THE UNIVERSITY OF HULL

Analogues of Bredinin: Synthesis of 5-Hydroxyimidazoles from Acyclic Precursors

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by

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Abstract

Compared to 1-substituted-5-aminoimidazoles relatively few 1-substituted-5-hydroxyimidazoles have been reported; however, an interesting 1-substituted-5-hydroxyimidazole is the immunosuppressive agent bredinin, which shows antiviral[1], antimalarial[2], antitumour[3,4] and antiarthritic[5] activities. Bredinin is obtained naturally by extraction from a fermentation process, and current synthetic strategies for the synthesis are ambiguous and don't easily allow for the synthesis of analogues. Surprisingly, relatively few analogues of bredinin are known hence a convenient route to such types of compounds would be useful in providing a library of compounds for structural activity studies to be made.

The aim of the present study was to develop a synthetic strategy which employed inexpensive and readily available acyclic precursors to synthesise a library of analogues of bredinin. An advantage of using acyclic precursors to synthesise imidazoles is that the introduction of substituents, in particular, those in the 1-position is unambiguous. Therefore, our approach has been to explore an efficient and general route to 1-substitued-5-hydroxyimidazoles employing inexpensive and readily available starting materials.

Our initial target was to synthesise ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates since such ester intermediates have the potential to be converted to a wide variety of related 4-substituted imidazoles. A variety of ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates were synthesised. Those synthesised using lipophilic amines at position 1 of the imidazole ring (structures 39-43), were obtained in high yields and easily purified. However, those synthesised using polar amines at position 1 (structures 43-45), were more difficult to isolate and purify due to their increased hydrophilic nature.

In addition, the adopted strategy offers the opportunity to introduce a variety of substituents in the 2-position of the imidazole ring (structures 48-49), however this increase in chain length at position 2 had a direct effect on the yield of the imidazole obtained. The longer the chain length, the lower the yields, due to the steric hindrance experienced in the cyclisation step of the synthetic sequence.

In summary a novel convergent synthetic strategy was devised and both ethyl 1,2-substituted-5-hydroxyimidazole-4-carboxylates, with both aryl and alkyl groups at position 1, and benzyl 1-benzyl-5-hydroxyimidazole-4-carboxylate were successfully synthesised. Both lipophilicity and steric bulk were found to play a crucial role in the successful cyclisation and subsequent isolation and purification of the imidazoles, and a compromise between the two must be achieved to give the optimal reaction conditions.

In an attempt to gain a greater understanding of these ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates systems, their reactivity under different reactions conditions was assessed. Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was used as the model system and was found to be stable to all targeted manipulation at position 2 and 4 of the imidazole ring. Additionally, all attempts to protect the 5-hydroxyl function, to allow the subsequent manipulation of the ethyl ester, were also unsuccessful. The unexpected benzylation of the nitrogen at position 3 of the imidazole ring, to form the quaternary ammonium salt, confirms that this is the most nucleophilic atom within the imidazole ring system.

A novel linear synthetic strategy using acyclic precursors was developed which enabled the addition of substituents in positions 1, 2, 4 and 5 of the imidazole ring unambiguously. A particular advantage of this route is that it would be possible to synthesise 4-substituted-5-hydroxyimidazoles.

An interesting difference between the two synthetic routes is that nucleosides of the 5-hydroxyimidazoles were successfully synthesised using the linear synthetic strategy, however they could not be synthesised using the convergent strategy. This indicates the important role nucleophilicity could play in the intramolecular cyclisation step of the convergent synthetic strategy.

The most important implications of the present study are the development of two separate, unambiguous synthetic routes to the 5-hydroxyimidazoles, employing inexpensive acyclic precursors. The routes could provide access to a variety of substituted 5-hydroxyimidazoles in order to build a library of these compounds.

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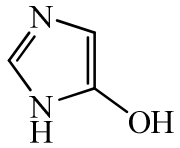
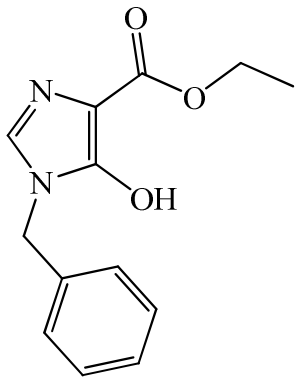
Nomenclature and Abbreviations

As each of the nitrogen atoms can bear a proton, imidazoles are tautomeric molecules and the nomenclature of its derivatives can be confusing. Thus, 5-hydroxyimidazole may also be called 4-hydroxyimidazole. To overcome this problem, the imidazole (I) would be referred to as 5(4)-hydroxyimidazole.



(I)

The numbering of N-substituted imidazoles commences at the nitrogen atom which bears the substituents. Thus, imidazole (II) is called ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate.



(II)

Enzyme Abbreviations

Inosine monophosphate dehydrogenase (IMP DHase)

Orotidine-5'-monophosphate decarboxylase (ODCase)

Purine and Pyrimidine Biosynthetic Pathway Intermediate Abbreviations

1-(5-Phosphoribosyl)-5-amino-4-carboxyimidazole (CAIR)

1-(5-Phosphoribosyl)-5-amino-4-imidazole carboxamide (AICAR)

1-(5-Phosphoribosyl)-5-formamido-4-imidazole carboxamide (FAICAR)

1-(5-Phosphoribosyl)-4-(*N*-succinocarboxamide)-5-aminoimidazole (SAICAR)

5-Phosphoribose-1-pyrophosphate (PRPP)

5-Phosphoribosylamine (PRA)

5-Phosphoribosyl-aminoimidazole (AIR)

5-Phosphoribosylglycinamide (GAR)

5-Phosphoribosyl-*N*-formylglycinamide (FGAR)

5-Phosphoribosyl-*N*-formylglycinamidine (FGAM)

Adenosine-5’-monophosphate (AMP)

Adenosine-5’-triphosphate (ATP)

Cytidine-5’-triphosphate (CTP)

Dihydroorotic acid (DHO)

Guanosine-5’-monophosphate (GMP)

Guanosine-5’-triphosphate (GTP)

Inosine-5’-monophsphate (IMP)

Orotic acid (OA)

Orotidine-5’-monophosphate (OMP)

Uridine-5’-monophosphate (UMP)

Uridine-5’-triphosphate (UTP)

Xanthosine-5’-monophosphate (XMP)

Schemes

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CHAPTER ONE

MALARIA

1.1 Introduction

Malaria is a major public health problem, and continues to afflict the poor nations of the world; it is the third biggest killer disease in the developing world.[6] Treatment and control of malaria has become more difficult with the spread of drug-resistant strains of the parasites, coupled with insecticide-resistant strains of mosquito vectors. Health education, better case management, better control tools and concerted action could all greatly alleviate the current burden of the disease; however, ultimately new antimalarial drugs are needed to combat the disease.

Despite more than a century of effects to eradicate or control malaria, the disease remains a major and growing threat to public health, particularly in the developing world.[7] Malaria is one of the world's most prevalent tropical parasitic diseases; it is responsible for more deaths than any other communicable disease except tuberculosis. The number of deaths and infections contributed to this parasitic infection are astonishing.[8,9]

- There are 300-515 million clinical cases of malaria each year.
- 1.5-2.7 Million deaths each year, either directly or resulting from acute respiratory infections and anaemia associated with the malaria infection.
- In over 100 countries, malaria is endemic.
- Over 90 % of all malaria deaths are in Africa.
- Malaria kills an African child every 30 seconds.

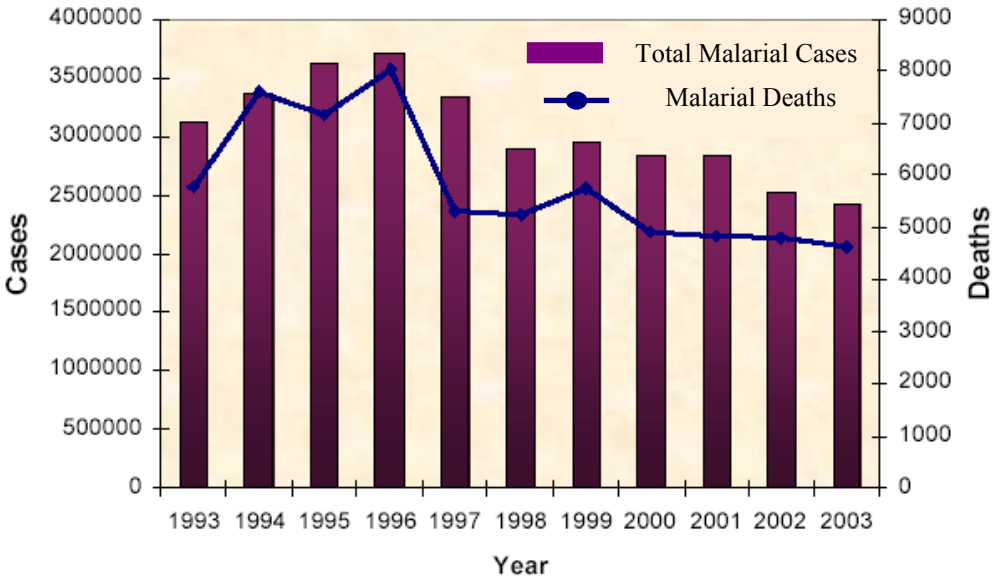


Figure 1 Reported malaria cases and deaths in South-East Asia region, 1993-2003.[10]

The clinical features of malaria vary, but the classic symptoms include persistent fever, shivering, joint pain, headache and repeated vomiting. Severe and complicated cases of malaria can result in renal failure, hypoglycaemia, anaemia, pulmonary oedema, shock and coma with fatal consequences.[10]

1.2 ***Plasmodium falciparum***

Malaria is caused by parasites that belong to the genus *Plasmodium*. The parasites are transmitted by the female Anopheline mosquito, which acts as a vector. There are over fifty species of *Plasmodium*, of which four can cause malaria in humans. These are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*, each of which presents slightly different clinical symptoms. *P. falciparum* is the most widespread of the four geographically and the most deadly causing the majority of malaria-related morbidity and mortality.[8] Other *Plasmodia* species specifically infect a variety of birds, reptiles, amphibians and mammals. In regions where malaria is endemic, several species are able to transmit the parasite and the exact species varies from area to area.[8]

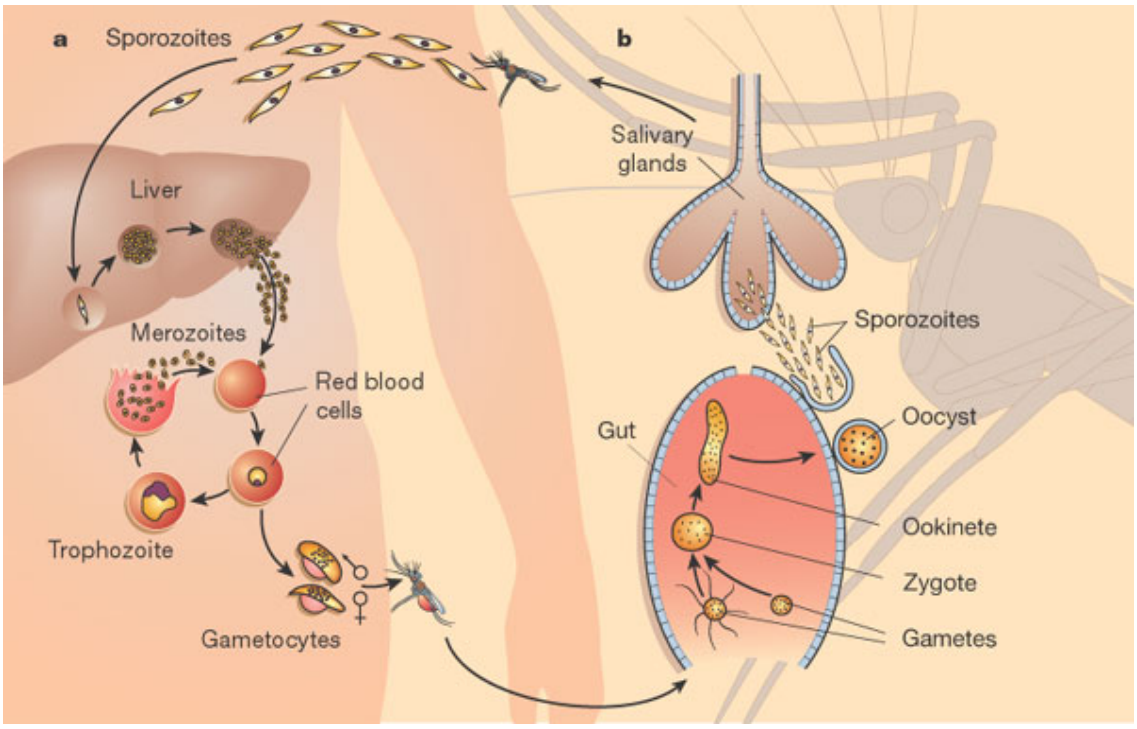


Figure 2 The life cycle of the parasite *Plasmodium falciparum*.[11]

The life cycle of the malaria parasite (figure 2) is complex involving an intermediate vertebrate host and the definitive host, the female Anopheline mosquito.[12] Infection of the intermediate host begins with a bite from an infected female mosquito, which requires a blood meal in order for her ovaries to develop. Sporozoites (infecting cells), released from the salivary glands of the mosquito, enter the intermediate host

bloodstream during feeding and quickly invade hepatocytes and multiply. Sporozoites are cleared from the circulation within 30 minutes of initial infection.

During the next 14 days the liver-stage parasites differentiate and undergo asexual multiplication resulting in tens of thousands of merozoites (daughter cells) which burst from the hepatocyte and return to the bloodstream. Individual merozoites invade erythrocytes and undergo an additional round of multiplication producing 12-16 merozoites within trophozoites (activated feeding cells) which develop into schizonts (asexual reproductive cells), this normally occurs within 48 hours. The clinical manifestations of malaria, such as the fever and chills experienced, are associated with the synchronous rupture of the schizonts, and therefore the erythrocyte, and the release of merozoites into the blood stream. The released merozoites go on to invade additional erythrocytes.

Not all of the merozoites divide into schizonts, some differentiate into sexual forms; male and female gametocytes. These gametocytes are taken up by another female mosquito during a blood meal. Within the mosquito mid-gut, the male gametocyte undergoes a rapid nuclear division, producing 8 flagellated microgametes which fertilise the female macrogamete. The resulting ookinete traverses the mosquito gut wall and encysts on the exterior of the gut wall as an oocyst. Soon the oocyst ruptures, releasing hundreds of sporozoites into the mosquito body cavity where they eventually migrate to the mosquito salivary gland.[8]

1.3 Current Therapeutic Approaches to the Treatment of *P. falciparum* Infections

Plasmodium falciparum		
	Generic drug name	**Comments**
Chloroquine sensitive areas (Haiti, the Dominican Republic, Central America, North Africa, west/central China and parts of the Middle East)	Chloroquine Quinine	Quinine and its derivatives are used in severe or complicated malaria.
Chloroquine resistant areas (Most of Africa, South America, Oceania and Asia)	Pyrimethamine/sulphadoxine Halofantrine Mefloquine Quinine Quinine plus pyrimethamine/sulphadoxine	Drug selection depends on the parasite susceptibility. Quinine and its derivatives are used in severe or complicated malaria.
Areas with multi-resistance (South East Asia)	Artemisinin derivatives Halofantrine Mefloquine Quinine Quinine plus tetracyclines	Quinine plus tetracyclines or artemisinin derivatives are used in severe or complicated malaria.

Table 1 Drug recommendations for *Plasmodium falciparum*.[13]

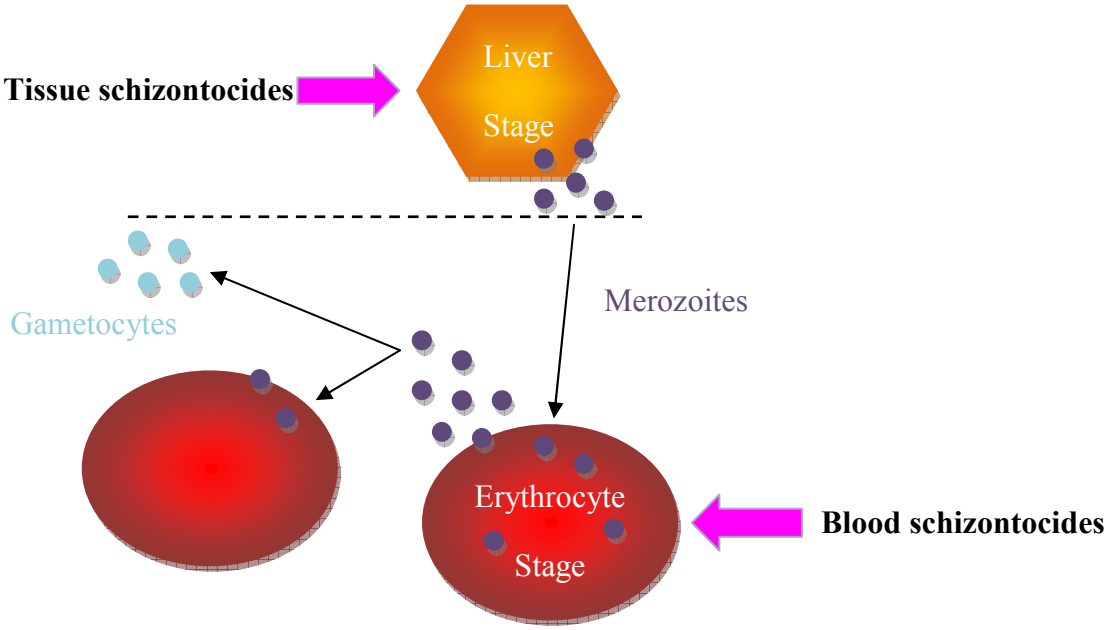


Figure 3 Stages within the plasmodium life cycle where some antimalarial drugs act.

1.3.1 Blood Schizonticides

These drugs act on the intraerythrocytic parasites and work by inhibiting formation of haemozoin and thereby terminate clinical attacks of malaria.

- 4-Aminoquinolines

Chloroquine is a 4-aminoquinoline that has been used extensively for the treatment and prevention of malaria. Widespread resistance has now rendered it virtually useless against *P. falciparum* infections in most parts of the world.[14] Chloroquine (figure 4) was first synthesised in 1934 and the initial clinical studies were all carried out in Germany. In 1944 the drug underwent extensive clinical studies in America where it was found to be an outstanding antimalarial compound.[15] Chloroquine is a racemate with two enantiomers with similar antimalarial activity. The mechanism of action of chloroquine is thought to be related to its inhibition of the enzyme that polymerises and detoxifies ferriprotoporphyrin IX in the parasites food vacuole.[16] It is a potent schizonticidal drug which was highly effective against the asexual forms of all four species of malaria,[17] however development of chloroquine-resistant *P. falciparum* is now becoming widespread.

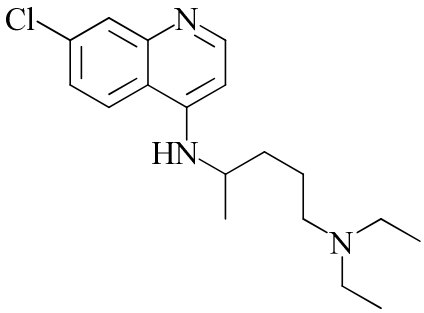


Figure 4 Chloroquine

Amodiaquine is a Mannich base 4-aminoquinoline (figure 5) with a mode of action similar to that of chloroquine. A systematic review of relevant studies on the treatment of uncomplicated falciparum malaria conducted over the past ten years in Africa showed that amodiaquine proved significantly more effective than chloroquine in clearing parasites, with a tendency for faster clinical recovery. This difference was also observed in areas with considerable chloroquine resistance.[18-20] It is effective against some chloroquine-resistant strains of *P. falciparum,* although there is cross-resistance.[14]

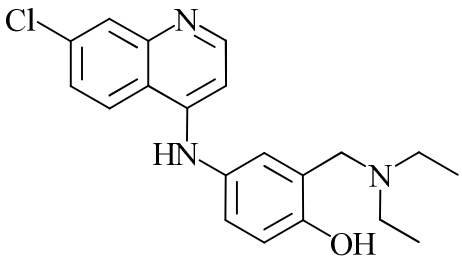


Figure 5 Amodiaquine.

- Arylamino alcohols

Quinine (figure 6) is the principal alkaloid of cinchona bark, and was first used against fever in Peru, around 1630, and soon after was introduced into Europe.[21] It is a potent schizonticidal agent against all human plasmodial species.[17]

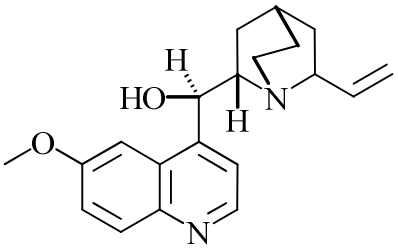


Figure 6 Quinine.

Mefloquine is a quinoline methanol derivative (figure 7), which is structurally related to quinine. It was synthesised and tested by the American Army in the 1960s. The available mefloquine preparation is a racemate with two enantiomers in equal proportions. It was introduced for the treatment of multi-resistant *P. falciparum* in the mid-1980s, however there has been a rapid development of resistance in the 1990s. The mechanism of action is not well established but it is a schizontocidal drug active against the erythrocytic stage of all species of malaria parasites.[17]

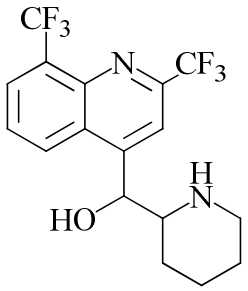


Figure 7 Mefloquine.

- Phenanthrene methanol compounds

Halofantrine is a phenanthrene methanol antimalarial drug developed by the American military (figure 8). The drug exists as a racemic mixture, but the two enantiomers have shown similar activity *in vitro*,[22,23] however the mechanism of action for the drug is not known. Halofantrine is a potent blood schizonticide against *P. falciparum* both *in vitro* and *in vivo*, but it is not effective against exoerythrocytic forms of the parasite.[24]

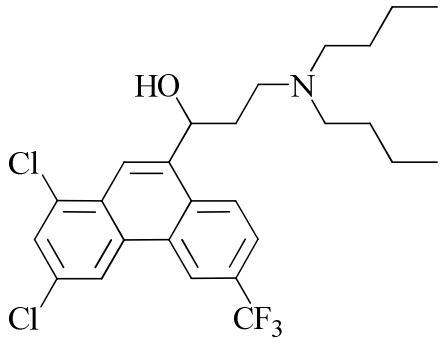


Figure 8 Halofantrine.

- Peroxides

Artemisinin is an antimalarial compound first isolated in pure form in 1972 by Chinese scientists from the herb *Artemisia annua*. This herb has been used in Chinese traditional medicine to control fever for over 2000 years.[25] Artemisinin has been shown in trails to demonstrate low toxicity and high efficacy even in chloroquine resistant *P. falciparum* malaria.[26] Artemisinin is a compound with an unusual structure (figure 9), unlike current antimalarial drugs that have a nitrogen-containing heterocyclic ring system, it is a sesquiterpene lactone with an endoperoxide linkage. Although the mechanism of action of artemisinin is not clearly understood, the endoperoxide linkage is thought to be essential for the antimalarial activity of the drug.[27] It is know that when the parasite infects a host red blood cell, it consumes haemoglobin and liberates free haeme, an iron-porphyrin complex. It is thought that the iron reduces the peroxide bond in artemisinin generating high-valent iron-oxo species, resulting in a cascade of reactions that produce reactive oxygen radicals which damage the parasite leading to its death.[28] Numerous studies have investigated the type of damage that these oxygen radicals may induce. For example, Pandey *et al.* have observed inhibition of digestive vacuole cysteine protease activity of malaria parasite by artemisinin.[29]

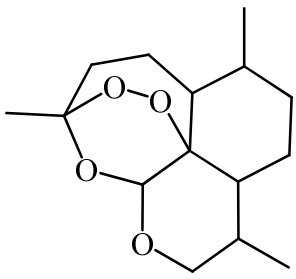
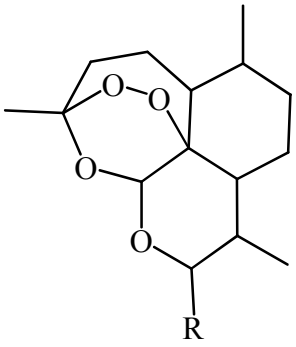


Figure 9 Artemisinin.

To improve artemisinin's bioavailability the derivatives artemether (lipid-soluble) and artesunate (water-soluble) have been developed (figure 10). When artemisinin, or one of its analogues, is used exclusively a high rate of treatment failures[30,31] has been reported so it is now being combined with mefloquine for the treatment of *P. falciparum* malaria.



R = OCH_3 - Artemether

R = $OCOCH_2CH_2CO_2Na$ - Artesunate

Figure 10 Artemisinin derivatives

1.3.2 Tissue Schizonticides

Hepatocytes are epithelial cells in the liver and make up 70-80 % of the cytoplasmic mass of the liver. In mammals, these cells are involved in protein synthesis, protein storage and transformation of carbohydrates, synthesis of cholesterol, bile salts and phospholipids, and detoxification, modification and excretion of exogenous and endogenous substances.

- 8-Aminoquinolines

Primaquine (figure 11) is active against primary exoerythrocytic stages of all malaria parasites; however the mechanism of action is unknown. Primaquine has no effect on the erythrocytic stages of plasmodia unless toxic concentrations are achieved.[17]

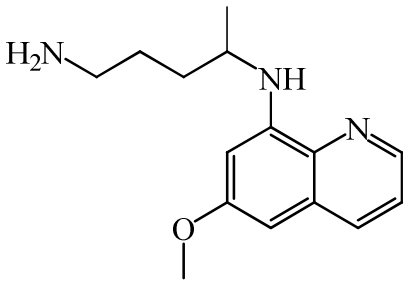


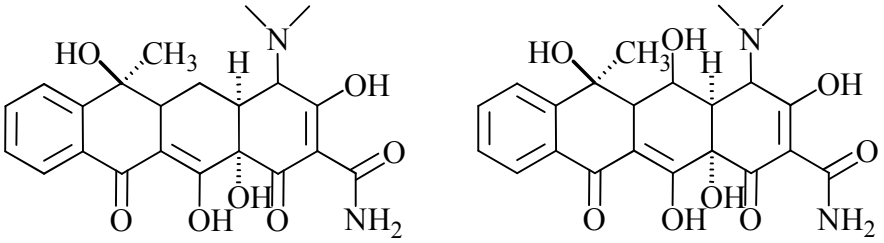
Figure 11 Primaquine.

1.3.3 Both Blood and Tissue Schizonticides

Blood schizonticides kill the erythocytic stages inside the red blood cells, while tissue schizonticides kill the liver stages of the parasite. In the case of *falciparum* malaria where there is no re-infection or relapse from the liver, a single blood schizonticidal drug is sufficient as treatment. In the case of infection by the other parasites, such treatment only suppresses the blood infection but does not lead to cure. Here a combination of both blood and tissue schizonticides has to be used.

- Antibiotics

Tetracyclines (figure 12) are broad spectrum antimicrobials, which have a potent but slow blood schizonticidal effect; due to this slow action tetracyclines should never be used alone in the treatment of malaria. They are not suitable for extended prophylactic use due to the possibility of the development of resistance, not only in the plasmodial parasites but also in a wide variety of susceptible bacteria which they are also used to treat.[32]



Tetracycline Deoxycycline

Figure 12 Antibiotic anti-malarial drugs.

Tetracyclines are employed primarily as a supplement to quinine in the treatment of *P. falciparum* malaria when resistance to quinine has been reported in patients in whom pyrimethamine/sulfadoxine is contraindicated because of hypersensitivity to sulfonamides. The mechanism of action of the tetracyclines is still unknown.

1.3.4 Nucleic Acid Inhibitors/Antimetabolites

Nucleotides are activated precursors of DNA and RNA and intermediates in many biosyntheses, and take part in many biochemical processes in the mammalian host, as well as in the parasite, especially those involving energy transfer and biosynthesis. However, major differences exist between plasmodial and corresponding human pathways (Table 2).

Pathway	***Plasmodium***	**Mammal**
Pyrimidines	Synthesizes pyrimidines *de novo*; cannot salvage bases/ nucleotides	Can either synthesize or salvage pyrimidine nucleotides
Purines	No *de novo* synthesis; relies on host-derived hypoxanthine as source of purine precursors	Can either synthesize or salvage purine nucleotides- hypoxanthine waste product
Folate cofactors	Can either synthesize or salvage folate precursors	No *de novo* synthesis; rely on external sources

Table 2 Differences between plasmodial and mammalian nucleic acid biosynthetic pathways.

The biosynthesis of pyrimidine nucleotides is essential for most living organisms, including the human parasite *P. falciparum*.[33] Studies by Büngener and Nielsen,[34,35] showed that infected erythrocytes incorporated tritiated hypoxanthine and adenosine, but not thymidine. From these findings as well as from correlated studies,[36-41] it became clear that plasmodia were unable to synthesise the purine ring *de novo*; that is, labelled formate and glycine were not incorporated into plasmodial purines derived from nucleic acids. Therefore, the purines necessary for the synthesis of nucleic acids and other metabolic functions had to be obtained by salvage pathways. By contrast, it was found that the plasmodia did have the capacity for the *de novo* fabrication of pyrimidines.[36-41]

In keeping with the incorporation of ^{14}C-labelled bicarbonate into plasmodial DNA and RNA has been the identification of about six enzymes associated with *de novo* pyrimidine synthesis, ribonucleoside monophosphate formation, and conversion of these compounds into deoxyribonucleotides. The parasite relies on *de novo* synthesis of folate cofactors, although exogenous folates can also be salvaged.[42-46] Contrary to this, mammalian cells can either salvage or synthesize *de novo* purine and pyrimidine nucleotides.[46-58]

Consequently, potent inhibitors of the *de novo* pathway of pyrimidine biosynthesis may have antimalarial activity with excellent selective toxicity: the parasite dies of a pyrimidine deficiency, while the infected human host is able to salvage required pyrimidine nucleotides from uridine or cytidine in the circulation.[59]

- Sulphonamides and sulphones

The efficacy of sulphadoxine (figure 13) for the treatment of human malaria was first reported in 1964.[60] Soon thereafter it was found that potentiation took place when sulphadoxine was combined with pyrimethamine for treatment for malaria and monotherapy was abandoned.[61] Malaria parasites synthesise their folate co-factors and cannot use dietary folic acid as the human host can. Sulphadoxine competes with para-aminobenzoic acid (PABA) for binding to the enzyme dihydropteroate synthetase in the synthesis of dihydropteroate which is an essential substance for the formation of folic acid.[61] It is active against asexual blood forms of *P. falciparum* but less effective against other species, however the action is too slow for sulphadoxine to be used as a monotherapy.[32]

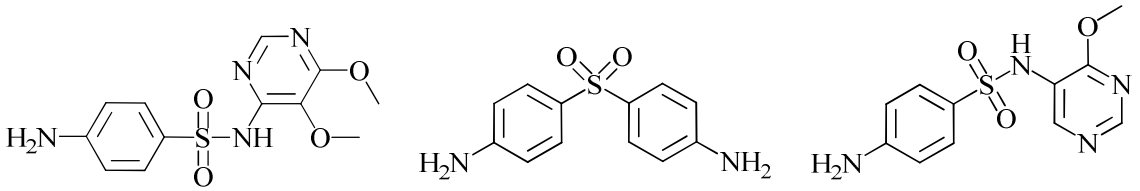


Figure 13 Sulphonamides and sulphones.

- Diaminopyrimidines

Proguanil (figure 14) was originally introduced as a prophylactic agent against malaria; it is a pyrimidine analogue that stops the parasite from reproducing once it is in the red blood cells. It does this by blocking the action the enzyme *dihydrofolate reductase*, which is involved in the reproduction of the parasite. Proguanil's success in treating humans led to further study of its chemical class and to the development of pyrimethamine. Resistance to the two monotherapies appeared quickly (within one year in the case of proguanil). Sulfones and sulfonamides were then combined with proguanil or pyrimethamine in hopes of increasing efficacy, however they have become less useful as the parasite develops resistance.[62]

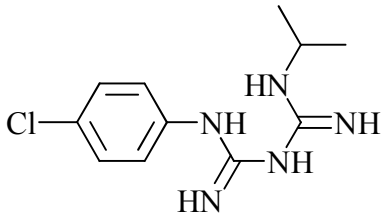


Figure 14 Proguanil.

Pyrimethamine is a diaminopyrimidine (figure 15), it is effective against the erythrocytic stage of *P. falciparum*, and also the sporogony in the mosquito resulting in a decrease of transmission of the infection.[17] The mechanism of pyrimethamine is related to its inhibition of dihydrofolic reductase necessary for the folic acid synthesis in the parasite.[63] However, pyrimethamine acts slowly and is not recommended as a monotherapy in acute malaria attacks. Resistance developed rapidly after the use of pyrimethamine as a monoprophylaxis.[17] However when combined with long acting sulphonomides (sulphadoxine), the effect of pyrimethamine is potentiated and the risk of developing resistant strains is far less.[15]

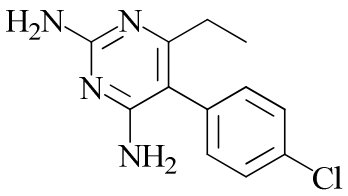


Figure 15 Pyrimethamine.

1.4 Future Outlook

The approach to antimalarial chemotherapy has been hampered in the past by a lack of understanding of the host parasite relationship. However, the development of *in vitro*[64,65] culture techniques that permit continuous growth of the human malaria parasite (*Plasmodium falciparum*) in human erythrocytes has now overcome this problem.

Bredinin is assumed to be inhibiting IMP dehydrogenase[66] based on the whole parasite studies carried out, at a concentration of 50 μM. This block in GMP biosynthesis was fatal to both a drug sensitive and drug resistant strain of the malaria parasite, at the trophozoite stage of development. It is thought that this subtle chemotherapeutic mechanism may offer a new approach to the clinical treatment of malaria (for more information on bredinin see chapter three).

Chemotherapy of malaria is available but is complicated by both drug toxicity and widespread resistance to most of the current antimalarial drugs. The need for more efficacious and less toxic chemotherapeutics, particularly rational drugs that exploit pathways and targets unique to the parasite, is therefore crucial.

