isocyanate hydrochloride (**31**) and *N*-methyl-4-piperidyl isocyanate hydrochloride (**33**) to react with PaTrin-2.

The alkoxycarbonyl analogues, O^6 -(4-bromophenyl)-9-(4-picolylloxycarbonyl)-guanine and O^6 -(4-bromophenyl)-9-(*N*-methyl-4-piperidylloxycarbonyl)guanine, prepared in Chapter 2, had been obtained in good yield by procedures involving 4-nitrophenyl carbonate intermediates. We had not attempted to synthesize these

derivatives by a procedure involving chloroformates. For comparative purposes we decided it would now be worthwhile. Following the procedure used in Chapter 2 (Section 2.10.1, pg 93), solutions of both 4-picolyl alcohol (**35**) and 4-hydroxy-*N*-methyl-piperidine (**36**) in dry DCM were added dropwise to solutions of BTC (**11**) in dry DCM at less than -5°C (Scheme 4.17). After 20 minutes, the solvent was removed, and the chloroformate hydrochlorides were characterized by infrared spectroscopy. Although the carbonyl stretching frequency of *N*-methyl-4-piperidyl chloroformate (**38**) was found at 1776 cm^{-1} , there was nothing to suggest the formation of 4-picolyl chloroformate (**37**).



Scheme 4.17

The “chloroformates” (**37**) and (**38**) were subsequently used without further purification. However, the reaction of both (**37**) and (**38**) with PaTrin-2, carried out in the presence of two equivalents of triethylamine, failed to afford any products.

We had attempted the above reactions for a comparison with the reactions involving 4-picolyl isocyanate (**31**) and *N*-methyl-4-piperidyl isocyanate (**33**). The negative result suggested that the chloroformate and isocyanate hydrochlorides derived from these alcohols and amines respectively encountered similar problems in attempted further reaction with PaTrin-2. As previously discussed, evidence suggests that both

chloroformates (see Chapter 2, Section 2.9.5, Scheme 2.29, pg 87) and isocyanates (see Section 4.5.2) react with the added base in these PaTrin-2 reactions to form reactive intermediates analogous to (18) (see Scheme 4.9). It could be proposed that the PaTrin-2 anion is hindered in its attack on such reactive intermediates. However, such a steric problem would not have been anticipated since we have successfully reacted structurally similar chloroformates and isocyanates with PaTrin-2.

The alkoxycarbonyl derivatives, O^6 -(4-bromophenyl)-9-(4-picolylloxycarbonyl)-guanine and O^6 -(4-bromophenyl)-9-(*N*-methyl-4-piperidylloxycarbonyl)guanine, had been prepared by a procedure involving 4-nitrophenyl carbonate intermediates, and we therefore decided that the problem of the synthesis of their elusive carbamoyl counterparts might be solved in a similar way.

4.7 An alternative procedure for the preparation of N-9 carbamoyl derivatives

It was important to investigate an alternative route to the synthesis of N-9 carbamoyl derivatives for two reasons. Firstly, to allow for the preparation of derivatives whose synthesis was not possible using the procedure involving isocyanates. Secondly, an alternative procedure might afford a higher yield of a derivative or allow for the more satisfactory isolation of a derivative from the reaction mixture.

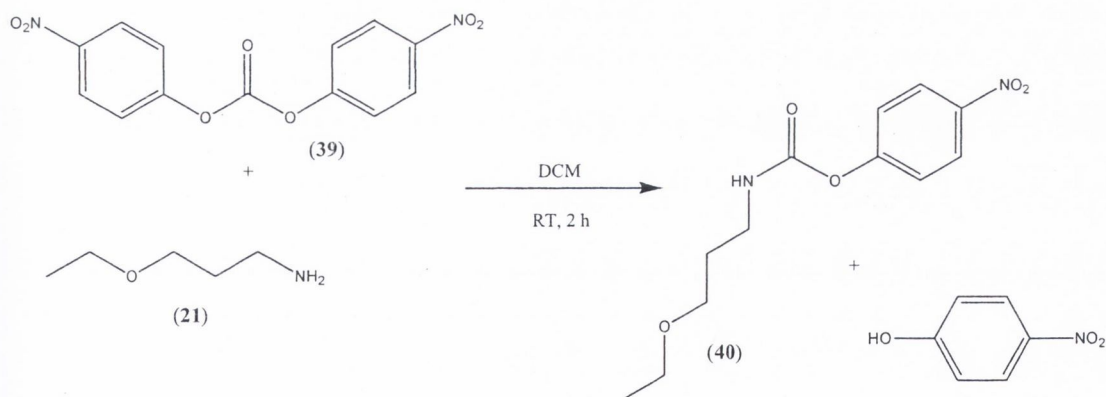
In Chapter 2 we discussed the usefulness of bis(4-nitrophenyl) carbonate in facilitating the synthesis of a variety of N-9 alkoxycarbonyl derivatives. Similarly, Izdebski *et al.*¹⁴ reported a method for the preparation of *N,N'*-disubstituted ureas in good yield by stepwise aminolysis of bis(4-nitrophenyl) carbonate.

We decided to investigate the reaction of primary amines with bis(4-nitrophenyl) carbonate, and the subsequent reaction of the resulting activated carbamate derivatives with PaTrin-2.

4.7.1 The reaction of bis(4-nitrophenyl) carbonate with primary amines

In Section 4.5.3 we discussed the preparation of O^6 -(4-bromophenyl)-9-[*N*-(3-ethoxypropyl)carbamoyl]guanine (**22**) using a procedure involving an isocyanate. Although the N-9 derivative (**22**) was synthesized in a quantitative yield, its isolation from the reaction mixture was not possible. We decided to examine if a pathway involving bis(4-nitrophenyl) carbonate would overcome this difficulty.

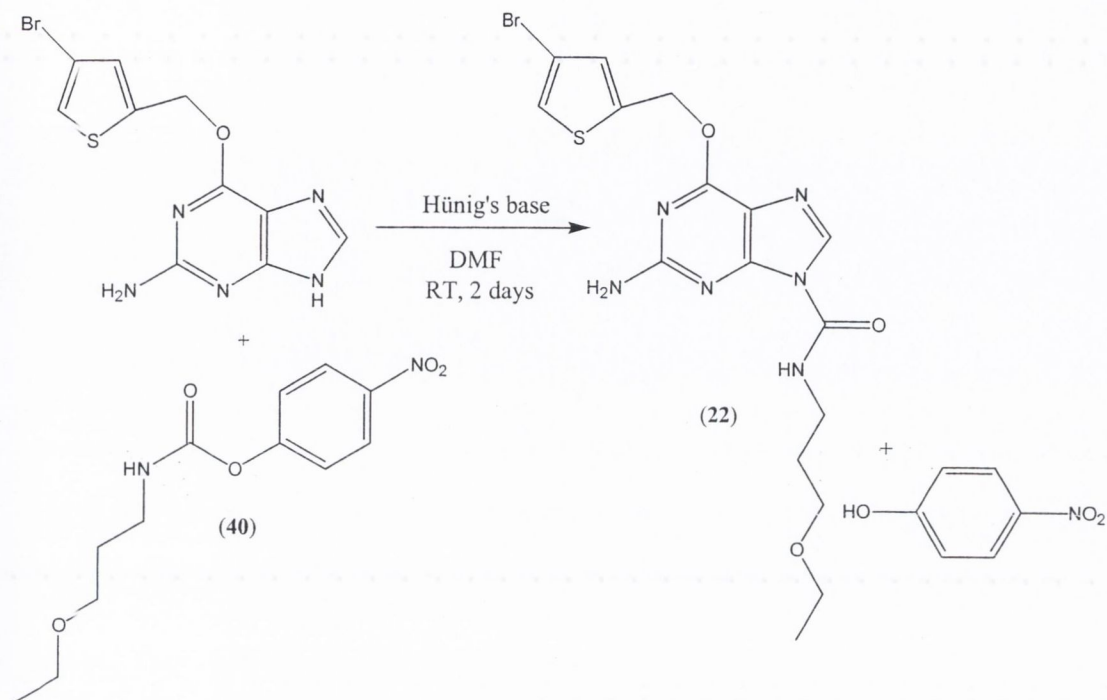
Following the procedure described by Izdebski *et al.*¹⁴, a solution of 3-ethoxypropylamine (**21**) in dry DCM was added dropwise to a solution of bis(4-nitrophenyl) carbonate (**39**) in dry DCM (Scheme 4.18). The reaction was carried out in the absence of base and was allowed to stand for 2 hours. The solution in DCM was washed repeatedly with a saturated aqueous solution of NaHCO_3 and then once with brine to remove traces of the liberated 4-nitrophenol. The solution was dried over MgSO_4 and evaporated to afford 4-nitrophenyl *N*-(3-ethoxypropyl)carbamate (**40**) in approximately 50% yield.



Scheme 4.18

The subsequent reaction of the 4-nitrophenyl carbamate (**40**) with PaTrin-2 was carried out in DMF in the presence of an equimolar amount of Hünig's base (Scheme 4.19). After 2 days, the solvent was removed and ethyl acetate was added to the residue. A solid precipitated upon the addition of hexane and was collected and dried

to afford *O*⁶-(4-bromophenyl)-9-[*N*-(3-ethoxypropyl)carbamoyl]guanine (**22**) in 80% yield.



Scheme 4.19

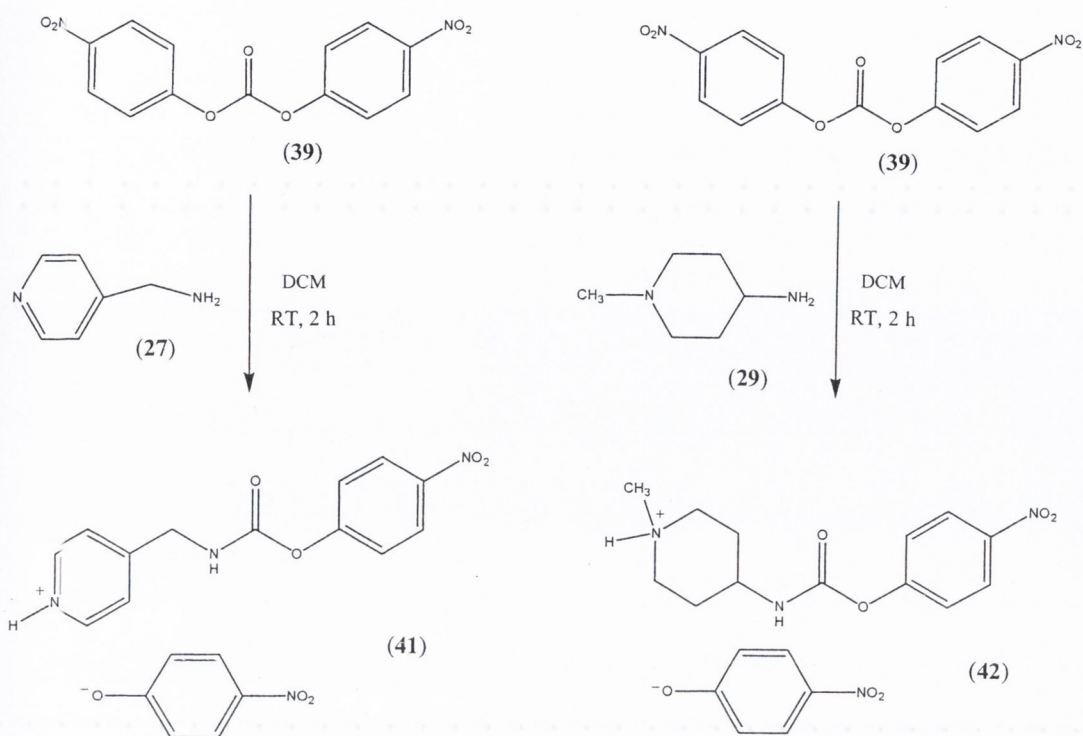
Stage one of this procedure had resulted only in a low yield of the 4-nitrophenyl *N*-(3-ethoxypropyl)carbamate (**40**) due to the aqueous work-up which resulted in the hydrolysis of this activated derivative. However, the reaction of (**40**) with PaTrin-2 afforded the *N*-9 derivative in high yield. For comparative purposes, the above procedure was repeated using hexylamine to prepare *O*⁶-(4-bromophenyl)-9-(*N*-hexylcarbamoyl)guanine (**8**), which we had also made by the isocyanate route. The reaction was analogous to that involving 3-ethoxypropylamine. The first stage was low-yielding, but the second stage resulted in an almost quantitative yield of the *N*-9 derivative.

We had demonstrated that bis(4-nitrophenyl) carbonate (**39**) could be used successfully to prepare *N*-9 carbamoyl derivatives that were derived from simple primary amines. In the following section we will discuss the reaction of bis(4-nitrophenyl) carbonate (**39**) with primary amines containing a tertiary amino group.

4.7.2 The reaction of bis(4-nitrophenyl) carbonate with primary amines containing a tertiary amino group

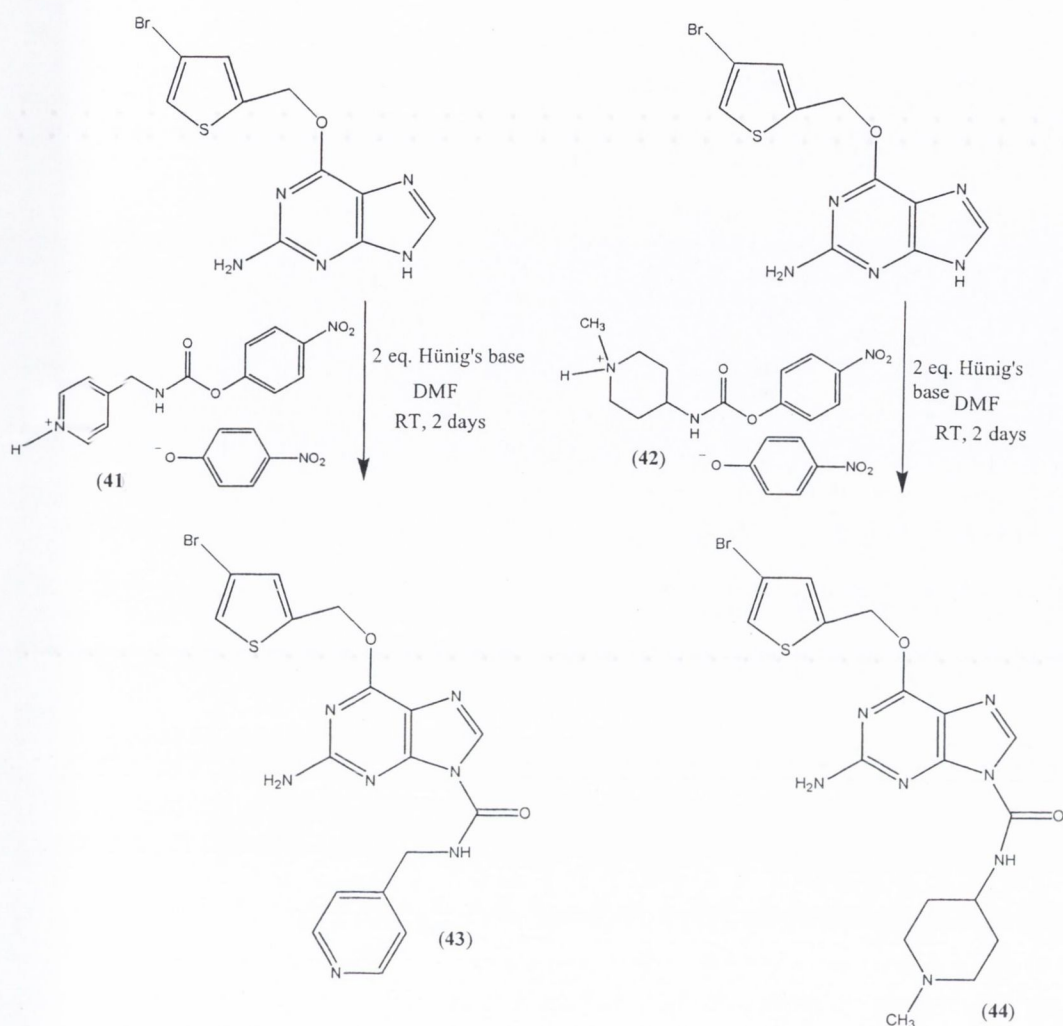
In Section 4.6.2 we discussed the unsatisfactory reaction of two isocyanates with PaTri-2. The isocyanates derived from 4-picolylamine (27) and 4-amino-*N*-methyl-piperidine (29) had failed to react with PaTri-2. We hoped that a procedure involving bis(4-nitrophenyl) carbonate (39) might provide a successful route to the preparation of *O*-(4-bromophenyl)-9-[*N*-(*N*-methyl-4-piperidyl)carbamoyl]guanine and *O*-(4-bromophenyl)-9-[*N*-(4-picolyl)carbamoyl]guanine.

The reaction of bis(4-nitrophenyl) carbonate (39) with both 4-picolylamine (27) and 4-amino-*N*-methyl-piperidine (29) was carried out in a similar way to the reactions discussed above (Section 4.7.1). A solution of the primary amine in dry DCM was added dropwise to a solution of bis(4-nitrophenyl) carbonate (39) in dry DCM (Scheme 4.20). After 2 hours, the solvent was removed and ethyl acetate was added to the residues. Hexane was added gradually to each mixture and solid precipitated. The precipitates were collected and dried to afford both the 4-nitrophenol salts (41) and (42) in approximately 50% yield. We attempted a similar reaction involving 3-dimethylaminopropylamine (28), but it was not possible to prepare the corresponding 4-nitrophenyl carbamate.



Scheme 4.20

The subsequent reaction of PaTrin-2 with both (41) and (42) was carried out in DMF in the presence of two equivalents of Hünig's base (Scheme 4.21). After 2 days, the solvent was removed and acetonitrile was added to each residue. Solid precipitated from both reaction mixtures and was collected and dried. The N-9 derivatives *O*⁶-(4-bromophenyl)-9-[*N*-(4-picolyl)carbamoyl]guanine (43) and *O*⁶-(4-bromophenyl)-9-[*N*-(*N*-methyl-4-piperidyl)carbamoyl]guanine (44) were obtained in 70% and 50% yield respectively. Using (44), we also demonstrated the successful preparation of a citrate salt (44a), made in the same way as its alkoxycarbonyl analogue (Chapter 2, Section 2.8.2, pg 75).

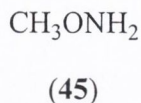


Scheme 4.21

Like Zdebski *et al.*¹⁴, we have found that activated derivatives containing a 4-nitrophenoxy group provided a useful alternative to isocyanates. Our initial investigation of the use of bis(4-nitrophenyl) carbonate (Chapter 2) had equipped us with the knowledge to employ this reagent successfully. We found 4-nitrophenol to be highly soluble in both hot ethyl acetate and hot acetonitrile. This provided an efficient method for the purification of both our N-9 alkoxycarbonyl and carbamoyl derivatives when prepared by procedures that liberated free 4-nitrophenol. This method proved essential for the isolation in good yield of derivatives, such as those discussed above, that were too susceptible to hydrolysis to be separated by flash chromatography.

4.8 Methoxyamine – a cytotoxic primary amine

In the following section we will report the synthesis of a more specialized PaTrin-2 carbamoyl derivative derived from the primary amine methoxyamine (45).

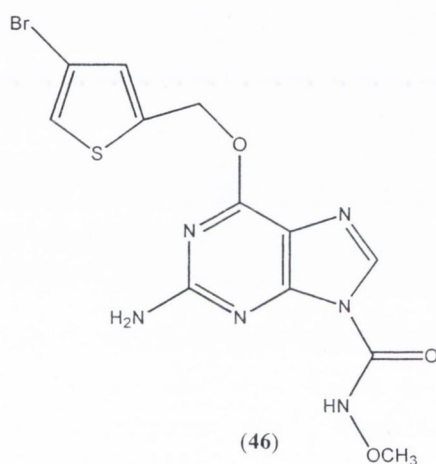


Liu *et al.*¹⁵ report that the cytotoxicity of the chemotherapeutic methylating agent temozolomide is dramatically enhanced when combined with the ATase inactivator *O*⁶-benzylguanine and methoxyamine (45). In Chapter 1 (Section 1.4.1, Scheme 1.1, pg 14) we discussed the mechanism by which temozolomide methylates the *O*⁶-position of guanine in DNA.¹⁶ The modified guanine can no longer hydrogen-bond at the *O*⁶-position with cytosine and instead it mispairs with thymine during DNA replication. This results in GC to AT transition mutations.¹⁷ ATase can repair methylation damage to the *O*⁶-position in guanine by removing the methyl group. However, the *O*⁶-position in guanine is not the only site methylated by temozolomide¹⁶ and ATase does not provide the only DNA repair process.

Temozolomide also gives rise to N-7 methylguanine and N-3 methyladenine adducts, and the base excision repair system (BER) is responsible for processing such adducts.¹⁸ The damage is repaired by BER and the resultant single-strand breaks are re-joined with the aid of the enzyme poly(ADP-ribose) polymerase (PARP).¹⁸ The methylated base is removed by methylpurine glycosylase and this creates an abasic or apurinic-apyrimidinic (AP) site. The phosphodiester bond is then hydrolyzed immediately by AP endonuclease and this initiates the repair of the AP site. The primary amine methoxyamine (45) can react with the aldehydic AP site preventing AP endonuclease cleavage and therefore disrupting DNA repair.¹⁵

PaTrin-2 has been shown to potentiate the cytotoxicity of temozolomide (Chapter 1, Section 1.7, pg 27).¹⁹ Therefore, the cytotoxicity of temozolomide should be further enhanced if combined with PaTrin-2 and methoxyamine (45). This is currently under investigation.

As illustrated by Scheme 4.1, the hydrolysis of N-9 carbamoyl derivatives, whether by the E1cB or B_{AC}2 pathway, results in the regeneration of the primary amine. The prodrug *O*⁶-(4-bromophenyl)-9-(*N*-methoxycarbamoyl)guanine (46), derived from methoxyamine, is a molecular combination which would undergo hydrolysis to release two active agents.

