ATP MIMICS AS GLUTAMINE SYNTHETASE INHIBITORS

—AN

EXPLORATORY SYNTHETIC STUDY

THESIS

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SHERIFF TOMILOLA SALISU

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ABSTRACT

Using a mechanism-based approach to drug discovery, efforts have been directed towards developing novel ATP mimics that can act as GS inhibitors. The purine-based systems, adenosine, adenine and allopurinol, were identified as possible scaffolds for potential ATP mimics, while various meta-disubstituted benzenoid compounds, 3-aminobenzonitrile, 3-aminophenol, resorcinol, 3-aminobenzyl alcohol, 3-hydroxybenzoic acid and 3-aminobenzoic acid have been explored as adenine analogues.

These compounds were treated with different alkylating and acylating agents. Allylation of all the substrates was achieved using allyl bromide and N-9 alkylation of protected allopurinol was effected using a number of specially prepared Baylis-Hillman adducts. Acylation of the benzenoid precursors with chloroacetyl chloride, acetoxyacetyl chloride, acryloyl chloride and specially prepared 2,3,4,5,6-pentaacetylgluconoyl chloride afforded the corresponding mono- and/or diacylated products in varying yields (4-96%). Elaboration of the alkylated and acylated products has involved the reaction of hydroxy systems with diethyl chloro phosphate and chloro derivatives with triethyl phosphite in Arbuzov-type reactions to afford phosphorylated products.

In all cases, products were fully characterized using 1- and 2-D NMR analysis and, where appropriate, high-resolution mass spectrometry. The application of Modgraph and ChemWindow NMR prediction programmes has been explored and the resulting data have been compared with experimental chemical shift assignments to confirm chemical structures and, in some cases, to establish the position of allylation or acylation. Experimental assignments were found to be generally comparable with the Modgraph data, but not always with the ChemWindow values.

The docking of selected products in the ‘active-site’ of GS and their structural homology with ATP, both in their free and bound conformations have been studied using the ACCELERYS Cerius² platform. All the selected ATP mimics exhibit some form of interaction with the ‘active-site’ residues, and a number of them appear to be promising GS ligands.
ABBREVIATIONS

\(^1\text{H}\) Proton NMR spectroscopy
\(\text{CH}_2\text{Cl}_2\) Dichloromethane
\(\text{CHCl}_3\) Chloroform
COSY Correlation spectroscopy (\(^1\text{H}-^1\text{H}\))
DABCO 1,4-Diazabicyclo[2.2.2.]octane
DEAD Diethylazodicarboxylate
DMF \(N,N'\)-Dimethylformamide
DHPA Dihydroxypropyl Adenine
DMSO Dimethyl sulfoxide
EtOAc Ethyl acetate
EtOH Ethanol
HMBC Heteronuclear multiple bond coherence (\(^1\text{H}-^{13}\text{C}\))
HPMPA 9-[2-Hydroxy-3-(phosphonomethoxy)propyl]adenine
HSQC Heteronuclear single quantum coherence (\(^1\text{H}-^{13}\text{C}\))
TBAF Tetrabutylammonium fluoride
THF Tetrahydrofuran
1. INTRODUCTION

1.1. Tuberculosis

Tuberculosis (TB) is a deadly disease that has killed millions of people for a very long time.\textsuperscript{1,2} It primarily affects the lungs, though it does affect other parts of the body, being characterized by the formation of tiny lumps otherwise referred to as tubercles in the affected part of the body.\textsuperscript{3} \textit{Mycobacterium tuberculosis} \textit{M.tb.}, a tubercle bacillus is the organism that causes TB.\textsuperscript{4,5} Infected individuals with pulmonary TB transmit the disease when they cough, sneeze or talk by propelling many bacilli into the air. Hence, inhalation of the airborne bacilli by healthy people may lead to infection.\textsuperscript{3,6,7} Approximately 1.6 million people fell casualty to this preventable disease in 2005 with the highest mortality rates in the poorest parts of the world. The World Health Organization (WHO) TB/HIV Working Group was established to formulate policies directed at combating the deadly combination of TB and HIV/AIDS.\textsuperscript{7}

1.1.1. History of tuberculosis in man

Archaeological evidence, art and literature strongly suggest that TB has been plaguing mankind from time immemorial. As a result of its widespread nature, the disease has been known by many different names. Some of these include: \textit{pulmonary TB}, described as lung sickness, white plague or consumption; \textit{Lupus vulgaris,} otherwise referred to as skin TB; and \textit{white swelling, i.e.} TB of the bones. Famous people that have fallen prey to TB include Voltaire (philosopher), Walter Scott (poet and novelist), Anton Chekov (playwright), Niccolo Paganini (violinist) and George Orwell (writer).\textsuperscript{1,8}

1.1.2 History of Tuberculosis Therapy

It was not until the 16\textsuperscript{th} century that tuberculosis was recognized as a contagious disease. The 19\textsuperscript{th} century witnessed the establishment of sanatoria in different parts of the world to cater for TB patients. These were treatment institutions located in the
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countryside where TB patients were believed to be cured by being exposed to fresh air, eating a healthy diet, exercising and resting under strict supervision. However, there is no scientific evidence that the sanatoria reduced the menace of TB. In the same century, organizations around the world, such as the National Association for the Prevention of Consumption and other forms of TB (NAPT) in the UK and the National Tuberculosis Association (NTA) in the USA, were also established to curb the menace of the then incurable and deadly disease.1,9

In 1890, an Italian physician Forlanini invented pneumothorax (otherwise referred to as lung collapse) therapy. This method involved compressing the part of the diseased lung(s) and then filling the pleural cavities with nitrogen through a needle. An extreme form of the method was thoracoplasty, which involved the removal of ribs from one side of the thorax to accomplish a permanent collapse of the diseased part of the lung; an obvious side effect was shortness of breath as a result of the decreased lung space.1

The ability to view the tubercle bacillus in 1882, which was made possible by the staining technique of Robert Koch10 and the invention of X-rays by Wilhelm Roentgen in 1889,1 gave a boost to the fight against TB. It was then realized that TB is not inherited as previously believed, but that transmission occurs from diseased to healthy individuals through the mycobacterium and that prevention is possible.1,3

1.1.2.1. Bacillus Calmette-Guerin BCG

BCG, a potent vaccine against TB, was developed10 in 1924 by two French scientists, Calmette and Guérin, from an attenuated strain of mycobacterium bovis. Though not particularly effective in preventing infection, BCG has been administered in areas with high TB prevalence and incidence. It has been impossible to judge the efficacy of BCG because the original stocks were not kept. Scientists are working on making a better vaccine that can prevent TB following the successful mapping of the M.tb. genome in 1997.11,12,13,14
1.1.2.2. The antibiotic era in tuberculosis treatment

In a method invented in 1885 by Cantani, an Italian bacteriologist, patients inhaled nonpathogenic bacteria so as to reduce the level of tuberculosis bacilli in the sputum. It was discovered in 1888 by Babes, a Romanian scientist, that products of certain *Gram positive* and *Gram negative* bacteria could inhibit the growth of *m.tb*.

Sanocrysin, a double thiosulphate of gold and sodium introduced by Holger Mollgaard in 1925 was used as an anti TB drug in 1925, but was discontinued because of its high toxicity. Streptomycin was first used for a human patient in 1944, but by 1947, *M. tb* had begun to show resistance to streptomycin. The search continued to find drug(s) that could treat the disease or be used together with streptomycin. In 1949 *para*-aminosalicyclic acid (PAS) became available and, in combination with streptomycin, afforded better treatment for TB.

![Chemical structures](image)

A new drug Isoniazid [INH] was discovered in 1951 and, in combination with PAS, proved to be more effective than the combination of streptomycin and PAS.
Introduction

Other drugs made to treat TB include pyrazinamide 4 (1954), cycloserine 5 (1955), ethambutol 6 (1962), and rifampicin 7 (1963).

\[ \text{Pyrazinamide} \]
\[ \text{Cycloserine} \]
\[ \text{Ethambutol} \]
\[ \text{Rifampicin} \]

1.1.3 The problem of drug resistance in TB chemotherapy

Although the pathogen \textit{M. tb}. does manage to evade the host-protective response in man’s alveolar macrophages to cause active TB disease in some infected individuals, chemotherapy has been effective in curing the disease. However, TB continues to be a major threat to life as a result of drug resistance to chemotherapy. Drug-resistant TB, otherwise referred to as multidrug resistant TB (MDR-TB), is defined as resistance to INH 3 and RIF 7, whether there is resistance to other antitubercular drugs or not. Extreme drug resistant TB (XDR) is caused by a new strain of \textit{M. tb}. and is not curable even with the current available combination therapy that effectively treats MDR-TB. Resistance to anti-TB agents is usually a consequence of poorly administered TB treatment. Mycolic acids such as compound 8 are essential components of the cell wall of the pathogen that causes TB. INH 3 is believed to act by inhibiting the synthesis of mycolic acids by mimicking nicotinamide 9 and being incorporated instead, thereby impairing the activity of the enzymes that require the latter.
However, replacement of nicotinamide as the natural substrate, as a consequence of mutation in the *katG* and *inhA* genes of the bacteria, led to strains that are resistant to INH. Similar mechanisms of resistance are encountered with other antitubercular drugs.

1.1.4. Current TB treatment

Both latent TB infection and active TB disease with the exception of XDR are curable with combination therapy. Latent TB infection can be diagnosed with the aid of the tuberculin skin test, while active TB, which may be drug-susceptible or drug-resistant, can be diagnosed by chest X-ray, sputum smear, culture tests, polymerase chain reaction (PCR) and the recently developed *M. tb*. direct test (MTD). The WHO and the International Union Against Tuberculosis and Lung disease (IUATLD) have formulated treatment regimens for all forms of TB plaguing society today, although there may be minor and major adverse reactions.

First-line TB drugs such as isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin suffice for the treatment of latent TB infection and drug-susceptible TB disease. However, treatment of drug-susceptible TB with combination therapy may take up to 18 months, and resistance often develops as individuals find it difficult to maintain their medication for such a long time. The mechanism of action of INH is not fully understood. However, INH has been observed to be bacteriostatic as it
stops replication of the mycobacterium. It is also bactericidal against semi-dormant populations as *M.* *tb.* loses its acid-fastness after being exposed to INH, which may be due to the drug interfering with the cell wall of the pathogen. New research has suggested that INH is activated by the *katG* enzyme, which exhibits catalase-peroxidase activity. The isonicotinoyl radical 14 or perisonicotinic acid 16 (Figure 1) are very reactive intermediates that may result from action of the *katG* enzyme on INH leading to the formation of isonicotinaldehyde 10, isonicotinic acid 11 and isonicotinamide 12. It has been proposed that these activated species from the oxidation of INH with the *katG* enzyme impair the activity of *inhA*, an enzyme that plays a vital role in the biosynthesis of the cell wall of *M.* *tb.*

![Proposed mechanism](image)

**Figure 1.** Catalase-peroxidase reaction of INH when administered.

Isonicotinic hydrazones 17 and isonicotinic hydrazides 18 are derivatives of INH that have been found to be less potent than INH. When resistance or intolerance to first-line TB agents occurs, second-line TB drugs are administered. These drugs, which are expensive, toxic and usually accompanied by severe side effects, include ethionamide 19, PAS 2, cycloserine 5, kanamycin 20 and fluoroquinolones 21.49
Ethionamide 19, a second line TB drug has been suggested to have a similar mechanism of action as INH. Ethionamide 19 is believed to be oxidized to ethionamide sulfoxide 22 by catalase-peroxidase, which impairs the activity of the \textit{inhA} enoyl reductase enzyme by acylating the protein at the Cys 243 residue (Figure 2), thus inhibiting the formation of compound 24. In addition to demonstrating activity against \textit{M.tb.}, fluoroquionolones are active against other mycobacteria such as \textit{M. xenopi} and \textit{M. avium-intracellulare}. Quinolones have the advantage of being active at low concentrations as well as having lower side effects when administered. It has been reported that non-fluorinated quinolones are not active against mycobacteria. However, quinolone derivatives 25, such as the piperazine analogues,
the pyrrolidine analogs and the 8-methoxy derivative, moxifloxacin 26, show better activity than the earlier quinolones.\textsuperscript{51,52}

![Chemical structures and reactions](image)

**Figure 2.** Mechanism of action of ethionamide.

It is unfortunate that XDR-TB has defied cure even by second-line TB drugs. A recent 98% mortality was recorded within 25 days in patients, including those on a nti-
retrovirals in KwaZulu-Natal, afflicted with this new strain. Hence, there is an urgent need to combat TB with a new approach, such as the target-based drug design.

1.1.5. The synergism between HIV/AIDS and tuberculosis

The HIV/AIDS pandemic is a major factor in the resurgence of the global TB epidemic. HIV weakens the immune system permitting the progression of latent TB infection to active TB disease in HIV positive individuals. Hence TB is the leading cause of death of HIV infected people. Research has also shown that extrapulmonary TB and MDR-TB are more common in people with compromised immune systems. The lethal combination of HIV and TB is also complicated by the difficulty in diagnosing TB in HIV-positive individuals. However, it is interesting to note that the treatment and prevention of TB using standard regimens prescribed by the WHO and IUATLD are effective in both HIV-positive and HIV-negative patients. The exception is that people taking protease inhibitors for HIV should substitute rifabutin for rifampin.

1.1.6. WHO TB controls

The WHO has adopted a practical approach to combat the TB epidemic. The Direct Observed Therapy (DOT) strategy seeks to ensure compliance by the patient through observation by a family member or health worker. The DOTS (directly observed therapy, short course) strategy, which is the main strategy adopted by the WHO and the IUATLD for effective TB control, consists of five elements: “Government commitment to a national tuberculosis programme as a specific health-system activity, integrated into comprehensive primary care, and supported technically at a national level; standardized, directly observed, short-course treatment prioritizing sputum smear-positive patients; case detection by means of a patient-friendly and clinically-efficient service based primarily on smear microscopy (passive case-finding); an ensured supply of all essential anti-tuberculosis drugs; and effective monitoring.”
1.2. Random drug discovery and rational drug design

1.2.1. Random drug discovery

Traditionally, different naturally occurring substances from plants, microbes, marine flora and fauna, arachnids and amphibians\textsuperscript{62,63} are explored for their potency to act as cures for different ailments. This often random, broad-screening process involves looking for direct evidence of a physiological reaction in animal tests. As a consequence, biological, chemical and clinical practice has produced considerable data that has guided pharmaceutical drug research.\textsuperscript{64,65,66,67} In many cases, of course, examination of the constituents of materials used as traditional medicines led to the isolation of active principles. For example, cocaine \textsuperscript{27}\textsuperscript{68} has local anesthetic properties and is extracted from the leaves of \textit{Erythroxylon coca}, while quinine \textsuperscript{28}\textsuperscript{68} is an antimalarial obtained from the bark of \textit{Cinchona} species.

![Chemical structures of cocaine and quinine](image)

The need to reduce or eliminate side effects as well as improve the potency of natural drugs prompted the development of derivatives of these natural substances. Knowledge of synthetic chemistry, medicinal chemistry and pharmacology as well as imagination was needed to relate drug discovery to synthetic practice. Hence, medicinal chemists usually try to make active compounds (otherwise referred to as ‘leads’) by elaborating or mimicking known potent substances to develop better drugs.\textsuperscript{69} Immediate examples are the hipolipidemics, Compactin \textsuperscript{29}\textsuperscript{65} and Pravastatin \textsuperscript{30}.\textsuperscript{65} Procaine \textsuperscript{31}\textsuperscript{68} and benzocaine \textsuperscript{32}\textsuperscript{68} are drugs that were synthesized to block the nerve activity like cocaine, but without the side-effects that lead to the abuse of
caine. Primaquine 33 and chloroquine 34 are two quinine analogues that became antimalarial standards for over five decades.68

Today, random drug discovery has been made much easier with the availability of high through-put screening and combinatorial chemistry using computer-controlled robotic systems. Hence, hundreds to thousands of compounds can be synthesized simultaneously and their preliminary bioassays conducted much more rapidly than in the past. The success of random screening depends on a good bioassay system for the pharmacological action of interest.69 One major criticism of random drug discovery is
that it is not guided by any specific biochemical theory of why some compounds work while others do not.\(^{66}\)

1.2.2. Enzyme Inhibition and Rational drug design

Advances in molecular biology have afforded a better understanding of therapeutic targets at the molecular level.\(^{53}\) Hence, it is now understood that the therapeutic targets of many medicinal agents extracted from natural products are specific enzyme systems. The bark of the willow tree, for example, has been known to have analgesic and antipyretic capabilities; the active ingredient, salicin, a glycoside, is metabolized \textit{in vivo} to salicylic acid \textbf{35}, which inhibits the formation of the prostaglandins that mediate pain and fever by inhibiting the enzyme, cyclooxygenase.\(^{70}\) Physostigmine \textbf{36}, a substance that is extracted from the West African Calabar bean, gives relief to glaucoma patients by inhibiting the enzyme, acetylcholinesterase, and, as a consequence, improving drainage and decreasing intra-ocular pressure.\(^{70}\)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{compound_images.png}
\caption{Structures of compounds \textbf{35} and \textbf{36}.}
\end{figure}

The antibacterial, prontosil \textbf{37}, inhibits the bacterial synthesis of folic acid \textbf{46} as a result of being metabolized into sulfanilamide \textbf{38} (Figure 3), a \textit{para}-aminobenzoic acid mimic.\(^{71}\) \textit{Para}-aminobenzoic acid \textbf{42} is an essential metabolite in the synthesis of tetrahydrofolic acid which, in turn, is vital in the \textit{in vivo} production of purines. The \textit{para}-aminobenzoic acid mimic, sulfanilamide \textbf{38}, selectively inhibits the bacterial enzyme, dihydropteroate synthase (Figure 4) production of dihydrofolic acid \textbf{45}.\(^{72}\) Dihydrofolic acid \textbf{45} is an important intermediate in the bacterial biosynthesis of folic acid (46). The rational approach to drug discovery has become more sophisticated with advances in molecular biology and information technology.\(^{73}\) Hence, modern drug discovery is often based on knowledge of the enzyme receptor.\(^{74,75}\) With a good understanding of the structure of a ligand and its receptor, it may be possible to propose molecules as lead compounds that will mimic the activity of a natural ligand.
Figure 3. Metabolic reduction of prontosil to sulfanilamide.

The combinatorial synthesis of lead analogues, high through-put screening, high-field NMR analysis, X-ray crystallography, molecular modelling, quantum mechanical calculations and quantitative structure activity relationship (QSAR) data may all contribute to the development of a rationally designed drug. However, it is important to note that drug discovery is a long and difficult process in which the design of an inhibitor is only the first step. The pharmacokinetic profile of the inhibitor, its toxicities and side effects and animal and pre-clinical studies are all hurdles the inhibitor has to cross before it can be entered into clinical studies as a new drug candidate. Ritonavir and saquinavir, peptidomimetic inhibitors of both HIV-1 and HIV-2 proteases, as well as zanamivir (a therapeutic for influenza) are examples of products of the rational approach to drug discovery. Perhaps rational drug design will provide a solution to the resurgence of the TB pandemic, especially with the evolution of XDR-TB as a result of resistance to the currently available treatment regimen for MDR-TB.
Figure 4. Inhibition of bacterial folic acid biosynthesis by sulfanilamide.
1.3. Glutamine synthetase GS

The survival of the *M. tb.* pathogen in the host to cause TB disease depends on its ability to protect itself in a kind of capsule that prevents its destruction in the phagocytic cells of human macrophages. Access to the cell is restricted and this capsule also determines what components are released into the host’s tissues. Research has shown that this pathogenic mycobacterium releases extracellular proteins that are vital for its replication and virulence in the host’s tissues. The enzyme, glutamine synthetase (GS), is one of these released proteins and it has been shown to be essential for the formation of the cell wall of *M. tb.* As well as catalyzing a number of other reactions, GS is an enzyme that is essential for nitrogen metabolism in living organisms. Different types of GS are present in man and bacteria. Man, like other eukaryotes, expresses GS II while *M. tb.* like other prokaryotes express GS I [otherwise referred to as (MTB-GS)]. The modes of regulation of GS I and GS II are different. Furthermore, pathogenic extracellular MTB-GS has been selectively inhibited by L-methionine-S-sulfoximine (MetSox) and by phosphinothricin. Hence, MTB-GS can be a therapeutic target.

1.3.1. Structure and function of MTB-GS and GS II

MTB-GS is a very large enzyme consisting of 12 identical subunits of two face-to-face hexameric rings forming a dodecamer of mass 640 kDa. Hydrogen bonding and hydrophobic interactions hold the dodecamer together. The junction between each pair of subunit forms an active site. There are thus 12 active sites and each of them is in the form of a bifunnel. The top of the bifunnel is the ATP binding site, while the bottom is the glutamate binding site. At the neck of the bifunnel are two divalent cation (Mn$^{2+}$ or Mg$^{2+}$) binding sites (referred to as n1 and n2, respectively) separated by 6Å. The n1 cation is essential in glutamate binding while the n2 cation is involved in phosphoryl transfer. The adenylylation loop is one of several loops with different functional importance resulting from the structure of the enzyme (Figure 5). GS II exists as an 8-subunit oligomer with a polypeptide chain of approximately 372 residues. Although there are similar residues in the active sites of GS I and GS II, performing similar functions, the adenylylation loop is absent in GS I. Glutamine is
an essential source of nitrogen in the biosynthesis of many organic compounds, such as purines, pyrimidines and other amino acids in living systems. GS catalyzes the biosynthetic reaction (Scheme 1) that produces glutamine 52 as the product of the combination of glutamate 50 and ammonia in the presence of adenosine triphosphate (ATP). GS activity is dependent on the n2 cation. The coordination of the n2 cation with the γ-phosphate oxygens of ATP permits phosphoryl transfer to the γ-carboxylate group of glutamate leading to the formation of the γ-glutamyl phosphate intermediate 51.88 Glutamine 52 is formed when ammonia displaces the phosphoryl group as a free phosphate anion.94

Figure 5. Trace display of reconstructed GS from *Salmonella typhimurium* downloaded from the PDB showing ADP in the ATP binding site and AMP in the adenylylation site. Downloaded at http://www.hort.purdue.edu/rhodcv/hort640c/ammonia/GSSi3D.htm
Scheme 1. Biosynthetic and transferase reactions

The transferase reaction is the reverse of the biosynthetic reaction, in which glutamine \textbf{52} is converted to $\gamma$-glutamylhydroxymate and ammonia in the presence of hydroxylamine.\textsuperscript{88,95}

\textbf{1.3.2. Regulation of MTB-GS: Feedback inhibition and covalent modification}

Allosteric control or feedback inhibition\textsuperscript{96,97} of MTB-GS occurs when there is sufficient glutamine in the system. A change in conformation at the relevant active site of the enzyme occurs so that other ligands, known as ‘effectors’, bind to the ATP and glutamate active sites.\textsuperscript{96} Crystal structures of GS have revealed allosteric regulation by serine, glycine and alanine at the glutamate substrate site, while GDP, ADP and AMP bind allosterically to the ATP site.\textsuperscript{88,98} MetSox \textbf{53} has been demonstrated to be a potent inhibitor of GS as it mimics glutamate and binds non-covalently but irreversibly with ATP at the glutamate binding site forming a tetrahedral intermediate.\textsuperscript{88,99}
Covalent modification by adenylylation,\textsuperscript{100,101,102,103} is another means of regulating the catalytic activity of GS I. An ATP dependent reaction, adenylylation, inactivates GS when ATP binds covalently to tyr\textsuperscript{397} \textbf{54} on GS I as (AMP-O-Tyr\textsuperscript{397}) \textbf{55} instead of the n1 metal ion in response to excess nitrogen in the cell (Figure 6). Each monomer on MTB-GS can be adenylylated and, as more sites are adenylylated, the catalytic activity of GS and the amount of glutamine produced both decrease. The reverse process, called deadenylylation, is the phosphorolytic removal of AMP as ADP. Both processes are catalyzed by adenylyl transferase (AT).\textsuperscript{104} Adenylylation does not occur in GS II due to the absence of the adenylylation loop.\textsuperscript{88} Consequently, this mode of regulation of GS I represents a therapeutic target.\textsuperscript{105} Efforts have been made to synthesize ATP mimics that will bind irreversibly to the relevant residues in the adenylylation site, preventing deadenylylation and the multiplication of mycobacterium in the host, which is man.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Covalent modification by adenylylation as a means or regulating MTB-GS.}
\end{figure}

Research has also shown the selective adenylylation of pathogenic MTB-GS in mixture of MTB-GS and nonpathogenic \textit{mycobacterium bovis} (BCG) on addition of glutamine.\textsuperscript{80} An enzymatic mechanism detailing the role of each residue in the bifunnel active site of GS during the biosynthetic reaction has been proposed and is summarized in Table 1.\textsuperscript{88}
Table 1: A summary of the mechanism of the biosynthetic reaction showing the role of each residue. Tyr is not in the bifunnel but lies in the adenylylation loop and is absent in GS II. Extracted from D. Eisenberg, H.S. Gill, G.M.U., Pfluegl, S.H. Rotstein, *Biochimica et Biophysica Acta*, 2000, **1477**, 122-145. Reproduced with permission from D. Eisenberg.

<table>
<thead>
<tr>
<th>Residue</th>
<th>Role in enzymatic mechanism</th>
<th>Effector</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp-50</td>
<td>Deprotonates ammonium substrate ion. Increases the affinity for ammonium binding</td>
<td>ADP</td>
<td>78, 106, 107</td>
</tr>
<tr>
<td>Ser-53</td>
<td>Increases intersubunit stability with Glu-327</td>
<td>ADP</td>
<td>78</td>
</tr>
<tr>
<td>Asp-64</td>
<td>Increases intersubunit stability with Arg-344</td>
<td>ADP</td>
<td>108</td>
</tr>
<tr>
<td>Glu-129</td>
<td>Coordinates the n2 ion and hydrogen bonds with His-271</td>
<td></td>
<td>78</td>
</tr>
<tr>
<td>Glu-131</td>
<td>Coordinates the amino group of glutamate and the n1 ion</td>
<td></td>
<td>78</td>
</tr>
<tr>
<td>Tyr-179</td>
<td>Coordinates the ammonium binding pocket</td>
<td></td>
<td>78, 106</td>
</tr>
<tr>
<td>Glu-212</td>
<td>Coordinates the ammonium binding pocket and the n1 ion</td>
<td></td>
<td>78</td>
</tr>
<tr>
<td>Glu 220</td>
<td>Coordinates the n1 ion</td>
<td></td>
<td>78</td>
</tr>
<tr>
<td>Asn-264</td>
<td>Coordinates the amino group of glutamate. Stabilizes the Glu-327 flap upon MetSox or PPT binding</td>
<td>Glu, Gln, Ser, Gly, Ala, TI, PPT, MetSox</td>
<td>78, 109</td>
</tr>
<tr>
<td>Gly-265</td>
<td>Coordinates the amino group of glutamate.</td>
<td></td>
<td>78</td>
</tr>
<tr>
<td>His-269</td>
<td>Coordinates the n2 ion</td>
<td></td>
<td>110, 111</td>
</tr>
<tr>
<td>His-271</td>
<td>Coordinates the α-phosphate group of ADP/AMPPMP and Glu-129</td>
<td></td>
<td>108</td>
</tr>
<tr>
<td>Arg-321</td>
<td>Coordinates the carboxylate of glutamate</td>
<td></td>
<td>78</td>
</tr>
<tr>
<td>Glu-327</td>
<td>Stabilizes the tetrahedral adduct at the transition state. Accepts a proton from the adduct to form glutamine. Closes active site and shields intermediate from hydrolysis.</td>
<td>Ser, TI, PPT, MetSox</td>
<td>112, 109, 78, 106, 107</td>
</tr>
<tr>
<td>Arg-339</td>
<td>Induces intersubunit stability by interacting with Asp-50</td>
<td>ADP</td>
<td>78</td>
</tr>
<tr>
<td>Arg-344</td>
<td>Coordinates the β-phosphate group of ADP/AMPPMP</td>
<td>ADP</td>
<td>78</td>
</tr>
<tr>
<td>Glu-357</td>
<td>Coordinates the n2 ion and Arg-344</td>
<td>ADP, AMPPMP</td>
<td>78</td>
</tr>
<tr>
<td>Arg-359</td>
<td>Coordinates the γ-phosphate group of glutamate</td>
<td></td>
<td>78</td>
</tr>
<tr>
<td>Tyr-397</td>
<td>Adenylylation site (seen in bacterial GS only)</td>
<td></td>
<td>113</td>
</tr>
</tbody>
</table>
1.4. The purine system

Many systems, of which ATP is one, containing the purine backbone are biologically important. DNA and RNA, the “building blocks of life”, are made of purine and pyrimidine bases. Hormones and neurotransmitters are made up of nucleosides and nucleotides, while energy transfers occurring in many metabolic systems as well as intercellular signaling are a consequence of the interconversion of mono-, di- and triphosphate esters of nucleosides. Pyrimidines are six membered heterocycles with two nitrogen atoms while purines are fused pyridine and imidazole heteroaromatic compounds with four nitrogen atoms.

Purine can alternate between the four tautomeric forms shown in Scheme 5. It exists in the 7-H tautomer in the crystalline state, while the 7- and 9- tautomers are predominant in solution.

Scheme 2. Tautomerism in purine

Adenine, adenosine and adenosine triphosphate ATP are examples of aminopurines.
1.4.1. Adenine

Adenine 61 is a molecule of important biological significance as it is a constituent of RNA and DNA\textsuperscript{114} as well as being an important component of ATP, a substance that is required to drive many biochemical reactions.\textsuperscript{124}

1.4.1.1. Synthesis and reactions of adenine

Adenine 61 has been synthesized in 43\% yield by treating formamide with POCl\textsubscript{3} at 120 °C in a closed vessel (Scheme 3).\textsuperscript{125}

\[ H\text{CONH}_2 + \text{POCl}_3, 120 \degree C \rightarrow \overset{43\%}{\text{H}_2\text{NCONHN}_3} \]


Alkylation and acylation can be carried out on the amino group on the pyrimidine ring as well as the nitrogen of the imidazole ring after deprotonation by a base. Synthesis of the diastereomeric acyclic nucleoside (65a and 65b) and nucleotide (74a and 74b) mimics have been reported according to schemes 4 and 5.\textsuperscript{126} α,β-Methylfuranoside 67a was obtained by methylating (+)-ribose with methanol in the presence of HCl. Tosylation of methylated ribose with tosyl chloride in pyridine at 4°C afforded the 5-tosylated derivative 68a in 90\% yield. In the presence of NaH, compound 68a was
coupled to adenine under anhydrous conditions to afford compound \(69a\). Oxidation of \(69a\) with sodium periodate at 4°C for one hour afforded the dialdehyde \(70a\) that was treated with aq. \(\text{NaBH}_4\) to afford the target compound \(65a\) in 85% yield. Synthesis of the stereoisomeric analogue \(65b\) in 15-30% yield was achieved with \((-)\)-ribose using the same approach.

\[
\begin{align*}
65a & \quad \text{Ade} \quad \text{OH} \quad \text{OMe} \\
65b & \quad \text{Ade} \quad \text{OH} \quad \text{OMe} \\
74a & \quad \text{Ade} \quad \text{OH} \quad \text{HOPO} \quad \text{OH} \\
74b & \quad \text{Ade} \quad \text{OH} \quad \text{HOPO} \quad \text{OH}
\end{align*}
\]

**Scheme 4.** Synthesis of adenine nucleosides \(65a\). Reagents and conditions: (i) AcCl/MeOH, rt, 16h; (ii) TsCl/Py, 4°C, 16h; (iii) Ade/DMF, 80°C, 5 days; (iv) \(\text{NaIO}_4\), 4°C, 1h; (v) \(\text{NaBH}_4\), 4°C, 3h.
The nucleotide 74b was made from the nucleoside 65b. Treatment of 65b with 60% formic acid or Dowex 50 (H⁺) afforded (R)-DHPA 71b. The iodomethylphosphonate 72b was obtained in 35% yield by reacting 71b with 1.5 eq. of iodomethylphosphonic acid in the presence of DCC in pyridine. Intramolecular cyclisation of the iodomethylphosphonate to afford intermediate 73b was achieved by treating 72b with 5 eq. of NaH in DMF. Hydrolysis did afford pure 73b; however, the nucleotide (R)-cyclo-HPMPA 74b was isolated in 90% yield after treating crude 73b with glacial acetic acid for 3 h. The corresponding (S)-DHPA 71a was made from nucleoside 65a affording access to (S)-cyclo-HPMPA 74a.126

**Scheme 5.** Synthesis of adenine nucleosides 74a and 74b. Reagents and conditions: (i) H⁺, 6h; (ii) ICH₂P(O)(OH)₂/DCC/Py, 16h; (iii) NaH/DMF, 6h; (iv) H₂O, 3 h; (v) glacial AcOH, 3h.

1.4.2. Adenosine

Adenosine is a nucleoside with a ribose sugar attached to the imidazole ring via an N-glycosidic bond.127 A molecule of great biological significance,128 adenosine exerts different physiological effects on the nervous, immune and cardiovascular systems by activating A₁, A₂A, A₂B, and A₃ receptors. Hence, heart muscles, platelets as well as coronary arteries depend on adenosine for their proper function. Adenosine prevents aggregation of platelets and modulates the immunity of the cell, through interaction with A₂A receptors.129,130 Research on blood cells has shown that adenosine and its
mimics may have a number of important roles, such as: (i) regulating normal immune responses; (ii) acting as anti-inflammatory and endogenous anti-aggregatory agents and (ii) as therapeutics. Research has also shown that adenosine, as a neurotransmitter, stimulates A₁ receptors in the brain thereby inducing sleep. Interestingly, sleep time is decreased when coffee is consumed because blockage of the central adenosine receptors is effected by the methylxanthines in coffee.

In the calf adenosine deamination reaction, adenosine is converted to inosine (Scheme 6) via the hemi-aminal. Enzyme-catalysed reactions such as the deamination reaction proceed very rapidly in the active site of the enzyme. However, transition-state mimics bind more strongly to the active site of the enzyme forming structurally and chemically similar structures to the transition state. Such stable intermediates provide information on the design of better inhibitors, and are thus very important in the mechanistic approach to drug discovery.

Scheme 6. The calf adenosine deamination reaction.