As a common therapeutic method, disruption of nucleic acid biosynthesis offers great potential for future antimalarial drug strategies. With differences occurring between the biosynthetic methods that the human host and parasite use to create their nucleic acids, it is hoped that biologically active compounds can be tailored to be more toxic to the parasite enzymes than the human enzymes in the purine and pyrimidine biosynthetic pathways. 5-Hydroxyimidazole-4-carboxylates are potential drug targets due to the fact that they are thought to act by disrupting nucleic acid biosynthesis.

CHAPTER TWO

THE BIOSYNTHESIS OF

PURINES AND PYRIMIDINES

2.1 Introduction

The metabolic requirements for nucleotides and their cognate bases can be met by either dietary intake or synthesis *de novo* from low molecular weight precursors. Indeed, the ability to salvage nucleotides from sources within the body alleviates any nutritional requirement for nucleotides, thus the purine and pyrimidine bases are not required in the diet. The salvage pathways are a major source of nucleotides for synthesis of DNA, RNA and enzyme co-factors.

Extracellular hydrolysis of ingested nucleic acids occurs through the concerted actions of endonucleases, phosphodiesterases and nucleoside phosphorylases. Endonucleases degrade DNA and RNA at internal sites leading to the production of oligonucleotides. Oligonucleotides are further digested by phosphodiesterases that act from the ends inward yielding free nucleosides. The bases are hydrolyzed from nucleosides by the action of phosphorylases that yield ribose-1-phosphate and free bases. If the nucleosides and/or bases are not re-utilised the purine bases are further degraded to uric acid and the pyrimidines to β-aminoiosobutyrate, NH_3 and CO_2.

2.2 Nucleosides, Nucleotides and Nucleic Acids

Nucleosides are N-glycosides in which the pyrimidine or purine nitrogen is bonded to the anomeric carbon in the sugar by a β-*N*-glycosidic bond (figure 16).

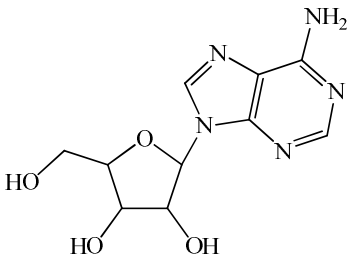


Figure 16 9-β-D-Ribofuranosyladenine or Adenosine.

Nucleotides are the 5'-phosphate esters of the corresponding nucleosides (figure 17).

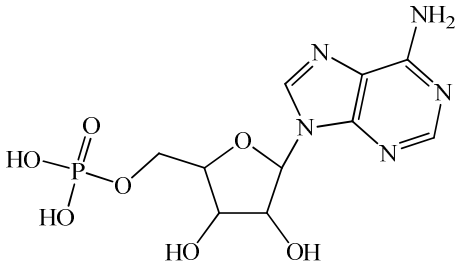


Figure 17 Adenosine 5'-monophosphate (AMP).

Nucleic acids are polymers built from nucleotide monomers (figure 18); the backbone of these polymers consists of alternating sugar and phosphate units, coupled at the 3'- and 5'- positions.

- In ribonucleic acids, RNA, the carbohydrate is D-ribose with cytosine, uracil, adenine and guanine bases.
- In deoxyribonucleic acids, DNA, the carbohydrate is 2-deoxy-D-ribose with cytosine, thymine, adenine and guanine bases.

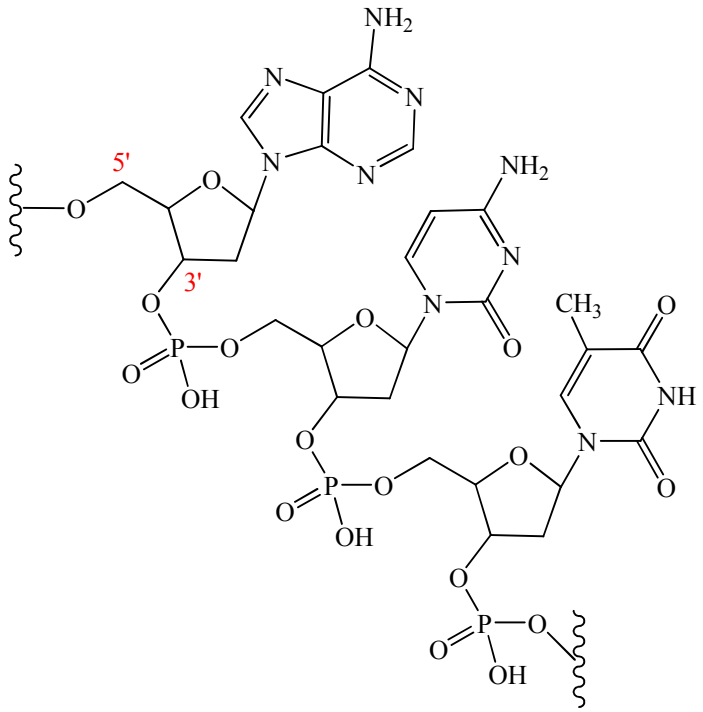


Figure 18 Single strand of nucleic acid.

The double stranded complex of both RNA and DNA are held together by hydrogen bonds between the pyrimidine and purine bases, known as the Watson-Crick base pairing.

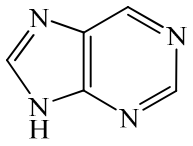
Purines and pyrimidines are heterocyclic, aromatic, organic compounds, which are central to cellular metabolism and growth. These nitrogenous bases are essential precursors in the synthesis of both DNA and RNA.[67] There are two pathways that exist for nucleic acid synthesis in eukaryotes; the first is the *de novo* pathway and the second is the salvage pathway.[68]

Purine biosynthesis is very different from pyrimidine biosynthesis. The increased complexity of purine biosynthesis derives largely from the fact that whereas the pyrimidine skeleton is constructed from two precursors, the nine atoms of the purine skeleton are derived from seven different precursor molecules, of which only three have a common primary substrate.[69]

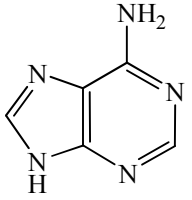
Another major difference is that the pyrimidine skeleton is assembled completely before the reaction with 5-phosphoribose-1-pyrophosphate (PRPP), whereas the first step in the

purine biosynthesis is the formation of a *N*-glycosidic bond between ribose-5-phosphate and ammonia.[70]

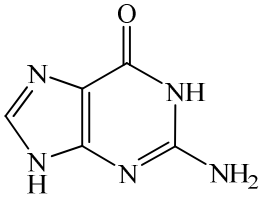
2.3 Purines



Basic purine skeleton



Adenine (A)



Guanine (G)

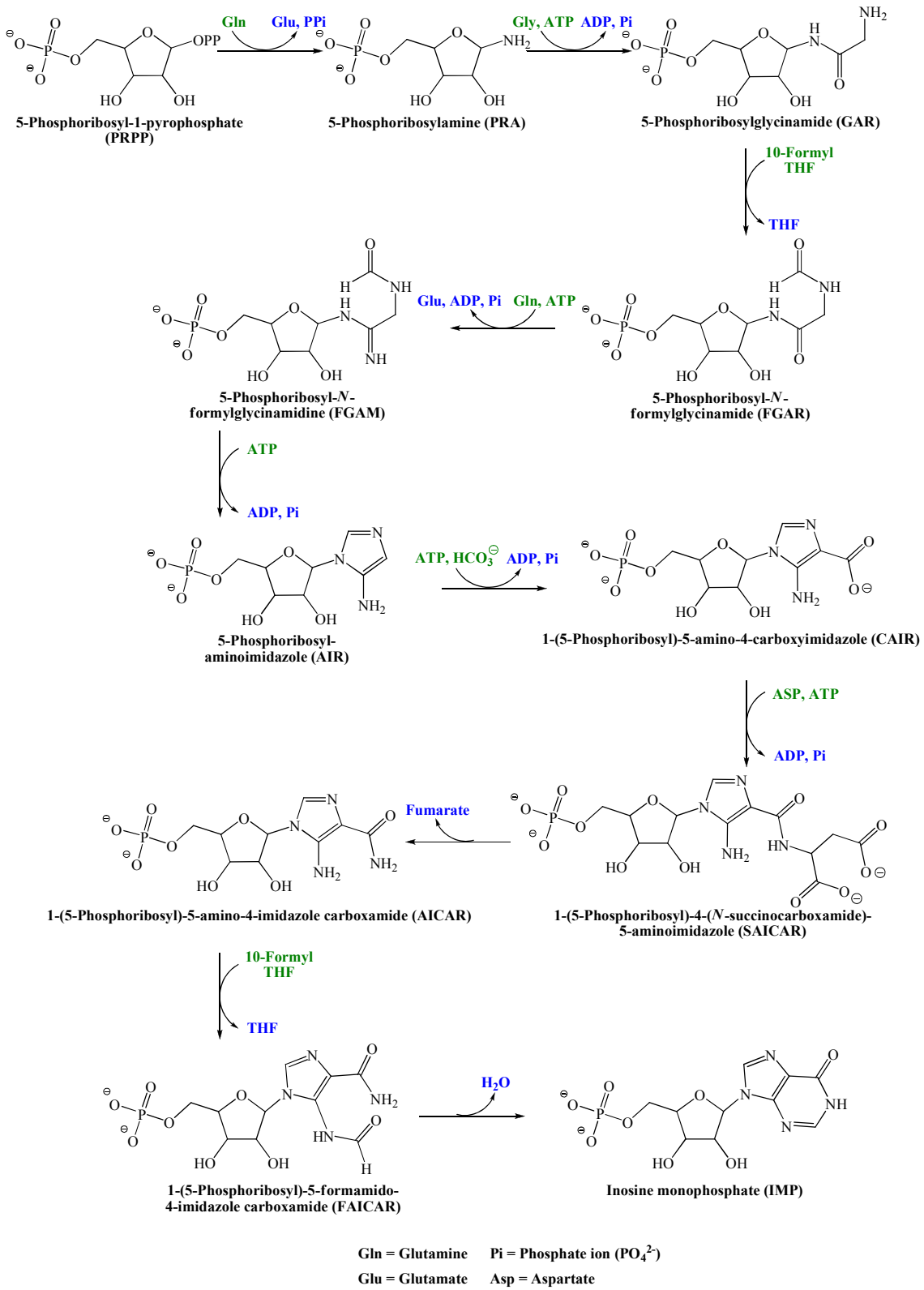
2.3.1 *De novo* Purine Biosynthetic Pathway

The initial step in the biosynthesis of purine nucleotides (scheme 1) is the glutamine-mediated amination of 5-phosphoribosyl-1-pyrophosphate (PPRP) to give 5-phosphoribosylamine (PRA); the biosynthetic pathway is regulated partly by the allosteric properties of the first enzyme responsible for this amination, *ammonia phosphoribotransferase*, which is separately inhibited by adenosine-5'-triphosphate (ATP) and guanosine-5'-triphosphate (GTP) nucleotides. The amination involves inversion at C-1 of the ribose unit to give the β-amino sugar, so the glycosidic link of the appropriate configuration is introduced at an early stage in the pathway.[71]

The second step in the process is the ATP-dependent conjugation of glycine with the amino acid group of the sugar to give 5-phosphoribosylglycinamide (GAR), which is then *N*-formylated in a step which involves one carbon atom transfer utilising a folic acid coenzyme, the resulting product being 5-phosphoribosyl-*N*-formylglycinamide (FGAR). A second ATP dependent glutamine mediated amination now takes place to give 5-phosphoribosyl-*N*-formylglycinamidine (FGAM) which has the structural basis of an imidazole. Ring closure follows via an ATP dependent dehydration to give 5-phosphoribosylaminoimidazole (AIR) which is the first heterocyclic nucleotide formed in the pathway.

The pyrimidine ring is now built on the imidazole nucleotide by a carboxylation with carbon dioxide in equilibrium with bicarbonate to give 1-(5-phosphoribosyl)-5-amino-4-carboxyimidazole (CAIR), followed by a two step amination to give the corresponding amide. The first step is an ATP dependent reaction with aspartate to give 1-(5-

phosphoribosyl)-4-(*N*-succinocarboxamide)-5-aminoimidazole (SAICAR) followed by elimination of fumarate to yield 1-(5-phosphoribosyl)-5-amino-4-imidazole carboxamide (AICAR). The ring system is completed by another one carbon transfer involving a folate coenzyme to give 1-(5-phosphoribosyl)-5-formamido-4-imidazole carboxamide (FAICAR), followed by a dehydration-cyclisation to give inosine monophosphate (IMP).

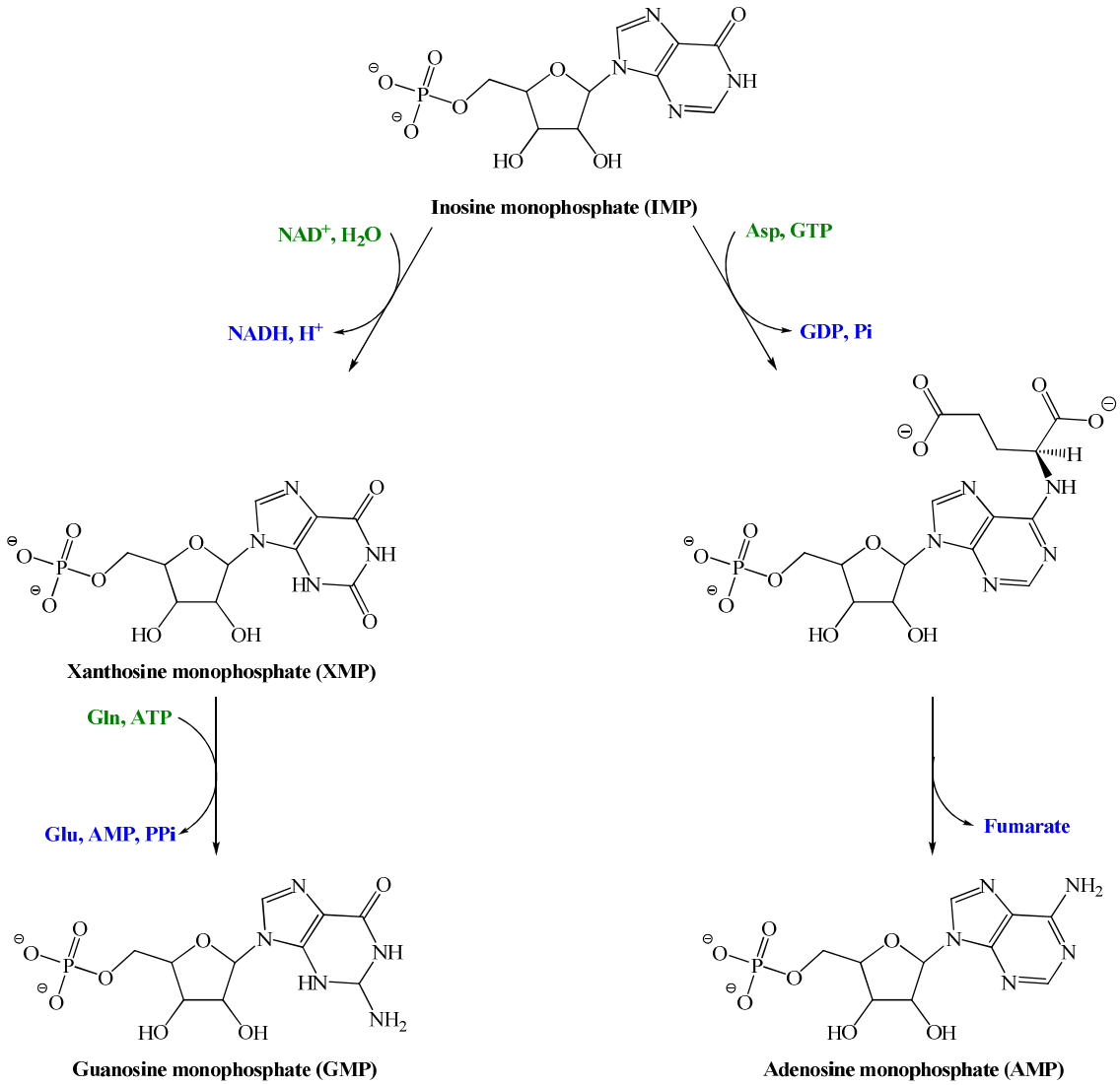


Scheme 1 Synthesis of inosine monophosphate in the *de novo* purine biosynthetic pathway.[72]

The amination of IMP to adenosine monophosphate (AMP) is a two step process (scheme 2) similar to that by which AICAR is formed. The conversion of IMP to

guanosine monophosphate (GMP) also involves a two-step process but is rather different to the synthesis of AMP. IMP is first oxidised to xanthosine monophosphate (XMP) in a reaction that uses NAD^+ as a coenzyme, and then XMP is aminated in a subsequent ATP dependent reaction to give GMP.

The balance between ATP and GTP is maintained partly by substrate availability (IMP to AMP requires GTP and conversely IMP to GMP requires ATP) and partly by the allosteric properties of the enzymes in the two branches.[72]



Gln = Glutamine **Pi = Phosphate ion (PO_4^{2-})**
Glu = Glutamate **Asp = Aspartate**

Scheme 2 Biosynthesis of purine nucleotides from inosine monophosphate.[72]

The intra-erythrocytic stages of the malaria parasite lack the *de novo* purine nucleotide biosynthetic pathway, and rely exclusively on the salvage of preformed purines for their nucleotide requirements.[43,73] As already discussed, the primary biosynthetic pathway is through adenosine uptake, which is then converted into IMP through inosine and hypoxanthine. IMP then serves as a precursor for AMP and GMP. However, parasite bypass mechanisms include the conversion of adenosine to AMP by adenosine kinase or the phosphoribosylation of adenine, generated from adenosine by a purine nucleoside phosphorylase (PNP).

Enzyme inhibitors can be classified for convenience as compounds which either inhibit the enzymes directly responsible for the formation of nucleic acids, or those that inhibit any of the variety of enzymes that catalyse different stages in the biosynthesis of pyrimidine and purine bases required for the formation of nucleic acids.[74]

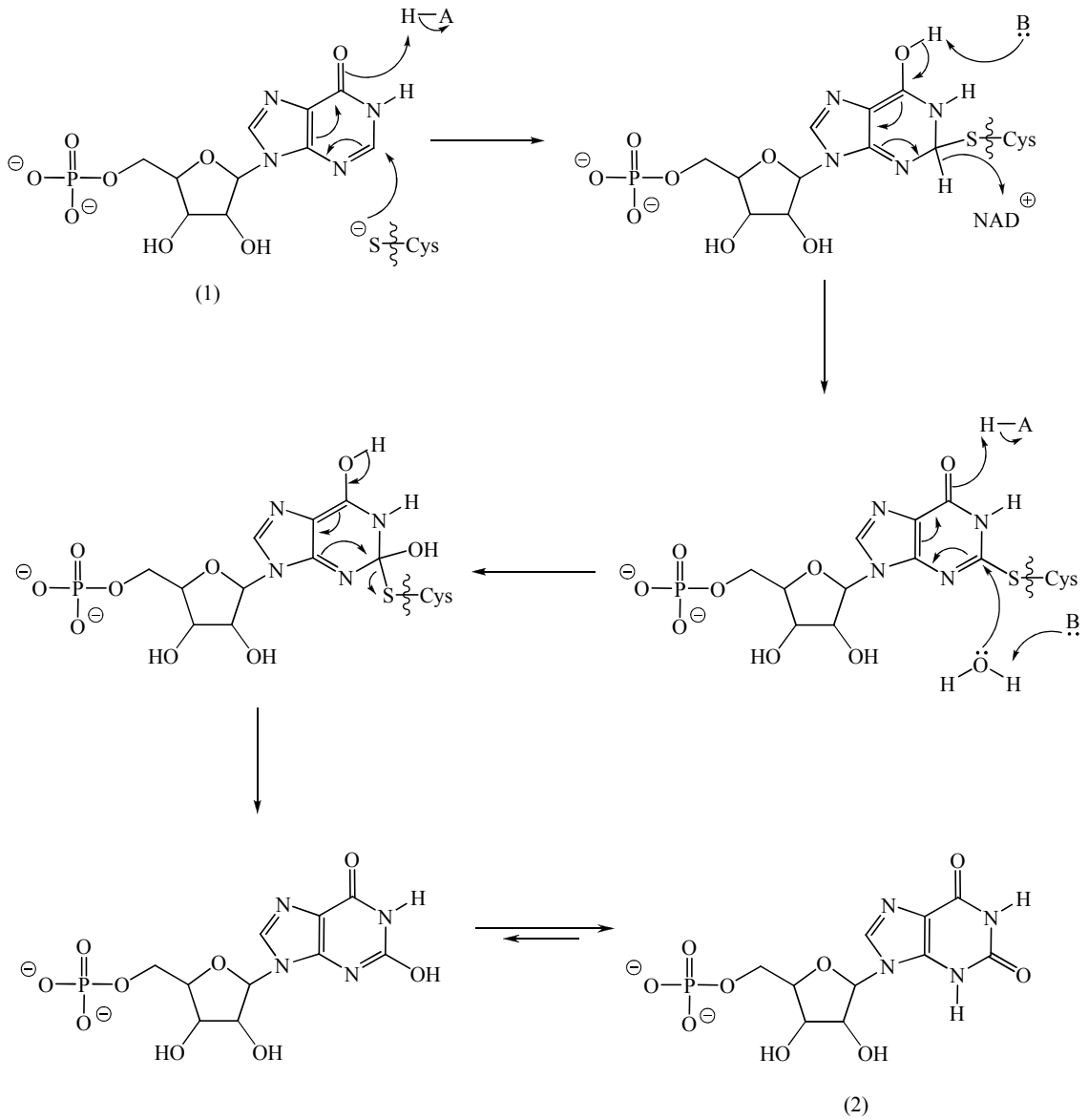
Wide ranges of compounds have been synthesised that are active against a number of the enzymes that are involved in the biosynthesis of purines and pyrimidines. The overall effect of inhibition of purine and pyrimidine synthesis is the inhibition of the synthesis of DNA.[74] Two of the enzymes of particular interest to this research are examined below.

2.3.2 *Inosine Monophosphate Dehydrogenase*

The 5'-monophosphates of the synthetic 5-ring heterocyclic nucleosides mimic the biosynthetic nucleoside monophosphate 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR, scheme 1) and as such inhibit *inosine monophosphate dehydrogenase*[75] (IMP DHase), one of the key rate controlling enzymes in the *de novo* biosynthesis of purine nucleotides. Inhibitors of IMP DHase have a broad spectrum of biological activity including antiviral,[76] anticancer,[77,78] antiarthritic[79,80] and immunosuppressive activities.[81]

The enzyme IMP DHase catalyzes the rate-limiting step in the *de novo* biosynthesis of guanine nucleotides[82] in mammalian cells. This reaction is the irreversible NAD dependent oxidation of inosine 5'-monophosphate (1) (IMP) to xanthosine 5'-monophosphate (2) (XMP).[83,84]

After substrate addition, nucleophilic attack of a thiol group in the enzyme active site with the purine ring of the IMP base forms a covalent intermediate (scheme 3). Binding of the cofactor NAD, results in a hydride transfer from the tetrahedral intermediate to give NAD^+. Addition of water then replaces the thiol group with a hydroxyl group, and tautomerisation of the product gives XMP (2).[85]



Scheme 3 Mechanism of the oxidation of inosine monophosphate to xanthosine monophosphate.

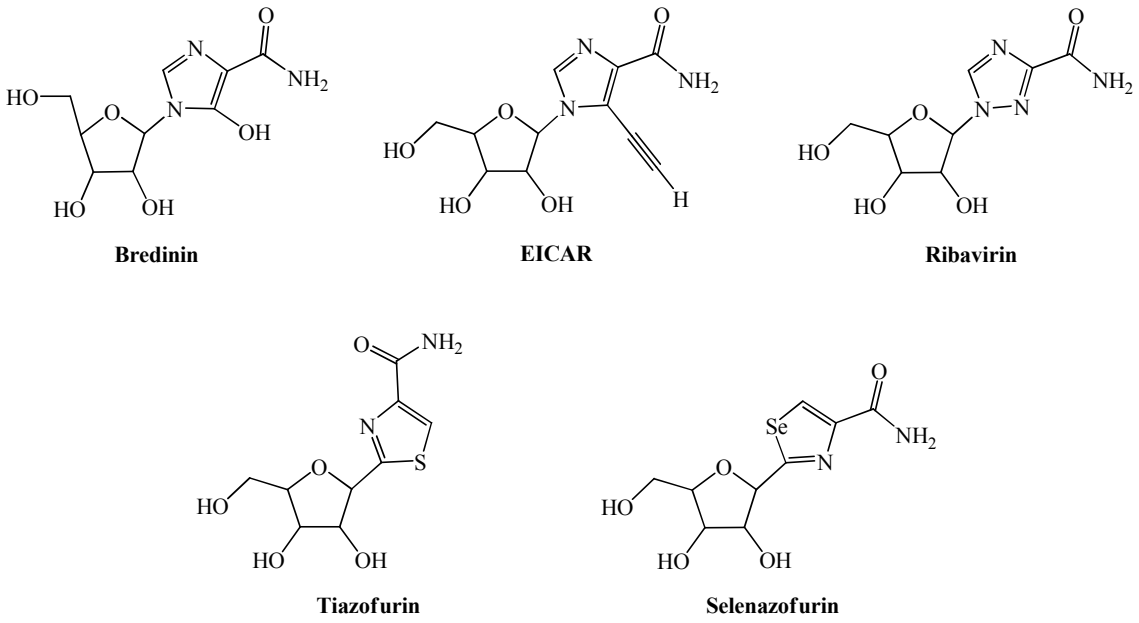
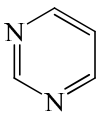
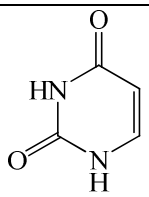
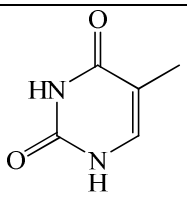
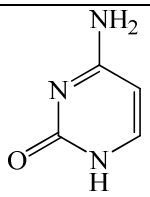


Figure 19 Some IMP DHase inhibitors.

Bredinin (figure 19) is an imidazole nucleoside that inhibits IMP DHase and depletes cells of guanine nucleotides.[86] EICAR inhibits the growth of human and mouse leukaemia cells and, as the 5'-monophosphate derivative, is a competitive inhibitor with respect to IMP of IMP DHase (K_i = 7 μM[87]). Ribavirin is a nucleoside analogue which is phosphorylated to the 5'-monophosphate derivative, a competitive inhibitor with respect to IMP of IMP DHase (K_i = 0.8 μM[88]). Tiazofurin is a structural variant of ribavirin with a different mechanism of action. This C-nucleoside is phosphorylated to the 5'-monophosphate derivative and then converted to an analogue of NAD.[89] Tiazofurin adenine dinucleotide (TAD) is a potent inhibitor of IMP DHase, binding at the NAD site with K_i = 0.13 μM.[90] Selenazofurin (SAD) is an analogue of tiazofurin where selenium replaces sulphur. Similarly, the NAD analogue SAD, is formed which is a competitive inhibitor with respect to NAD (K_i = 55 nM[91]).

2.4 Pyrimidines

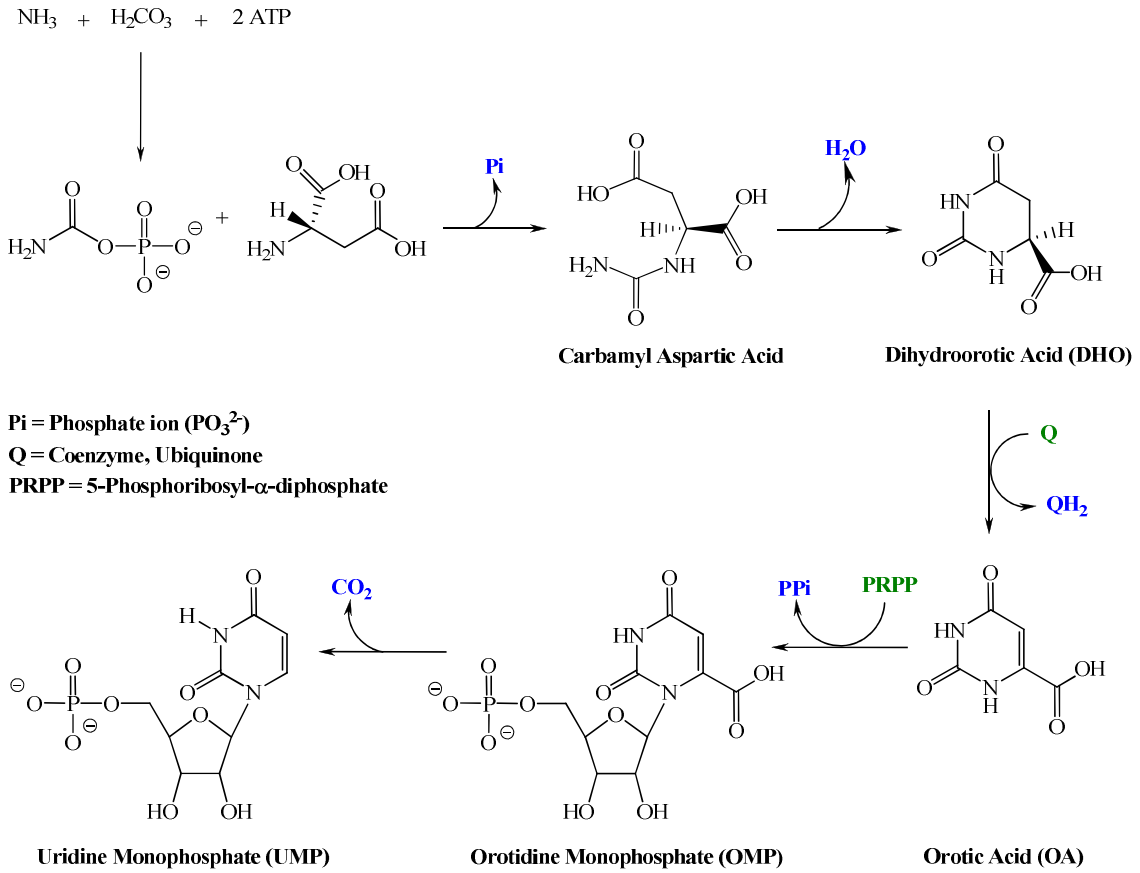
 Basic pyrimidine skeleton	 Uracil (U)	 Thymine (T)	 Cytosine (C)

2.4.1 *De novo* Pyrimidine Biosynthetic Pathway

In the biosynthesis of pyrimidine nucleotides the pyrimidine ring is preformed before the formation of the glycosidic link. The pathway (scheme 4) is generally considered to consist of six enzymes, which sequentially transform the primary substrate L-glutamine into uridine-5'-monophosphate (UMP).[72]

Carbamoyl phosphate and aspartic acid combine under the catalytic influence of *aspartate carbamoyl transferase* (ATCase) to form carbamoyl aspartate. Formation of the pyrimidine ring is then affected by the action of *dihydroorotase* giving the reduced pyrimidine dihydroorotic acid (DHO). In eukaryotes, CPSase, ATCase and DHOase are all part of a single multifunctional protein (CAD) that has a molecular weight of 200,000 and it is cytosolic.[69] Oxidation of DHO by *dihydroorotic dehydrogenase* produces the important pyrimidine intermediate, orotic acid (OA).

A phosphoribosyltransferase reaction then follows in which OA accepts a ribose 5-phosphate group from PRPP to give orotidine 5'-monophosphate (OMP) and an inorganic pyrophosphate. The final step is an irreversible enzymatic decarboxylation to UMP. In humans, these last two enzymes also form a multifunctional protein (*UMP synthase*), which is found in the cytosol. UMP can then undergo a series of enzymatic transformations to give up uridine-5'-triphosphate (UTP) and cytidine-5'-triphosphate (CTP) the precursors of RNA, and dCTP, dUTP and dTTP the precursors of DNA.[92]



Scheme 4 Biosynthesis of pyrimidines.

The synthesis of the pyrimidine nucleotides described above requires a significant quantity of energy for each step in the reaction sequence. It is therefore not surprising that organisms have evolved a way of salvaging the complex nitrogen bases generated during the breakdown of DNA and RNA.[70] Uracil can be converted to UMP by direct reaction with PRPP catalysed by *uracil phosphoribosyl transferase*, which is present in both animal and microbial cells. UMP can also be generated from uridine by the *uridine-cytidine kinase* reaction. Two further reactions that occur include the conversion of cytidine into uridine, and the conversion of thymine into deoxythymidine.[70]

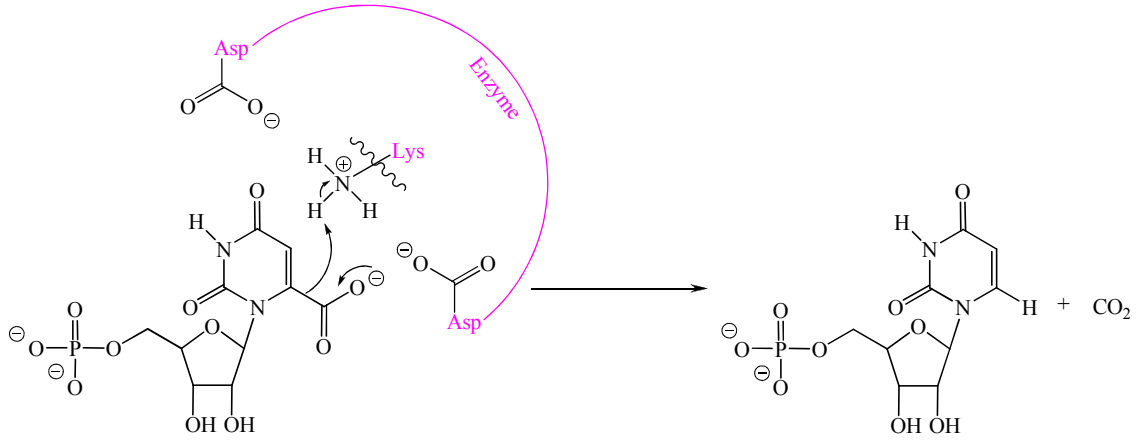
In contrast to the purine nucleotide pathway, the malaria parasite derives its entire pyrimidine nucleotide pool via the *de novo* biosynthetic pathway. The salvage enzymes have been found to be absent in the parasite.[40]

2.4.2 *Orotidine 5'-Monophosphate Decarboxylase*

To date, *orotidine 5'-monophosphate decarboxylase*[93] (ODCase) is the most proficient enzyme that has been analysed. It functions without a cofactor, and facilitates the final step in the *de novo* biosynthesis of uridine-5'-monophosphate (UMP). ODCase catalyses the decarboxylation of orotidine-5'-monophosphate (OMP) (3) to form UMP (4). The active form of the enzyme is a dimer of identical subunits.[94] Most decarboxylases use cofactors to delocalize the negative charge that is generated by elimination of CO_2 from the substrate. This enzyme contains no cofactors and is one of the most proficient enzymes currently known.[95-100] The half-life of the substrate in neutral aqueous solution is about 78 million years, but when catalyzed by ODCase, this changes to 18 ms. The enzyme's proficiency is ca. 10^{23} M^{-1},[98] for the catalysed versus uncatalysed reaction.