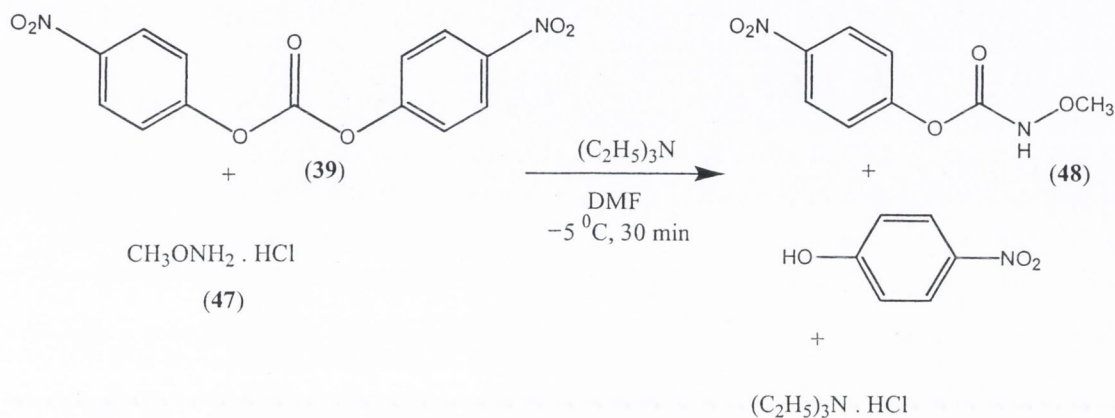


4.8.1 Synthesis of *O*⁶-(4-bromophenyl)-9-(*N*-methoxycarbamoyl)guanine

Methoxyamine (45) is a very reactive base but it is commercially available as a hydrochloride (47). Since the corresponding isocyanate is even more unstable^{20,21} we attempted the synthesis of this specialized prodrug by the procedure involving bis(4-nitrophenyl) carbonate (39).

Bis(4-nitrophenyl) carbonate (39) and an equimolar amount of triethylamine were added to a solution of methoxyamine hydrochloride (47) in DMF at -5°C (Scheme 4.22). After 30 minutes at this temperature, the solvent was removed and DCM was added to the residue. The solution was washed with water to remove the triethylamine

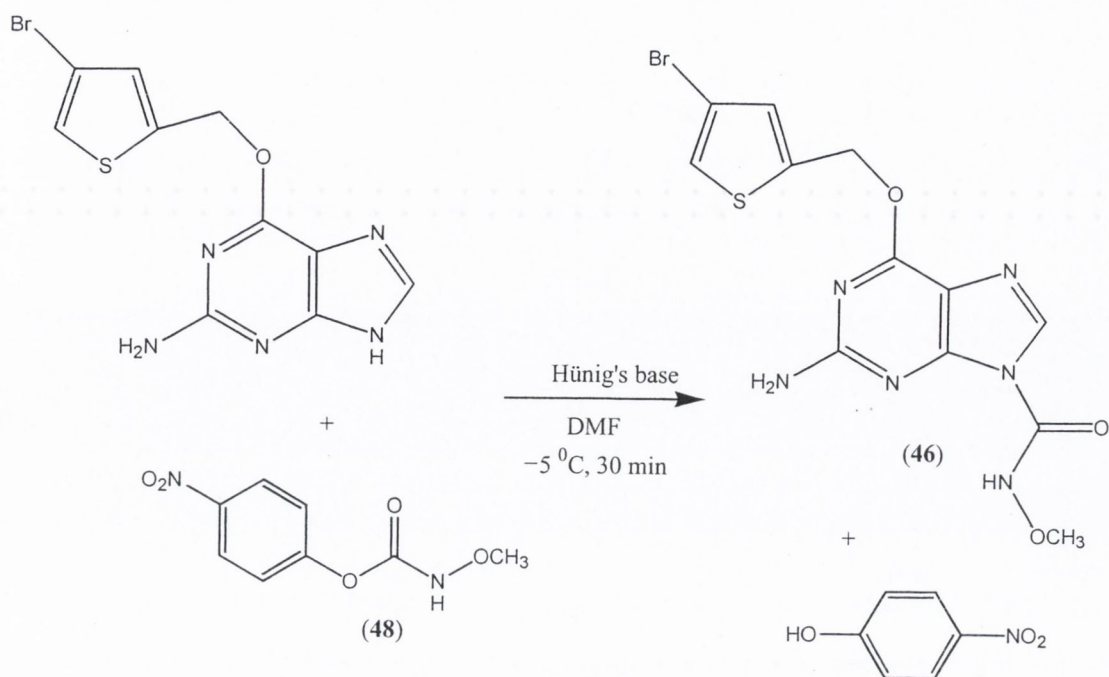
hydrochloride, dried over MgSO_4 and evaporated. Ethyl acetate (in which free 4-nitrophenol is soluble) was added to the residue and a solid precipitated upon the addition of hexane. The precipitate was collected and dried to afford 4-nitrophenyl *N*-methoxycarbamate (**48**) in approximately 60% yield.



Scheme 4.22

This reaction was unsuccessful when carried out at room temperature due to the volatility of the methoxyamine (**45**) that was liberated upon the addition of triethylamine. The subsequent reaction of PaTrin-2 with (**48**) was also found to require low temperatures.

The 4-nitrophenyl carbamate (**48**) was added to a solution of PaTrin-2 and Hünig's base in DMF at $-5\text{ }^\circ\text{C}$ (Scheme 4.23). The reaction was set aside at this temperature for 30 minutes. The solvent was removed and analysis of the ^1H NMR of the crude residue showed a quantitative yield of the N-9 derivative. Again ethyl acetate was added to the residue and hexane was added to precipitate a solid. The precipitate was collected and dried to afford *O*⁶-(4-bromophenyl)-9-(*N*-methoxycarbamoyl)guanine (**46**) in 80% yield.



Scheme 4.23

This specialized prodrug, *O*⁶-(4-bromophenyl)-9-(*N*-methoxycarbonyl)guanine (**46**), should provide interesting pharmacological results following hydrolysis to generate two active agents.

4.9 Chapter summary

The synthetic route used above in the preparation of these potential prodrugs was established in Chapter 2 and involves the treatment of PaTrin-2 with an activated derivative. Two categories of reagent have proved to be useful in the synthesis of N-9 carbamoyl derivatives.

Isocyanates provided a quick and high yielding route to the synthesis of several derivatives. We demonstrated the preparation of isocyanates from bis(trichloromethyl) carbonate (**11**). However, we found that the subsequent reaction of the isocyanate with PaTrin-2 was dependent on the type of base used in its preparation. Reports in the literature suggest the existence of precursors to isocyanates prepared using bis(trichloromethyl) carbonate (**11**). We proposed that an equilibrium

exists between the isocyanate and a precursor (Scheme 4.9, pg 234). These findings, reported in Section 4.5.2, again highlight the complexity of reactions involving PaTrin-2.

The unique electronic effects of the purine ring system must also be considered when attempting to rationalise problems with reactions involving PaTrin-2. In Section 4.6.2 we proposed that our failure to prepare a derivative derived from 3-dimethylaminopropylamine might be explained by an intramolecular decomposition reaction that was further facilitated by the ability of the PaTrin-2 anion to behave as an excellent leaving group. Experiments reported in previous chapters strongly support this theory.

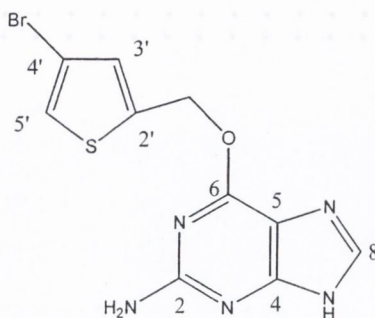
The unique characteristics of PaTrin-2 cannot explain all problems encountered in its reactions. In Section 4.6.2 we found that it was not possible to prepare two other prodrugs by procedures involving isocyanates. However, these PaTrin-2 derivatives derived from 4-picolylamine (27) and 4-amino-*N*-methylpiperidine (29) were eventually prepared by an alternative procedure involving bis(4-nitrophenyl) carbonate (39). There is no apparent explanation as to why these reactions proceed with 4-nitrophenyl carbamates but not isocyanates.

4-Nitrophenyl carbamates provide a useful alternative to isocyanates. We have demonstrated in previous chapters, that finding the best routes and reagents for the successful synthesis of a large variety of prodrugs within each category often involves considerable experimentation. However, although it is eventually possible to establish a general synthetic procedure, it is valuable to have an alternative procedure to allow the preparation of derivatives whose synthesis or isolation proves difficult by the standard method. The difficulty that is at times experienced in bringing PaTrin-2 into

reaction with certain reagents highlights the importance of alternative routes and reagents that may allow for the more facile synthesis of its derivatives.

4.10 Experimental*

Numbering system followed for *O*⁶-(4-bromophenyl)guanine:



***O*⁶-(4-Bromophenyl)-9-(*N*-hexylcarbamoyl)guanine (8)** – Hexyl isocyanate (15 μ l, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and triethylamine (1.4 μ l, 0.01 mmol) in DMF (0.1 ml) under nitrogen. After 2 h the solvent was evaporated and DCM was added to the residue, followed by hexane, to precipitate (**8**) (43 mg, 95%) as a white solid.

$\nu_{\max}$: 1614, 1743(C=O) cm^{-1}

$\lambda_{\max}$: 279nm

m.p. 140 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 0.88 (3H, br s, 6''-CH₃), 1.32 (6H, m, 3'', 4'', 5''-CH₂), 1.60 (2H, br s, 2''-CH₂), 3.34 (2H, br s, 1''-CH₂), 5.66 (2H, s, 6-OCH₂), 7.02 (2H, s, NH₂), 7.36 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.23 (1H, s, 8-CH), 8.86 (1H, s, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 13.9 (6''-CH₃), 22.1 (5''-CH₂), 26.0 (3''-CH₂), 30.0 (2''-CH₂), 31.0 (4''-CH₂), 40.5 (1''-CH₂), 60.6 (6-OCH₂), 108.1 (4'-C), 113.9 (5-C), 125.1 (5'-CH), 130.7 (3'-CH), 136.8 (8-CH), 140.7 (2'-C), 148.7 (C=O), 152.3 (4-C), 159.4 (2-C), 158.9 (6-C).

Found: **C**, 44.22; **H**, 4.79; **N**, 17.60 %. C₁₇H₂₁BrN₆O₂S·0.5 H₂O requires **C**, 44.19; **H**, 4.80; **N**, 18.19 %.

* For experimental details see Chapter 2, Section 2.13, pgs. 122-123.

MS (ES) $[M+H]^+$ Calcd m/z for $C_{17}H_{22}BrN_6O_2S$ 453.0708, Found 453.0705.

O⁶-(4-Bromophenyl)-9-(N-cyclohexylcarbamoyl)guanine (9) – This compound (27 mg, 60%) was prepared following the above procedure, but using cyclohexyl isocyanate (13 μ l, 0.1 mmol) instead of hexyl isocyanate.

$\nu_{\max}$: 1600, 1626, 1718(C=O) cm^{-1}

$\lambda_{\max}$: 283nm

m.p. 208 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 1.22 (1H, m, 4_{ax}''-CH), 1.35 (4H, m, 2_{ax}'', 3_{ax}'', 5_{ax}'', 6_{ax}''-CH), 1.57 (1H, m, 4_{eq}''-CH), 1.70 (2H, m, 3_{eq}'', 5_{eq}''-CH), 1.94 (2H, 2_{eq}'', 6_{eq}''-CH), 3.72 (1H, br s, 1''-CH), 5.68 (2H, s, 6-OCH₂), 7.05 (2H, s, NH₂), 7.35 (1H, s, 3'-CH), 7.70 (1H, s, 5'-CH), 8.23 (1H, s, 8-CH), 8.85 (1H, d, $J=7.5$, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 24.4 (3'', 5''-CH₂), 25.3 (4''-CH₂), 33.3 (2'', 6''-CH₂), 47.5 (1''-CH), 60.5 (6-OCH₂), 103.1 (4'-C), 108.1 (5-C), 125.1 (5'-CH), 130.7 (3'-CH), 138.0 (8-CH), 140.7 (2'-C), 148.0 (C=O), 156.6 (4-C), 159.4 (2-C), 159.5 (6-C).

Found: C, 45.42; H, 4.18; N, 18.35 %. $C_{17}H_{19}BrN_6O_2S$ requires C, 45.23; H, 4.21; N, 18.63 %.

MS (ES) $[M+H]^+$ Calcd m/z for $C_{17}H_{20}BrN_6O_2S$ 451.0552, Found 451.0515.

O⁶-(4-Bromophenyl)-9-(N-propylcarbamoyl)guanine (10) – This compound (36 mg, 88%) was prepared following the above procedure, but using propyl isocyanate (9 μ l, 0.1 mmol) instead of hexyl isocyanate.

$\nu_{\max}$: 1616, 1631, 1745(C=O) cm^{-1}

$\lambda_{\max}$: 281nm

m.p. 178 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 0.94 (3H, t, $J=7.5$, 3''-CH₃), 1.61 (2H, m, 2''-CH₂), 3.31 (2H, m, 1''-CH₂), 5.67 (2H, s, 6-OCH₂), 7.02 (2H, s, NH₂), 7.36 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.24 (1H, s, 8-CH), 8.87 (1H, s, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 11.2 (3''-CH₃), 22.3 (2''-CH₂), 41.3 (1''-CH₂), 60.1 (6-OCH₂), 108.0 (4'-C), 113.9 (5-C), 125.3 (5'-CH), 131.0 (3'-CH), 136.8 (8-CH), 139.9 (2'-C), 148.7 (C=O), 152.3 (4-C), 159.5 (2-C), 159.8 (6-C).

Found: C, 40.60; H, 3.41; N, 20.14 %. C₁₄H₁₅BrN₆O₂S requires C, 40.88; H, 3.65; N, 20.44 %.

MS (ES) [M+H]⁺ Calcd m/z for C₁₄H₁₆BrN₆O₂S 411.0239, Found 411.0257.

O⁶-(4-Bromophenyl)-9-[N-(3-morpholinopropyl)carbamoyl]guanine hydrochloride

(**25**) – A solution of 4-(3-aminopropyl)morpholine (15 μl , 0.1 mmol) in dry DCM (0.5 ml) was added dropwise to an ice-cold solution of bis(trichloromethyl) carbonate (10 mg, 0.034 mmol) in dry DCM (0.5 ml). After 30 min the DCM was evaporated and a solution of PaTrin-2 (33 mg, 0.1 mmol) in DMF was added to the residue and the reaction was set aside with stirring for 20 h. The precipitate was collected and dried to afford the hydrochloride (**25**) (35 mg, 65%).

ν_{max} : 1592, 1637, 1711(C=O) cm⁻¹

λ_{max} : 282nm

m.p. 185 °C

$\delta^1\text{H}$ (400MHz, DMSO): 2.05 (2H, br s, 2''-CH₂), 3.07 (2H, br s, 3_{ax}''', 5_{ax}'''-CH), 3.19 (2H, br s, 3''-CH₂), 3.41 (4H, br s, 1''-CH₂, 3_{eq}''', 5_{eq}'''-CH), 3.75 (2H, br s, 2_{ax}''', 6_{ax}'''-CH), 3.95 (2H, br s, 2_{eq}''', 6_{eq}'''-CH), 5.68 (2H, s, 6-OCH₂), 7.05 (2H, s, NH₂), 7.37 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.23 (1H, s, 8-CH), 9.01 (1H, s, NH), 10.46 (1H, s, HCl).

$\delta^{13}\text{C}$ (100MHz, DMSO): 23.2 (2''-CH₂), 37.2 (1''-CH₂), 51.0 (3''', 5'''-CH₂), 53.5 (3''-CH), 61.2 (6-OCH₂), 63.2 (2''', 6'''-CH₂), 108.1 (4'-C), 113.9 (5-C), 125.4 (5'-CH), 131.1 (3'-CH), 136.8 (8-CH), 140.0 (2'-C), 149.0 (C=O), 152.4 (4-C), 159.6 (2-C), 159.9 (6-C).

Found: C, 40.14; H, 4.51; N, 17.94 %. C₁₈H₂₃BrN₇O₃SCl·0.5 H₂O requires C, 39.89; H, 4.46; N, 18.09 %.

MS(ES) Calcd *m/z* for C₁₈H₂₃BrN₇O₃S 496.0766, Found 496.0796.

***O*⁶-4-Bromophenyl)-9-[N-(3-morpholinopropyl)carbamoyl]guanine**

naphthoquinone-sulfonate salt (26) – A solution of *O*⁶-(4-bromophenyl)-9-[N-(3-morpholinopropyl)carbamoyl]guanine hydrochloride (26.8 mg, 0.05 mmol) in methanol (2 ml) was added to a solution of the sodium salt of 1,2-naphthoquinone-4-sulphonic acid (13 mg, 0.05 mmol) in methanol (1 ml). After 15 min the solvent was evaporated and ether added to the residue. A solid separated and was collected to afford the salt (**26**) (35 mg, 95%).

ν_{max} 1593, 1687(C=O) cm⁻¹

λ_{max} 283nm

m.p decomp. > 110 °C

$\delta^1\text{H}$ (400MHz, DMSO): 2.06 (2H, br s, 2''-CH₂), 3.06 (2H, br s, 3_{ax}''', 5_{ax}'''-CH), 3.18 (2H, br s, 3''-CH₂), 3.41 (4H, br s, 1''-CH₂, 3_{eq}''', 5_{eq}'''-CH), 3.73 (2H, br s, 2_{ax}''', 6_{ax}'''-CH), 3.95 (2H, br s, 2_{eq}''', 6_{eq}'''-CH), 5.68 (2H, s, 6-OCH₂), 6.72 (1H, s, NQS 3-CH), 7.05 (2H, s, NH₂), 7.36 (1H, s, 3'-CH), 7.54 (1H, t, *J*=7.5, NQS 7-CH), 7.71 (2H, m, 5'-CH, NQS 6-CH), 7.95 (1H, d, *J*=8.0, NQS 5-CH), 8.26 (1H, s, 8-CH), 8.42 (1H, d, *J*=8.0, NQS 8-CH), 9.01 (1H, br s, NH), 10.59 (1H, br s, *H*).

$\delta^{13}\text{C}$ (100MHz, DMSO): 23.3 (2''-CH₂), 37.0 (1''-CH₂), 51.0 (3''', 5'''-CH₂), 53.6 (3''-CH₂), 61.1 (6-OCH₂), 63.2 (2''', 6'''-CH₂), 108.1 (4'-C), 113.8 (5-C), 124.0 (NQS 3-CH), 125.3 (5'-CH), 128.7 (NQS 5-CH), 129.9 (NQS 7-CH), 130.4 (NQS 8-CH), 131.1 (3'-CH), 131.7 (NQS 9-C), 131.9 (NQS 10-C), 134.3 (NQS 6-CH), 136.7 (8-CH), 140.0 (2'-C), 149.0 (C=O), 152.3 (4-C), 156.6 (NQS 4-C), 159.6 (2-C), 159.9 (6-C), 178.4 (NQS 1-C=O), 181.6 (NQS 2-C=O).

Found: C, 40.82; H, 3.86; N, 11.52 %. C₂₈H₂₈BrN₇O₈S₂·5 H₂O requires C, 40.81; H, 4.65; N, 11.90 %.

4-Amino-N-methylpiperidine (29)^{13*} – A mixture of sodium methoxide (6.48 g, 120 mmol) and *N*-methyl-4-piperidone oxime hydrochloride (19.8 g, 120 mmol) in methanol (240 ml) was stirred at room temperature for 1 h. The solvent was evaporated, water added to the residue and the product extracted into ether. The solvent was dried over MgSO₄ and evaporated to afford the free base of the oxime (4.5 g 30%) as a white solid.

Sodium (7 g) was added to a refluxed solution of *N*-methyl-4-piperidone oxime (3.84 g, 30 mmol) in dry ethanol (60 ml). Upon addition, the solution was concentrated, and K₂CO₃ was added and the product was extracted into ether. The solvent was dried by passing through phase separatory paper and was evaporated to afford a brown liquid. The liquid was distilled to afford the product (29) (2.16 g, 63%), as a colourless liquid.

$\delta^1\text{H}$ (400MHz, DMSO): 1.17 (2H, m, 3_{ax}, 5_{ax}-CH), 1.41 (2H, br s, NH₂), 1.61 (2H, d, *J*_{gem}=12.6, 3_{eq}, 5_{eq}-CH), 1.82 (2H, m, 2_{ax}, 6_{ax}-CH), 2.12 (3H, s, NCH₃), 2.47 (1H, m, 4-CH), 2.63 (2H, d, *J*_{gem}=12.0, 2_{eq}, 6_{eq}-CH).

* Compound (29) was kindly prepared by Dr. D.J. Donnelly.

$\delta^{13}\text{C}$ (100MHz, DMSO): 34.2 (3, 5- CH_2), 45.6 (CH_3), 47.3 (4- CH), 53.9 (2, 6- CH_2).

4-Nitrophenyl N-(3-ethoxypropyl)carbamate (40) – 3-Ethoxypropylamine (120 μl , 1 mmol) was added to a solution of bis(4-nitrophenyl) carbonate (304 mg, 1 mmol) in dry DCM (3 ml). After 2 h the DCM solution was washed three times with a 10% NaHCO_3 solution and then once with brine, before being dried over MgSO_4 . The DCM was evaporated to afford the 4-nitrophenyl carbamate (**40**) (153 mg, 57%).

ν_{max} : 1591, 1610, 1718($\text{C}=\text{O}$) cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 1.10 (3H, t, $J=7.0$, CH_3), 1.70 (2H, m, 2'- CH_2), 3.13 (2H, m, 1'- CH_2), 3.40 (4H, m, 1'', 3'- CH_2), 7.39 (2H, d, $J=9.0$, arom. 2, 6- CH), 8.03 (1H, br s, NH), 8.25 (2H, d, $J=9.0$, arom. 3, 5- CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 15.0 (CH_3), 29.4 (2'- CH_2), 65.3 (1''- CH_2), 65.4 (3'- CH_2), 67.6 (1'- CH_2), 122.4 (arom. 2, 6- CH), 125.1 (arom. 3,5- CH), 144.1 (arom. 1- C), 153.1 ($\text{C}=\text{O}$), 156.3 (arom. 4- C).

O⁶-(4-Bromophenyl)-9-[N-(3-ethoxypropyl)carbamoyl]guanine (22) – 4-Nitrophenyl N-(3-ethoxypropyl)carbamate (**40**) (27 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and Hünig's base (17 μl , 0.1 mmol) in DMF (0.5 ml). After 2 days the solvent was evaporated and ethyl acetate was added to the residue. Hexane was added gradually to precipitate (**22**) (36.5 mg, 80%) as a white solid.