Nucleosides such as adenosine have been synthesized by various routes. Stemming from the therapeutic properties of natural hexopyranosyl nucleosides, the synthesis of ring-expanded mimics of adenosine has afforded many compounds with antiviral and anticancer activity. The asymmetric synthesis of the nucleoside was recently reported by researchers at West Virginia University, USA (Scheme 7). The synthesis commenced by protecting the two hydroxyl groups on commercially available furfuryl alcohol in a two-step reaction to afford the Boc-protected pyranone. The protected alcohol was then coupled to 6-chloropurine in the presence of a palladium catalyst to afford the glycosylated 6-chloropurine pyranone in 86%
yield. The alcohol 80 was obtained by exposing the pyranone 79 to NaBH4 at -78 °C. Conversion of the alcohol to the olefin 81 was achieved with the reagent system (NBSH, PPh3/DEAD, NMM). The diol 82 was obtained enantioselectively by dihydroxylating the olefin 81 with (OsO4/NMO). Treatment of the diol 82 with ammonia at room temperature gave the amino derivative 83. The desired hexopyranosyl nucleoside 84 was finally obtained in 94% yield by deprotection of the diol with TBAF at room temperature. This procedure can be extended to the synthesis of other compounds such as adenosine.

Scheme 7. Synthesis of the hexopyranosyl nucleoside 84: (i) 0.5% Pd2(dba)3.CHCl3, 2.5%PPh3, THF, 0 °C, 86%; (ii) NaBH4, MeOH/CH2Cl2, -78 C; (iii) NBSH, PPh3, DEAD, NMM, -30 C; (iv) OsO4, NMO, 95%; (v) NH3, MeOH, 77%; (vi) TBAF, THF, rt, 94%.

1.4.3. Adenosine triphosphate ATP

Adenosine triphosphate 6381,82,83,84 consists of an adenine base, a ribose sugar and a phosphoryl ester group (Figure 7). The adenine base in ATP is linked to the ribose
sugar via a glycosidic bond which, in turn, is linked to phosphate groups through a phosphoester linkage. The phosphate groups are linked by phosphoanhydride linkages.\textsuperscript{123}

![Diagram of ATP showing glycosidic, phosphoester, and phosphoanhydride linkages.](image)

**Figure 7.** Adenosine triphosphate showing the glycosidic, phosphoester and phosphoanhydride linkages.

ATP \textit{63} is a nucleotide formed (Scheme 8) as a result of the successive addition of phosphate groups to adenosine monophosphate (AMP) \textit{85}. AMP is itself the produced by the esterification of adenosine \textit{62} with phosphoric acid (Scheme 8).\textsuperscript{141}

![Scheme 8. Synthesis of ATP.](image)
ATP is one of the most important molecules in living systems. Referred to as “energy currency”, the energy released from hydrolysis of ATP is used to drive many biochemical reactions that are otherwise not thermodynamically feasible. ATP plays a very important role in mediating endergonic anabolic and exergonic catabolic processes as shown in Figure 8.\(^{142}\)

![Figure 8. Coordination of anabolic and catabolic processes by ATP.](image)

ATP is able to drive many of these biochemical reactions because of the high negative free energy that accompanies its hydrolysis. Hydrolysis of ATP is a spontaneous reaction since ATP is less stable than the hydrolysis products, adenosine diphosphate (ADP) and free phosphate which exert mutual electrostatic repulsion as a result of both being negatively charged; the terminal phosphoryl group in ATP is not resonance-stabilized as effectively as the inorganic free phosphate anion (Figure 9).\(^{142}\)

![Figure 9. Resonance stabilisation of the terminal phosphoryl group in ATP and free inorganic phosphate.](image)
1,3,7-Trimethylxanthine (caffeine) 87 and theobromine 88 are natural stimulants present in coffee and chocolate respectively. Synthetically, both natural products may be obtained by methylating xanthine with a reagent like methyl iodide.\textsuperscript{143}

![Chemical structures of 1,3,7-Trimethylxanthine (87) and Theobromine (88)]

However, a more complicated methylation process is involved \textit{in vivo}. Each of the phosphate groups and the methylene carbon of the sugar moiety in ATP can be attacked by hard and soft nucleophiles, respectively. A typical biochemical reaction in living systems is the nucleophilic attack by the amino acid methionine 89 to form \textit{S}-adenosyl methionine (SAM) 90 (Scheme 9) in an \textit{S}\textsubscript{N}2 displacement\textsuperscript{144} of the triphosphate moiety. SAM is a highly reactive methylating agent and, in plants, caffeine 88 is produced by SAM-mediated methylation of theobromine 88 (Scheme 10).\textsuperscript{144}

![Scheme 9. Methylation of ATP by methionine](image)

\textbf{Scheme 9. Methylation of ATP by methionine}
Introduction

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

Scheme 10. Formation of caffeine via methylation of theobromine by SAM

1.4.4. Other purine systems

1.4.4.1. Medicinal uses of purine systems

Purine systems with medicinal potential include: - (i) dideoxynosine (DDI) 91, a therapeutic agent for AIDS; (ii) acyclovir 92, an antiviral agent for Herpes; and (iii) 6-mercaptopurine 93 that is administered to cancer and leukemia patients.\(^{145}\)

\[
\begin{align*}
91 & \\
92 & \\
93 & \\
94 & \\
\end{align*}
\]

Allopurinol 94 is used for patients afflicted with gout,\(^{146}\) while Sildenafil\(^{146}\) (Viagra\(^{\text{TM}}\)) 95 is an effective therapeutic for impotence. Oxanosine 96 and tubercidin 97 are natural products, obtained from Streptomyces, and are used for treating cancerous and bacterial infections.\(^{146}\)
1.4.4.2. Synthesis of purine systems

Various routes to purine derivatives have been developed. In the Traube synthesis, 8-substituted purines 100 can be obtained from di-aminopyrimidines 98 and an appropriate acylating agent as shown in Scheme 11.\textsuperscript{147,148} The reaction proceeds via the amide 99 that is isolable.

\begin{center}
\includegraphics[width=\textwidth]{Scheme_11.png}
\end{center}

**Scheme 11.** Synthesis of 8-substituted purines.

The 8,9-dimethylated xanthine derivative 104 has been synthesized successfully from the 5-aminimidazole 102 that was generated \textit{in situ} as outlined in Scheme 12.\textsuperscript{149}

\begin{center}
\includegraphics[width=\textwidth]{Scheme_12.png}
\end{center}

**Scheme 12.** Synthesis of dimethylated xanthine.

It is also possible to synthesize purines from the corresponding pyrimidine and imidazole derivatives in a cycloaddition reaction as outlined in Scheme 13.\textsuperscript{150}

\begin{center}
\includegraphics[width=\textwidth]{Scheme_13.png}
\end{center}

**Scheme 13.** Synthesis of purines from pyrimidines and imidazoles.
1.4.4.3. Synthesis of Viagra

The pyrazole precursor 109 used in the synthesis of Viagra (Scheme 14) can be obtained by treating the ester 108 with aq. hydrazine. Methylation at the N-1 nitrogen followed by nitration then affords the intermediate 111 the pyrazole equivalent of 5-aminoimidazole-4-carboxylic acid (AICA) derivatives which are the starting materials for the synthesis of purines from imidazole. Reduction of the nitro group in the intermediate amide 112 and treatment of the resulting amine 113 with an aromatic acid chloride affords the amide 114. Hydrolysis and cyclisation affords compound 115, which on treatment with chlorosulphonic acid and N-methylpiperazine affords Viagra 95.151


1.4.4.4. Reactions of purine systems

Alkylation of the nitrogen atoms in both the pyrimidine and imidazole moieties is possible. Thus the 7,9-dimethylpurinium salt 116 is the product of the reaction between purine 56 and iodomethane (Scheme 15).152
Scheme 15. Alkylation at nitrogen atoms in the imidazole ring of purine.

A mixture of 3,6-dimethylpurine and 6,9-dimethylpurine is obtained on methylating 6-methylpurine with dimethyl sulfate in alcoholic KOH (Scheme 16).\(^{153}\)

Scheme 16. Alkylation of nitrogen atoms in the pyrimidine and imidazole rings of purine.

Both the 7- and 9- substituted products (121 and 122 respectively) are produced in basic solution when 6-chloropurine is alkylated with a benzyl halide.\(^{154}\) However, substitution at N-9 (compound 123) results from the reaction of 6-chloropurine with a dihydrofuran-derived carbocation (Scheme 17).\(^{155}\)

Scheme 17. Substitution at the 7- and 9- position in 6-chloropurine.

Reductive alkylation of adenine with formaldehyde (Scheme 18)\(^{156}\) yields the monomethylated substitution product while treatment of adenine with the Mannich
product formed from the reaction of formaldehyde with morpholine (Scheme 19) affords the dimethylated derivative.\textsuperscript{157}

![Scheme 18. Alkylation of adenine at the NH$_2$ position with HCHO](image1)

![Scheme 19. Alkylation of adenine at the NH$_2$ position with Mannich product from morpholine and HCHO.](image2)
1.5. Aims of this research

With a detailed knowledge of the role of glutamine synthetase in pathogenic *Mycobacterium tuberculosis*, our goal has been to prepare novel ATP mimics that will bind to the active site of MTB-GS, thereby inhibiting normal enzymatic function. In general we have focused on establishing access to a series of structural scaffolds and, in some cases, their elaboration to potential ATP mimics.

More specifically, the research has involved:

(i) the synthesis and characterization of novel adenosine-, adenine- and allopurinol-based and truncated adenine derivatives;

(ii) the application of C-13 NMR shift prediction programmes to confirm experimental assignments; and

(iii) a study of the conformational preferences and receptor docking of potential ATP mimics into the MTB-GS active site using the ACCELERYS Cerius² platform.
2. DISCUSSION

The discussion will be focused on: - the syntheses of novel adenosine derivatives (Section 2.1), adenine derivatives (Section 2.2), allopurinol and Baylis-Hillman electrophiles (Section 2.3) and truncated systems (Section 2.4) as novel ATP analogues; the application of NMR prediction programme to confirm experimental data assignments (Section 2.5); computer modelling and docking of selected compounds into the GS active site (Section 2.6) and conclusions (Section 2.7)

ATP 63 comprises three parts: an adenine base, a ribose sugar and a phosphoryl ester group\(^\text{158,159}\). In our approach, each of these parts was altered in attempts to access a range of compounds as potential ATP mimics.

Adenosine triphosphate ATP

![ATP with proposed moieties for derivatives.](image)

**Figure 10.** ATP with proposed moieties for derivatives.
2.1. **Novel ATP analogues from adenosine**

The method employed was to replace the primary hydroxyl hydrogen of adenosine with various groups through acylation and alkylation reactions (Figure 11).

![Figure 11. General approach for the synthesis of adenosine derivatives.](image)

There are three hydroxyl groups on the ribose ring of adenosine and, although the primary hydroxyl group is more susceptible to acylation and alkylation, competition from the two secondary hydroxyl groups cannot be ruled out. However, the 1,2-dihydroxy groups can be selectively protected as a cyclic acetal. Acetals are not difficult to make, they are stable compounds and their removal does not generally pose any difficulty. Protection of the vicinal 1,2-diol was successfully achieved by treating adenosine with acetone in the presence of an acid catalyst following previous work in our research group by Hall. The method involved stirring adenosine in dry acetone in the presence of para-toluenesulfonic acid monohydrate as catalyst at room temperature overnight to produce adenosine 3',4'-acetonide in 93% yield (Scheme 20). The structure of the acetal obtained was confirmed by NMR and low-resolution mass spectroscopy. The two acetonide methyl singlets were observed at 1.22 and 1.38 ppm respectively in the $^1$H NMR spectrum (Figure 12); while the quaternary carbon of the acetal was found to resonate at 114.0 ppm in the $^{13}$C NMR spectrum (Figure 13).
Scheme 20. Protection of adenosine with acetone.

Figure 12. 400 MHz $^1$H NMR spectrum of adenosine 3',4'-acetonide 127 in CDCl$_3$. 
With the acetonide 127 in hand, a number of novel adenosine derivatives were synthesized in low to moderate yields by treating adenosine 3',4'-acetonide 127 with different alkylating and acylating agents\textsuperscript{164} following deprotonation of the primary hydroxyl group using either NaH or BuLi. These reactions afforded a set of primary substitution products which could then be elaborated further (Figure 14). In a number of cases the acylating agent had to be specially prepared.

Figure 13. 100 MHz $^{13}$C NMR spectrum of adenosine 3',4'-acetonide 127 in CDCl$_3$.  

C(CH$_3$)$_2$ at 114.0 ppm
2.1.1. Preparation of 2-(6-aminopurin-9-yl)-3,4-dihydroxy-5-[(2,3,4,5,6–penta-acetoxyhexanoyloxy)methyl]tetrahydrofuran 3,4-acetonide 132

The route to the pentaacetoxy derivative 132 is shown in Scheme 21. The required acylating agent, 2,3,4,5,6-pentaacetylgluconoyl chloride 131, was synthesized by stepwise acetylation of the primary and secondary hydroxyl groups in D-gluconolactone 128. In the first step, the lactone 128 was treated with acetic anhydride in the presence of ZnCl2 catalyst followed by hydrolysis with ice-water to open the lactone ring. In the second step, 2,3,4,6-tetraacetylgluconic acid 129 was treated with the same reagent and catalyst to afford 2,3,4,5,6-pentaacetylgluconic acid 130. Pentacetylation was confirmed by the series of signals integrating for the 15 acetyl protons in the $^1H$ NMR spectrum and 5 methyl signals and 6 carbonyl carbon signals in the $^{13}C$ NMR spectrum. An initial attempt to make the acid chloride by stirring 2,3,4,5,6-pentaacetylgluconic acid 130 with PCl5 in diethyl ether at room temperature was unsuccessful. However, 2,3,4,5,6-pentaacetylgluconoyl chloride 131 precipitated out as a white solid in 50% yield on stirring 2,3,4,5,6-pentaacetylgluconic acid 130 with oxalyl chloride under nitrogen at room temperature for ca. 0.5 h. 2,3,4,5,6-D-pentaacetylgluconic acid 130 melted at 142-144°C while the acid chloride
The adenosine acetonide 127 was treated immediately with the acid chloride 131, using pyridine as both solvent and base\(^{167,168}\) to obtain 2-(6-aminopurin-1-yl)-3,4-dihydroxy-5-[(2,3,4,5,6-pentaacetoxyhexanoyloxy)methyl]tetrahydrofuran 3,4-acetonide 132 as a yellow oil in 10% yield. Evidence for the formation of compound 132 was largely provided by NMR, mid-IR and mass spectrometric data.

**Scheme 21.** Synthesis of the acetylated derivative 132.

The amino nitrogen on the adenine ring was clearly not acylated in the isolated product as the NH\(_2\) proton signal integrates for 2 protons at 5.88 ppm in the \(^1\)H NMR spectrum (Figure 15). There is also no correlation between NH and gluconoyl C=O (C-1") in the HMBC spectrum (Figure 16).
Figure 15. 400 MHz $^1$H NMR spectrum of the acetylated derivative 132 in CDCl$_3$. 

NH$_2$ at 5.88 ppm
Figure 16. HMBC NMR spectrum of the acetylated derivative 132 in CDCl₃ showing the absence of any correlation between NH₂ and C-1''.

2.1.2. Preparation of 2-(6-aminopurin-1-yl)-3,4-dihydroxy-5-[(allyloxy)methyl]-tetrahydrofuran 3,4-acetonide 133

The O-allylated derivative 133 was expected to provide access to the diol 134 and the bis-phosphorylated derivative 136 as outlined in Scheme 22. On treating the acetonide 127 with NaH and allyl bromide in refluxing THF, the allylated derivative 133 was obtained in 9% yield. In the ¹H NMR spectrum (Figure 17), the 1''-methylene protons resonate as a pair of doublets at ca. 4 ppm; while the vinylic methylene protons resonate as overlapping multiplets at ca. 5.78 ppm, each of the allylic protons resonates as a dd (CHₐ at 4.98 ppm and CHₐ at 5.29 ppm respectively).

Scheme 22. Proposed route to the nucleotide 136.


**Figure 17.** 400 MHz $^1$H NMR spectrum of the allylated derivative 133 in CDCl$_3$.

Attempts to improve the efficiency of this reaction were made using different reaction conditions; the highest yield was 24% (Table 2). Unfortunately, attempts at dihydroxylating the allylated derivative 133 with various reagents including NaIO$_4$ in the presence of RuCl$_3$ catalyst, KMnO$_4$ and CTAP were unsuccessful.

**Table 2.** Yields of the allylated derivative 134 under various reaction conditions.

<table>
<thead>
<tr>
<th>Conc. of 134/[M]</th>
<th>Solvent</th>
<th>Base</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>THF</td>
<td>NaH</td>
<td>9.0</td>
</tr>
<tr>
<td>0.1</td>
<td>THF</td>
<td>NaH</td>
<td>21</td>
</tr>
<tr>
<td>0.01</td>
<td>THF</td>
<td>NaH</td>
<td>24</td>
</tr>
<tr>
<td>0.5</td>
<td>Pyridine</td>
<td>NaH</td>
<td>9.2</td>
</tr>
<tr>
<td>0.5</td>
<td>THF</td>
<td>Na</td>
<td>7.2</td>
</tr>
<tr>
<td>0.5</td>
<td>DMF</td>
<td>NaH</td>
<td>3.5</td>
</tr>
<tr>
<td>0.03</td>
<td>Acetone</td>
<td>$K_2CO_3$</td>
<td>0.0</td>
</tr>
</tbody>
</table>
2.1.3. Preparation of 2-(6-aminopurin-9-yl)-3,4-dihydroxy-5-(7-hydroxy-2,5-dioxaheptyl)tetrahydrofuran 3,4-acetonide 137 and 2-(6-aminopurin-9-yl)-5[7-diethylphosphoryloxy]-2,5-dioxaheptyl]-3,4-dihydroxytetrahydrofuran 3,4-acetonide 138

The deprotonated acetonide 127 was alkylated with 2-(2-chloroethoxy)ethanol in refluxing THF to afford 2-(6-aminopurin-9-yl)-3,4-dihydroxy-5-(7-hydroxy-2,5-dioxaheptyl)tetrahydrofuran 3,4-acetonide 137 in 72% yield (Scheme 23). In the $^1$H NMR spectrum (Figure 18), the methylene protons (7", 6", 4", 3" and 1"-Ha) resonate as a series of multiplets at ca. 3.6-3.8 ppm. FAB mass spectrometric analysis showed the presence of the M+1 peak at $m/z$ 396 with an intensity of 3% relative to the base peak at $m/z$ 136.

An initial attempt to phosphorylate compound 137 with tetrabutylammonium dihydrogen phosphate under reflux in an inert atmosphere of argon was not successful. However, 2-(6-aminopurin-9-yl)-5[7-diethylphosphoryloxy]-2,5-dioxaheptyl]-3,4-dihydroxy - tetrahydrofuran 3,4-acetonide 138 was successfully isolated pure on treating compound 137 with BuLi and diethyl chlorophosphate. The methylene protons (7", 6", 4", 3" and 1") now resonate at slightly higher chemical shifts ca. 4.0-4.3 ppm (Figure 18). The $^{31}$P signal is at 0.8 ppm (Figure 19) compared with 6.3 ppm in the diethyl chlorophosphate.

Figure 18. 400 MHz $^1$H NMR spectra of the alkylated derivative 137 and phosphonate 138 in CDCl$_3$. 
Figure 19. 162 MHz $^{31}\text{P}$ NMR spectrum of 138 in CDCl$_3$.

The mid-IR absorption spectrum of 2-(6-aminopurin-9-yl)-5[7-diethylphosphoryl-oxy]-2,5-dioxaheptyl]-3,4-dihydroxytetrahydrofuran 3,4-acetonide 138 shows a phosphate ester absorption band at 1028 cm$^{-1}$ (Figure 20).

Figure 20. Mid-IR absorption spectrum of the phosphorylated derivative 138 as a thin film.
2.1.4. Preparation of 2-(6-aminopurin-9-yl) -3,4-dihydroxy- 5-(10-hydroxy-2,5,8-trioxadecyl)tetrahydrofuran 3,4-acetonide 139 and 2-[6-(8-hydroxy-3,6-dioxaoctylamino)purin-9-yl]-3,4-dihydroxy-5-(10-hydroxy-2,5,8-trioxadecyl)tetrahydrofuran 3,4-acetonide 139A

On treating the acetonide 127 with NaH\textsuperscript{169} and 2-[2-(2-chloroethoxy)ethoxy]ethanol, in refluxing THF, the desired monoalkylated derivative 2-(6-aminopurin-9-yl)-3,4-dihydroxy-5-(10-hydroxy-2,5,8-trioxadecyl)tetrahydrofuran 3,4-acetonide 139 was isolated in 2% yield as well as the competing di-alkylated product, 2-[6-(8-hydroxy-3,6-dioxaoctylamino)purin-9-yl]-3,4-dihydroxy-5-(10-hydroxy-2,5,8-trioxadecyl)tetrahydrofuran 3,4-acetonide 139A, in 3% yield (Scheme 24). In the \textsuperscript{1}H NMR spectra of compound 139 and 139A (Figure 22), the methylene signals of compound 139 can be observed at ca. 3.58-3.96 ppm while those of the competing product 139A can be observed in a similar region ca. 3.51-3.81 ppm. The 2'-H methine proton of 139, however, resonates at 7.83 ppm while that of 139A resonates downfield at 8.50 ppm as a consequence of the presence of the 8-hydroxy-3,6-dioxaoctylamino substituent on the purine ring.

\[ \text{Scheme 24. Synthesis of compounds 139 and 139A} \]
Figure 21. Comparism of $^1$H NMR spectra of compound 139 and compound 139A in CDCl$_3$.

2.1.5. Preparation of 2-(6-aminopurin-9-yl)-5-acetoxyacetyl-3,4-dihydroxytetrahydrofuran 3,4-acetonide 140 and 2-[6-(acetoxyacetylamino)purin-9-yl]-5-acetoxyacetyl 3,4-dihydroxytetrahydrofuran 3,4-acetonide 140A

On treating the acetonide 127 with NaH and acetoxyacetyl chloride$^{169,178}$ in refluxing THF, the ester 2-(6-aminopurin-9-yl)-5-acetoxyacetyl-3,4-dihydroxytetrahydrofuran 3,4-acetonide 140 was isolated in 34% yield as well as the di-acylated product, 2-[6-(acetoxyacetylamino)purin-9-yl]-5-acetoxyacetyl-3,4-dihydroxytetrahydrofuran 3,4-acetonide 140A in 40% yield (Scheme 25). There is one CH$_3$CO signal at 2.13 ppm in $^1$H NMR spectrum of compound 140 while there are (2 × CH$_3$CO) signals at 2.11 and 2.22 ppm respectively in the $^1$H NMR spectrum of compound 140A (Figure 22). The signal of the NH$_2$ on C-6' of the purine ring of compound 140 can be seen at 5.71 ppm while NH signal of compound 140A can be seen at 9.4 ppm. The methylene protons of the acetoxyacetyl moiety attached to the ribose sugar of both products resonate as singlets at ca. 4.5 ppm while the methylene protons of the amide group of the di-acylated product resonate as a singlet at 5.30 ppm. FAB mass spectrometric
analysis of the ester 140 showed the presence of the M+1 peak at m/z 408 with an intensity of 96% relative to the base peak at m/z 136, while FAB mass spectrometric analysis of the di-acylated product showed the presence of the M+1 peak at m/z 508 with an intensity of 100% as the base peak.

Scheme 25. Synthesis of the esters mono-ester 140 and di-ester 140A.

Figure 22. Comparison of $^1$H NMR spectra of compounds (a) 140 and (b) 140A in CDCl$_3$. 
2.1.6. Preparation of 2-(6-aminopurin-9-yl)-5-[(2-chloroacetoxy)methyl]-3,4-dihydroxyltetrahydrofuran 3,4-acetonide 141 and 2-[6-(2-chloroacetamido)purin-9-yl]- 5-[(2-chloroacetoxy)methyl]-3,4-dihydroxyltetrahydrofuran 3,4-acetonide 141A

Scheme 26. Synthesis of compounds 141 and 141A

The ester 2-(6-aminopurin-9-yl)-5-[(2-chloroacetoxy)methyl]-3,4-dihydroxyltetrahydrofuran 3,4-acetonide 141 was synthesized in 94% yield by stirring the acetonide 127 with chloroacetyl chloride \(^{179}\) under argon at room temperature for ca. 0.5 h (Scheme 26). In a separate reaction, on treating 127 with the same acid chloride under the same reaction conditions, compound 141A was obtained as well as the desired product 141. While each of the \(CH_2Cl\) methylene signals were observed at 4.00 ppm as shown in Figure 23, the methylene protons next to the amide group on the purine ring of 141A resonate as a singlet at 4.70 ppm. The 8’-H methine proton of compound 141 resonates at 7.88 ppm, while the corresponding proton in compound 141A resonates at 8.72 ppm. Attempts to synthesize compound 142 by refluxing compound 141 with triethyl phosphite (Arbuzov reaction)\(^{180}\) were unsuccessful.
Figure 23. Comparison of 400MHz $^1$H NMR spectra of compounds 141 and 141A in CDCl$_3$.

2.1.7. Preparation of 5-[(acryloyloxy)methyl]-2-(6-aminopurin-9-yl)-3,4-dihydroxytetrahydrofuran 3,4-acetonide 143

The acrylic ester 143 was obtained as light yellow oil in 4% yield on treating deprotonated 127 with acryloyl chloride under reflux (Scheme 27).

Scheme 27. Synthesis of the acrylic ester 143.
Formation of the acrylic ester 143 was confirmed by the downfield shift of the 1"-methylene proton signals in compound 143 relative to the precursor 127 (Figure 24). Both methine protons on the heteroaromatic ring of the acrylic ester 143 resonate at about the same chemical shifts (7.84 and 8.34 ppm) as in the precursor 127 confirming that there was no acylation of the amino group on the purine ring. The NH$_2$ protons also integrate to approximately 2 at 5.87 ppm in the $^1$H NMR spectrum of the acrylic ester product. The formation of the acrylic ester was further confirmed by the presence of the molecular ion at $m/z$ 361.137825 with 30% abundance relative to the base peak at $m/z$ 55.018601 in the high-resolution mass spectrometric analysis.

**Figure 24.** 400 MHz $^1$H NMR spectrum of acrylic ester 143 in CDCl$_3$.

The mid-IR spectrum of compound 143 showed an ester carbonyl absorption band at 1714 cm$^{-1}$.

### 2.1.8. Preparation of 4-bromomethyl-2,2-dimethyl[1,3]dioxolane 145

The vicinal 1,2-diol moiety of 3-bromo-1,2-dihydroxypropane 144 was protected by stirring it with acetone in the presence of para-toluenesulfonic acid monohydrate.
catalyst\textsuperscript{163} to obtain 4-bromomethyl-2,2-dimethyl[1,3]dioxolane \textbf{145} in 65\% yield (Scheme 28). The protection was confirmed by the observation of two clearly defined methyl singlets at 1.35 and 1.43 ppm respectively in the \textsuperscript{1}H NMR spectrum (Figure 25) as well as six carbon signals in the \textsuperscript{13}C NMR spectrum (Figure 26). However, attempts at alkylating\textsuperscript{169} the deprotonated acetonide \textbf{127} with the dioxolane \textbf{145} was unsuccessful.

\textbf{Scheme 28}. Proposed synthesis of the derivative \textbf{146}.

\textbf{Figure 25}. 400 MHz \textsuperscript{1}H NMR spectrum of the dioxolane \textbf{145} in CDCl\textsubscript{3}.

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Figure 26. 100 MHz $^{13}$C NMR spectrum of the dioxolane 145 in CDCl$_3$.

2.2. Novel ATP analogues from adenine

The approach employed was to replace the proton on the N-9 nitrogen of adenine with various electrophiles after deprotonation of adenine to afford primary products (Figure 27) that could be elaborated further. In one case the electrophile had to be specially prepared.
Discussion

Figure 27. General approach for the synthesis of adenine derivatives.

2.2.1. Preparation of 3,4,5-triacetoxy-6-acetoxymethyl-2-(6-aminopurin-9-yl)-pyran 149

Acetylated glucose (3,4,5,6-tetraacetoxy-2-acetoxymethylpyran) 148 was synthesized in 87% yield by heating glucose 147 with acetic anhydride in the presence of ZnCl₂ catalyst₁₈¹ (Scheme 29). ¹H NMR analysis (Figure 28) indicated the presence of the desired product. Thus the anomeric proton (2-H) resonates as a doublet at 6.3 ppm with a coupling constant of 3.7 Hz, a down-field shift of 0.1 ppm from its value in glucose, while the five acetoxymethyl groups resonate between 1.94 and 2.23 ppm. Five methyl carbons and five C=O carbons can be observed in the ¹³C NMR spectrum (Figure 29). Low-resolution mass spectrometric analysis reveals the presence of the molecular ion at m/z 390 with 3% abundance relative to the base peak at m/z 98, while mid-IR absorption at 1750 cm⁻¹ was observed for the ester carbonyl group.
Scheme 29. Synthesis of the adenine derivative 149.

Figure 28. 400MHz $^1$H NMR spectrum of compound 148 in CDCl$_3$. 
The acetylated glucose 148 was then coupled with adenine in a tin tetrachloride catalyzed\textsuperscript{182} glycosidation reaction using acetonitrile\textsuperscript{183} as solvent to afford of 3,4,5-triacetoxy-6-acetoxymethyl-2-(6-aminopurin-9-yl)pyran \textbf{149} in 30\% yield. In the $^1$H NMR spectrum (Figure 30), the anomeric proton (2-H) now resonates at 5.88 ppm with a coupling constant of 5.9 Hz \textit{ca.} 0.4 ppm upfield of the pentaacetylated precursor, while the anomeric carbon has a chemical shift of 80.3 ppm in the $^{13}$C NMR spectrum (Figure 30) - an upfield shift of 8.8 ppm. Low-resolution mass spectrometric analysis of compound \textbf{149} showed the presence of the M-1 peak at $m/z$ 464 as the base peak. Mid-IR absorption at 1744 cm$^{-1}$ was observed for the ester carbonyl groups.

\textbf{Figure 29.} 100MHz $^{13}$C NMR spectrum of the acetylated product 148 in CDCl$_3$. 

\begin{center}
\includegraphics[width=0.5\textwidth]{FIGURE_29.png}
\end{center}
Figure 30 (i) 400MHz $^1$H NMR spectrum of acetylated derivative 149 and (ii) 100MHz $^{13}$C NMR spectrum of acetylated derivative 149 in CDCl$_3$. 
2.2.2. Preparation of 6-\((N\text{-allylamino})\)purine 150 and 9-allyl-6-\((N\text{-allylamino})\)purine 150A

Deprotonation of adenine in DMF followed by treatment with allyl bromide\(^{169}\) under reflux yielded the mono- and di-allylated products 150 and 150A (Scheme 30). NMR as well as high-resolution mass spectroscopy confirmed the formation of both allylated products. All protons are accounted for in the \(^1\)H NMR spectra of both compounds (Figures 31 and 32). The NH proton of the mono-allylated product resonates as a singlet at 11.12 ppm while its 2'-H methine proton resonates at 6.06 ppm as a tdd. The molecular ion peak of the mono-allylated product 150 was clearly visible at \(m/z\) 175 as the base peak while the spectrum of the di-allylated product 150A exhibited a molecular ion at \(m/z\) 215 with 95% abundance relative to the base peak at \(m/z\) 200.

![Scheme 30. Synthesis of the adenine derivatives 150 and 150A](image)

**Figure 31.** 400MHz \(^1\)H NMR spectrum of the adenine derivative 150 in CDCl\(_3\).
Figure 32. 400MHz ¹H NMR spectrum of the adenine derivative 150A in CDCl₃.

2.2.2. Attempted Preparations

Attempts were made to couple deprotonated adenine with each of 2-(chloroethoxy)ethanol, acetoxyacetyl chloride and chloroacetyl chloride (Scheme 31). Unfortunately all these acylation reactions were unsuccessful. The reason for these failed reactions may be the steric bulk of the purine nucleophile in the case of chloroacetyl chloride and acetoxyacetyl chloride. 2-(chloroethoxy)ethanol, on the other hand, might have undergone intramolecular cyclisation in the presence of NaH (Figure 33) hence preventing its reaction with the nucleophilic deprotonated adenine.
Scheme 31. Attempted preparations of adenine derivatives.

Figure 34. Possible intramolecular cyclisation of 2-(chloroethoxy)ethanol in the presence of NaH.

2.3. ATP mimics from allopurinol and Baylis-Hillman electrophiles

The approach was to replace the hydrogen of the imidazole ring in allopurinol with different electrophiles, after deprotonation by a base, to afford novel ATP mimics. Hence, the hydroxyl group on allopurinol was protected with dihydropyran\(^ {184}\) (Scheme 32) to avoid competition between the NH and OH moieties in the molecule. \(^ {1}H\) NMR analysis (Figure 34) showed the formation of compound 157 with all the protons accounted for in their expected positions, and the 2'-methine proton having moved upfield by 0.4 ppm relative to its position in dihydropyran. In the \(^ {13}C\) NMR spectrum (Figure 35) the C-3' carbon has been shifted upfield by 71.2 ppm as a
consequence of being converted from an sp$^2$ centre in dihydropyran to an sp$^3$ carbon in the protected allopurinol.

Scheme 32. Protection of allopurinol.

Figure 34. 400MHz $^1$H NMR spectrum of the protected allopurinol 157 in CDCl$_3$.  
Figure 35. 100MHz $^{13}$C NMR spectrum of the protected allopurinol 157 in CDCl$_3$.

With the protected allopurinol in hand, an attempt was made to synthesize the range of primary substitution products illustrated in Figure 38.

Figure 36. General approach to allopurinol derivatives.
2.3.1. Preparation of 9-allyl-4-(tetrahydropyran-2-yloxy)-1H-pyrazolo[3,4-
d]pyrimidine 161

The protected allopurinol 157 was deprotonated using sodium hydride and then treated with allyl bromide\textsuperscript{160} (Scheme 33) under reflux and the allylated derivative 158 was obtained in 87% yield. The $^1$H NMR and $^{13}$C NMR (Figure 37) spectra both confirm the formation of the product. The 2"-methine proton resonates at 5.94 ppm as a multiplet due to coupling with adjacent vinylic and methylene protons, while all 13 carbon atoms are accounted for (Figure 38). Mass spectrometric analysis shows the presence of the molecular ion at $m/z$ 260 relative to the base peak at $m/z$ 177.