Mechanistically, the decarboxylation is unusual because the substrate is not a β-keto acid and has no obvious electron sink nearby to accept electrons as CO_2 leaves. The catalytic mechanism remains undefined despite numerous mechanistic proposals.[99]

One proposal, the electrostatic stress mechanism (scheme 5) has found limited support in crystallographic studies,[101,102] it involves activation of the bound substrate's carboxyl moiety via electrostatic stress. It is envisaged that binding of the substrate's critical phosphoryl group drives into juxtaposition the labile carboxylate group and a negatively charged active site aspartate residue. Neutralization of lysine leads to increased electrostatic repulsion between two apartate residues, which were originally held together by two salt bridges from the lysine residue. This causes conformational changes in the enzyme active site expelling the products from the binding pocket. The lysine residue is then re-protonated by the solvent, restoring the catalytic cycle.



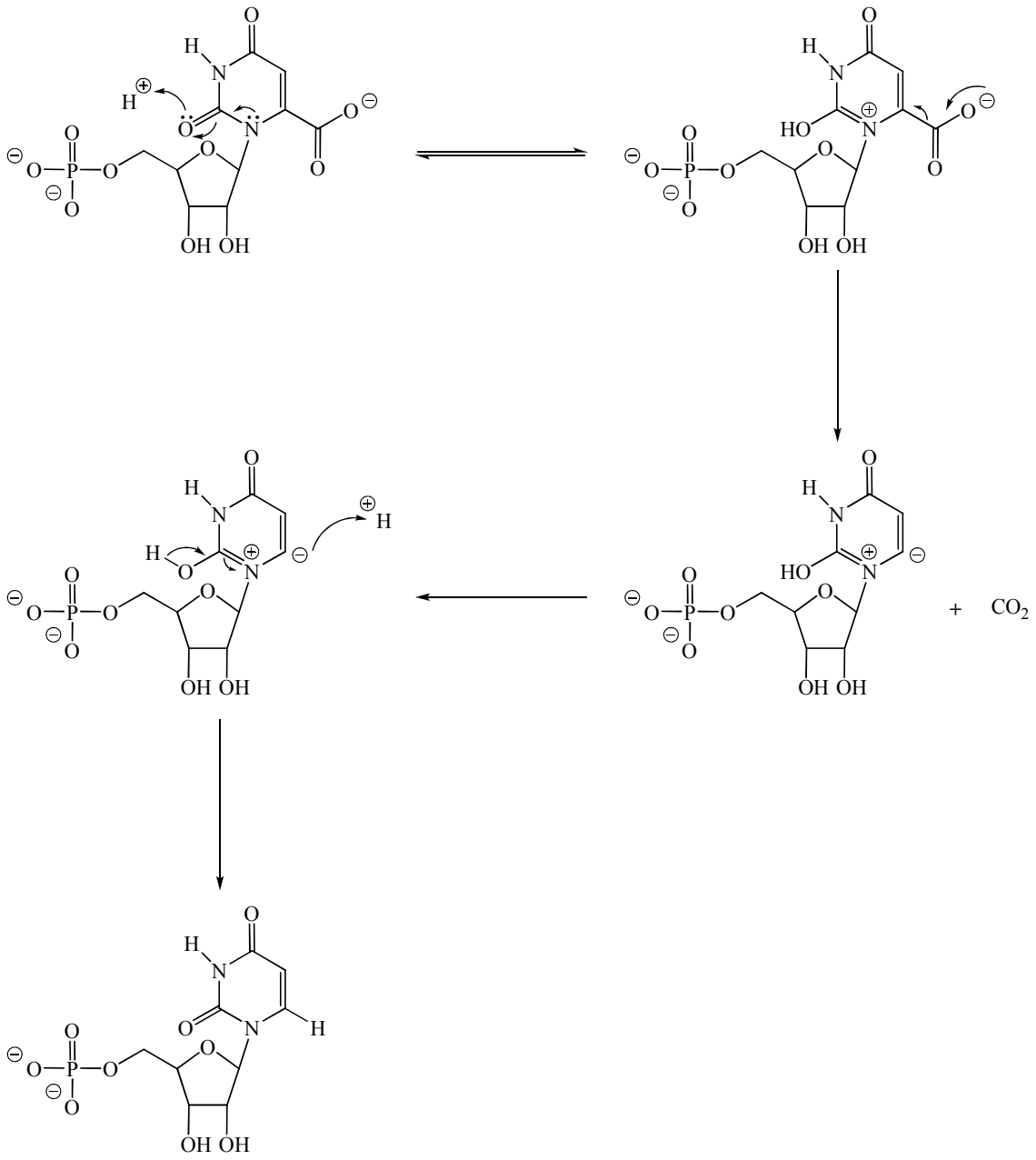
(3) (4)

Scheme 5 Electrostatic stress mechanism of action of ODCase.

Results of computational analyses predict that electrostatic stress furnishes the majority of the catalytic driving force for OMP decarboxylation. However, independent experimental and computational studies, along with careful thermodynamic considerations, have provided circumstantial evidence questioning the significance of the electrostatic stress mechanism.[103,104]

Another proposed mechanism, the zwitterions mechanism, is one in which protonation of OMP at the pyrimidine oxygen at position 4 or 2 occurs prior to decarboxylation, leading to formation of an ylide involving the nitrogen at position 1 (scheme 6).[105] This mechanism is supported by the findings of a proton inventory study indicating the existence of an isotope sensitive rate-limiting step.[106] The X-ray structure of some enzyme-inhibitor complexes provides strong evidence that the proton at position 6 of UMP is derived from the terminal NH_3^+ group of Lys.[107] The product isotope effect of unity for ODCase-catalyzed decarboxylation of OMP in 50/50 (v/v) H_2O/D_2O eliminates a mechanism[108] in which proton transfer from Lys to C-6 provides 'electrophilic push' to the loss of CO_2 in a concerted reaction.[109] This result also provides strong evidence for the formation of a short-lived enzyme-bound carbanion intermediate that shows no discrimination between H and D in the proton-transfer step.[109] This is further supported by the findings of a recent study[110] which found the pKa for the proton at position 6 of enzyme-bound UMP to be at least 10 units lower than the estimated values of the p*K*a for the proton at position 6 of 1,3-dimethyluracil in water.[100,111,112] It was concluded that ODCase provides substantial stabilization of the

late carbanion-like transition state for the decarboxylation of OMP, and that this transition state stabilization constitutes a large fraction of, but probably not the entire, enzymatic rate acceleration.



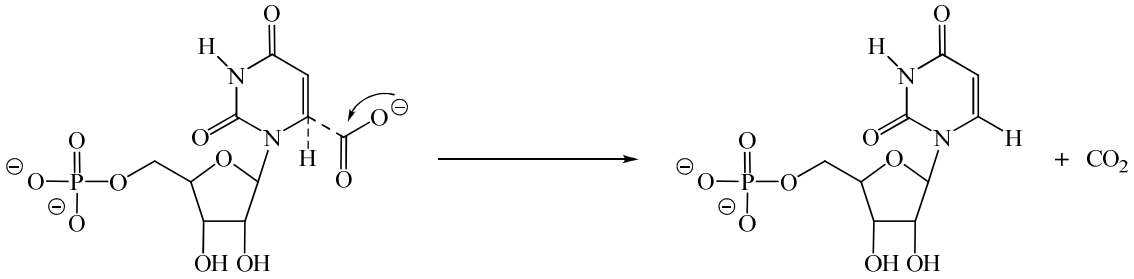
Scheme 6 Zwitterion mechanism of action of ODCase with protonation on oxygen at position 2.

There are however, arguments against protonation, the most serious of which seems to be the absence of a suitably positioned proton donor, with the exception of amide groups, in the crystal structures of ligand-bound ODCase. In addition, results of ^{15}N

kinetic isotope experiments effectively exclude ylide generation as a rate-limiting step in the ODCase reaction.[113]

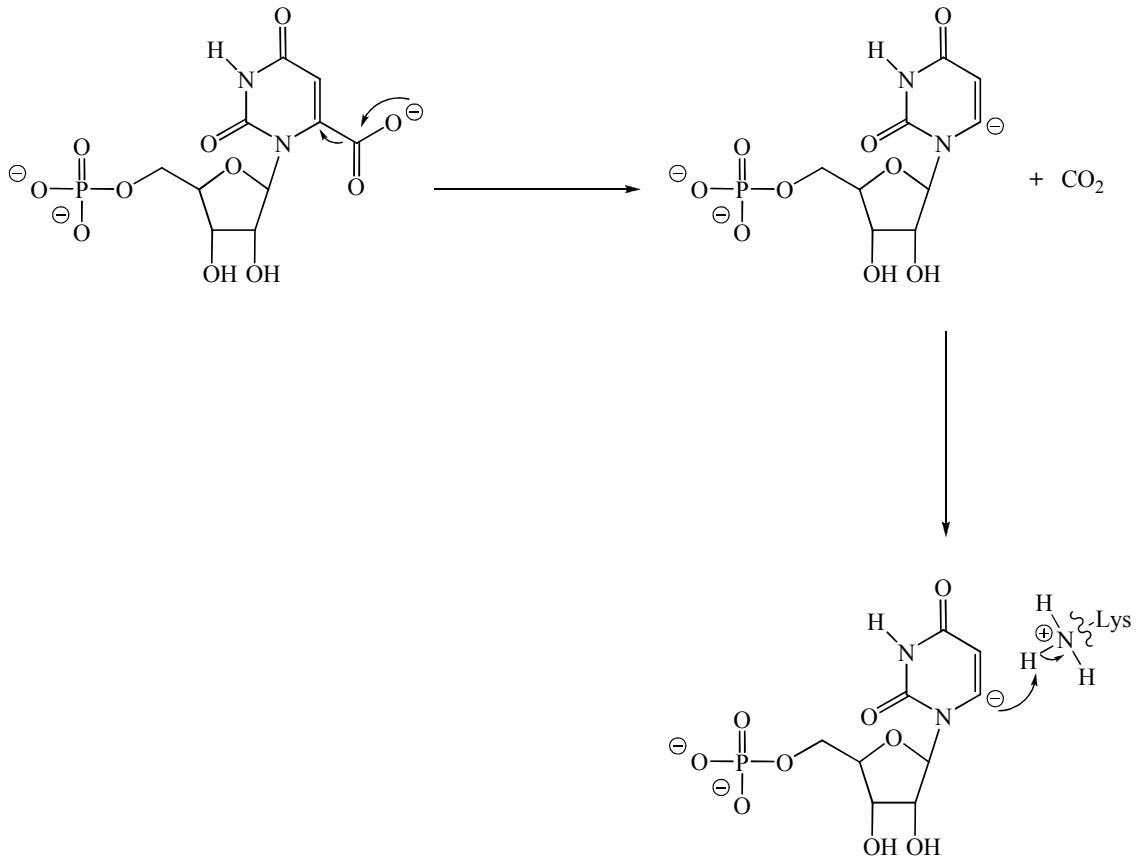
Doubts about the viability of a protonated intermediate, as well as the absence of electron stabilizing cofactors, prompted several investigators to question the very existence of negative charge at the transition state in the ODCase reaction.[114]

An alternative pathway that was proposed by Begley *et al*. involved the synchronous addition of a proton at the carbon in position 6 as the C-6–C-7 bond ruptures, bypassing formation of a discrete carbanion (scheme 7).[101,108] Whilst seemingly at odds with the enzyme's preference for nucleotide inhibitors possessing electronegative substituents at the C-6 position,[99] it finds precedent in other enzyme catalysed reactions.



Scheme 7 Synchronous addition mechanism of action of ODCase.

In contrast, some other experimental evidence supports the formation of a carbanionic intermediate species upon decarboxylation (scheme 8). The observation of a favourable interaction between the negatively charged oxygen of the inhibitor BMP in the complex may imply a high affinity between the enzyme and the negative charge generated on the carbon at position 6.[99,107,115] Kinetic studies have unambiguously identified lysine as the residue that protonates the C6 centre.[99,101,102]



Scheme 8 Simple direct decarboxylation mechanism.

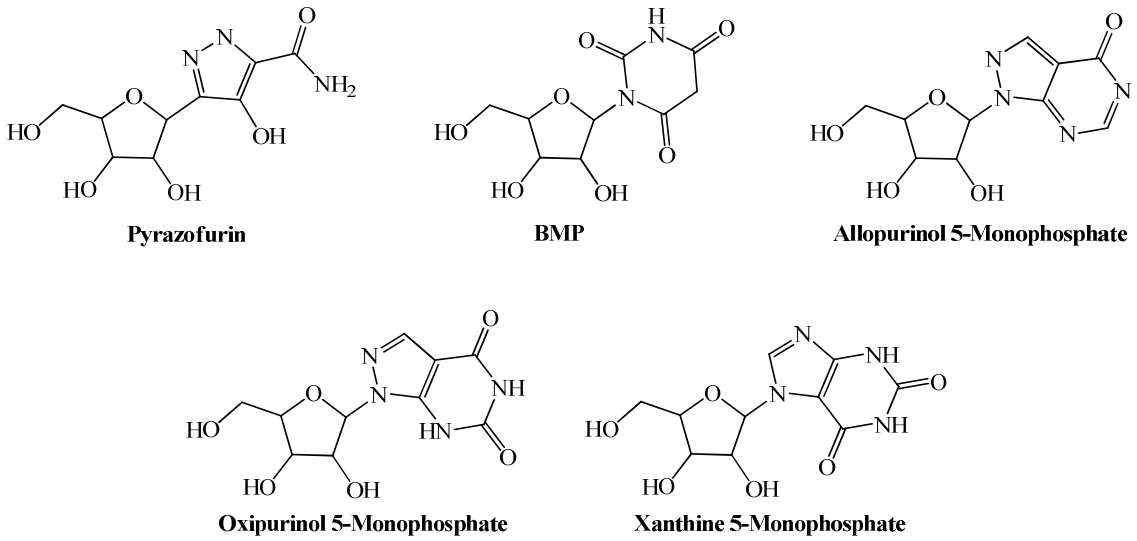


Figure 20 Some ODCase inhibitors.

Pyrazofurin (figure 20) is a C-nucleoside readily taken up by mammalian cells and phosphorylated to the 5'-monophosphate derivative, a potent inhibitor of ODCase (K_i = 5 nM).[116] The nucleoside-5'-monophosphate derivative of barbiturate (BMP) is an extremely potent inhibitor of yeast ODCase (K_i = 8.8 pM),[117] but it may not be

sufficiently stable to inhibit this enzyme in intact cells.[118] The nucleoside 5'-monophosphate derivatives of allopurinol, oxipurinol, and xanthine,[119] with the ribose linked at the 3- or 9-position of the purine ring are potent inhibitors of ODCase.

2.5 Summary

Human erythrocytes have very limited nucleotide requirements and therefore, lack the complete nucleotide biosynthetic pathway. However, the rapidly dividing intra-erythrocytic parasite has dramatically increased nucleotide requirements that have to be met through parasite-encoded pathways. Purine nucleotides, in most protozoan parasites, are derived through the salvage of preformed host purines, while pyrimidine nucleotides are synthesised through the *de novo* biosynthetic pathway.[41] Flux through both of the pathways is essential with little or no means of bypass. The two pathways therefore, present a range of essential enzymes that can be targeted for therapeutic intervention.[120]

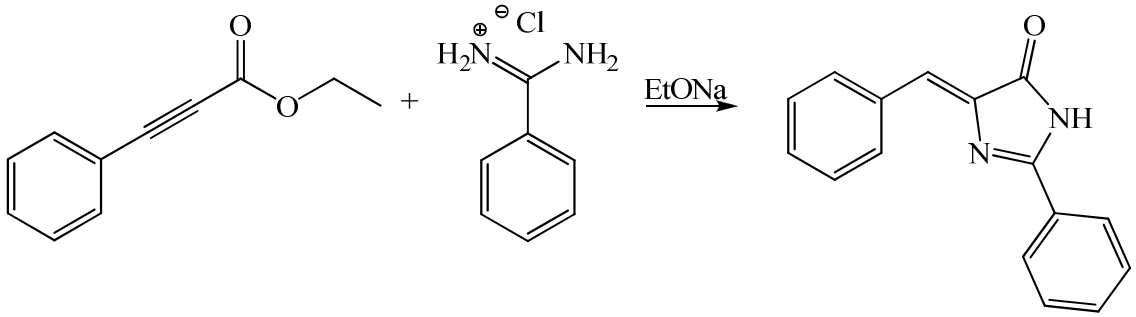
CHAPTER THREE

THE CHEMICAL SYNTHESIS AND PROPERTIES OF A SELECTION OF 5-HYDROXYIMIDAZOLES

3.1 Introduction

The chemistry of imidazole compounds has been of much interest due to the presence of such heterocycles in a large variety of biologically important molecules.[121] Indeed, the close structural similarity of most of the C-nucleosides to components of nucleic acids or to intermediates in purine or pyrimidine *de novo* nucleotide biosynthesis is remarkable, and adds weight to the search for synthetic chemotherapeutically active compounds in this area.[122] In addition to the growing interest in imidazole compounds as mimetics of naturally occurring compounds of importance, they also offer an alternative synthetic route to purine nucleosides by various cyclisation procedures which may allow the preparation of compounds not readily obtainable by more conventional synthetic methods.

One of the earliest reported syntheses of an imidazole was achieved by Ruhemann and Cunnington[123] in 1899, who obtained 2-phenyl-4-benzylidene-3,5-dihydroimidazol-4-one (scheme 9) by reacting ethyl phenylpropiolate with benzamidine hydrochloride in the presence of sodium ethoxide.[124]



Scheme 9 The first synthesised imidazole, 2-phenyl-4-benzylidene-3,5-dihydroimidazol-4-one.

Currently the most promising compounds in the class of imidazole nucleosides are believed to be bredinin and other 5-substituted derivatives.

3.2 Bredinin

Interest in imidazole compounds grew with the isolation and subsequent chemical synthesis of bredinin, 1-β-D-ribofuranosyl-5-hydroxyimidazole-4-carboxamide.[76] Bredinin (also referred to as Mizoribine®) is an imidazole nucleoside antibiotic isolated from *Eupenicillium brefeldianu*, M-2166.[76,125] The chemical structure of bredinin was determined by the use of X-ray crystallographic studies (figure 21).[125]

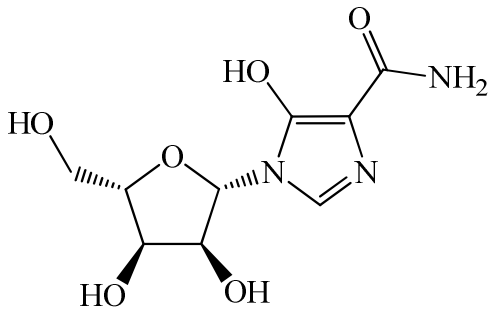


Figure 21 Bredinin.

The core structural motif thought to be responsible for the broad-spectrum of biological activities is the imidazole aglycone, viz. 5-hydroxyimidazole-4-carboxamide, which is known to bring about competitive inhibition of the enzymes in the purine biosynthetic pathway.[126]

Studies[76,127,128] have revealed bredinin to be ineffective against experimental mouse candidiasis, and most other microorganisms, except for *Candida albicans*. It is currently being used clinically in Japan as an immunosuppressant for post transplant patients,[129] because of lesser side effects, both biochemical and histopathological, but greater potency when compared to azathioprine (figure 22).[81,130,131] Azathioprine is routinely used as the choice of immunosuppressant drug in the West.

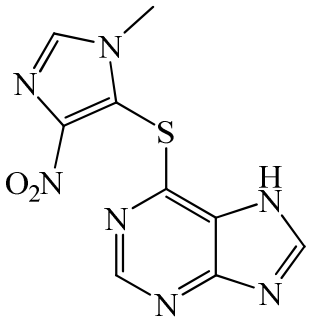
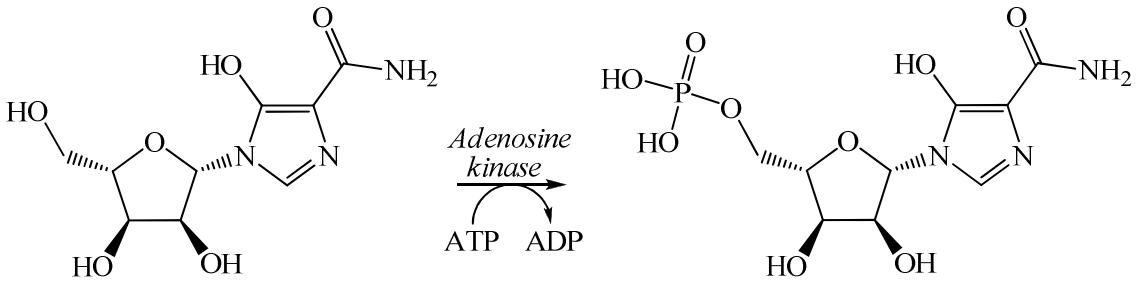


Figure 22 Azathioprine.

Bredinin is a structural analogue of the 1-β-D-ribofuranosyl-5-aminoimidazole-4-carboxamide (AICAR) (figure 24) which is an intermediate found in the *de novo* purine biosynthetic pathway. Unlike AICAR, bredinin is not taken up by nucleic acids in the cell. Instead, after phosphorylation by *adenosine kinase*[80] to bredinin-5'-monophosphate (scheme 10), it inhibits GMP synthesis by the antagonistic blocking of IMP dehydrogenase (Ki = 10^{-8} M) and GMP synthetase (Ki =10^{-5} M)[132] and depletes cells of essential guanine nucleotides.[133]



Scheme 10 Activation of bredinin to its monophosphate form.

Despite having interesting biological activity, few studies on the synthesis of derivatives of bredinin have been reported. Bredinin, as well as reported analogues, have antitumour effects in experimental tumour models[134-136] and recently a significant antiviral effect was also reported.[76,137] Additional biological activities found for bredinin are as an antiarthritic[80,138] and antimalarial[139] drug. Therefore, chemical modification studies of bredinin may result in the development of useful compounds with desired pharmacological effects. Unfortunately previously reported syntheses have facilitated only modest yields and are not easily adapted to the synthesis of derivatives and analogues.[140] However, of the syntheses reported,[141-143] the direct Vorbrüggen

coupling of silylated 4(5)-carbamoyl imidazolium-5(4)-olate with an acylated ribose resulted in the best overall yield (scheme 11).[143]

3.3 Interesting Biological Properties of Bredinin

3.3.1 Immunosuppressive

Bredinin has been found to exhibit potent immunosuppressive activity.[144] The first clinical trial[81] of bredinin in 1993, studied fourteen patients who had undergone renal transplants (many of them had an adverse reaction to azathioprine). The dosage used was 4-5 mg kg^{-1} daily, which was gradually decreased to a level of 1-2 mg kg^{-1} daily. The conclusion of this early trial was that bredinin appeared to possess full immunosuppressive efficacy for renal transplant patients but none of the adverse reactions that were found when azathioprine had been administered to patients originally.

The greater potency of bredinin compared with azathioprine is demonstrated in the study conducted by Inou[145] who by 1982, in a randomized clinical trial, had administered more than 300 patients with bredinin immunosuppressive therapy. At a dose of 50-300 mg daily, bredinin produced an 80.7 % one year graft survival rate, whereas the control group treated with azathioprine only had a 63.9 % one year graft survival rate. Bredinin was also found to have fewer side effects, such as hepatoxicity and agranulocytosis, than azathioprine.

Due to the immunosuppressive property of bredinin, it has also been investigated in the treatment of human glomerulonephritis, which is thought to be induced by an immunological mechanism (more than 80 % of renal disease is thought to be triggered by immunological reactions).[146] Immunosuppressive agents have been widely used within the clinical setting for the treatment of human glomerulonephritis. Interestingly in rats,[147,148] rabbits[149] and mice[150] bredinin administered at doses that were ¼ or ⅓ of the usual dose of azathioprine showed a more potent effect in preventing histological lesions and dysfunctions in the kidney glomerular that usually accompany glomerulonephritis. These results indicate that bredinin might be a useful drug in the treatment of any rapidly progressing cases of the disease.

3.3.2 Antitumour

Bredinin has been shown[77] to inhibit the multiplication of several mammalian cell lines in tissue culture. In particular, at a concentration of 1.2 x 10^{-5} M bredinin was found to strongly inhibit L5178Y mouse leukaemia cells, whilst at a concentration of 0.5 x 10^{-5} M, 100 % survival rate was observed. Also, bredinin was found[78] to be cytocidal at concentrations above 2 x 10^{-5} M, however at 5 x 10^{-5} M in the presence of excess GMP (4 x 10^{-5} M) it was found to be cytostatic. However, at the higher concentration inhibition was not reversed even when the concentration of GMP was increased. This reversal of inhibition is also seen[78] with guanosine and guanine but not by any other purine or pyrimidine nucleotides, nucleosides or bases. These results suggest that the point of enzyme inhibition is beyond IMP in the *de novo* purine biosynthesis, although the exact point of inhibition hasn't yet been established.[77,78]

The cytostatic effect of bredinin when combined with GMP was found to be completely reversed by cyclic AMP, although other cyclic nucleotides were ineffective. The reversing effect of cyclic AMP on cell survival was found to depend on the concentration of GMP, but this reversing effect was not observed in the absence of GMP. It can be concluded therefore, that cyclic AMP influences the secondary cytostatic effect of bredinin, but not the primary.

Although there is good evidence[77,78] that bredinin strongly inhibits nucleic acid biosynthesis, there is none to support its incorporation into nucleic acids. The antitumour potential of bredinin is apparently limited, since it was found to only be slightly active in prolonging the survival period of *in vitro* experimental mice, inoculated with leukaemia L1210 cells, and was ineffective against Ehrlich ascites tumour.

In an attempt to study the cytotoxic mechanism of bredinin and its activity as a potent inhibitor of purine biosynthesis in mammalian tissue, a number of chemically induced mutant cells resistant to 10 μM bredinin were isolated from cultured mouse mammary carcinoma.[80] These resistant cells were shown to be 15-19 times less sensitive to bredinin than wild type cells, and they retained their resistant phenotype even in the absence of the drug for more than three months. Enzyme assays using cell free extracts

revealed that all the bredinin mutants had less than 3 % of the adenosine kinase activity found in wild type cells. These results demonstrated that bredinin resistance is attributed to defective adenosine kinase activity, and therefore, that bredinin is phosphorylated in the cell to the corresponding cytotoxic nucleotide. This is supported by the findings of earlier cytotoxicity studies[151] comparing bredinin with its 5'-phosphate derivative, which revealed that the nucleotide showed enhanced cytotoxicity over the parent nucleoside. Since nucleotides don't normally enter the cell, it was suggested[151] that an exogenous phosphatase hydrolysed the nucleotide to bredinin, allowing penetration into the cell, followed by re-phosphorylation by a kinase once inside the cell. However, since bredinin 5'-phosphate showed greater activity, it was later suggested that the nucleotide could transverse the cell membrane directly, on the basis that if it were subject to hydrolysis, the cytotoxcity observed would be the same as that of bredinin. In agreement with earlier studies,[78,148] bredinin sensitive wild type cells treated with exogenously added guanine, showed a reversal in the cytotoxic effect of bredinin.[152]

It has also been shown that the bredinin aglycone (4-carbamoylimidazolium-5-olate, SM-108), displays similar cytotoxicity to bredinin,[152] presumably by enzymatic glycosylation with PRPP to the nucleotide. Recently, a new bredinin aglycone derivative, 5-carbamoylimidazol-4-yl piperonylate (SL-1250, figure 23), has been shown[153,154] to have broad antitumour activity when examined with certain carcinomas, leukaemias, and melanomas.

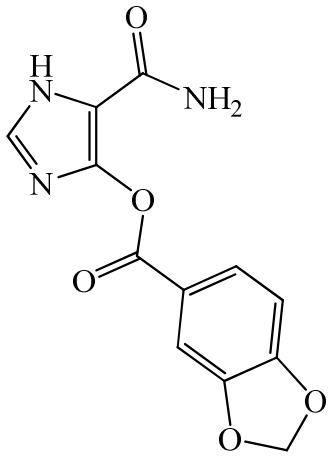


Figure 23 5-Carbamoylimidazol-4-yl piperonylate.

3.3.3 Antiviral and Antimicrobial

The antiviral activity of bredinin was assayed *in vitro*. The cells, treated with various concentrations of the drug, were challenged with vaccinia virus, and after 72 hours, virus-induced cytopathogenic effects as a parameter for antiviral evaluation were determined by microscopic evaluation. Bredinin prevented the proliferation of the vaccinia virus at a concentration of more than 0.8 mg ml^{-1} but did not inhibit any other viruses tested.[76]

Bredinin has been found to inhibit the growth of vaccinia virus but not that of bacteria or fungi except for *Candida albicans in vitro*.[76] The partial activity of bredinin against *C. albicans* was specific among candida species. Against other fungi, such as *Saccharomyces cerevisiae*, *Trichophyton asteroids*, *Aspergillus fumigates* and *Penicillium chrysogenum*, it showed no inhibitory effect. Attempts to protect against experimental candidiasis of mice with bredinin were unsuccessful.

3.3.4 Antiarthritic

It is known that the development of rheumatoid arthritis involves an immunological mechanism; consequently it has been shown that some immunosuppressive agents have beneficial effects in the treatment of the disease. In view of bredinin's known immunosuppressive activity, its potential was investigated[155] as an inhibitor of adjuvant-induced polyarthritis in rats (the arthritic syndrome was induced by intradermal injection of heat killed *Mycobacterium butyricum* in the foot). Bredinin was shown not to inhibit the primary phase of inflammation (3 days after injection), however the secondary phase of inflammation (20-30 days after injection) was suppressed. Only the highly cytotoxic drug 6-mercaptopurine has been shown to have similar activity. It was suggested bredinin's mechanism of action could be due to the inhibition of antibody formation and/or multiplication of sensitized lymphocytes possibly by an antigen in adjuvant polyarthritis. Preliminary studies have shown the potential clinical application of bredinin in the treatment of rheumatoid arthritis.

3.3.5 Antimalarial

The approach to antimalarial chemotherapy has been hampered in the past by a lack of understanding of the host-parasite relationship. However, with the sequencing of the parasite genome[156] and the development of *in vitro* culture techniques that permit continuous growth of the human malaria parasite *Plasmodium falciparum* in human erythrocytes, this problem has now been overcome.

The plasmodium parasite has a requirement for hypoxanthine, which is used for the biosynthesis of both guanosine and adenosine nucleotides, whereas the mature human erythrocytes, where the parasites reside, do not. Bredinin has been found[157] to strongly inhibit IMP dehydrogenase, at a concentration of 50 μM. This block in GMP biosynthesis proved to be fatal to both drug resistant and drug sensitive strains of the parasite at the trophozoite stage of development. It is hoped that this observed subtle chemotherapeutic mechanism might offer a new approach to the design of new antimalarial drugs and ultimately the clinical treatment of malaria.

3.4 Comparison of some Bredinin Analogues Currently Known

3.4.1 AICAR

As already mentioned AICAR is a natural nucleotide intermediate in the biosynthesis of purines and has an imidazole riboside structure (figure 24). Particular attention has recently been paid to AICAR since its 5'-phosphorylated derivative, a key biosynthetic precursor of purine nucleotides, is an activator of AMP-activated protein kinase (AMPK).[158] The extracellular role of adenosine and other nucleosides as endogenous cell function modulators indicates adenosine receptors as significant targets with wide therapeutic potential. In this context AICAR has been indicated as a promising pro-drug which induces benefits in patients suffering from autism, cerebral palsy, insomnia, schizophrenia and other neuropsychiatric symptoms generally thought to be associated with a chronic low level of adenosine.[159] However, AICAR has a short half-life in the cell; it does not efficiently cross the blood-brain barrier, and is poorly absorbed from the gastrointestinal tract.

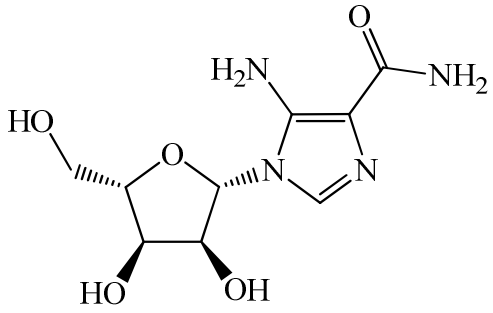


Figure 24 AICAR.

Unfortunately studies have found that AICAR has only modest biological efficacy when used as an antiviral agent.[160] Gong *et al.*[161] investigated the effects of AICAR on phosphorylation and antiretroviral activity of purine nucleoside analogues. Cells pre-treated with AICAR (0.5 mM) were found to be less susceptible to human immunodeficiency virus (HIV) infection. These results show that AICAR modulates the anabolism and antiviral activity of particular purine nucleoside analogues; this has implications for possible therapies with these types of analogues.

Consequently, the production of new AICAR derivatives is an appealing objective in the field of medicinal chemistry. Over the last twenty five years, the synthesis of a number of AICAR derivatives such as the 2-aryl,[162] 4-N-benzyl,[163] 4-substituted,[164] 5-substituted,[165,166] 5-hydroxyl (bredinin),[76,144] triazolyl-riboside (ribavirin),[167,168] 2',3'-secoriboside[169] derivatives have been reported.

3.4.2 Ribavirin

Effectively a guanosine analogue,[74] ribavirin (figure 25) is a ribosyl purine analogue with an incomplete purine 6-membered ring. It is a pro-drug, activated by cellular kinases which give the 5'-triphosphate nucleotide form.