ν_{max} : 1598, 1633, 1722($\text{C}=\text{O}$) cm^{-1}

λ_{max} : 280nm

m.p. 138 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 1.08 (3H, t, $J=7.0$, CH_3), 1.81 (2H, m, $2''\text{-CH}_2$), 3.40 (6H, m, $1'''$, $1''$, $3''\text{-CH}_2$), 5.67 (2H, s, 6-OCH_2), 7.02 (1H, s, $3'\text{-CH}$), 7.37 (1H, s, $5'\text{-CH}$), 8.24 (1H, s, 8-CH), 8.87 (1H, br s, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 15.1 (CH_3), 29.3 ($2''\text{-CH}_2$), 37.3 ($1''\text{-CH}_2$), 61.2 (6-OCH_2), 65.4 ($1'''\text{-CH}_2$), 67.3 ($3''\text{-CH}_2$), 108.1 ($4'\text{-C}$), 114.0 (5-C), 125.4 ($5'\text{-CH}$), 131.1 ($3'\text{-CH}$), 136.9 (8-CH), 140.1 ($2'\text{-C}$), 148.8 (C=O), 152.4 (4-C), 159.6 (2-C), 159.9 (6-C).

Found: C, 41.96; H, 4.07; N, 18.36 %. $\text{C}_{16}\text{H}_{19}\text{BrN}_6\text{O}_3\text{S}$ requires C, 42.20; H, 4.21; N, 18.46 %.

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{16}\text{H}_{20}\text{BrN}_6\text{O}_3\text{S}$ 455.0501, Found 455.0470.

4-Nitrophenol salt of 4-nitrophenyl N-(4-picolyl)carbamate (41) – 4-Picolylamine (102 μl , 1 mmol) was added to a solution of bis(4-nitrophenyl) carbonate (304 mg, 1 mmol) in dry DCM (3 ml). The reaction was set aside with stirring for 2 h. The solvent was evaporated and ethyl acetate was added to the residue. Hexane was added gradually to precipitate the 4-nitrophenol salt (**41**) (214 mg, 52%).

ν_{max} : 1591, 1615, 1722(C=O) cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 4.35 (2H, d, $J=5.5$, CH_2Ar), 6.88 (2H, d, $J=9.0$, 4-nitrophenol $2''$, $6''\text{-CH}$), 7.35 (2H, d, $J=5.5$, picolyl $3'$, $5'\text{-CH}$), 7.45 (2H, d, $J=9.0$, arom. 2, 6-CH), 8.09 (2H, d, $J=9.0$, 4-nitrophenol $3''$, $5''\text{-CH}$), 8.27 (2H, d, $J=9.0$, arom. 3, 5-CH), 8.54 (2H, d, $J=5.5$, picolyl $2'$, $6'\text{-CH}$), 8.67 (1H, br s, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 43.1 (CH_2Ar), 115.8 (4-nitrophenol $2''$, $6''\text{-CH}$), 122.0 (picolyl $3'$, $5'\text{-CH}$), 122.6 (arom. 2, 6-CH), 125.1 (arom. 3, 5-CH), 126.2 (4-nitrophenol $3''$, $5''\text{-CH}$), 141.0 (4-nitrophenol $1''\text{-C}$), 144.3 (arom. 1-C), 147.7 (picolyl $4'\text{-C}$), 149.6 (picolyl $2'$, $6'\text{-CH}$), 153.6 (C=O), 156.1 (arom. 4-C), 164.0 (4-nitrophenol $4''\text{-C}$).

4-Nitrophenol salt of 4-nitrophenyl N-(N-methyl-4-piperidyl)carbamate (42) – This compound (230 mg, 55%) was obtained following the above procedure, but using 4-amino-N-methylpiperidine (114 mg, 1 mmol) instead of 4-picolylamine.

$\nu_{\max}$: 1524, 1705(C=O) cm^{-1}

$\delta^1\text{H}$ (400MHz, DMSO): 1.50 (2H, m, 3'_{ax}, 5'_{ax}-CH), 1.79 (2H, d, $J_{\text{gem}}=12.5$, 3'_{eq}, 5'_{eq}-CH), 1.93 (2H, m, 2'_{ax}, 6'_{ax}-CH), 2.15 (3H, s, NCH₃), 2.72 (2H, d, $J_{\text{gem}}=11.5$, 2'_{eq}, 6'_{eq}-CH), 3.31 (1H, br s, 4'-CH), 6.87 (2H, d, $J=9.0$, 4-nitrophenol 2'', 6''-CH), 7.39 (2H, d, $J=9.0$, arom. 2, 6-CH), 8.02 (1H, d, $J=7.0$, NH), 8.09 (2H, d, $J=9.0$, 4-nitrophenol 3'', 5''-CH), 8.25 (2H, d, $J=9.0$, arom. 3, 5-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 31.3 (3', 5'-CH₂), 45.8 (NCH₃), 48.0 (4'-CH), 54.0 (2', 6'-CH₂), 116.0 (4-nitrophenol 2'', 6''-CH), 122.4 (arom. 2, 6-CH), 125.2 (arom. 3, 5-CH), 126.3 (4-nitrophenol 3'', 5''-CH), 138.9 (4-nitrophenol 1''-C), 144.1 (arom. 1-C), 152.3 (C=O), 156.2 (arom. 4-C), 165.1 (4-nitrophenol 4''-C).

O⁶-(4-Bromophenyl)-9-[N-(4-picolyl)carbamoyl]guanine (43) – The 4-nitrophenol salt (41) (27 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and Hünig's base (34 μl , 0.2 mmol) in DMF (0.5 ml). After 2 days the solvent was evaporated, acetonitrile added to the residue and (43) (32 mg, 70%), a white solid, separated.

$\nu_{\max}$: 1601, 1630, 1724(C=O) cm^{-1}

$\lambda_{\max}$: 281nm

m.p. 180 °C

$\delta^1\text{H}$ (400MHz, DMSO): 4.65 (2H, s, NHCH₂), 5.69 (2H, s, 6-OCH₂), 7.02 (2H, s, NH₂), 7.38 (1H, s, 3'-CH), 7.39 (2H, br s, arom. 3'', 5''-CH), 7.72 (1H, s, 5'-CH), 8.29 (1H, s, 8-CH), 8.55 (2H, s, arom. 2'', 6''-CH), 9.39 (1H, s, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 42.1 (NHCH₂), 61.1 (6-OCH₂), 108.0 (4'-C), 113.9 (5-C), 121.8 (arom. 3'', 5''-CH), 125.3 (5'-CH), 131.1 (3'-CH), 136.8 (8-CH), 140.0 (2'-C), 147.1 (C=O), 149.2 (arom. 4''-C), 149.7 (arom. 2'', 6''-CH), 152.4 (4-C), 159.5 (2-C), 159.9 (6-C).

Found: C, 43.87; H, 2.93; N, 20.54 %. C₁₇H₁₄BrN₇O₂S·0.5 H₂O requires C, 43.53; H, 3.22; N, 20.91 %.

MS (ES) [M+H]⁺ Calcd *m/z* for C₁₇H₁₅BrN₇O₂S 460.0191, Found 460.0179.

O⁶-(4-Bromophenyl)-9-[N(N-methyl-4-piperidyl)carbamoyl]guanine (44) – This compound (23 mg, 50%) was obtained following the above procedure, but using the 4-nitrophenol salt (**42**) (41 mg, 0.1 mmol) instead of (**41**).

ν_{max} : 1601, 1626, 1718(C=O) cm⁻¹

λ_{max} : 281nm

m.p. 190 °C

$\delta^1\text{H}$ (400MHz, DMSO): 1.64 (2H, m, 3_{ax}'', 5_{ax}''-CH), 1.91 (2H, m, 3_{eq}'', 5_{eq}''-CH), 2.03 (2H, m, 2_{ax}'', 6_{ax}''-CH), 2.20 (3H, s, NCH₃), 2.72 (2H, m, 2_{eq}'', 6_{eq}''-CH), 3.67 (1H, br s, 4''-CH), 5.67 (2H, s, 6-OCH₂), 7.06 (2H, s, NH₂), 7.36 (1H, s, 3'-CH), 7.71 (1H, s, 5'-CH), 8.23 (1H, s, 8-CH), 8.89 (1H, d, *J*=7.5, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 31.5 (3'', 5''-CH₂), 45.9 (NCH₃), 46.9 (4''-CH), 53.8 (2'', 6''-CH₂), 61.2 (6-OCH₂), 108.1 (4'-C), 114.0 (5-C), 125.4 (5'-CH), 131.1 (3'-CH), 136.8 (8-CH), 140.0 (2'-C), 148.0 (C=O), 152.4 (4-C), 159.4 (2-C), 159.5 (6-C).

Found: C, 43.99; H, 4.44; N, 20.72 %. C₁₇H₂₀BrN₇O₂S requires C, 43.78; H, 4.32; N, 21.02 %.

MS (ES) [M+H]⁺ Calcd *m/z* for C₁₇H₂₁BrN₇O₂S 466.0661, Found 466.0626.

***O*⁶-(4-Bromophenyl)-9-[N(N-methyl-4-piperidyl)carbamoyl]guanine citrate (44a) –**

Citric acid (10 mg, 0.05 mmol) was added to a solution of *O*⁶-(4-bromophenyl)-9-[N(N-methyl-4-piperidyl)carbamoyl]guanine (23 mg, 0.05 mmol) in methanol (3 ml). After 10 min the solvent was evaporated and ether added to the residue. A white solid separated, was collected and dried to afford the citrate (28 mg, 85%).

ν_{max} : 1563, 1624, 1722(C=O) cm⁻¹

λ_{max} : 262, 280nm

m.p. decomp. > 100 °C

$\delta^1\text{H}$ (400MHz, DMSO): 1.80 (2H, m, 3_{ax}'', 5_{ax}''-CH), 2.06 (2H, m, 3_{eq}'', 5_{eq}''-CH), 2.51 – 2.73 (9H, m, citric acid-CH₂, NCH₃, 2_{ax}'', 6_{ax}''-CH), 3.11 (2H, m, 2_{eq}'', 6_{eq}''-CH), 3.84 (1H, br s, 4''-CH), 5.68 (2H, s, 6-OCH₂), 7.11 (2H, s, NH₂), 7.37 (1H, s, 3'-CH), 7.72 (1H, s, 5'-CH), 8.26 (1H, s, 8-CH), 8.91 (1H, d, *J*=7.5, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 29.0 (3'', 5''-CH₂), 43.4 (NCH₃), 43.9 (citric acid-CH₂), 52.2 (4''-CH), 54.9 (2'', 6''-CH₂), 61.1 (6-OCH₂), 71.5 (citric acid-C), 108.1 (4'-C), 113.9 (5-C), 125.5 (5'-CH), 131.2 (3'-CH), 136.8 (8-CH), 140.0 (2'-C), 148.1 (C=O), 152.4 (4-C), 159.5 (2-C), 159.9 (6-C), 171.3 (citric acid-COOH), 176.4 (citric acid-C).

Found: C, 40.99; H, 4.34; N, 13.94 %. C₂₃H₂₈BrN₇O₉S·1.25 H₂O requires C, 40.56;

H, 4.33; N, 14.40 %.

4-Nitrophenyl N-methoxycarbamate (48)* – Bis(4-nitrophenyl) carbonate (304 mg, 1 mmol) was added to a solution of methoxyamine hydrochloride (84 mg, 1 mmol) and triethylamine (140 μ l, 1 mmol) in DMF (2 ml) at -5°C . The reaction was set aside at this temperature for 30 min. The solvent was evaporated and DCM was added to the residue. The DCM solution was washed with water to remove the triethylamine hydrochloride and dried over MgSO_4 . The solvent was removed and ethyl acetate was added to the residue. Hexane was added gradually to precipitate (48) (127 mg, 60%) as a white solid.

ν_{max} : 1725(C=O) cm^{-1}

(This compound was too unstable in deuterated DMSO to allow for analysis by NMR)

O⁶-(4-Bromophenyl)-9-(N-methoxycarbamoyl)guanine (46) – 4-Nitrophenyl N-methoxycarbamate (48) (21 mg, 0.1 mmol) was added to a solution of PaTrin-2 (33 mg, 0.1 mmol) and Hünig's base (17 μ l, 0.1 mmol) in DMF at -5°C . The reaction was set aside at this temperature for 30 min. The solvent was evaporated and ethyl acetate added to the residue. Hexane was added gradually to precipitate (46) (32 mg, 80%) as a white solid.

ν_{max} : 1600, 1626, 1734(C=O) cm^{-1}

λ_{max} : 280 nm

m.p. decomp. $> 150^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 3.82 (3H, s, OCH_3), 5.67 (2H, s, 6- OCH_2), 7.07 (2H, s, NH_2), 7.37 (1H, s, 3'-CH), 7.72 (1H, s, 5'-CH), 8.26 (1H, s, 8-CH), 11.56 (1H, s, NH).

* Demoute *et al.*²² reported the preparation of (47) by a reaction involving methoxyamine and 4-nitrophenyl chloroformate.

$\delta^{13}\text{C}$ (100MHz, DMSO): 61.2 (6-OCH₂), 64.4 (OCH₃), 108.1 (4'-C), 113.6 (5-C), 125.4 (5'-CH), 131.1 (3'-CH), 136.4 (8-CH), 140.0 (2'-C), 148.1 (C=O), 152.0 (4-C), 159.6 (2-C), 159.9 (6-C).

Found: **C**, 36.09; **H**, 2.78; **N**, 20.06 %. $\text{C}_{12}\text{H}_{11}\text{BrN}_6\text{O}_3\text{S}\cdot 0.25 \text{ EtOH}$ requires **C**, 36.57; **H**, 3.07; **N**, 20.48 %.

(This compound proved too unstable to allow for the measurement of accurate mass)

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CHAPTER 5

Chapter 5: Biological results

5.1 Introduction

The Christie Hospital in Manchester is one of the most important cancer hospitals in the United Kingdom. The Paterson Institute for Cancer Research is a part of this complex and is one of the largest laboratories in the U.K. that is devoted exclusively to cancer research. Each ATase inactivator prepared in our laboratory is tested at the Paterson Institute.

We would like to thank Dr. Geoffrey P. Margison and his colleagues at the Paterson Institute for releasing the results of assays carried out to determine the ATase inactivating ability of each of the PaTrin-2 N-9 derivatives that we have prepared. We are also appreciative of their current work to establish the potential of these derivatives as prodrugs.

The ATase inactivating ability is reported as the I_{50} , which is the concentration of inactivator required to give a 50% reduction in ATase activity. Hence, the lower the I_{50} value, the better the inactivator. Details of the experimental procedure involved in measuring the I_{50} value are given in the following section.

5.2 Determination of the ATase-depleting ability (I_{50}) of inactivators

The standard procedure to assay ATase (O^6 -alkylguanine-DNA alkyltransferase) activity in any solution involves the use of a radiolabelled [^3H]-methylated DNA substrate, in which one of the protons of the methyl group is replaced by tritium. The [^3H]-methylated DNA is added to a buffer containing the purified protein ATase and the mixture is then incubated at 37 $^{\circ}\text{C}$ for 1 hour. During this time reaction occurs, and the [^3H]-methyl group is irreversibly transferred to the cysteine group at the

ATase active site. The DNA is then hydrolysed and the [^3H]-methylated ATase is recovered and its concentration measured by liquid scintillation counting.

The I_{50} value of an inactivator is determined in a competition assay. The procedure is similar to that discussed above, but a fixed amount of ATase is incubated with varying amounts of the inactivator at 37°C for 1 hour. The [^3H]-methylated DNA substrate is then added and incubated at 37°C for a further hour. The radiolabelled methylated DNA reacts with the remaining ATase. By determining the amount of [^3H]-methylated ATase at the end of the reaction, and comparing it to the actual amount of ATase used in the experiment, the amount of ATase inactivated by the inactivator can be measured.

Samples are typically assayed in duplicate and the I_{50} value of an inactivator is determined graphically from plots of the percentage of ATase activity remaining versus inactivator concentration (μM).

5.3 Inactivating ability of potential prodrugs

The I_{50} value has been determined for each of the prodrugs whose synthesis we have discussed. The I_{50} values for each of the three categories of prodrug are given in individual tables (Table 5.1 – 5.3) and also a sample of the graphs from which these values were determined is given also (Fig. 5.2 – 5.4).

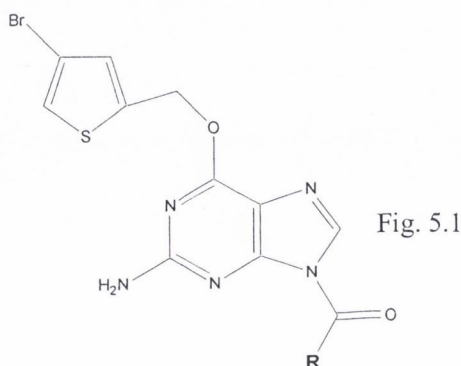
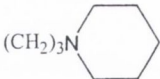
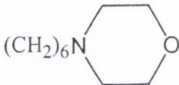
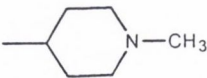


Table 5.1a

Category 1: **R** = OR

Compound Test No.	Substituent (R)	I ₅₀ value (μM)
B. 4346	CH ₃	0.0048
B. 4638	(CH ₂) ₂ CH ₃	0.0036
B. 4627	(CH ₂) ₃ OEt	0.0043
B. 4587	CH(CH ₃) ₂	0.008
B. 4581	Cyclohexyl	0.004
B. 4589	2-Adamantyl	0.008
B. 4531	Benzyl	0.007
B. 4633	4-Nitrobenzyl	0.0047
B. 4538	Piperonyl	0.007
B. 4575	Phenyl	0.005
B. 4579 ^a	Phenyl	0.009
B. 4635		0.0047
B. 4634	(CH ₂) ₆ N(CH ₃) ₂	0.0046
B. 4588		0.006
B. 4619		0.004
	Citrate	0.007
B. 4569	4-Picolyl	0.01

^a C=S for C=O

Table 5.1b

Category 1: **R** = OR

(Steroid derivatives)

Compound Test No.	Substituent (R)	I ₅₀ value (μM)
B. 4358	Dihydrotestosterone	0.025
B. 4599 [♦]	Dihydrotestosterone	0.003
B. 4629	Oestradiol	0.017
B. 4639 [♦]	Oestradiol	0.0065

[♦] N-7 derivative

Table 5.2

Category 2: $R = NR_2$

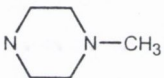
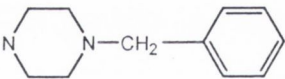
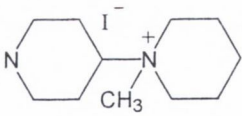
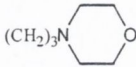
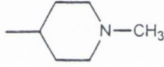
Compound Test No.	Substituent (R)	I_{50} value (μM)
B. 4543	<i>N,N</i> -Dimethyl	0.03
B. 4608	<i>N,N</i> -Diphenyl	0.12
B. 4609		0.28
B. 4542		0.2
B.4601		0.05
	Hydrochloride	0.2
	Maleate	0.25
	Citrate	0.08
	Pamoate	0.08
B. 4612		0.28
B. 4632		0.27

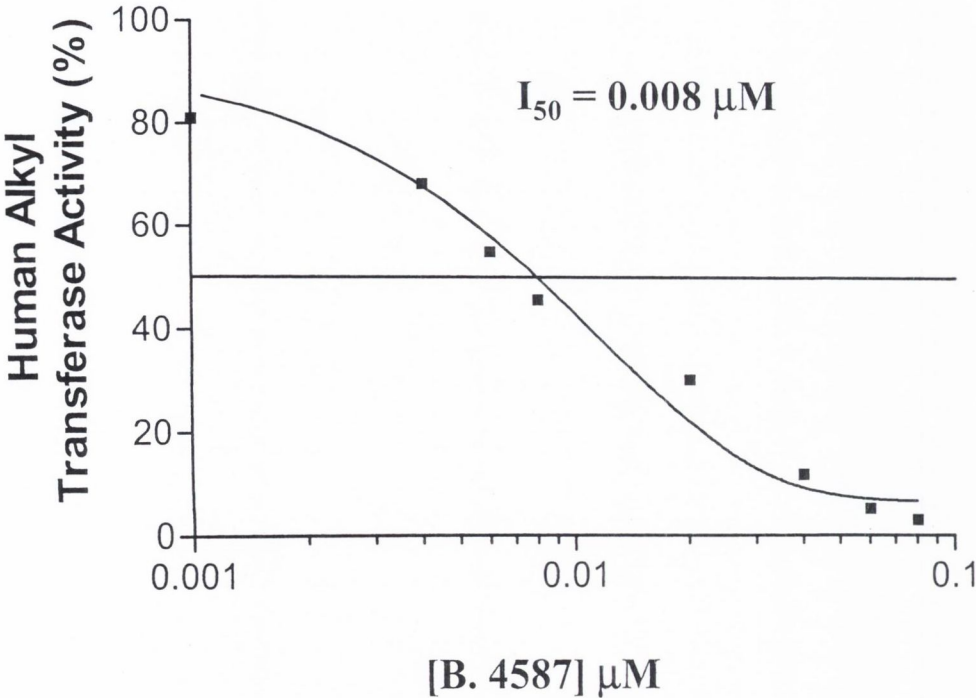
Table 5.3

Category 3: **R** = NHR

Compound Test No.	Substituent (R)	I ₅₀ value (μM)
B. 4571	(CH ₂) ₂ CH ₃	0.05
B. 4544	(CH ₂) ₅ CH ₃	0.013
B. 4628	(CH ₂) ₃ OEt	0.03
B. 4564	Cyclohexyl	0.095
B. 4636	OCH ₃	0.026
B. 4616		
	Hydrochloride	0.015
	NQS [♦]	0.03
B. 4625		0.005
	Citrate	0.0096
B. 4572	4-Picolyl	0.006

[♦] NQS = 1,2-Naphthoquinone-4-sulphonate

Inactivation of ATase by B. 4587 (see Table 5.1a)