![Scheme 33. Preparation of the allopurinol derivative 158.](image-url)

**Figure 37.** 600MHz $^1$H NMR spectrum of the allopurinol derivative 158 in CDCl$_3$. 

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\textsuperscript{160} The reaction conditions were optimized to ensure high yield and selectivity. The use of sodium hydride as a base was necessary to facilitate the deprotonation step, while the allyl bromide was chosen for its reactivity towards the nucleophilic allopurinol derivative. Additionally, the reaction was conducted under a nitrogen atmosphere to prevent the reaction from being affected by air oxidation. The product was purified by column chromatography to obtain a high-purity sample.
 Attempts to acylate the pyrazole nitrogen\textsuperscript{178} of protected allopurinol with various acylating agents (Scheme 34) were, also unsuccessful - paralleling the difficulties encountered in acylating adenine at the N-9 nitrogen.

\textbf{Scheme 34}. Attempted acylations of compound 157.
2.3.2. Preparation of *tert*-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylene propanoate 165a and *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylene propanoate 165b

In separate reactions, 5-bromo-2-hydroxybenzaldehyde 163a and salicylaldehyde 163b were stirred with *tert*-butyl acrylate in the presence of DABCO as catalyst to obtain 165a and 165b, respectively (Scheme 35), following previous work done in the research group by Musa. Each of these Baylis-Hillman adducts was then reacted with deprotonated allopurinol. $^1$H NMR and other spectroscopic analysis confirmed the formation of the Baylis-Hilman products. The intramolecular cyclisation product 166b was also obtained as side product in the reaction between salicylaldehyde and *tert*-butyl acrylate. This product arises from a conjugate addition-elimination (or $S_N^1$ displacement) reaction affording a chromene instead of the coumarin analogue. The vinylic protons of compound 165b, 3"-H$_a$ and 3"-H$_b$, resonate at 5.49 ppm and 6.23 ppm, respectively (Figure 39). The 3-H methine proton singlet at 5.69 ppm and the 3-OH and 2'-OH signals at 4.36 ppm and 8.14 ppm disappear in the spectrum of the compound 166b to give way for the 2-methylene singlet (Figure 40) at 4.94 ppm as a consequence of the intramolecular cyclisation of 165b. The 3-H methine proton at 5.69 ppm on a sp$^3$ carbon in compound 165b moves downfield to 7.32 ppm (4-H in 166b) since it now resides on a sp$^2$ carbon.

![Scheme 35. General preparation of Baylis-Hilman products.](attachment:image.png)

There is also a considerable shift upfield in the position of the carbonyl carbon in the $^{13}$C NMR spectra from 166.7 ppm in 165b (Figure 41) to 163.8 ppm in 166b (Figure 42). High-resolution mass spectrometric analysis of compound 166b shows the presence of the M-1 ion at $m/z$ 231.101945 with 30% abundance relative to the base.
peak at $m/z$ 57.070785, while the mid-IR spectrum shows carbonyl absorption at 1700 cm$^{-1}$.

**Figure 39.** 400MHz $^1$H NMR spectrum of the B-H product 166b in CDCl$_3$

**Figure 40.** 400MHz $^1$H NMR spectrum of the chromene 165b in CDCl$_3$. 
Figure 41. 100MHz $^{12}$C NMR spectrum of the Baylis-Hillman product $165b$ in CDCl$_3$.

Figure 42. 100MHz $^{13}$C NMR spectrum of the Baylis-Hillman product $166b$ in CDCl$_3$. 

$\text{C=O}$
2.3.2. Preparation of 6-bromo-3-(chloromethyl)coumarin 167, 6-bromo-3-(acetoxyethyl)coumarin 168 and 6-bromo-3-(hydroxymethyl)coumarin 169

Cyclisation of the Baylis-Hillman adduct 165a in a refluxing mixture of HCl/CH$_3$COOH$^{185}$ yielded the desired 6-bromo-3-(chloromethyl)coumarin product 167 in 31% yield together with two side-products, 6-bromo-3-(acetoxyethyl)coumarin 168 (1.8%) and 6-bromo-3-(hydroxymethyl)coumarin 169 (7.3%) as shown in Scheme 36. The chemical structures of the side-products were confirmed by spectroscopic methods. The formation of the ester 168 resulted from direct interception by acetic acid (165a $\rightarrow$ 168) and/or the reaction of the excess acetate ion with the desired alkyl halide product 167, while the formation of the alcohol 169 is attributed to the hydrolysis of the ester 168. All the protons and carbons of the ester 168 are accounted for as shown in Figures 43 and 44. The methyl protons resonate at 2.16 ppm and the 1'-methylene protons resonate as a singlet at 5.06 ppm, while the 4-H proton resonates as a singlet at 7.94 ppm. In the $^{13}$C NMR spectrum the lactone carbonyl and the acetyl carbonyl carbons resonate at 159.5 and 170.4 ppm, respectively. Mass spectrometric analysis of the ester 168 shows the presence of the molecular ion at $m/z$ 297 with 18% abundance relative to the base peak at $m/z$ 43. Mid-IR spectroscopic analysis of the compound 168 shows the lactone carbonyl absorption band overlapping the coumarin carbonyl band at 1720 cm$^{-1}$.

Figure 43 400MHz $^1$H NMR spectrum of compound 168 in CDCl$_3$.

Figure 44 100MHz $^{13}$C NMR spectrum of compound 168 in CDCl$_3$. 
Figure 45. Mid-IR absorption of compound 168.

All the protons and carbons of the alcohol 169 are accounted for as shown in Figures 46 and 47. The hydroxymethylene protons (CH$_2$OH) signal is a multiplet at 4.36 ppm while the hydroxyl proton resonates as a triplet at 5.52 ppm. All the carbon signals are accounted for in the $^{13}$C NMR spectrum with the sp$^2$ C-4 carbon resonating at 135.6 ppm while the lactone carbonyl signal appears at 159.1 ppm. Mass spectrometric analysis of the alcohol 169 shows the presence of the molecular ion peak at $m/z$ 255 with 83% abundance relative to the base peak at $m/z$ 225. Mid-IR spectroscopic analyses of the compound 169 show a hydroxyl absorption band at 3409 cm$^{-1}$ and a coumarin carbonyl absorption band at 1630 cm$^{-1}$.
The Baylis-Hillman adducts 165a and 165b each have two electrophilic sites (Figure 48) at which they may be susceptible to attack by a nucleophile. The 2-
(chloromethyl)coumarin 167, on the other hand has three different electrophilic sites, as shown in Figure 48, at which it may be susceptible to nucleophilic attack.

![Figure 48. Nucleophilic sites in compounds 165a,b and compound 167](image)

2.3.2.3. Reaction of protected allopurinol 157 with 2-(chloromethyl)coumarin 167

The 2-(chloromethyl)coumarin 167 was refluxed with deprotonated protected allopurinol 157 to afford the coumarin derivative 170a in 31% yield (Scheme 37). The structure of the coumarin derivative 170a was confirmed by spectroscopic methods. The significant downfield shift of the 1'-methylene protons from 4.53 ppm in the precursor 167 to 5.02 ppm in the product 170a (Figure 49) and the downfield shift of the 1'-methylene carbon signal from 40.8 ppm in the precursor to 45.7 ppm in the product are clear evidence that the nucleophilic attack occurred at the 1'-centre of the coumarin. Furthermore there was no significant change in the chemical shift of the carbonyl carbon [159.4 ppm in the alkyl halide compared with 160.7 ppm in the product derivative (Figure 50)]. All other protons and carbon signals are accounted for in the respective spectra (Figures 49 and 50). High-resolution mass spectrometric analysis of 170a shows the presence of the M-1 ion at $m/z$ 456.043762 with 19% abundance relative to the base peak at $m/z$ 84.048546.
Scheme 37. Synthesis of the coumarin derivative 170a.

Figure 49. 400MHz $^1$H NMR spectra in CDCl$_3$ of compound 167 and compound 170a in CDCl$_3$. 
2.3.2.4. Reaction of protected allopurinol with Baylis-Hillman adducts 165a and 165b

The Baylis-Hillman adducts 165a and 165b were refluxed separately with deprotonated protected allopurinol to afford products 170a and 170b respectively (Scheme 38). In the presence of NaH, intramolecular cyclisation (Figure 51) seems to have occurred in both 165a and 165b before the nucleophilic S_N^1 attack by the deprotected allopurinol to yield 170a and 170b. The chemical structure of each of these allopurinol derivatives was elucidated by spectroscopic methods. The only difference between 170a and 170b is the presence of the Br atom on C-6 of the coumarin moiety. Hence, a comparison of the proton NMR spectra of both compounds indicate the presence of 6 protons in the aromatic region of compound 170a while compound 170b has 7 protons in the same region (Figure 52). MS analysis of 170a shows the presence of the molecular ion at m/z at 378.132008 with 35%...
abundance relative to the base peak at \( m/z \) 84.057287, while mid-IR spectroscopic analysis of the compound shows carbonyl absorption at 1698 cm\(^{-1}\).

**Scheme 38.** Synthesis of allopurinol derivatives 170a and 170b.

**Figure 51.** Proposed mechanism for the formation of 170a and 170b
Figure 52. Comparison of 400MHz $^1$H NMR spectra of compounds \textbf{170a} and \textbf{170b} in CDCl$_3$. 
2.4. Truncated adenine mimics

It was envisaged that benzenoid compounds with suitable \textit{meta} substituents could serve as truncated analogues of the 6-aminopurine (adenine) nucleus. Drugs in chemical use often have relatively simple structures and when treated with base, followed by reaction with various electrophiles (as outlined in the Figure 53), it was hoped that such systems would provide convenient access to novel and effective ATP analogues.

![Diagram of truncated adenine mimics](image)

\textbf{Figure} 53. General approach to novel truncated ATP analogues.

2.4.1. ATP mimics from 3-aminobenzonitrile 173

3-Aminobenzonitrile was identified as a synthon for the construction of truncated ATP analogues (Figure 54). Deprotonation and treatment with a range of electrophiles
was expected to afford a number of intermediates that could be elaborated further (Figure 54).

Figure 54. Construction of truncated ATP mimics from 3-aminobenzonitrile.

Figure 55. Proposed preparation of derivatives of 3-aminobenzonitrile 173.
2.4.1.1. N-Allyl-3-cyanoaniline 177 and N,N-diallyl-3-cyanoaniline 177a

The alkene 177 was expected to provide access\textsuperscript{177} to the phosphorylated derivative 180 via the dihydroxylated derivative 179 as shown in Scheme 39. Treatment of 173 with 1 equivalent of NaH at 0°C followed by allyl bromide\textsuperscript{169} under reflux yielded the mono-allylated product 177 in 45% yield (Scheme 39). Using 2 equivalent of NaH and the same reaction conditions, both the mono-allylated derivative 177 (31%) and the di-allylated derivative 177a (8%) were obtained. NMR, mass spectrometric and mid-IR spectroscopy were employed to confirm the formation of these products. Figures 56 and 57 show the $^1$H NMR spectra of compounds 177 and 177a respectively. The vinylic methine proton in compound 177 resonates as a multiplet at 5.89 ppm while the two vinylic methine protons in the di-allylated product resonate as a multiplet at 5.81 ppm. The NH signal for the mono-allylated product 177 is evident as a singlet at 4.13 ppm. MS analysis of compound 177 indicates the presence of the molecular ion at m/z 158 as the base peak, while high-resolution MS analysis of 177a indicates the presence of the M+1 peak at m/z 199.119390 with 9% abundance relative to the base peak at m/z 41.040152. Mid-IR analysis of compound 177 (Figure 58) showed an NH absorption band at 3391 cm$^{-1}$ and the cyanide absorption band at 2228 cm$^{-1}$, while the di-allylated analogue 177a (Figure 59) show only the cyanide absorption band at 2228 cm$^{-1}$. In attempts directed at improving the yield, the reaction was also carried out under various reaction conditions. The maximum yield obtained was 45% using 1 equivalent of NaH in THF (Scheme 39). Unfortunately, repeated attempts to either dihydroxylate compound 177 with CTAP,\textsuperscript{174} KMnO$_4$\textsuperscript{174} or NaIO$_4$\textsuperscript{170} or to make the epoxide\textsuperscript{186} 178 were unsuccessful, precluding access to the targeted diphosphate 180.
Scheme 39. Synthesis of compounds 177 and 177a.

Figure 56. 400MHz $^1$H NMR spectrum of compound 177 in CDCl$_3$. 
Figure 57. 400MHz $^1$H NMR spectrum of compound 177a in CDCl$_3$.

Figure 58. Mid-IR absorption of spectrum of compound 177.
Figure 59. Mid-IR absorption of spectrum of 177a.

2.4.1.2. Preparation of 2-chloro-N-(3-cyanophenyl)acetamide 181 and diethyl 3-(3-cyanophenyl)carbamoylmethyl phosphonate 182

The chloroacetamide 181 was obtained in high yield of 95% by stirring 3-cyanoaniline 173 with chloroacetyl chloride179 (Scheme 40). 1H NMR analysis of the product 181 (Figure 60) reveals the CH2Cl signal at 4.20 ppm as a singlet, while high-resolution mass spectrometric analysis revealed the molecular ion peak at \( m/z \) 194.02431 with 45% abundance relative to the base peak at \( m/z \) 118.05162. Phosphorylation of the chloroacetamide 181 with triethyl phosphite in an Arbuzov reaction180 afforded the phosphonate 182 in 84% yield. The protons and carbon signals corresponding to the CH2P methylene group are both observed to be split by the coupling to the \( ^{31} \)P nucleus in the \( ^{1} \)H and \( ^{13} \)C NMR spectra respectively (Figures 61 and 62). The carbonyl carbon is also observed to be split by the \( ^{31} \)P nucleus with a coupling constant of 4.5 Hz. The \( ^{31} \)P NMR spectrum (Figure 63) shows a signal at 18.30 ppm corresponding to the phosphorous atom. The high-resolution mass spectrum of the phosphonate 182 shows the molecular ion at \( m/z \) 296.01963 with 65% abundance relative to the base peak at \( m/z \) 179.08244. Attempts to effect catalytic hydrogenation of the nitrile moiety in compound 182, in the presence of platinum on carbon catalyst, however, were unsuccessful.
Scheme 40. Synthesis of compounds 181 and 183.

Figure 60. 400 MHz $^1$H NMR spectrum of the chloroacetamide 181 in CDCl$_3$. 
Figure 61. 400 MHz $^1$H NMR spectrum of compound 182 in CDCl$_3$.

Figure 62. 100 MHz $^{13}$C NMR spectrum of compound 182 in CDCl$_3$, showing splitting of the CH$_2$P and C=O signals by $^{31}$P.
Figure 63. 162 MHz $^{31}$P NMR of the phosphonate 182.

2.4.1.3. Preparation of 2-acetoxy-N-(3-cyanophenyl)acetamide 184

Treatment of deprotonated 3-aminobenzonitrile 173 with acetoxyacetyl chloride afforded 2-acetoxy-N-(3-cyanophenyl)acetamide 184 in 85% yield (Scheme 41). Formation of the product 184 was confirmed by spectroscopic methods. The HMBC NMR spectrum (Figure 64) shows the correlation between the NH proton and the carbonyl carbon of the carbamoyl group while the mid-IR spectrum showed an amide C=O absorption band at 1675 cm$^{-1}$ and an ester C=O absorption band at 1735 cm$^{-1}$. Mass spectrometric analysis revealed the M+1 peak at $m/z$ 219 with 60% abundance relative to the base peak at $m/z$ 177. Attempted reduction of the nitrile group, in this case with lithium aluminium hydride was also unsuccessful, and the difficulties associated with this seemingly straightforward transformation will have to be addressed in future studies.

Scheme 41. Synthesis of 2-acetoxy-N-(3-cyanophenyl)acetamide 185.
**Figure 64.** HMBC NMR spectrum of compound **184** in CDCl₃.

**Figure 65.** Mid-IR spectrum of compound **185** as solid deposit.
2.4.1.4. Preparation of 2,3,4,5,6-pentaacetyl-N-(3-cyanophenyl)-gluconamide 186

3-Aminobenzonitrile 173 was stirred with 2,3,4,5,6-pentaacetylgluconoyl chloride\textsuperscript{167,168} 131 in pyridine to afford the gluconamide derivative 186 in 41\% yield (Scheme 42), formation of which was confirmed by spectroscopic methods. The HMBC NMR spectrum (Figure 66) again shows the correlation between the NH proton and the carbonyl of the carbamoyl group. Mass spectrometric analysis of the gluconamide 186 shows the presence of the molecular ion at \( m/z \) 506 with 24\% abundance relative to the base peak at \( m/z \) 505.

![Scheme 42. Synthesis of the acetylated derivative 187.](image)

**Figure 66.** HMBC NMR spectrum of the gluconamide 186 in CDCl\textsubscript{3}.  

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Discussion
2.4.2. ATP mimics from resorcinol 187

Deprotonation of one of the hydroxyl groups of resorcinol with 1 equivalent of NaH,\(^{169}\) followed by treatment with different alkylating and acylating agents,\(^ {169,178}\) afforded a set of primary substitution products as outlined in Scheme 43.

Scheme 43. ATP mimics derived from resorcinol.

Resorcinol 187 in dry THF was deprotonated with 1 equivalent of NaH and then treated with 1 equivalent of allyl bromide\(^ {169}\) under reflux to afford the allylated derivative 188 in 28% yield (Scheme 43). However, all attempts at dihydroxylating 188 with oxidizing agents, such as CTAP, KMnO\(_4\) and NaIO\(_4\) in the presence of RuCl\(_3\) catalyst were unsuccessful.

Resorcinol 187 was similarly deprotonated and boiled under reflux with 1 equivalent of chloroacetyl chloride.\(^ {179}\) The reaction was repeated several times but each time the di-acylated product 189A was obtained instead of the desired mono-acylated product.
When 1 equivalent acetoxyacetyl chloride was used instead of chloroacetyl chloride, the di-acylated product 190A was obtained repeatedly instead of the desired mono-acylated product.

However, when resorcinol 187 was stirred with 2,3,4,5,6-pentaacetylgluconoyl chloride 131 in pyridine,\textsuperscript{167,168} the desired mono-ester 191 was obtained in 24\% yield (Scheme 43). The \textsuperscript{1}H NMR spectrum of compound 191 (Figure 68) shows the expected acetyl and methine protons, while MS analysis revealed the molecular ion peak at \textit{m/z} 498.

Resorcinol was again deprotonated with 1 equivalent of NaH and then treated with 1 equivalent of acryloyl chloride\textsuperscript{169} (Scheme 43). In this case however, both the mono-acrylic and di-acrylic esters were obtained in very low yields. Attempts to improve the yield of the monoester by using 1 equivalent of KOH in ethanol as base appeared to result in the di-acrylic ester alone. Spectroscopic analysis confirmed the formation of both products. \textsuperscript{1}H NMR spectra of both esters are shown in Figures 69 and 70 and it is clearly evident from integration data that both products have been isolated. Mass spectrometric analysis of the mono-ester 192 shows the presence of the molecular ion at \textit{m/z} 164 in 97\% abundance relative to the base peak at \textit{m/z} 27, while the mass spectrum of the di-ester 192A shows the presence of the molecular ion at \textit{m/z} 218 with 80\% abundance relative to a base peak at \textit{m/z} 27.

Generally the desired mono-acylation proved difficult to achieve, except in the case of gluconoylation. Acylation of one of the resorcinol hydroxyl groups would increase the acidity of the remaining hydroxyl group relative to resorcinol itself thus favouring further deprotonation and formation of the di-acylated product (Figure 67). Formation of the mono-gluconoyl derivative can be argued to be due to steric hindrance by the first gluconoyl moiety on the molecule making the now more acidic second hydroxyl group less accessible to acylation.
**Figure 67.** Explanation for di-acylation in resorcinol derivatives.

**Figure 68.** 400 MHz $^1$H NMR spectrum of compound 191 in CDCl$_3$. 
2.4.3. ATP mimics from 3-aminophenol

Deprotonation of the amino substituent on 3-aminophenol 193 with NaH and treatment with different alkylating and acylating agents was expected to afford a set of primary substitution products (Figure 71). While regioselectivity might have been expected to be a major problem, the use of one equivalent of NaH\textsuperscript{169} for deprotonation
of the substrate worked very well in achieving mono-alkylation and mono-acylations at the amino nitrogen.

**Figure 71.** General approach to 3-aminophenol derivatives.

**2.4.3.1. Preparation of N-allyl-3-hydroxyaniline 194, N-allyl-3-allyloxyaniline 194A and N,N-diallyl-3-hydroxyaniline 194B**

Treatment of 3-aminophenol 193 with NaH and allyl bromide\(^{160}\) afforded 194, the desired allylated intermediate with two other allylated products 194A and 194B.

**Scheme 44.** Synthesis of compounds 194, 194A and 194B.

In the comparative \(^1\)H NMR spectra (Figure 72) of N-allyl-3-hydroxyaniline 194, N-allyl-3-allyloxyaniline 194A and N,N-diallyl-3-hydroxyaniline 194B, the 1'-methylene
protons have similar chemical shift values (ca. 3.8 ppm), while the methyleneoxy protons in the $O$-allylated moiety 194A resonate downfield at 4.51 ppm because of the greater de-shielding effect of the oxygen atom. It is also observed that the 2'-H (5.97 ppm) and 2''-H (6.07 ppm) vinylic methine protons in the $N,O$-di-allylated product 194A resonate as multiplets at slightly different chemical shifts, while the corresponding methine protons in the $N,N$-di-allylated product 194B resonate as a multiplet at 5.58 ppm being in the same chemical environment. The C-3' vinylic carbons in the $N$-allylated systems 194 and 194A (Figure 73) resonate at similar chemical shift values (ca. 116 ppm), while the corresponding nuclei in the $N,O$-di-allylated system resonate at different chemical shift values. MS analysis of the products gave the following results: compound 194 showed the presence of the molecular ion at $m/z$ 149 as the base peak, compound 194A showed the presence of the molecular ion at $m/z$ 189 with 95% abundance relative to the base peak at $m/z$ 121, while 194B showed the presence of the molecular ion at $m/z$ 189 as the base peak.

**Figure 72.** Comparative 400 $^1$H NMR spectra of 194, 194A and 194B, in CDCl3.
Figure 73. Comparative partial 100 MHz $^{13}$C NMR spectra of compounds 194, 194A and 194B in CDCl$_3$.

Attempts to di-hydroxylate the mono-allylated product 194 with different agents such as CTAP, KMnO$_4$ and NaIO$_4$ were unsuccessful - a pattern observed with earlier systems.

2.4.3.2. Preparation of 2-chloro-N-(3-hydroxyphenyl)acetamide 195 and diethyl {[(3-hydroxy-phenyl)carbamoyl)methyl]phosphonate 196

2-Chloro-N-(3-hydroxyphenyl)acetamide 195 was obtained in 76% yield by treating 3-aminophenol in dry THF under nitrogen with NaH followed by chloroacetyl chloride$^{179}$ under reflux (Scheme 45). The correlation between the NH proton and the carbonyl carbon can be observed in the HMBC spectrum of compound 195, confirming that the substitution had occurred at the amino group and not the hydroxyl group (Figure 74).
Phosphorylation of 195 with triethyl phosphite (Arbuzov reaction)\textsuperscript{180} afforded the diethyl phosphonate ester 196 in 48% yield. The protons and carbon of the $\text{CH}_2\text{P}$ group both resonate as doublets due to coupling to the $^{31}\text{P}$ nucleus in the $^1\text{H}$ and $^{13}\text{C}$ NMR spectra, respectively (Figure 75). The $\text{C}=\text{O}$ is also observed to be split by the $^{31}\text{P}$ with a coupling constant of 4.1Hz $^{31}\text{P}$. The phosphorous $^{31}\text{P}$ signal is at 24.2 ppm.
Figure 75. Coupling of $^{31}$P to (a) $CH_2$ and (b) $CH_2$ and C=O in compound 196.

Interestingly, the phosphonate ester 196 was found to inhibit the DOX-P reductoisomerase enzyme of the malaria parasite *Plasmodium falciparum* (PfDXR).
both in vitro (in Biochemistry Department at Rhodes) and from Saturation Transfer Difference (STD)\textsuperscript{188,189} NMR spectroscopy experiments (in Chemistry). The protein-ligand interaction was confirmed by the presence of ligand proton signals in the \( PfDXR \) protein spectrum (Figure 76). The implication of this observation is that although, the phosphonate ester \textbf{196} was made to inhibit the \textit{Mycobacterium tuberculosis} enzyme glutamine synthetase, it is to be explored in our group as a potential therapeutic for malaria, and analogues are currently being made!

\textbf{Figure 76.} 600 MHz \( ^1 \text{H} \) STD NMR spectrum of \textbf{196} and \( PfDXR \) showing interaction between protein and ligand.

\textbf{2.4.3.3. Preparation of 2-acetoxy-N-(3-hydroxyphenyl)acetamide 197}

2-Acetoxy-N-(3-hydroxyphenyl)acetamide \textbf{197} was isolated pure on treating deprotonated 3-aminophenol \textbf{193} with acetoxyactyl chloride\textsuperscript{178} (Scheme 46). All the proton and carbons are accounted for in the \( ^1 \text{H} \) NMR and \( ^{13} \text{C} \) NMR spectra, respectively. The methyl signal is visible at 2.11 ppm while the methylene carbon resonates at 62.5 ppm The OH and NH protons resonate at 9.39 ppm and 9.92 ppm, respectively, while the amide and ester carbonyls resonate at 165.2 ppm and 169.9 ppm, respectively, as determined from the correlations between corresponding proton
and carbon signals in the HMBC spectrum (Figure 77). High-resolution mass spectroscopic analysis shows the presence of the molecular ion at $m/z$ 209.068446 with 40% abundance relative to the base peak at $m/z$ 109.053492.

Scheme 46. Route to the ester 197.

Figure 77. HMBC NMR spectrum of compound 197 in DMSO-$d_6$. 
2.4.3.4. Preparation of 2,3,4,5,6-pentaacetyl-N-(3-hydroxyphenyl)gluconamide 198

2,3,4,5,6-Pentaacetyl-N-(3-hydroxyphenyl)gluconamide 198 was isolated successfully from the reaction between 3-aminophenol 193 and acid chloride178 131 (Scheme 47). The five acetoxyethyl groups are accounted for by the fifteen protons corresponding to the series of singlets that resonate between 2.24 and 2.01 ppm in the $^1$H NMR spectrum (Figure 78). In the $^{13}$C NMR spectrum, the presence of only four carbon signals corresponding to five methyl carbons is attributed to the coincidence of two of the signals. Mass spectrometric analysis confirmed the formation of 198 as the molecular ion is prominent at $m/z$ 497.450192 as the base peak.

Scheme 47. Route to the acetylated derivative 198.

Figure 78. 400 MHz $^1$H NMR spectrum of compound 198 in CDCl$_3$. 
2.4.3.5. Reaction of 3-aminophenol with acryloyl chloride

Treatment of 3-aminophenol 193 in dry THF with 1 equivalent of NaH at 0°C, followed by 1 equivalent of acryloyl chloride,\textsuperscript{169} afforded both the mono-acylated and di-acylated products 199 and 199A. The products were separated and fully characterized but the yield of the acrylamide 199 was too small for the reaction to be carried further.

Figure 79. 100 MHz $^{13}$C NMR spectrum of compound 198 in CDCl$_3$. 

\[ 5 \times \text{CH}_3 \]
Scheme 48. Route to acrylic amide 199 and acrylic ester 199A

2.4.4. ATP mimics from 3-aminobenzyl alcohol

Deprotonation of the amino substituent of 3-aminobenzyl alcohol 199 with NaH and treatment with different alkylating and acylating agents was expected to afford a further set of primary substitution products as scaffolds for elaboration to ATP mimics (Figure 80).

Figure 80. General approach to 3-aminobenzyl alcohol derivatives.
2.4.4.1. Reaction of 3-aminobenzylalcohol with allyl bromide

Treatment of 3-aminobenzyl alcohol 200 with NaH and allyl bromide\(^{169}\) afforded the desired product \(N\)-allyl-3-(hydroxymethyl)aniline 201 in 10% yield together with \(N\)-allyl-3-(allyloxymethyl)aniline 201A, \(N,N\)-diallyl-3-(hydroxymethyl)aniline 201B and 3-(allyloxymethyl)aniline 201C (Scheme 49). The structures of all four products were confirmed by spectroscopic methods, the assignments being based largely on a comparison of the allyl group signals in each compound. Thus, from the inspection of the spectra of all four products (Figure 81), it is apparent that the \(N\)-allyl methylene protons resonate at \(ca.\) 3.8 ppm, the \(O\)-allyl methylenes at \(ca.\) 4.0 ppm and the hydroxymethylene protons at \(ca.\) 4.6 ppm. The structural assignments are consistent with the \(^{13}\)C, DEPT135, HSQC and HMBC data and are supported by the \(^{13}\)C NMR predicted values (Section 2.5).

\[\text{Scheme 49. Allylation of 3-aminobenzylalcohol 200.}\]
Figure 81. Comparison of 400 MHz $^1$H NMR spectra of compounds 201, 201A, 201B and 201C in CDCl$_3$.

2.4.4.2. Reaction of 3-aminobenzyl alcohol with chloroacetyl chloride and acryloyl chloride

The $N,O$-bis-chloroacetylated product 202A was obtained in 95% yield on stirring 3-aminobenzyl alcohol 200 with chloroacetyl chloride$^{179}$ under nitrogen. In another reaction a mixture of both the amide 202 and the bis-chloroacetylated product 202A were obtained on refluxing deprotected (using NaH) 3-aminobenzyl alcohol 200 with 1 equivalent of chloroacetyl chloride (Scheme 50). In the HMBC spectrum of the amide 202 (Figure 82), the chloromethylene protons were observed to correlate with the carbonyl carbon only, while hydroxymethylene protons correlate with aromatic carbons. The hydroxymethylene protons resonate as a singlet at 4.50 ppm while the chloromethylene proton singlet is at 4.42 ppm. The two pairs of chloromethylene protons in the bis-acylated product 202A may be differentiated by examining the HMBC spectrum (Figure 83). The carbonyl carbon signal at 167.1 ppm correlates with the 1'-methylenoxy signal at $\delta$ 5.19 ppm as well as with the 4'-chloromethylene signal at 4.01 ppm, making it possible to distinguish between the amide and ester methylene signals as well as the corresponding carbonyl carbon signals. The Mid-IR spectrum of compound 202A shows both an ester C=O absorption band at 1747 cm$^{-1}$
and an amide C=O absorption band at 1664 cm\(^{-1}\) (Figure 84). MS analysis showed the presence of the molecular at \(m/z\) 199 in 10% abundance relative to the base peak at \(m/z\) 198 for compound 202, while the molecular ion for compound 202A was observed at \(m/z\) 276 as the base peak.

**Scheme 50.** Routes to the Arbuzov product 203, and acryloyl chloride derivatives 204 and 204A.

Phosphorylation of the mono-acylated product 202 (Scheme 50) with triethyl phosphite (Arbuzov reaction)\(^{180}\) afforded the expected phosphonate ester 203 in 37% yield. The protons and carbon signals for the \(CH_2P\) moiety are both split due to coupling with the \(^{31}\)P nucleus (Figures 85 and 86), while the C=O signal exhibits a doublet with a coupling constant of 129.9 Hz due to coupling with \(^{31}\)P. The phosphorous \(^{31}\)P signal is observed at 23.5 ppm. High-resolution MS analysis of 203 showed the presence of the molecular at \(m/z\) 301.275911 as the base peak.
Figure 82. HMBC spectrum of the mono-acylated product 202 in CDCl₃.

Reaction of 3-aminobenzyl alcohol 200 in dry THF with 1 equivalent NaH at 0°C, followed by 1 equivalent acryloyl chloride, afforded a mixture of both the acrylamide 204 and the acrylate ester 204A (Scheme 50). The products were separated and characterized spectroscopically, but the yields were too low for the reactions to be carried further in the available time.
Figure 83. Partial HMBC spectrum of the di-acylated product 202A in CDCl₃.

Figure 84. Mid-IR spectrum of 202A as solid deposit.
Figure 85. 400MHz $^1$H NMR of compound 203 in CDCl$_3$.

Figure 86. 100 MHz $^{13}$C NMR spectrum of compound 203 in CDCl$_3$. 
2.4.4.3. Reaction of 3-aminobenzyl alcohol with acetoxyacetyl chloride and 2,3,4,5,6-pentaacetylgluconoyl chloride

2-Acetoxy-N-[(3-hydroxymethyl)phenyl]acetamide 205 was isolated pure on treating deprotonated 3-aminobenzyl alcohol 200 with acetoxyacetyl chloride\textsuperscript{179} (Scheme 51). In the HMBC spectrum (Figure 87), correlation is obtained between the methyl signal at 2.19 ppm and the ester carbonyl signal at 169.6 ppm, while the amide C=O carbon resonating at 165.3 ppm correlates with the 2-methylene signal at 4.63 ppm. High-resolution mass spectrometric analysis shows the presence of the molecular ion at \textit{m/z} 223.08369 as the base peak.

The 2,3,4,5,6-pentaacetyl-N-(3-hydroxyphenyl)gluconamide derivative 206 was also isolated successfully from the reaction between 3-aminobenzyl alcohol 200 and the gluconoyl chloride\textsuperscript{167,168} 131 (Scheme 51). The five acetoxyethyl groups are accounted for by the fifteen protons resonating as a series of singlets between 2.02 and 2.24 ppm in the \textsuperscript{1}H NMR spectrum (Figure 88); the overlapping carbon-13 signals at \textit{ca.} 20 ppm correspond to the five methyl groups in the \textsuperscript{13}C NMR spectrum of 206, but six discrete signals are evident in the carbonyl carbon region at \textit{ca.} 163-171 ppm (Figure 89). MS analysis confirmed the formation of compound 206 with the molecular ion appearing at \textit{m/z} 511.16685 as the base peak.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Scheme51.png}
\caption{Scheme 51. Routes to amide ester 205 and acetylated derivative 206}
\end{figure}
Figure 87. Partial HMBC spectrum of compound 205 in CDCl₃.

Figure 88. 400 MHz ¹H NMR spectrum of compound 206.
Issues of regioselectivity also arose in the reaction of 3-hydroxy- and 3-aminobenzoic acid with allyl bromide. Treatment of both substrates with 1 equivalent NaH, followed by allyl bromide yielded the mono-allylated products 208 and 210 respectively, while use of 2 equivalents of NaH yielded the di-allylated products 208A and 210A, respectively, (Scheme 52). The position of the methylene proton signals in the $^1$H NMR spectra of all these allylated compounds as well as comparisons of experimental and predicted $^{13}$C NMR data were used to confirm their structures. In the $^1$H NMR spectra of compounds 208 and 210, the 1'-methylene protons resonate at 4.81 ppm and 3.74, respectively, (Figure 90), confirming that these methylene groups are in different chemical environments in the mono-allylated compounds.