Ribavirin is a broad spectrum antiviral agent with activity against at least 12 DNA and 40 RNA viruses. It is a member of the nucleoside antimetabolite class of drugs, and has been found to interfere with duplication of viral genetic material.[170] A 99.3 % loss in viral genome infectivity was observed after a single round of virus infection; however the exact mechanism of action of ribavirin has not yet been fully elucidated.

Ribavirin, in combination with interferons, is licensed for the treatment of hepatitis C virus infection.[171] Ribavirin therapy, however, is associated with a number of adverse side effects, particularly haemolytic anaemia which results from transport and retention of phosphorylated ribavirin within red blood cells.[172]

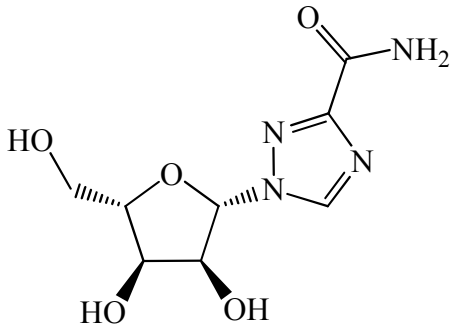


Figure 25 Ribavirin.

In an effort to minimise the high level of toxicity that ribavirin exhibits, several attempts have been made to synthesise analogues. Most notably levovirin, the L-enantiomer of

ribofuranosyl carboxamide, and viramidine, a pro-drug of ribavirin.[173] Both drugs seem to retain the immunomodulatory properties of the parent compound but are less toxic.

3.4.3 Levovirin

Levovirin is an L-isomer of ribavirin (figure 26), it is not phosphorylated intracellularly and therefore exhibits reduced red blood cell toxicity.[174] Importantly however, the compound has not shown genotoxic effects in animals and cell-line studies and it does not seem to cause hemolytic anaemia which is the major dose limiting side effect of ribavirin.

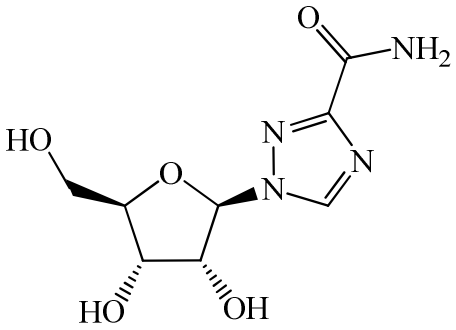


Figure 26 Levovirin.

3.4.4 Viramidine

Viramidine (figure 27) is rapidly converted to ribavirin *in vivo* by *adenosine deaminase* but has a higher liver-to-erythrocyte drug ratio with reduced uptake and storage in red blood cells and therefore reduced toxicity,[175] possibly owing to the 3-carboximidamide group which is protonated at physiological pH.

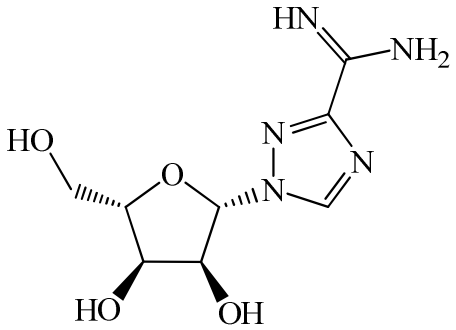


Figure 27 Viramidine.

3.4.5 EICAR

5-Ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR, figure 28) can be considered a structurally related analogue of ribavirin, in which the nitrogen at position 2 of the triazole ring has been replaced by a carbon with an alkyne group attached. The antiviral activity spectrum of EICAR has been found to be similar to that of ribavirin, however EICAR is more potent than ribavirin.[176]

From the antiviral activity spectrum of EICAR, it can be inferred that its mode of action must be similar to that of ribavirin. Although the primary site for the antiviral action of ribavirin has remained a point of some controversy, it had been originally identified as the enzyme IMP dehydrogenase,[177] if this was proved to be true IMP dehydrogenase may be postulated as a target for the cytostatic action of EICAR also.

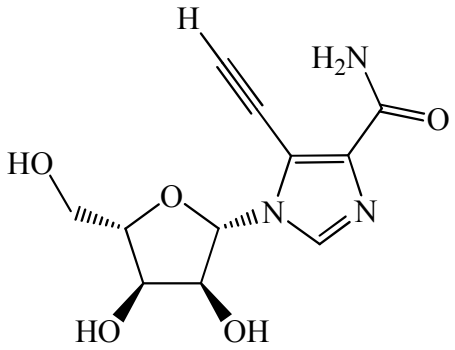


Figure 28 EICAR.

3.4.6 Pyrazofurin

The naturally occurring azole-carboxamide nucleoside antibiotic, pyrazofurin (figure 29), is a biologically active carbon-linked nucleoside isolated from the broth of a strain of *Streptomyces candidus*.[178] Pyrazofurin as the nucleoside inhibits malarial OPRTase (orotic acid → orotidine 5'-monophosphate), while the 5'-monophosphate derivative inhibits ODCase.[179] Pyrazofurin inhibits *de novo* pyrimidine biosynthesis at orotidine-5'-monophosphate decarboxylase, and this compound also inhibits *5-aminoimidazole-4-carboxamide ribotide transformylase* of the purine pathway.[180] Pyrazofurin induces accumulation of orotate and orotidine in malaria, consistent with inhibition of ODCase with subsequent dephosphorylation of the OMP accumulated.[181]

Pyrazofurin is an effective antimalarial, which works by impeding the maturation of trophozoites to schizonts; observed toxicity is not affected by the addition of uracil or uridine to the culture because the parasite lacks the ability to salvage preformed pyrimidines.[46] However, the toxicity experienced for pyrazofurin means it cannot be used clinically as an antimalarial drug.

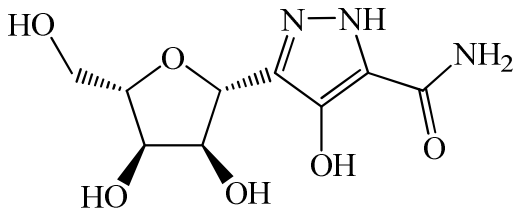


Figure 29 Pyrazofurin.

It can be seen that all of the analogues mentioned have structural similarities, however, there are particularly close structural similarities found between both bredinin and pyrazofurin. With this in mind, it can be postulated that any bredinin analogues synthesized might also have a similar biological activity to pyrazofurin and be potent inhibitors of ODCase in the *de novo* pyrimidine biosynthesis. If this was the case, it is also possible that this biological activity could be in addition to inhibiting IMP DHase in the purine biosynthetic pathway, as bredinin has been found to do.

Should any analogues be found to possess biological activity against the synthesis of pyrimidines, then these drug candidates would allow for selective toxicity; thus selectively lethal to the parasite, due to a pyrimidine deficiency because they can only synthesise pyrimidines *de novo*, whilst the host continues to salvage the pyrimidines required.

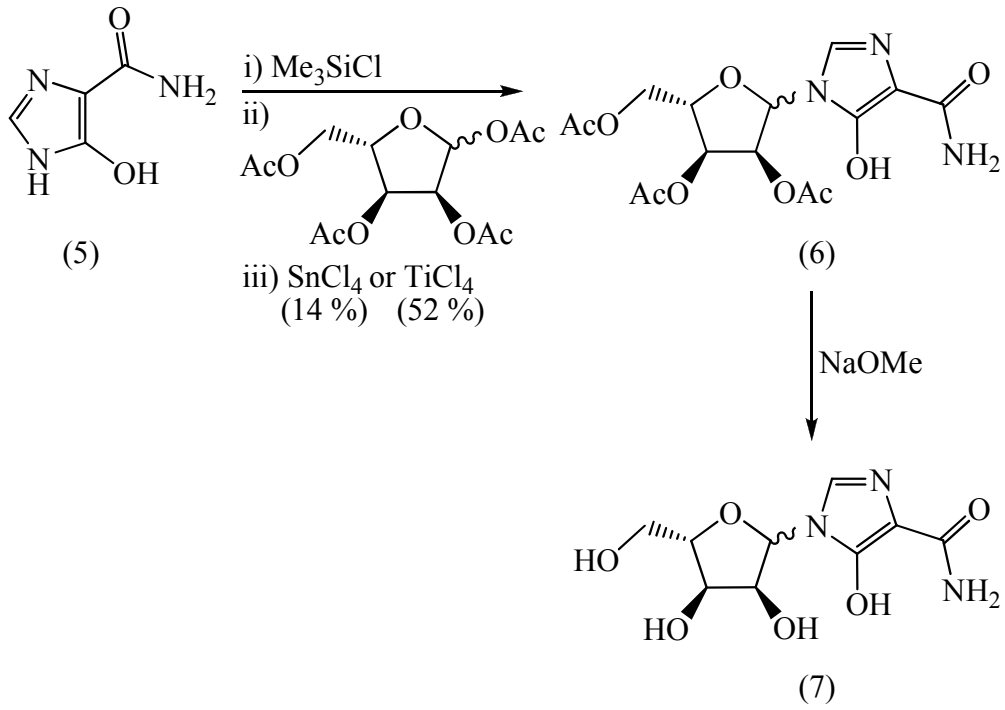
3.5 Methods of Synthesis of 5-Hydroxyimidazole-4-Carboxylates

3.5.1 Natural Product Synthesis

Bredinin is a naturally occurring 5-hydroxyimidazole-4-carboxamide; the producing organism was isolated from the culture media of *Eupenicillium brefeldianu* (M-2166) obtained from a soil sample. The imidazole nucleoside was originally isolated with the hope of it being a potential antibiotic,[76,77,182] but it was found to be ineffective against most microorganisms except *Candida albicans*, which it was partially active against.

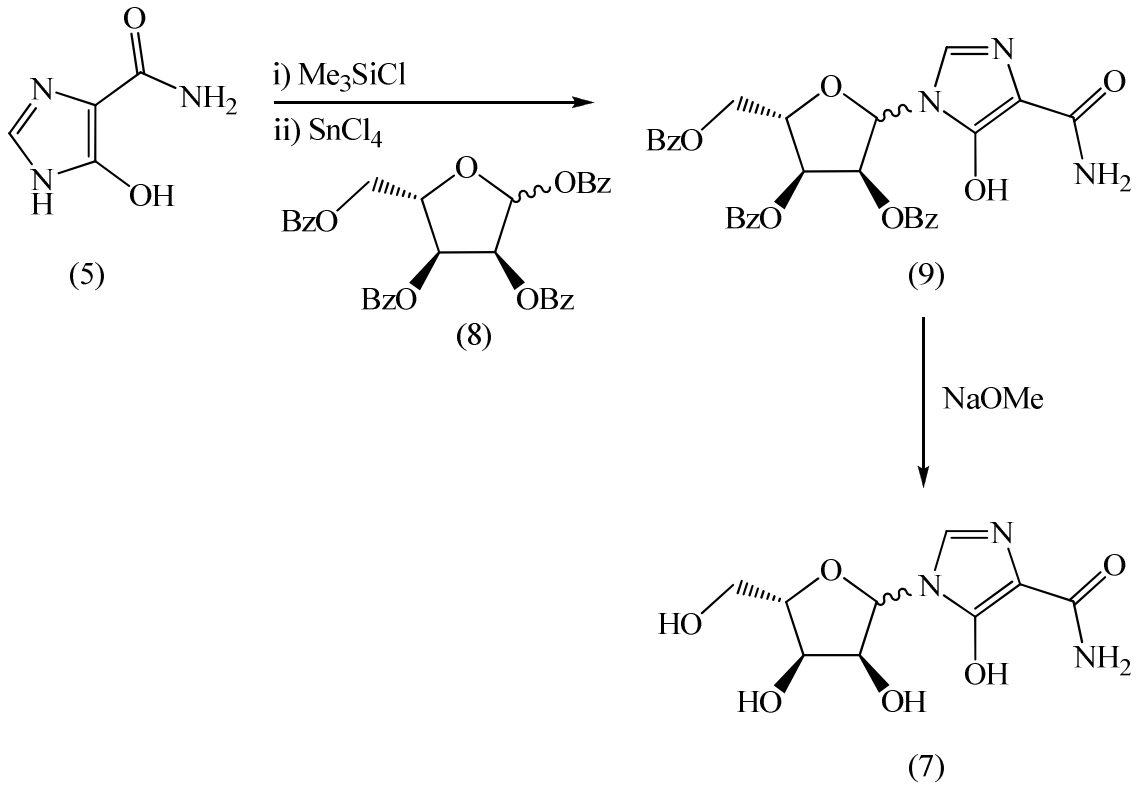
3.5.2 Direct Glycosylation of Preformed Imidazoles

The first reported[142] synthesis of bredinin (7) involved the direct glycosylation of the trimethylsilyl derivative of 5-hydroxyimidazole-4-carboxamide (5) with peraceylated-α-D-ribofuranose, in the presence of a Lewis acid catalyst (scheme 11). The choice of catalyst, either $SnCl_4$ or $TiCl_4$, and its effect on the yield of bredinin triacetate (6) was found to be significant, 14 % and 52 % respectively.



Scheme 11 First reported synthesis of bredinin by glycosylation.

Another reported synthetic route to bredinin (scheme 12) involved glycosylation of the trimethylsilylated bredinin aglycone using 1-acetyl-2,3,5-tribenzoyl-β-D-ribofuranose[183] (8), obtained by degradation of 2,3,5-tribenzoylated inosine, as a convenient source of the protected sugar moiety (9).

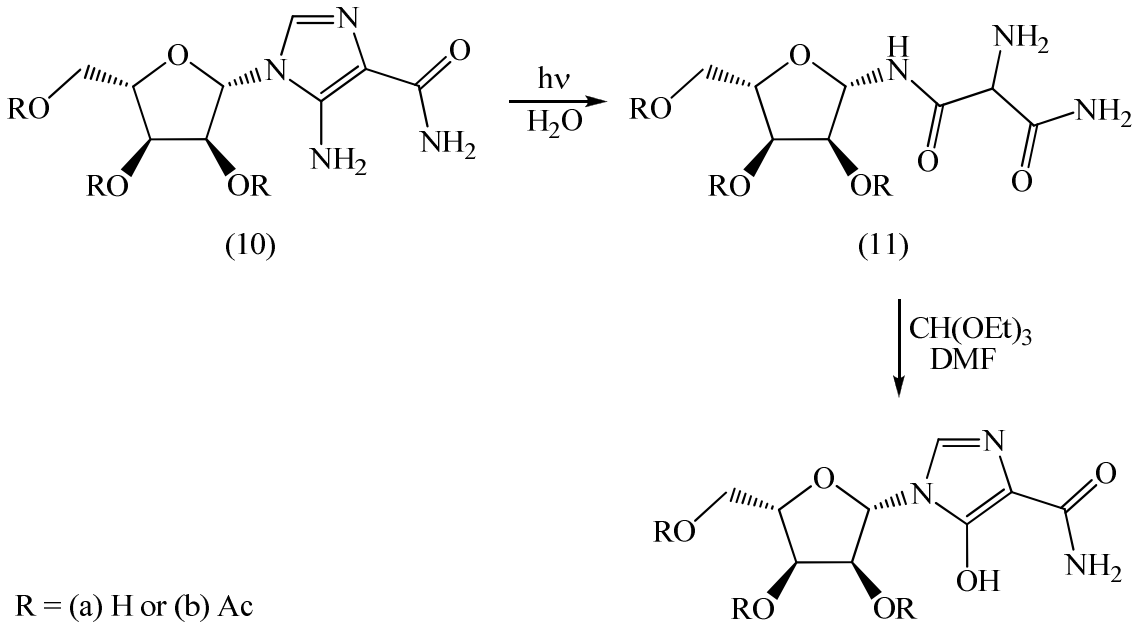


(5) (8) (9) (7)

Scheme 12 Synthesis of bredinin from the glycosylation of 1-acetyl-2,3,5-tribenzoyl-β-D-ribofuranose.

3.5.3 Degradation/Ring Opening of Purine Nucleosides or Nucleotides

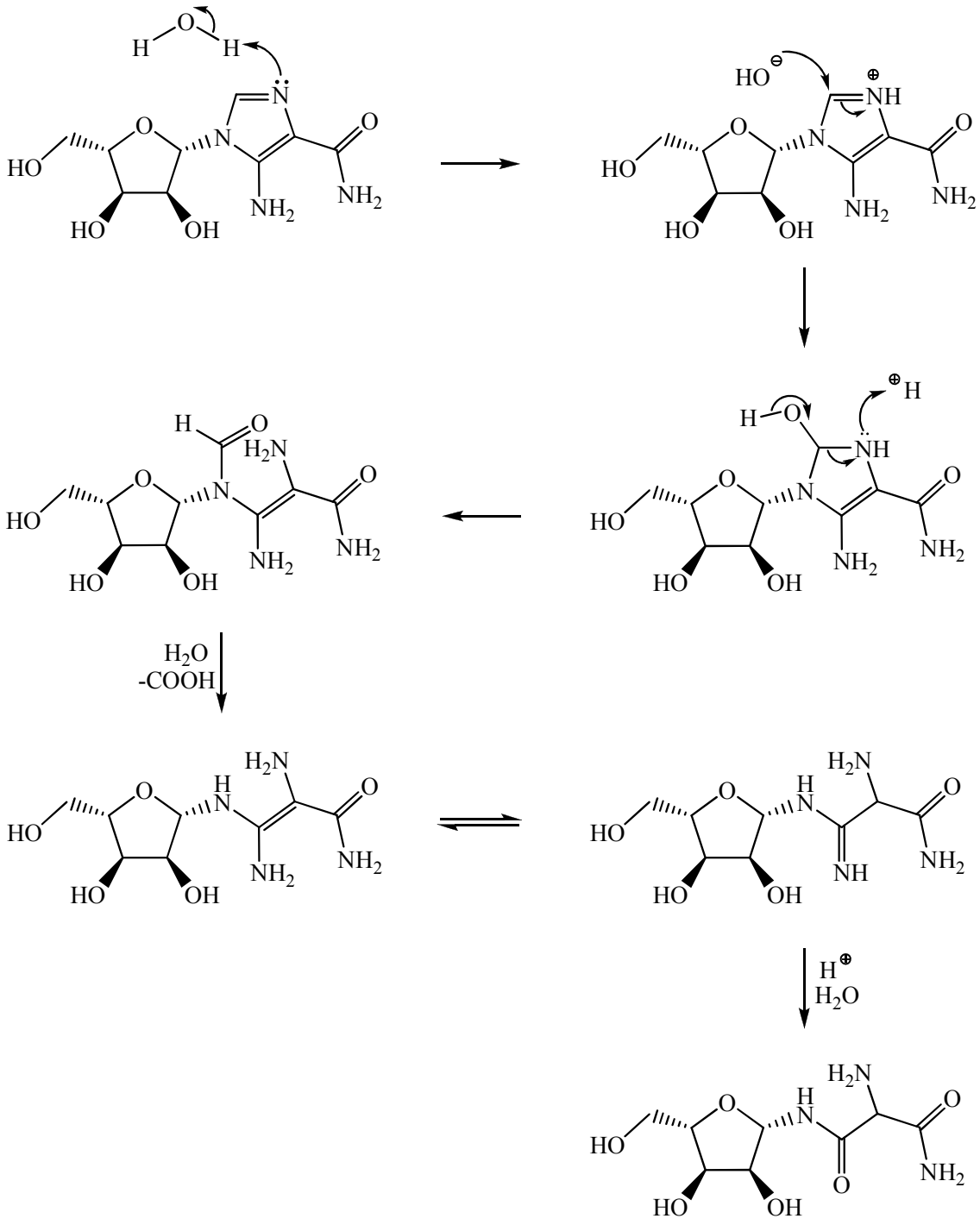
An alternative synthesis of bredinin reported, involved the photochemical cleavage[143,184] of 1-β-D-ribofuranosyl-5-aminoimidazole-4-carboxamide (AICA-riboside) (10a) or more conveniently the triacetylated derivative (10b), to the acyclic product 2-amino-N-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-malondiamide (11b), followed by cyclisation with triethyl orthoformate in dry DMF (scheme 13). However, the use of an expensive preformed imidazole nucleoside, although commercially available, is an expensive starting point for large-scale conversion of the 5-amino group to the 5-hydroxy of bredinin. To buy 5.00 g of the AICA-hydrochloride salt from Sigma Aldrich costs £64.10 (correct on the 1st June 2008).[185]



(10) (11)

R = (a) H or (b) Ac

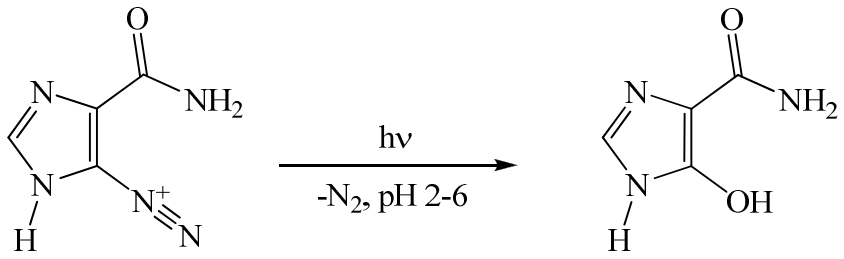
Scheme 13 Synthesis of bredinin from AICA-riboside.



(11)

Scheme 14 Proposed mechanism of photolytic hydrolysis of AICA-riboside.[143,184]

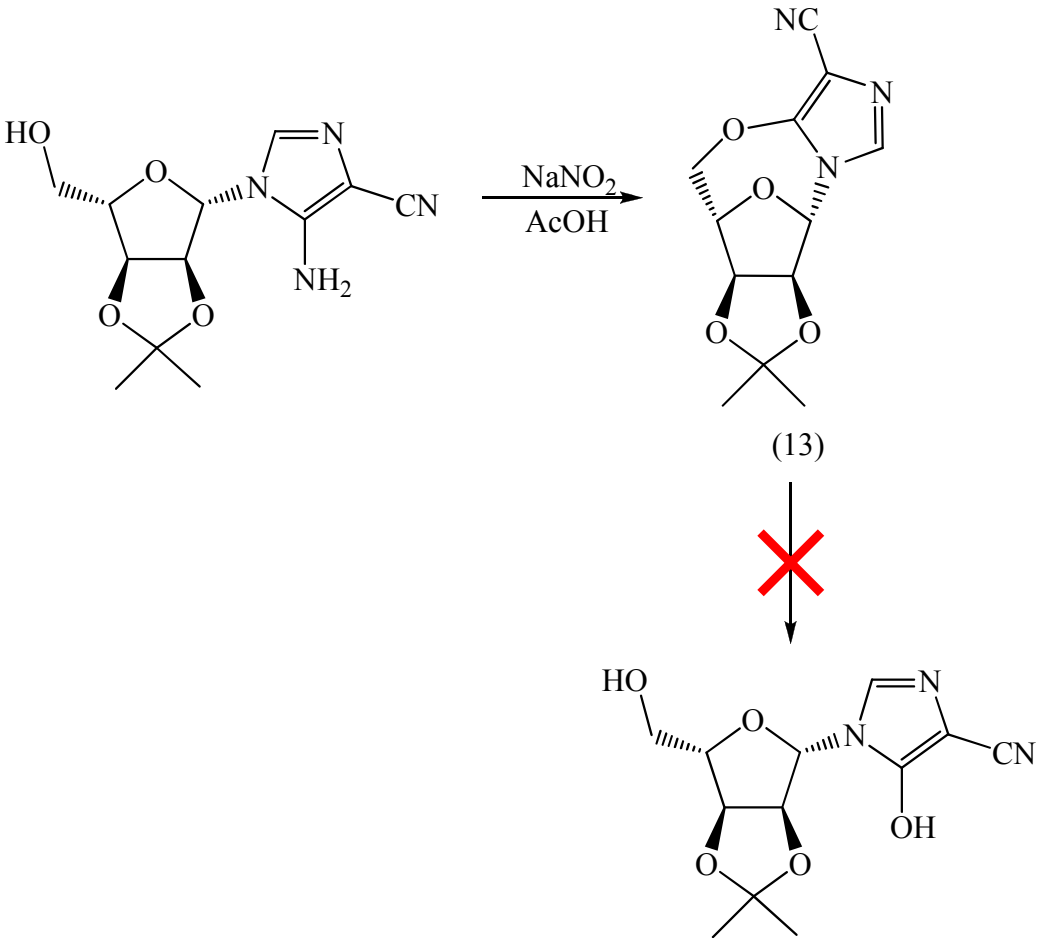
The synthesis of 5-hydroxyimidazole-4-carboxylate (5) from 5-diazoimidazole-4-carboxamide (12) by photolysis under acidic conditions has also been reported.[186]



(12) (5)

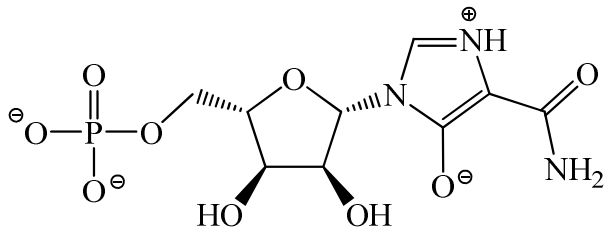
Scheme 15 Synthesis of 5-hydroxyimidazole-4-carboxylate.

A further approach to the synthesis of bredinin involved an attempted hydrolytic cleavage of a 5,5'-*O*-anhydro derivative of bredinin (13).[184] However, the nucleoside proved stable to this treatment without yielding the desired product.

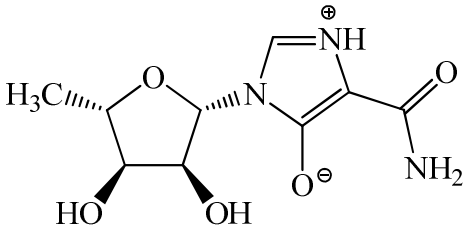


Scheme 16 Attempted hydrolytic cleavage of the 5,5'-*O*-anhydro derivative of bredinin.

Shuto *et al.*[187] planned to synthesize the currently biologically important 5'-modified derivatives of bredinin, the 5'-phosphate (14), the 5'-deoxy derivative (15), and the 5'-*O*-(3-aminopropyl)carbamate (16) (figure 30).



(14)



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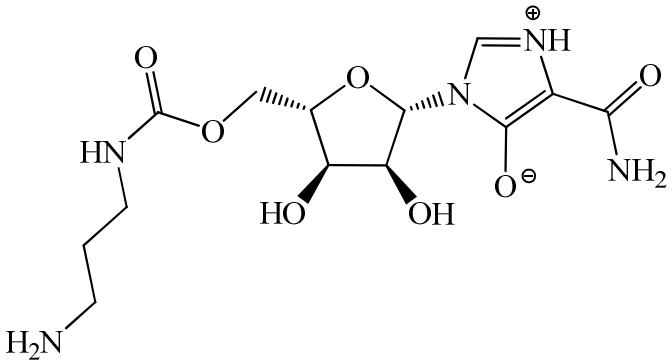
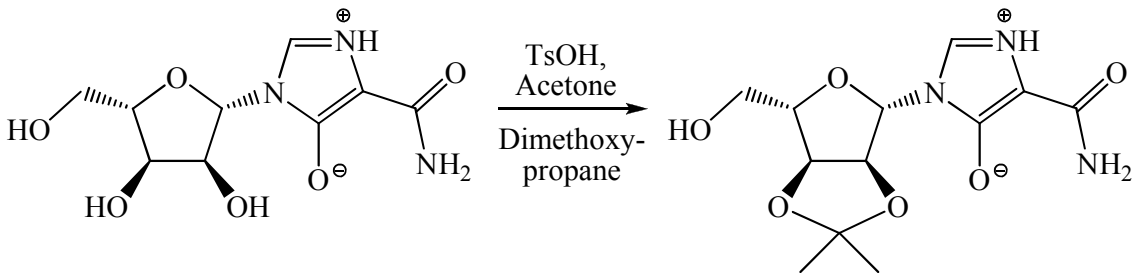


(16)

Figure 30 Targeted 5'-modified derivatives of bredinin.

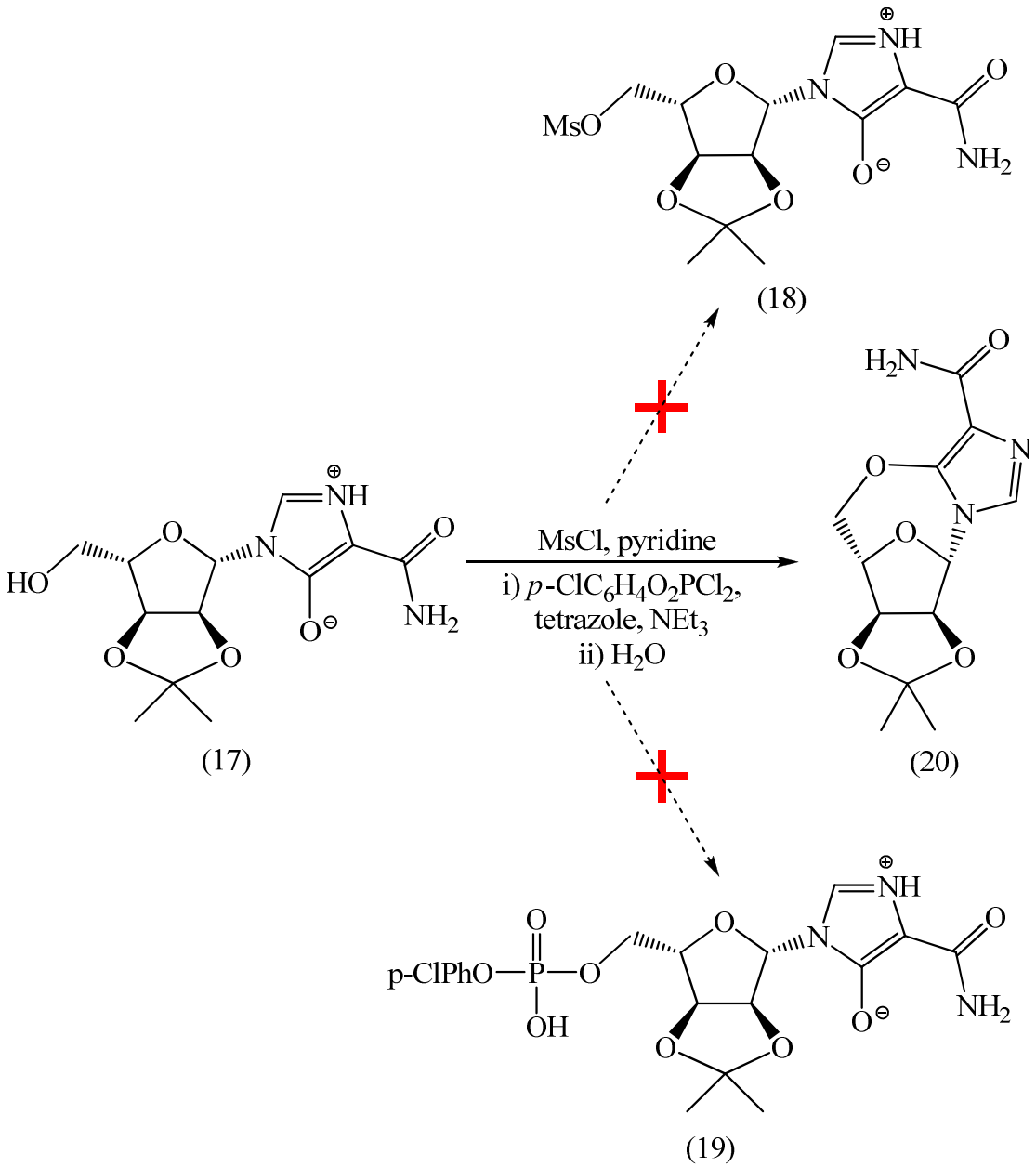
However, their first attempt at synthesising the target compounds from 2',3'-*O*-isopropylidenebredinin (17), which was readily obtained by treating bredinin with TsOH–acetone (scheme 17), was unsuccessful.



(7) (17)

Scheme 17 Synthesis of 2',3'-*O*-isopropylidenebredinin.

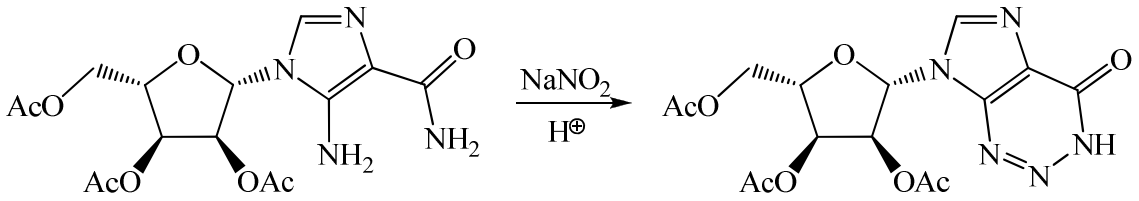
The study found that when an electron-withdrawing group was introduced at the 5'-position, intramolecular attack by the 2-oxygen of the base moiety quickly occurred. For example, treatment of (17) under the usual mesylation conditions or reaction of (17) using the phosphotriester method produced none of the desired 5'-*O*-mesylester (18) or 5'-phosphate (19). Instead it was found that both reactions gave the 5,5'-*O*-anhydro derivative (20) as the major product (Scheme 18).



Scheme 18 The attempted phosphotriester reaction and mesylation of 2',3'-*O*-isopropylidenebredinin.

3.5.4 Diazotization of Purine Nucleosides or Nucleotides

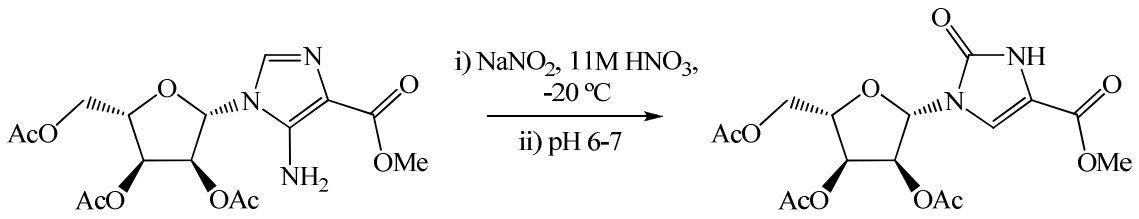
It might appear that logically the most direct method to the synthesis of bredinin is the diazotization of the 5-amino group of AICA-riboside. Nevertheless, attempts at diazotization of this nucleoside in its triacetylated derivative, resulted in the unexpected formation of 2-azainosine (21) as the main product.[188]



(10b) (21)

Scheme 19 Formation of 2-azainosine, the unexpected product resulting from the diazotization of the 5-amino group of AICA-riboside.