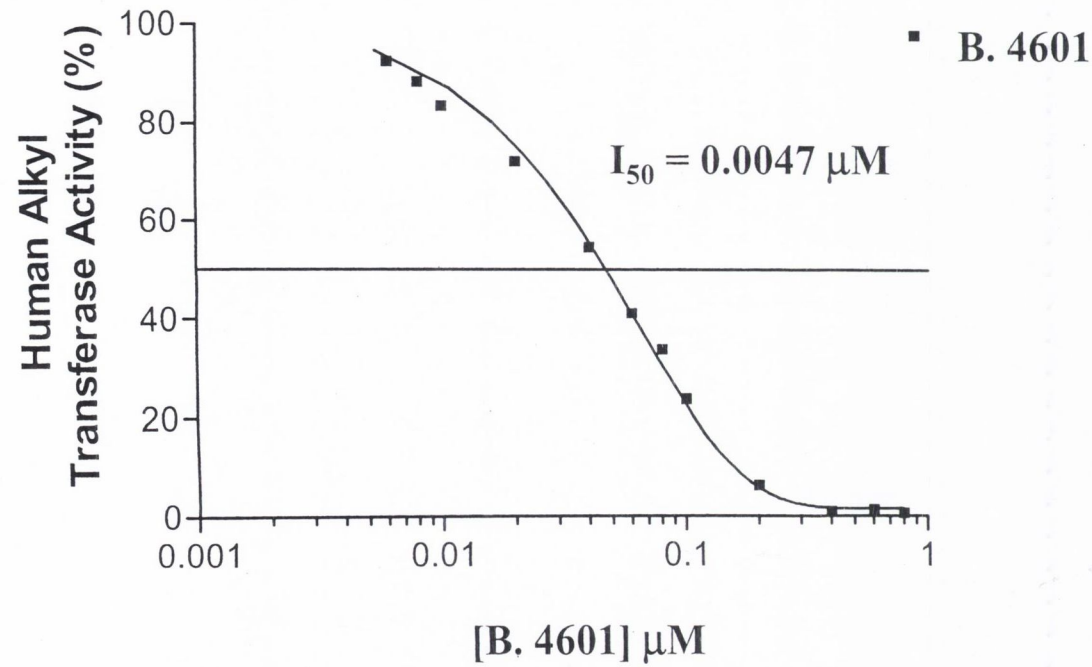


▪ B. 4587

[B4587]uM	% Activity of ATase
0.00	100.0
1.00e-003	81.0
4.00e-003	68.4
6.00e-003	55.0
8.00e-003	45.7
2.00e-002	30.1
4.00e-002	11.9
0.06	5.2
0.08	3.0

Fig. 5.2

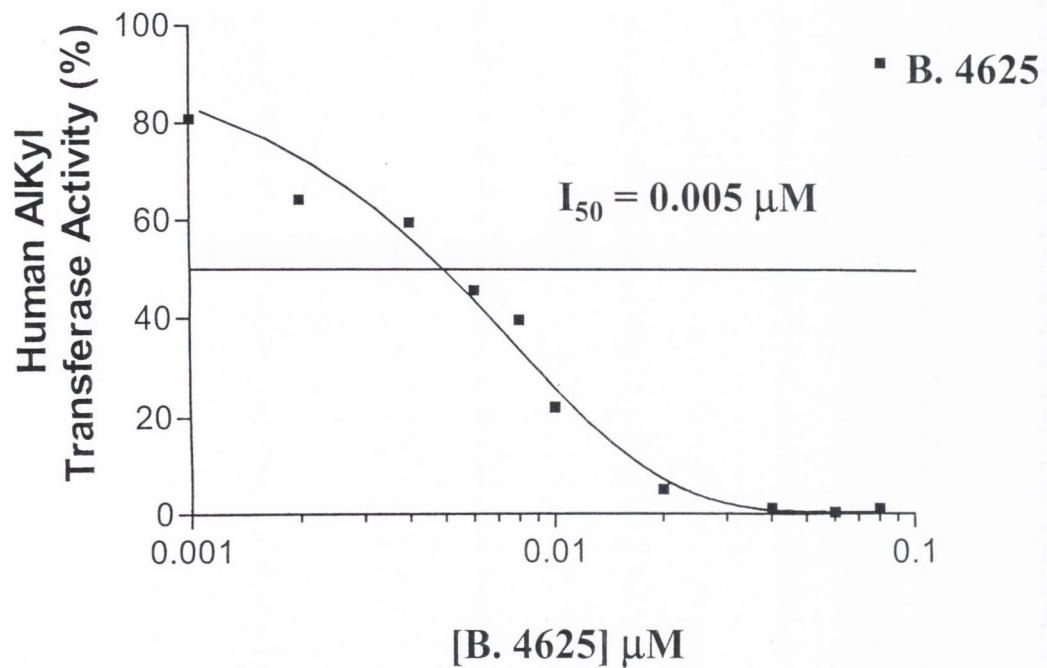
Inactivation of ATase by B. 4601 (see Table 5.2)



[B4601] μ M	% Activity of ATase
0.00	100.0
1.00e-003	102.2
2.00e-003	105.9
4.00e-003	103.1
6.00e-003	92.7
8.00e-003	88.6
1.00e-002	83.7
2.00e-002	72.4
4.00e-002	54.7
0.06	41.2
0.08	33.7
0.10	23.7
0.20	6.3
0.40	1.0
0.60	1.4
0.80	0.6

Fig. 5.3

Inactivation of ATase by B. 4625 (see Table 5.3)



[B4625]uM	% Activity of ATase
0.000	100.0
0.001	80.9
0.002	64.4
0.004	59.7
0.006	45.7
0.008	39.7
0.010	22.0
0.020	5.0
0.040	1.1
0.060	0.3
0.080	1.0

Fig. 5.4

5.4 Analysis of I_{50} values

The I_{50} value of PaTrin-2 is 0.0034 μM . Many of the I_{50} values reported above (Table 5.1 – 5.3) are comparable with this value. However, the activity recorded may not reflect the activity of the prodrug, as substantial hydrolysis may have occurred to liberate free PaTrin-2. As a result, recent work at the Paterson Institute has focused on determining the stability of many of the prodrugs under the conditions of the assay. This has helped to establish whether the observed I_{50} value reflects the ATase-inactivating ability of each prodrug or merely that of the PaTrin-2 formed. The results of this investigation are discussed in the following section.

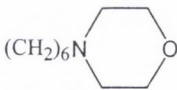
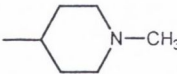
5.4.1 Investigation of prodrug stability

As discussed in Section 5.2, the standard procedure to determine the I_{50} value involves the incubation of the inactivator with ATase for 1 hour at 37 $^{\circ}\text{C}$ and at pH 8.3, the pH required to maintain the absolute integrity of the ATase in the assay. The procedure also uses a solution of the inactivator in DMSO diluted with buffer solution. Upon receiving the compounds for testing, stock solutions in DMSO are prepared and stored at -20°C until their subsequent use. As previously mentioned, we have found that some of the prodrugs are somewhat unstable in solutions in deuterated DMSO (prepared for NMR analysis) due to traces of water in the solutions. To establish whether the I_{50} value recorded for each prodrug is a measure of the activity of the prodrug, or reflects the presence of a substantial proportion of PaTrin-2, researchers at the Paterson Institute have investigated the stability of many of the prodrugs under the conditions discussed above. This was done by measuring the percentage of free PaTrin-2 present in the stock DMSO solution diluted with buffer at pH 8.3. This measurement corresponded to a time zero (t_0). This stock

solution (at pH 8.3) was then incubated for 1 hour at 37 °C, after which time (t_{60}) the percentage of PaTrin-2 present was again determined. The results of this investigation are given below in individual tables (Table 5.4 – 5.6) corresponding to each of the three categories of prodrug. HPLC (UV detection) was used to measure the percentage of PaTrin-2 present by a comparison of the peak heights of PaTrin-2 and the prodrug. We are aware that the margin of error associated with this technique may be quite significant, but have no estimate of its value.

Table 5.4

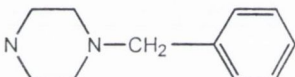
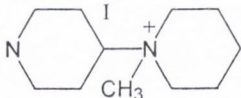
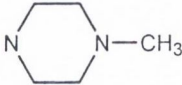
Category 1: R = OR

Test No.	Substituent (R)	% PaTrin-2 (t_0)	% PaTrin-2 (t_{60})
B. 4346	CH ₃	ND	46
B. 4531	Benzyl	29	48
B. 4575	Phenyl	ND	8.3
B. 4587	CH(CH ₃) ₂	ND	6.5
B. 4633	4-Nitrobenzyl	ND	19
B. 4588		ND	10
B. 4619		7.5	70
B. 4572	4-Picolyl	14	41

ND = Degradation not detected (*i.e.* <5% degradation)

Table 5.5

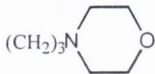
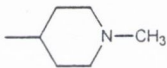
Category 2: $R = NR_2$

Test No.	Substituent (R)	% PaTrin-2 (t_0)	% PaTrin-2 (t_{60})
B. 4543	<i>N,N</i> -Dimethyl	6.3	6.3
B. 4608	<i>N,N</i> -Diphenyl	ND	ND
B. 4542		ND	ND
B. 4609		ND	ND
B. 4612		ND	ND
B. 4632		ND	ND
B. 4601		9.6	9.6
	Citrate	ND	6

ND = Degradation not detected (*i.e.* <5% degradation)

Table 5.6

Category 3: **R** = NHR

Test No.	Substituent (R)	% PaTrin-2 (t ₀)	% PaTrin-2 (t ₆₀)
B. 4571	(CH ₂) ₂ CH ₂	ND	ND
B. 4564	Cyclohexyl	ND	ND
B. 4628	(CH ₂) ₃ OEt	ND	61
B. 4616			
	Hydrochloride	10	25
	NQS [*]	ND	7.5
B. 4625		ND	ND
	Citrate	ND	16
B. 4572	4-Picolyl	14	41

ND = Degradation not detected (*i.e.* <5% degradation)^{*}NQS = 1,2-Naphthoquinone-4-sulphonate

5.4.2 Analysis of prodrug stability results

The results reported above (Table 5.4 – 5.6) indicate the general chemical stability of each prodrug. Overall, the majority of prodrugs maintain at least 50% stability following incubation at 37 °C for 1 hour. The stability of these prodrugs towards hydrolysis can be rationalised by a consideration of their chemical structure. The carbamates should be most susceptible to hydrolysis at pH 8.3 and should hydrolyse *via* the standard Bac2 mechanism. The simple methyl carbamate (B. 4346, Table 5.4)

is found, as expected, to be the most labile. However, the degree of hydrolysis of the benzyl derivative (B. 4531, Table 5.4) is unexpectedly large, and as such, the result may need checking. It is possible that this derivative reacts *via* an Aa11 mechanism, which can occur in some specialized cases at weakly alkaline pHs. The degree of hydrolysis of the 4-nitrobenzyl analogue (B. 4633, Table 5.4) is lower than that for the benzyl derivative, as expected if the Aa11 mechanism operates, as the 4-nitrobenzyl cation is less stable than the benzyl. The effect of a basic substituent in the N-9 side chain is varied, having little effect in compounds like O^6 -(4-bromophenyl)-9-(6-morpholinohexyloxycarbonyl)guanine (B. 4588, Table 5.4) where the amino group is well removed from the carbonyl. However, in O^6 -(4-bromophenyl)-9-(dimethylaminohexyloxycarbonyl)guanine (B. 4634) the effect is large and at t_0 total hydrolysis was found to have occurred. The chemical instability of this compound may be due to the interaction of the amino group and the carbonyl group to form an intermediate involving a six-membered ring (see Section 2.10.3, Scheme 2.39, pg 102). This interaction may assist hydrolysis.

The *N*-monosubstituted ureas ($R = NHR$, see Fig. 5.1) are more stable to hydrolysis, with no decomposition shown for the alkyl-substituted derivatives (Table 5.6). The higher degree of hydrolysis found with the basic derivative O^6 -(4-bromophenyl)-9-[*N*-(3-morpholinopropyl)carbamoyl]guanine hydrochloride (B. 4616, Table 5.6) may also be due to an internal interaction between amino and carbonyl groups, similar to that discussed above. However, the result from the 4-picolyl derivative (B. 4572, Table 5.6) is clearly anomalous, while the presence of a tertiary amino group in O^6 -(4-bromophenyl)-[*N*-(*N*-methyl-4-piperidyl)carbamoyl]guanine (B. 4625, Table 5.6) has no effect.

As anticipated, the *N,N*-disubstituted ureas ($R = NR_2$, see Fig. 5.1) are the most stable category of prodrug, with no hydrolysis detected for the majority of derivatives.

By observing the amount of free PaTrin-2 present at t_{60} , we can comment on the degree to which the reported I_{50} value reflects the activity of each prodrug. The majority of the I_{50} values measured do reflect the activity of the actual prodrug, while those of the less stable compounds must reflect the activity of both prodrug and parent drug. However, since these I_{50} values are still comparable with PaTrin-2, we can propose that these prodrugs are themselves also good ATase inactivators.

The variation in the chemical stability of prodrugs within categories (as well as between categories) appears to suggest that the stability of these PaTrin-2 derivatives is dependent as much on the nature of the substituent (R) as it is on the category (*i.e.* $R = OR, NR_2, NHR$, see Fig. 5.1). For example, preliminary results suggest that those prodrugs (both N-7 and N-9) containing a steroidal residue (see Table 5.1b) are extremely stable, and perhaps the most stable of all the alkoxycarbonyl derivatives prepared. The decreased susceptibility to hydrolysis may be associated with steric hindrance at the 17-position in the steroid.

The above results reflect chemical stability, however the stability of these compounds *in vivo* may be significantly different. The presence of esterases or amidases may increase the rate of hydrolysis and change the order of stability of these derivatives. The focus of current PaTrin-2 research at the Paterson Institute is to assess the potential of these compounds as prodrugs and to determine the type of N-9 derivative most suitable to deliver PaTrin-2 as desired *in vivo*.

5.5 Further analysis of I_{50} values

While awaiting the results of the prodrug stability tests from the Paterson Institute we attempted to use a different approach to allow us to comment further on the stability of the prodrugs. This approach involved the preparation of several prodrugs identical to the N-9 guanine derivatives discussed above, but with a different O^6 -substituent. As well as allowing for the further analysis of the I_{50} values, these derivatives would enable us to compare the stabilities of prodrugs of O^6 -(4-bromophenyl)guanine with those of other O^6 -substituted guanines.

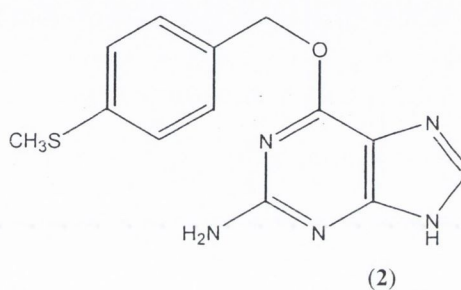
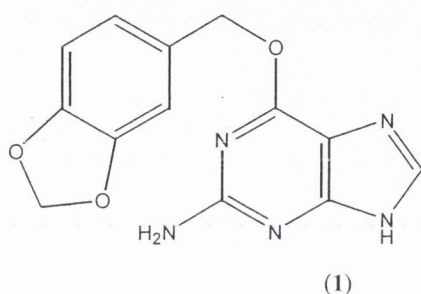
5.5.1 Interactions of active site and substrate

An enzyme is not a rigid structure. When a substrate interacts with the active site of an enzyme, it can induce structural changes within that active site. These changes permit maximum interaction energies between the amino acid residues in the active site and the substrate, and this in turn lowers the energy of the transition state of the chemical reaction between the two.

The interactions of the amino acid residues in the ATase active site and the N-9 substituent in the PaTrin-2 derivative can increase the stabilization of the guanyl moiety in the binding pocket. The “anchoring” of the inactivator into the active site lowers the energy of the transition state of the reaction in which the 4-bromophenyl is transferred from the O^6 -position to the cysteine residue (Cys 145). Lower I_{50} values, and therefore good inactivators, are associated with higher stabilization energies.

If the O^6 -substituent of the prodrug is changed, then the shape of the substrate is changed. The active site will interact differently not only with the O^6 -substituent, but with the guanyl moiety and the N-9 substituent. We decided to prepare several potential prodrugs from two other powerful inactivators, O^6 -piperonylguanine (**1**) and

*O*⁶-(4-methylthiobenzyl)guanine (2). These prodrugs would orientate themselves differently in the active site when compared to prodrugs of *O*⁶-(4-bromophenyl)guanine. Therefore if the active site is interacting with the prodrug, and not the parent compound, then the N-9 substituent may be orientated so that it hinders the effective anchoring of the substrate into the binding pocket, resulting in a much higher *I*₅₀ value than that of the parent compound.



It was hoped that a comparison of the *I*₅₀ values of prodrugs of PaTrin-2 and prodrugs of *O*⁶-piperonylguanine (1) and *O*⁶-(4-methylthiobenzyl)guanine (2) would provide valuable information on what the *I*₅₀ values actually reflect.

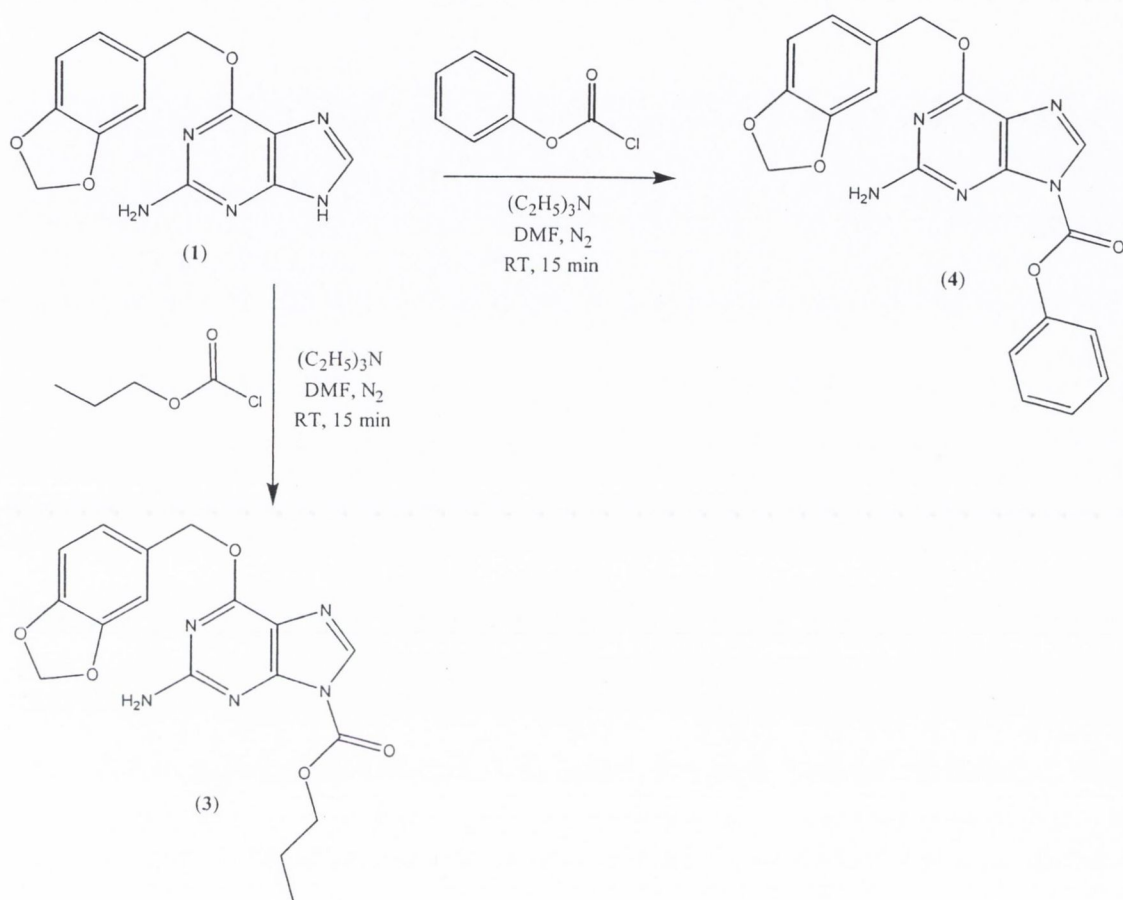
5.6 Prodrugs of *O*⁶-piperonylguanine and *O*⁶-(4-methylthiobenzyl)guanine

Using *O*⁶-piperonylguanine (1) and *O*⁶-(4-methylthiobenzyl)guanine (2) as starting materials[♦], we prepared six derivatives that were representative of the three categories of prodrug. Each of the derivatives was prepared in a similar manner to their *O*⁶-(4-bromophenyl)guanine analogue.

The N-9 alkoxycarbonyl derivatives *O*⁶-piperonyl-9-(propoxycarbonyl)guanine (3) and 9-phenoxy carbonyl-*O*⁶-piperonylguanine (4) were prepared by adding the respective chloroformate to a solution of the *O*⁶-substituted guanine and an equimolar

[♦] Samples of *O*⁶-piperonylguanine (1) and *O*⁶-(4-methylthiobenzyl)guanine (2) were kindly provided by Dr. D.J. Donnelly.

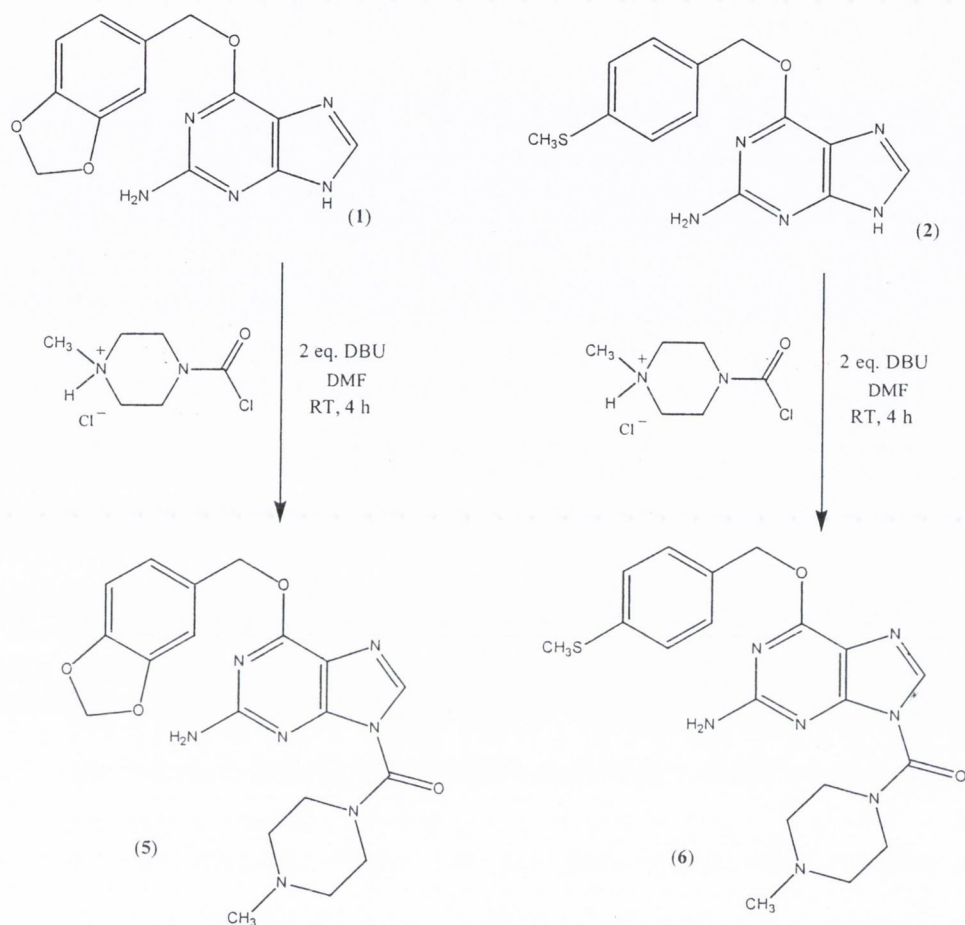
amount of triethylamine in DMF (Scheme 5.1). After 15 minutes, evaporation and addition of ethanol precipitated the derivatives (3) and (4) in good yield.