**Figure 89.** 100 MHz $^{13}$C NMR spectrum of compound 206.

**2.4.5. ATP mimics from 3-hydroxy- and 3-aminobenzoic acid**
Scheme 52. Synthesis of compounds 208, 208A, 210 and 210A.

These values are very similar to the predictions by the Modgraph NMR predict programme (Section 2.5) which indicate their chemical shifts to be 4.92 ppm and 3.73 ppm, respectively, confirming that the amino group was mono-allylated in compound 210 while the carboxylic acid group was allylated in the mono-allylated product 208. In the di-allylated compound 210A, the 2' - (at δ 5.94 ppm) and 2"- (at δ 6.02 ppm) methine protons are clearly observed as separate multiplets confirming that there is no di-substitution on the amine nitrogen (Figure 91). Unfortunately, attempts at dihydroxylating these alkenes were again unsuccessful.
Figure 90. Comparison of 400 MHz $^1$H NMR spectra of mono-allylated derivatives 208 and 210.
Figure 91. 400 MHz $^1$H NMR spectra of di-allylated derivative 210A in CDCl$_3$. 
2.5. Application of NMR chemical shift prediction programmes

The Modgraph NMRPredict programme (version 3.2.2) was used to verify chemical shift assignments of selected systems. This programme can be used to predict both $^1$H and $^{13}$C chemical shifts. The Hierarchical Organization of Spherical Environments (HOSE) code and the Neural Network (Nnet) algorithm are the two approaches used for prediction. The HOSE code looks at a carbon atom relative to its spherical environments and uses this information based on the NMR spectral data base of thousands of compounds it contains as references. The draw-back of this approach is that if the query compound is not well represented in the data base, the prediction is not reliable as the programme can only make a prediction to a maximum of two spheres. Another disadvantage of this method is that it uses the contents of the data base and, hence, it is prone to inherent errors or bias. The Nnet is a general and more reliable approach as it is much more error tolerant and more accurate in predicting the chemical shifts of atoms not encountered before. The Modgraph data presented in this section is a combination of both the HOSE and Nnet approaches. For comparative purposes, use was also made of the ChemWindow C-13 prediction module. The relevant $^{13}$C data is tabulated on the following pages and, while comment is generally limited to particular assignments, significant discrepancies between experimental and predicted data are highlighted in the various tables.

2.5.1. NMR chemical shift prediction for adenosine analogues

2-(6-Aminopurin-9-yl)-5[7-diethylphosphoryloxy)-2-5-dioxaheptyl]3,4-dihydroxy-tetrahydrofuran 3,4-acetonide 138

The experimental chemical shifts assigned for compound 138 were compared with shift values provided by both NMR predict programmes, Modgraph and ChemWindow. Generally the Modgraph data is more comparable to the experimental data than the ChemWindow data. The CH$_3$CH$_2$OP protons resonate as superimposed triplets at 1.38 ppm in the experimental spectrum while Modgraph predicts them to be at 1.29 ppm. The $^{13}$C signals for the polyoxyalkyl centres, C-1", C-3", C-4", C-6" and C-7", are present in the $^{13}$C NMR spectrum between 66.6 and 66.7 ppm, while both ChemWindow and Modgraph predict them to be between 66.5 and 71.2 ppm (Table 3). However, the ChemWindow predictions tend to vary significantly from the
experimentally established values, over-estimating certain aromatic carbons by as much as 9 ppm and under-estimating alkyl carbons by as much as 13 ppm (data highlighted in yellow).

![Chemical Structure](image)

Table 3. Experimental and predicted $^{13}$C NMR data for compound 138

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a = Experimental values, b = Modgraph predict, c = ChemWindow predict
2-(6-Aminopurin-9-yl)-5-[(2-chloroacetoxy)methyl]-3,4-dihydroxytetrahydrofuran 3,4-acetonide 141 and 2-[6-(2-chloroacetamido)purin-9-yl]-5-[(2-chloroacetoxy)methyl]-3,4-dihydroxytetrahydrofuran 3,4-acetonide 141A

Table 4. Experimental and predicted $^{13}$C NMR data for compounds 141 and 141A

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In the $^1$H NMR spectrum of compound 141 the 3''-CH$_2$ protons and 2'-H aromatic proton resonate as singlets at 4.00 ppm and 8.32 ppm, respectively in the experimental spectrum while Modgraph predicts them to be at 4.34 ppm and 8.58 ppm. The signals for the C-8' and carbonyl $^{13}$C signals observed at 139.4 ppm and 166.8 ppm are predicted to be at 139.4 ppm and 167.5 ppm by Modgraph and at 147.9 ppm and 166.4 ppm respectively by ChemWindow. The ChemWindow $^{13}$C value for C-8' is clearly not

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Discussion
comparable with the Modgraph and experimental data values. The ChemWindow values for the tetrahydrofuran carbons, C-2, C-3, C-4 and C-5, similarly exhibit large discrepancies from the experimental values. In compound 141A, the di-acylated derivative, the 3''-H and 2'''-H protons resonate at 4.01 ppm and 4.68 ppm respectively and are predicted by Modgraph to be resonate at 4.34 ppm and 3.63 ppm respectively. The C-3'' carbon signal at 40.5 ppm is predicted to resonate at 40.6 ppm and 48.3 ppm by Modgraph and ChemWindow respectively while the C-2'' signal at 44.6 ppm is predicted by Modgraph and ChemWindow to resonate at 43.8 ppm and 48.6 ppm. In fact, going down Table 4, the discrepancies between the ChemWindow values, on one hand, and the Modgraph and experimental values, on the other, continue to be significant; in some cases, the ChemWindow values are over-estimated by up to 9 ppm or under-estimated by up to 14 ppm.

2-(6-Aminopurin-9-yl)-5-acetoxyacetyl-3,4-dihydroxytetrahydrofuran 3,4-acetonide 140 and 2-[6-(acetoxyacetamino)purin-9-yl]-5-acetoxyacetyl-3,4-dihydroxytetrahydrofuran 3,4-acetonide 140A

There is a generally good corellation between the experimental assignments and the Modgraph $^{13}$C predict values for compounds 140 and 140A (Table 5). ChemWindow values however show considerable differences from the other two values in some instances as indicated by the highlighted data. Again the discrepancies are far greater for the ChemWindow data which over-estimates by as much as 8 ppm and under-estimates by as much as 13 ppm.
### Table 5. Experimental and predicted $^{13}$C NMR data for compounds 140 and 140A

<table>
<thead>
<tr>
<th>140</th>
<th>140A</th>
</tr>
</thead>
<tbody>
<tr>
<td>13C nuclei</td>
<td>a</td>
</tr>
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<td>7''</td>
<td>20.4</td>
</tr>
<tr>
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</tr>
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<td>C.CH$_{3}$</td>
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<td>4''</td>
<td>60.5</td>
</tr>
<tr>
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<td>4</td>
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<td>90.9</td>
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<tr>
<td>C(CH$_{3})$_2</td>
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</tr>
<tr>
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<td>120.4</td>
</tr>
<tr>
<td>8'</td>
<td>139.8</td>
</tr>
<tr>
<td>4'</td>
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<tr>
<td>2'</td>
<td>153.2</td>
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<td>6'</td>
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<td>3'</td>
<td>167.4</td>
</tr>
<tr>
<td>6'</td>
<td>170.3</td>
</tr>
<tr>
<td>C(CH$_{3})$_2</td>
<td>114.9</td>
</tr>
<tr>
<td>5</td>
<td>122.0</td>
</tr>
<tr>
<td>8'</td>
<td>142.7</td>
</tr>
<tr>
<td>4</td>
<td>148.7</td>
</tr>
<tr>
<td>2'</td>
<td>150.7</td>
</tr>
<tr>
<td>6'</td>
<td>152.3</td>
</tr>
<tr>
<td>1''</td>
<td>167.3</td>
</tr>
<tr>
<td>3''</td>
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<td>4''</td>
<td>170.3</td>
</tr>
<tr>
<td>6''</td>
<td>170.3</td>
</tr>
</tbody>
</table>

a = Experimental values, b = Modgraph predict, c = ChemWindow predict

#### 2.5.2 NMR chemical shift prediction for adenine analogues

**6-(N-Allylamino)purine 150 and 1-allyl-[6-amino(N-allylamino)]purine 150A**

![Diagram of 6-(N-Allylamino)purine 150](image1)

![Diagram of 1-allyl-[6-amino(N-allylamino)]purine 150A](image2)

The chemical shift predictions for the 1'-methylene carbons in compound 150 are quite different; Modgraph under-estimates by 4 ppm and ChemWindow over-estimates by 9 ppm. The predictions for the C-3' carbon compare well with each other.
(ca. 114 ppm) but both under-estimate the experimental value by 5 ppm. The experimental assignments for the pyrimidine carbons are observed to correlate well with the Modgraph prediction values (Table 6). The predictions for the C-3' and the C-3" methylene carbons in compound 150A correlate reasonably with experimental values but, generally, the ChemWindow values tend to be considerably less accurate.

Table 6. Experimental and predicted $^{13}$C NMR data for compounds 150 and 150A

<table>
<thead>
<tr>
<th>$^{13}$C nuclei</th>
<th>150</th>
<th>150A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>1'</td>
<td>46.1</td>
<td>42.0</td>
</tr>
<tr>
<td>3'</td>
<td>119.6</td>
<td>114.8</td>
</tr>
<tr>
<td>5</td>
<td>120.4</td>
<td>126.2</td>
</tr>
<tr>
<td>2'</td>
<td>131.2</td>
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<td>144.2</td>
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<td>150.0</td>
</tr>
</tbody>
</table>

a = Experimental values, b = Modgraph predict, c = ChemWindow predict

2.5.3 NMR chemical shift prediction for truncated adenine analogues

While the discussion in Sections 2.5.1 and 2.5.2 has focused on an assessment of the relative accuracy of the two prediction approaches, Sections 2.5.3 – 2.5.5 will illustrate their application in supporting structural assignments.
Discussion

3-Hydroxyphenyl acrylate 192 and 3-(acyloyloxy)phenyl acrylate 192A

Table 7. Experimental and predicted $^{13}$C NMR data for compounds 192 and 192A

<table>
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<th>b</th>
<th>c</th>
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<td>154.5</td>
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</tr>
<tr>
<td>C=O</td>
<td>164.0</td>
<td>164.8</td>
<td>162.0</td>
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</table>

<table>
<thead>
<tr>
<th>$^{13}$C nuclei</th>
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<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
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<td>113.5</td>
<td>114.3</td>
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<tr>
<td>4</td>
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<td>1'C=O</td>
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<tr>
<td>1''C=O</td>
<td>164.1</td>
<td>164.8</td>
<td>162.0</td>
</tr>
</tbody>
</table>

a = Experimental values, b = Modgraph predict, c = ChemWindow predict

There is a generally good correlation between the experimental assignment, the Modgraph predict values and the ChemWindow $^{13}$C values for compound 192, with the C-1 quartenary and C-2' methine carbons experimental assignments varying from prediction values by ca. 3 ppm (Table 7). Similar patterns are observed for compound 192A, with the C-1 and C-3 quartenary carbons being indistinguishable and, hence, having the same chemical shifts and confirming formation of the di-substituted product.
N- Allyl-3-hydroxyaniline 194, N-allyl-3-allyloxyaniline 194A and N,N-diallyl-3-hydroxyaniline 194B

Comparison of the 1'- and 1''- methylene assignments was used to establish the position of allylation in compounds 194, 194A and 194B. In all the compounds the experimental 1'-methylene carbon shifts compare very favourably with the Modgraph values (Tables 8 and 9); ChemWindow, however, over-estimates the chemical shifts by ca. 8 ppm. In compound 194A, the 1''-methylene carbon resonates at the higher chemical shift value of 68.6 ppm (Modgraph 69.0 ppm), consistent with acylation of the phenolic group. The aromatic carbon predictions for these compounds generally show good correlation with the experimental assignments as indicated in the Tables.
Table 8. Experimental and predicted $^{13}$C NMR data for compounds 194 and 194A

<table>
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<tr>
<th></th>
<th>195</th>
<th>195A</th>
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</tr>
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</tbody>
</table>

a = Experimental values, b = Modgraph predict, c = ChemWindow predict

Table 9. Experimental and predicted $^{13}$C NMR data for compound 194B

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<tr>
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</tr>
<tr>
<td>1''-CH$_2$</td>
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<td>59.6</td>
</tr>
<tr>
<td>2</td>
<td>99.6</td>
<td>151.1</td>
<td>100.3</td>
</tr>
<tr>
<td>6</td>
<td>103.5</td>
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<td>3''</td>
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<td>114.9</td>
</tr>
<tr>
<td>5</td>
<td>129.9</td>
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<tr>
<td>2'</td>
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<td>2''</td>
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<td>156.4</td>
<td>160.2</td>
<td>158.2</td>
</tr>
</tbody>
</table>
a = Experimental values, b = Modgraph predict, c = ChemWindow predict

N-Allyl-3-(hydroxymethyl)aniline 201, N-allyl-3-[(allyloxy)methyl]aniline 201A,
N,N-di-allyl-3-(hydroxymethyl)aniline 201B and 3-[(allyloxy)methyl]aniline 201C

\[
\begin{align*}
N & \text{Allyl-3-(hydroxymethyl)aniline} & 201 \\
N & \text{Allyl-3-[(allyloxy)methyl]aniline} & 201A \\
N,N & \text{di-Allyl-3-(hydroxymethyl)aniline} & 201B \\
3 & \text{-[(allyloxy)methyl]aniline} & 201C
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \\
\text{3} & \text{2} & \text{1} & \text{NH} & \\
\text{4} & \text{5} & \text{6} & \text{1' & 2'} & \text{3'}
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \\
\text{3} & \text{2} & \text{1} & \text{NH} & \\
\text{4} & \text{5} & \text{6} & \text{1' & 2'} & \text{3'}
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \\
\text{3} & \text{2} & \text{1} & \text{NH} & \\
\text{4} & \text{5} & \text{6} & \text{1' & 2'} & \text{3'}
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \\
\text{3} & \text{2} & \text{1} & \text{NH} & \\
\text{4} & \text{5} & \text{6} & \text{1' & 2'} & \text{3'}
\end{align*}
\]

**Table 10.** Experimental and predicted $^{13}$C NMR data for compounds 201 and 201A

<table>
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</tr>
<tr>
<td>3</td>
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<td>143.5</td>
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</table>

<table>
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<th>13C nuclei</th>
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<th>c</th>
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<td>135.3</td>
<td>134.6</td>
<td>134.3</td>
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<tr>
<td>3</td>
<td>139.4</td>
<td>142.2</td>
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</tr>
</tbody>
</table>
Comparison of the experimental and predicted 1'- and 1''- methylene $^{13}$C chemical shifts was also used to establish the position of allylation in compounds 201, 201A, 201B and 201C (Tables 10 and 11). The Modgraph predicted values, in particular, correlate closely with the assignment of experimental data. Thus in compound 201, the signals at 46.5 and 65.6 ppm correspond to the $N$-allyl- and hydroxymethylene carbons, respectively. Allylation of the hydroxyl group (compound 201A) results in a downfield shift of the latter carbon signal to 72.3 ppm. In the $N,N$-di-allylated product 201B, both $N$-allyl carbons resonate at 52.7 ppm – a signal which is absent resonate in the spectrum of the $O$-allylated product 201C.
2.5.4 NMR chemical shift prediction for allopurinol-derived Baylis-Hillman analogues

1-(6-Bromo-3-methyl-2H-chromen-2-one)-4-(tetrahydropyran-2-yloxy)pyrazolo[3,4-d]pyrimidine 170A

Table 12. Experimental and predicted $^{13}$C NMR data for compound 170A

<table>
<thead>
<tr>
<th>$^{13}$C nuclei</th>
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<th>b</th>
<th>c</th>
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<td>4'</td>
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<td>3'</td>
<td>29.3</td>
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<td>32.7</td>
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<tr>
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<td>57.1</td>
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<td>C=O</td>
<td>160.7</td>
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Comparison of the experimental and Modgraph predicted data for the \(N\)-methylene carbon was used to confirm the formation of the coumarin **170A**. If the chromene had been formed, the methylene carbon would have been in the lactone ring and resonated at a higher chemical shift (> 65 ppm; Modgraph prediction: 68.7 ppm). There is a good correlation between the experimental assignment and the Modgraph predicted values for most of the pyran carbons except for the C-2\(^{\prime}\) carbon. Some of the purine and coumarin ring predictions, however, show significant differences from the experimental data due, perhaps, to mutual shielding or deshielding of the linked, magnetically anisotropic aromatic systems.

### 2.5.5. NMR chemical shift prediction for 3-amino- and 3-hydroxybenzoic acid derivatives 208, 208A, 210 and 210A

![Chemical structures](image1)

Tables 13 and 14 show the agreement between the \(^{13}\)C NMR experimental assignments and the predictions for the 1\(^{\prime}\)-CH\(_2\) and 1\(^{\prime}\)\(^{\prime}\)-CH\(_2\) carbons as well as other carbon atoms in each of the mono- and di-allylated products 208, 208A, 210 and 210A. The data also confirms the structures proposed for the respective compounds. The argument is similar to that for compounds 194, 194A and 194B, a critical point being the resonance of the 1\(^{\prime}\)-CH\(_2\) group at ca. 65 ppm and the ester carbonyl carbon at ca. 166 ppm in each of the allyl esters 208, 208A and 210A.
Table 13. Experimental and predicted $^{13}$C NMR data for compounds 208 and 210

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a = Experimental assignment, b = Modgraph prediction, c = ChemWindow prediction

Table 14. Experimental and predicted $^{13}$C NMR data for compounds 208A and 210A.

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a = Experimental assignment, b = Modgraph prediction, c = ChemWindow prediction

2.6. Computer Modelling of ATP Mimics

A vital tool in rational drug design is the use of computer modelling to explore enzyme-receptor interactions. A number of successes have been recorded in this rational approach to drug discovery in finding therapeutic agents such as ritonavir and its analogues that are administered to AIDS patients\textsuperscript{191} and relenza\textsuperscript{192} for treating flu. Computer modelling at the molecular mechanics level has been used to explore the GS receptor-binding potential of compounds prepared in this study. The first step was to find the global minima of selected deprotected ATP analogues by subjecting each to a molecular dynamics routine, the assumption being that, \textit{in vivo}, accessible ester moieties would be hydrolysed prior to binding. The most stable conformation, in each case, was subjected to various docking options in the receptor cavity of glutamine synthetase \textit{in silico}, each option involving a different orientation and/or conformational change in the ligand. The crystal structure data for 1F52, the glutamine synthetase of \textit{Salmonella typhimurium} co-crystallized with ADP reported by Eisenberg and co-workers (Figure 92)\textsuperscript{193,194,195} was used for this study. The relevant residues with their catalytic roles both in the biosynthetic reaction as well as the allosteric control of the enzyme by adenylylation, as identified by Eisenberg, have been confirmed, \textit{in silico} and are summarized below.

2.6.1. Docking of ATP and ATP mimics into the MTB-GS receptor site

The computer modelling studies of the selected, synthesized ATP mimics were conducted on the ACCELERYS Cerius\textsuperscript{2} platform. The OFF METHODS and OFF SET UP modules were used for the conformational search while the LIGANDFIT module was used for receptor-docking. A reconstructed model of the crystal structure of MTB-GS determined by Eisenberg and co-workers was used and attention was focused on the ‘active site’ which is a region defined by amino acid residues within 5Å of the ATP ligand site on GS. The divalent metal ions (Mg\textsuperscript{2+} or Mn\textsuperscript{2+}) were adequately taken into consideration because of the vital role they play in phosphoryl transfer during nitrogen biosynthesis. Particular attention was given to residues which might interact with ATP, and we used the B-subunit (in light brown colour) of the
1F52 *Salmonella* GS, which contains one ATP binding site (Figure 93). The conformational homology and ‘active-site’ docking of ATP and selected mimics will be illustrated in the following sub-sections.

![Figure 92](http://www.rcsb.org/pdb/explore.do?structureId=1F52)

**Figure 92.** Crystal structure of 1F52, the glutamine synthetase of *Salmonella typhimurium* co-crystallized with ADP. The structure is made up of 25 chains containing 9180 residues. Downloaded from the pdb site at [http://www.rcsb.org/pdb/explore.do?structureId=1F52](http://www.rcsb.org/pdb/explore.do?structureId=1F52).

The following interactions were observed between ADP and amino acid residues in the ATP site in the B-subunit (Figure 94).

i. Ser 273 acting as donor in hydrogen-bonding to the amino group on adenine;

ii. Glu 129 interacting with ribose of ADP *via* donor hydrogen-bonding;
Figure 93. Reconstructed 1F52 *Salmonella typhimurium* GS with A-subunit (in light blue), B-subunit (in light brown), G-subunit (in yellow) and H-subunit (in light-green), showing ADP molecules in the ATP sites and AMP molecules in the adenylylation sites.

iii. Thr 223 interacting with ribose hydroxyl group *via* donor hydrogen-bonding;

iv. Arg 355 acting as an acceptor *via* hydrogen-bonding with an oxygen of the β-phosphate group and the pyrimidine ring of ADP;

v. His 271, Arg 344, His 210 and Glu 220 interacting with the phosphate groups of ADP *via* acceptor hydrogen-bonding.
2.6.1.1. Modelling of ATP in the GS active site

ATP plays a vital role in biosynthesis and also in the covalent modification of GS by adenylylation, and it is important to explore its interaction with the active site in silico since no X-ray structure is available of ATP-bound GS. Hence, the global minimum of ATP was found by conducting a molecular dynamics conformational search at 600K. ATP was then docked into the ‘B-sub-unit’ active site of GS (Figure 93) and its interaction with active-site residues and its binding potential were analyzed. The non-bonded intermolecular interaction between the ligand and protein is measured in terms of the ‘Dock score’. The ‘Ligscore’ is a measure of the protein-ligand interaction,
while ‘vdW’ is the Leonard-Jones soft potential. Table 15 shows the scores for the best twenty, bound conformations of ATP in descending order of the ‘dock score’. There are few differences between the interactions of ATP with active-site amino acid residues compared with ADP, but it was observed that ATP interacts with Lys 354 via acceptor hydrogen-bonding and with Arg 354 via donor hydrogen-bonding – interactions not exhibited by ADP. Figure 95 shows the interaction of ATP with active site residues.

Figure 95. ATP interaction with amino acid residues within 3Å in the ‘active-site’ of GS, showing potential hydrogen-bonds in yellow, dashed lines.
Table 15. Summary of scores of the docking of ATP into the ‘B-subunit’ GS active-site.

<table>
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<tr>
<th>Conformer</th>
<th>Dock score (kcal/mol)</th>
<th>Ligscore (kcal/mol)</th>
<th>vDW energy (kcal/mol)</th>
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<td>1</td>
<td>87.41</td>
<td>-3.06</td>
<td>58.10</td>
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<tr>
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2.6.1.2. **Computer modelling of adenosine, adenine and allopurinol derivatives in the GS active site**

The homology of selected synthetic ATP mimics and their interaction with active-site residues were explored. A ‘target model’ alignment of each of the mimics with ATP was conducted using the DRUG DISCOVERY module in Cerius². The global minimum conformation of each of the analogues was aligned with ATP to check their *in vacuo* similarities with ATP. Docking of the energy-minimized mimics into the active-site of the enzyme *in vacuo* was undertaken and docking scores were examined to assess binding with the amino acid residues in the GS ‘active-site’ (Table 16).

**Table 16.** Scores for the best binding conformations of ATP and selected adenosine, adenine and allopurinol derivatives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dock score (kcal/mol)</th>
<th>Ligscore (kcal/mol)</th>
<th>vdW (kcal/mol)</th>
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</table>

The ‘target model’ alignment of compound 132 with ATP (in yellow) shown in Figure 96 illustrates good structural homology and the ligand 132 was found to have common interactions with amino acid residues in common with those exhibited by ADP and ATP (Figure 98). In addition, the following interactions were observed.

i. Lys 352 interacts with the 6-amino group *via* donor hydrogen-bonding;

ii. Asn 280 and Arg 354 interacts with the 6-amino group *via* acceptor hydrogen-bonding;

iii. Mn 469 was found to be at a distance of 2.64Å to the terminal acetyl group of ligand 132.

Figure 97 shows the alignment of the binding conformation of ATP with the binding conformation of ligand 132. While compound 132 exhibits good homology with ATP
when comparing both global minima (Figure 96) and fair homology when comparing binding conformations (Figure 97), it has a very low ‘dock score’.

**Figure 96.** ‘Target model’ alignment of ATP (in yellow) with ligand 132.

**Figure 97.** ‘Binding conformation’ alignment of ATP with ligand 132 (in yellow).
Figure 98. Interaction of ligand 132 with active-site-residues within 3Å in the active-site of GS, showing potential hydrogen-bonds in yellow, dashed lines.

On the other hand, although the global minimum and bound c onformations of compound 138 show poor structural homology with ATP (Figures 99 and 100), the ligand appears to bind very well to the active-site of the protein! Its dock score of 91.09 kcal/mole is better than the value for ATP and is an indication of high affinity for the GS active site. Compound 138 appears to interact (Figure 101) with almost all the amino acid residues that ATP interacts with and exhibits the following additional interactions: - (i) acceptor hydrogen-bond interaction via one of the amino hydrogens
Discussion

on adenine with Lys 352; (ii) a donor hydrogen-bond interaction between Gly 127 and the ribose moiety; and (iii) the side-chain that contains the alkyl phosphate group interacts with Ile 345 via an acceptor hydrogen-bond. The metal atoms, however, are too far off in this case to have any significant interaction with the ligand.

Figure 99. ‘Target model’ alignment of ATP with ligand 138 (in yellow).
Figure 100. ‘Binding conformation’ alignment of ATP with ligand 138 (in yellow).

Figure 101. Interaction of ligand 138 with active-site-residues within 3Å in the active-site of GS, showing potential hydrogen-bonds in yellow, dashed lines.

2.6.1.3. Computer modelling of truncated adenine derivatives in the active site of GS

Molecular dynamics to find the global minimum conformation was conducted for each of the truncated mimics listed in Table 17 and their alignment with ATP explored. While the global minimum conformations of the truncated systems exhibit fair to poor structural homology with ATP, several appear to interact with the GS active-site residues via both acceptor and donor hydrogen-bonds, indicating their
binding potential. Figures 102 and 103 illustrate the ‘target model alignment’ and the ‘binding conformation’ alignment of compound 203 and ATP, while Figure 104 summarises the interaction of the ligand 203 with active-site residues. It is interesting to note the significant conformational differences between the global-minimum (Figure 102) and bound conformations (Figure 103) of the ligand 203.

**Table 17.** Comparison of the ligands scores of truncated adenine mimics with ATP

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dock score (kcal/mol)</th>
<th>Ligscore (kcal/mol)</th>
<th>vDW (kcal/mol)</th>
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<td>9.07</td>
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</table>
Discussion

**Figure 102.** ‘Target model’ alignment of ATP with ligand 203 (in yellow).

**Figure 103.** ‘Binding conformation’ alignment of ATP with ligand 203 (in yellow).
**Figure 104.** Interaction of ligand 203 with active-site-residues within 3Å in the active-site of GS, showing potential hydrogen-bonds in yellow, dashed lines.

Figures 105 and 106 illustrate the ‘target model’ alignment and the ‘binding conformation’ alignment of compound 196 with ATP, while Figure 107 summarises the interaction of the ligand 196 with active-site residues. While the global minimum conformations (Figure 105) exhibit poor structural homology, the alignment of the binding conformations (Figure 106) is clearly much better.
**Figure 105.** ‘Target model’ alignment of ATP with ligand **196** (in yellow).

**Figure 106.** ‘Binding conformation’ alignment of ATP with ligand **196** (in yellow).
Figure 107. Interaction of ligand 196 with active-site-residues within 3Å in the active-site of GS, showing potential hydrogen-bonds in yellow, dashed lines.

The *in silico* data obtained thus far suggest a measure of complementarity between various synthetic ligands and the GS-ATP active-site and some degree of conformational homology with ATP. Issues of enzyme inhibition and selectivity, aqueous solubility, and free energies of solvation/desolvation and binding have yet to be explored. However, on the basis of modelling data, it seems that a number of the ATP mimics have potential as GS inhibitors and will be investigated further.
2.7. Conclusions

This research has formed part of a collaborative programme aimed at developing glutamine synthetase inhibitors as potential therapeutics for TB – a disease for which drug resistance to current treatment regimens makes it an even greater threat to life.

A mechanism-based approach to drug discovery has been pursued in attempts to discover better drugs. Hence, attention has been focused on synthesizing novel ATP mimics that can act as inhibitors of bacterial glutamine synthetase – an enzyme that is essential for the virulence of *Mycobacterium tuberculosis*, the organism that causes tuberculosis. Consideration has been given to issues of structural homology in designing potential ATP mimics. Initially various purine derivatives were identified as scaffolds for the construction of novel ATP analogues and a range of adenosine, adenine and allopurinol derivatives have been prepared as primary targets *via* alkylation and acylation reactions. Attention was then turned to developing “truncated” ATP analogues and a range of 3-aminobenzonitrile, 3-aminophenol, resorcinol, 3-aminobenzyl alcohol, 3-hydroxybenzoic acid and 3-aminobenzoic acid derivatives have been successfully prepared. A number of alkylating and acylating agents have also been prepared and then reacted, with mixed success, with the purine substrates and the truncated analogues.

Allylation of the hydroxyl, amino or N-9 centres of the various substrates was achieved readily in low to high yield (10-87%). Mono- and di-substituted derivatives were isolated in almost all of these allylation reactions, but attempted dihydroxylation of the resulting terminal alkenes of the mono-allylated derivatives with CTAP, KMnO₄ and NaIO₄ in the presence of RuCl₃ were frustratingly unsuccessful; in one case, attempted epoxidation with *meta*-chloroperbenzoic was also unsuccessful.

Alkyations of the N-9 nitrogen of protected allopurinol with specially prepared Baylis-Hillman products, *tert*-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylene propanoate, *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate and 6-bromo-3-(chloromethyl)chromen-2-one were successful and provided access to interesting products.
Acylations were generally successful with substrates having free hydroxyl and/or amino groups but not at the N-9 nitrogen of adenine or protected allopurinol. Mono- and di-acylations were recorded in almost all cases except with 2,3,4,5,6-pentaacetylgluconoyl chloride probably because of steric hindrance in the mono-acylated product. Mono-acylation of resorcinol however, proved difficult as the second hydroxyl group becomes more susceptible to acylation as a result of increased acidity. While tin tetrachloride-catalyzed glycosylation of adenine with acetylated glucose in an inert atmosphere of argon was successfully achieved, the corresponding reaction with 3-aminobenzonitrile as substrate was, surprisingly, unsuccessful. Phosphorylation of a number of hydroxy and chloro derivatives was effected using diethyl chlorophosphate and triethyl phosphite, respectively.

One- and two-dimensional NMR spectroscopic methods were used extensively to confirm the chemical structures of synthesized compounds. Experimental chemical shift data were compared with predicted values using NMR prediction programmes and this approach proved useful in assigning structures in cases of competing reaction centres as well with isomeric di-substituted products.

Molecular modelling with the ACCELERYS Cerius² platform was used to conduct in silico receptor-ligand docking in the ‘active site’ of bacterial glutamine synthetase in order to identify potential glutamine synthetase inhibitors. The modelling results certainly indicate the binding potential of certain ligands, in silico at least.

The results of this study have provided a basis for future research and for addressing challenges such as:

i. facilitating N-9 allylation and acylation in purine systems;

ii. effective perhydroxylation of various α,β-unsaturated carbonyl derivatives; and

iii. achieving better regio-control in reactions of bifunctional substrates.
3. EXPERIMENTAL

Reagents were supplied by manufacturers. Solvents such as DMF, THF, actonitrile, pyridine, etc were redistilled by literature methods before use. Dry reactions were carried out in an inert atmosphere either under argon or nitrogen. Plastic TLC plates as supplied by Merck – (precoated with silica gel 60 F254) were used for thin layer chromatography and visualization effected under UV light. Silica gel 60 (particle size 0.040-0.063 nm) was employed as stationary phase for flash column chromatography while silica gel 60 PF254 was employed as stationary phase for radial chromatography. For normal phase HPLC a Whatman Partisol\textsuperscript{10} semi-preparative column was used, while a Phenomenex C-18 LUNA semi-preparative column was used for reverse phase HPLC.

\(^1\)H, \(^{13}\)C and \(^{31}\)P NMR experiments were conducted on Bruker AVANCE 400 MHz and 600 MHz spectrometers. Chemical shifts were reported relative to the solvent peaks i.e. \(\delta_H : 7.25\) ppm for CDCl\(_3\), \(4.81\) for D\(_2\)O, \(3.30\) for CD\(_3\)OD, \(2.50\) ppm for DMSO-\(d_6\), and \(20.5\) ppm for (CD\(_3\))\(_2\)C=O; \(\delta_C : 77.0\) ppm for CDCl\(_3\), \(49.0\) ppm for CD\(_3\)OD, \(39.4\) ppm for DMSO-\(d_6\) and \(30.8\) ppm for (CD\(_3\))\(_2\)C=O.

Low resolution mass spectra recorded on a Finnigan-MAT GCQ mass spectrometer while high resolution mass spectrometric data was obtained by Martin Brits using APCI (Atmospheric Pressure Chemical Ionization) and Tommie van de Walt (University of Witwatersrand Mass Spectrometry Unit).

Mid-IR spectra recorded on Perkin-Elmer spectrum 2000FT-IR spectrometer as thin films and solid deposits between CsI discs.