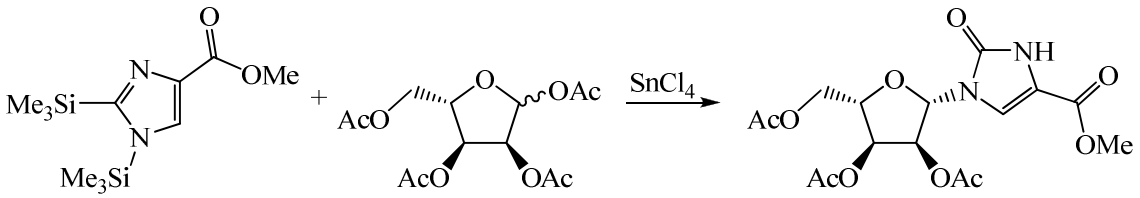
Other attempts made to diazotize the 5-amino function, resulted in an unusual rearrangement reaction.[189] Treatment of methyl 5-amino-1, (2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (22), with sodium nitrite in the presence of 11 M nitric acid at -20 °C resulted in the production of methyl 1-(2,3,5-tri-O-acetyl- β-D-ribofuranosyl)-2-oxo-Δ4-imidazoline-4-carboxylate (23).



(22) (23)

Scheme 20 Synthesis of methyl 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-oxo-Δ4-imidazoline-4-carboxylate.

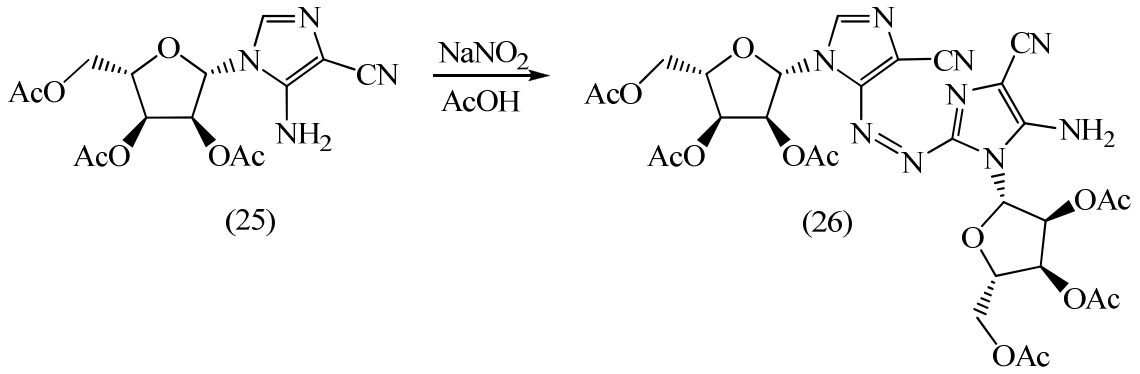
The product was initially mistakenly identified as bredinin (based on elemental analysis) but the actual structure was later confirmed by comparison with authentic bredinin and total synthesis. The total synthesis involved direct condensation of the bistrimethylsilyl derivative of methyl 2-oxo-Δ4-imidazoline-4-carboxylate (24) with peracetylated α-D-ribofuranose in the presence of stannic chloride.



(24) (23)

Scheme 21 Total synthesis of methyl 1-(2,3,5-tri-O-acetyl- β-D-ribofuranosyl)-2-oxo-Δ4-imidazoline-4-carboxylate.

In order to avoid cyclisation of the 5-diazonium intermediate, the 4-amido group of acetylated AICA-riboside was dehydrated to the 4-nitrile. Several attempts to diazotize the 4-cyano derivative of AICA-riboside (25), afforded a complex mixture and a red precipitate.[190] Analysis of the product by IR, UV, NMR spectroscopies, mass spectrometry, and elemental analysis, and determination of the melting point of the precipitate showed it to be an intermolecular azo coupled product, involving positions 5 and 2 of the starting material (26).

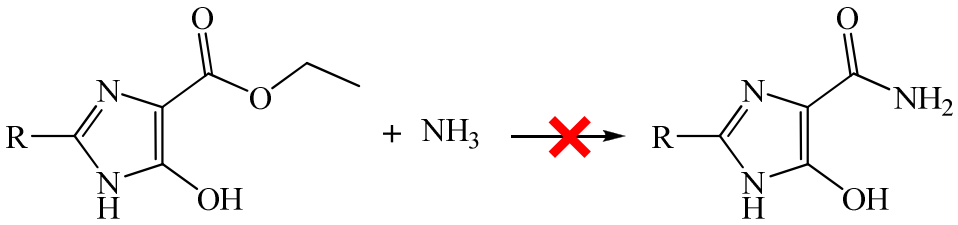


(25) (26)

Scheme 22 Diazotization of the 4-cyano derivative of methyl 2-oxo-Δ4-imidazoline-4-carboxylate.

3.5.5 Cyclisation of Acyclic Precursors

Miller *et al.*[191] used the method developed by Finger[192] to synthesise 2-substituted-5-hydroxyimidazole-4-carboxylic acid ethyl esters by condensation of the appropriate iminoesters and amino acid esters. Derivatisation of the carbethoxy group to the corresponding amide directly by reaction with ammonia was then attempted, however this proved to be unsuccessful (scheme 23).

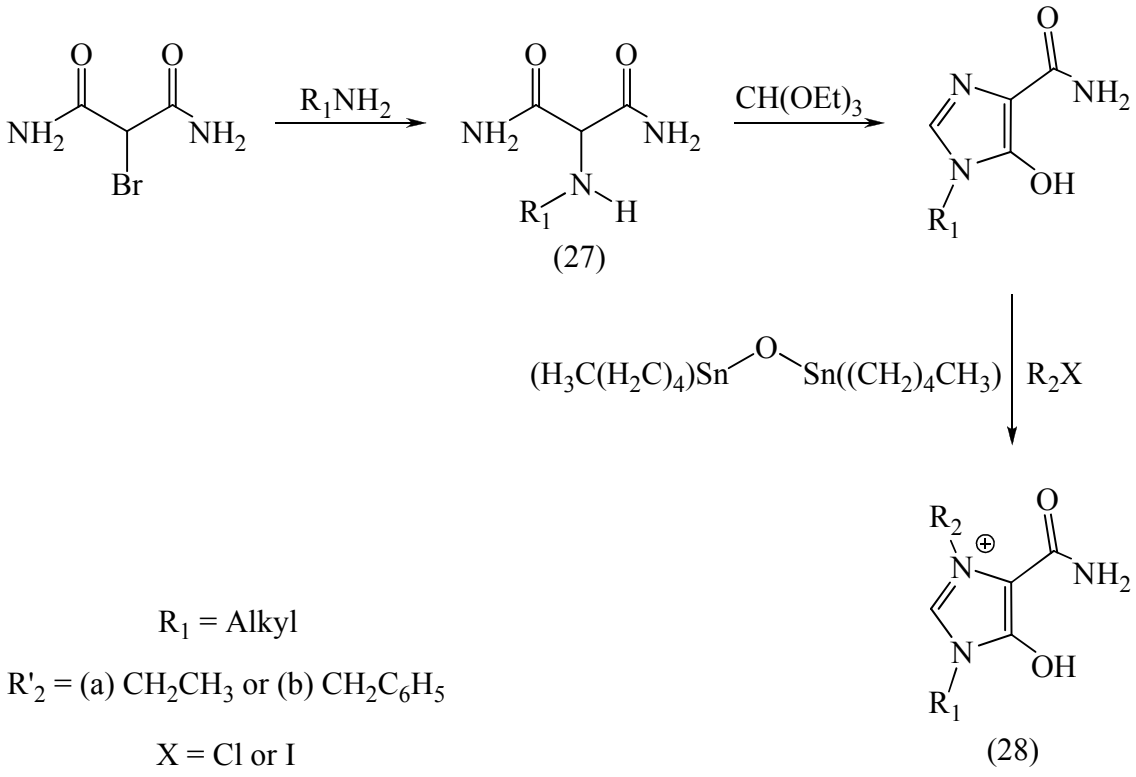


Where R = H or CH_3

Scheme 23 Attempted derivatisation of 2-substituted-5-hydroxyimidazole-4-carboxylic acid ethyl esters.

A more recent series of publications[154,193,194] described the synthesis of mono, and dialkyl-substituted derivatives of the bredinin aglycone.[194] In addition, an improved synthesis of bredinin by direct glycosylation, was also reported.[154]

The N-1 alkyl derivatives of 5-hydroxyimidazoles were prepared by cyclisation of the 2-alkyl-aminomalonamides (27) with triethyl orthoformate. This series was extended by the preparation of N-3 alkylated derivatives by successive treatment with bis(tri-n-butyltin)oxide and ethyl iodide or benzyl chloride, to yield the ethyl (28a) and benzyl (28b) substituent respectively (scheme 24).



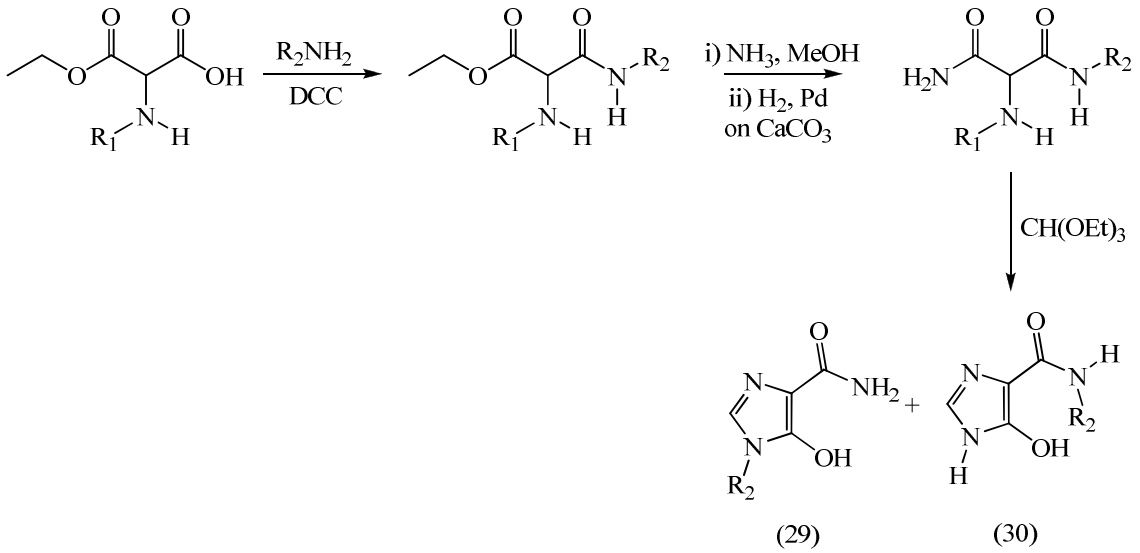
R_1 = Alkyl

R'_2 = (a) CH_2CH_3 or (b) $CH_2C_6H_5$

X = Cl or I

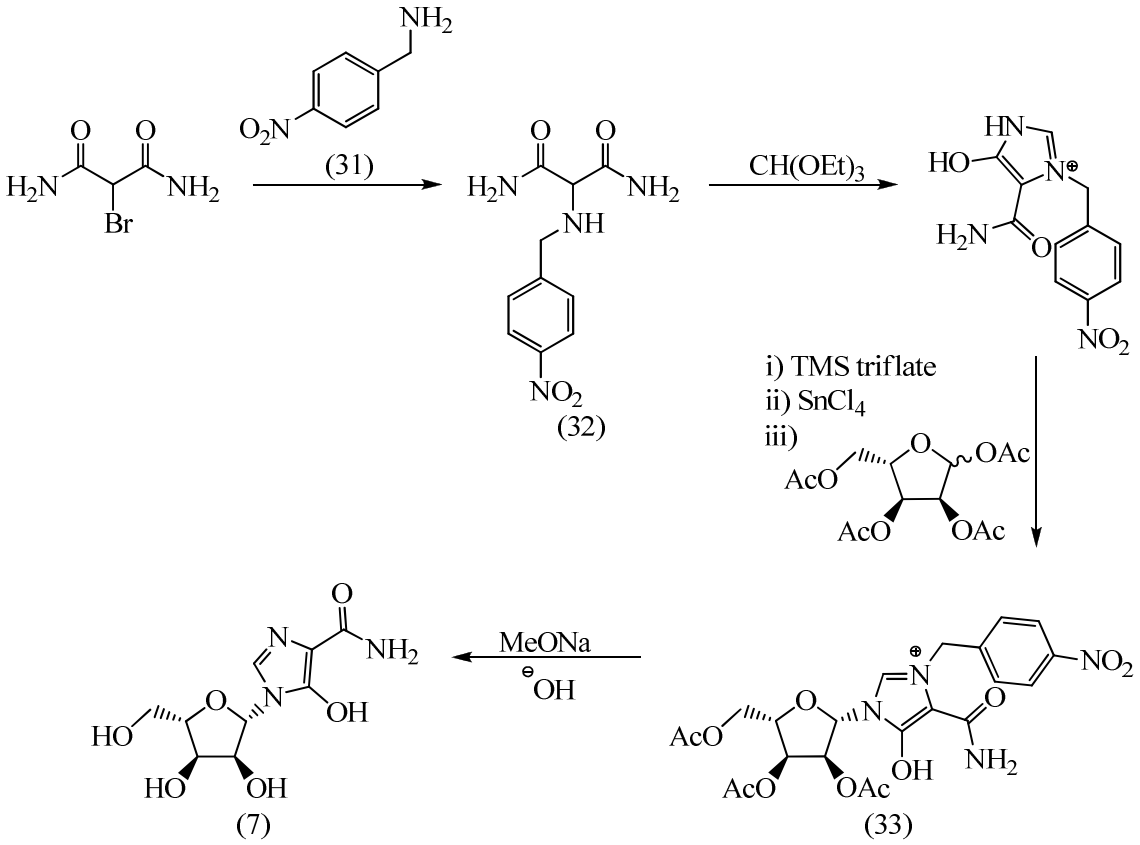
Scheme 24 Synthesis of monoalkyl- and dialkyl-substituted derivatives of bredinin.

The preparation of the N-1 (29) and the N-amido (30) derivatives was achieved by use of a similar route, but it employed the use of the aminomalonic acid half ethyl ester as a starting material instead (scheme 25).[194]



Scheme 25 Synthesis of N-1 and N-amido derivatives of bredinin.

An unambiguous synthesis[154] of bredinin (with respect to the position of glycosylation) was achieved by the protection of the N-3 position with a *p*-nitrobenzyl group (31). The protected imidazole was prepared by cyclisation of the 2-(4-nitrobenzyl)aminopropanediamide (32) with triethyl orthoformate (5 mol equivalents) in dry ethanol, using p-toluene sulphonic acid as a catalyst. Glycosylation with 1,2,3,5-tetra-O-acetyl-α-D-ribofuranose in the presence of stannic chloride and trimethylsilyl trifluoromethanesulphonate (TMS triflate) afforded the correspondingly protected bredinin (33). Interestingly, glycosylation was not observed in the absence of stannic chloride.



Scheme 26 Synthesis of bredinin by protection at the N-3 position.

3.6 Summary

Compared to 1-substituted-5-aminoimidazoles relatively few 1-substituted-5-hydroxyimidazoles have been reported; however, an interesting 1-substituted-5-hydroxyimidazole is the immunosuppressive agent bredinin, which shows antiviral,[76] antimalarial,[157] antitumour[77,78] and antiarthritic[80] activities.

An advantage of using acyclic precursors to synthesise imidazoles is that the introduction of substituents, in particular, those in the 1-position is unambiguous. Therefore, our approach has been to explore an efficient and general route to 1-substitued-5-hydroxyimidazoles employing inexpensive and readily available starting materials (chapter four).

In order to provide a greater understanding of some of the chemistry of these 5-hydroxyimidazole systems, some reactions of the ethyl 5-hydroxyimidazole-4-carboxylates have been studied (chapter five).

In addition to the ethyl 5-hydroxyimidazole-4-carboxylates, parallel studies were carried out into the synthesis of 5-hydroxyimidazole-4-carbonitriles and 5-hydroxyimidazole-4-carboxamides (chapter six).

CHAPTER FOUR

THE CHEMICAL SYNTHESIS OF ETHYL 5-HYDROXYIMIDAZOLE-4-CARBOXYLATES

4.1 Introduction

5-Membered heterocyclic nucleosides structurally derived from 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR, figure 24) by modification at position 5 such as bredinin and EICAR (figure 28), or by modification of the ring skeleton such as ribavirin (figure 25), are known to exhibit a broad spectrum of biological activity.

Recently, the production of large analogue libraries has emerged as an important synthetic goal to chase the high efficiency and velocity of the current biological screenings. Synthetic chemists have been expected to increase their rate of production of chemical entities with an ever-increasing demand for new and novel compounds. The current trend in compound library generation is towards well-designed, individual and pure compounds, which have a yield within a reasonable range (at least 20 mg).[195]

In the present study, the development of a synthetic route to a library of 5-hydroxyimidazoles was attempted. This library would allow thorough investigation of the effect of different functional groups, present on the imidazole aglycone, on the biological activity of these compounds.

4.2 Rationale

The eventual aim of the research was to make analogues of bredinin, this meant finding a suitable methodology to allow the synthesis of 5-hydroxyimidazole-4-carboxamide derivatives (figure 31) either directly or through modification of other 5-hydroxyimidazoles.

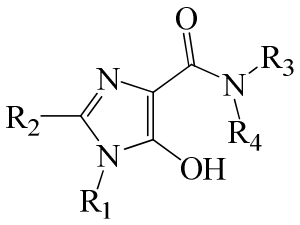


Figure 31 Target secondary amide 5-hydroxyimidazoles.

A number of primary amide derivatives of the 5-hydroxyimidazole-4-carboxamides have already been synthesised, however secondary amide derivatives are more scarce and the synthesis of those known[194] is not adaptable for other analogues. Tarumi's route is ambiguous with respect to substituents, and it is for this reason that it couldn't be used to easily produce a library of secondary amide derivatives of the 5-hydroimidazole-4-carboxamides for the purpose of biological analysis.

The need for an accessible route to such secondary amide derivatives is due to the fact that these compounds hold a greater structural resemblance to the intermediates in the nucleic acid biosynthetic pathways. As a result they are believed to be more appealing targets for the production of a library of compounds for biological testing. The attractiveness of the present research is that if a synthetic strategy was developed to these secondary amide derivatives of the 5-hydroxyimidazole-4-carboxamides, it could provide a route to a variety of analogues with no ambiguity of substituents. Therefore, the approach used was to explore an efficient and general route to substituted 5-hydroxyimidazoles employing inexpensive and readily available starting materials.

The initial synthetic targets were the ethyl-1-sustituted-5-hydroxyimidazole-4-carboxylates since such ester intermediates have the potential to be converted to a wide variety of related 4-substituted imidazoles.

These target ethyl 5-hydroxyimidazole-4-carboxylate derivatives were used to develop a model synthetic route for the synthesis of 5-hydroxyimidazoles from acyclic precursors, with an additional aim being the manipulation of the functional groups present on the imidazole system to produce a library of analogues. In turn, the chemistry of the 5-hydroxyimidazole systems could then be explored once the synthetic route had been perfected.

Having such a library of compounds would allow the opportunity for all the 5-hydroxyimidazoles derivatives synthesised to be sent for biological analysis thus affording us the chance to assess the structure-activity relationships. This information could then be used when designing any future synthetic strategies for the preparation of potentially biologically active compounds.

4.3 Retrosynthetic Analysis

To determine the best possible general synthetic route, to a variety of 5-hydroxyimidazoles starting from acyclic precursors, a retrosynthetic analysis was conducted. Retrosynthesis involves working backwards from the target molecule in order to devise a suitable synthetic route. E.J Corey[196] defined retrosynthesis as:

"*a problem solving technique for transforming the structure of a synthetic target molecule to a sequence of progressively simpler structures along the pathway which ultimately leads to simple or commercially available starting materials for a chemical synthesis*."

Retrosynthesis allows possible synthetic routes for complex molecules to be devised from simple precursors. In the case of the present study, the retrosynthetic analysis revealed that there were two possible routes to explore when synthesising 5-hydroxyimidazoles:

- The first was a convergent route, involving several basic 'building blocks', joined together relatively late on in the synthesis to give the final product.
- The second was a linear route, involving the sequential addition of reagents to slowly build up the final product 'piece by piece'.

Each route had its own advantages and disadvantages, but both were deemed plausible, and indeed as proof of concept, syntheses using both the designed routes was attempted.

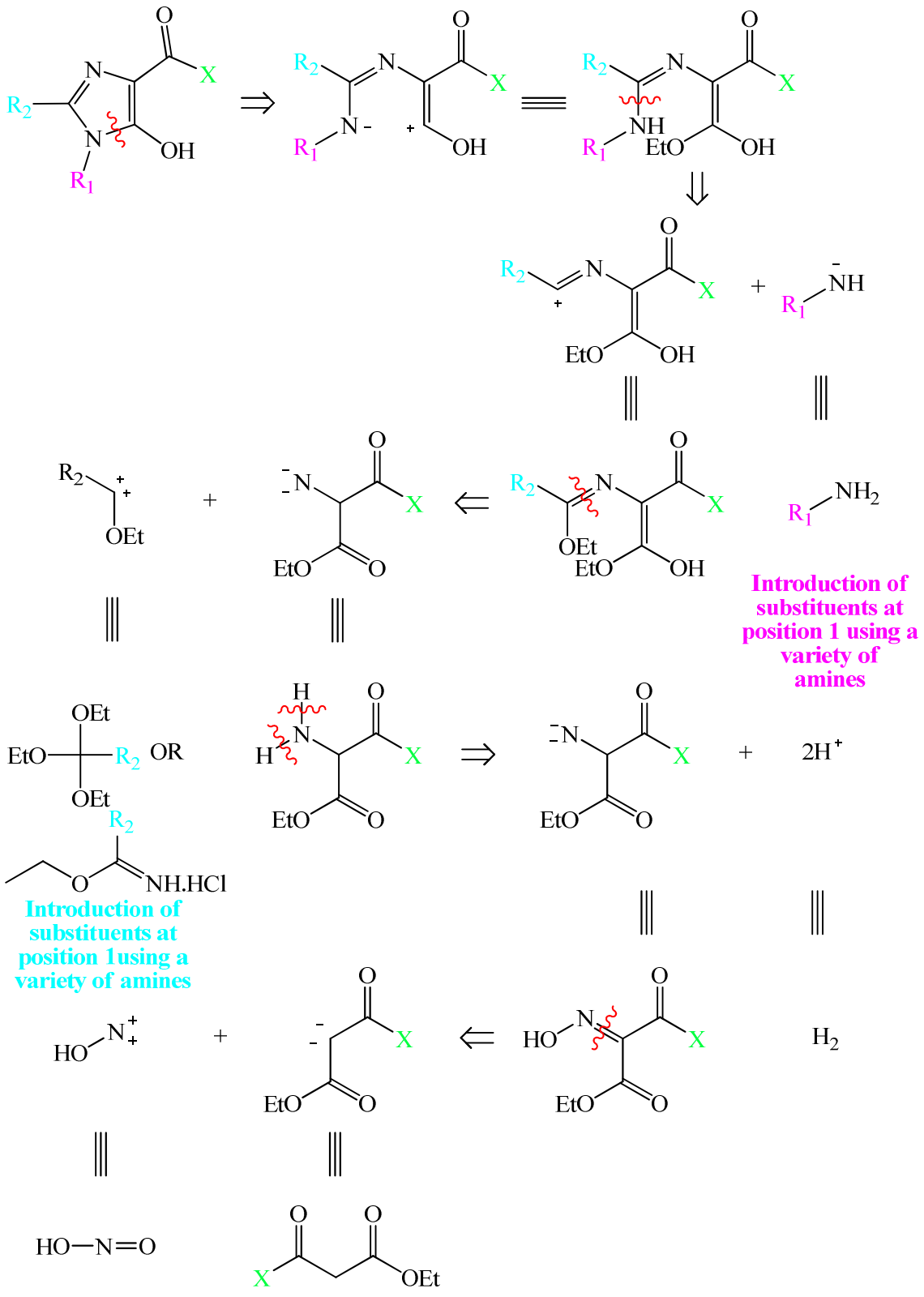
4.4 Convergent Synthetic Route

For a convergent synthesis, individual parts of the molecule are synthesised separately and then the final compound is made by assembling all respective parts towards the end of the synthetic route.

This type of synthesis has its advantages, it generally results in a higher overall yield of the product when compared with linear routes, by reducing the number of linear steps required thus allowing for key components of the final product to be optimized individually. This means that structural changes can be made to one part of the molecule independently of other parts, this is particularly useful when making non-natural products because it allows the exploration of structure-activity relationships.

Retrosynthetic analysis and convergent synthesis are complementary and are often employed together when planning a new synthetic approach. Together retrosynthetic analysis and convergent synthesis generally aid in the establishment of an efficient synthetic scheme.

From the retrosynthesis of the 5-hydroxyimidazole in scheme 27 it can be seen that it requires 5 key 'building blocks'. It should also be noted that in addition, the adopted strategy also offers the opportunity to introduce a variety of substituents in positions 1 and 2 on the imidazole ring unambiguously.

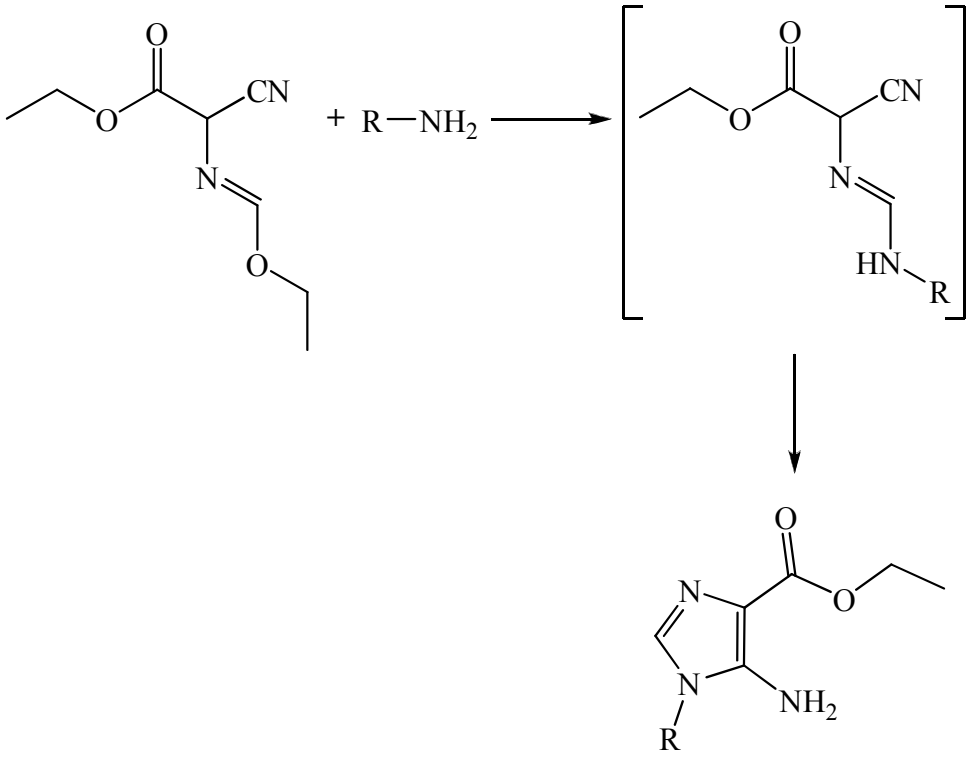


Scheme 27 Convergent retrosynthetic analysis of 5-hydroxyimidazoles.

4.4.1 Synthetic Strategy

Various methods of imidazole synthesis have been reported,[197] however these methods aren't easily adaptable to the synthesis of analogues. In the present study attempts were made to overcome this and design a synthetic strategy that allowed for a library of substituted 5-hydroxyimidazoles to be synthesised, for this a modification of the Shaw synthesis[198] was used.

The route was originally developed by Shaw *et al*.[199-209] as a general method for the synthesis of 1-substitued-5-aminoimidazoles, additionally, it closely follows the proposed convergent retrosynthetic route. The Shaw synthesis proceeds via condensation of a primary amine with an acyclic imidate to give an amidine. This amidine can then undergo intramolecular cyclisation to furnish the desired 5-aminoimidazole (scheme 28).



Scheme 28 Shaw synthesis of 5-aminoimidazoles.

In the case of the present study, where the synthetic targets were the ethyl 5-hydroxyimidazole-4-carboxylates (scheme 29), the final developed synthetic strategy involved the reaction of diethyl malonate (34) with aqueous sodium nitrite in the

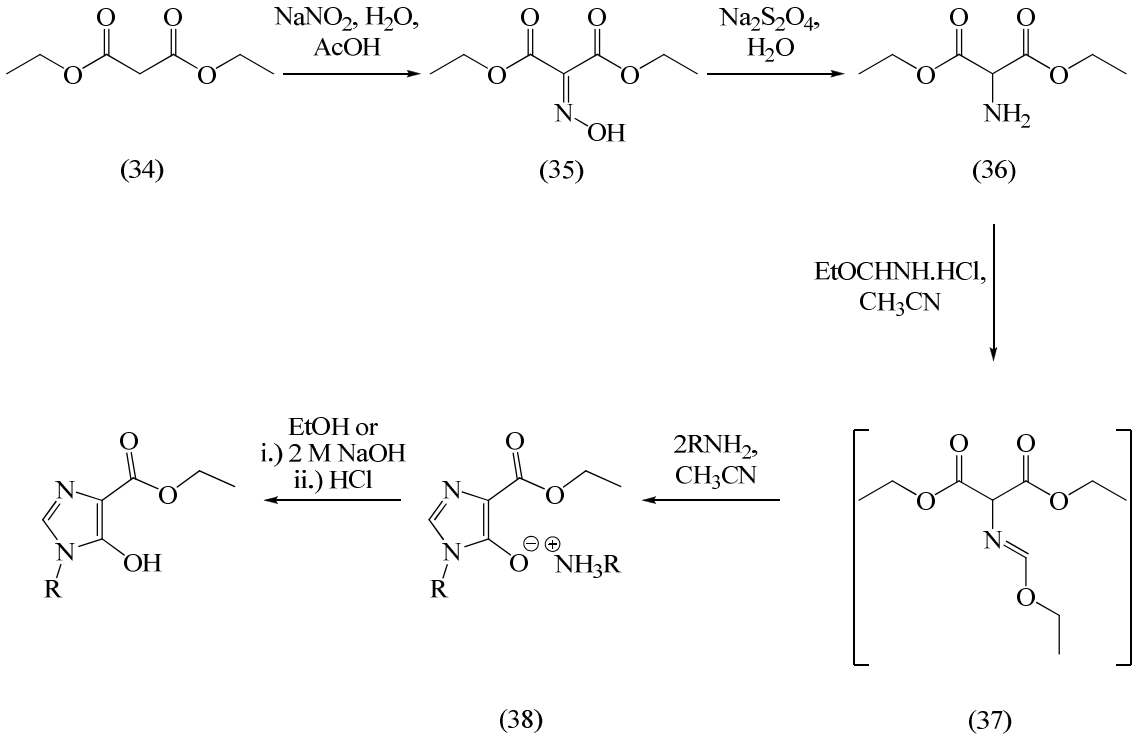
presence of acetic acid to give diethyl 2-(hydroxyimino)malonate (35) as a yellow gum which would not crystallise from bench solvents.

Reaction of diethyl 2-(hydroxyimino)malonate with aqueous sodium dithionite in the presence of sodium hydrogen carbonate (35 ºC, 4 h) gave diethyl 2-aminomalonate (36). The imidate, diethyl 2-(ethoxymethyleneamino)malonate (37), was then prepared by reaction of diethyl 2-aminomalonate with ethyl formimidate hydrochloride in acetonitrile at room temperature for 1 h, followed by filtration of the ammonium chloride by-product.

Finally addition of a 2 mol equivalent of a primary amine to diethyl diethyl 2-(ethoxymethyleneamino)malonate results in the formation of an amidine which can undergo intramolecular cyclisation to furnish the desired ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates as a salt of the primary amine added (38).

This salt could then be purified providing the ‘free’ 5-hydroxyimidazole by either recrystallisation from ethanol or by dissolving the salt in 2 M sodium hydroxide and then adjusting the pH to 7 with concentrated hydrochloric acid.

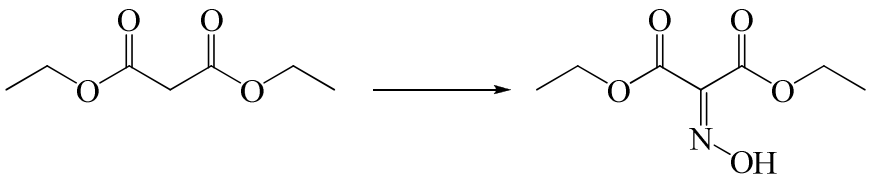
An important point to be noted for the synthesis is that the imidate was not isolated due to its highly hygroscopic nature, however its presence can be inferred by its ability to react smoothly with the primary amines at room temperature.



Scheme 29 Final synthetic strategy.

4.4.2 Development of the Synthetic Strategy

The first objective of the research was to optimise the synthetic scheme for the synthesis of the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylate derivatives. To do this each step in the synthetic scheme was developed individually and optimised in order to keep the yields of the imidazoles synthesised as high as possible.

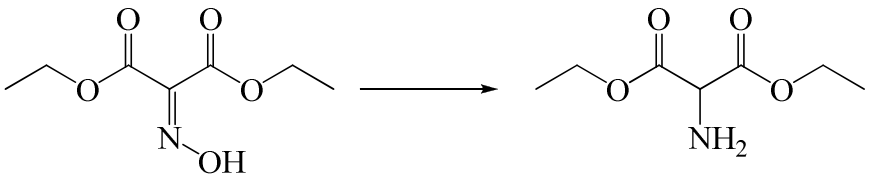


Scheme 30 Formation of diethyl-2-hydroximino malonate.

A method previously developed within the research group was used initially in the synthesis of diethyl 2-(hydroxyimino)malonate. It involved addition of an aqueous solution of sodium nitrite to a stirring suspension of diethyl malonate in acetic acid and tetrahydrofuran (THF). The desired oxime was then extracted in toluene and the organic phase washed with water, dried ($MgSO_4$) and the solvent was removed under

reduced pressure. However, NMR analysis of the product obtained showed the oxime was also contaminated with a number of by-products which could not be excluded through any subsequent washing procedures.