Scheme 5.1

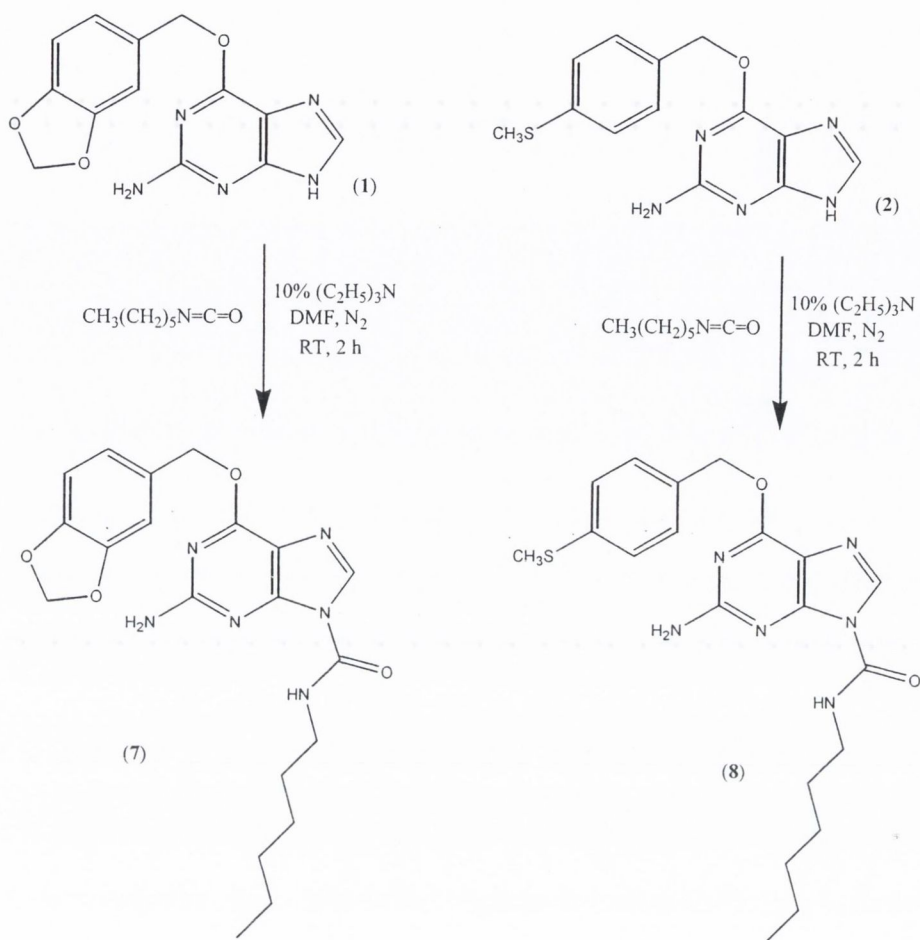
The N-9 dialkylcarbamoyl derivatives 9-(4-methyl-1-piperazinylcarbonyl)-*O*⁶-piperonylguanine (5) and 9-(4-methyl-1-piperazinylcarbonyl)-*O*⁶-(4-methylthiobenzyl)guanine (6) were prepared by adding 4-methylpiperazine-1-carbonyl chloride hydrochloride to a solution of the *O*⁶-substituted guanine and two equivalents of DBU in DMF (Scheme 5.2). After 4 hours, the solvent was evaporated and ¹H NMR analysis of the crude residues showed almost complete reaction. Ethanol

was added to each residue and the N-9 derivatives (**5**) and (**6**) isolated by filtration, in low yield.



Scheme 5.2

We also synthesized the N-9 monoalkylcarbamoyl derivatives 9-(*N*-hexylcarbamoyl)-*O*⁶-piperonylguanine (**7**) and 9-(*N*-hexylcarbamoyl)-*O*⁶-(4-methylthiobenzyl)guanine (**8**) by adding hexyl isocyanate to a solution of the *O*⁶-substituted guanine and a catalytic amount of triethylamine in DMF (Scheme 5.3). The solvent was replaced by DCM after 2 hours and gradual addition of hexane precipitated the crystalline derivatives (**7**) and (**8**) in good yield.

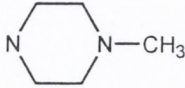
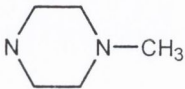


Scheme 5.3

5.6.1 Analysis of I_{50} values and prodrug stability

These N-9 derivatives of *O*⁶-piperonylguanine and *O*⁶-(4-methylthiobenzyl)guanine (compounds 3-8) were tested for their ATase-inactivating ability. Their I_{50} values (Table 5.7) can be compared with these of *O*⁶-piperonylguanine (1) and *O*⁶-(4-methylthiobenzyl)guanine (2) which are 0.02 μM and 0.024 μM respectively.

Table 5.7

Compound Test No.	Category	Substituent (R)	I ₅₀ value (μM)
B. 4624 ^a	OR	(CH ₂) ₂ CH ₃	0.02
B. 4620 ^a	OR	Phenyl	0.03
B. 4614 ^a	NR ₂		8
B. 4615 ^b	NR ₂		3
B. 4618 ^a	NHR	(CH ₂) ₅ CH ₃	0.84
B. 4617 ^b	NHR	(CH ₂) ₅ CH ₃	0.6

^a O⁶-substituent = piperonyl^b O⁶-substituent = 4-CH₃SC₆H₄CH₂

The I₅₀ values of the N-9 carbamoyl derivatives B. 4614 and B. 4615 are significantly different from those of compounds (1) and (2). The presence of the N-9 substituent substantially lowered the ATase-depleting ability of both O⁶-piperonylguanine (1) and O⁶-(4-methylthiobenzyl)guanine (2). A comparison of the I₅₀ values of 9-(4-methyl-1-piperazinylcarbonyl)-O⁶-piperonylguanine (B. 4614) and O⁶-piperonylguanine (1) show that they differ by a factor of four hundred. This suggests that the I₅₀ values of these compounds are a measure of the activity of the prodrugs and not the parent compound. This is consistent with the results of the PaTrin-2 prodrug stability tests for this category of prodrug.

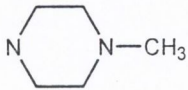
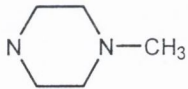
The I₅₀ values of the N-9 carbamoyl derivatives B. 4618 and B. 4617 also differ from those of the parent compounds O⁶-piperonylguanine (1) and O⁶-(4-methylthiobenzyl)guanine (2). The difference is not as great as that for the derivatives above (R = NR₂, see Fig. 5.1), but the comparatively higher I₅₀ does suggest that the

entire prodrug has not decomposed and that the I_{50} value is reflective of the activity of both the prodrug and parent compound. This finding would also appear to be in line with the PaTrin-2 prodrug stability tests.

It transpired that the I_{50} values of the alkoxycarbonyl derivatives B. 4624 and B. 4620 are almost the same as that of the parent drug (**1**). This would suggest that either these prodrugs are excellent ATase inactivators or that their I_{50} values merely reflect the activity of the parent drug (**1**). A similar relationship was found between the I_{50} values of PaTrin-2 and the corresponding derivatives B. 4638 and B. 4575 (see Table 5.1a), however, the prodrug stability results for B. 4575 (see Table 5.4) indicate that this prodrug is very stable to hydrolysis under the conditions of the ATase assay.

To measure the susceptibility of prodrugs of O^6 -piperonylguanine (**1**) and O^6 -(4-methylthiobenzyl)guanine (**2**) to hydrolysis and to observe the influence, if any, of the O^6 -substituent on their stability, researchers at the Paterson Institute have also determined the chemical stability of several of these compounds. The results are given below (Table 5.8) and provide a useful comparison with their PaTrin-2 analogues.

Table 5.8

Test No.	Substituent (R)	% PaTrin-2 (t_0)	% PaTrin-2 (t_{60})
B. 4620 ^a	Phenyl	8.8	70
B. 4618 ^a	(CH ₂) ₅ CH ₃	ND	ND
B. 4614 ^a		ND	ND
B. 4615 ^b		ND	ND

ND = Degradation not detected (*i.e.* <5% degradation)

^a *O*⁶-substituent = piperonyl

^b *O*⁶-substituent = 4-CH₃SC₆H₄CH₂

A comparison of the stability of 9-phenoxy-carbonyl-*O*⁶-piperonyl-guanine (B. 4620) with that of the PaTrin-2 analogue *O*⁶-(4-bromophenyl)-9-(phenoxy-carbonyl)guanine (B. 4575, see Table 5.4) shows that while both prodrugs are of similar stability in the stock solution at pH 8.3 (t_0), following the incubation period (t_{60}) the *O*⁶-piperonyl-guanine derivative (B. 4620) has largely decomposed, while the PaTrin-2 derivative remains mostly stable. The dramatic influence of the *O*⁶-substituent on the stability of the substituent in the 9-position was unexpected and needs to be checked.* A similar dramatic difference is found with the stabilities of 9-(*N*-hexylcarbamoyl)-*O*⁶-piperonyl-guanine (B. 4618) and *O*⁶-(4-bromophenyl)-9-(*N*-hexylcarbamoyl)-guanine (B. 4544). Preliminary results suggest that the PaTrin-2 derivative is unstable in the stock solution at pH 8.3 (t_0), with none of the prodrug detected. In this case it is

* This result had not been checked at the time of submission of this thesis.

the O^6 -piperonylguanine derivative (B. 4618) that is unexpectedly more stable. This result may also need checking.*

The chemical stabilities of the dialkylcarbamoyl derivatives B. 4614 and B. 4615 were found to be similar to that of O^6 -(4-bromophenyl)-9-(4-methyl-1-piperazinylcarbonyl)guanine (B. 4601).

Further investigation of the influence of the O^6 -substituent on prodrug stability is most certainly required.

5.7 Biological results – conclusion

We have designed a variety of second-generation drugs. Although further biological investigation is required to determine how these prodrugs will behave *in vivo*, the initial results are encouraging. Determination of the rates of hydrolysis of these compounds under physiological conditions will suggest which prodrug is most suitable to deliver PaTrin-2 *in vivo*. We hope that the biological results will indicate several advantages of these prodrugs over PaTrin-2, particularly with regard to solubility and selectivity. We have prepared compounds in which we have incorporated a tertiary amino group into the N-9 substituent, making possible the synthesis of water-soluble salts, which will improve formulation and therefore drug delivery.

We have also synthesized N-9 alkoxycarbonyl derivatives with tumour targeting ability. These prodrugs, consisting of PaTrin-2 coupled to either a dihydrotestosterone or an oestradiol moiety, are good ATase inactivators (Table 5.1b) and, from preliminary results, not as susceptible to hydrolysis as other alkoxycarbonyl derivatives. Another oestradiol derivative of PaTrin-2 (B. 4342), coupled through a

* This result had not been checked at the time of submission of this thesis.

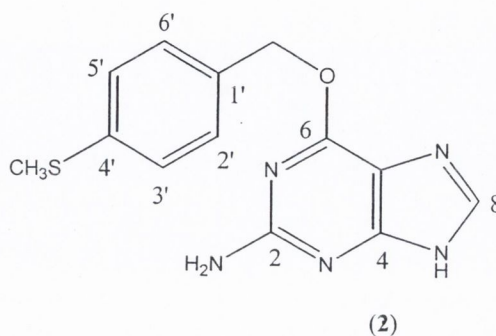
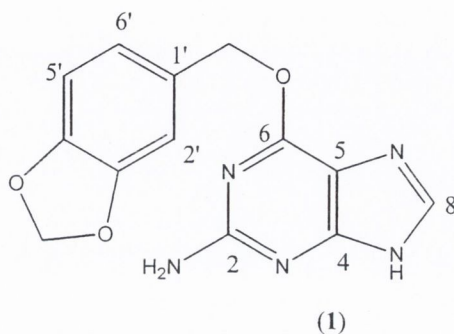
9-CH₂COOR ester bond, has been shown both to inactivate ATase in the human breast tumour cell line MCF-7 and to bind to oestrogen receptors. We hope that our steroid-containing prodrugs will have similar capabilities and once concentrated in the steroid-dependent tumour will undergo gradual hydrolysis to release PaTrin-2.

Prodrugs specifically targeted to tumour cells of various types will most certainly reduce the toxicity that is associated with PaTrin-2, which itself shows limited selectivity. It is hoped that even a simple prodrug may reduce toxicity to some extent. The slow release of an active agent by a prodrug has been shown to be an effective and less toxic way of administering a drug. Often, a drug requires administration in large and toxic doses, due to rapid plasma clearance of the agent *in vivo*.

Without doubt, the biological results will determine the course of further research in the area of PaTrin-2 prodrug development. However, it is hoped that our thorough investigation of the routes and reagents that make possible the synthesis of these derivatives will facilitate preparation of further prodrugs of the types discussed above.

5.8 Experimental[♦]

Numbering system followed for *O*⁶-piperonylguanine (1) and *O*⁶-(4-methylthiobenzyl)guanine (2):



9-Phenoxycarbonyl-*O*⁶-piperonylguanine (4) – Phenyl chloroformate (13 μ l, 0.1 mmol) was added to a solution of *O*⁶-piperonylguanine (28.5 mg, 0.1 mmol) and triethylamine (14 μ l, 0.1 mmol) in DMF (0.5 ml) under nitrogen. After 15 min, the solvent was evaporated, ethanol added to the residue and (4) (34 mg, 85%) separated as a white solid.

$\nu_{\max}$: 1572, 1632, 1782(C=O) cm^{-1}

$\lambda_{\max}$: 283nm

m.p. decomp. > 160⁰C

$\delta^1\text{H}$ (400MHz, DMSO): 5.42 (2H, s, 6-OCH₂), 6.04 (2H, s, OCH₂O), 6.89 (2H, s, NH₂), 6.93 (1H, d, $J=8.0$, 5'-CH), 7.03 (1H, d, $J=8.0$, 6'-CH), 7.13 (1H, s, 2'-CH), 7.36 (1H, m, arom. 4''-CH), 7.42 (2H, m, arom. 2'', 6''-CH), 7.50 (2H, m, arom. 3'', 5''-CH), 8.40 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 67.2 (6-OCH₂), 101.1 (OCH₂O), 108.2 (5'-CH), 109.5 (2'-CH), 114.1 (5-C), 121.7 (arom. 2'', 6''-CH), 122.8 (6'-CH), 126.7 (arom. 4''-CH),

[♦] For experimental details see Chapter 2, Section 2.13, pgs. 122-123.

129.7 (arom. 3'', 5''-CH), 130.0 (1'-C), 137.9 (8-CH), 146.4 (arom. 1''-C), 147.2 (3'-C), 147.3 (4'-C), 149.9 (C=O), 153.7 (4-C), 160.5 (2-C), 161.0 (6-C).

Found: C, 58.15; H, 3.63; N, 16.60 %. C₂₀H₁₅N₅O₅·0.5 H₂O requires C, 58.02; H, 3.90; N, 16.92 %.

MS (ES) [M+H]⁺ Calcd *m/z* for C₂₀H₁₆N₅O₅ 406.1151, Found 406.1190.

O⁶-Piperonyl-9-(propoxycarbonyl)guanine (3) – This compound (33 mg, 88%) was prepared following the above procedure, but using propyl chloroformate (13 µl, 0.1 mmol) instead of phenyl chloroformate.

$\nu_{\max}$: 1580, 1622, 1780(C=O) cm⁻¹

$\lambda_{\max}$: 283nm

m.p. 140 °C

$\delta^1\text{H}$ (400MHz, DMSO): 0.98 (3H, t, *J*=7.5, 3''-CH₃), 1.74 (2H, m, 2''-CH₂), 4.32 (2H, t, *J*=6.5, 1''-CH₂), 5.38 (2H, s, 6-OCH₂), 6.03 (2H, s, OCH₂O), 6.82 (2H, s, NH₂), 6.92 (1H, d, *J*=8.0, 5'-CH), 7.02 (1H, d, *J*=8.0, 6'-CH), 7.12 (1H, s, 2'-CH), 8.21 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 10.1 (3''-CH₃), 21.4 (2''-CH₂), 67.1 (6-OCH₂), 69.0 (1''-CH₂), 101.0 (OCH₂O), 108.1 (5'-CH), 109.4 (2'-CH), 114.0 (5-C), 122.7 (6'-CH), 129.9 (1'-C), 137.5 (8-CH), 147.1 (3'-C), 147.2 (4'-C), 147.6 (C=O), 153.5 (4-C), 160.3 (2-C), 160.8 (6-C).

Found: C, 54.09; H, 4.80; N, 18.49%. C₁₇H₁₇N₅O₅·0.25 H₂O requires C, 54.37; H, 4.70; N, 18.66 %.

MS (ES) [M+H]⁺ Calcd *m/z* for C₁₇H₁₈N₅O₅ 372.1308, Found 372.1310.

9-(4-Methyl-1-piperazinylcarbonyl)-O⁶-piperonylguanine (5) – 4-Methylpiperazine-1-carbonyl chloride hydrochloride (20 mg, 0.1 mmol) was added to a solution of O⁶-piperonylguanine (28.5 mg, 0.1 mmol) and DBU (30 μ l, 0.2 mmol) in DMF (1 ml). After 4 hours, the solvent was evaporated, ethanol added to the residue and **(5)** (16 mg, 40%) separated as a white solid.

$\nu_{\max}$: 1606, 1691(C=O) cm^{-1}

$\lambda_{\max}$: 286nm

m.p. 172 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 2.26 (3H, br s, CH_3), 2.46 (4H, br s, 3'', 5''- CH_2), 3.47 (4H, br s, 2'', 6''- CH_2), 5.39 (2H, s, 6-O CH_2), 6.03 (2H, s, O CH_2O), 6.68 (2H, s, NH_2), 6.92 (1H, d, $J=8.0$, 5'-CH), 7.02 (1H, d, $J=8.0$, 6'-CH), 7.11 (1H, s, 2'-CH), 8.03 (1H, s, 8-CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 42.4 (2'', 6''- CH_2), 43.5 (N CH_3), 52.3 (3'', 5''- CH_2), 67.2 (6-O CH_2), 96.2, 101.1 (O CH_2O), 108.2 (5'-CH), 109.5 (2'-CH), 114.0 (5-C), 122.8 (6'-CH), 130.1 (1'-C), 138.2 (8-CH), 147.2 (C=O), 147.3 (3'-C), 147.4 (4'-C), 153.0 (4-C), 160.0 (2-C), 160.3 (6-C).

Found: C, 54.71; H, 5.23; N, 22.82 %. $\text{C}_{19}\text{H}_{21}\text{N}_7\text{O}_4 \cdot 0.5 \text{ H}_2\text{O}$ requires C, 54.33; H, 5.28; N, 23.35 %

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{19}\text{H}_{22}\text{N}_7\text{O}_4$ 412.1733, Found 412.1754.

9-(4-Methyl-1-piperazinylcarbonyl)-O⁶-(4-methylthiobenzyl)guanine (6) – This compound (12 mg, 30%) was prepared following the above procedure, but using O⁶-(4-methylthiobenzyl)guanine (29 mg, 0.1 mmol) instead of O⁶-piperonylguanine.

$\nu_{\max}$: 1599, 1618, 1680(C=O) cm^{-1}

$\lambda_{\max}$: 280nm

m.p. 160 °C

$\delta^1\text{H}$ (400MHz, DMSO): 2.23 (3H, s, NCH_3), 2.41 (4H, br s, 3'', 5''- CH_2), 2.50 (3H, s, CH_3SAr), 3.38 (4H, br s, 2'', 6''- CH_2), 5.46 (2H, s, 6- OCH_2), 6.67 (2H, s, NH_2), 7.28 (2H, d, $J=8.5$, 2', 6'- CH), 7.46 (2H, d, $J=8.0$, 3', 5'- CH), 8.04 (1H, s, 8- CH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 14.7 (CH_3SAr), 43.0 (2'', 6''- CH_2), 45.4 (NCH_3), 54.0 (3'', 5''- CH_2), 66.6 (6- OCH_2), 112.9 (5- C), 125.8 (2', 6'- CH), 129.3 (3', 5'- CH), 132.9 (4'- C), 137.8 (8- CH), 138.1 (1'- C), 148.6 ($\text{C}=\text{O}$), 153.2 (4- C), 160.1 (2- C), 160.2 (6- C).

Found: C, 55.02; H, 5.55; N, 23.48 %. $\text{C}_{19}\text{H}_{23}\text{N}_7\text{O}_2\text{S}$ requires C, 55.19; H, 5.61; N, 23.71 %

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{19}\text{H}_{24}\text{N}_7\text{O}_2\text{S}$ 414.1712, Found 414.1712.

9-(N-Hexylcarbamoyl)- O^6 -piperonylguanine (7) – Hexyl isocyanate (15 μl , 0.1 mmol) was added to a solution of O^6 -piperonylguanine (28.5 mg, 0.1 mmol) and a catalytic amount of triethylamine (1.4 μl , 0.01 mmol) in DMF (0.5 ml) under nitrogen. The reaction was set aside with stirring for 2 h. The solvent was evaporated and DCM was added to the residue, followed by hexane to precipitate (7) (25 mg, 60%) as a white solid.