Melting points were determined on a Reichert hot-stage apparatus and Gallenkamp melting point apparatus and are not corrected.
Adenosine 3',4'-acetonide 127

A solution of adenosine 62 (51 mg, 0.19 mmol) and p-toluenesulphonic acid (150 mg, 0.79 mmol) in acetone (20 mL) was stirred at room temperature overnight. The reaction was quenched with aqueous Na₂CO₃ (10%, 7 mL). The solvent was removed in vacuo and the aqueous residue extracted with CH₂Cl₂ (3 × 10 mL). The combined organic extracts were dried (anhdr. MgSO₄) and evaporated in vacuo to afford adenosine 3',4'-acetonide 127 as a white powder, (55 mg, 93%), m.p. 215-216°C, (Found: [M+H]⁺, 308.14706. C₁₃H₁₇N₅O₄ requires: M⁺, 307.30518); νmax (solid deposit/cm⁻¹) 3256 (OH), 3095 (NH₂); δH/ppm (400MHz; CDCl₃) 1.36 and 1.64 (6H, 2 × s, 2 × C₃H₃), 3.78 (1H, dd, J = 12.8 and 1.9 Hz, CH₃OH), 3.96 (1H, dd, J = 12.8 and 1.4 Hz, CH₂OH), 4.53 (1H, d, J = 1.1 Hz, 4'-H), 5.10 (1H, dd, J = 5.9 and 0.9 Hz, 5'-H), 5.19 (1H, t, J = 5.4 Hz, 3'-H), 5.85 (1H, d, J = 4.9 Hz, 2'-H), 5.94 (2H, s, NH₂), 7.84 (1H, s, 8- H) and 8.30 (1H, s, 2- H); δC/ppm (100MHz; CDCl₃) 25.2 and 27.6 (2 × CH₃), 63.4 (CH₂), 81.7 (C-5'), 83.0 (C-3'), 86.2 (C-4'), 94.3 (C-2'), 114.0 [C(CH₃)₂], 121.0 (C-5), 140.4 (C-8), 148.4 (C-4), 152.2 (C-2) and 155.7 (C-6).

2,3,4,6-Tetraacetylgluconic acid 129
Crushed zinc chloride (8.2 g, 0.060 mol) was dissolved in acetic anhydride (100 mL). The temperature of this solution was reduced to 0°C, and D-glucono-D-lactone (20 g, 0.11 mol) was added slowly with stirring. The reaction mixture was stirred at 0°C for 1 h, and then at room temperature for 24 h, before adding water (400 mL) and stirring for a further 1 h. The product which crystallized out on standing at 0°C for 24 h was 2,3,4,6-tetraacetyl-gluconic acid 129 as white crystals (8.2 g, 20%), m.p. 84-88°C (lit.165 113-117°C); \( \nu_{\text{max}} \) (solid deposit/cm\(^{-1}\)) 3409 (OH) and 1735 (C=O); \( \delta_{\text{H}}/\text{ppm} \) [600 MHz; \((\text{CD}_3)_2\text{C}=\text{O}\)] 2.00 (3H, s, 6-\(\text{CH}_3\)), 2.03 (3H, 2 \times s, 3-\(\text{CH}_3\) and 4-\(\text{CH}_3\)), 2.10 (3H, s, 2-\(\text{CH}_3\)), 3.98 (1H, dd, \(J = 12.0\) and 4.8 Hz, 5-H), 4.08 (2H, d, \(J = 4.9\) Hz, 6-H), 4.72 (1H, d, \(J = 0.5\) Hz, 5-OH), 5.28 (1H, dd, \(J = 7.6\) and 5.0 Hz, 4-H), 5.33 (1H, d, \(J = 3.6\) Hz, 2-H) and 5.78 (1H, dd, \(J = 4.9\) and 3.7 Hz, 3-H); \( \delta_{\text{C}}/\text{ppm} \) [150 MHz; \((\text{CD}_3)_2\text{C}=\text{O}\)] 21.4 (6-O\(\text{C}=\text{O.}\text{CH}_3\)), 21.5 and 21.7 (3-O\(\text{C}=\text{O.}\text{CH}_3\) and 4-O\(\text{C}=\text{O.}\text{CH}_3\)), 21.8 (2-\(\text{CH}_3\)), 66.7 (C-6), 69.7 (C-5), 71.4 (C-2), 72.8 (C-3), 73.0 (C-4), 169.5 (C=O), 171.0 (2-C=O), 171.17 (3-C=O), 171.20 (4-C=O) and 171.9 (6-C=O).

2,3,4,5,6-Pentaacetylgluconic acid 130

2,3,4,6-Tetraacetyl-gluconic acid (3.0 g, 9.6 mmol) was slowly added to a chilled solution (0°C) of zinc chloride (1.3 g, 9.5 mmol) in acetic anhydride (13 mL, 0.14 mol) in a 50 mL three necked round bottomed flask equipped with a condenser and a CaCl\(_2\) guard tube. The reaction mixture was kept at 0°C for 1 h and then stirred at room temperature for 24 h. The reaction was quenched with water (70 mL). The aqueous reaction mixture was extracted with CHCl\(_3\) (4 × 25 mL). Toluene (75 mL) was added to the combined extract and the volume reduced to 75 mL in vacuo. More toluene (75 mL) was added and the volume reduced to 12 mL. Pure 2,3,4,5,6-Pentaacetylgluconic acid 130 crystallized out at 0°C as white crystals (3.0 g, 77%), m.p. 108-112 °C (lit.165 110-111°C); \( \nu_{\text{max}} \) (solid deposit/cm\(^{-1}\)) 3445 (OH) and 1747 (C=O); \( \delta_{\text{H}}/\text{ppm} \) (400MHz; CDCl\(_3\)) 2.05 (12H, m, 4 \times OAc), 2.18 (3H, s, 6-OAc), 4.11 (1H, dd, \(J = 12.2\) and 5.5 Hz, 6-\(\text{H}_a\)), 4.29 (1H, dd, \(J = 12.3\) and 4.0 Hz, 6-\(\text{H}_b\)), 5.06
(1H, dd, \( J = 10.3 \) and 5.6 Hz, 5–H), 5.29 (1H, d, \( J = 3.8 \) Hz, 2–H), 5.49 (1H, m, 4–H), 5.61 (1H, m, 3–H), and 6.58 (1H, b, OH); \( \delta_C/\text{ppm} \) (100MHz; CDCl3) 20.3, 20.4, 20.6, 20.65 and 20.7 (5 × OAc), 61.4 (C–6), 68.49 (C–3), 68.51 (C–5), 69.3 (C–4), 70.1 (C–2), 169.76 (C–1), 169.83 (C–5’), 169.91 (C–4’), 169.94 (C–3’), 170.4 (C–2’) and 170.7 (C–6’); \( m/z = 406 \) (M\(^+\), 0.52%) and 43 (100%).

2,3,4,5,6-pentaacetylgluconoyl chloride 131

\[
\begin{array}{c}
\text{AcO} \\
5
\end{array}
\begin{array}{c}
\text{OAc} \\
4
\end{array}
\begin{array}{c}
\text{OAc} \\
3
\end{array}
\begin{array}{c}
\text{OAc} \\
2
\end{array}
\begin{array}{c}
\text{Cl} \\
1
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\]

To a solution of 2,3,4,5,6-pentaacetylgluconic acid 130 (1.0 g, 2.5 mmol) in a dry 50 mL round bottomed flask under nitrogen was added oxalyl chloride (0.55 mL, 6.3 mmol) through a septum. There was immediate effervescence and the product precipitated out within 30 minutes. Excess oxalyl chloride was evaporated \textit{in vacuo} to afford 2,3,4,5,6-pentaacetylgluconoyl chloride 131 as a white precipitate (0.52 g, 50%), m.p. 42-44°C (lit. 68-71°C); \( \nu_{\text{max}} \) (solid deposit/cm\(^{-1}\)) 1748 (C=O); \( \delta_H/\text{ppm} \) (400MHz; CDCl3) 2.02-2.10 (12H, overlapping singlets, 4 × CH\(_3\)), 2.19 (3H, s, 6-OAc), 4.12 (1H, dd, \( J = 12.2 \) and 5.5 Hz, 6-H\(_b\)), 4.30 (1H, dd, \( J = 12.3 \) and 4.1 Hz, 6-H\(_b\)), 5.06 (1H, dt, \( J = 5.6 \) and 4.2 Hz, 5-H), 5.31 (1H, d, \( J = 3.9 \) Hz, 2-H), 5.50 (1H, dd, \( J = 6.4 \) and 5.0 Hz, 4-H) and 5.62 (1H, dd, \( J = 4.9 \) and 3.9 Hz, 3-H); \( \delta_C/\text{ppm} \) (100MHz; CDCl3) 20.3, 20.4, 20.6, 20.66 and 20.7 (5 × CH\(_3\)), 61.5 (C-6), 68.5 (C-3), 68.55 (C-5), 69.3 (C-4), 70.0 (C-2), 169.7 (C-1), 169.76 (C-6-C=O), 169.8 (4-O,C=O), 169.86 (3-O,C=O), 170.1 (2-O,C=O) and 170.6 (6-O,C=O); \( m/z = 424 \) (M\(^+\), 0.08%) and 361 (100%).
A solution of adenosine 3′,4′-acetonide (0.14 g, 0.47 m mol) and 2,3,4,5,6-pentaacetylgluconoyl chloride (0.20 g, 0.47 mmol) in pyridine (4 mL) was stirred for 36 h. The solvent was evaporated in vacuo and the residue dissolved in CHCl₃ (30 mL). The solution was washed with 5% aq. NaHCO₃ (2 × 30 mL), water (2 × 30 mL) and brine (2 × 30 mL). The organic layer was then dried (anhyd. MgSO₄) and the solvent was evaporated in vacuo and the yellow residue was chromatographed [radial chromatography; elution with EtOAc] to afford 2-(6-aminopurin-9-yl)-3,4-dihydroxy-5-[(2,3,4,5,6-pentaacetoxyhexanoyloxy)methyl]tetrahydrofuran-3,4-acetonide 132 as a yellow oil (46 mg, 14%), ν max (thin film/cm⁻¹) 3345 (NH₂), 1749 (C=O) and 1598 (aromatic C=N) and 1216 (C–N); δH/ppm (400MHz; CDCl₃) 1.38 and 1.60 (6H, 2 × s, 2 × CH₃, 2.03 (12H, series of singlets, 4 × CH₃), 2.15 (3H, s, 6-CH₃)), 4.10 (1H, dd, J = 12.3 and 5.6 Hz, 6″-Ha), 4.28 (2H, m, 6-Hb and CH₂a), 4.38 (1H, dd, J = 11.6 and 6.1 Hz, CH₂a), 4.45 (1H, m, 4-H), 5.05 (2H, m, 5″-H and 5-H), 5.18 (1H, d, J = 3.6 Hz, 2″-H), 5.45 (2H, m, 3-H and 4″-H), 5.54 (1H, dd, J = 4.8 and 3.7 Hz, 3″-H), 5.93 (2H, s, NH₂), 6.10 (1H, d, J = 2.1 Hz, 2-H), 7.94 (1H, s, 8′-H) and 8.34 (1H, s, 2′-H); δC/ppm (100MHz; CDCl₃) 20.3, 20.34, 20.56, 20.6 and 20.7 (5 × CH₃), 27.1 and 29.7 (2 × CH₃), 61.5 (C-6″), 65.1 (CH₂), 68.4 (C-3″), 68.6 (C-5″), 69.3 (C-4″), 70.8 (C-2″), 81.6 (C-5), 84.2 (C-3), 84.8 (C-4), 90.8 (C-2), 114.6 [C(CH₃)₂], 120.2 (C-5″), 140.0 (C-8′), 149.2 (C-4″), 152.7 (C-2′), 155.3 (C-6′), 166.4 (C=O), 169.5 (5-O.C=O),
169.6 (4-O.C=O), 169.67 (3-O.C=O), 169.9 (2-O.C=O) and 170.5 (6-O.C=O); m/z = 696 ([M+1]⁺, 16%) and (694, 100%).

2-(6-Aminopurin-9-yl)-3,4-dihydroxy-5-[(allyloxy)methyl]tetrahydrofuran-3,4-acetonide 133

To a stirred solution of adenosine 3',4'-acetonide 127 (0.50 g, 1.6 mmol) in THF (5 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 42 mg, 1.8 mmol) in small portions to permit controlled evolution of hydrogen. Allyl bromide (0.20 mL, 1.8 mmol) was added through a septum and the resulting solution was refluxed for ca. 6h. The reaction was quenched by the addition of water (10 mL). The solvent was evaporated in vacuo and the aqueous residue was extracted with CH₂Cl₂ (2 × 20 mL). The organic extract was washed sequentially with water (2 × 40 mL) and brine (2 × 40 mL). The aqueous washings were extracted with CH₂Cl₂ and the organic layers combined and dried (anhdr. MgSO₄). Evaporation of the solvent in vacuo to afforded a yellow residue, chromatography of which [radial chromatography; elution with 100% EtOAc] afforded 2-(6-aminopurin-9-yl)-3,4-dihydroxy-5-[(allyloxy)methyl]-tetrahydrofuran 3,4-acetonide 133 as a yellow oil (51 mg, 9%). (Found [M+1]⁺, 348.16276, C₁₆H₂₁N₅O₄ requires 347.36904) νmax (thin film/cm⁻¹) 3388 (NH₂); δH/ppm (400MHz; CDCl₃) 1.38 and 1.62 (6H, 2 × s, 2 × CH₃), 3.59 (1H, dd, J = 10.5 and 4.4 Hz, 1''-CH₃), 3.66 (1H, dd, J = 10.5 and 3.5 Hz, 1''-CH₆), 3.94 (1H, dd, J = 4.6 and 1.2 Hz, 3''-H), 4.50 (1H, dd, J = 6.6 and 3.7 Hz, 4'-H), 4.98 (1H, dd, J = 6.1 and 2.5 Hz, 5''-H₆), 5.17 (2H, m, 5'-H and 8'-H₉), 5.29 (1H, dd, J = 6.1 and 2.4 Hz, 5'-H₆), 5.78 (1H, tdd, J = 16.2, 10.4 and 5.8 Hz, 4''-H), 5.98 (2H, s,
NH₂), 6.19 (1H, d, J = 2.4 Hz, 2-H), 8.06 (1H, s, 8'-H) and 8.35 (1H, s, 2'-H); δC/ppm (100MHz; CDCl₃) 25.4 and 27.4 (2 × CH₃), 70.2 (C-1''), 72.4 (C-3''), 81.8 (C-5), 85.0 (C-3), 86.0 (C-4), 91.4 (C-2), 114.2 [C(CH₃)₂], 117.8 (C-5''), 119.9 (C-5') 133.8 (C-4''), 139.6 (C-8'), 149.5 (C-4'), 152.4 (C-2') and 155.1 (C-6').

2-(6-Aminopurin-9-yl)-3,4-dihydroxy-5-(7-hydroxy-2,5-dioxaheptyl)tetrahydrofuran 3,4-acetonide 137

To a stirred solution of adenosine 3',4'-acetonide 127 (0.50 g, 1.6 mmol) in THF (33 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 0.043 g, 1.8 mmol) in small portions to permit controlled evolution of hydrogen. 2-(2-chloroethoxy)ethanol (0.20 mL, 1.8 mmol) was added through a septum and the resulting solution was refluxed for ca. 6h. The reaction was quenched by the addition of water (40 mL). The solvent was evaporated in vacuo and the aqueous residue was extracted with CH₂Cl₂ (2 × 40 mL). The organic extract was washed sequentially with water (2 × 80 mL) and brine (2 × 80 mL). The aqueous washings were extracted with CH₂Cl₂ and the organic layers combined and dried (anhydr. MgSO₄). Evaporation of the solvent in vacuo afforded 2-(6-aminopurin-9-yl)-3,4-dihydroxy-5-(7-hydroxy-2,5-dioxaheptyl)tetrahydrofuran 3,4-acetonide 137 as a white powder (0.46 g, 72%), m.p. 174-176°C. νmax (solid deposit/cm⁻¹) 3219 (NH₂) and 3112 (OH); δH/ppm (400MHz; CDCl₃) 1.38 and 1.60 (6H, 2 × s, 2 × CH₃), 2.03-2.64 (1H, br s, OH), 3.63 (4H, dd, J = 10.4 and 5.3 Hz, 6''-H and 4''-H), 3.76 (5H, m, 1''–Ha, 7''-H and 3''-H), 3.96 (1H, dd, J = 12.8 and 1.4 Hz, 1''-Hb), 4.53 (1H, d, J = 1.1 Hz, 4-H), 5.10 (1H, dd, J = 5.9 and 0.9 Hz, 5-H), 5.19 (1H, t, J = 5.4 Hz, 3-H), 5.85 (1H, d, J = 4.9 Hz, 2-H), 5.94 (2H, s, NH₂), 7.84 (1H, s, 8'-H) and 8.30 (1H, s, 2'-H); δC/ppm (100MHz; CDCl₃) 25.2 and 27.6 (2 × CH₃), 42.9 (C-6''), 61.6 (C-7''), 63.4 (C-1''),
Experimental

71.1 (C-3"), 72.4 (C-4"), 81.7 (C-8"), 83.0 (C-3), 86.1 (C-4), 94.3 (C-2), 114.0 [C(CH₃)₂], 121.0 (C-5"), 140.3 (C-8"), 148.4 (C-4"), 152.3 (C-2") and 155.8 (C-6"); m/z = 395 (M⁺, 0.39%) and 218 (100%).

2-(6-Aminopurin-9-yl)-5[7-diethylphosphoryoxy)-2-5-dioxaheptyl]-3,4-dihydroxytetrahydrofuran 3,4-acetonide 138

![Chemical Structure](image)

A solution of 2-(6-aminopurin-9-yl)-3,4-dihydroxy-5-(7-hydroxy-2,5-dioxaheptyl)-tetrahydrofuran 3,4-acetonide 137 (260 mg, 0.67 mmol) in THF (10 mL) at room temperature was cooled (-1 ºC). Buthyllithium (300 µL, 0.74 mmol) was added and after stirring for 2 h diethyl chlorophosphate (110 µL, 0.72 mmol) was added and the resulting mixture stirred at room temperature for 24 h. The reaction was quenched by the addition of satd. aq. NH₄Cl solution (10 mL). The THF was evaporated in vacuo and the aqueous residue extracted with CH₂Cl₂ (2 × 15 mL). The organic extract was washed with aq. NH₄Cl (2 × 60 mL), water (2 × 60 mL) and brine (2 × 60 mL). The aqueous fractions were extracted with CH₂Cl₂ and the organic layers combined and dried (anhdr. MgSO₄). The brown oil obtained after evaporation of the solvent in vacuo was washed with hexane (3 × 15 mL) to afford 2-(6-aminopurin-9-yl)-5[7-diethylphosphoryoxy)-2-5-dioxaheptyl]-3,4-dihydroxytetrahydrofuran 3,4-acetonide 138 as a yellow oil, (130 mg, 37%). νmax (solid deposit/cm⁻¹) 3337 (NH₂) and 1028 (P=O); δH/ppm (400MHz; CDCl₃) 1.28 (6H, td, J_P–H = 16.5 and 7.1 Hz, 2 × C_H₃), 1.38 and 1.61 (6H, 2 × s, 2 × C_H₃), 4.06 (5H, m, 2 × C_H₂ and 1"-Hₐ), 4.21 (9H, series of multiplets, 1"-H₉, 7"-H, 6"-H, 4"-H and 3"-H), 4.48 (1H, m, 4-H), 5.06 (1H, dd, J = 6.2 and 2.9 Hz, 5-H), 5.35 (1H, dd, J = 6.2 and 2.3 Hz, 3-H), 6.14 (1H, d, J = 4.9 Hz, 2-H), 6.76 (2H, s, NH₂), 8.05 (1H, s, 2'-H) and 8.32 (1H, s, 8'-H); δC/ppm
Experimental

(100MHz; CDCl₃) 16.05 (d, J_P-C = 6.5 Hz, 2 × CH₃), 25.3 and 27.1 (2 × CH₃), 64.1 (dd, J_P-C = 5.8 and 2.1 Hz, 2 × CH₂), 66.6 and 66.7 (5C, series of singlets, C-1", C-3", C-4", C-6" and C-7"), 81.3 (C-5'), 84.4 (C-3'), 85.4 (C-4'), 91.0 (C-2), 114.7 [C(CH₃)₂], 119.9 (C-5'), 140.2 (C-8'), 149.0 (C-4'), 150.8 (C-2') and 154.4 (C-6'); δ_P/ppm (162 MHz; CDCl₃) 0.8 (P=O); m/z 532.31 (M⁺+1,3%) and 444.25 (100%).

**2-(6-Aminopurin-9-yl)-3,4- dihydroxy-5-(10-hydroxy-2,5,8-trioxadecyl)tetrahydrofuran 3,4-acetonide 139**

![Diagram](image1)

To a stirred solution of adenosine 3',4'-acetonide **127** (0.50 g, 1.6 mmol) in THF (33 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 43 mg, 1.8 mmol) in small portions to permit controlled evolution of hydrogen. 2-[2-(2-chloroethoxy)ethoxy]ethanol (0.30 mL, 1.8 mmol) was then added through a septum and the resulting solution refluxed for ca. 5h. The reaction was quenched by the addition of water (40 mL). The solvent was taken off in vacuo and the aqueous
residue extracted with CH₂Cl₂ (2 × 40 mL). The organic extract was washed sequentially with satd. NaHCO₃ (2 × 80 mL), water (2 × 80 mL) and brine (2 × 80 mL). The aqueous washings were extracted with CH₂Cl₂ and the organic layers combined and dried (anhydr. MgSO₄). Evaporation of the solvent in vacuo afforded a yellow residue that was chromatographed [column chromatography; elution with EtOH:CHCl₃:hexane = 1:2:2] to afford two products:

(i) 2-(6-Aminopurin-9-yl)-3,4-dihydroxy-5-(10-hydroxy-2,5,8-trioxadecy)tetrahydrofuran 3,4-acetonide 139 as yellow oil (23 mg, 3%). ν<sub>max</sub> (thin film/cm<sup>-1</sup>) 3414 (NH₂ and OH); δ<sub>H</sub>/ppm (600MHz; CDCl₃) 1.37 and 1.64 (6H, 2 × s, 2 × CH₃), 3.58-3.82 (13H, series of multiplets, 1"-CH₃, 3"-H, 4"-H, 6"-H, 7"-H, 9"-H and 10"-H), 3.67 (1H, d, J = 13.0 Hz, 1"-CH₃), 4.53 (1H, s, 4"-H), 5.10 (1H, d, J = 5.9 Hz, 5-H), 5.19 (1H, t, J = 5.4 Hz, 3-H), 5.74 (2H, d, J = 0.9 Hz, NH₂), 5.84 (1H, d, J = 4.9 Hz, 2-H), 7.83 (1H, s, 8'-H) and 8.31 (1H, s, 2'-H); δ<sub>C</sub>/ppm (150MHz; CDCl₃) 25.2 and 27.7 (2 × CH₃), 63.4 (1"-CH₃), 61.5-72.8 (C-3", C-4", C-5", C-6", C-7", C-9" and C-10"), 81.7 (C-5), 82.9 (C-3), 86.1 (C-4), 94.4 (C-2), 114.0 [C(CH₃)₂], 121.1 (C-5'), 140.3 (C-8'), 148.4 (C-4'), 152.7 (C-2') and 155.9 (C-6').

(ii. 2-[6-(8-hydroxy-3,6-dioxaoctylamino)-purin-9-yl]-3,4-dihydroxy-5-(10-hydroxy-2,5,8-trioxadecy)tetrahydrofuran 3,4-acetonide 139A as a pale yellow oil (43 mg, 5%). ν<sub>max</sub> (thin film/cm<sup>-1</sup>) 3393 (NH and OH); δ<sub>H</sub>/ppm (400MHz; CDCl₃) 1.38 and 1.64 (6H, 2 × s, 2 × CH₃), 3.51-3.81 (26H, series of multiplets, 1"-CH₂, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 1"'-H, 2"'-H, 4"'-H, 5"'-H, 6"'-H, 7"'-H and 8"'-H), 4.52 (1H, d, J = 2.1 Hz, 4-H), 4.94 (1H, dd, J = 5.8 and 1.6 Hz, 5-H), 5.08 (1H, dd, J = 5.8 and 3.3 Hz, 3-H), 6.29 (1H, b, NH), 6.31 (1H, d, J = 3.3 Hz, 2-H), 8.34 (1H, s, 2'-H) and 8.50 (1H, s, 8'-H); δ<sub>C</sub>/ppm (100MHz; CDCl₃) 25.4 and 27.4 (2 × CH₃), 61.2-73.1 (1"-CH₂, C-3",C-4", C-6", C-7", C-9", C-10", C-1"", C-2"", C-4"", C-5", C-7"" and C-8""), 81.7 (C-5), 84.9 (C-3), 85.4 (C-4), 90.6 (C-2), 114.0 [C(CH₃)₂], 118.8 (C-5'), 139.5 (C-8'), 149.8 (C-4'), 153.2 (C-2') and 155.3 (C-6').
NaH (60% dispersion in mineral oil; 43 mg, 1.8 mmol) was added in small portions to a stirred solution of adenosine 3',4'-acetonide 127 (0.50 g, 1.6 mmol) in THF (33 mL) under nitrogen. Acetoxyacetyl chloride (0.20 mL, 1.8 mmol) was then added through a septum and the resulting solution was refluxed for ca. 5 h. The reaction was quenched by the addition of water (50 mL). The solvent was evaporated *in vacuo* and the aqueous residue extracted with CH$_2$Cl$_2$ (2 × 50 mL). The combined organic extract was washed sequentially with satd. aq. NaHCO$_3$ (2 × 100 mL), water (2 × 100 mL) and brine (2 × 100 mL). The aqueous washings were extracted with CH$_2$Cl$_2$ and the organic layers combined and dried (anhydr. MgSO$_4$). Evaporation of the solvent *in vacuo* afforded a brownish white residue that was chromatographed [radial chromatography; elution with EtOAc] to afford two products:

i. **2-(6-aminopurin-9-yl)-5-acetoxyacetyl-3,4-dihydroxytetrahydrofuran 3,4-acetonide 140**, light yellow oil (230 mg, 35%). $\nu_{\text{max}}$ (thin film/cm$^{-1}$) 3326 (NH$_2$), 1750 (CH$_3$C=O) and 1644 (aromatic C=N ); $\delta_{\text{H}}$/ppm (400MHz; CDCl$_3$) 1.39 and
1.61 (6H, 2 × s, 2 × CH₃), 2.13 (3H, s, 7"-H), 4.32 (1H, m, 1"-Hₐ), 4.46 (2H, m, 1"-H₉ and 4-H), 4.55 (2H, s, 4"-H), 5.07 (2H, dd, J = 6.3 and 3.3 Hz, 5-H), 5.46 (1H, dd, J = 6.3 and 2.0 Hz, 3-H), 5.71 (2H, s, NH₂), 6.08 (1H, d, J = 2.0 Hz, 2-H), 7.88 (1H, s, 8'-H) and 8.34 (1H, s, 2'-H); δC/ppm (100MHz; CDCl₃) 20.4 (C-7"), 25.3 and 27.1 (2 × CH₃), 60.5 (C-4"), 64.6 (C-1"), 81.6 (C-5), 84.1 (C-3), 84.7 (C-4), 90.9 (C-2), 114.7 [(C(CH₃)₂)], 120.4 (C-5'), 139.8 (C-8'), 149.2 (C-4'), 153.2 (C-2'), 155.5 (C-6'), 167.4 (C-3") and 170.3 (C-6"); m/z = 407 (M⁺-1, 2.44%) and 408 (100%).

ii. 2-[6-(Acetoxyacetylamino)purin-9-yl]-5-acetoxyacetyl 3,4-dihydroxytetrahydrofuran 3,4-acetonide, 140A light yellow oil (330 mg, 40%). νmax (thin film/cm⁻¹) 3422 (NH), 2957 (CH), 1725 (CH₃C=O) 1610 (aromatic C=N) and 1589 (NH=O); δH/ppm (400MHz; CDCl₃) 1.39 and 1.62 (6H, 2 × s, 2 × CH₃), 2.11 (3H, s, 7"-H), 2.22 (3H, s, 5"-H), 4.34 (1H, dd, J = 11.7 and 6.1 Hz, 1"-Ha), 4.48 (4H, m, 4–H, 1"–Hb and 4"-H), 5.04 (1H, dd, J = 6.3 and 3.4 Hz, 5–H), 5.30 (2H, d, J = 3.0 Hz, 2"–H), 5.43 (1H, dd, J = 6.3 and 2.11 Hz, 3–H), 6.15 (1H, d, J = 2.1 Hz, 2–H), 8.20 (1H, s, 2–H), 8.66 (1H, s, 8’–H) and 9.40 (1H, s, NH); δC/ppm (100MHz; CDCl₃) 20.4 (C-7"), 20.6 (C-5"), 25.3 and 27.1 (2 × CH₃), 60.4 (C-4"), 64.4 (C-1"), 64.6 (C-2"), 81.4 (C-5), 84.1 (C-3), 84.7 (C-4), 91.1 (C-2), 114.9 [(C(CH₃)₂)], 122.0 (C-5'), 142.7 (C-8'), 148.7 (C-4'), 150.7 (C-2'), 152.3 (C-6'), 167.3 (C-1" and C-3"), 170.3 (C-4") and 170.3 (C-6"); m/z = 507 (M⁺, 17.32%) and 157 (100%).

2-(6-Aminopurin-9-yl)-5-[(2-chloroacetoxy)methyl]-3,4-dihydroxytetrahydrofuran 3,4-acetonide 141 and 2-[6-(2-chloroacetamido)purin-9-yl]-5-[(2-chloroacetoxy)-methyl] 3,4-dihydroxytetrahydrofuran 3,4-acetonide 141A
**Experimental**

A mixture of adenosine 3',4'-acetonide 127 (0.50 g, 1.6 m mol) in chloroacetyl chloride (1.4 mL, 1.8 mmol) was stirred under argon. The crude ester crystallized out within 0.5 h. The excess acid chloride was evaporated in vacuo and the residue was dissolved in CHCl₃ (2 × 50 mL). This solution was washed sequentially with satd. aq. NaHCO₃ (2 × 100 mL) and brine (2 × 100 mL). The aqueous washings were extracted with CHCl₃ and the organic layers combined and dried (anhydr. MgSO₄). Evaporation of the solvent in vacuo afforded the pure 2-(6-aminopurin-9-yl)-5-[(2-chloroacetoxy)methyl]-3,4-dihydroxytetrahydrofuran 3,4-acetonide 141 as light yellow crystals, (0.59 g, 94%), m.p. 150-151°C. ν max (solid deposit/cm⁻¹) 3398 (NH₂), 1738 (C=O); δ H/ppm (400MHz; CDCl₃) 1.38 and 1.59 (6H, 2 × s, 2 × CH₃), 4.00 (2H, s, 4''-H), 4.34 (1H, dd, J = 13.0 and 7.6 Hz, 1''-CH₃), 4.48 (2H, m, 1''-CH₂ and 4-H), 5.08 (1H, dd, J = 6.3 and 3.2 Hz, 5-H), 5.46 (1H, dd, J = 6.3 and 1.9 Hz, 3-H), 6.09 (1H, d, J = 1.9 Hz, 2-H), 6.14 (2H, s, NH₂), 7.88 (1H, s, 8'-H) and 8.32 (1H, s, 2'-H); δ C/ppm (100MHz; CDCl₃) 25.3 and 27.1 (2 × CH₃), 40.5 (C-4''), 65.4 (C-1''), 81.5 (C-5), 84.1 (C-3), 84.6 (C-4), 90.9 (C-2), 114.7 [C(CH₃)₂], 120.3 (C-5''), 139.8 (C-8'), 149.1 (C-4'), 153.1 (C-2'), 155.7 (C-6') and 166.8 (C=O); m/z = 384.22 (M⁺+1, 9%) and 346 (100%).

In a separate reaction the acetonide 127 was treated with the same reagent following the procedure above. Work up afforded (i) 141 and (ii) 2-(6-(2-chloroacetamido)purin-1-yl)-5-[(2-chloroacetoxy)methyl]-3,4-dihydroxytetrahydrofuran 3,4-acetonide 141A as a yellow oil.
ii 2-[6-(2-chloracetamido) purin-9-yl]-5-[(2-chloroacetoxy)methyl] 3,4-dihydroxy tetra-hydrofuran 3,4-acetonide 141A as light yellow crystals. $\nu_{\text{max}}$ (solid deposit/cm$^{-1}$) (NH), (C=O); $\delta$H/ppm (400MHz; CDCl$_3$) 1.39 and 1.62 (6H, 2 × s, 2 × CH$_3$), 4.01 (2H, s, 4''-CH$_2$), 4.45 (4H, m, 1''-CH$_2$, 4-H and NH), 4.68 (1H, s, 2''-CH$_2$), 5.06 (1H, dd, $J = 6.2$ and 3.4 Hz, 5-H), 5.45 (1H, dd, $J = 6.2$ and 1.9 Hz, 3-H), 6.18 (1H, d, $J = 1.9$ Hz, 2-H), 8.25 (1H, s, 2'-H) and 8.72 (1H, s, 8'-H); $\delta$C/ppm (100MHz; CDCl$_3$) 25.3 and 27.1 (2 × CH$_3$), 40.5 (C-4''), 44.6 (C-2'''), 65.3 (C-1''), 81.3 (C-5), 84.1 (C-3), 84.6 (C-4), 91.1 (C-2), 115.0 [C(CH$_3$)$_2$], 122.5 (C-5'), 142.8 (C-8'), 148.5 (C-4'), 152.4 (C-2'), 166.0 (C-6'), 166.8 (NH-C=O) and 166.83 (O-C=O).