Since distillation of these types of oxime derivatives is not possible due to their highly explosive nature, an alternative route to the synthesis of the desired oxime was found.[210] The method used the same reagents as the previous reaction, but the sequence of addition and the work-up were different. The method involved the drop-wise addition of acetic acid to a stirring solution of diethyl malonate, water and sodium nitrite. Sodium nitrite was added as a solid instead of as an aqueous solution due to the instability of sodium nitrite solutions, and the crude diethyl 2-(hydroxyimino)malonate was extracted into diethyl ether. Diethyl ether was found to be more selective for the extraction than toluene, and was more easily removed due to its lower boiling point. TLC examination of the gum revealed a green spot when visualised with 5 % copper sulphate solution and IR examination of the gum showed ν C=NOH 1656 cm^{-1}, ν C=O 1744 cm^{-1} and ν N-OH 3342 cm^{-1}. The structure of this oxime was further confirmed by NMR and mass spectroscopy.



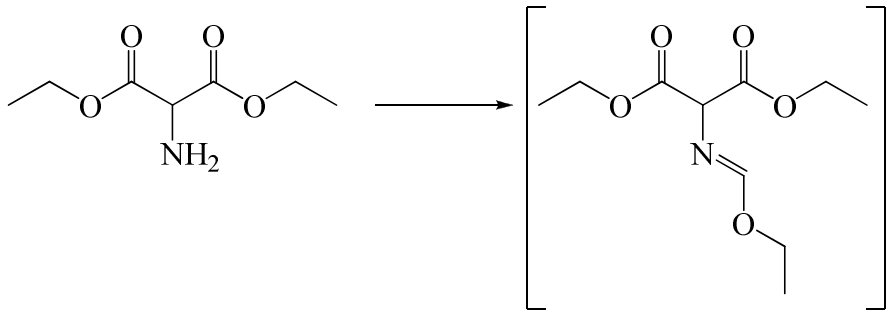
Scheme 31 Formation of diethyl 2-aminomalonate.

One approach that had been widely used within the literature was to use an aluminium/mercury amalgam for the reduction of an oxime to an amine, however the use of mercury would be strictly prohibited in the production of any pharmaceuticals. So, if any of the compounds synthesised were found to have desirable biological activity, an alternative method of reduction would need to be found before the compounds could go on to clinical trials. It was for this reason that an alternative method of reduction was sought.

A novel approach was developed using sodium dithionite and a saturated solution of sodium hydrogen carbonate. The method involves adding sodium dithionite portion-

wise to a rapidly stirring solution of diethyl 2-(hydroxyimino)malonate and saturated sodium hydrogen carbonate. The mixture was warmed to 40 ºC for 4 h, extracted into diethyl ether, washed with water, dried ($MgSO_4$) and the solvent was removed under reduced pressure. TLC examination of the gum revealed a brown spot when visualised with 5 % copper sulphate solution. The structure of this amine was further confirmed by NMR spectroscopy and mass spectrometry.

If necessary, a stream of dry hydrochloric acid could be blown over a solution of the amine in diethyl ether to obtain the hydrochloride salt of the amine as a white precipitate which could be easily stored for several months without any degradation. The structure of this amine hydrochloride was confirmed by NMR spectroscopy and mass spectrometry.



Scheme 32 Formation of diethylethoxyethyl-2-amino malonate.

Although the isolation of the imidate intermediate, diethyl 2-(ethoxymethyleneamino)malonate, was never attempted due to its highly hygroscopic nature, there were two reported methods which could be used for its synthesis:

- Reaction of diethyl 2-aminomalonate with triethyl orthoformate in refluxing acetonitrile for 4 h.
- Reaction of diethyl 2-aminomalonate with ethyl formimidate hydrochloride in acetonitrile at room temperature for 1 h.

Assessment of both aforementioned methods revealed the use of ethyl formimidate hydrochloride as the most convenient and it also resulted in less by-product formation because no heating was required. This method was the quicker of the two used, and due

to the hygroscopic nature of the imidate being formed it was decided that ethyl formimidate would be used in all future syntheses.

Once the synthetic route to all intermediate compounds had been optimised, the synthesis of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was attempted.

Many previously reported synthetic routes to imidazoles gave low to moderate yields of the final targeted compounds (32 - 65 %).[142,148,154,184,187,194,211] This was also found to be a problem initially in the modified Shaw synthesis that was used in the present study.

One reason for the low yields that were being obtained, could be attributed to the highly hygroscopic nature of the imidate being formed, if any moisture was able to get into the reaction mixture some degradation of the imidate through hydrolysis could occur. Therefore, the synthetic strategy was adapted so that all of the moisture sensitive steps of the synthetic sequence were carried out under a nitrogen atmosphere to exclude moisture, limiting its possible interference.

In addition to this, a comparative study was devised to try and understand why the synthesis was achieving such low yields in the final cyclisation step. A possible explanation for the low yields of the final product being obtained was that the 5-hydroxyimidazole could be forming a salt in which an additional molecule of the amine was ionically bonded to the oxygen at position 5 of the imidazole aglycone (figure 32).

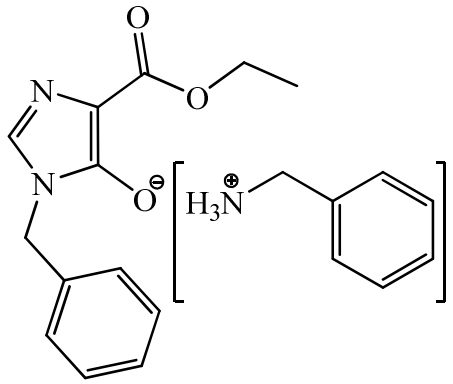


Figure 32 Phenylmethanaminium 1-benzyl-4-(ethoxycarbonyl)-1*H*-imidazol-5-olate, the salt formed when benzylamine was used as the amine in the final step of the synthesis.

It was apparent from ^{1}H-NMR analysis that the crude imidazoles that precipitated out of solution were forming this salt, and only after dissolving the salt in a base and acidifying it to pH 7 was the free 5-hydroxyimidazole obtained.

Taking this observation into account, it is understandable that the yields of the desired 5-hydroxyimidazoles would be affected if the final cyclisation step of the synthesis was carried out based on a 1:1 mol equivalent ratio of the imidate to the selected primary amine. In this case the amine would become the rate limiting step in the reaction, thus restricting the yield of the final product. Tamuri *et al*.[194] also reported the formation of a salt when attempting the synthesis of a 5-hydroxyimidazole-4-carboxamide. However, no attempt was ever made to explore this result and its possible implications on the overall effect of the yields of imidazoles being obtained, which consequently were low.

The devised three part parallel study involved synthesising ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate using exactly the same reagents and under identical reaction conditions:

- The first reaction flask was a control containing a 1:1 mol equivalent ratio of imidate to benzylamine.
- The second reaction flask had a 1:2 mol equivalent ratio of imidate to benzylamine.
- The third reaction flask had a 1:1:1 mol equivalent ratio of imidate to benzylamine to triethylamine.

As expected, the second reaction flask gave the benzylamine salt of the 5-hydroxyimidazole, confirmed by NMR analysis, in a good yield (79 %). Rather more surprisingly however, the first and third reaction flasks gave low but comparable yields and NMR analysis revealed that the products from both reaction flasks were also the benzylamine salt of the 5-hydroxyimidazole. From this analysis it was deduced that benzylamine had become the limiting factor in both reaction flasks and this also explains the comparably low yields obtained for both reactions.

The reason that the result found for the third reaction flask was surprising was because a one mole equivalent of triethylamine was also added to this reaction flask. In this particular case the aim was to synthesise the triethylamine salt of the 5-hydroxyimidazole, subsequently 'freeing up' the benzylamine for use in the cyclisation step. Benzylamine has a conjugate acid pK_a of ~9.51, whereas triethylamine has a conjugate acid pK_a of ~10.21 this means that triethylamine is actually a stronger base than benzylamine. The assumption was made that the 5-hydroxyimidazole would preferentially form the triethylamine salt instead of the benzylamine salt previously observed, however, this was not the case.

A possible suggestion for this anomaly could be that although triethylamine is the stronger of the two bases, due to steric hindrance the benzylamine salt of the 5-hydroxyimidazole was more favoured sterically than the triethylamine derivative.

From the results of this study it was concluded that the best synthetic route for the synthesis of the imidazoles was one in which a 2 mol equivalent of the amine was added in the final cyclisation step of the synthesis. This allowed the salt to form, which not only meant that the reaction was able to proceed more efficiently but that the resulting crude product was then more easily isolated and purified, to give the 5-hydroxyimidazole.

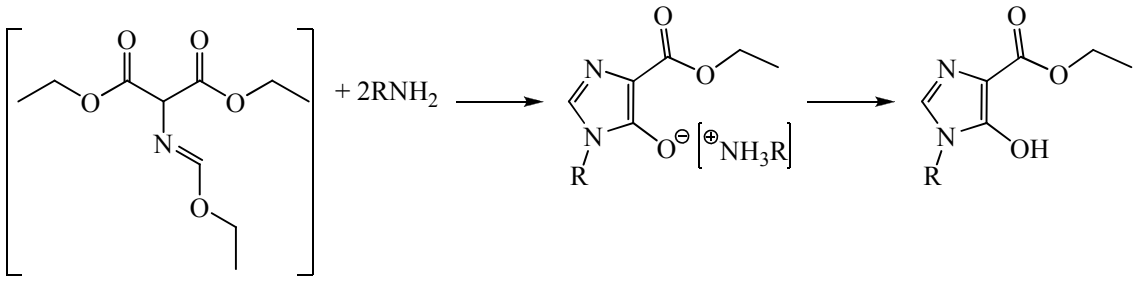
Each crude product was then dissolved in a 2 M solution of sodium hydroxide and the pH was adjusted to 7 with the addition of concentrated hydrochloric acid to yield the desired 'free' 5-hydroxyimidazole as a bright, white crystalline solid. When the product obtained was checked by NMR analysis it revealed the absence of any extra benzylic protons which had been observed in the NMR spectrum of the crude compound.

4.4.3 Ethyl 1-Substituted 5-Hydroxyimidazole-4-carboxylates

Having developed optimised reaction conditions using benzylamine, the use of other primary amines in the reaction sequence was then examined. The initial investigations involved using a selection of both aryl and alkyl amines in the final step of the synthesis to allow for the variation of the functional group present at position 1 of the imidazole aglycone (structures 39-46). This is an important aspect of the synthetic route which needed to be explored, results would then need to be taken into account when designing any future compounds.

A quantitative study of amine basicity and nucleophilicity was investigated, however no correlation could be found between imidazole synthesis and either factor. Therefore, a qualitative study was conducted which examined, which of the amines used were able to cyclise, and form the imidazole as well as which resulted in the highest yields of the desired ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates.

As with the previous three part parallel study, each experiment was carried out under identical reaction conditions in which the imidate was reacted with a 2 mol equivalent excess of the primary amine to imidate. The reaction mixtures were left at room temperature overnight, the product was collected by filtration and the crude ethyl 1-substituted-5-hydroxyimidazole-4-carboxylate was purified by either recrystallisation from ethanol or by an acid-base workup (as previously described).



Structure	Amine Used	Yield of Salt of 5-Hydroxyimidazole	Yield of Purified 5-Hydroxyimidazole
39	Benzylamine	79 %	61 %
40	Phenethylamine	82 %	64 %
41	4-Methoxbenzylamine	84 %	63 %
42	4-Nitrobenzylamine	67 %*	N/A
43	3-Aminopropan-1-ol	23 %	69 %
44	Ethanolamine	41 %	72 %
45	Diethyl 2-(hydroxyimino)malonate	46 %	58 %
46	Allylamine	56 %	71 %

Table 3 Primary amines used to successfully synthesise ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates. *Crude salt couldn't be purified to yield the corresponding 5-hydroxyimidazole.

The results of the study with the primary amines revealed that the more lipophilic amine derivatives resulted in the imidazoles that were the easiest to isolate and purify. Additionally it was found that those amine derivatives with polar functional groups present, such as ethanolamine, resulted in lower yields. The difference observed is believed to be down to the polarity of the final imidazole formed. As the polarity of the amine derivatives used in the final step of the synthesis increased, the more difficult the imidazole products became to isolate and purify because of the increase in solubility that the polar groups provided.

One particular point to be noted here is that the ethyl 5-hydroxyimidazole-4-carboxylate, produced using 4-nitrobenzylamine (42) as the primary amine in the final

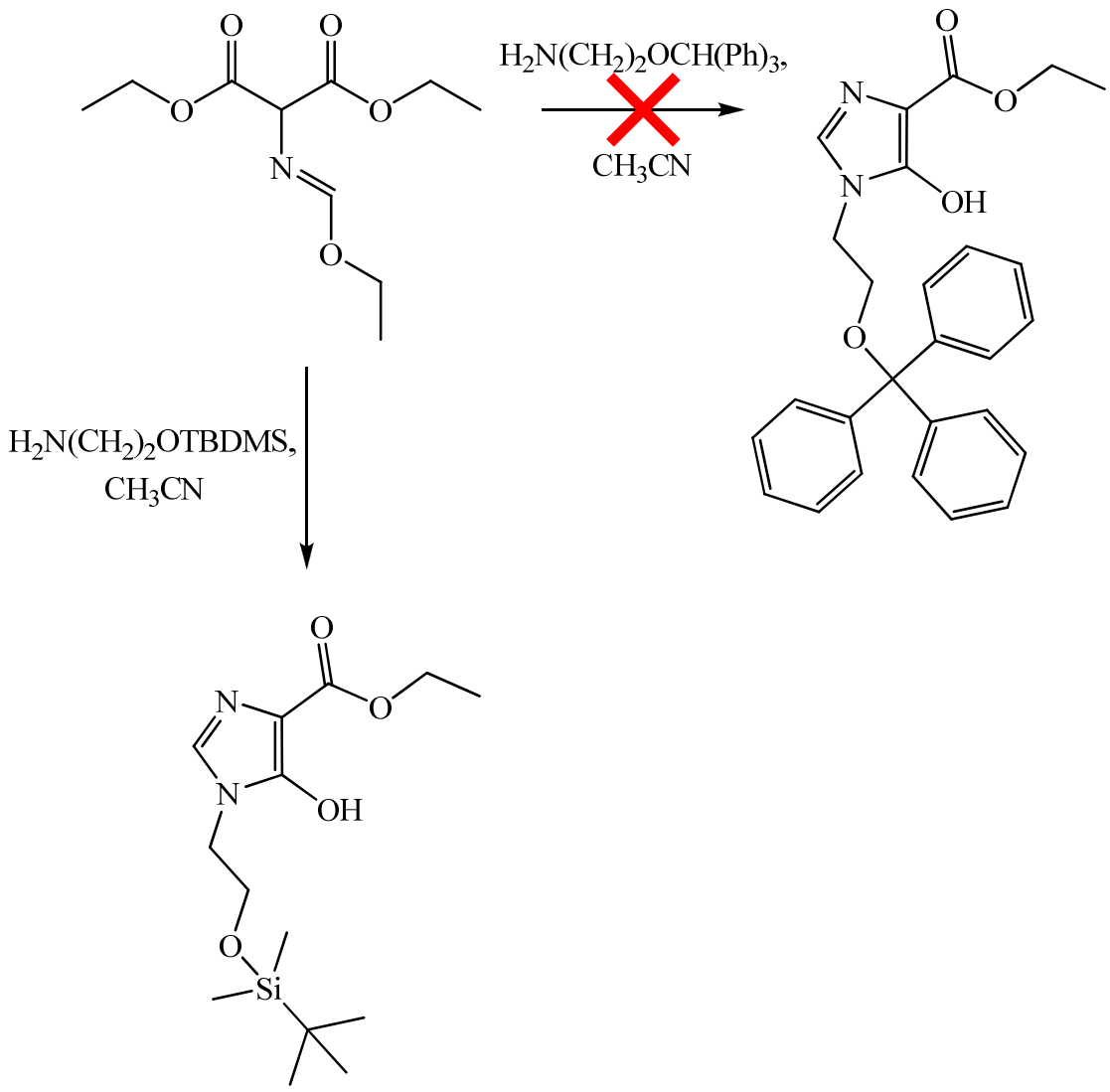
step of the synthesis of the imidazole, did successfully form the amine salt of the imidazole, however it was difficult to purify the product efficiently. All attempts at purification, such as recrystallisation and the acid-base workup were unsuccessful.

It became clear that the yields of the imidazoles being synthesised with the less lipophilic amines were being affected by the hydrophilicity of the substituent at position 1 of the imidazole aglycone, particularly the acyclic sugar analogues. Taking this into account, the synthetic route was adapted and the highly polar, hydrophilic groups of the acyclic amino-alcohol derivatives were protected.

Using ethanolamine as a model system, silylation of the free alcohol group was initially attempted. Silylation was accomplished using the method reported by Padwa *et al*.[212] who used *tert*-butylsilyl protected 3-aminopropan-1-ol in the synthesis of hexahydroindolinones. The resulting silylated amine was used in the synthesis of the imidazole (scheme 33). However the yield of the ethyl 1-(2-(tert-butyldimethylsilyloxy)ethyl)-5-hydroxyimidazole-4-carboxylate (47) obtained wasn't greatly increased, this could be due to the fact that the siloxanes are hydrophobic but aren't greatly lipophilic. The importance of using a lipophilic group in the synthesis of these 5-hydroxyimidazoles has already been shown from the qualitative study conducted.

An alternative protecting group for the alcohol of ethanolamine was sought and a more lipophilic group was introduced. Subsequently, the choice of protection for the alcohol was a common protecting group for hydroxyls, the trityl group. This was achieved via a method[213] that used a 3 mol excess of ethanolamine hydrochloride to trityl chloride in pyridine at room temperature for 72 h. The reaction mixture was quenched with water and the resulting hydrochloride salt of the product was formed by treatment of the filtered solid, dissolved in diethyl ether, with a 3 M solution of hydrochloric acid. To regenerate the desired *O*-tritylethanolamine, the salt was dissolved in diethyl ether and a saturated solution of sodium hydrogen carbonate. The clear organic phase was washed with water, dried ($MgSO_4$) and the solvent removed under reduced pressure to give the *O*-tritylethanolamine as a white solid.

The *O*-tritylethanolamine was subsequently used in the synthesis of the imidazole in order to try to increase the yields obtained. However no imidazole formation was observed on TLC analysis, possibly owing again to the steric hindrance caused by the presence of the trityl group, which meant that the amidine formed was unable to cyclise to give the imidazole.

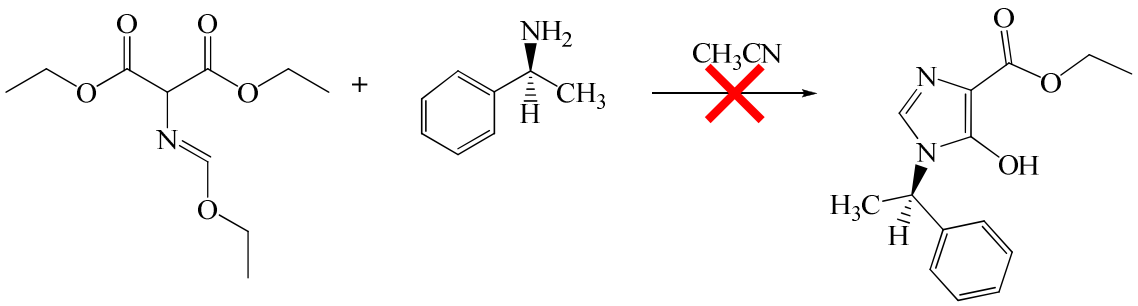


(47)

Scheme 33 Attempted synthesis of suitably protected acyclic sugar analogues of ethyl 5-hydroxyimidazole-4-carboxylates.

It was evident that steric bulk was an important factor in the final cyclisation step of the synthetic route. To further confirm this finding, the synthesis was attempted using the sterically hindered but lipophilic amine, methylbenzylamine (scheme 34). As before,

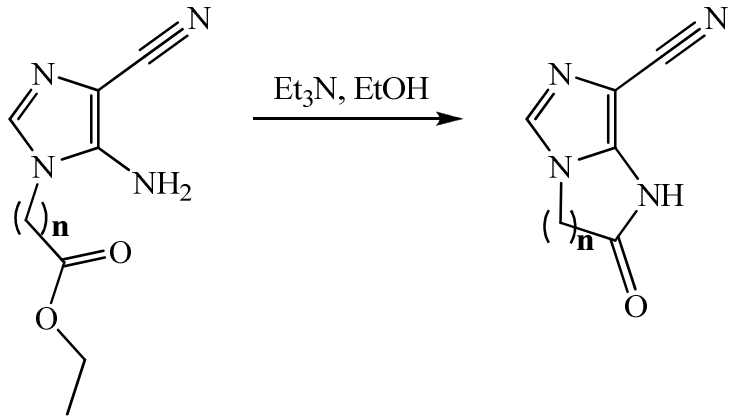
diethyl 2-(ethoxymethyleneamino)malonate was reacted with a 2 mol equivalent of the amine and left at room temperature for 24 h, however no reaction was observed by TLC analysis. More forcing conditions were subsequently tried, yet still no reaction was observed.



Scheme 34 Attempted synthesis of ethyl 5-hydroxy-1-(1-phenylethyl)-imidazole-4-carboxylate.

It was now evident that a compromise between lipophilicity and steric bulk must be met to achieve a synthesis which yields ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates that are easily isolated and purified. This in turn helps to keep the yields of the final 5-hydroxyimidazole obtained higher. This is an important point which must be taken into consideration in any future synthetic strategies. However, it must also be noted here that the use of these more lipophilic amines does not necessarily make the synthesis of the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates more efficient, instead it means that the isolation and purification of the product synthesised is easier.

A relevant publication[214] investigating the reactivity of the 5-aminoimidazoles reported the unusual intramolecular cyclisation reaction of appropriately substituted 5-aminoimidazoles to form fused imidazo-imidazoles (scheme 35).



Scheme 35 Synthesis of fused imidazo-imidazoles using 5-aminoimidazoles.

A similar series of experiments were devised, which would use appropriately substituted 5-hydroxyimidazoles, with the hope of producing the corresponding imidazo-oxazoles (figure 33). If successful, application of the strategy could be used to give a range of purine analogues derived from naturally occurring amino acids.

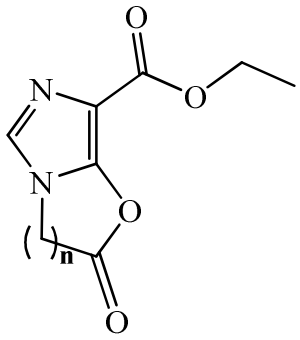
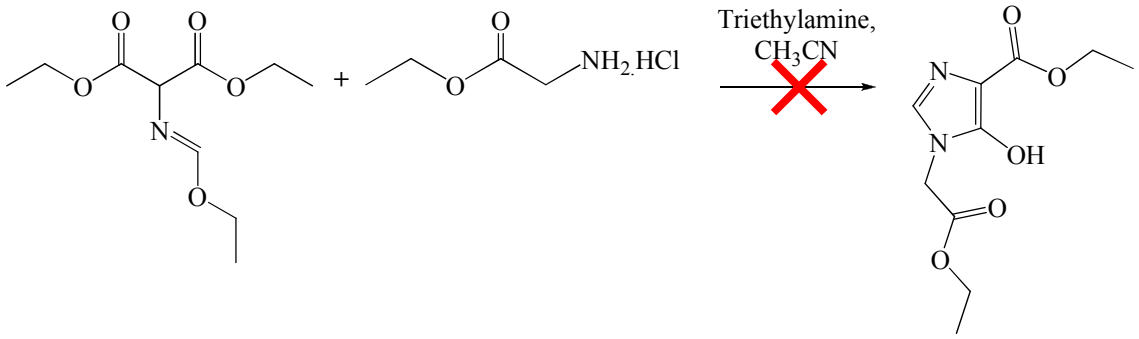


Figure 33 An imidazo-oxazole.

An additional advantage of this devised synthetic route is that it would allow for the protection of the 5-hydroxyl group present at position 5 of the imidazole ring. This is desirable because of the need to manipulate the ethyl ester group at position 4 of the imidazole ring in order to obtain the desired secondary amide derivatives of the 5-hydroxyimidazoles. Modification studies of the 5-hydroxyimidazole system, and the functional groups bonded to it, are covered in more detail in chapter five.

Before these intramolecular cyclisation experiments could be attempted, a series of appropriately substituted 5-hydroxyimidazoles needed to be synthesised. To do this diethyl 2-(ethoxymethyleneamino)malonate, a 2 mol equivalent of glycine ethyl ester

hydrochloride and a 2 mol equivalent of triethylamine were reacted together (scheme 36).



Scheme 36 Attempted synthesis of ethyl 1-(2-ethoxy-2-oxoethyl)-5-hydroxyimidazole-4-carboxylate.

However this reaction resulted in problems isolating any synthesised ethyl 1-(2-ethoxy-2-oxoethyl)-5-hydroxyimidazole-4-carboxylate from the triethylamine hydrochloride salt also obtained as a by-product from the reaction. Both compounds are water soluble so the 5-hydroxyimidazole derivative couldn't be extracted, and purification via silica gel column chromatography also couldn't be used because the 5-hydroxyimidazole system readily decomposes on silica gel. This is due to the fact that silica gel is slightly acidic, so protonation of the nitrogen at position 3 of the imidazole ring can occur, allowing degradation from the subsequent nucleophilic attack on position 2.

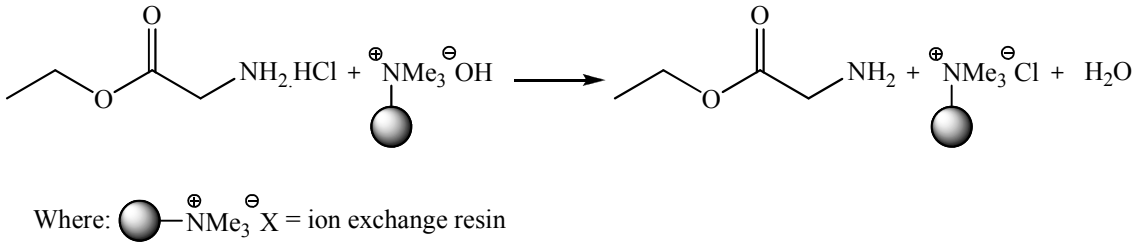
To overcome the problems experienced, attempts were made to isolate the 'free' glycine ethyl ester for subsequent use in the synthetic route. To do this, glycine ethyl ester hydrochloride was stirred with triethylamine at ambient temperature in acetonitrile. The resulting solution was filtered to remove any triethylamine hydrochloride formed and the filtrate was added to diethyl 2-(ethoxymethyleneamino)malonate, no reaction was observed by TLC analysis.

It was believed that the difficulties experienced were due to the solubility of the reagents in acetonitrile, and that when the solution was filtered to remove the triethylamine hydrochloride salt, the majority of the glycine ethyl ester was also filtered off. This in itself posed another problem, a subsequent solubility study revealed that the glycine ethyl ester was insoluble in all bench organic solvents but fully soluble in water.

However, due to the hygroscopic nature of the imidate intermediate, water could not be considered as a solvent for the reaction.

A further attempt to 'free' the glycine ethyl ester from the hydrochloride salt involved the use of Amberlyst A-26 OH polymeric ion exchange resin. If successful, it would overcome the problems associated with isolation and purification of the amino acid ethyl ester.

For this the ion exchange resin was soaked in a 2 M solution of sodium hydroxide for 1 h, filtered, washed with ethanol followed by diethyl ether. The resin was then added to a solution of glycine ethyl ester hydrochloride in acetonitrile and left to stir for 1 h. Diethyl 2-(ethoxymethyleneamino)malonate was then added to the reaction mixture and the solution was left overnight at ambient temperature (scheme 37). The resin was filtered off and a TLC performed on the filtrate, however again no reaction was observed.

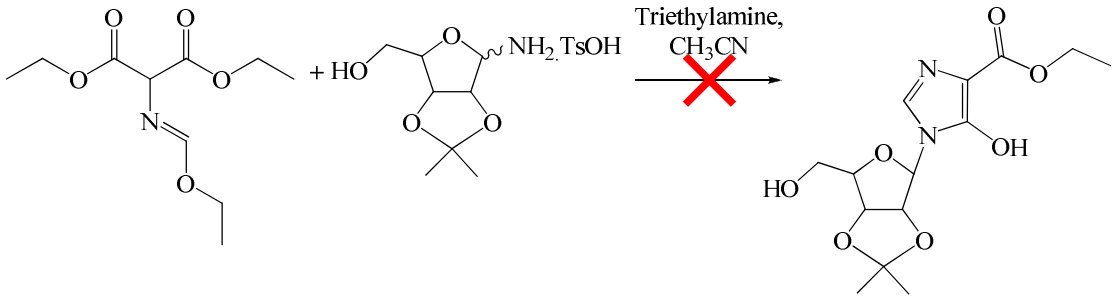


Scheme 37 Schematic of the ion exchange process to give the free glycine ethyl ester.

The purpose of the ion exchange resin in the reaction was to exchange the hydrochloric acid on the glycine ethyl ester. However, a mole of water is generated in the exchange which again would degrade the diethyl 2-(ethoxymethyleneamino)malonate when added, so this problem needed to be rectified.

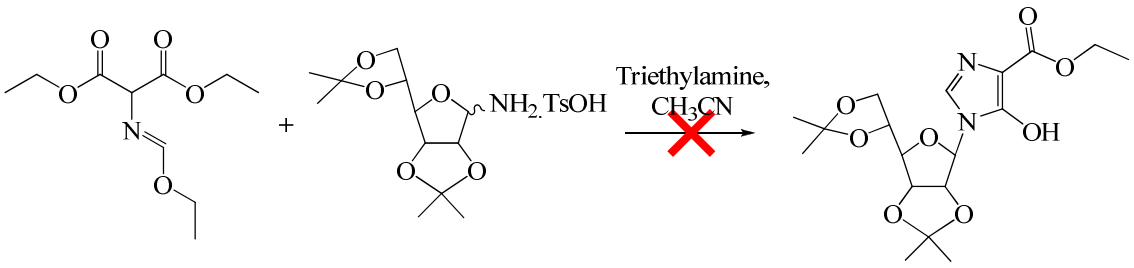
The reaction was repeated again as above, but in an attempt to remove any water formed in the exchange process activated 5 Å molecular sieves were added to the reaction mixture with the aim of 'mopping up' any excess water present. Once again a TLC was performed on the filtrate but no reaction was observed.

In addition to synthesising ethyl 1-subtituted-5-hydroxyimidazole-4-carboxylates using aryl and alkyl groups, synthesis using a sugar amine was also attempted with the hope of forming a nucleoside (scheme 38). Protected ribose has been used several times before in the literature[215-219] to produce 5-aminoimidazoles, so was chosen to be used initially in the present study. However, after several attempts, each time changing the reaction conditions, no reaction was ever observed using the ribose. It was thought that this could be due to interference of the free hydroxyl group present on the sugar amine in the reaction.



Scheme 38 Attempted synthesis of a 5-hydroxyimidazole nucleoside using protected ribose.

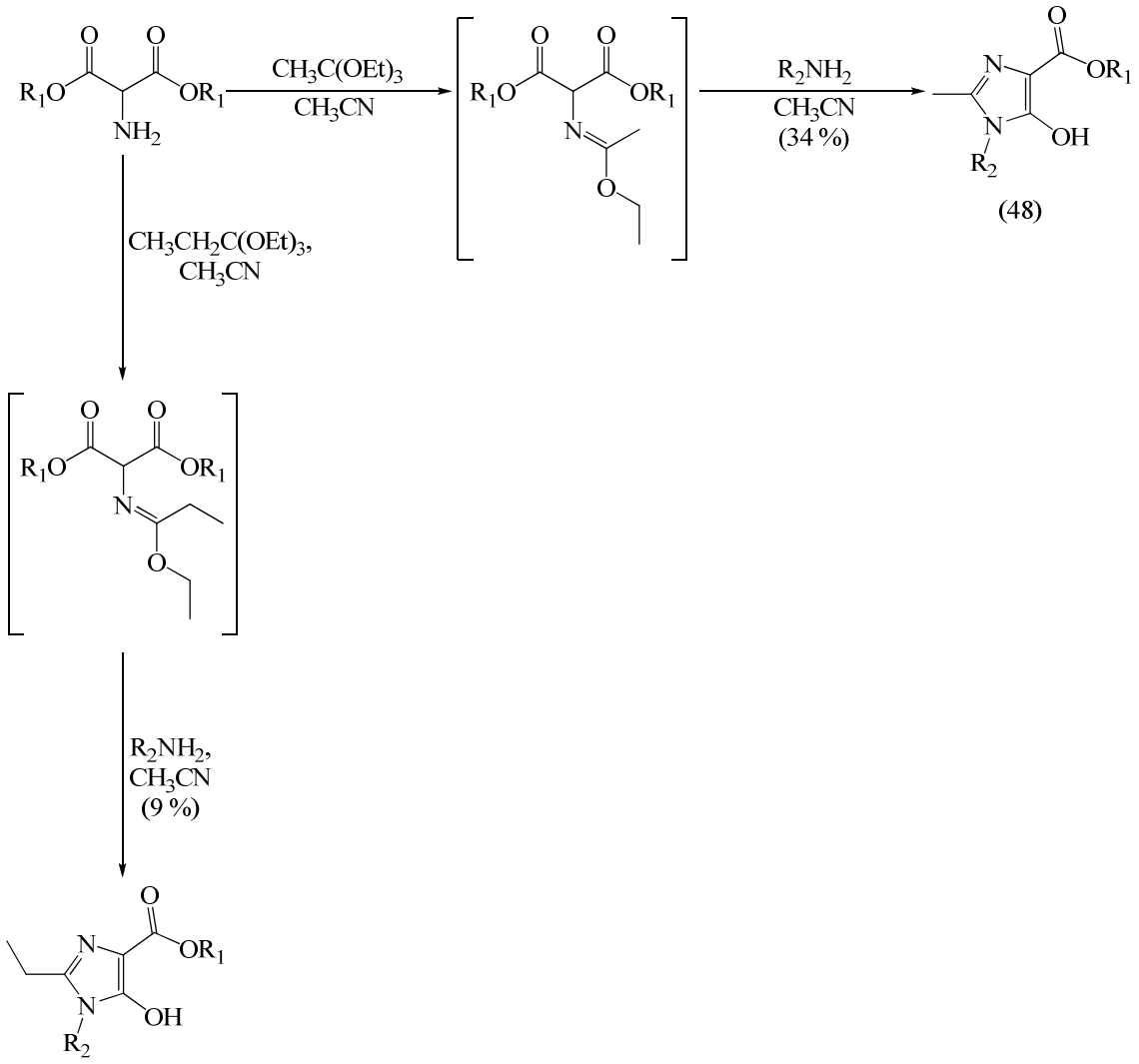
In an attempt to overcome this perceived problem, the reaction was repeated using 2,3;5,6-di-*O*-isopropylidene-D-mannofuranosylamine (scheme 39), in which all of the free hydroxyls are fully protected with isopropylidene groups. The product could then be fully deprotected using acid once the proposed cyclisation had occurred, unfortunately no reaction was observed. TLC examination showed the presence of separate spots after development in iodine ($R_f = 0.22$ and $R_f = 0.35$), one of which also showed charring after development with ethanolic sulphuric acid. With no hydroxyl groups free to interfere with the reaction, it was thought that the unidentified charring spot on the TLC could be as a result of the amidine forming but subsequent cyclisation not occurring. This is possibly due to steric effects but primarily this is thought to be due to the less nucleophilic nature of the amine on the sugar. Even further attempts to force the reaction by heating it at reflux failed.