ν_{max} : 1558, 1630, 1709($\text{C}=\text{O}$) cm^{-1}

λ_{max} : 292nm

m.p. 168 °C

$\delta^1\text{H}$ (400MHz, DMSO): 0.86 (3H, br s, CH_3), 1.31 (6H, m, 3'', 4'', 5''- CH_2), 1.61 (2H, br s, 2''- CH_2), 3.34 (2H, m, 1''- CH_2), 5.41 (2H, s, 6- OCH_2), 6.03 (2H, s, OCH_2O), 6.92 – 7.12 (4H, m, 5', 6'- CH , NH_2), 7.12 (1H, s, 2'- CH), 8.23 (1H, s, 8- CH), 8.90 (1H, s, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 13.9 (CH_3), 22.0 ($5''\text{-CH}_2$), 26.0 ($3''\text{-CH}_2$), 29.0 ($2''\text{-CH}_2$), 30.9 ($4''\text{-CH}_2$), 39.7 ($1''\text{-CH}_2$), 67.4 (6- OCH_2), 101.1 (OCH_2O), 108.1 ($5'\text{-CH}$), 109.5 ($2'\text{-CH}$), 114.1 (5- C), 122.8 (6'- CH), 129.9 (1'- C), 136.5 (8- CH), 147.3 (3', 4'- C), 148.8 (C=O), 152.2 (4- C), 159.8 (2- C), 160.6 (6- C).

Found: C, 58.09; H, 5.91; N, 20.29 %. $\text{C}_{20}\text{H}_{24}\text{N}_6\text{O}_4$ requires C, 58.24; H, 5.87; N, 20.83 %.

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{20}\text{H}_{25}\text{N}_6\text{O}_4$ 413.1937, Found 413.1959.

9-(N-Hexylcarbamoyl)- O^6 -(4-methylthiobenzyl)guanine (8) – This compound (19 mg, 45%) was prepared following the above procedure, but using O^6 -(4-methylthiobenzyl)guanine (29 mg, 0.1 mmol) instead of O^6 -piperonylguanine.

ν_{max} : 1562, 1632, 1713(C=O) cm^{-1}

λ_{max} : 292nm

m.p. 160 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 0.88 (3H, br s, CH_3), 1.31 (6H, m, 3'', 4'', 5''- CH_2), 1.61 (2H, m, 2''- CH_2), 2.49 (3H, s, CH_3S), 3.34 (2H, m, 1''- CH_2), 5.48 (2H, s, 6- OCH_2), 6.97 (2H, s, NH_2), 7.28 (2H, m, 2', 6'- CH), 7.46 (2H, m, 3', 5'- CH), 8.23 (1H, s, 8- CH), 8.90 (1H, br s, NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 13.9 (CH_3), 14.7 (S- CH_3), 22.0 ($5''\text{-CH}_2$), 25.0 ($3''\text{-CH}_2$), 29.0 ($2''\text{-CH}_2$), 30.9 ($4''\text{-CH}_2$), 40.2 (1''- CH_2), 67.1 (6- OCH_2), 114.1 (5- C), 125.9 (2', 6'- CH), 129.4 (3', 5'- CH), 132.8 (4'- C), 136.6 (8- CH), 138.3 (1'- C), 148.8 (C=O), 152.2 (4- C), 159.8 (2- C), 160.6 (6- C).

Found: C, 57.45; H, 6.25; N, 20.20 %. $\text{C}_{20}\text{H}_{26}\text{N}_6\text{O}_2\text{S}$ requires C, 57.95; H, 6.32; N, 20.27 %.

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{20}\text{H}_{27}\text{N}_6\text{O}_2\text{S}$ 415.1916, Found 415.1888.

CHAPTER 6

Chapter 6: Conclusion

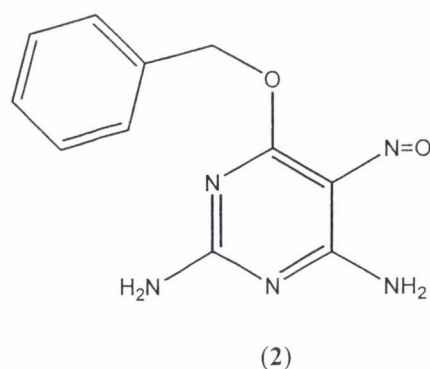
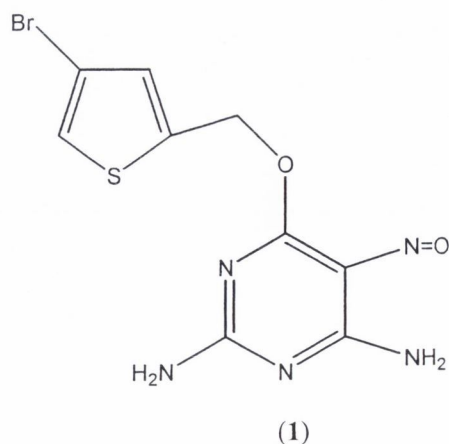
6.1 The prodrug approach – other systems

PaTrin-2 is not alone in having special characteristics and requiring finely-tuned reaction conditions. In this concluding chapter we will discuss briefly some other ATase-inactivating systems, the prodrugs of which we have either prepared or currently are being investigated in our laboratory.

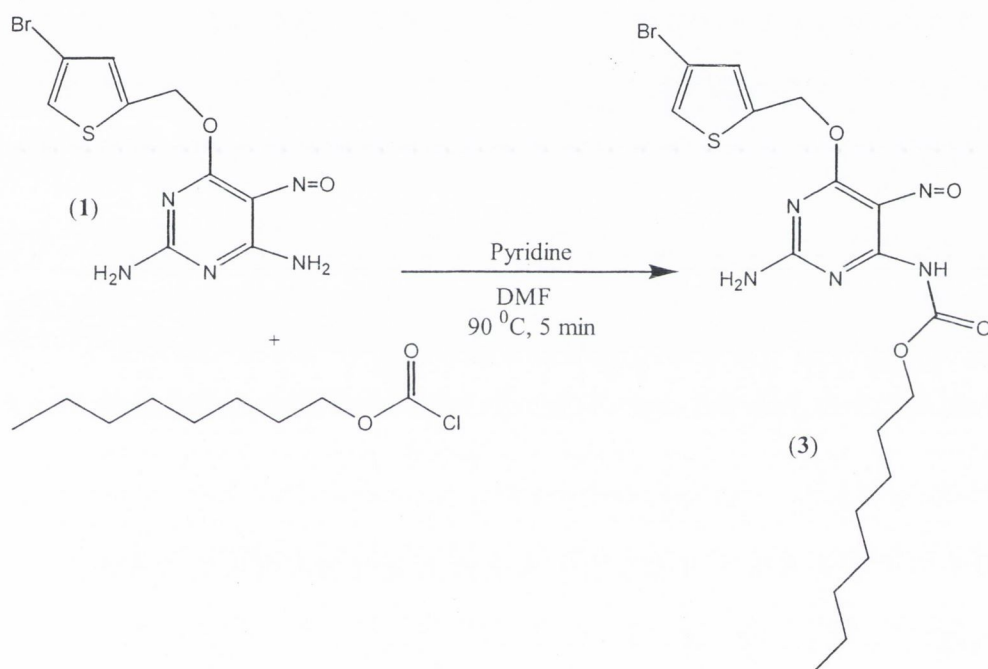
Although *O*⁶-(4-bromophenyl)guanine and *O*⁶-benzylguanine are the only two ATase inactivators to have entered clinical trials, other ATase inactivators have comparable activities. The disadvantages of these inactivators are similar to those of PaTrin-2 (*i.e.* poor solubility, limited selectivity *etc.*), and therefore these compounds are also candidates for the prodrug approach. Because of the different physical and electronic properties of these systems, they require different reagents and reaction conditions to form equivalent prodrugs to those formed from PaTrin-2.

6.1.1 Prodrugs of 6-(4-bromophenyl)-2,4-diamino-5-nitrosopyrimidine

Both 6-(4-bromophenoxy)-2,4-diamino-5-nitrosopyrimidine (**1**) and 6-benzyloxy-2,4-diamino-5-nitrosopyrimidine (**2**)¹⁻³ are good ATase inactivators having *I*₅₀ values of 0.045 μ M and 0.06 μ M respectively.



Following our successful synthesis of N-9 alkoxy carbonyl prodrugs from PaTrin-2 we attempted to prepare an alkoxy carbonyl derivative of 6-(4-bromophenoxy)-2,4-diamino-5-nitrosopyrimidine (**1**)[†] as a potential prodrug. Kempter *et al.*⁴ reported the synthesis of a series of N-4 alkoxy carbonyl pyrimidines. Using this procedure we prepared 2-amino-6-(4-bromophenoxy)-5-nitroso-4-(octyloxycarbonylamino)-pyrimidine (**3**). The procedure involved the addition of octyl chloroformate to a solution of the pyrimidine (**1**) and an equimolar amount of pyridine in DMF (Scheme 6.1).



Scheme 6.1

The reaction mixture was heated at 90 °C for 5 minutes. The solution was allowed to cool and addition of methanol precipitated a blue solid. The solid was collected and dried to afford 2-amino-6-(4-bromophenoxy)-5-nitroso-4-(octyloxycarbonylamino)-pyrimidine (**3**) in good yield. The I_{50} value of (**3**) was found to be 5.3 μ M. This value is over one hundred-fold that of the parent drug 6-(4-bromophenoxy)-2,4-diamino-5-

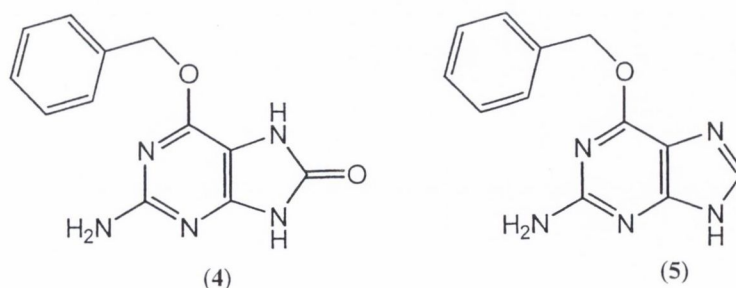
[†] A sample of 6-(4-bromophenoxy)-2,4-diamino-5-nitrosopyrimidine (**1**) was provided by Dr. D.J. Donnelly.

nitrosopyrimidine (1) (I_{50} 0.045 μ M), and implies that (3) is stable to hydrolysis. We are awaiting the results of further pharmacological evaluation.

Although the synthesis of the alkoxycarbonyl derivative (3) was straightforward and high-yielding we were unable to prepare any carbamoyl derivatives. Under a variety of conditions 6-(4-bromophenoxy)-2,4-diamino-5-nitrosopyrimidine (1) failed to react with either a carbamoyl chloride or an isocyanate. While we have established procedures for the synthesis of any PaTrin-2 carbamoyl derivative, it is apparent that substituent amino in the pyrimidine ring system is significantly different from nuclear imino in the purine ring system, and the synthesis of certain derivatives may not be possible. Research in this area is ongoing.

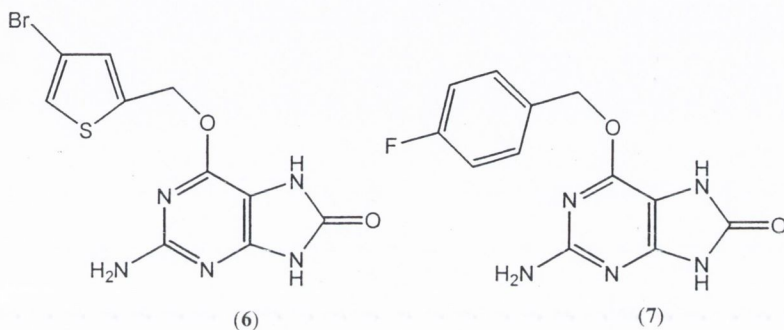
6.1.2 Prodrugs of O^6 -(4-fluorobenzyl)-8-oxoguanine

O^6 -Benzyl-8-oxoguanine (4) has been reported to be a major metabolite of the ATase inactivator O^6 -benzylguanine (5).^{1,5} Since the ATase-inactivating ability of the metabolite (4) is of comparable potency to (5), the direct administration of this compound has been considered as a way of minimizing polymorphic variations in the metabolism of O^6 -benzylguanine (5). However, O^6 -benzyl-8-oxoguanine (4) is very insoluble resulting in problems with the direct administration of this agent.^{1,5}



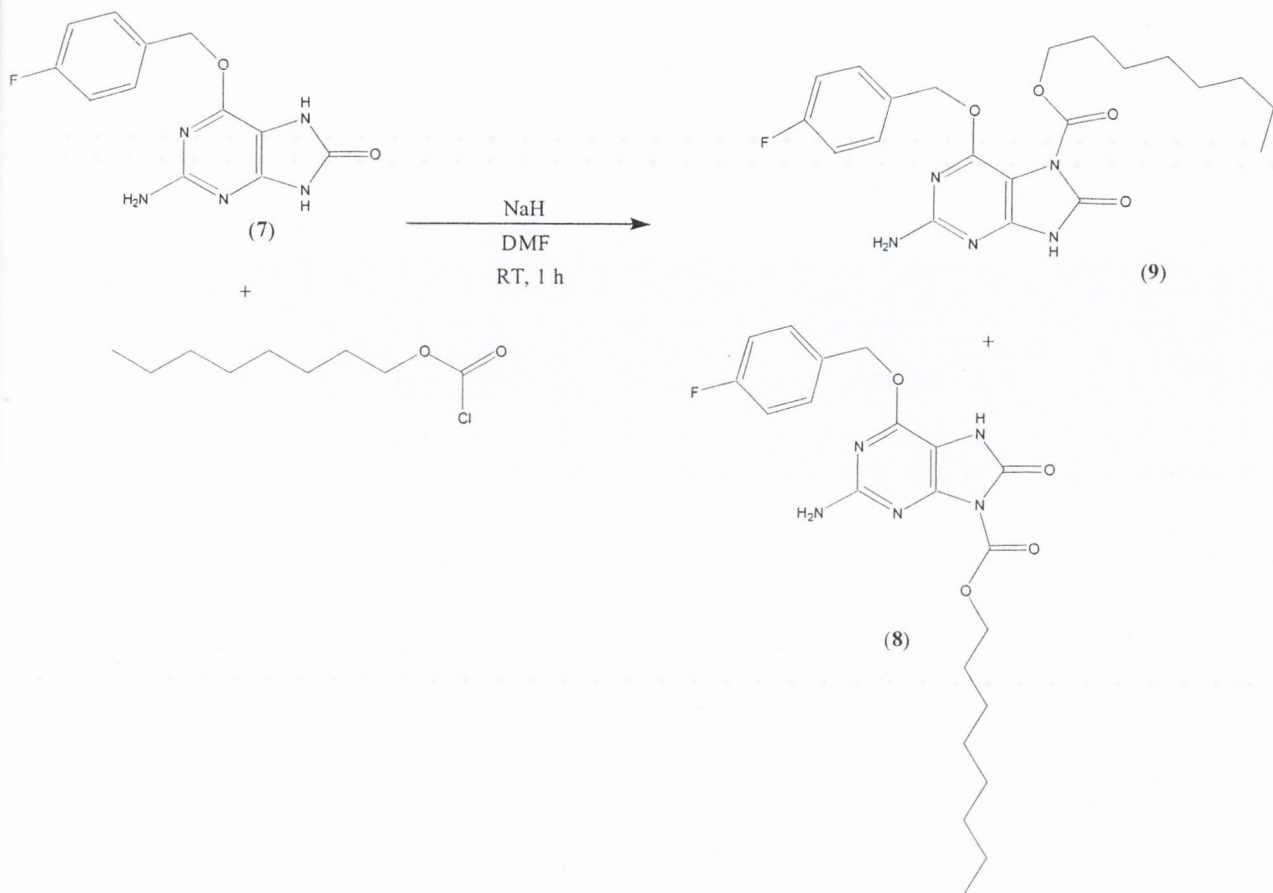
Similarly, O^6 -(4-bromophenyl)-8-oxoguanine (6) is a principal metabolite of PaTrin-2, but has only one-fifteenth the activity of PaTrin-2.⁶ Prodrugs of O^6 -benzyl-8-

oxoguanine (4) would offer some advantages, particularly with regards to improving solubility. A sample of the 8-oxo derivative O^6 -(4-fluorobenzyl)-8-oxoguanine (7) was available[♦] and we used this as a model to examine the suitability of the N-9 position in this system as a point for bioreversible derivatization and the preparation of prodrugs similar to those derived from PaTrin-2.



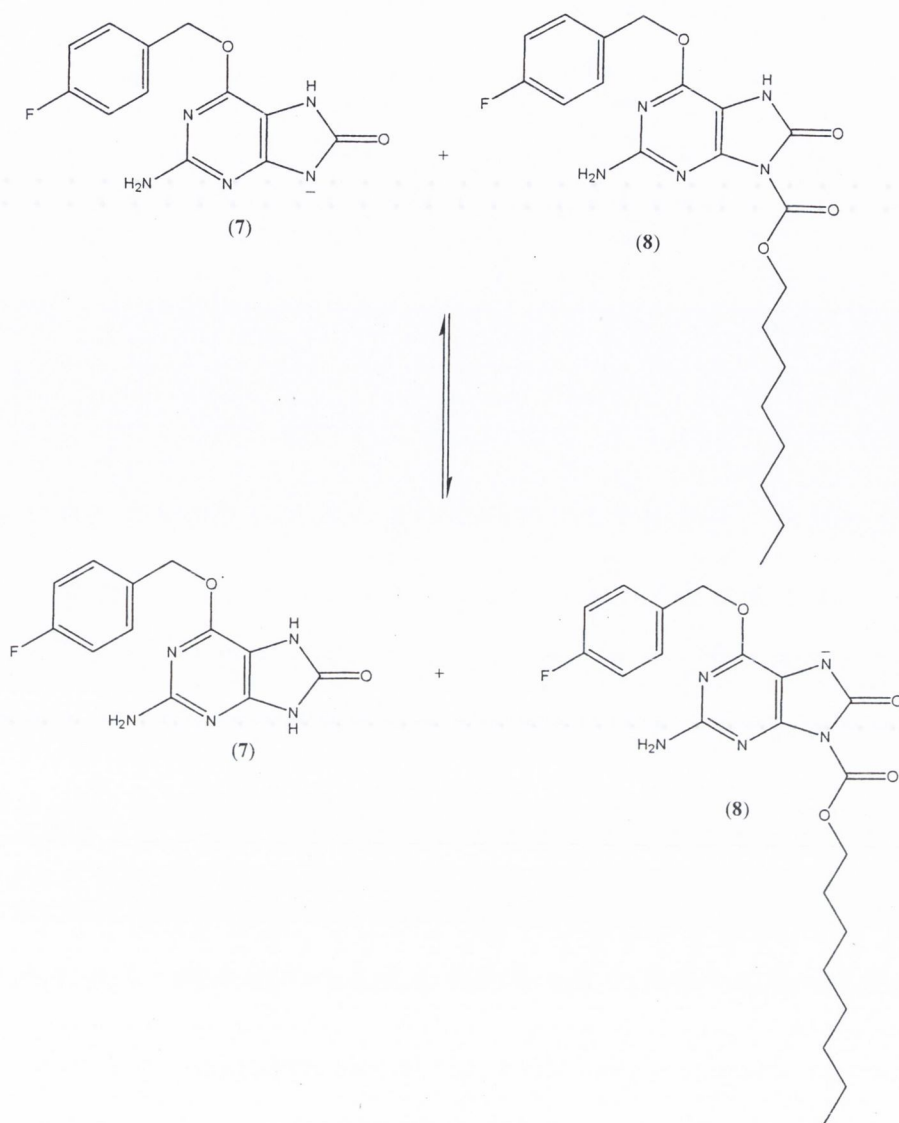
We attempted to prepare the N-9 alkoxy carbonyl derivative O^6 -(4-fluorobenzyl)-9-(octyloxycarbonyl)-8-oxoguanine (8). The procedure required the use of a strong inorganic base. An equimolar amount of sodium hydride was added to a solution of O^6 -(4-fluorobenzyl)-8-oxoguanine (7) in DMF and the suspension stirred for 5 minutes before the addition of octyl chloroformate (Scheme 6.2).

[♦] A sample of O^6 -(4-fluorobenzyl)-8-oxoguanine (7) was provided by Dr. D.J. Donnelly.



Scheme 6.2

After 1 hour, the solvent was removed and analysis of the ¹H NMR spectrum of the crude residue showed approximately equal quantities of unreacted *O*⁶-(4-fluorobenzyl)-8-oxoguanine (7) and the *N*-9 and *N*-7 isomers. Increases in the reaction time did not decrease the amount of unreacted *O*⁶-(4-fluorobenzyl)-8-oxoguanine (7). This may be explained by an equilibrium reaction, such as that shown in Scheme 6.3, in which *O*⁶-(4-fluorobenzyl)-8-oxoguanine (7) is regenerated following the removal of the *N*-7 proton in *O*⁶-(4-fluorobenzyl)-9-(octyloxycarbonyl)-8-oxoguanine (8) by the *O*⁶-(4-fluorobenzyl)-8-oxoguanine anion.



Scheme 6.3

The three compounds *O*⁶-(4-fluorobenzyl)-8-oxoguanine (**7**) *O*⁶-(4-fluorobenzyl)-9-(octyloxycarbonyl)-8-oxoguanine (**8**) and *O*⁶-(4-fluorobenzyl)-7-(octyloxycarbonyl)-8-oxoguanine (**9**) were of similar solubility and could not be separated by recrystallisation from a variety of solvents. Attempts to isolate the N-9 and N-7 derivatives by flash chromatography also failed due to their similar *R_F* values. Preparative thick layer chromatography proved to be a more successful separation technique. One isomer, to which we assigned the N-7 substituted structure (**9**), was isolated in good yield. However, the other derivative (**8**), to which we assigned a N-9

structure, was very susceptible to hydrolysis and we were unable to isolate a pure sample. We assigned the two structures on the basis of their NMR data, using as an analogy the N-7 and N-9 substituted PaTrin-2 derivatives. In the ^1H NMR spectrum of (9) (Fig. 6.1), the 2-NH₂ peak occurs at 6.34 ppm, while the same peak in the ^1H NMR spectrum of (8) (Fig. 6.2) occurs at 6.77 ppm.* The NH₂ peak in N-9 alkoxycarbonyl PaTrin-2 derivatives always appears further downfield, although this analogy might not hold in the 8-oxo series. Examination of the elution of the compounds (8) and (9) in TLC also suggests that (9) is the N-7 isomer. In PaTrin-2 alkoxycarbonyl derivatives the N-9 isomer always has a greater R_F value than the N-7 isomer, and probable analogy for the R_F values of (8) and (9) would suggest that (9) is the N-7 isomer.