5-[(Acryloyloxy)methyl]-2-(6-aminopurin-9-yl)-3,4-dihydroxytetrahydrofuran 3,4-acetonide 143

To a stirred solution of adenosine 3',4'-acetonide 127 (1.0 g, 3.3 mmol) in THF (30 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 0.15 g, 3.3 mmol) in small portions to permit controlled evolution of hydrogen. Acryloyl chloride (0.27 mL, 3.3 mmol) was then added with a septum and the resulting solution was refluxed for ca. 5h. The reaction was quenched by the addition of water (50 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with CH$_2$Cl$_2$ (2 × 50 mL). The organic extract was washed sequentially with satd. aq. NaHCO$_3$ (2 × 100 mL) and brine (2 × 100 mL). The aqueous washings were extracted with CH$_2$Cl$_2$ and the organic layers combined and dried (anhyd. MgSO$_4$). Evaporation of the solvent in vacuo afforded a pale yellow residue. Column chromatography on silica [elution with EtOH-EtOAc (1:20)] afforded 5-[(acryloyloxy)methyl]-2-(6-aminopurin-9-yl)-3,4-dihydroxytetrahydrofuran 3,4-acetonide 143 as a light brown solid (49 mg, 4.1%), m.p. 188-192°C. (Found: M$^+$, 361.137825. C$_{16}$H$_{19}$N$_5$O$_5$ requires: M$^+$, 361.138619);
Experimental

ν_{max} (thin film/cm^{-1}) 3413 (NH_{2}) and 1714 (C=O); δ_{H}/ppm (400MHz; CDCl_{3}) 1.39 and 1.61 (6H, 2 × s, 2 × CH_{3}), 4.30 (1H, dd, J = 11.8 and 6.0 Hz, 1"-H_{a}), 4.44 (1H, dd, J = 11.9 and 4.5 Hz, 1"-H_{b}), 4.50 (1H, d, J = 1.1 Hz, 4-H), 5.08 (1H, dd, J = 6.3 and 3.4 Hz, 5-H), 5.48 (1H, dd, J = 6.3 and 2.0 Hz, 3-H), 5.80 (1H, dd, J = 10.5 and 0.8 Hz, 5"-H_{E}), 5.87 (2H, s, NH_{2}), 6.02 (1H, dd, J = 17.3 and 10.4 Hz, 4"-H), 6.10 (1H, d, J = 1.9 Hz, 2-H), 6.36 (1H, dd, J = 17.3 and 0.9 Hz, 5"-H_{Z}), 7.87 (1H, s, 8'-H) and 8.33 (1H, s, 2'-H); δ_{C}/ppm (100MHz; CDCl_{3}) 25.4 and 27.1 (2 × CH_{3}), 64.0 (C-1"), 81.7 (C-5), 84.2 (C-3), 85.0 (C-4), 91.0 (C-2), 114.6 [C(CH_{3})_{2}], 120.3 (C-5"), 127.6 (C-4"), 131.6 (C-5"), 139.7 (C-8'), 149.2 (C-4'), 153.2 (C-2'), 155.6 (C-6') and 165.5 (C=O).

4-Bromomethyl-2,2-dimethyl-[1,3]dioxolane 145

$$\begin{align*}
\text{Br} & \\
4 & \\
3 & \\
2 & \\
1 & \\
\end{align*}$$

A solution of 3-bromo-1,2-propanediol (0.50 mL, 5.7 mmol) and p-toluenesulphonic acid (4.1 g, 24 mmol) in acetone (600 mL) was stirred at room temperature overnight. The reaction was quenched with aqueous Na_{2}CO_{3} (10%, 210 mL). The solvent was removed in vacuo and the aqueous residue extracted with CH_{2}Cl_{2} (3 × 250 mL). The combined organic extracts were dried (anhydr. MgSO_{4}) and evaporated in vacuo to afford 4-bromomethyl-2,2-dimethyl-[1,3]dioxolane 145 as a yellow oil (730 mg, 66%). δ_{H}/ppm (400MHz; CDCl_{3}) 1.34 and 1.43 (6H, 2 × s, 2 × CH_{3}), 3.30 (1H, dd, J = 9.9 and 8.3 Hz, CH_{a}), 3.42 (1H, dd, J = 10.0 and 4.7 Hz, CH_{b}), 3.86 (1H, dd, J = 8.7 and 5.2 Hz, 5-H_{a}), 4.12 (1H, dd, J = 8.7 and 6.1 Hz, 5-H_{b}) and 4.33 (1H, ddd, J = 11.0, 8.2 and 5.4 Hz, 4-H); δ_{C}/ppm (100MHz; CDCl_{3}) 25.4 and 27.0 (2 × CH_{3}), 32.7 (CH_{2}), 63.3 (C-5), 75.3 (C-4) and 110.3 (C-2).
(3,4,5,6-Tetraacetoxy 2-acetoxymethyl)pyran 148

A mixture of zinc chloride (0.5 g, 3.7 mmol) in acetic anhydride (13 mL, 13.0 mmol) was heated for *ca.* 10 minutes. D-glucose (2.5 g, 1.4 mmol) was then added slowly a little at a time to control the evolution of hydrogen. The mixture was then heated without reflux for *ca.* 1 h. Ice-water (125 mL) was added with gentle stirring to quench the reaction in a period of *ca.* 30 minutes. Further stirring was done for 30 minutes by which time the crude product had started crystallizing out of solution. The crude product mixture was allowed to stand at 0°C for 24 h and the white solid filtered off and recrystallised from methanol to afford pure pentaacetylated α-D-glucopyranose 148 as a white crystalline solid (4.7 g, 87%), m.p. 84-86°C (lit.181 110-111°C). ν<sub>max</sub> (solid deposit/cm<sup>-1</sup>) 1750 (C=O); δ<sub>H</sub>/ppm (400MHz; CDCl<sub>3</sub>) 2.01 (9H, series of singlets, 3 × CH<sub>3</sub>), 2.08 (3H, s, 2-CH<sub>3</sub>), 2.16 (3H, s, 6-CH<sub>3</sub>), 4.09 (2H, m, CH<sub>a</sub> and 2-H,), 4.25 (1H, dd, J = 12.5 and 4.1 Hz, CH<sub>b</sub>), 5.11 (2H, m, 3-H and 5-H), 5.45 (1H, t, J = 9.9 Hz, 4-H) and 6.31 (1H, d, J = 3.7 Hz, 6-H); δ<sub>C</sub>/ppm (100MHz; CDCl<sub>3</sub>) 20.4, 20.5, 20.6, 20.7 and 20.9 (series of singlets, 5 × CH<sub>3</sub>), 61.5 (CH<sub>2</sub>), 67.9 (C-3), 69.2 (C-5), 69.8 (C-2 and C-4), 89.1 (C-6), 168.7 (6-C =O), 169.4, 169.6 and 170.2 (3 × C =O), and 170.6 (2-C=O); m/z = 390 (M<sup>+</sup>, 0.03%) and 98 (100%).
Experimental

3,4,5-Triacetoxy-6-acetoxymethyl-2-(6-aminopurin-9-yl)pyran 149

Adenine 61 (0.5 g, 3.7 mmol) and acetylated glucose 148 (1.4 g, 3.5 mmol) were placed in a 100 mL dried 3-neck flask equipped with a reflux condenser. Acetonitrile (20 mL) was added through a septum and the reaction mixture stirred. Tin tetrachloride (1.0 mL, 9.3 mmol) was then added and the reaction mixture refluxed under argon for ca. 6 h. The reaction was quenched with satd. NaHCO₃ (50 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with EtOAc (2 × 25 mL). The organic extract was washed sequentially with satd. aq. NaHCO₃ (2 × 50 mL) and brine (2 × 50 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO₄). Evaporation of the solvent in vacuo afforded a pale yellow residue. Column chromatography on silica [elution with EtOH-CHCl₃ (1:19)] afforded 2-[(6-aminopurin-9-yl)-6-acetoxymethyl-3,4,5-triacetoxy] pyran 149 as yellow crystals (510 mg, 30%), m.p. 63-65°C. (Found: M⁺, 465.14892. C₁₀H₁₃N₅O₉ requires: M⁺, 465.41498); δ max (solid deposit/cm⁻¹) 3339 (NH₂) and 1744 (C=O); δH/ppm (400MHz; CDCl₃) 2.00 – 2.08 (12H, series of singlets, 4 × CH₃), 4.02 (1H, ddd, J = 10.1, 4.8 and 2.1 Hz, 6-H), 4.14 (1H, dd, J = 12.6 and 2.1 Hz, CH₉a), 4.29 (1H, dd, J = 12.6 and 4.9 Hz, CH₉b) 5.27 (1H, t, J = 9.8 Hz, 5-H), 5.44 (1H, t, J = 9.5 Hz, 4-H), 5.58 (1H, t, J = 9.5 Hz, 3-H), 5.76 (1H, s, NH₂), 5.88 (1H, d, J = 9.5 Hz, 2-H), 8.00 (1H, s, 8'-H) and 8.36 (1H, s, 2'-H); δC/ppm (100MHz; CDCl₃) 20.1, 20.5, 20.6 and 20.7 (series of singlets, 5 × CH₃), 61.6 (CH₂), 67.9 (C-5), 70.4 (C-3), 72.9 (C-4), 75.1 (C-6), 80.3 (C-2), 119.2 (C-5'), 138.3 (C-8'), 150.7 (C-4'), 153.4 (C-2'), 155.4 (C-6'), 169.0 (3-C=O), 169.4(5-C=O), 169.8(4-C=O) and 170.5 (6-C=O).
6-(N-Allylamino)purine 150 and 9-Allyl-[6-amino(N-allylamino)] purine 150A

NaH (60% dispersion in mineral oil; 100 mg, 4.0 mmol) was added in small portions permit controlled evolution of hydrogen, to a stirred solution of adenine 61 (300 mg, 2.2 mmol) in dry DMF (20 mL) under nitrogen at 0°C. Allyl bromide (230 µL, 2.7 mmol) was then added with through a septum and the resulting solution refluxed for ca. 6 h. The reaction was quenched by the addition of water (25 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with CHCl₃ (2 × 25 mL). The combined organic extracts were washed sequentially with satd. NaHCO₃ (2 × 50 mL), water (2 × 50 mL) and brine (2 × 50 mL). The aqueous washings were extracted with CHCl₃ and the organic layers combined and dried (anhydr. MgSO₄). The solvent was evaporated in vacuo and the residue chromatographed on silica [column chromatography; elution with EtOH-EtOAc (1:20)] to afford two fractions.

(i) 6-(N-allylamino)purine 150 as a yellow solid (45 mg, 12%), m.p. 165-167°C. (Found: M⁺, 175.085842. C₈H₉N₅ requires: M⁺, 175.085795); νmax (solid deposit/cm⁻¹) 3382 (NH); δH/ppm (400MHz; CDCl₃) 4.91 (2H, d, J = 5.8 Hz, 1'-H), 5.25 (1H, d, J = 17.1 Hz, 3'-Ha, CH=CH₂), 5.35 (1H, d, J = 10.2 Hz, 3'-Hb CH=CH₂), 6.07 (1H, tdd, J = 16.1, 10.4 and 5.8 Hz, 2'-H), 8.43 (1H, s, 8-H), 8.70 (1H, s, 2-H), 10.01 (1H, d, J = 10.0 Hz, 6-NH) and 11.12 ( 1 H, d, J = 9.4 Hz, 1-NH); δC/ppm (100MHz; CDCl₃) 46.1 (C-1'), 119.6 (C-3'), 120.4 (C-5), 131.2 (C-2'), 144.2 (C-8), 149.3 (C-4), 151.9 (C-6) and 152.5 (C-2).
(ii) 9-allyl-[6-amino(N-allylamino)] purine 150A as a yellow solid (8.0 mg, 2.0%), m.p. 103-105°C. (Found: M⁺, 215.118412. C_{11}H_{13}N_{5} requires: M⁺, 215.117096); ν_max (thin film/cm⁻¹) 3383 (NH); δH/ppm (400MHz; CDCl₃) 4.80 (4H, d, J = 5.27 Hz, 1'-CH₂ and 1''-CH₂), 5.19 (2H, m, 3''-H), 5.30 (1H, m, 3'-H), 6.02 (2H, m, 2'-H and 2''-H), 7.77 (1H, s, 2-H) and 8.40 (1H, s, 8-H); δC/ppm (100MHz; CDCl₃) 45.7 (C-1' and C-1''), 116.4 (C-3'), 118.9 (C-3''), 119.6 (C-5), 131.9 (C-2''), 134.3 (C-2'), 139.7 (C-2), 152.5 (C-4), 153.2 (C-2) and 154.7 (C-6).

4-(Tetrahydropyran-2-yloxy)-1H-pyrazolo[3,4]pyrimidine 157

A stirred mixture of allopurinol (1.0 g, 7.4 mmol) in 50 mL reagent grade DMF (50 mL) was heated to ca. 75 °C to dissolve the substrate. To the resulting solution was added dihydropyran (20 mL) followed by the addition of para-toluene sulphonic acid [16 mg in benzene (4 mL) i.e. 0.4% p-TSA in dry benzene]. The reaction mixture was stirred for 24 h. at room temperature. The reaction was quenched with a 4% pyridine in benzene solution (0.5 mL) at end of which the solvent was evaporated in vacuo at 40°C and the crude product dissolved in petroleum ether (100 mL) and allowed to crystallize overnight at 0°C. Recrystallisation from EtOAc-acetone afforded 4-(tetrahydropyran-2-yloxy)-1H-pyrazolo[3,4]pyrimidine 157 as white crystals, (0.40 g, 25%); m.p. 190-193°C (lit. 184 200-201°C). ν_max (solid deposit/cm⁻¹) 3403 (NH); δH/ppm (400MHz; CDCl₃) 1.62 (1H, dd, J = 7.0 and 4.6 Hz, 4'-CH₃), 1.77 (2H, m, 4'-CH₂ and 5'-CH₃), 1.94 (1H, dd, J = 12.8 and 2.7 Hz, 3'-CH₃), 2.13 (1H, m, 5'-CH₂), 2.55 (1H, m, 3'-CH₂), 3.76 (1H, dt, J = 11.4, 11.3 and 2.5 Hz, 6'-CH₃), 4.12 (1H, dd, J = 7.6 and 6.0 Hz, 6'-CH₂), 5.89 (1H, dd, J = 10.2 and 2.5 Hz, 2'-H), 8.03 (1H, s, 7-H), 8.15 (1H, s, 2-H) and 12.22 (1H, s, NH); δC/ppm (100MHz; CDCl₃) 22.7 (C-5'), 28.3 (C-4'), 33.2 (C-3'), 33.3 (C-3''), 44.0 (C-2'), 44.3 (C-2''), 69.9 (C-6''), 112.3 (C-1), 116.7 (C-3'), 119.4 (C-5), 119.5 (C-3''), 127.5 (C-2), 130.4 (C-2') and 158.7 (C-2'').
To a stirred solution of protected allopurinol 157 (200 mg, 0.91 mmol) in dry THF (50 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 40 mg, 0.91 mmol) in small portions to permit controlled evolution of hydrogen. Allyl bromide (80 µL, 0.91 mmol) was then added through a septum and the resulting solution was refluxed for ca. 6h. The reaction was quenched by the addition of water (50 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with CH₂Cl₂ (2 × 50 mL). The combined organic extracts were washed sequentially with water (2 × 100 mL) and brine (2 × 100 mL). The aqueous washings were extracted with CH₂Cl₂ and the organic layers combined and dried (anhydr. MgSO₄). Evaporation of the solvent in vacuo afforded 1-allyl-4-(tetrahydropyran-2-yloxy)-1H-pyrazolo[3,4-d]pyrimidine 158 as a pale yellow solid (210 mg, 87%), m.p. 60-62°C. (Found: M⁺, 260.12774. C₁₃H₁₆N₄O₂ requires: M⁺, 260.29174); δH/ppm (400MHz; CDCl₃) 1.61 (1H, m, 4’-CH₃), 1.75 (2H, m, 4’-CH₂ and 5’-CH₃), 1.93 (1H, m, 3’-CH₃), 2.13 (1H, m, 5’-CH₂), 2.54 (1H, m, 3’-CH₂), 3.75 (1H, dt, J = 11.4 and 2.5 Hz, 6’-CH₃), 4.10 (1H, m, 6’-CH₂), 4.61 (2H, m, 3’-CH₂), 5.21 (1H, d, J = 17.1 Hz, CH₂-CH=CH₂), 5.28 (1H, dd, J = 10.3 and 0.8 Hz, CH₂-CH=CH₂), 5.83 (1H, dd, J = 10.3 and 2.6 Hz, 2”-H), 5.94 (1H, tdd, J = 16.1, 10.4 and 5.7 Hz, 2”-H), 7.94 (1H, s, 7-H) and 8.12 (1H, s, 2-H). δC/ppm (100MHz; CDCl₃) 22.7 (C-5’), 24.9 (C-4’), 29.3 (C-3’), 47.8 (C-3”), 68.2 (C-6’), 82.9 (C-2’), 106.3 (C-8), 118.9 (C-1”), 132.1 (C-2”), 135.8 (C-7), 149.0 (C-2), 151.7 (C-6) and 156.8 (C-5).
Tert-Butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylene propanoate 165a

To a solution of tert-butyl acrylate 164 (3.2 mL, 22 mmol) in CHCl₃ (3 mL) was added 1,4-Diazabicyclo[2.2.2]octane (DABCO) (1.3 g, 12 mmol). The solution was stirred for ca. 5 mins. 5-bromo-2-hydroxybenzaldehyde 163 (3.0 g, 15 mmol) was then added and the reaction mixture stirred for ca. 4 days. The resultant viscous yellow material was eluted through silica with acetone. The solvent was taken evaporated in vacuo and the residue recrystallised from CHCl₃ to obtain 2-[(5-Bromo-2-hydroxyphenyl) hydroxymethyl] acrylic acid tert-butyl ester 165a as a white powder (4.3 g, 88%), m.p. 185-187°C (lit. 185-186-188°C); ν_max (solid deposit/cm⁻¹) 3294 OH, 1684 C=O, δ_H/ppm (400MHz; DMSO-d₆) 1.32 (9H, s, But), 5.59-5.36 (1H, br s, CH-OH), 5.65 (1H, dd, J = 5.2 and 3.6 Hz, 3-H₃), 5.66 (1H, s, CH), 6.05 (1H, s, 3-H₆), 6.75 (1H, d, J = 8.33 Hz, 4'-H), 7.21 (2H, m, 3'-H and 6'-H) and 9.81–9.53 (1H, b, 2' -OH); δ_C (100MHz; DMSO-d₆) 27.4 C(CH₃)₃, 64.5 (C-HOH), 80.0 C(CH₃)₃, 109.5 (C-2'), 117.0 (C-4'), 122.8 (C-3), 129.7 (C-6'), 130.3 (C-5'), 131.8 (C-3'), 144.8 (C-2), 153.7 (C-1') and 164.8 (C=O).

Tert-Butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylene propanoate 165b and tert-butyl 2-H-chromene-2-carboxylate 166b
A solution of tert-butyl acrylate 164 (5.5 mL, 38 m mol) and 1,4-Diazabicyclo-[2.2.2]octane (DABCO) (2.2 g, 20 mmol) in CHCl₃ (3 mL) was stirred for ca. 5 mins. Salicylaldehyde 163b (2.6 mL, 25 mmol) was then added and the reaction mixture stirred for ca. 3 weeks. The solvent was evaporated in vacuo and the reddish residue chromatographed [gravity chromatography; elution with hexane:EtOAc = 7:1] to afford two products.

i. **Tert-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylene propanoate 165b** as a white powder (0.25 g, 4.1%), m.p. 107-111°C (lit. 108-110°C); (Found: [M-1]⁺, 231.10945. C₁₄H₁₆O₃ requires: M⁺, 232.109945); νmax (solid deposit/cm⁻¹) 3414 (OH) and 1687 (C=O); δH/ppm (400MHz; CDCl₃), 1.50 (9H, s, But), 4.36 (1H, d, J = 3.9 Hz, 3-0H), 5.49 (1H, s, 3"-Ha), 5.69 (1H, d, J = 3.3 Hz, 3'-H), 6.23 (1H, s, 3"'-Hb), 6.83 (1H, t, J = 7.4 Hz, 5'-H), 6.90 (1H, d, J = 8.0 Hz, 3'-H), 6.96 (1H, dd, J = 7.6 and 1.5 Hz, 6'-H), 7.20 (1H, m, 4'-H) and 8.14 (1H, s, 2'-OH); δC (100MHz; CDCl₃) 28.0 C(C₃H₃), 73.6 (C-3), 82.6 C(CH₃)₃, 117.5 (C-3'), 119.8 (C-5'), 124.1 (C-1'), 126.9 (C-3'), 127.8 (C-6'), 129.5 (C-4'), 140.8 (C-2), 155.9 (C-2') and 166.7 (C=O).

ii. **Tert-Butyl 2-H-chromene-3-carboxylate 166b**

**Tert-Butyl 2-H-chromene-3-carboxylate 166b** (21 mg, 0.37%) as a yellow oil, νmax (thin film/cm⁻¹) 1699 (C=O); δH/ppm (400MHz; CDCl₃), 1.52 (9H, s, But), 4.94 (2H, s, 2-H), 6.82 (1H, d, J = 8.1 Hz, 5-H), 6.90 (1H, t, J = 7.4 Hz, 6-H), 7.11 (1H, d, J = 7.4 Hz, 8-H), 7.20 (1H, m, 7-H), and 7.32 (1H, s, 4-H); δC (100MHz; CDCl₃) 28.1 (3 × C(CH₃)₃), 64.6 (C-2), 81.2 C(CH₃)₃, 116.0 (C-5), 121.1 (C-10), 121.6 (C-6), 124.2 (C-3), 128.7 (C-8), 131.5 (C-7), 132.5 (C-4), 155.0 (C-9) and 163.8 (C=O).

**Tert-butyl 6-bromo-2H-chromene-3-carboxylate 166A**

NaH (60% dispersion in mineral oil; 0.03 g, 0.61 mmol) added in small portions, to permit controlled evolution of hydrogen, to a stirred solution of 3-aminobenzonitrile
**Experimental**

173 (72 mg, 0.61 mmol) in dry THF (10 mL) under nitrogen at 0°C. The resulting solution was refluxed for 1 h and then allowed to cool. Tert-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylene propanoate 165A (200 mg, 0.61 mmol) was added and the mixture refluxed for ca. 6 h. The reaction was quenched by the addition of water (15 mL). The solvent was evaporated in vacuo and the aqueous residue was extracted with EtOAc (2 × 30 mL). The combined organic extracts were washed sequentially with water (2 × 60 mL) and brine (2 × 60 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO₄). Flash chromatography [on silica gel; elution with hexane-EtOAc (8:2)] afforded tert-butyl 6-bromo-2H-chromene-3-carboxylate 166a, as a yellow oil, (47 mg, 25%). (Found: M⁺, 310.020638. C₁₄H₁₅⁷⁹BrO₃ requires: M⁺, 310.185966); νmax (solid deposit/cm⁻¹) 1703 (C=O); δ_H/ppm (400MHz; CDCl₃), 1.52 (9H, s, Bu²), 4.94 (2H, d, J = 1.3 Hz, 2-H), 6.71 (1H, d, J = 8.6 Hz, 7-H), 7.23 (2H, m, 5-H and 8-H) and 7.27 (1H, dd, J = 8.6 and 2.4 Hz, 4-H); δC (100MHz; CDCl₃) 28.1 (C(CH₃)₃, 64.8 (C-2), 81.6 C(CH₃)₃, 113.5 (C-6), 117.8 (C-7), 122.9 (C-10), 125.5 (C-3), 130.9 (C-5), 131.1 (C-8), 134.0 (C-4), 154.0 (C-9) and 163.4 (C=O).

6-Bromo-3-(chloromethyl)chromen-2-one 167, 6-Bromo-3-(acetoxymethyl)coumarin 168 and 6-Bromo-3-(hydroxymethyl)coumarin 169

A mixture of tert-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylene propanoate (2.0 g, 6.1 mmol), conc. HCl (10 mL, 32%) and glacial acetic acid (10 mL) was boiled under reflux for 2.5 h. The reaction mixture was allowed to cool to
room temperature. Ice water (40 mL) was then added and the reaction mixture stirred for 30 mins. The product mixture was allowed to stand at 0ºC for 24 h and the pink solid was filtered off and chromatographed [flash chromatography; elution with hex:EtOAc=7:3] to afford 3 products.

i. 6-Bromo-3-(chloromethyl)chromen-2-one 167 as a white powder (0.52 g, 31%), m.p. 102-104°C. (Found: M⁺, 178.064011. C₁₀H₁₀O₃ requires: M⁺, 178.062994); ν_max (solid deposit/cm⁻¹) 1724 C=O, δ_H/ppm (400MHz; CDCl₃), 4.53 (2H, s, CH₂), 7.23 (1H, d, J = 8.8 Hz, 7-H), 7.62 (1H, dd, J = 8.8 and 2.3 Hz, 8-H), 7.65 (1H, d, J = 2.3 Hz, 5-H) and 7.80 (1H, s, 4-H); δ_C (100MHz; CDCl₃) 40.8 (CH₂), 117.3 (C-6), 118.4 (C-7), 120.3 (C-10), 126.3 (C-3), 130.3 (C-5), 134.7 (C-8), 139.5 (C-4), 152.3 (C-9) and 159.4 (C=O).

ii. 6-Bromo-3-(acetoxymethyl)coumarin 168 as a white powder (33 mg, 1.8%), m.p. 119-121°C. (Found: M⁺, 297.966202. C₁₂H₉BrO₄ requires: M⁺, 298.114208); ν_max (solid deposit/cm⁻¹) 1720 (C=O), δ_H/ppm (400MHz; CDCl₃) 2.16 (3H, s, CH₃), 5.06 (1H, d, J = 1.1 Hz, CH₂), 7.22 (1H, d, J = 8.7 Hz, 7-H), 7.61 (1H, dd, 8.7 and 2.3 Hz, 8-H) and 7.64 (2H, m, 5-H and 4-H); δ_C (100MHz; CDCl₃) 20.8 (CH₃), 60.9 (CH₂), 117.2 (C-6), 118.4 (C-7), 120.3 (C-10), 125.0 (C-3), 130.2 (C-5), 134.5 (C-8), 138.9 (C-4), 152.3 (C-9), 159.4 (2-C=O) and (CH₃C=O).

iii. 6-Bromo-3-(hydroxymethyl)coumarin 169 as a white powder (0.11 g, 7.3%), m.p. 147-149°C. (Found: M⁺, 253.959816. C₁₀H₇⁷⁹BrO₃ requires: M⁺, 254.079497); ν_max (solid deposit/cm⁻¹) 3409 (OH) and 1632 (C=O); δ_H/ppm (400MHz; DMSO-d₆) 4.36 (1H, d, J = 5.4 Hz, CH₂), 5.52 (1H, dt, J = 5.3 and 1.3 Hz, OH), 7.37 (1H, d, J = 8.8 Hz, 7-H), 7.71 (1H, dd, 8.8 and 2.3 Hz, 8-H), 7.94 (1H, d, J = 1.1 Hz, 4-H) and 8.05 (1H, d, J = 2.2 Hz, 5-H); δ_C (100MHz; DMSO-d₆) 58.1 (CH₂), 116.1 (C-6), 118.2 (C-7), 121.0 (C-10), 130.1 (C-5), 130.5 (C-3), 133.3 (C-8), 135.6 (C-4), 151.3 (C-9) and 159.1 (C=O).
1-(6-Bromo-3-methyl-2H-chromen-2-one)-6-(tetrahydropyran-2-yloxy)pyrazolo[3,4-d] pyrimidine 170A

Method 1

To a stirred solution of protected allopurinol 157 (120 mg, 0.55 mmol) in dry THF (10 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 24 mg, 0.99 mmol) in small portions to permit controlled evolution of hydrogen. The resulting solution was refluxed for 1 h and then cooled to room temperature before adding of 6-bromo-3-(chloromethyl)chromen-2-one 167 (150 mg, 0.55 mmol). The reaction mixture was refluxed for ca. 6 h at the end of which water (30 mL) was added to quench the reaction. The organic solvent was evaporated in vacuo and the aqueous residue extracted with CH₂Cl₂ (2 × 30 mL). The combined organic extracts were washed sequentially with water (2 × 60 mL) and brine (2 ×60 mL). The aqueous washings were extracted with CH₂Cl₂ and the organic layers combined and dried (anhyd. MgSO₄). Evaporation of the solvent in vacuo afforded pale yellow solid that was chromatographed [flash chromatography; elution with hexane-EtOAc (1:1)] to afford 1-(6-Bromo-3-methyl-2H-chromen-2-one)-4-(tetrahydropyran-2-yloxy)pyrazolo[3,4-d] pyrimidine 170A as a white powder (77 mg, 31%), m.p. 210-212°C. (Found: M⁺, 458.041200. C₂₀H₁₇BrN₄O₄ requires: M⁺, 458.290594); νmax (solid deposit/cm⁻¹) 1720 (C=O); δH/ppm (400MHz; CDCl₃) 1.60 (1H, m, 4''-Hₐ), 1.73 (2H, m, 4''-Hₐ and 5''-Hₐ), 1.90 (1H, m, 3''-Hₐ), 2.11 (1H, m, 5''-Hₐ), 2.50 (1H, m, 3''-Hₐ), 3.75 (1H, m, 6''-Hₐ), 4.10 (1H, m, 6''-Hₐ), 5.02 (1H, s, CH₂), 5.83 (1H, dd, J = 10.4 and 2.5 Hz, 2''-H), 7.19 (1H, d, J = 8.8 Hz, 8-H), 7.60 (1H, dd, J = 8.8 and 2.3 Hz, 7-H), 7.66 (1H, d, J = 2.3 Hz, 5-H), 8.00 (1H, s, 3'-H), 8.08 (1H, s, 2'-H) and 8.42 (1H, s, 4-H). δC/ppm (100MHz; CDCl₃) 22.7 (C-5''), 24.8 (C-4''), 29.3 (C-3''), 45.7 (CH₂), 68.3 (C-
Experimental

6°, 82.8 (C-2°), 106.1 (C-3), 117.4 (C-6), 118.3 (C-8), 120.2 (C-5°), 123.0 (C-10), 130.7 (C-5), 135.0 (C-7), 135.6 (C-2°), 143.2 (C-7°), 150.0 (C-4), 151.7 (C-6°), 152.4 (C-9), 157.3 (C-4°) and 160.7 (C=O).

Method 2

In another reaction 157 was reacted with 165A using the same procedure to afford 170A in 14% yield.

1-(3-methyl-2H-chromen-2-one)-6-(tetrahydropyran-2-yloxy)pyrazolo[3,4-d] pyrimidine 170B

To a stirred solution of protected allopurinol 157 (110 mg, 0.49 mmol) in dry THF (10 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 21 mg, 0.88 mmol) in small portions to permit controlled evolution of hydrogen. The resulting solution was refluxed for 1 h and then cooled to room temperature before adding of tert-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylene propanoate 165B (120 mg, 0.49 mmol). The reaction mixture was refluxed for ca. 6h. Water (30 mL) was added to quench the reaction. The organic solvent was evaporated in vacuo and the aqueous residue extracted with CH₂Cl₂ (2 × 30 mL). The combined organic extracts were washed sequentially with water (2 × 60 mL) and brine (2 × 60 mL). The aqueous washings were extracted with CH₂Cl₂ and the organic layers combined and dried (anhydr. MgSO₄). Evaporation of the solvent in vacuo afforded pale yellow solid that was chromatographed [flash chromatography; elution with hexane-EtOAc (3:7)] to afford 1-(2H-chromen-3-carbonyl)-4-(tetrahydropyran-2-yloxy)pyrazolo[3,4] pyrimidine 170B as a white powder (45 mg, 24%), m.p. 210-212°C. (Found: M⁺,
378.132008. C_{20}H_{18}N_4O_4 requires: M^+, 378.382244; ν_{max} (solid deposit/cm\(^{-1}\)) 1698 (C=O); δ\(_H/\)ppm (600MHz; CDCl\(_3\)) 1.59 (1H, m, 4''-H\(_a\)), 1.74 (2H, m, 4''-H\(_b\) and 5''-H\(_a\)), 1.90 (1H, m, 3''-H\(_a\)), 2.11 (1H, m, 5''-H\(_b\)), 2.51 (1H, m, 3''-H\(_b\)), 3.74 (1H, m, 6''-H\(_b\)), 4.10 (1H, m, 6''-H\(_b\)), 5.03 (2H, s, C\(_2\)H\(_2\)), 5.84 (1H, dd, \(J = 10.4\) and 2.4 Hz, 2''-H), 7.29 (2H, m, 6-H and 8-H), 7.52 (2H, m, 5-H and 7-H), 8.07-8.09 (2H, 2 × s, 2'-H and 7'-H) and 8.46 (1H, s, 4-H). δ\(_C/\)ppm (150MHz; CDCl\(_3\)) 22.7 (C-5''), 24.8 (C-4''), 29.4 (C-3''), 45.7 (CH\(_2\)), 68.3 (C-6''), 82.8 (C-2''), 106.2 (C-3), 116.6 (C-6), 118.7 (C-7), 121.9 (C-10), 124.8 (C-8), 128.5 (C-5''), 132.3 (C-5), 135.6 (C-4), 144.5 (C-7'), 150.2 (C-2''), 151.8 (C-4''), 153.7 (C-9), 157.4 (C-6') and 161.4 (C=O).

**N- Allyl-3-cyanoaniline 177 and N,N-Diallyl-3-cyanoaniline 177A**

![Structure diagram](image)

To a stirred solution of 3-aminobenzonitrile 173 (0.50 g, 4.2 mmol) in dry THF (42 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 0.10 g, 4.2 mmol) in small portions to permit controlled evolution of hydrogen. Allyl bromide (0.37 mL, 4.2 mmol) was then added through a septum and the resulting solution was refluxed for ca. 48 h. The reaction was quenched by the addition of water (45 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with EtOAc (2 × 90 mL). The combined organic extracts were washed sequentially with water (2 × 180 mL) and brine (2 × 180 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO\(_4\)). Radial chromatography [on silica gel; elution with hexane-EtOAc (4:1)] afforded N- Allyl-3-cyanoaniline 177 as a yellow oil (300 mg, 45%). (Found: M^+, 158.085475. C\(_{10}\)H\(_{10}\)N\(_2\) requires: M^+, 158.200253); ν_{max} (thin film/cm\(^{-1}\)) 3391 (NH) 2228 (CN); δ\(_H/\)ppm (400MHz; CDCl\(_3\)) 3.76 (2H, m, 1'-CH\(_2\)), 4.13 (1H, s, NH), 5.18 (1H, ddd, \(J = 10.3, 2.8\) and 1.4 Hz, CH=CH\(_E\)), 5.26 (1H, ddd, \(J = 17.2, 3.1\) and 1.6 Hz, CH=CH\(_Z\)), 5.89 (1H, tdd, \(J = 17.2, 3.1\) and 1.6 Hz, CH=CH\(_Z\)), 5.89 (1H, tdd, \(J = 17.2, 3.1\) and 1.6 Hz, CH=CH\(_Z\)).
17.1, 10.4 and 5.2 Hz, 2'-H), 6.78 (2H, m, 4-H and 6-H), 6.92 (1H, m, 2-H) and 7.19 (1H, m, 5-H); δC/ppm (100MHz; CDCl₃) 45.9 (C-1'), 112.7 (CN), 114.9 (C-6), 116.6 (C-3'), 117.3 (C-4), 119.4 (C-3), 120.6 (C-2), 129.7 (C-5), 134.1 (C-2') and 148.2 (C-1).