Scheme 39 Attempted synthesis of a 5-hydroxyimidazole nucleoside using protected mannose.

4.4.4 2-Substituted Ethyl 1-Benzyl-5-hydroxyimidazole-4-carboxylates

In an adaptation of the synthetic route being used, triethyl orthoacetate and triethyl orthopropionate were used instead of triethyl orthoformate. This allowed the assessment of what effect increasing the chain length at position 2 on the imidazole ring might have on the overall yields of the imidazoles obtained, and ultimately the biological activity of these compounds (scheme 40).



Scheme 40 Synthesis of 2-substituted imidazole-4-carboxylate ethyl esters.

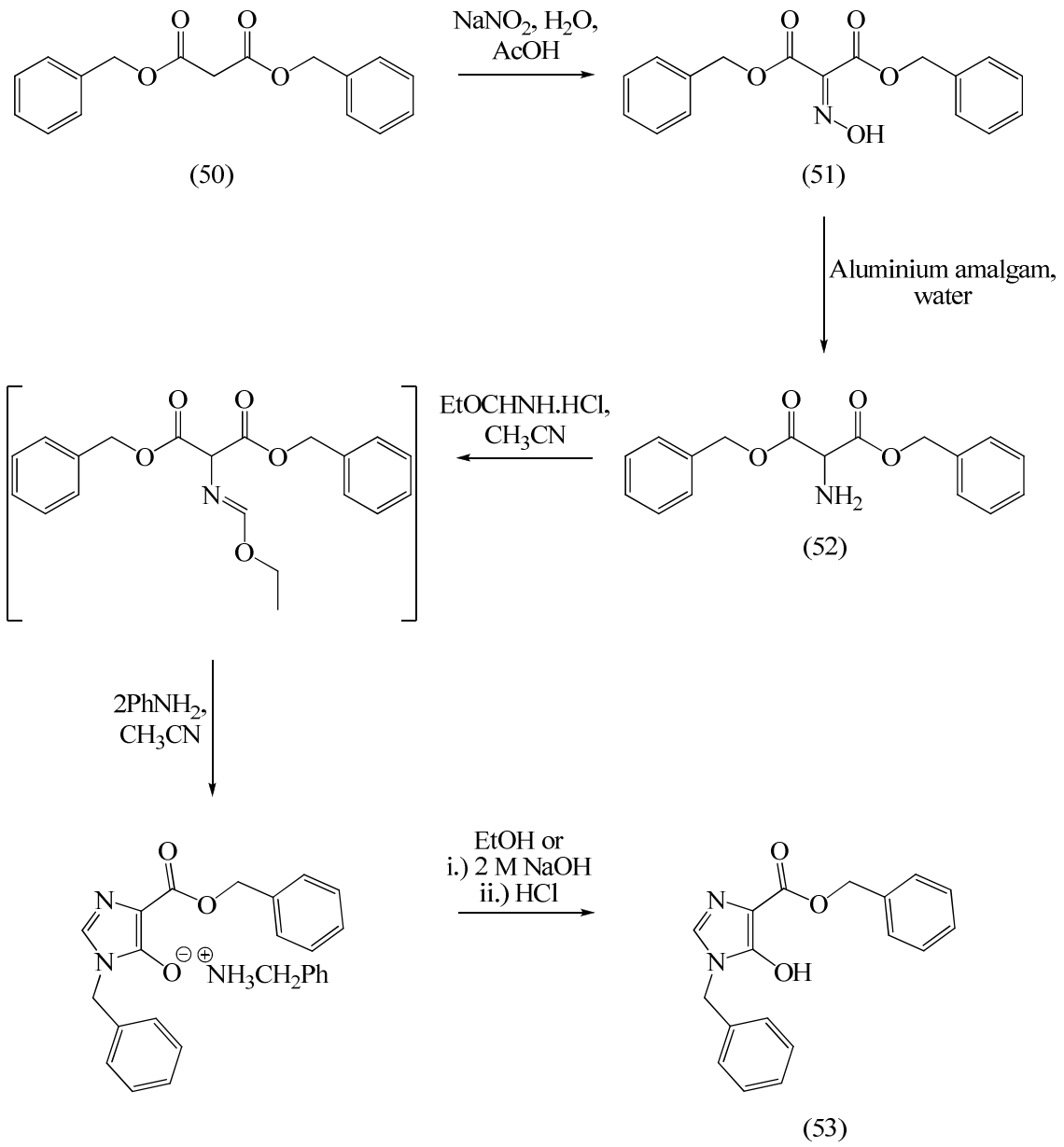
Although both the imidazoles could be successfully synthesised, yields began to dramatically decrease as the chain length at position 2 increased. This may be due to steric hindrance experienced in the final step of the synthesis where the amidine formed, after addition of the amine, must undergo intramolecular cyclisation to form the imidazole ring.

An alternative method for producing 5-hydroxyimidazoles with a variety of substituents at position 2 of the imidazole ring involves the reaction of the imidazole with bromine and the subsequent Suzuki coupling of the product with a variety of aryl substituents. This is discussed in more detail in chapter five, section 5.3.

4.4.5 Benzyl 1-Benzyl-5-hydroxyimidazole-4-carboxylate

In addition to the use of diethyl malonate in the synthetic route, dibenzyl malonate (50) was also used to assess if the route could be applied more generally to the synthesis of a variety of 4-substituted-5-hydroxyimidazoles (scheme 41).

Dibenzyl malonate was chosen specifically due to the fact that the benzyl ester functional group can be removed under neutral conditions, such as hydrogenation. This could prove to be particularly useful when considering further modification of the 5-hydroxyimidazoles produced (a concept that is discussed in further detail in chapter five).



Scheme 41 Synthesis of benzyl 1-benzyl-5-hydroxyimidazole-4-carboxylate.

On the whole, the route proved to be relatively successful, however there were problems experienced with one particular step of the synthetic route. This step was the reduction of dibenzyl 2-(hydroxyimino)malonate (51) to give dibenzyl 2-aminomalonate (52). Dibenzyl 2-(hydroxyimino)malonate was found to be insoluble in water which is the solvent system used in the sodium dithionite reduction. Instead dibenzyl 2-(hydroxyimino)malonate was reduced to give dibenzyl 2-aminomalonate using an aluminium amalgam, and the subsequent steps of the synthetic procedure were carried out as previously discussed for the synthesis of ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates.

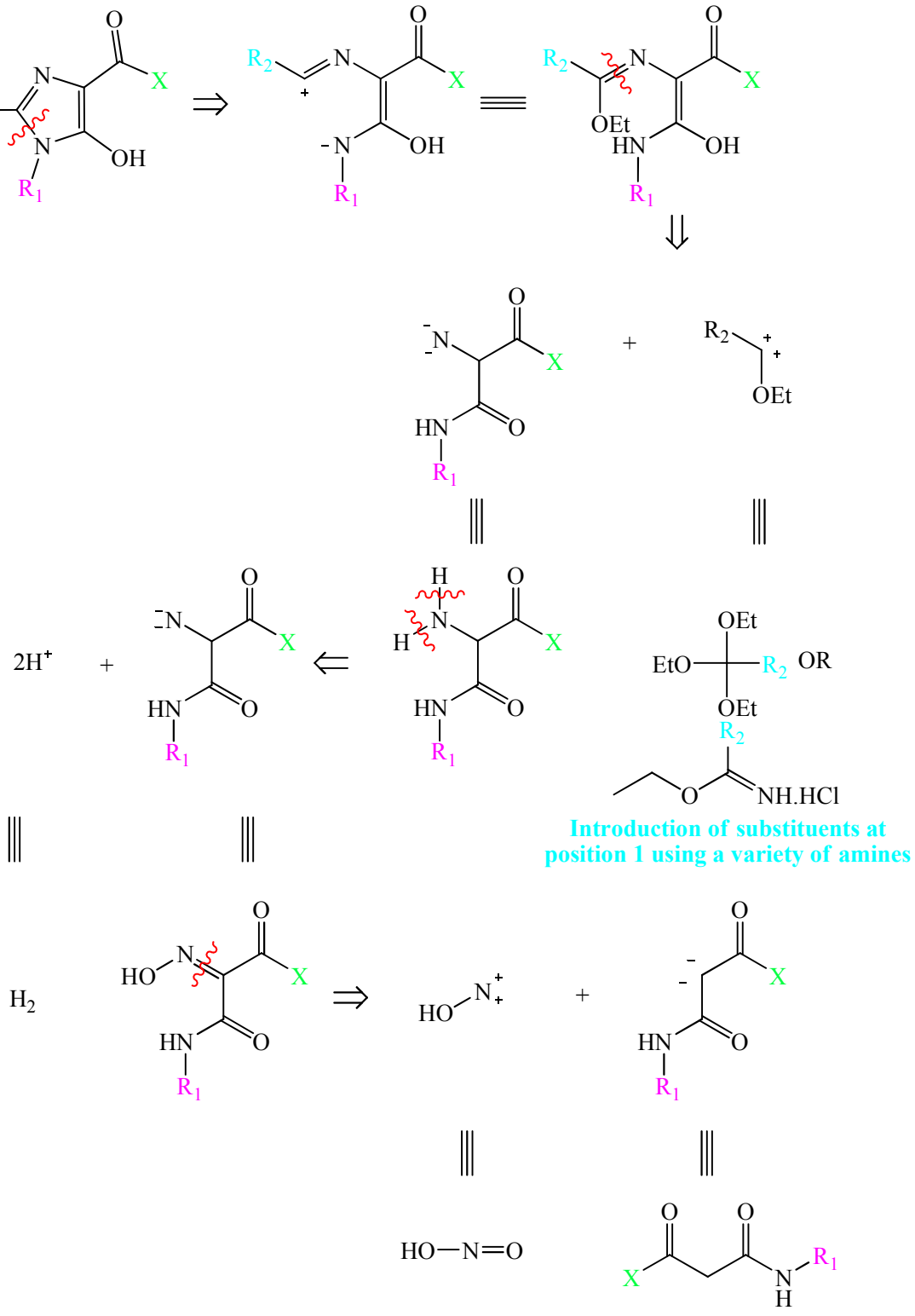
Again the benzyl 1-benzyl-5-hydroxyimidazole-4-carboxylate (53) was produced as the salt of the imidazole which could be easily purified by dissolving it in 2 M sodium hydroxide and adjusting the pH to 7 using concentrated hydrochloric acid, to give the product as a bright white crystalline solid.

4.5 Linear Synthetic Route

The retrosynthetic analysis conducted also revealed that a linear synthetic strategy was possible for the formation of the 5-hydroxyimidazoles from acyclic precursors.

The disadvantage with a linear synthetic route is that if any step is mishandled the amount of material that can be safely carried though is decreased. In addition, each step depends heavily on the reliability of the step preceding it.

From the retrosynthesis of the 5-hydroxyimidazole in scheme 42 it can be seen that it requires 4 key building blocks. It should also be noted that in addition, the adopted strategy also offers the opportunity to introduce a variety of substituents in the 1, 2 and 4-positions on the imidazole ring.



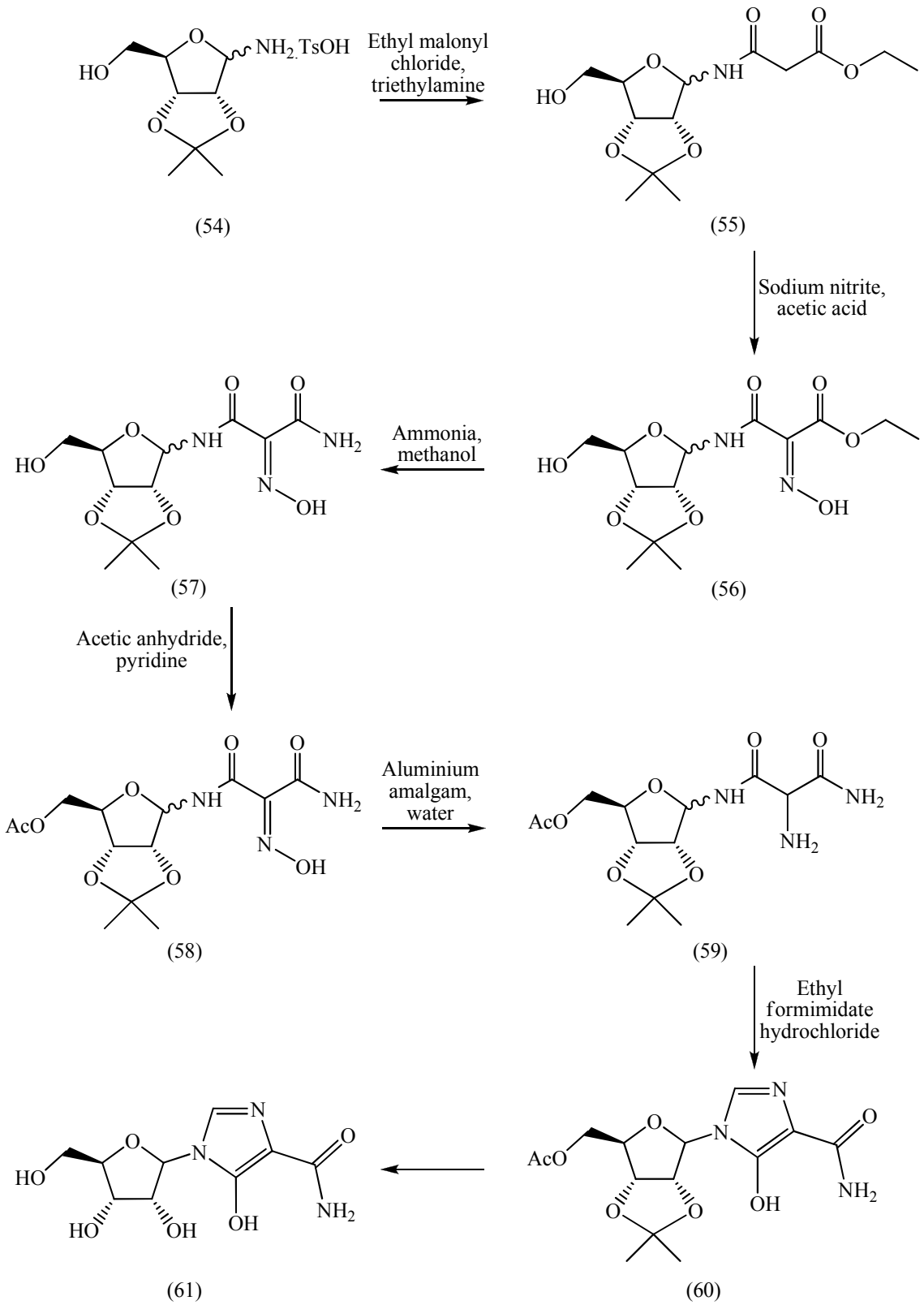
Scheme 42 Linear retrosynthetic analysis of 5-hydroxyimidazoles.

4.5.1 Synthetic Strategy

Previously reported chemical syntheses of bredinin[140,142,143,154,194,211,220,221] have facilitated only modest yields and are not easily adapted to analogues which could potentially also have interesting biological activities.

As part of some ongoing work within the research group, a novel, linear synthetic route to the synthesis of bredinin was developed. This strategy has the advantage that the reagents employed are relatively inexpensive and can be easily modified to introduce a wide variety of substituents at positions 1, 2, and 4 of the 5-hydroxyimidazole and hence provide a route to a large number of bredinin analogues.

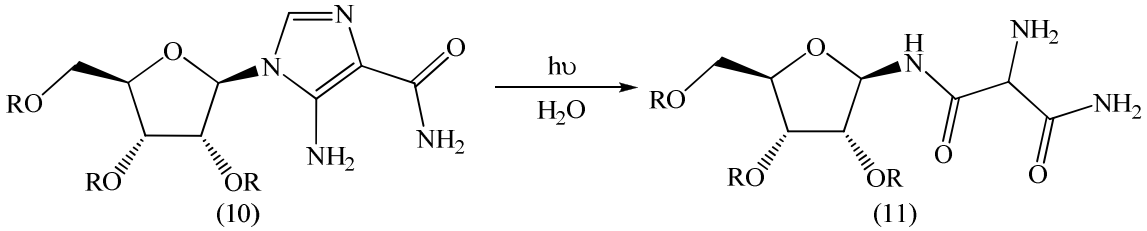
In the case of the present study, where the synthetic target was bredinin (scheme 43), the final developed synthetic strategy involved the reaction of protected ribose with ethyl malonyl chloride. The product was then reacted with sodium nitrite and acetic acid to give the corresponding oxime. Reaction of the oxime with ammonia to gave the corresponding amide derivative which then underwent protection of the ribose free hydroxyl to give the 5-*O*-acetyl derivative, this aided with subsequent purification. The resulting 2-amino-*N*-(5-*O*-acetyl-2,3-*O*-isopropylidene-D-ribofuranosyl)malondiamide was reduced with an aluminium amalgam to give the corresponding amine which was then cyclised with ethyl formimidate hydrochloride to give the protected 5-hydroxyimidazole-4-carboxamide derivative. The sugar was then deprotected to give bredinin.



Scheme 43 Linear synthesis of bredinin.

4.5.2 Development of Synthetic Strategy

As previously mentioned (chapter three, section 3.5.3), Fukukawa *et al*.[143,220] prepared both the acyclic intermediates, 2-amino-*N*-β-D-ribofuranosyl malondiamide (11a) and 2-amino-*N*-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)malondiamide (11b) by photolytic cleavage of AICA-riboside (10a) and per-acetylated AICA-riboside (10b) respectively. These intermediates were key molecules in the degradative synthesis of bredinin and bredinin triacetate.



Where R = H (a) or Ac (b)

Scheme 44 Photolytic cleavage of AICA-riboside.

Similarly for the present study, 2-amino-*N*-β-D-ribofuranosyl malondiamide, or an alternative suitably protected derivative, was initially chosen as a target intermediate in the attempted synthesis of bredinin from simple, acyclic precursors using a linear synthetic strategy.

A novel strategy was developed in which the 5-hydroxyimidazole moiety of bredinin was formed by the cyclisation of its D-ribofuranosyl amine derivative. This strategy is again comparable to that devised by Shaw *et al*. for the synthesis of the 5-aminoimidazoles where the heterocycle is formed from acyclic intermediates.[222]

In the present study, 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-p-sulphonate (54) was reacted with ethyl malonyl chloride in the presence of triethylamine to give an anomeric mixture of ethyl *N*-(2,3-*O*-isopropylidene-D-ribofuranosyl)malonamate (55). The product was obtained as a gum which would not crystallise from bench solvents. IR examination of the gum showed υ C=O 1735 cm^{-1}, υ C=O 1682 cm^{-1} and υ gem dimethyl 1372 cm^{-1}. The structure was further confirmed by NMR spectroscopy and mass spectrometry.

Reaction of the ethyl *N*-(2,3-*O*-isopropylidene-D-ribofuranosyl)malonamate in aqueous sodium nitrite in acetic acid gave an anomeric mixture of ethyl 2-hydroxyimino-*N*-(2,3-*O*-isopropylidene-D-ribofuranosyl) malonamate (56). Again IR examination showed υ O-H (oxime) 3341 cm^{-1}, υ C=O 1731 cm^{-1}, υ C=O 1690 cm^{-1}, υ C=N 1666 cm^{-1}, υ gem dimethyl 1374 cm^{-1}, υ N-O 976 cm^{-1}, and the structure was further confirmed by NMR spectroscopy and mass spectrometry.

Reaction of ethyl 2-hydroxyimino-*N*-(2,3-*O*-isopropylidene-D-ribofuranosyl)malonamate with ethanolic ammonia (4 ºC, 18 h) gave 2-hydroxyimino-*N*-(2, 3-*O*-isopropylidene-D-ribofuranosyl)malondiamide (57).

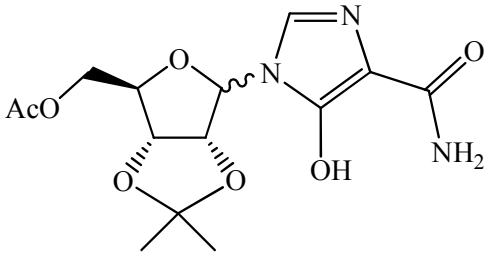
To facilitate chromatographic purification of later products in the reaction route, 2-hydroxyimino-*N*-(2,3-*O*-isopropylidene-D-ribofuranosyl)malondiamide was acetylated (Ac_2O in pyridine) to give the corresponding 5-*O*-acetyl derivative (58), which in turn was reduced with an aluminium/mercury amalgam to give a separable (silica gel column chromatography eluted with $CHCl_3$-MeOH) anomeric mixture of 2-amino-*N*-(5-*O*-acetyl-2,3-*O*-isopropylidene-D-ribofuranosyl)malondiamide (59) α (10 %) and β (19 %).

2-Amino-*N*-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)malondiamide is analogous to (11a) and (11b), previously obtained by the photochemical cleavage[143,184] of (10a) and (10b), respectively. The low yields of 2-amino-*N*-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)malondiamide obtained are thought to be due to the absorption of the product onto the amalgam 'cake' produced by the reagents in the reduction of 2-hydroxyimino-*N*-(2,3-*O*-isopropylidene-D-ribofuranosyl)malondiamide. An alternative route to the amine was sought in an attempt to increase the yields of the product obtained, this involved treating ethyl *N*-(2,3-*O*-isopropylidene-D-ribofuranosyl)malonamate with *O*-mesitylenesulphonylhydroxylamine, however it proved to be unsuccessful.

It was found that the reaction conditions required for clean cyclisation without the formation of by-products were highly specific. Thus, 2-amino-*N*-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)malondiamide was reacted with ethylformimidate hydrochloride (1 mol. eq.) in DMF at 110 ºC for 5 min. under nitrogen to give 4-

carbamoyl-(5'-*O*-acetyl-2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-5-hydroxyimidazole-4-carboxylate (60).

When an excess (1.3 mol eq.) of ethyl formimidate hydrochloride was used, competition between different modes of cyclisation was observed resulting in the formation of 60β, 62β and 63β. The synthesis of α-bredinin did not demonstrate a similar selectivity since the reaction using an equivalent or excess amount of ethyl formimidate hydrochloride resulted in a mixture 60α, 62α and 63α.



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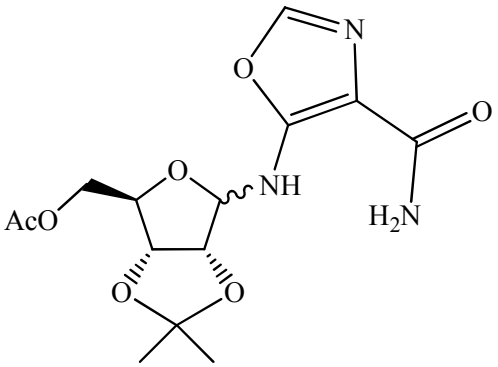
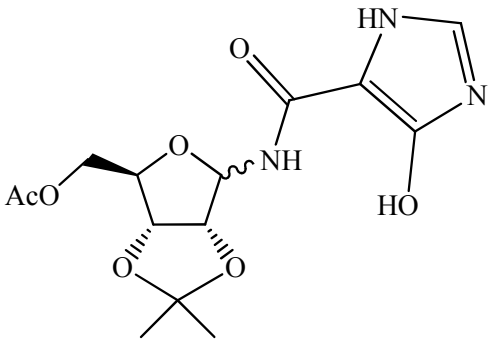


(62)



(63)

Figure 34 Products from alternative modes of cyclisation.

A comparison was made of 60β with an authentic specimen of bredinin that was purchased from Japan. The physical constants, such as melting point and NMR analysis, were identical to those of the authentic specimen.

4.6 Summary

A novel convergent route to substituted 5-hydroxyimidazoles using simple acyclic precursors was developed which allowed the introduction of substituents in both position 1 and 2 of the imidazole ring unambiguously.

A qualitative study conducted, revealed that both lipophilicity and steric demand play crucial roles in the final cyclisation step of the imidazole synthesis, a compromise between the two must be achieved to give the optimal reaction conditions. Both need to be taken into account when planning new target compounds.

A novel linear route to bredinin using acyclic precursors was developed which enabled the addition of substituents in positions 1, 2, 4 and 5 of the imidazole ring unambiguously. A particular advantage of this route is that it would be possible to synthesise secondary amide derivatives of 5-hydroxyimidazole-4-carboxamides. This can be achieved before cyclisation has occurred and therefore means that there would be no interference from other functional groups present on the imidazole ring, a particular issue that has to be addressed when using the convergent synthetic strategy for the synthesis of these 5-hydroxyimidazoles.

Sugar analogues could be successfully synthesised using the linear synthetic strategy but could not be synthesised using the convergent strategy.

CHAPTER FIVE

THE REACTIVITY OF ETHYL 5-HYDROXYIMIDAZOLE-4-CARBOXYLATES

5.1 Introduction

Modification of the heterocyclic base moiety of nucleosides has been less extensively studied than sugar modifications; however research conducted so far in this area has lead to many compounds with potent activity (covered in more detail in chapter three, section 3.4).

Modification studies of the imidazole system would allow the opportunity to gain a greater understanding of the chemistry of these 5-hydroxyimidazole systems.

In an attempt to further understand the chemistry of these heterocyclic systems, several studies were carried out to assess their general reactivity, as well as the reactivity at specific positions on the imidazole ring.

The results of these studies could possibly allow the findings to be used in future syntheses as a guide as to how the 5-hydroxyimidazole system may react under particular conditions.

5.2 Reactivity Study of Ethyl 1-Benzyl-5-hydroxyimidazole-4-carboxylate

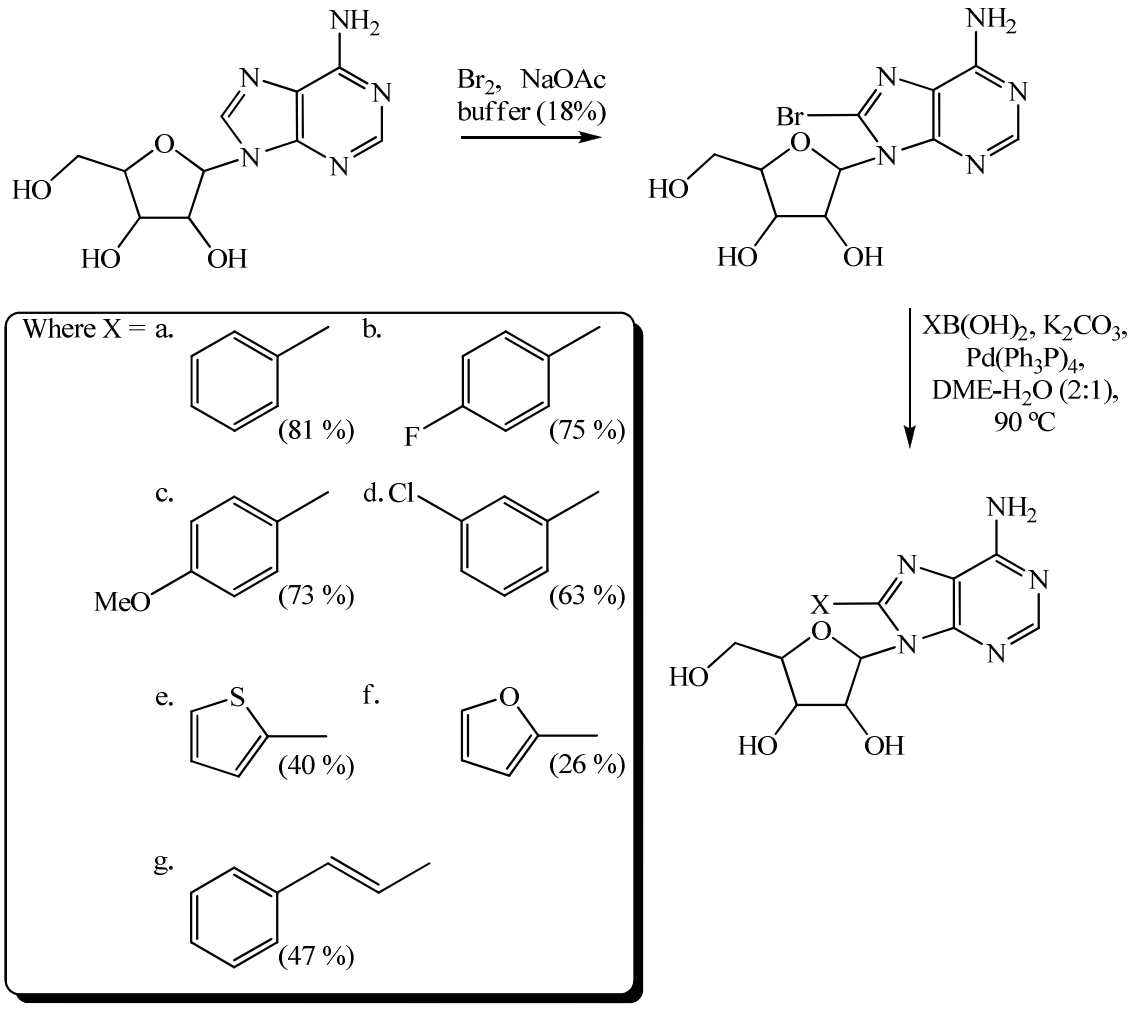
5.2.1 Reactivity at C-2

As previously discussed (chapter four, section 4.4.4), the use of substituted orthoimidates in the reaction scheme to form the imidate, and then addition of the desired primary amine to such imidates results in the formation of 5-hydroxyimidazoles with substituents of varied chain length at position 2 of the imidazole.

However, as stated the yields of the 5-hydroxyimidazoles obtained was found to decrease dramatically as the chain length of the substituent at position 2 increased, owing to steric hindrance in the amidine cyclisation step of the imidazole synthesis.

An alternative approach, to overcome the problem experienced with decreasing yields, could be a nucleophilic substitution reaction at position 2 of the imidazole ring. Bromination at position 2 of the imidazole ring has been shown to be successful for the 5-aminoimidazole nucleosides to give the corresponding 2-bromo-5-aminoimidazole nucleosides.[223,224]

The resulting product could then be substituted to produce a 5-hydroxyimidazole product with the desired substituent at position 2 of the imidazole ring. One possible strategy to achieve this goal is bromination followed by a Suzuki-type coupling, similar to that attempted by Kohyama.[80] Kohyama *et al*. examined the use of Suzuki coupling to introduce a selection of aryl groups to 8-bromoadenosine (scheme 45). 8-Bromoadenosine was obtained from adenosine in 81 % yield, then a Suzuki coupling was carried out using a catalytic amount of tetrakis(triphenylphosphine)palladium and potassium carbonate as a base.



Scheme 45 Bromination of benzimidazoles followed by a Suzuki coupling to give a variety of 8-substituted benzimidazoles.

Bromination of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was attempted. Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was suspended in dichloromethane and a mole equivalent of 2 M bromine in dichloromethane was added. The solution was then stoppered and stirred at room temperature overnight. The solvent was removed under reduced pressure to give a quantitative yield of a white precipitate, TLC analysis indicated that no reaction had occurred. NMR analysis of this crude compound revealed that only the starting material had been obtained from the reaction. The proton signal for the C-H at position 2 of the imidazole ring was still present and no upfield shifting of other signals was observed, as would be expected had bromine been incorporated into the imidazole ring.

5.2.2 Reactivity of 5-Hydroxyl Group

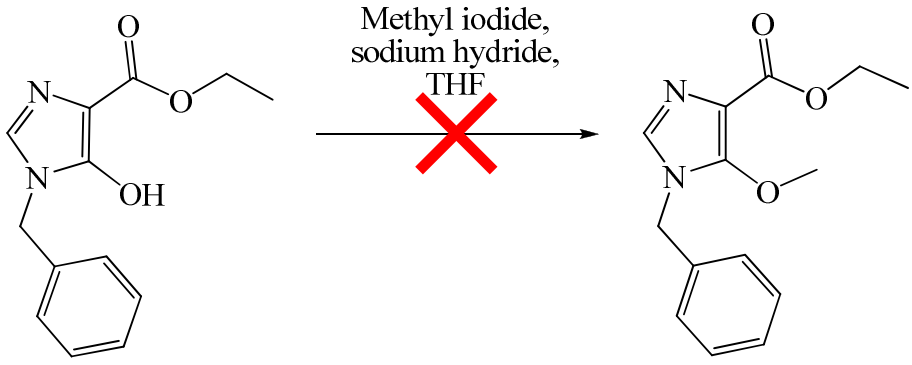
Before any reactivity studies of the 5-hydroxyl function were carried out, it was believed that the hydroxyl group should essentially react in a similar way to the hydroxyl function present in a phenolic system. This is for two reasons:

- Phenolic systems are slightly acidic and unlike aliphatic alcohols form the water soluble phenoxide ion due to the resonance stabilisation provided by the aromatic ring. The 5-hydroxyimidazole system bears close structural similarities to a phenol, it is an aromatic system in which ionisation of the hydroxyl function can be stabilised by the imidazole ring.
- Additionally, both phenol and the 5-hydroxyimidazole have similar pKa values. Phenol has a conjugate acid pKa of approximately 10.02, whereas the 5-hydroxyimidazole system has a conjugate acid pKa of approximately 9.63; this means that the 5-hydroximidazole system is slightly more acidic than the phenolic system.