* The signal associated with the 2-NH₂ of *O*⁶-(4-fluorobenzyl)-8-oxoguanine (7) appears at 6.09 ppm in the ^1H NMR spectrum.

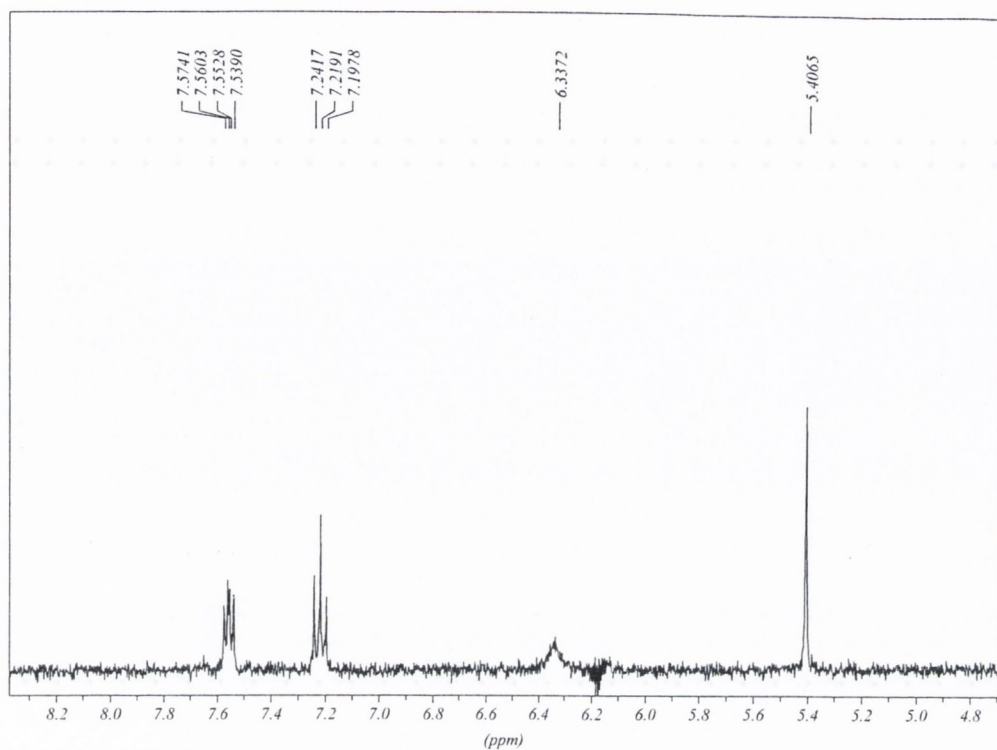


Fig. 6.1

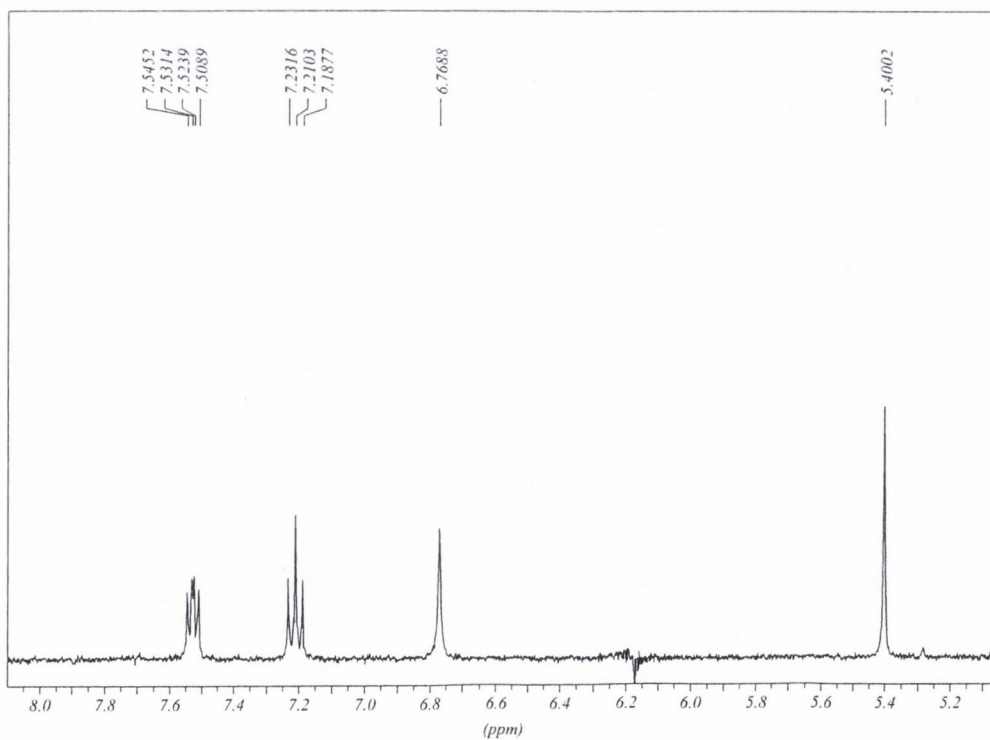
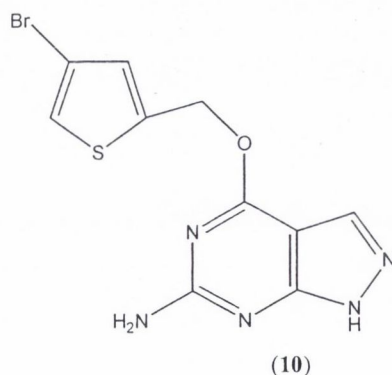


Fig. 6.2

The N-7 derivative O^6 -(4-fluorobenzyl)-7-(octyloxycarbonyl)-8-oxoguanine (**9**) was quite stable when dissolved in deuterated DMSO (for NMR analysis), apparently more so than N-7 and N-9 PaTrin-2 alkoxycarbonyl derivatives. The I_{50} values of both O^6 -(4-fluorobenzyl)-8-oxoguanine (**7**) and O^6 -(4-fluorobenzyl)-7-(octyloxycarbonyl)-8-oxoguanine (**9**) were measured by researchers at the Paterson Institute and found to be 0.075 μM and 0.085 μM respectively. We await further pharmacological evaluation of O^6 -(4-fluorobenzyl)-7-(octyloxycarbonyl)-8-oxoguanine (**9**), including a determination of the rate of hydrolysis. This will indicate whether the synthesis and pharmacological investigation of further prodrugs of this type is worthwhile.

6.1.3 Prodrugs of allo-PaTrin-2

The design and synthesis of prodrugs of O^6 -(4-bromophenyl)-8-aza-7-deazaguanine (**10**) or “allo-PaTrin-2” is another focus of current research in our laboratory.



The ATase-inactivating ability of allo-PaTrin-2 (**10**) (I_{50} , 0.007 μM) is comparable to that of PaTrin-2. However, like PaTrin-2, allo-PaTrin-2 has disadvantages *i.e.* poor water-solubility, limited selectivity *etc.* The allo-PaTrin-2 prodrugs which have been prepared by Úna Hanafin are analogues of the prodrugs of PaTrin-2 that we have

discussed in the previous chapters, and consist of a group “**R**” coupled to an allo-PaTrin-2 moiety *via* a C=O linker (Fig. 6.3). Her research is aimed at the preparation of prodrugs of the three categories (*i.e.* **R** = OR, NR₂ and NHR) based on the allo-PaTrin-2 skeleton.

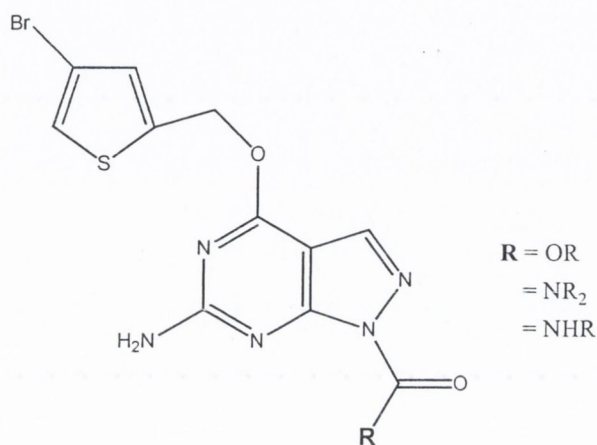


Fig. 6.3

Allo-PaTrin-2 (**10**) has different physical and electronic properties to PaTrin-2. The N-9 derivatization of (**10**) has proved significantly more difficult than the preparation of the corresponding derivatives of PaTrin-2. Examples of prodrugs from two of the categories (**R** = OR and NR₂) have been prepared, but so far it has not been possible to prepare an example from the third category (**R** = NHR). The preparation of prodrugs of allo-PaTrin-2 has required a different approach to that employed in the synthesis of the corresponding PaTrin-2 prodrugs.

Further pharmacological evaluation of the allo-PaTrin-2 prodrugs is awaited, including a determination of their rates of hydrolysis and the measurement of their ATase inactivating ability under physiological conditions. These results will provide a useful comparison to those obtained for the PaTrin-2 prodrugs.

6.2 Summary of PaTrin-2 prodrug synthesis

We have discussed in detail the development of definitive procedures to allow the synthesis of PaTrin-2 prodrugs of three categories (Fig. 6.4).

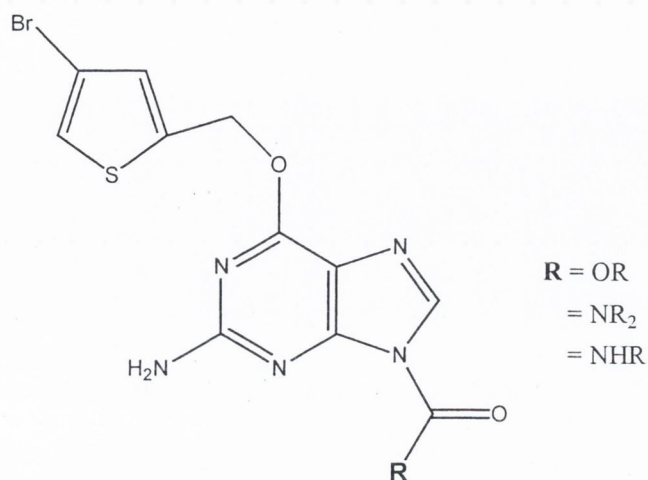


Fig. 6.4

The preparation of such derivatives presented us with a challenge since, to our knowledge, no purine carbamates or ureas were known. We have carried out a comprehensive investigation of routes and reagents for the synthesis and isolation of carbamate and urea type prodrugs of PaTrin-2. The development of satisfactory procedures for the preparation of such prodrugs has allowed us to synthesize a large number of compounds within each of the three categories.

In Chapter 2 we discussed the use of activated carbonic acid derivatives for the preparation of N-9 alkoxy carbonyl derivatives of PaTrin-2. We found that procedures involving bis(4-nitrophenyl) carbonate gave the preferred route to the synthesis of such derivatives, while in cases where this failed, the use of chloroformates provided an alternative route. It was also found that the reaction of PaTrin-2 with 4-nitrophenyl esters provided a regio-selective route to N-9 derivatives. No N-7 isomers were detected in these reactions.

Our investigation provided a general approach to the synthesis of alkoxy carbonyl derivatives. However, as discussed in Chapter 3, although it was possible to prepare

disubstituted N-9 carbamoyl derivatives ($\mathbf{R} = \text{NR}_2$, see Fig. 6.4) using a similar approach, we were more limited in the type of activated reagent employed. We found that carbamoyl chlorides were the best reagents in the preparation of such N-9 carbamoyl derivatives. Although we used them to synthesize all prodrugs of this type, separation problems in one case rendered this route impractical. This demonstrated the importance of having an alternative procedure. The reaction of PaTrin-2 with a carbamoyl imidazolium salt derived from *N,N'*-carbonyldiimidazole was comparatively slow and incidentally yielded the quaternary salt of a PaTrin-2 derivative. We had prepared the corresponding free base by the standard procedure (using the carbamoyl chloride), but could not isolate it from the reaction mixture.

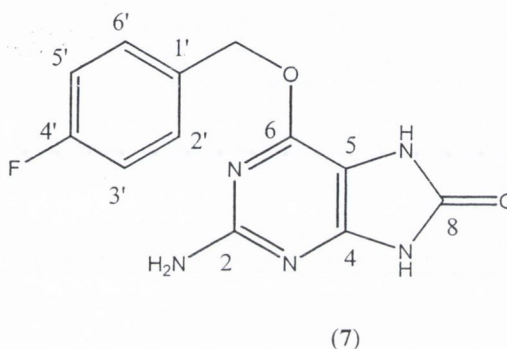
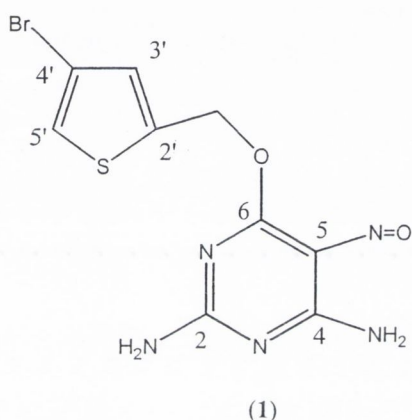
In Chapter 4 we reported the usefulness of isocyanates in making possible the synthesis of monosubstituted N-9 carbamoyl derivatives ($\mathbf{R} = \text{NHR}$, see Fig. 6.4). Although isocyanates were the preferred reagents for speed and simplicity, a different procedure involving bis(4-nitrophenyl) carbonate proved useful in cases where this method failed.

Although there is nothing novel about the general synthesis of carbamates and ureas, incorporation of the purine ring system into such derivatives often requires unique reaction conditions. The problems encountered in reactions involving PaTrin-2 have been discussed in detail, and we have attributed the unpredictable behaviour of PaTrin-2 to the steric and electronic effects of the purine ring system. We have proposed that the susceptibility of certain PaTrin-2 derivatives to decomposition is associated with these unique electronic effects, which make the PaTrin-2 anion an excellent leaving group. We have found that the success of many reactions involving the PaTrin-2 anion and an activated reagent is often dependent on the degree of steric hindrance about the site. We have shown that a comprehensive investigation of routes

and reagents is both necessary and useful in permitting the synthesis of almost any derivative of this type.

6.3 Experimental[♦]

Numbering system followed for 6-(4-bromophenoxy)-2,4-diamino-5-nitrosopyrimidine (**1**) and *O*⁶-(4-fluorobenzyl)-8-oxoguanine (**7**):



2-Amino-6-(4-bromophenoxy)-5-nitroso-4-(octyloxycarbonylamino)pyrimidine (3)

Octyl chloroformate (22 μ l, 0.11 mmol) was added to a solution of 6-(4-bromophenoxy)-2,4-diamino-5-nitrosopyrimidine (33 mg, 0.1 mmol) and pyridine (8.3 μ l, 0.1 mmol) in DMF (0.2 ml). After stirring at 90 $^{\circ}$ C for 5 min and cooling, methanol was added gradually to the reaction mixture and **(3)** (32 mg, 66%), a blue solid, precipitated.

ν_{max} : 1616, 1667, 1758(C=O) cm^{-1}

λ_{max} : 249, 287, 347nm

m.p. decomp. > 78 $^{\circ}$ C

[♦] For experimental details see Chapter 2, Section 2.13, pgs. 122-123.

$\delta^1\text{H}$ (400MHz, DMSO): 0.85 (3H, br s, CH_3), 1.27 (10H, br s, 3'', 4'', 5'', 6'', 7''- CH_2), 1.64 (2H, br s, 2''- CH_2), 4.10 (2H, t, $J=6.5$, 1''- OCH_2), 5.78 (2H, s, 6- OCH_2), 7.41 (1H, s, 3'- CH), 7.74 (1H, s, 5'- CH), 8.76 (2H, d, $J=20.0$, NH_2), 12.28 (1H, s, 4-NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 13.9 (CH_3), 22.1 (7''- CH_2), 25.2 (3''- CH_2), 28.0 (2''- CH_2), 28.5 (5''- CH_2), 28.6 (4''- CH_2), 31.2 (6''- CH_2), 62.3 (6- OCH_2), 65.7 (1''- CH_2), 108.0 (4'- C), 125.7 (5'- CH), 131.5 (3'- CH), 133.5 (4- C), 139.4 (2'- C), 150.8 (2- C), 163.0 (5- C), 180.0 (6- C), 195 ($\text{C}=\text{O}$).

Found: C, 43.35; H, 5.26; N, 14.62 %. $\text{C}_{18}\text{H}_{24}\text{BrN}_5\text{O}_4\text{S}\cdot 0.5 \text{H}_2\text{O}$ requires C, 43.64; H, 5.09; N, 14.14 %

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{18}\text{H}_{25}\text{BrN}_5\text{O}_4\text{S}$ 486.0811, Found 486.0825.

*O*⁶-(4-Fluorobenzyl)-7-(octyloxycarbonyl)-8-oxoguanine (9) – NaH (60% in oil, 16 mg, 0.4 mmol) was added to a solution of *O*⁶-(4-fluorobenzyl)-8-oxoguanine (110 mg, 0.4 mmol) in DMF (4 ml). The mixture was stirred for 5 min and octyl chloroformate (78 μl , 0.4 mmol) was added. After 1 h, the solvent was evaporated, and the N-7 isomer (9) was isolated (40 mg, 23%) by preparative thick layer chromatography (5% EtOH in DCM).

ν_{max} : 1722 (8- $\text{C}=\text{O}$), 1764 ($\text{C}=\text{O}$) cm^{-1}

λ_{max} : 256, 299nm

m.p. 150 $^{\circ}\text{C}$

$\delta^1\text{H}$ (400MHz, DMSO): 0.84 (3H, br s, CH_3), 1.26 (8H, br s, 4'', 5'', 6'', 7''- CH_2), 1.41 (2H, br s, 3''- CH_2), 1.67 (2H, m, 2''- CH_2), 4.28 (2H, t, $J=6.5$, 1''- CH_2), 5.41 (2H, s, 6- OCH_2), 6.34 (2H, s, NH_2), 7.20 (2H, t, $J=8.8$, 3', 5'- CH), 7.54 (2H, dd, $J=8.8$, 5.5, 2', 6'- CH), 11.0 (1H, br s, 9-NH).

$\delta^{13}\text{C}$ (100MHz, DMSO): 13.9 (CH_3), 22.1 ($7''\text{-CH}_2$), 25.1 ($3''\text{-CH}_2$), 28.0 ($2''\text{-CH}_2$), 28.5 ($5''\text{-CH}_2$), 28.6 ($4''\text{-CH}_2$), 31.2 ($6''\text{-CH}_2$), 66.1 (6-OCH₂), 67.1 ($1''\text{-CH}_2$), 98.0 (5-C), 115.1 (3', 5'-CH), 130.4 (2', 6'-CH), 132.7 (4'-C), 148.4 (C=O), 149.2 (4-C), 152.8 (1'-C), 158.5 (8-C=O), 163.1 (2-C), 163.4 (6-C).

$\delta^{19}\text{F}$ (376MHz, DMSO): -114.7

Found: C, 56.78; H, 5.99; N, 15.65 %. $\text{C}_{21}\text{H}_{26}\text{FN}_5\text{O}_4\cdot\text{H}_2\text{O}$ requires C, 56.12; H, 6.28; N, 15.58 %

MS (ES) $[\text{M}+\text{H}]^+$ Calcd m/z for $\text{C}_{21}\text{H}_{27}\text{FN}_5\text{O}_4$ 432.2047, Found 432.2043.

6.4 References

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APPENDIX 1

Glossary

Amino acid

Organic molecule containing both an amino group and a carboxyl group. Those that serve as the building blocks of proteins are α -amino acids, that is, the amino group is on the α -carbon atom, the one adjacent to the carbonyl group. There are twenty α -amino acids commonly found in proteins; glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), serine (Ser), threonine (Thr), cysteine (Cys), methionine (Met), proline (Pro), phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), glutamine (Gln), lysine (Lys), arginine (Arg) and histidine (His).

Antimetabolites

Drugs structurally similar to the natural components of certain important biochemical pathways in a cell. They are incorporated into these biochemical pathways and usually lead directly or indirectly to the inhibition of DNA or RNA synthesis.

Antineoplastic

Against neoplasm (an abnormal tissue growth which can be either benign or malignant).

Apoptosis

Form of cell death (also known as programmed cell death), in which a 'suicide' program is activated within the cell leading to fragmentation of DNA, shrinkage of

the cytoplasm, membrane changes and cell death without lysis or damage to neighbouring cells.

Carcinogen

Any agent that causes cancer.

Cyclin-dependent

kinase

(CDK) A protein kinase that has to be complexed with a cyclin protein in order to act. Different CDK-cyclin complexes trigger different steps in the cell division cycle by phosphorylating specific target proteins.

The so-called CDK4 is known to have increased activity in a wide variety of solid tumours and this may be associated with the overexpression of the protein cyclin D1. Inhibition of CDK4 should block entry into the cell cycle.

Cytotoxic

Destructive to living cells. The term is applied particularly to a class of drugs that inhibit cell division, and therefore are used to destroy cancer cells.

Cytochrome P450

Is a mitochondrial oxidase involved in the hydroxylation of steroids in the adrenal cortex. Also may be found on smooth endoplasmic reticulum (*e.g.* of liver cells) and is involved in the metabolism of xenobiotics.

Differentiation

Process by which a cell undergoes a change to an overtly specialized cell type.

Eukaryote

Organism composed of one or more cells with a distinct nucleus and cytoplasm.

<i>Gene</i>	Region of DNA that controls a discrete hereditary characteristic usually corresponding to a single protein or RNA. (From Greek; <i>genos</i> = parentage or origin)
<i>Glutathione</i>	Tripeptide composed of glutamic acid, cysteine and glycine. Functions as a coenzyme in some enzymes and is an important antioxidant in the biochemical defense reactions of cells.
<i>Hypoxia</i>	A deficiency of oxygen in body tissues which can result from living in an oxygen deficient environment.
<i>I₅₀</i>	The concentration of inactivator required to give a 50% reduction in enzyme activity.
<i>Proliferation</i>	The growth or reproduction of a tissue by active cell division. (From Latin; <i>proles</i> = offspring, <i>ferre</i> = to bear)
<i>Messenger RNA</i>	(mRNA) Type of single-stranded ribonucleic acid molecule arising from genetic transcription of DNA. The nucleotide sequence of mRNA is complementary to the DNA strand and is translated into the sequence of amino acid residues in protein synthesis.
<i>Metastasis</i>	Spread of cancer cells from their site of origin to other sites in the body.
<i>Microfilaria</i>	A minute larval stage of parasitic filarial worms living in the blood and tissues of a host animal. (From Latin; <i>filum</i> = thread)

Mismatch Repair

(MMR) DNA repair process that corrects mismatched nucleotides inserted during DNA replication. A short stretch of newly synthesized DNA including the mismatched nucleotide is removed and replaced with the correct sequence with reference to the template strand.