In a separate reaction 3-aminobenzonitrile 173 (1.0 g, 8.5 mmol) in THF (20 mL) under nitrogen was NaH (60% dispersion in mineral oil; 370 mg, 15 mmol) and allyl bromide (0.74 mL, 8.5 mmol). Radial chromatography [on silica gel; elution with hexane-EtOAc (4:1)] afforded (i) N-Allyl-(3-cyano)aniline 177 as a yellow oil (410 mg, 32%) and (ii) N,N-Diallyl-3-cyanoaniline 177A as yellow oil (130 mg, 7.6%). (Found: [M-1]⁺, 197.107123. C₁₃H₁₄N₂ requires: M⁺, 198.115699); νmax (thin film/cm⁻¹) 2228 (CN); δH/ppm (400MHz; CDCl₃) 3.92 (4H, m, 1'-CH₂ and 1''-CH₂), 5.16 (4H, m, 3'-CH₂ and 3''-CH₂), 5.81 (2H, m, 2'-H and 2''-H), 6.85 (2H, m, 4-H and 6-H), 6.91 (1H, d, J = 7.36 Hz, 2-H) and 7.22 (1H, m, 5-H); δC/ppm (100MHz; CDCl₃) 52.7 (C-1' and C-1''), 112.7 (CN), 114.9 (C-4 or C-6), 116.2 (C-4 or C-6), 116.4 (C-3' and C-3''), 119.4 (C-2), 119.7 (C-3), 129.6 (C-5), 132.6 (C-2' and C-2''), and 148.6 (C-1).

2-Chloro-N-(3-cyanophenyl)acetamide 181

A mixture of 3-aminobenzonitrile (0.50g, 4.2 mmol) 173 and chloroacetyl chloride (3.3 mL, 42 mmol) was stirred under argon at room temperature for ca. 4 days. Excess chloroacetyl chloride was evaporated in vacuo and the residue dissolved in EtOAc (50 mL). The solution was washed sequentially with satd. aq. NaHCO₃ (2 × 50 mL) and brine (2 × 50 mL). The aqueous washings were extracted with EtOAc and the organic layers were combined and dried (anhydr. MgSO₄). The solvent was evaporated in vacuo to afford 2-chloro-N-(3-cyanophenyl)acetamide 181 as light brown flakes (0.79
g, 95%), m.p. 141–144 °C (lit.\textsuperscript{1} 144–146 °C) (Found: \( M^+ \), 194.02431. Calc. for \( \text{C}_9\text{H}_7\text{ClN}_2\text{O} \) \( M^+ \), 194.02469); \( \nu_{\text{max}} \) (solid deposit/cm\(^{-1}\)) 3451 (NH), 2232 (C≡N) and 1676 (NHC=O); \( \delta_H/\text{ppm} \) (400MHz; CDCl\(_3\)) 4.20 (2H, s, CH\(_2\)), 7.45 (1H, d, \( J = 1.0 \) Hz, 4'-H), 7.46 (1H, m, 6'-H), 7.73 (1H, m, 5'-H), 7.98 (1H, s, 2'-H) and 8.35 (1H, s, NH); \( \delta_C \) (100MHz; CDCl\(_3\)) 42.7 (CH\(_2\)), 113.3 (C-3'), 118.1 (C-1'), 123.1 (C-2'), 124.1 (C-5'), 128.6 (C-6'), 130.0 (C-4'), 137.5 (C≡N) and 164.1(C=O).

Diethyl [(3-cyanophenyl)carbamoyl]methyl phosphonate 182

![Diethyl [(3-cyanophenyl)carbamoyl]methyl phosphonate 182](image)

2-Chloro-N-(3-cyanophenyl)acetamide 181 (1.0 g, 5.1 mmol) was placed in an oven-dried round bottomed flask equipped with a reflux condenser. Triethyl phosphite (4.4 mL, 26 mmol) was added under nitrogen through a septum. The resulting mixture was refluxed until the substrate 181 was shown by TLC to have been completely consumed (ca. 5.5h). The cooled mixture was then stirred with hexane (20 mL) for ca. 10 minutes followed by decantation of the hexane layer to remove the excess phosphite. This procedure was repeated twice. The crude solid was transferred to a Buchner funnel and washed with more hexane to afford \textit{diethyl [(3-cyanophenyl)carbamoyl]methyl phosphonate} 182 as a cream powder (1.3 g, 84%), m.p. 119-125ºC (Found: \( M^+ \), 296.09163. C\(_{13}\)H\(_{17}\)N\(_2\)O\(_4\)P requires: \( M^+ \), 296.09260); \( \nu_{\text{max}} \) (solid deposit/cm\(^{-1}\)) 3423 (NH), 2231 (C≡N), 1685 (C=O), 1220 (P=O) and 1024 (P–O–C\(_2\)H\(_5\)); \( \delta_H/\text{ppm} \) (400MHz; CDCl\(_3\)) 1.39 (6H, t, \( J = 7.1 \) Hz, 2 × CH\(_3\)), 3.09 (2H, d, \( J_{\text{P–H}} = 21.6 \) Hz, CH\(_2\)P), 4.22 (4H, p, \( J = 7.1 \) Hz, 2 × CH\(_3\)CH\(_2\)H), 7.21 (2H, m, 1\textsuperscript{1}\text{http://www.acros.com/DesktopModules/Acros_Search_Results/Acros_Search_Results.aspx?search_type=CatalogSearch&SearchString=BTB02751}}
Experimental

4'-H and 5'-H), 7.52 (1H, td, J = 6.9 and 2.3 Hz, 6'-H), 7.87 (1H, s, 2'-H) and 9.92 (1H, s, NH); δC (100MHz; CDCl₃) 16.3 (d, Jᵣ₋₋C = 24.8 Hz, 2 × CH₃), 36.3 (d, Jᵣ₋₋C = 129.6 Hz, CH₂P), 63.3 (d, Jᵣ₋₋C = 26.8 Hz, 2 × CH₂CH₃), 112.6 (C-1'), 118.4 (C-3'), 122.4 (C-2'), 123.2 (C-6'), 127.3 (C-5'), 129.5 (C-4'), 138.9 (C≡N) and 162.6 (C=O, d, Jᵣ₋₋C = 4.5); δP/ppm (162 MHz; CDCl₃) 18.3 (P=O).

2-Acetoxy-N-(3-cyanophenyl)acetamide 184

NaH (60% dispersion in mineral oil; 0.17 g, 8.1 mmol) in small portions to permit controlled evolution of hydrogen, to a stirred solution of 3-aminobenzonitrile 176 (0.50 g, 4.2 mmol) in dry THF (43 mL) under nitrogen at 0ºC. Acetoxyacetyl chloride (0.50 mL, 4.2 mmol) was then added through a septum and the resulting solution refluxed and monitored for ca. 5 h during which time the reaction was monitored for the disappearance of the substrate 173. The reaction was quenched by the addition of water (40 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with CH₂Cl₂ (2 × 40 mL). The combined organic extracts were washed sequentially with 10% aq. NaHCO₃ (2 × 80 mL), water (2 × 80 mL) and brine (2 × 80 mL). The aqueous washings were extracted with CH₂Cl₂ and the organic layers combined and dried (anhydr. MgSO₄). Evaporation of the solvent in vacuo afforded 2-acetoxy-N-(3-cyanophenyl)acetamide 184 as light yellow crystals (0.78 g, 85%), m.p. 96-100ºC. (Found M⁺, 218.06803, C₁₁H₁₀N₂O₃ requires M⁺, 218.20920), v_max (solid deposit/cm⁻¹) 3344 (NHC=O), 2230 (CN) and 1735 (CH₃C=O); δH/ppm (400MHz;CDCl₃) 2.20 (3H, s, CH₃), 4.68 (2H, s, C-2), 7.40 (2H, m, 5'-H and 6'-H), 7.74 (1H, td, J = 7.6 and 2.0 Hz, 4'-H) and 7.94 (1H, s, 2'-H); δC/ppm (100MHz; CDCl₃) 20.6 (CH₃), 63.0 (C-2), 112.7 (C-3'), 118.3 (C-1'), 123.2 (C-2'), 124.3 (C-4'), 128.1 (C-5'), 129.9 (C-6'), 137.7 (C≡N), 165.6 (NHC=O) and 169.6 (CH₃C=O); m/z = 217.10 (M⁺-1, 4%) and 294.12 (100%).
**Experimental**

**2,3,4,5,6-Pentaacetyl-N-(3-cyanophenyl)gluconamide 186**

![Chemical Structure]

To a solution of 3-aminobenzonitrile (0.10 g, 0.85 mmol) in pyridine (2 mL) was added NaH (60% dispersion in mineral oil; 0.035 g, 0.85 mmol) in small portions to permit controlled evolution of hydrogen. 2,3,4,5,6-pentaacetylgluconoyl chloride 131 (0.36 g, 0.85 mmol) was then added and the reaction mixture stirred at room temperature for 2.5 days. The solvent was evaporated in vacuo and the residue dissolved in EtOAc (30 mL). The solution was washed with water (2 × 30 mL) and brine (2 × 30 mL), dried (anhyd. MgSO₄) and evaporated in vacuo to afford a yellow residue, chromatography [radial chromatography; elution with hexane-EtOAc (1:1)] of which afforded 2,3,4,5,6-pentaacetyl-N-(3-cyanophenyl)gluconamide 186 as yellow crystals (180 mg, 41%), m.p. 103-105°C. (Found: M⁺, 506.15293. C₂₃H₂₆N₂O₁₁ requires: M⁺, 506.46033); νmax (solid deposit/cm⁻¹) 3431 (NH), 2231 (CN), 1747 (CH₃C=O) and 1600 (NHC=O); δH/ppm (400MHz; CDCl₃) 2.05-2.11 (12H, series of singlets, 4 × OAc), 2.23 (3H, s, CH₃, 6-OAc), 4.12 (1H, dd, J = 12.5 and 5.3 Hz, 6-Hₐ), 4.36 (1H, dd, J = 12.5 and 3.2 Hz, 6-Hₐ), 5.05 (1H, ddd, J = 6.6, 5.3 and 3.2 Hz, 5-H), 5.28 (1H, dd, J = 5.9 Hz, 2-H), 5.47 (1H, ddd, J = 6.7 and 4.6 Hz, 4-H), 5.72 (1H, dd, J = 5.81 and 4.58 Hz, 3-H), 7.41 (2H, m, 4'-H and 6'-H), 7.72 (1H, m, 5'-H), 7.89 (1H, s, 2'-H) and 8.23 (1H, s, NH); δC/ppm (100MHz; CDCl₃) 20.4, 20.6, 20.67, 20.70 and 20.73 (5 × CH₃), 61.8 (C-6), 68.6 (C-3), 68.9 (C-5), 69.1 (C-4), 71.6 (C-2), 113.1 (C-3'), 118.2 (C-1'), 123.1 (C-2'), 124.2 (C-5'), 128.4 (C-6'), 130.0 (C-4') 137.7 (C-7'), 164.6 (NH-C=O), 169.4 (2-C=O), 169.9 (3-C=O), 170.0 (5-C=O), 170.3 (4-C=O) and 170.9 (6-C=O); m/z = 507.07 (M⁺+1, 100%).
Experimental

3-(Allyloxy)phenol 188

NaH (60% dispersion in mineral oil; 0.36 g, 9.1 mmol) was added in small portions to permit controlled evolution of hydrogen, to a stirred solution of 3-aminophenol (1.0 g, 9.1 mmol) in dry THF (20 mL) under nitrogen at 0°C. Allyl bromide (0.8 mL, 9.1 mmol) was then added with through a septum and the resulting solution refluxed for ca. 6 h. The reaction was quenched by the addition of water (50 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed sequentially with water (2 × 100 mL) and brine (2 × 100 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO₄). The solvent was evaporated in vacuo and the residue chromatographed [radial chromatography; elution with hexane-EtOAc (7:3)] to afford 3-(allyloxy)phenol 188 as a reddish brown oil (0.38 g, 28%). (Found: M⁺, 150.067020. C₉H₁₀O₂ requires: M⁺, 150.068080); νmax (thin film/cm⁻¹) (OH); δH/ppm (400MHz; CDCl₃) 4.50 (2H, dt, J = 5.2 and 1.5 Hz, 1'-CH₂), 5.28 (1H, qd, J = 10.5 and 1.4 Hz, CH=CH₂), 5.40 (2H, m, HC=CH₂ and OH), 6.04 (1H, m, 2'-H), 6.43 (2H, m, 2-H and 6-H), 6.52 (1H, ddd, J = 8.3, 2.2 and 1.1 Hz, 4-H) and 7.12 (1H, t, J = 8.5, 5-H); δC/ppm (100MHz; CDCl₃) 68.9 (C-1'), 102.4 (C-2), 107.3 (C-4), 108.0 (C-6), 117.8 (C-3'), 130.2 (C-5), 133.1 (C-2'), 156.6 (C-1) and 159.8 (C-3).
**Experimental**

1,3-Bis(chloroacetoxy)phenol 189A

NaH (60% dispersion in mineral oil; 0.22 g, 9.1 mmol) was added in small portions to permit controlled evolution of hydrogen, to a stirred solution of resorcinol 187 (1.0 g, 9.1 mmol) in dry THF (20 mL) under nitrogen at 0 ºC. Chloroacetyl chloride (0.71 mL, 9.1 mmol) was then added through a septum and the resulting solution refluxed for ca. 6 h. The reaction was quenched by the addition of water (20 mL). The solvent was evaporated *in vacuo* and the aqueous residue extracted with EtOAc (2 × 40 mL). The combined organic extracts were washed sequentially with water (2 × 80 mL) and brine (2 × 80 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO₄). The solvent was evaporated *in vacuo* and the residue chromatographed [radial chromatography; elution with hexane-EtOAc (6:4)] to afford 1,3-(bischloroacetoxy)phenol 189A as a yellow oil. (570 mg, 24%), (Found: M⁺, 261.978798 C₁₀H₈Cl₂O₄ requires: M⁺, 262.106210); νmax (thin film/cm⁻¹) 1776 (C=O); δH/ppm (400MHz; CDCl₃) 4.27 (4H, s, 2'-H and 2"-H), 7.03 (1H, t, J = 2.2 Hz, 2-H), 7.06 (1H, d, J = 2.3 Hz, 4-H or 6-H), 7.08 (1H, d, J = 2.15 Hz, 4-H or 6-H) and 7.41 (1H, t, J = 8.2 Hz, 5-H); δC/ppm (100MHz; CDCl₃) 40.7 (C-2' and C-2"), 114.7 (C-2), 119.1 (C-4 and C-6), 130.1 (C-5), 150.7 (C-3 and C-1) and 165.3 (C-1' and C-1").
**Experimental**

Resorcinol bis [acetyloxy)acetate] 190A

![Chemical Structure](image)

NaH (60% dispersion in mineral oil; 48 mg, 2.0 mmol) was added in small portions to permit controlled evolution of hydrogen, to a stirred solution of resorcinol 187 (220 mg, 2.0 mmol) in dry THF (15 mL) under nitrogen at 0°C. Acetoxyacetyl chloride (0.22 mL, 2.0 mmol) was then added through a septum and the resulting solution refluxed for ca. 5 h during which time the reaction was monitored for the disappearance of the substrate resorcinol 187. The reaction was quenched by the addition of water (20 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed sequentially with water (2 x 40 mL) and brine (2 x 40 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO₄). Evaporation of the solvent in vacuo and chromatography of the residue [column chromatography with EtOH:CHCl₃:hexane = 0.5:1.5:2.5] afforded resorcinol bis[acetyloxy)acetate] 190A as a yellow oil (25 mg, 40%). (Found M⁺, 310.067879, C₁₄H₁₄O₈ requires 310.068868); νmax (solid deposit) 1746 δH/ppm (400MHz; CDCl₃) 2.18 (6H, 2 x s, 2 x CH₃), 4.81 (4H, 2 x s, 2 x CH₂), 6.96 (1H, t, J = 2.2 Hz, 2-H), 7.03 (2H, dd, J = 8.2 and 2.2 Hz, 4-H and 6-H) and 7.38 (1H, t, J = 8.2 Hz, 5-H); δC/ppm (100MHz; CDCl₃) 20.4 (2 x CH₃), 60.7 (2 x CH₂), 115.0 (C-2), 119.2 (C-4 and C-6), 129.9 (C-5), 150.4 (C-1 and C-3), 167.8 (2 x CH₂C=O) and 170.3 (2 x CH₃C=O).
2,3,4,5,6-Pentaacetyl-O-(3-hydroxyphenyl)gluconate 191

To a solution of resorcinol (200 mg, 0.91 mmol) in pyridine (2 mL) was added NaH (60% dispersion in mineral oil; 41 mg, 1.7 mmol) in small portions to permit controlled evolution of hydrogen. 2,3,4,5,6-pentaacetylgluconoyl chloride 131 (400 mg, 0.94 mmol) was then added and the reaction mixture stirred at room temperature for ca. 2.5 days. The solvent was taken off in vacuo and the residue dissolved in EtOAc (2 × 25 mL). This solution was washed with water (2 × 50 mL) and brine (2 × 50 mL). The organic layer was then dried (anhydr. MgSO4) and the solvent evaporated in vacuo to afford a reddish brown oil, flash chromatography [on silica gel; elution with EtOH:CHCl3:hexane (0.5:1.5:2.5)] of which afforded 2,3,4,5,6-pentaacetyl-O-(3-hydroxyphenyl)-gluconate 191 as a yellow oil, (64 mg, 14%).

(Found: M⁺, 498.137550, C22H26O13 requires: M⁺, 498.434913); νmax (thin film/cm⁻¹) 3419 (OH) and 1747 (C=O); δH/ppm (400MHz; CDCl3) 2.08 (12H, series of singlets, 4 × OAc), 2.22 (3H, s, CH3, 6-OAc), 4.16 (1H, dd, J = 12.2 and 5.3 Hz, 6-Ha), 4.22 (1H, dd, J = 12.3 and 4.0 Hz, 6-Hb), 5.14 (1H, m, 5-H), 5.16 (1H, d, J = 3.53 Hz, 2-H), 5.58 (1H, dd, J = 6.7 and 4.6 Hz, 4-H), 5.74 (1H, t, J = 4.10 Hz, 3-H), 6.59 (1H, t, J = 2.0 Hz, 2'-H), 6.65 (1H, dd, J = 8.0 and 1.8 Hz, 4'-H), 6.71 (1H, dd, J = 8.2 and 1.8 Hz, 6'-H) and 7.20 (1H, t, J = 8.2 Hz, 5'-H); δC/ppm (100MHz; CDCl3) 20.4, 20.45, 20.6, 20.68 and 20.8 (5 × OAc), 61.6 (C-6), 68.3 (C-5), 68.4 (C-3), 69.2 (C-4), 70.8 (C-2), 108.8 (C-2'), 113.2 (C-4'), 113.7 (C-4'), 130.2 (C-5'), 150.8 (C-1'), 156.7 (C-3'), 165.4 (1'-C=O), 169.7 (5-C=O), 169.8 (3-C=O), 169.9 (4-C=O), 170.1 (2-C=O) and 170.6 (6-C=O).
Resorcinol monoacrylate 192 and resorcinol diacrylate 192A

NaH (60% dispersion in mineral oil; 400 mg, 9.1 mmol) was added in small portions permit controlled evolution of hydrogen, to a stirred solution of resorcinol 187 (1.0 g, 9.1 mmol) in dry THF (20 mL) under nitrogen at 0°C. Acryloyl chloride (0.74 mL, 9.1 mmol) was then added with through a septum and the resulting solution refluxed for ca. 6 h. The reaction was quenched by the addition of water (25 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed sequentially with water (2 × 50 mL) and brine (2 × 50 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO₄). The solvent was evaporated in vacuo and the residue chromatographed [preparative TLC; elution with hexane-EtOAc (1:1)] to afford two fractions.

(i) **Resorcinol monoacrylate 192** as a yellow oil (120 mg, 8%). (Found: M⁺, 164.046723. C₉H₈O₃ requires: M⁺, 164.047344); ν max (thin film/cm⁻¹) 3393 (OH) 1721 (C=O); δ H/ppm (400MHz; CDCl₃) 5.68 (1H, s, OH), 6.02 (1H, d, J = 10.5 Hz, CH=CH₂), 6.31 (1H, dd, J = 17.3 and 10.5 Hz, 2'-H), 6.60 (1H, m, 2-H and CH=CH₂), 6.66 (1H, d, J = 2.2 Hz, 4-H), 6.68 (1H, d, J = 2.1 Hz, 6-H) and 7.21 (1H, t, J = 8.2 Hz, 5-H); δ C/ppm (100MHz; CDCl₃) 109.1 (C-2), 113.2 (C-6), 113.5 (C-4), 127.8 (C-2'), 130.1 (C-5), 133.0 (C-3'), 151.3 (C-1), 156.6 (C-3) and 164.8 (C=O).

(ii) **Resorcinol diacrylate 192A** as a yellow oil (260 mg, 13%). (Found: M⁺, 218.058102. C₁₂H₁₀O₄ requires: M⁺, 218.057909.); ν max (thin film/cm⁻¹) 1743 (C=O);
Experimental

δ_H/ppm (400MHz; CDCl_3) 6.01 (1H, 2 × dd, J = 10.4 and 0.8 Hz, 2 × CH=CH_2), 6.30 (1H, dd, J = 17.3 and 10.4 Hz, 2'-H and 2''-H), 6.60 (2H, 2 × dd, J = 17.3 and 0.8 Hz, 2 × CH=CH_2), 7.01 (1H, m, 2-H), 7.03 (1H, d, J = 2.1 Hz, 4-H), 7.05 (1H, d, J = 2.1 Hz, 6-H) and 7.39 (1H, t, J = 8.1 Hz, 5-H); δ_C/ppm (100MHz; CDCl_3) 115.4 (C-2), 119.0 (C-4 and C-6), 127.6 (C-2' and C-2''), 129.7 (C-5), 132.9 (C-3' and C-3''), 150.9 (C-1 and C-3) and 164.1 (2 × C=O).

_N- Allyl-(3-hydroxy)aniline 194, N- Allyl-3-(allyloxy)aniline 194A and N,N-diallyl-3-hydroxyaniline 194B_

To a stirred solution of 3-aminophenol 193 (3.0 g, 28 mmol) in dry THF (30 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 1.1 g, 28 mmol) in small portions to permit controlled evolution of hydrogen. Allyl bromide (2.4 mL, 28 mmol) was then added through a septum and the resulting solution was refluxed for ca. 4.5 h. The reaction was quenched by the addition of water (50 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed sequentially with water (2 × 100 mL) and brine (2 × 100 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO_4). Flash chromatography [on silica gel; elution with hexane-EtOAc (8:2)] afforded 3 fractions.

(i) _N- Allyl-(3-hydroxy)aniline 194_ a dark brown oil (680 mg, 17%). (Found M^+, 149.083104. C_9H_{11}NO requires 149.084064); ν_{max} (thin film/cm\(^{-1}\)) 3388 (NH); δ_H/ppm (400MHz; CDCl_3) 3.70 (2H, dt, J = 5.4 and 1.6 Hz, 1'-CH_2), 5.16 (1H, qd, J = 10.3 and 1.40 Hz, CH=CH_E), 5.26 (1H, qd, J = 17.2 and 1.6 Hz, CH=CH_E), 5.92 (1H, m, 2'-H), 6.10 (1H, t, J = 2.3 Hz, 2'-H), 6.20 (1H, dd, J = 8.0 and 2.3 Hz, 6-H), 6.23 (1H, dd, J = 8.1 and 2.1 Hz, 4-H) and 7.02 (1H, t, J = 8.0 Hz, 5-H); δ_C/ppm
Experimental

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(ii) *N*-Allyl-3-(allyloxy)aniline 194A a dark brown oil (270 mg, 5.2%). (Found M+, 189.115840. C_{12}H_{15}NO requires 189.115364); ν_{max} (thin film/cm^{-1}) 3406 (NH_{2}); δ_{H}/ppm (400MHz; CDCl_{3}) 3.77 (2H, dt, \( J = 5.4 \) and 1.6 Hz, 1'-CH_{2}), 4.51 (2H, td, \( J = 5.3 \) and 1.5 Hz, 1''-CH_{2}), 5.18 (1H, m, 3'-Ha), 5.30 (2H, m, 3'-Hb and 3''-Ha), 5.42 (1H, m, 3''-Hb), 5.97 (1H, m, 2'-H), 6.07 (1H, m, 2''-H), 6.23 (1H, t, \( J = 2.3 \) Hz, 2-H), 6.26 (1H, dd, \( J = 8.0 \) and 2.2 Hz, 6-H), 6.31 (1H, dd, \( J = 8.1 \) and 2.3 Hz, 4-H) and 7.08 (1H, t, \( J = 8.1 \) Hz, 5-H); δ_{C}/ppm (100MHz; CDCl_{3}) 46.5 (C-1'), 68.6 (C-1''), 99.7 (C-2), 103.4 (C-4), 106.3 (C-6), 116.1 (C-3'), 117.3 (C-3''), 129.8 (C-5), 133.5 (C-2''), 135.3 (C-2'), 149.4 (C-1) and 159.8 (C-3).

(iii) *N,N*-Diallyl-3-(hydroxy)aniline 194B as a dark brown oil (830 mg, 16%). (Found M+, 189.114166. C_{12}H_{15}NO requires 189.115364); ν_{max} (thin film/cm^{-1}) 3363 (NH); δ_{H}/ppm (400MHz; CDCl_{3}) 3.89 (4H, m, 1'-CH_{2} and 1''-CH_{2}), 5.18 (4H, m, 3'-CH_{2} and 3''-CH_{2}), 5.85 (2H, m, 2'-H and 2''-H), 6.19 (1H, m, 6-H), 6.22 (1H, s, 2-H), 6.32 (1H, m, 4-H), and 7.06 (1H, t, \( J = 8.1 \) Hz, 5-H); δ_{C}/ppm (100MHz; CDCl_{3}) 52.7 (C-1' and C-1''), 99.6 (C-2), 103.5 (C-6), 105.3 (C-4), 116.0 (C-3' and C-3''), 129.9 (C-5), 133.8 (C-2' and C-2''), 150.2 (C-1) and 156.4 (C-3).

2-Chloro-*N*-(3-hydroxyphenyl)acetamide 195

![Structure](attachment:structure.png)

To a stirred solution of 3-aminophenol (3.0 g, 28 mmol) in THF (30 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 1.2 g, 50 mmol) in small portions to permit controlled evolution of hydrogen. Chloroacetyl chloride (2.2 mL,
28 mmol) was then added through a septum and the resulting solution was stirred for ca. 6 h. The solvent was evaporated in vacuo and the residue dissolved in EtOAc (2 x 50 mL). The organic extract was washed sequentially with satd. aq. NaHCO₃ (2 x 100 mL), water (2 x 100 mL) and brine (2 x 100 mL). The aqueous washings were extracted with EtOAc and the combined organic solutions were (anhydr. MgSO₄). Evaporation of the solvent in vacuo afforded 2-chloro-N-(3-hydroxyphenyl)acetamide 195 as a grey solid (3.9 g, 76%) m.p. 119-120°C. νₓmax (solid deposit/cm⁻¹) 3360 (OH) and 1642 (CO); δH/ppm (400MHz; DMSO-d₆) 4.22 (2H, s, CH₂), 6.48 (1H, dd, J = 8.0 and 2.0 Hz, 6'-H), 6.94 (1H, d, J = 8.5 Hz, 4'-H), 7.09 (1H, t, J = 8.1 Hz, 5'-H), 7.17 (1H, t, J = 2.0 Hz, 2'-H), 9.48 (1H, s, OH) and 10.18 (1H, s, NH); δC/ppm (100MHz; DMSO-d₆) 43.6 (CH₂), 106.3 (C-2'), 110.0 (C-6'), 110.9 (C-4'), 129.5 (C-5'), 139.5 (C-1'), 157.6 (C-3') and 164.4 (C=O).

[(3-Hydroxyphenyl)carbamoyl)methyl]phosphonate 196

To 2-Chloro-N-(3-hydroxyphenyl)-acetamide 195 (500 mg, 30 mmol) in an oven-dried round bottomed flask equipped with a reflux condenser was added triethyl phosphite (2.6 mL, 15 mmol) under nitrogen through a septum. The resulting mixture was refluxed for ca. 9 h during which time the reaction was monitored by TLC. The cooled mixture was then stirred with hexane (20 mL) for ca. 10 minutes followed by decantation of the hexane layer to remove the excess phosphite; this was repeated twice. Evaporation of the solvent in vacuo afforded [(3-hydroxyphenyl)-carbamoyl)methyl]phosphonate 196 as white crystals (370 mg, 48%), m.p. 115-117°C. (Found: M⁺, 287.093363. C₁₂H₁₈NO₅P requires: M⁺, 287.092261); νₓmax (solid deposit/cm⁻¹) 3263 (OH), 1667 (C=O), 1230 (P=O) and 1024 (O=O=PH₃);
\[ \delta_H/\text{ppm} \ (400\text{MHz}; \text{CDCl}_3) \ 1.32 \ (6\text{H}, \text{t}, \ J = 6.8 \text{ Hz}, 2 \times \text{CH}_3), \ 3.05 \ (2\text{H}, \text{d}, J_{\text{P-H}} = 21.1 \text{ Hz}, 1-\text{CH}_2), \ 4.17 \ (4\text{H}, \text{p}, J_{\text{P-H}} = 7.1 \text{ Hz}, 2 \times \text{CH}_3\text{CH}_2), \ 6.60 \ (1\text{H}, \text{d}, J = 8.0 \text{ Hz}, 4'-\text{H}), \ 6.80 \ (1\text{H}, \text{d}, J = 7.9 \text{ Hz}, 6'-\text{H}), \ 7.09 \ (1\text{H}, \text{t}, J = 8.0 \text{ Hz}, 5'-\text{H}), \ 7.46 \ (1\text{H}, \text{s}, 2'-\text{H}), \ 7.99 \ (1\text{H}, \text{s}, \text{OH}) \text{ and } 8.96 \ (1\text{H}, \text{s}, \text{NH}); \ \delta_C \ (100\text{MHz}; \text{CDCl}_3) \ 16.3 \ (2\text{C}, \text{d}, J_{\text{P-C}} = 6.0 \text{ Hz}, 2 \times \text{CH}_3), \ 36.4 \ (1\text{C}, \text{d}, J_{\text{P-C}} = 130.4 \text{ Hz}, 1-\text{CH}_2), \ 63.3 \ (2\text{C}, \text{d}, J_{\text{P-C}} = 6.6 \text{ Hz}, 2 \times \text{CH}_2), \ 107.3 \ (\text{C-2'}), \ 111.1 \ (\text{C-6'}), \ 112.1 \ (\text{C-4'}), \ 129.8 \ (\text{C-5'}), \ 138.5 \ (\text{C-1'}), \ 157.3 \ (\text{C-3'}) \text{ and } 162.7 \ (1\text{C}, \text{d}, J_{\text{P-C}} = 4.1 \text{ Hz}, \text{C}=\text{O}); \ \delta_P/\text{ppm} \ (162 \text{MHz}; \text{CDCl}_3) \ 24.2 \ (\text{P}=\text{O}).\]

**Experimental**

2-Acetoxy-N-(3-hydroxyphenyl)acetamide 197

\[
\begin{align*}
\text{NaH} \ (60\% \text{ dispersion in mineral oil; } 60 \text{ mg, 2.5 mmol}) \text{ was added in small portions to a stirred solution of 3-aminophenol (150 mg, 1.4 mmol) in dry THF (10 mL) under nitrogen at 0\degree \text{C}, to permit controlled evolution of hydrogen. Acetoxyacetyl chloride (160 } \mu\text{L, 2.5 m mol) was then added through a septum and the resulting solution refluxed for } & \text{ca. } 5.0 \text{ h. The reaction was quenched by the addition of water (20 mL). The solvent was evaporated } \text{in vacuo} & \text{and the aqueous residue was extracted with EtOAc (2 } \times \ 40 \text{ mL). The combined organic extracts were washed sequentially with water (2 } \times \ 80 \text{ mL) and brine (2 } \times \ 80 \text{ mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO}_4). \text{Evaporation of the solvent } & \text{in vacuo} & \text{and chromatography of the residue [column chromatography with EtOH:CHCl}_3:\text{hex} = (0.5:1.5:2.5)] \text{ afforded 2-acetoxy-N-(3-hydroxyphenyl)acetamide } 197 & \text{as a grey powder (70 mg, 24\%), m.p. 154-156\degree \text{C}. (Found } M^+ \text{, 209.068446, C}_{10}H_{11}NO_4 \text{ requires 209.068808), } & \text{v}_{\text{max}} \ (\text{solid deposit/cm}^{-1}) \ 3424 \ (\text{OH}), \ 1734 \ (\text{CH}_3\text{C}=\text{O}), \ 1678 \ (\text{NHC}=\text{O}); & \delta_H/\text{ppm} \ (400\text{MHz}; \text{DMSO-}d_6) \ 2.11 \ (3\text{H, s, CH}_3), \ 4.61 & \text{(2H, s, } \text{CH}_2), \ 6.46 \ (1\text{H, dd, } J = 7.9 \text{ and } 2.2 \text{ Hz, } 6'-\text{H}), & 6.93 \ (1\text{H, d, } J = 8.3 \text{ Hz, } 4'-\text{H}), \end{align*}
\]
Experimental

7.07 (1H, t, J = 8.06 Hz, 5'-H), 7.13 (1H, t, J = 2.0 Hz, 2'-H), 9.39 (1H, br s, OH) and 9.92 (1H, s, NH); δC/ppm (100MHz; DMSO-d₆) 20.4 (CH₃), 62.5 (CH₂), 106.3 (C-2'), 109.9 (C-4'), 110.5 (C-6'), 129.3 (C-5'), 139.3 (C-1'), 157.5 (C-3'), 165.2 (C-1) and 169.9 (CH₃C=O).