Mitosis

The division of the nucleus of a eukaryotic cell, resulting in two nuclei which are genetically identical with each other and with the mother nucleus. (From Greek; *mitos* = a thread; referring to the threadlike appearance of the condensed chromosomes)

Mutagen

Any physical or chemical agent which increases the number of mutations above the spontaneous background level. (From Greek; *gennan* = to produce)

Mutation

A sudden random change in the genetic material of a cell that may cause it and all cells derived from it to differ from the normal type.

Neoplasm

Abnormal tissue growth that can be either benign or malignant.

Oncogene

Typically a mutant form of a normal gene (proto-oncogene) involved in the control of cell growth or division and is capable of transforming a normal cell into a cancerous cell.

Prokaryote

Single-celled microorganism whose cells lack a well-defined membrane-enclosed nucleus.

<i>Protein</i>	Natural polymer composed of amino acid units joined one to another by amide (or peptide) bonds.
<i>Protein Kinase</i>	Enzyme that transfers the terminal phosphate group of ATP to a specific amino acid of a target protein.
<i>Proto-oncogene</i>	Gene that controls the growth, differentiation and proliferation of the cells under its control.
<i>Ribosome</i>	Particle composed of ribosomal RNAs and ribosomal proteins that associates with mRNA and catalyses the synthesis of protein.
<i>RNA</i>	(Ribonucleic acid) Polymer of ribonucleotide monomers which is concerned with protein synthesis.
<i>Topoisomerase</i>	Types I and II; are enzymes that stepwise catalyse the breaking of DNA, the transfer of a DNA segment to this break and the reseal of the DNA break. By doing this, these enzymes alter the linking number of DNA, which is defined as the number of times one DNA strand winds around another.
<i>Transcription</i>	Copying of one strand of DNA into a complementary RNA sequence by the enzyme RNA polymerase.
<i>Translation</i>	Process by which the sequence of nucleotides in a strand of mRNA directs the incorporation of amino acids into protein. Process occurs on a ribosome.

Tumour suppressor

gene

Gene that codes for enzymes that control DNA transcription, DNA repair and other (mainly) nucleus-located functions.

Tyrosine Kinase

A protein kinase that plays an important role in signal transduction pathways regulating a number of cellular functions such as cell growth, differentiation and cell death.

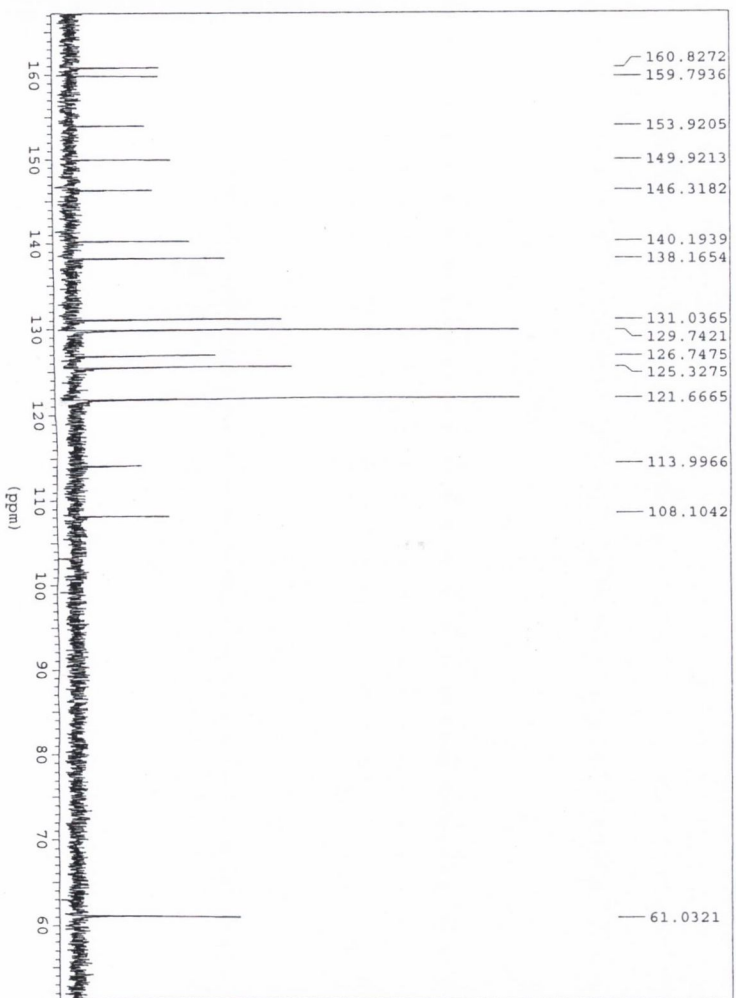
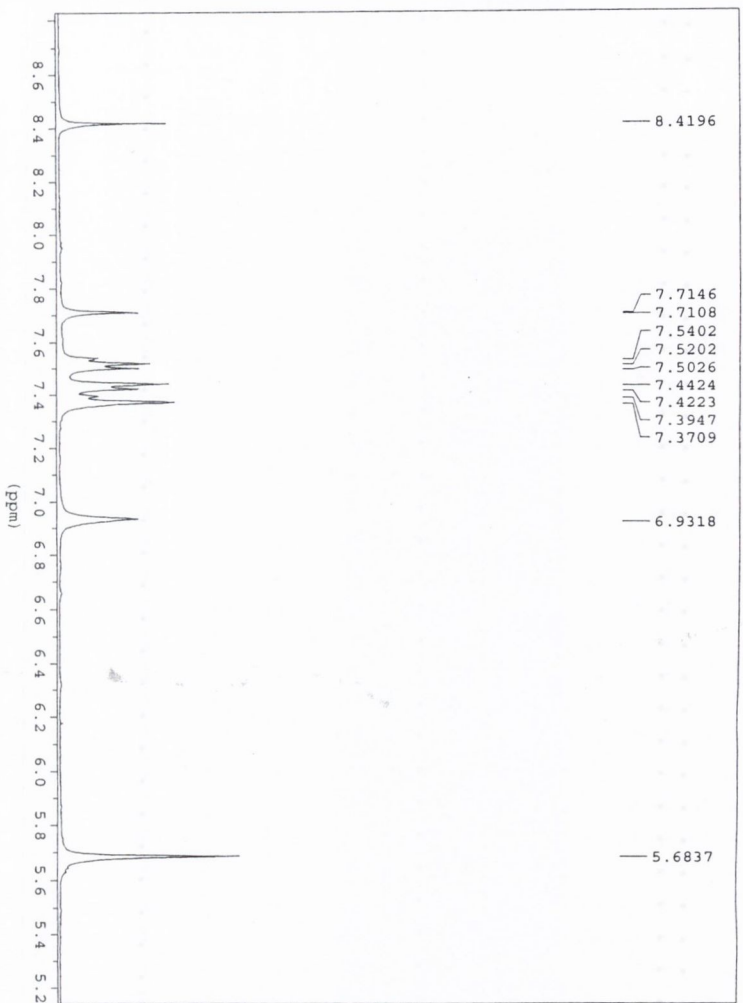
Chronic myeloid leukaemia (CML) is characterized by the presence of an abnormal chromosome (the Philadelphia chromosome). Patients with CML produce a unique fusion tyrosine kinase. This kinase transfers a phosphate from ATP to substrate proteins. These phosphorylated substrates activate a series of intracellular pathways that are involved in malignant transformation. Thus, this tyrosine kinase is a target for rational drug design.

Xenobiotics

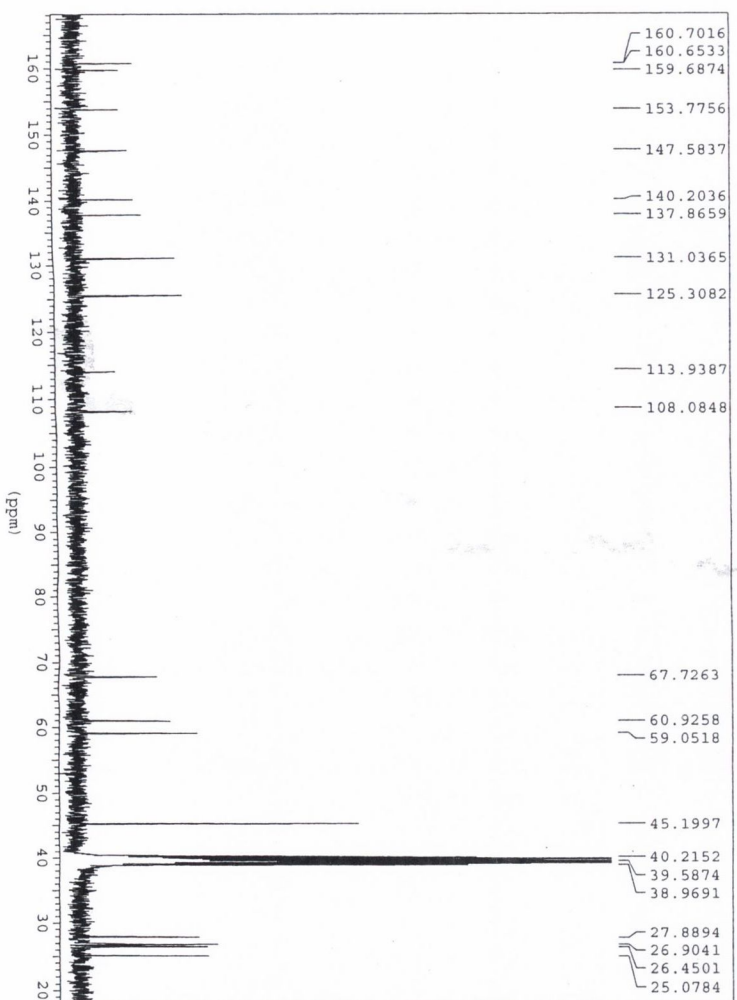
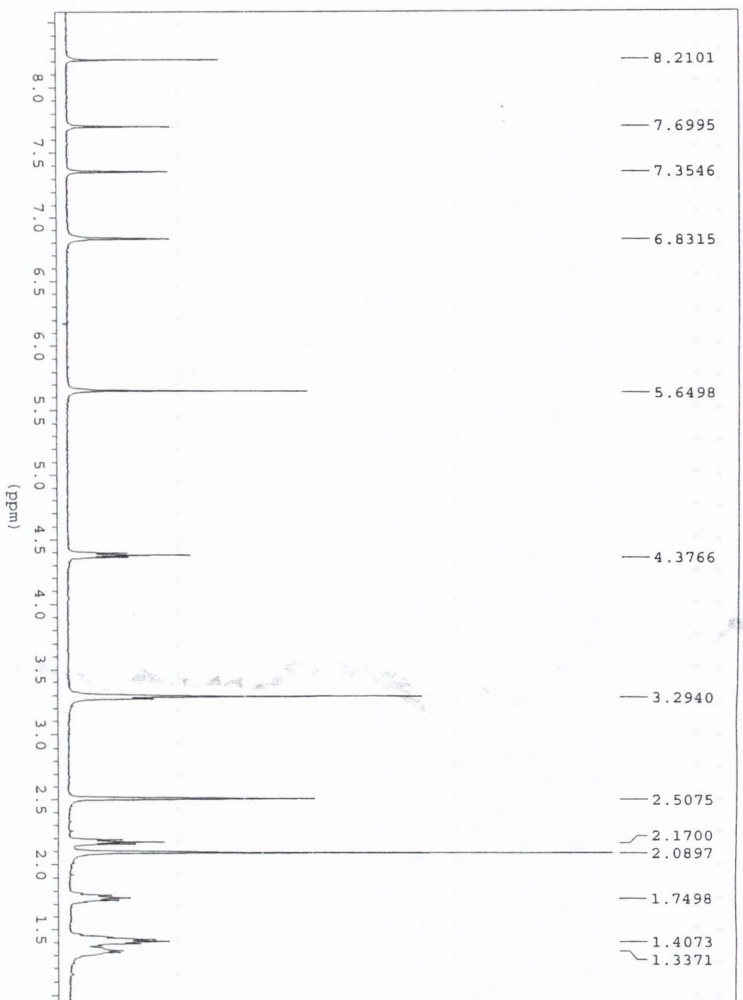
Chemicals foreign to organisms.

APPENDIX 2

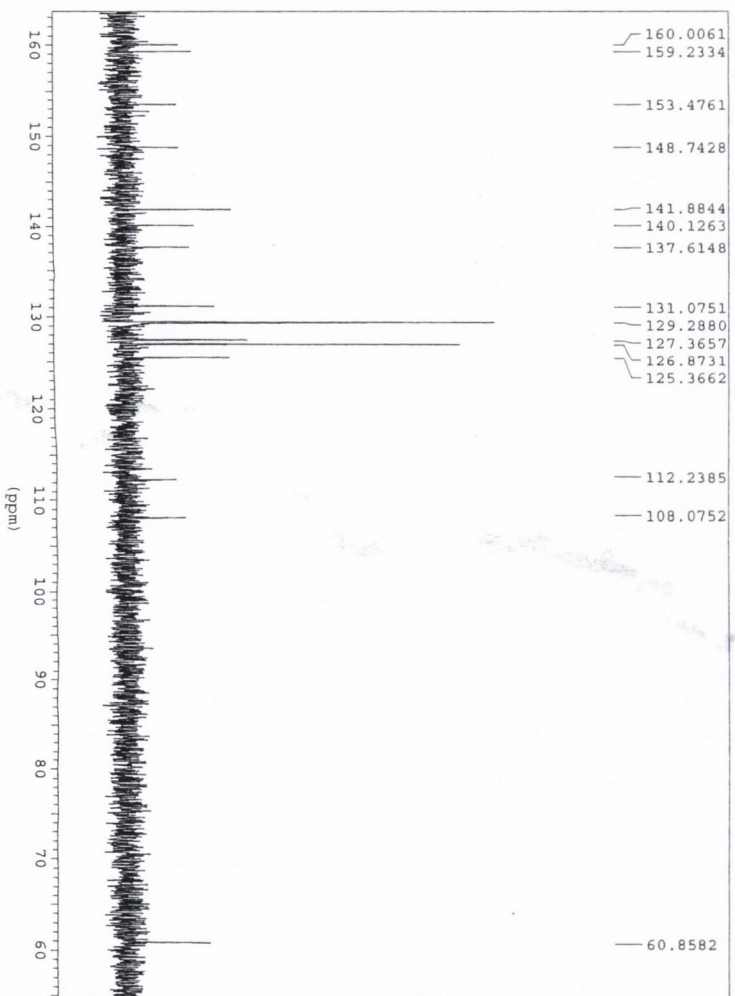
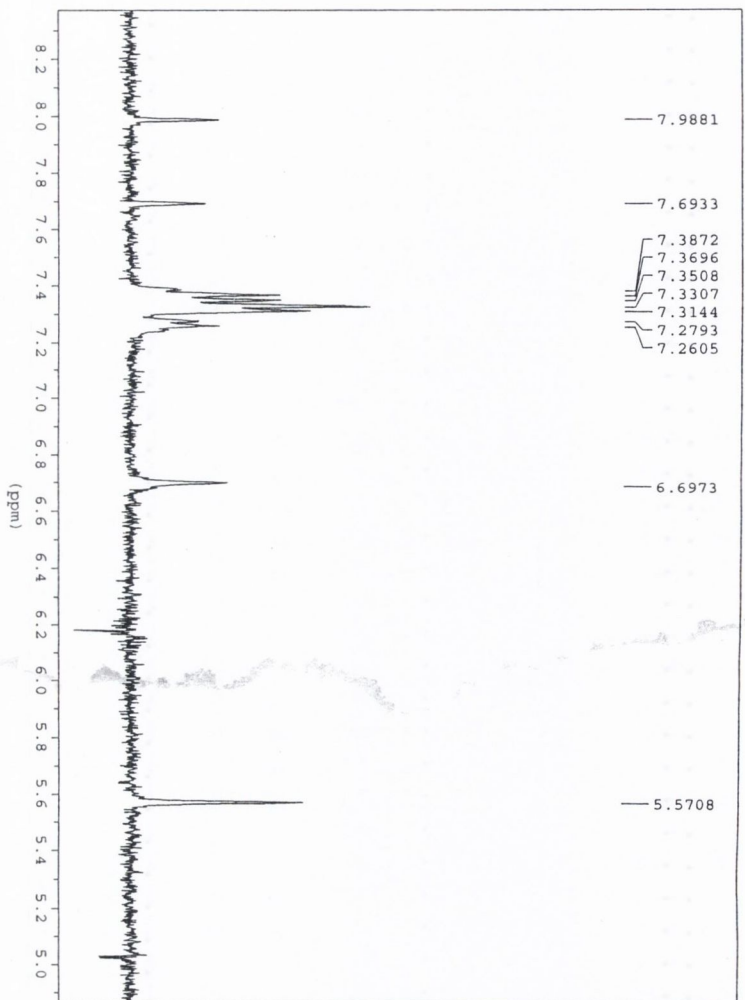
O⁶-(4-Bromophenyl)-9-(phenoxy carbonyl)guanine



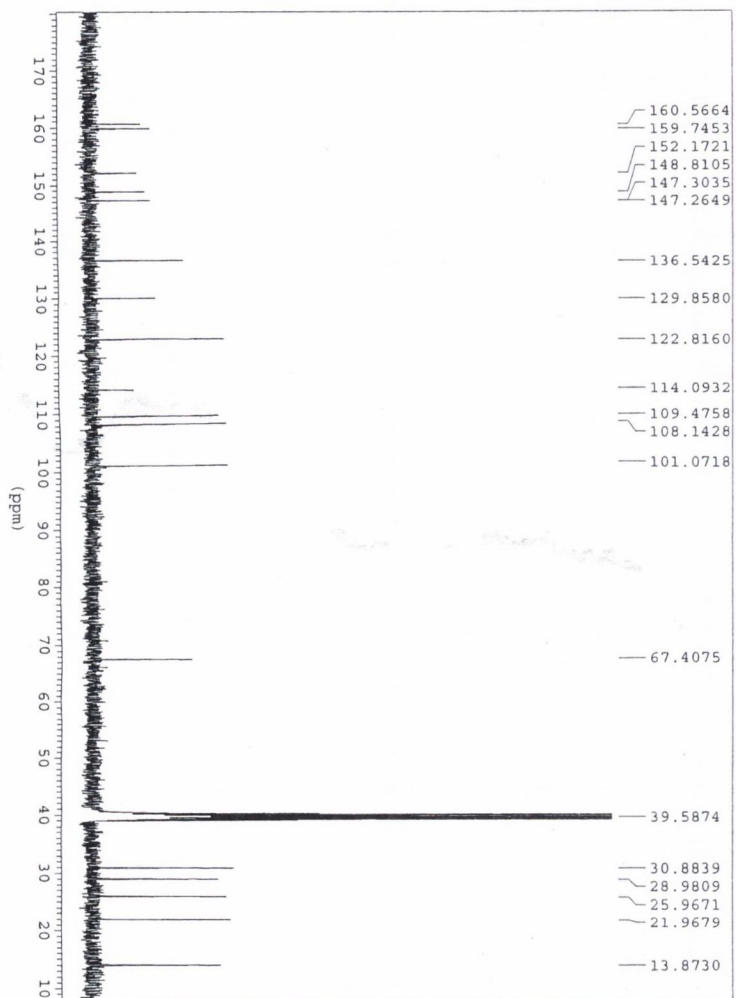
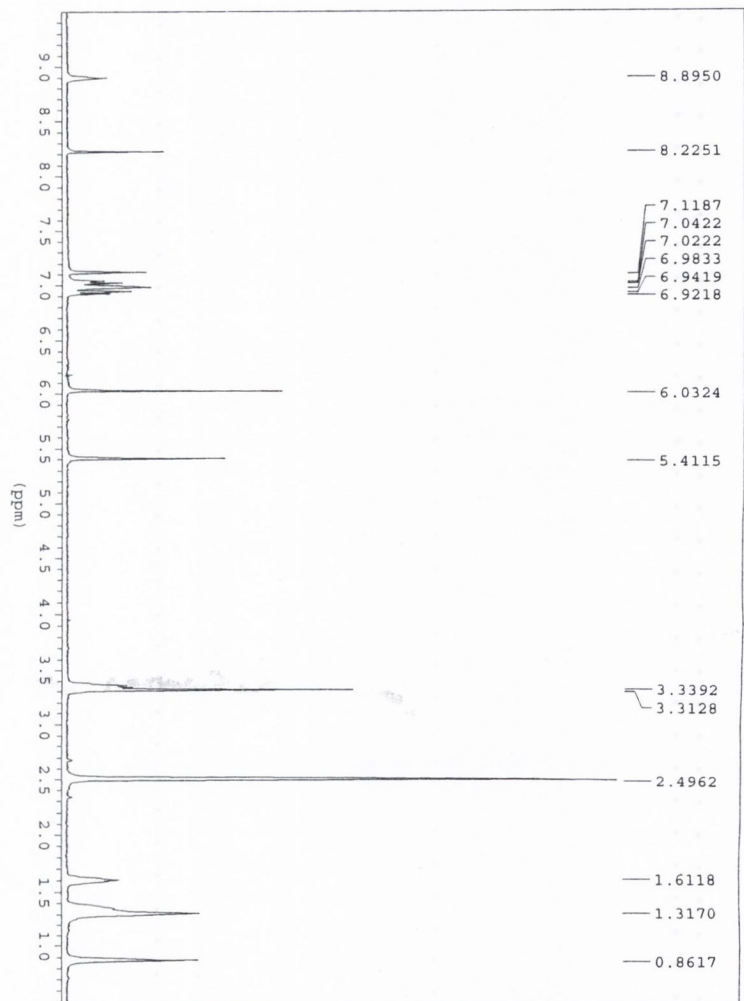
O⁶-(4-Bromophenyl)-9-(6-dimethylaminohexyloxycarbonyl)guanine



O⁶-(4-Bromophenyl)-9-(N,N-diphenylcarbamoyl)guanine



9-(N-Hexylcarbamoyl)-O⁶-piperonylguanine



9-(N-Hexylcarbamoyl)-O⁶-(4-methylthiobenzyl)guanine