2,3,4,5,6-Pentaacetyl-N-(3-hydroxyphenyl)-gluconamide 198

![Chemical Structure](image)

To a solution of 3-aminophenol (0.10 g, 0.92 mmol) in pyridine (2 mL) was added NaH (60% dispersion in mineral oil; 0.041 g, 0.92 mmol) in small portions to permit controlled evolution of hydrogen. 2,3,4,5,6-pentaacetylgluconoyl chloride 131 (0.40 g, 0.92 mmol) was then added and the reaction mixture stirred at room temperature for ca. 2.5 days. The solvent was taken off *in vacuo* and the residue dissolved in EtOAc (30 mL). This solution was washed with water (2 × 30 mL) and brine (2 × 30 mL). The organic layer was then dried (anhydr. MgSO₄) and the solvent was evaporated *in vacuo* to afford a reddish brown oil, flash chromatography [on silica gel; elution with hexane-EtOAc (4:6)] of which afforded 2,3,4,5,6-pentaacetyl-N-(3-hydroxyphenyl)-gluconamide 198 as a reddish brown oil, (93 mg, 20%). (Found: M⁺, 497.15132. C₂₂H₂₇NO₁₂ requires: M⁺, 497.45019); νmax (solid deposit/cm⁻¹) 3427 (OH), 1740 (CH₃C=O) and 1605 (NH-C=O); δH/ppm (400MHz; CDCl₃) 2.06 (12H, series of singlets, 4 × OAc), 2.22 (3H, s, CH₃, 6-OAc), 4.12 (1H, dd, J = 12.4 and 5.5 Hz, 6-Ha), 4.34 (1H, dd, J = 12.4 and 3.3 Hz, 6-Hb), 5.06 (1H, dt, J = 6.0, 5.8 and 3.4 Hz, 5-H), 5.34 (1H, d, J = 5.4 Hz, 2-H), 5.47 (1H, dd, J = 6.5 and 4.7 Hz, 4-H), 5.70 (1H, t, J = 5.00 Hz, 3-H), 6.63 (1H, dd, J = 8.1 and 2.0 Hz, 4'-H), 6.81 (1H, dd, J = 8.0 and 1.1 Hz, 6'-H), 7.13 (1H, t, J = 8.1 Hz, 5'-H), 7.33 (1H, s, 2'-H) and 8.10 (1H, s, NH); δC/ppm (100MHz; CDCl₃) 20.4, 20.6, 20.66 and 20.72 (5 × OAc), 61.7 (C-6), 68.9 (C-3), 69.0 (C-5), 69.2 (C-4), 71.8 (C-2), 107.4 (C-2¹), 111.6 (C-6'), 112.4 (C-4'), 130.0 (C-5'), 137.6 (C-3'), 156.9 (C-1'), 164.6 (NH-C=O), 169.6 (5-C=O), 169.9 (3-
\[ C=O \], 170.0 (2-C=O), 170.3 (4-C=O) and 170.9 (6-C=O); \( m/z = 496.11 \) (\( M^+ - 1 \), 28%) and 412.25 (100%).

**N-(3-Hydroxyphenyl) acryloyl amine 199** and **3-(acryloylamino)phenyl acrylate 199A**

To a stirred solution of 3-aminophenol (0.50 mg, 4.6 mmol) in dry THF (15 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 0.11 g, 4.6 mmol) in small portions to permit controlled evolution of hydrogen. Acryloyl chloride (0.37 mL, 4.6 mmol) was then added through a septum and the resulting solution was refluxed for ca. 6 h. The reaction was quenched by the addition of water (20 mL). The solvent was evaporated *in vacuo* and the aqueous residue extracted with EtOAc (2 × 40 mL). The combined organic extracts were washed sequentially with water (2 × 80 mL) and brine (2 × 80 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO\(_4\)). Preparative thin layer chromatography [on silica gel; elution with hexane-EtOAc (4:6)] afforded two fractions.

(i) **N-(3-hydroxyphenyl)acryloyl amine 199** as a yellow oil, (5.4 mg, 0.72%). (Found: \( M^+ \), 163.076095. \( \text{C}_9\text{H}_9\text{NO}_2 \) requires: \( M^+ \), 163.063329); \( \nu_{\max} \) (thin film/cm\(^{-1}\)) 3419 (OH) 1667 (C=O); \( \delta_{\text{H}}/\text{ppm} \) (400MHz; CDCl\(_3\)) 6.01 (1H, d, \( J = 10.5 \) Hz, 3'-Ha), 6.30 (1H, dd, \( J = 17.3 \) and 10.5 Hz, 2'-H), 6.60 (1H, d, \( J = 17.3 \) Hz, 3'-Hb), 6.89 (1H, d, \( J = 7.0 \) Hz), 7.34 (2H, m, 5-H and 6-H) and 7.50 (1H, s, 2'-H); \( \delta_{\text{C}}/\text{ppm} \) (150MHz; CDCl\(_3\)) 113.5 (C-2), 117.1 (C-6), 17.6 (C-4), 127.8 (C-2'), 129.7 (C-5), 132.9 (C-3'), 138.5 (C-1), 150.9 (C-3) 164.6 and 167.7 (H-N-C=O).
(ii) 3-(Acryloylamino)phenyl acrylate **199A** as yellow oil, (12 mg, 1.2%). (Found: \( M^+ \), 217.072324. \( C_{12}H_{11}NO_3 \) requires: \( M^+ \), 217.073893); \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 1731(C=O) and 1663 (NHC=O); \( \delta_H / \text{ppm} \) (400MHz; CDCl\(_3\)) 5.70 (1H, d, \( J = 10.2 \) Hz, 3"-H\(_a\)), 6.03 (1H, d, \( J = 10.4 \) Hz, 3'-H\(_a\)), 6.16 (1H, dd, \( J = 16.8 \) and 10.3 Hz, 2"-H), 6.32 (2H, m, 2'-H and 3"-H\(_b\)), 6.60 (1H, d, \( J = 17.3 \) Hz, 3'-H\(_b\)), 6.82 (1H, d, \( J = 7.09 \) Hz, 6-H), 7.24 (2H, m, 4-H and 5-H), 7.58 (1H, s, 2-H) and 7.95 (1H, s, NH); \( \delta_C / \text{ppm} \) (100MHz; CDCl\(_3\)) 113.5 (C-2), 117.2 (C-4), 117.3 (C-6), 127.7 (C-2'), 127.9 (C-3"), 129.6 (C-5), 130.9 (C-1), 133.1 (C-3'), 139.1 (C-3), 150.7 (C-1), 163.7 (C=O) and 165.0 (NHC=O).

**N-allyl-3-(hydroxymethyl) aniline 201, N-allyl-3-[(allyloxy)methyl] aniline 201A, N,N-diallyl-3-(hydroxymethyl)aniline 201B and 3-[(allyloxy)methyl]aniline 201C**

\[\text{HO} \quad 4 \quad 3 \quad 2 \quad 1' \quad 2' \quad 3' \quad 6 \quad 5 \quad \text{NH} \quad 1'' \quad 2'' \quad 3'' \]

\[\text{O} \quad 4 \quad 3 \quad 2 \quad 1' \quad 2' \quad 3' \quad 6 \quad 5 \quad \text{NH}_2 \quad 1'' \quad 2'' \quad 3'' \]

To a stirred solution of 3-aminobenzyl alcohol **200** (1.0 g, 8.1 mmol) in dry THF (41 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 0.30 g, 8.1 mmol) in small portions to permit controlled evolution of hydrogen. Allyl bromide (0.80 mL,
8.1 mmol) was then added through a septum and the resulting solution was refluxed for ca. 6 h. The reaction was quenched by the addition of water (50 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed sequentially with water (2 × 100 mL) and brine (2 × 100 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO₄). Flash chromatography [on silica gel; elution with hexane-EtOAc (7:3)] afforded four fractions.

i. **N-Allyl-3-(hydroxymethyl) aniline 201** as a reddish brown oil (130 mg, 10%). (Found M⁺, 163.098763. C₁₀H₁₃NO requires M⁺, 163.099714); νmax (thin film/cm⁻¹) 3347 (OH and NH); δ_H/ppm (400MHz; CDCl₃) 3.76 (2H, td, J = 5.4 and 1.6 Hz, 1'-CH₂), 4.58 (2H, s, 7-CH₂), 5.16 (1H, ddd, J = 10.3, 3.0 and 1.5 Hz, CH=CH₂), 5.28 (1H, ddd, J = 17.2, 3.3 and 1.6 Hz, CH=CH₂), 5.95 (1H, m, 2'-H), 6.54 (1H, dd, J = 8.0 and 2.2 Hz, 6-H), 6.62 (1H, s, 2-H), 6.68 (1H, d, J = 7.47 Hz, 4-H) and 7.15 (1H, t, J = 7.8 Hz, 5-H); δC/ppm (100MHz; CDCl₃) 46.5 (C-1'), 65.6 (C-7), 111.4 (C-2), 112.2 (C-6), 116.0 (C-4), 116.2 (C-3'), 129.4 (C-5), 135.3 (C-2'), 142.1 (C-1) and 148.3 (C-3).

ii. **N-Allyl-3-[allyloxy)methyl] aniline 201A** as reddish brown oil (140 mg, 8.5%). (Found: M⁺, 203.130341. C₁₃H₁₇NO requires: M⁺, 203.131014); νmax (thin film/cm⁻¹) 3393 (NH); δ_H/ppm (400MHz; CDCl₃) 3.78 (2H, td, J = 5.4 and 1.5 Hz, 1'-CH₂), 4.02 (1H, td, J = 5.6 and 1.4 Hz, 3-H), 4.46 (2H, s, CH₂), 5.18 (1H, m, 3'-H), 5.29 (2H, qdd, J = 17.2, 9.8 and 1.6 Hz, 3''-H), 5.96 (2H, m, 2''-H and 2'-H), 6.55 (1H, dd, J = 8.0 and 2.3 Hz, 6-H), 6.64 (1H, s, 2-H), 6.68 (1H, d, J = 7.5 Hz, 4-H), 7.14 (1H, dd, J = 9.6 and 5.9 Hz, 5-H); δC/ppm (100MHz; CDCl₃) 46.5 (C-1'), 71.0 (C-1''), 72.3 (C₂H₂), 112.15 (C-2), 112.20 (C-6), 116.2 (C-3'), 116.91 (C-3''), 116.93 (C-4), 129.2 (C-5), 134.8 (C-2''), 135.3 (C-2''), 139.4 (C-3) and 148.2 (C-1).

iii. **N,N-bis allyl-3-(hydroxymethyl) aniline 201B** as a reddish brown oil, (82 mg, 6.2%). (Found: M⁺, 203.131507. C₁₀H₁₇NO requires: M⁺, 203.131014); νmax (thin film/cm⁻¹) 3360 (OH) and (NH); δ_H/ppm (400MHz; CDCl₃) 3.93 (4H, m, 1'-H and 1''-H), 4.60 (2H, s, CH₂), 5.17 (4H, m, 3'-H and 3''-H), 5.86 (2H, tdd, J = 17.1, 10.1 and 4.9 Hz, 2'-H and 2''-H), 6.63 (1H, dd, J = 8.3 and 2.5 Hz, 6-H), 6.67 (1H, d, J = 7.4 Hz, 4-H), 6.71 (1H, d, J = 1.1 Hz, 2-H) and 7.18 (1H, t, J = 7.9 Hz, 5-H); δC/ppm (100MHz; CDCl₃) 52.7 (C-1' and C-1''), 65.9 (CH₂), 110.8 (C-2), 111.7 (C-6), 114.9...
(C-4), 116.0 (C-3' and C-3'"), 129.3 (C-5), 133.8 (C-2' and C-2'"), 141.9 (C-3) and 148.9 (C-1).

iv. 3-[(allyloxy)methyl]aniline 201C as a reddish brown oil (140 mg, 11%). (Found: M⁺, 163.099525. C₁₀H₁₃NO requires: M⁺, 163.099714); νmax (thin film/cm⁻¹) 3356 (NH₂); δ H/ppm (400MHz; CDCl₃) 3.50 (2H, b, NH₂), 4.02 (2H, td, J = 5.6 and 1.2 Hz, 1'-CH₂), 4.44 (2H, s, 7-CH₂), 5.20 (1H, dd, J = 10.4 and 1.3 Hz, CH₂−CH=CHₑ), 5.30 (1H, qd, J = 17.2 and 1.5 Hz, CH₂-CH=CH₂), 5.95 (1H, tdd, J = 17.2, 10.5 and 5.6 Hz, 2'-H), 6.60 (1H, dd, J = 7.9 and 1.7 Hz, 6-H), 6.69 (1H, s, 2-H), 6.72 (1H, d, J = 7.2Hz, 4-H) and 7.11 (1H, dd, J = 9.6 and 5.8 Hz, 5-H); δC/ppm (100MHz; CDCl₃) 71.0 (C-1'), 72.1 (C-7), 114.2 (C-2), 114.3 (C-6), 117.0 (C-3'), 117.9 (C-4), 129.2 (C-5), 134.8 (C-2'), 139.5 (C-3) and 146.5 (C-1).

2-Chloro-N-[3-(hydroxymethyl)phenyl]acetamide 202 and 3-(Chloroacetamido)benzyl chloroacetate 202A

NaH (60% dispersion in mineral oil; 0.20 g, 8.1 mmol) was added in small portions to permit controlled evolution of hydrogen, to a stirred solution of 3-aminobenzyl alcohol 200 (1.0 g, 8.1 mmol) in dry THF (20 mL) under nitrogen at 0°C. Chloroacetyl chloride (0.64 mL, 8.1 mmol) was then added through a septum and the resulting solution refluxed for ca. 8h. The reaction was quenched by the addition of water (30 mL). The solvent was evaporated in vacuo and the aqueous residue was extracted with EtOAc (2 × 30 mL). The combined organic extracts were washed sequentially with water (2 × 60 mL) and brine (2 × 60 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr.
MgSO₄). The solvent was evaporated in vacuo and the residue was chromatographed [Column chromatography; elution with hexane-EtOAc (1:1)] to afford two products:

i. 2-Chloro-N-[3-(hydroxymethyl)phenyl]acetamide 202 as a light brown solid (0.60 g, 37%) m.p. 88-90°C. (Found: M⁺, 199.03915. C₉H₁₀NO₂³⁵Cl requires: M⁺, 199.15044); νmax (solid deposit/cm⁻¹) 3378 (OH), 1681 (C=O) and 1615 (NHCO); δH/ppm (400MHz; DMSO-d₆) 4.23 (2H, s, 1"-H), 4.48 (2H, d, J = 5.2 Hz, CH₂Cl), 5.17 (1H, d, J = 5 Hz, OH), 7.03 (1H, d, J = 7.3 Hz, 6'-H), 7.27 (1H, t, J = 7.7 Hz, 5'-H), 7.47 (1H, d, J = 7.8 Hz, 4'-H), 7.56 (1H, s, 2'-H) and 10.23 (1H, s, NH); δC/ppm (100MHz; DMSO-d₆) 43.4 (CH₂OH), 62.6 (CH₂Cl), 117.3 (C-2'), 117.6 (C-4'), 121.7 (C-6'), 128.3 (C-5'), 138.2 (C-1'), 143.2 (C-3') and 164.4 (C=O); m/z = 199 (M⁺, 10%) and 198 (100%).

ii. 3-(Chloroacetamido)-benzyl chloroacetate 202A as a yellow solid (0.41 g, 18%), m.p. 84-86°C. (Found: M⁺, 275.01078. C₁₁H₁₁NO₃³⁵Cl₂ requires: M⁺, 275.14811); νmax (solid deposit/cm⁻¹) 3411 (NH), 1747 (O.C=O), 1664 (C=O) and 1617 (NHC=O); δH/ppm (400MHz; CDCl₃) 4.10 (2H, s, 2-CH₂), 4.18 (2H, s, 2"-CH₂), 5.19 (2H, s, 7'-H), 7.17 (1H, d, J = 7.6 Hz, 4'-H), 7.36 (1H, t, J = 7.9 Hz, 5'-H), 7.52 (1H, d, J = 8.14 Hz, 6'-H), 7.60 (1H, s, 2'-H) and 8.28 (1H, s, NH); δC/ppm (100MHz; CDCl₃) 40.8 (C-2), 42.8 (C-2"), 67.4 (C-7'), 119.9 (C-6'), 120.2 (C-2'), 125.0 (C-4'), 129.5 (C-5'), 136.1 (C-1'), 137.0 (C-3'), 163.9 (C-1") and 167.1 (C-1); m/z = 274.12 (M⁺-1, 50%) and 314.02 (100%).
**Experimental**

**Diethyl \([3-(\text{hydroxymethyl})\text{phenyl}\text{carbamoyl}\text{methyl}]\) phosphonate 203**

![Chemical Structure](image)

Triethyl phosphite (1.6 mL, 9.3 mmol) was added under nitrogen through a septum to 2-chloro-\(N\)-(3-hydroxymethylphenyl)acetamide 202 (150 mg, 0.75 mmol) in an oven-dried round bottomed flask equipped with a reflux condenser. The resulting mixture was refluxed for *ca.* 5.5h during which time the reaction was monitored by TLC. The cooled mixture was then stirred with hexane (20 mL) for *ca.* 10 minutes followed by decantation of the hexane layer to remove the excess phosphite; this was repeated twice. The residual brown oil that was chromatographed [column chromatography; elution with EtOAc:EtOH = 20:1] to afford *diethyl \([3-(\text{hydroxymethyl})\text{phenyl}\text{carbamoyl}\text{methyl}]\) phosphonate 203* as a light yellow oil (77 mg, 34%). (Found: \(M^+\), 301.10706. \(C_{13}H_{20}NO_2P\) requires: \(M^+\), 301.27591); \(\nu_{\text{max}}\) (thin film/cm\(^{-1}\)) 3400 (OH), 1670 NH\(=\)O (1613), 1232 (P=O) and 1023 (O=P−O−C\(_2\)H\(_5\)); \(\delta_H/\text{ppm}\) (400MHz; CDCl\(_3\)) 1.35 (6H, t, \(J = 7.1\) Hz, 2 × CH\(_3\)), 1.78 (1H, br s, OH), 3.04 (2H, d, \(J_{P-H} = 21.1\) Hz, CH\(_2\)P), 4.18 (4H, m, 2 × CH\(_3\)CH\(_2\)), 4.57 (2H, s, 7-H), 6.98 (1H, d, \(J = 7.5\) Hz, 6-H), 7.18 (1H, t, \(J = 7.8\) Hz, 5-H), 7.39 (1H, s, 2-H), 7.43 (1H, d, \(J = 8.1\) Hz, 4-H) and 9.27 (1H, s, NH); \(\delta_C/\text{ppm}\) (100MHz; CDCl\(_3\)) 16.3 (2C, d, \(J_{P-C} = 6.1\) Hz, 2 × CH\(_3\)), 36.3 (d, \(J_{P-C} = 129.9\) Hz, CH\(_2\)P), 63.1 (d, \(J_{P-C} = 6.5\) Hz, 2 × CH\(_2\)CH\(_3\)), 64.8 (C-7), 118.1 (C-2), 118.7 (C-4), 122.6 (C-6), 128.9 (C-5), 138.1 (C-1), 141.9 (C-3) and 162.3 (C=O, d, \(J_{P-C} = 3.9\)); \(\delta_P/\text{ppm}\) (162 MHz; CDCl\(_3\)) 23.5 (P=O).
**Experimental**

*N-Acryloyl-3-(hydroxymethyl)aniline 204 and 3-acrylamidobenzyl acrylate 204A*

![Structural formulas](image)

To a stirred solution of 3-aminobenzyl alcohol 200 (0.50 g, 4.1 mmol) in dry THF (20 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 0.098 g, 4.1 mmol) in small portions to permit controlled evolution of hydrogen. Acryloyl chloride (0.33 mL, 4.1 mmol) was then added through a septum and the resulting solution was refluxed for ca. 6 h. The reaction was quenched by the addition of water (30 mL). The solvent was evaporated *in vacuo* and the aqueous residue extracted with EtOAc (2 × 30 mL). The combined organic extracts were washed sequentially with water (2 × 60 mL) and brine (2 × 60 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhyd. MgSO₄). Preparative thin layer chromatography [on silica gel; elution with hexane-EtOAc (4:6)] afforded two fractions.

(i) **N-Acryloyl-3-(hydroxymethyl)aniline 204** as a yellow oil, (7.2 mg, 1%). Found: M⁺, 177.078091. C₁₀H₁₁NO₂ requires: M⁺, 177.078979; νmax (thin film/cm⁻¹) 3296 (OH) and 1711 (C=O); δH/ppm (600MHz; CDCl₃) 5.17 (1H, s, 7'-CH₂), 5.85 (1H, d, J = 10.5 Hz, 3'-H₉), 6.16 (1H, dd, J = 17.3 Hz and 10.5 Hz, 2'-H₁), .45 (1H, d, J = 17.3 Hz, 3'-H₁b), 7.14 (1H, d, J = 7.7 Hz, 4'-H₁), 7.32 (1H, t, J = 7.8 Hz, 5'-H₂), 49 (1H, d, J = 7.7 Hz, 6'-H₃) and 7.56 (1H, s, 2-H); δc/ppm (150MHz; CDCl₃) 66.0 (C-7), 119.6 (C-2), 119.9 (C-6), 124.3 (C-4), 128.2 (C-2'), 129.5 (C-5), 131.3 (C-3'), 137.0 (C-1), 137.7 (C-3) and 167.7 (C=O).

(ii) **3-acrylamidobenzyl acrylate 204A** as a yellow oil, (0.019, 2%). (Found: M⁺, 231.088347. C₁₃H₁₃NO₃ requires: M⁺, 231.089543; νmax (thin film/cm⁻¹) 1713
(C=O) and 1665 NHC=O; δH/ppm (400MHz; CDCl₃) 5.18 (1H, s, 7-CH₂) , 5.78 (1H, dd, J = 10.2 and 1.1 Hz, 3"-Hₐ), 5.85 (1H, dd, J = 10.4 and 1.3 Hz, 3'-Hₐ), 6.20 (2H, m, 2'-H and 2"'-H), 6.42 (1H, dd, J = 5.2 and 1.2 Hz, 3"'-Hₐb), 6.46 (1H, dd, J = 5.7 and 1.1 Hz, 3'-Hₐb), 7.14 (1H, d, J = 7.5 Hz, 6-H), 7.33 (1H, t, J = 7.8 Hz, 5-H), 7.55 (1H, d, J = 6.8 Hz, 4-H) and 7.61 (1H, s, 2-H); δC/ppm (100MHz; CDCl₃) 66.0 (7-C₈H₂), 119.8 (C-4), 124.2 (C-6), 128.2 (C-2' and C-2"'), 129.3 (C-5), 131.3 (C-3' and C-3") , 136.9 (C-1 and C-3) and 166.0 (2 × C=O).

2-Acetoxy-N-[(3-hydroxymethyl)phenyl]-acetamide 205

NaH (60% dispersion in mineral oil; 0.33 g, 8.1 mmol) was added in small portions to a stirred solution of 3-aminobenzyl alcohol (1.0 g, 8.1 mmol) in dry THF (20 mL) under nitrogen at 0 ºC, to permit controlled evolution of hydrogen. Acetoxyacetyl chloride (0.90 mL, 8.1 mmol) was then added through a septum and the resulting solution refluxed for ca. 5.0 h. The reaction was quenched by the addition of satd. aq. NaHCO₃ (50 mL). The solvent was evaporated in vacuo and the aqueous residue was extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed sequentially with water (2 × 100 mL) and brine (2 × 100 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhydr. MgSO₄). Evaporation of the solvent in vacuo and chromatography of the residue [radial chromatography with hexane-EtOAc (1:9)] to afforded 2-acetoxy-N-[(3-hydroxymethyl)phenyl]-acetamide 205 as a light yellow oil (0.26 g, 14%). (Found M⁺, 223.08369, C₁₁H₁₃NO₄ requires 223.22569). ν max (solid deposit/cm⁻¹) 3354 (OH), 1741 (CH₃C=O), 1678 (NHC=O); δH/ppm (400MHz; CDCl₃) 2.19 (3H, s, C₃H₃), 4.62 (2H, s, CH₂OH), 4.63 (2H, s, CH₂), 5.28 (1H, s, OH), 7.09 (1H, d, J = 7.6 Hz, 4'-H), 7.27 (1H, t, J = 7.8 Hz, 5'-H), 7.43 (1H, d, J = 8.40 Hz, 6'-H), 7.47 (1H, s, 2'-H) and
To a solution of 3-aminobenzyl alcohol 200 (100 mg, 0.81 mmol) pyridine (2 mL) was added NaH (60% dispersion in mineral oil; 37 mg, 0.81 mmol) was added in small portions to permit controlled evolution of hydrogen. 2,3,4,5,6-pentaacetoxyhexanoyl chloride 131 (350 mg, 0.81 mmol) was then added and the reaction mixture stirred at room temperature for 2.5 days. The solvent was evaporated in vacuo and the residue dissolved in EtOAc (30 mL). This solution was washed with satd. aq. NaHCO₃ (2 × 30 mL) and brine (2 × 30 mL). The organic layer was then dried (anhdr. MgSO₄) and the solvent was evaporated in vacuo to afford a reddish brown oil of which flash chromatography [on silica gel; elution with hexane-EtOAc (4:6)] afforded 2,3,4,5,6-pentaacetyl-N-[3-(hydroxymethylphenyl)]-gluconamide 206 as a light brown oil, (58 mg, 14%). (Found: M⁺, 511.16685. C₂₃H₂₉NO₁₂ requires: M⁺, 511.47681) νmax (solid deposit/cm⁻¹) 3413 (OH), 1747 (CH₃C=O) and 1602 (NHC=O); δH/ppm (400MHz; CDCl₃) 2.06 (12H, series of singlets, 4 × OAc), 2.23 (3H, s, 6-OAc), 4.11 (1H, dd, J = 12.4 and 5.5 Hz, 6-Hₐ), 4.31 (1H, dd, J = 12.3 and 3.5 Hz, 1-Hₐ), 4.63 (1H, s, CH₂), 5.06 (1H, dt, J = 5.7, 5.6 and 3.8 Hz, 5-H), 5.34 (1H, d, J = 5.7 Hz, 2-H), 5.48 (1H, dd, J = 6.7 and 4.5 Hz, 4-H), 5.70 (1H, m, 3'-H), 7.10 (1H, d, J = 7.7 Hz, 6'-H), 7.25 (1H, s, OH), 7.29 (1H, m, 5'-H), 7.40 (1H, d, J = 8.2 Hz, 4'-H), 7.47 (1H, s, 2'-H) and 8.07 (1H, s, NH); δC/ppm (100MHz; CDCl₃) 20.4,
Experimental

20.6, and 20.7 (5 × OAc), 61.6 (C-6), 64.8 (C-7'), 68.7 (C-3), 68.8 (C-5), 69.1 (C-4), 71.8 (C-2'), 118.5 (C-2'), 119.3 (C-4'), 123.4 (C-6'), 129.2 (C-5'), 136.9 (C-1'), 142.1 (C-3'), 164.3 (NH-C=O), 169.4 (3-C=O), 169.8 (2-C=O), 169.9 (5-C=O), 170.1 (4-C=O) and 170.7 (6-C=O).

Allyl (3-hydroxy)benzoate 208 and allyl 3-(allyloxy)benzoate 208A

To a stirred solution of 3-hydroxybenzoic acid 207 (0.50 g, 3.6 mmol) in dry DMF (36 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 170 mg, 7.2 mmol) in small portions to permit controlled evolution of hydrogen. Allyl bromide (0.34 mL, 4.0 mmol) was then added through a septum and the resulting solution was refluxed for ca. 48 h. The reaction was quenched by the addition of water (40 mL). The solvent was evaporated in vacuo and the aqueous residue extracted with EtOAc (2 × 80 mL). The combined organic extracts were washed sequentially with water (2 × 160 mL) and brine (2 × 160 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhdr. MgSO₄). Radial chromatography [on silica gel; elution with hexane-EtOAc (4:1)] afforded two fractions.

(i) Allyl 3-hydroxybenzoate 208 as a yellow oil that crystallized at 0°C. (330 mg, 52%), m.p. 43-45°C. (Found: M⁺, 178.04011. C₁₃H₁₄O₃ requires: M⁺, 178.04044); vmax (thin film/cm⁻¹) 3410 (OH) and 1693 (CO); δH/ppm (400MHz; CDCl₃) 4.82 (2H, td, J = 5.6 and 1.3 Hz, 1'-CH₂), 5.28 (1H, dd, J = 10.5 and 1.24 Hz, CH=CH₂) 5.40 (1H, ddd, J = 17.2, 2.9 and 1.43 Hz, CH=CH₂), 5.84 (1H, br s, OH), 6.02 (1H, tdd, J = 16.1, 10.6 and 5.6 Hz, 2'-H), 7.07 (1H, ddd, J = 8.1, 2.6 and 0.9 Hz, 4-H), 7.31 (1H, t, J = 7.9 Hz, 5-H) and 7.61 (2H, m, 2-H and 6-H); δc/ppm (100MHz; CDCl₃) 65.8
(C-1’), 116.4 (C-2), 118.4 (C-3’), 120.4 (C-4), 122.0 (C-6), 129.7 (C-5), 131.4 (C-2’),
131.9 (C-1), 155.9 (C-3) and 166.4 (C=O).

(ii) *Allyl 3-(allyloxy)benzoate* 208A as light yellow oil (150 mg, 19%). (Found: M⁺,
218.093606. C₁₃H₁₄NO₃ requires: M⁺, 218.094294); ν_max (thin film/cm⁻¹) 1691 (CO);
δH/ppm (400MHz; CDCl₃) 4.58 (2H, d, J = 5.3 Hz, 1’-CH₂), 4.81 (2H, d, J = 5.6 Hz,
1”-CH₂), 5.35 (4H, m, 3’-Ha and 3”-Ha), 6.04 (2H, m, 2’-H and 2”-H), 7.11 (1H, dd, J =
8.2 and 2.0 Hz, 4-H), 7.33 (1H, t, J = 8.0 Hz, 5-H), 7.59 (1H, s, 2-H), 7.65 (1H, d, J =
7.7 Hz, 6-H); δC/ppm (100MHz; CDCl₃) 65.6 (C-1”), 69.0 (C-1’), 115.1 (C-2),
117.9 (C-3”), 118.2 (C-3’), 120.2 (C-4), 122.2 (C-6), 129.4 (C-5), 131.5 (C-1), 132.2
(C-2”), 132.9 (C-2’), 158.6 (C-3) and 166.1 (C=O).

In a separate reaction 3-hydroxybenzoic acid 207 (2.0 g, 14 mmol) in DMF (60 mL)
under nitrogen was treated with NaH (60% dispersion in mineral oil; 360 mg, 150
mmol) and allyl bromide (1.2 mL, 15 mmol). Radial chromatography [on silica gel;
elution with hexane-EtOAc (4:1)] afforded only *allyl 3-hydroxybenzoate* 208 (1.2 g,
45%).

### 3-(Allylamino)benzoic acid 210 and Allyl 3-(N-allylamino)benzoate 210A

To a stirred solution of 3-aminobenzoic acid 209 (500 mg, 3.7 mmol) in dry DMF
(36 mL) under nitrogen was added NaH (60% dispersion in mineral oil; 9.7 mg, 4.1
mmol) in small portions to permit controlled evolution of hydrogen. Allyl bromide
(0.32 mL, 3.7 mmol) was then added through a septum and the resulting solution was
refluxed for *ca.* 48 h. The reaction was quenched by the addition of water (40 mL).
The solvent was evaporated *in vacuo* and the aqueous residue extracted with EtOAc
Experimental

(2 × 80 mL). The combined organic extracts were washed sequentially with water (2 × 160 mL) and brine (2 × 160 mL). The aqueous washings were extracted with EtOAc and the organic layers combined and dried (anhyd. MgSO₄). Radial chromatography [on silica gel; elution with hexane-EtOAc (4:1)] afforded.

(i) **3-(Allylamino)benzoic acid 210** as a reddish brown oil (160 mg, 25%). (Found: M⁺, 177.077749. C₁₀H₁₁NO₂ requires: M⁺, 177.200260); νmax (thin film/cm⁻¹) 3436 (NH) and 1710 (CO); δH/ppm (400MHz; CDCl₃) 3.76 (1H, d, J = 5.2 Hz, 1'-CH₂), 5.19 (1H, dd, J = 10.3 and 1.3 Hz, CH=CH₂), 5.60 (1H, dd, J = 17.2 and 1.4 Hz, CH=CH₂), 5.89 (1H, tdd, J = 17.1, 10.4 and 5.2 Hz, 2'-H), 6.78 (2H, m, 2-H and 4-H), 6.93 (1H, d, J = 7.4 Hz, 6-H) and 7.20 (1H, m, 5-H); δC/ppm (100MHz; CDCl₃) 46.0 (C-1'), 112.9 (C-3), 115.0 (C-4), 116.8 (C-3'), 117.3 (C-2), 119.4 (C-1), 120.8 (C-6), 129.8 (C-5), 134.2 (C-2') and 148.2 (C=O).

In a separate reaction 3-aminobenzoic acid 209 (1.0 g, 7.3 mmol) in DMF (20 mL) under nitrogen was treated NaH (60% dispersion in mineral oil; 320mg, 13 mmol) and allyl bromide (0.63 mL, 7.3 mmol). Two fractions were obtained.

(i) **210** (220 mg, 17%) and

(ii) **Allyl 3-(N-allylamino)benzoate 210A** as a yellow oil (160 mg, 10%). (Found: M⁺, 217.109334. C₁₃H₁₅NO₂ requires: M⁺, 217.264231); νmax (thin film/cm⁻¹) 3391 (OH) and 1711 (CO); δH/ppm (400MHz; CDCl₃) 3.80 (2H, td, J = 5.3 and 1.5 Hz, 1'-CH₂), 3.94 (1H, s, NH), 4.79 (2H, td, J = 5.6 and 1.4 Hz, 1"-CH₂), 5.17 (1H, dd, J = 10.3 and 1.4 Hz, 3'-Hₐ), 5.27 (2H, m., 3'-Hₐ and 3"-Hₐ), 5.40 (1H, m, 3"-Hₐ), 5.94 (1H, tdd, J = 17.1, 10.5 and 5.3 Hz, 2'-H), 6.02 (1H, ddd, J = 22.8, 10.8 and 5.6 Hz, 2"-H), 6.79 (1H, dd, J = 7.8 and 2.2 Hz, 4-H), 7.22 (1H, t, J = 7.9, 5-H), 7.30 (1H, s, 2-H) and 7.40 (1H, d, J = 7.7 Hz, 6-H); δC/ppm (100MHz; CDCl₃) 46.4 (C-1'), 65.4 (C-1") 113.6 (C-2), 116.5 (C-3'), 117.8 (C-4), 118.0 (C-3"), 118.7 (C-6), 129.1 (C-5), 131.0 (C-1), 132.3 (C-2"), 134.9 (C-2'), 150.0 (C-3) and 166.6 (C=O).
4. REFERENCES


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