With this in mind a series of reactions were attempted to test this theory and assess the reactivity of the 5-hydroxyl function.

5.2.2.1 Alkylation

Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was initially reacted in a Williamson ether-type synthesis (scheme 46). This basic methylation was carried out following the method of Jung *et al*.,[225] where ethyl 1-benzyl-5-hydroxy-1*H*-imidazole-4-carboxylate was reacted with methyl iodide in the presence of sodium hydride. However, the reaction did not proceed to the desired protected imidazole and only starting material was obtained from the reaction mixture.

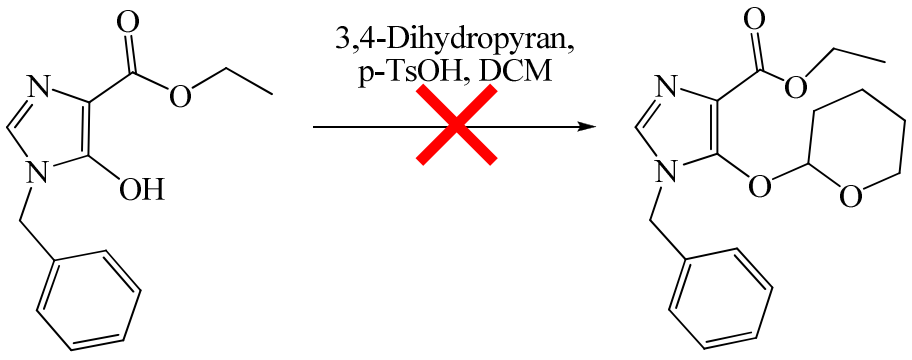


(39) (63)

Scheme 46 Methylation of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate.

3,4-Dihydropyran is used extensively in organic synthesis as a hydroxyl protecting group because of its low cost, general chemical stability and general ease of installation and removal. Generally, it can be thought that most acid catalysts, or reagents that generate an acid *in situ*, can be used to introduce the 3,4-dihydropyran group into a compound.

With this in mind it was decided to try reacting ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with 3,4-dihydropyran in the presence of *p*-toluene sulphonic acid (scheme 47) following the method of Bernady *et al*.[226] to give a gum which would not crystallise from bench solvents and could not be purified by silica gel column chromatography.

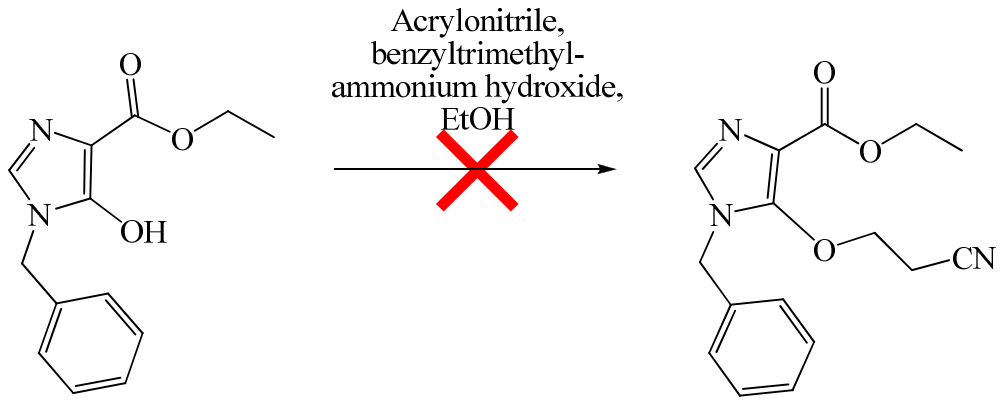


(39) (64)

Scheme 47 Reaction of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with 3,4-dihydropyran.

The introduction of a 2-cyanoethyl moiety was then attempted following the method of Liu *et al*.[227] If successful, the reaction should involve the conjugate addition of ethyl 1-

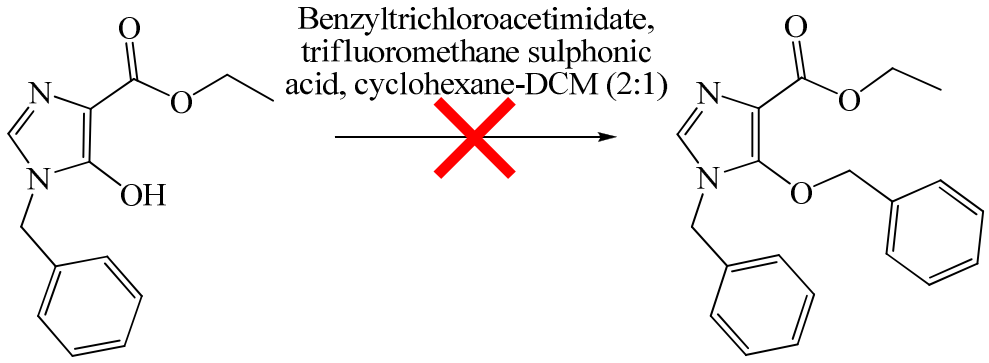
benzyl-5-hydroxyimidazole-4-carboxylate to acrylonitrile, in the presence of a catalytic amount of benzyltrimethylammonium hydroxide, to yield the desired ethyl 1-benzyl-5-(2-cyanoethoxy)-imidazole-4-carboxylate (scheme 48). However, the reaction was unsuccessful and once again only starting material was recovered from the solution.



(39) (65)

Scheme 48 Reaction of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with acrylonitrile.

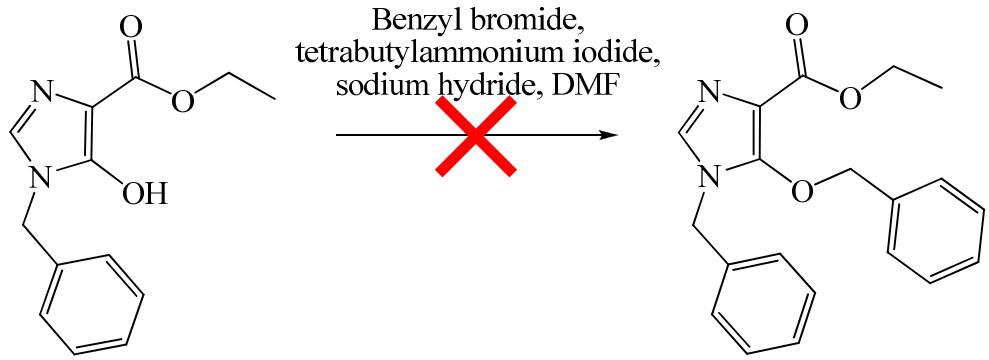
Benzylation of the hydroxyl group was also attempted using benzyl trichloroacetimidate, following the method of Stampe Jensen *et al*.[228] The reaction was carried out under slightly acidic conditions (a catalytic amount of trifluoromethane sulphonic acid) in a non-polar solvent system (scheme 49). However problems were experienced with the reaction due to insufficient solubility of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate in the non-polar solvents system required for the reaction. Acetimidates can rearrange in polar solvents, therefore a different benzylating agent was required that wouldn't be affected by the polarity of the solvent needed to dissolve ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate.



(39) (66)

Scheme 49 Benzylation of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with benzyl trichloroacetimidate.

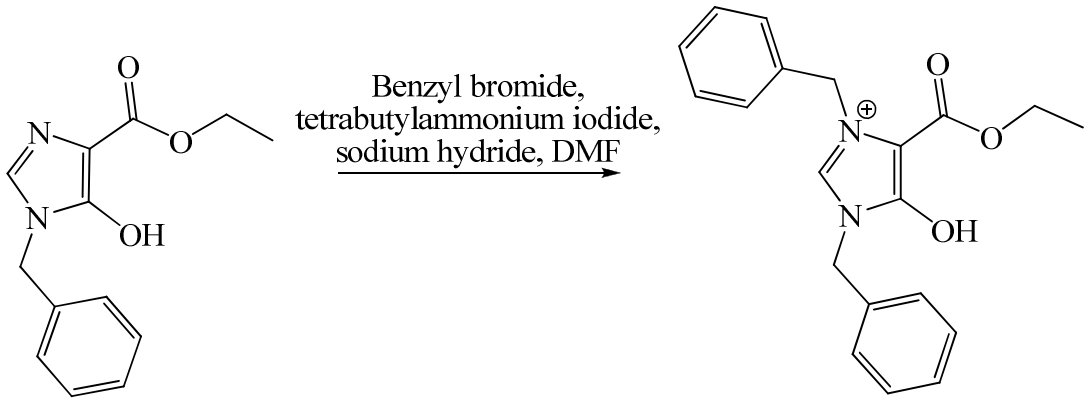
Kanai *et al.*[229] successfully benzylated glycosides with benzyl bromide in the presence of a strong base, sodium hydride (scheme 50). However under identical conditions, the reaction with ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate did not proceed to the desired product.



(39) (67)

Scheme 50 Benzylation of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with benzyl bromide.

Although the desired product wasn't obtained from this reaction, an interesting result was observed. In this reaction, instead of benzylation occurring on the 5-hydroxyl function as would be expected, nucleophilic addition occurred on the nitrogen at position 3 of the imidazole system, resulting in the formation of the quaternary ammonium salt (scheme 51).



(39) (68)

Scheme 51 Unexpected product from the reaction of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with benzyl bromide.

Initially, reaction at the hydroxyl function was thought to have been successful. NMR analysis indicated that the benzyl group had been incorporated in the product without any degradation of the imidazole itself. However, because the hydroxyl proton is rarely seen by ^{1}H-NMR another possibility was that the product could be a result of benzylation of the nitrogen at position 3 of the imidazole ring. Mass spectrometry of the product revealed a mass ion peak of 337 (M^{+}), indicating that benzylation had occurred on the nitrogen as opposed to the oxygen, this was further confirmed by TLC analysis which revealed a homogenous spot which was both UV and ferric chloride active, indicating that the hydroxyl group was still present in the product.

In light of the findings described so far, it was believed that the product isolated should be the halide salt of 1,3-dibenzyl-4-(ethoxycarbonyl)-5-hydroxyimidazol-3-ium (68). With this in mind, a silver nitrate test was conducted to confirm this theory; the result of the investigation was positive, indicating the presence of a bromide ion.

Though the quaternary ammonium salt (1,3-dibenzyl-4-(ethoxycarbonyl)-5-hydroxyimidazol-3-ium bromide) obtained was not the desired product, it had never been reported in the literature. Benzylation is believed to have occurred here because the nitrogen at position 3 of the imidazole ring is particularly nucleophilic. A similar finding was also reported by Brown *et al*.[230] who observed methylation at position 3 of the imidazole ring with ethyl 5-aminoimidazole-4-carboxylate nucleosides.

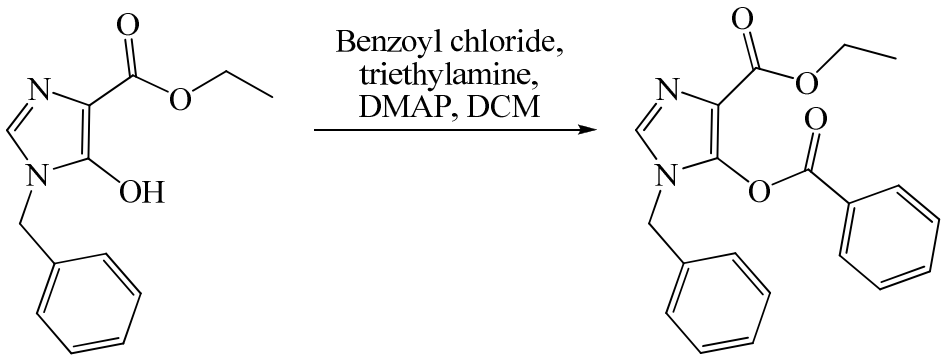
Brown *et al*.[230] found that basic methylation of ethyl 5-aminoimidazole-4-carboxylate using methyl iodide gave a crystalline product which could be readily isolated in good yield. The mass spectrum revealed a peak corresponding to a mono-methylated aglycone. The compound, however, was readily diazotised and gave a high colour yield in the Bratton-Marshall assay indicating that actually the 5-amino-group had not been methylated as first thought. Accordingly the compound was reassigned as the 3-substituted quaternary ammonium salt of ethyl 1-benzyl-5-aminoimidazole-4-carboxylate. It has been reported that alkylation at position 3 of the imidazole system is favoured because this is the most nucleophilic nitrogen atom of the system.[231]

5.2.2.2 Acylation

In a series of reactions conducted by Shuto *et al*.[187] the protection of the phenolic hydroxy group of the base moiety of bredinin by using both silyl and acyl groups was attempted, however they observed no reaction.

Similarly, silylation of the 5-hydroxyl function of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was attempted here but all attempts were unsuccessful; however, contrary to the findings by Shuto *et al*., in the present study, reaction of the 5-hydroxyl group of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with three acylating agents was successfully achieved under mild reaction conditions. Unfortunately Shuto didn't give any indication of the reaction conditions used in their attempts of silylation or acylation, so no useful comparisons can be made at this stage between the reactivity of the 5-hydroximidazole base moiety of bredinin and the 5-hydroxyimidazole system of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate used in the present study.

Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was suspended in dichloromethane and reacted with benzoyl chloride in the presence of triethylamine and DMAP, overnight at room temperature (scheme 52). The resulting solution was washed with saturated sodium hydrogen carbonate and water, the organic phase was extracted, dried ($MgSO_4$) and the solvent removed under reduced pressure to give the product (69) in good yield as a white crystalline solid.



(39) (69)

Scheme 52 Acylation of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with benzoyl chloride.

Similarly, ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was suspended in dichloromethane and reacted with acetyl chloride in the presence of triethylamine and DMAP, overnight at room temperature (scheme 53). Again the resulting solution was washed, dried and the solvent removed under reduced pressure to give the product (70) in good yield as white crystals.

(39) (70)

Scheme 53 Acylation of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with acetyl chloride.

Reaction of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was also attempted with pivaloyl chloride using the same condition as previously used (scheme 54). Although TLC analysis revealed that an additional product spot had appeared, the resulting product (71) was found to be particularly difficult to isolate and the yields were much lower for this acylating reagent than the benzoyl and acetyl derivatives.

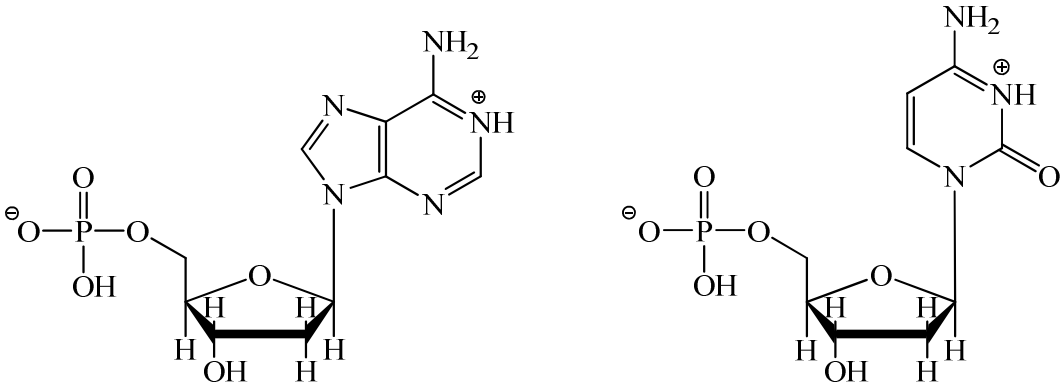
(39) (71)

Scheme 54 Acylation of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with pivaloyl chloride.

Shuto *et al.*[187] reasoned that one explanation that the attempted reactions of the 5-hydroxyl group of bredinin had been unsuccessful could be that the base moiety of bredinin could exhibit a zwitterionic nature. It has been established that the free acids of adenosine 5'- and 3'-monophosphates (AMP),[232-234] cytidine 5'- and 3'-monophosphates (CMP),[235-237] guanosine 5'-monophosphate (GMP) and inosine 5'-monophosphate (IMP)[238] have zwitterionic character from their X-ray crystal structures.

In AMP the protonation of the base is on the nitrogen at position 1 and in CMP it is at the analogous pyrimidine ring site, on the nitrogen at position 3. Whereas in GMP and IMP the zwitterionic character of the free acids occurs with protonation of the base on the nitrogen at position 7 site of the imidazole moiety (figure 35). The fourth common nucleotide, containing uracil in RNA and thymine in DNA, is unusual in that it appears to exist in the neutral form.

As a consequence of the zwitterionic character of the heterocycle, one of the phosphate hydrogen atoms is ionized. This means that in the resulting molecule, the phosphate group is negatively charged and that the base is positively charged.



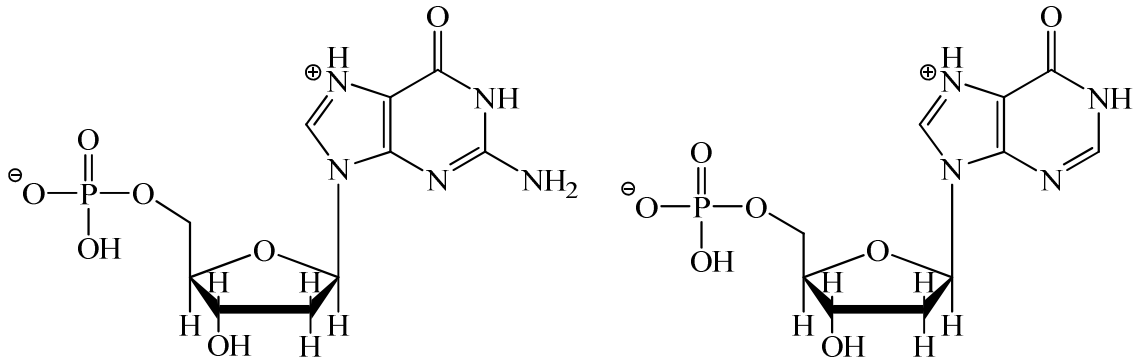


Figure 35 Zwitterionic structures of some nucleotides.

Several imidazoles have also been shown to demonstrate zwitterionic character from their X-ray crystal structures. From this it could be inferred that, depending on the conditions for crystal growth, the imidazole system for the ethyl ester 5-hydroxyimidazoles could also exhibit a zwitterionic nature.

This is further supported, in the case of the ethyl 5-hydroxyimidazole-4-carboxylates, by the finding that in some of the ^{13}C-NMR spectra, there are two peaks that correspond to the carbonyl carbon. It is believed that this result is the consequence of the imidazole existing in the two tautomeric forms (figure 36).

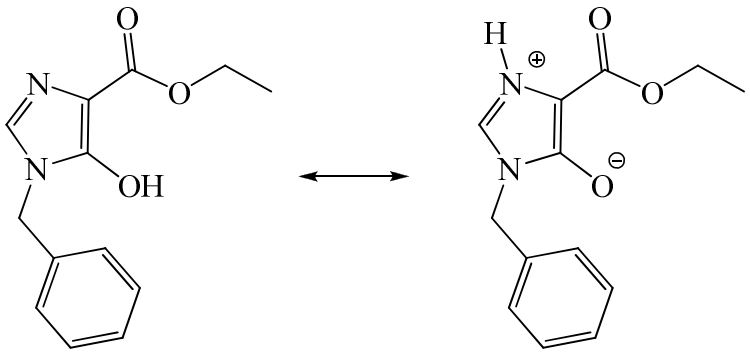
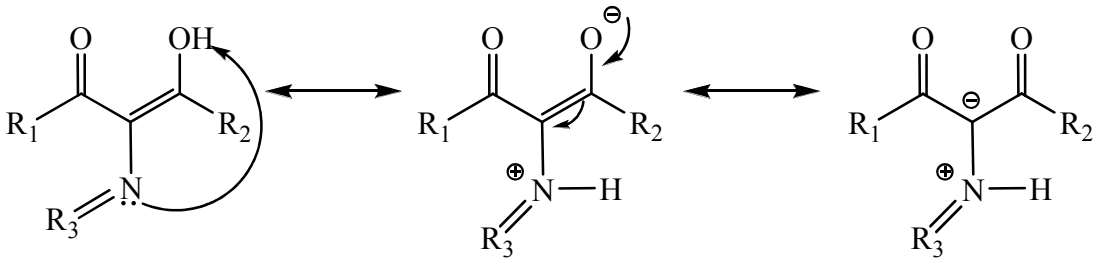


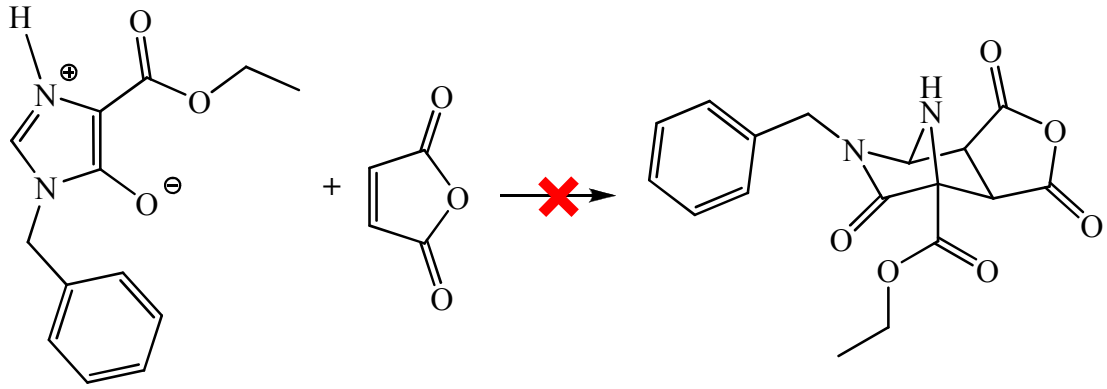
Figure 36 Zwitterionic form of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate.

If this were found to be true, then it could be a useful indicator as to the susceptibility of the 5-hydroxyimidazole system to particular types of reactions. If the 5-hydroxyimidazoles exhibited the discussed zwitterionic nature, it would mean that the ethyl ester 5-hydroxyimidazole-4-carboxylate aglycone could be thought of as chemically similar to a compound with a 1,3-dipole (scheme 55), and therefore could undergo 1,3-dipolar cycloaddition.[239,240]



Scheme 55 1,3-Dipole system.

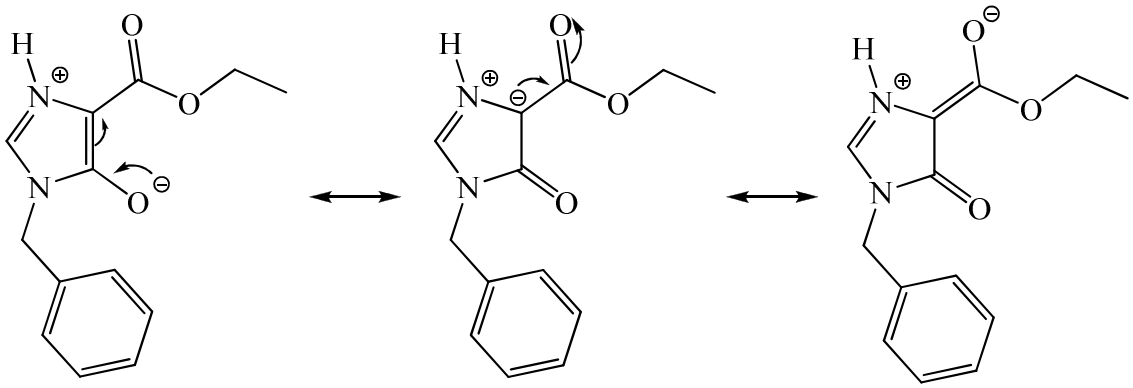
In an effort to test this theory and try and explain the reason for limited reactivity at the 5-hydroxyl group, a 1,3-dipolar cycloaddition reaction was attempted between ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate and maleic anhydride in toluene (scheme 56).



(72)

Scheme 56 1,3-Dipolar addition of maleic anhydride with ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate.

Usually in a 1,3-dipolar cycloaddition, a dipolarophile, is reacted with a 1,3-dipole to result in the formation of a heterocyclic ring. In this particular reaction, maleic anhydride was acting as the dipolarophile, however the desired product could not be obtained from the reaction mixture. Only starting material was recovered from the reaction indicating that even if two tautomeric forms of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate did exist as suspected, the keto form may be experiencing additional stability *via* conjugation through the imidazole ring as well as the carbonyl group (scheme 57).



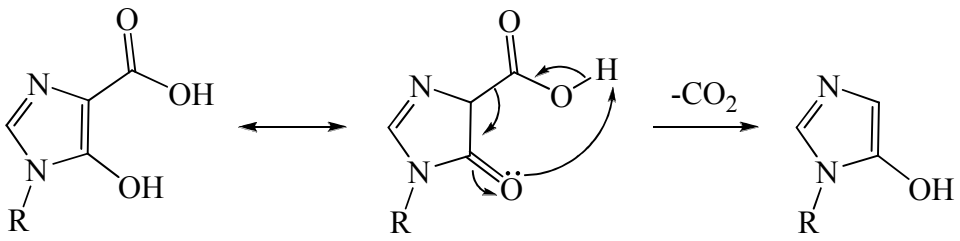
Scheme 57 Conjugation of the keto tautomer of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate.

Had the reaction been successful and the product isolated, this 1,3-dipolar cycloaddition reaction wouldn't necessarily be a useful tool in producing the desired bredinin analogue library; however the results would certainly have been useful in further

developing the present understanding of the chemistry of these 5-hydroxyimidazole systems and how they react.

5.2.3 Reactivity of the Ethyl Ester and C-4

Providing that there wasn't any interference from any other group present on the imidazole aglycone, the ethyl ester group present at position 4 could be hydrolysed to the corresponding carboxylic acid which then has the potential to be easily manipulated into a variety of other functional groups. However, carboxylic acid analogues of the 5-hydroxyimidazoles are known to be very unstable and will readily decarboxylate (scheme 58).



Scheme 58 Mechanism of decarboxylation.

As previously discussed (chapter four, section 4.2), the group of 5-hydroxyimidazoles of particular interest in the present study is the secondary amide derivatives because of their close structural resemblance to intermediates within the nucleic acid biosynthetic pathways. The primary aim for the present research was to develop a general and efficient route to the 5-hydroximidazoles using acyclic precursors which was easily adaptable to producing large libraries of analogues of bredinin.

The initial target compounds were the ethyl 5-hydroxyimidazole-4-carboxylates because the ethyl ester group present at position 4 of the imidazole ring had the possibility of being converted into a wide variety of 5-hydroxyimidazoles.

For manipulation of the ethyl ester to be successful, decarboxylation has to be prevented. This would require the use of a 5-hydroxyl protecting group, which is orthogonal to the ester derivative on the 4-carboxylate group. If an orthogonal

protecting group could be found, it would mean that the protecting group would be retained whist derivatisation of the ester was attempted (figure 37).

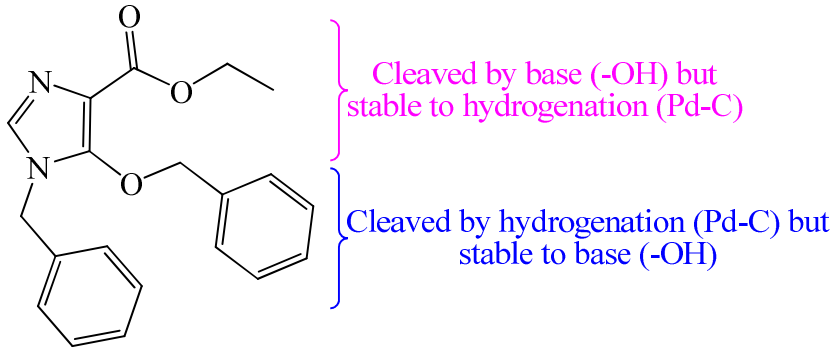
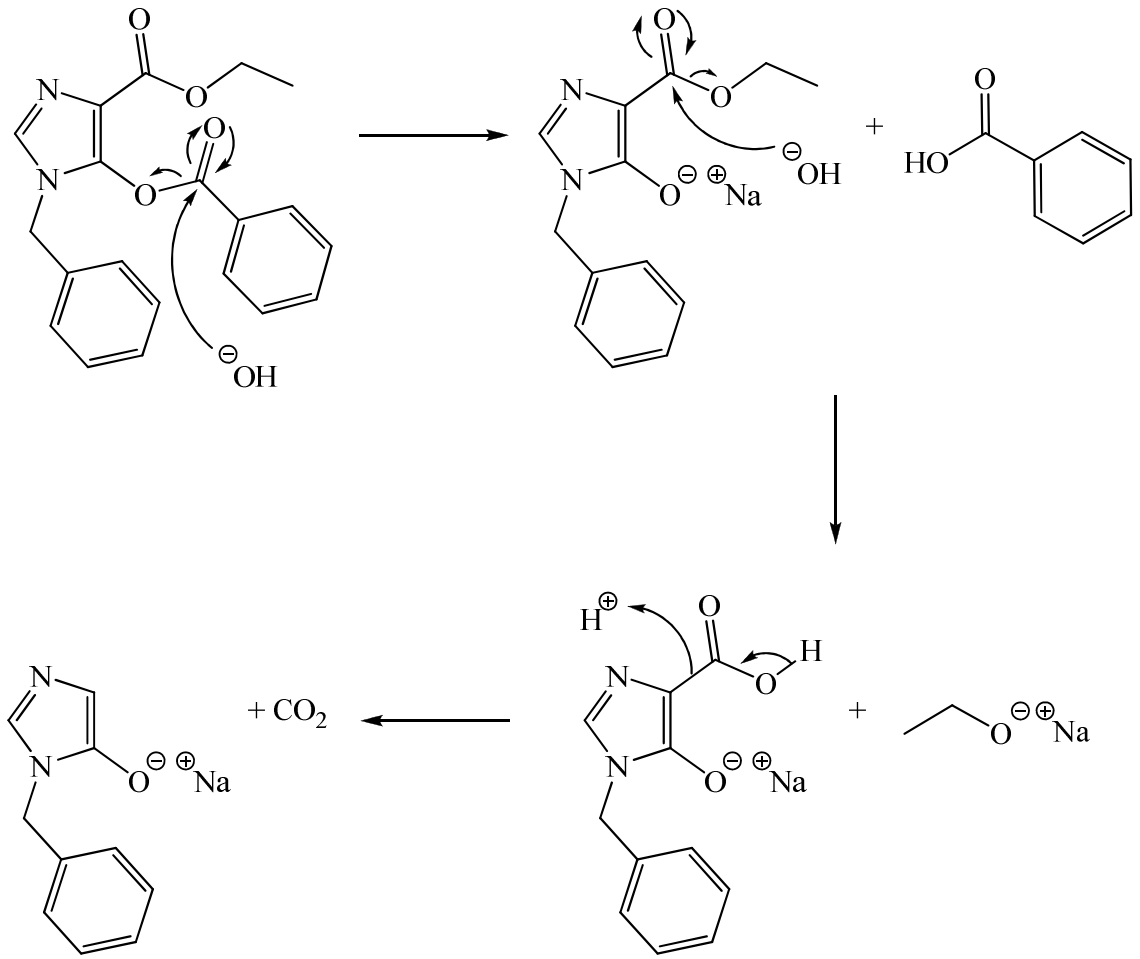


Figure 37 Ethyl 1-benzyl-5-benzylimidazole-4-carboxylate.

With this in mind, the results of the general reactivity investigations carried out on ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate, and more specifically the 5-hydroxyl function, posed a problem.

It was found that the 5-hydroxyl could be successfully acylated, however the acyloxy groups are not orthogonal to the ethyl ester group present at position 4 of the imidazole system. This means that manipulation of the ethyl ester group couldn't be carried out independently of the acyloxy group present at position 5. The hydrolysis of the ethyl ester to the respective carboxylic acid has to be carried out under basic conditions, in which the acyloxy group would also be cleaved, subsequently resulting in decarboxylation and ultimately degradation of the imidazole ring system.

As a proof of concept, an NMR study of the hydrolysis of the benzoyl protected imidazole was conducted. Benzoates are less readily hydrolysed than acetates,[241,242] so the benzoyloxy derivative was chosen for the NMR study. 5-Benzoyloxy-1-benzyl-imidazole-4-carboxylic acid ethyl ester was dissolved in DMSO-d_6 and a few drops of 2 M NaOH in D_2O was added and the reaction was monitored. The analysis clearly showed the rapid cleavage of both the benzoyl protecting group and the ethyl ester functional groups (scheme 59).



Scheme 59 Base hydrolysis of a benzoyl group.

It was also evident from the NMR that just 5 min after the addition of sodium hydroxide, *N*-benzyl formamide had already started forming. This was indicated by the presence of a splitting (doublet) of the benzylic -CH_2- which is a result of coupling to the -NH- in *N*-benzyl formamide. Degradation of the imidazole is a consequence of protonation of the nitrogen at position 3 and subsequent nucleophilic attack of a hydroxide ion at position 2, leading to the formation of *N*-benzyl formamide.

Had a derivative of the imidazole been synthesised with a suitable alkoxy group present at position 5, the alkoxy group could also have served as a good method of protecting this position to allow manipulation of the ethyl ester present at position 4. This is because an alkoxy group would have been orthogonal to the ethyl ester, so manipulation of one wouldn't affect the other.

Manipulation of the ethyl ester couldn't be attempted without the use of a protecting group at position 5 because it would result in degradation of the imidazole itself. So in addition to the parallel synthetic studies that were carried out into the synthesis of other 4-substituted 5-hydroxyimidazoles (chapter six), the reactivity of the carbon at position 4 of the imidazole ring was also investigated.

A recent publication[243] detailed a new intermolecular ene reaction using oxazolones under very mild conditions without the use of any catalyst. The study found that methyl ester substituted oxazolones reacted rapidly (~20 min.) with butyl vinyl ether at room temperature in near quantitative yields, with no degradation of the product.

Due to the close structural similarities between the methyl ester oxazolones used in the study and the imidazole-4-carboxylate ethyl esters (figure 38), a similar experiment was attempted using ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate, to provide analogues with structural differences at the 4 position of the imidazole ring. This study[243] also found that by reacting methyl ester oxazolones with 3,4-dihydro-(2*H*)-pyran (THP) gave the desired quaternary amino esters in good yields, albeit with longer reaction times (~22 h).

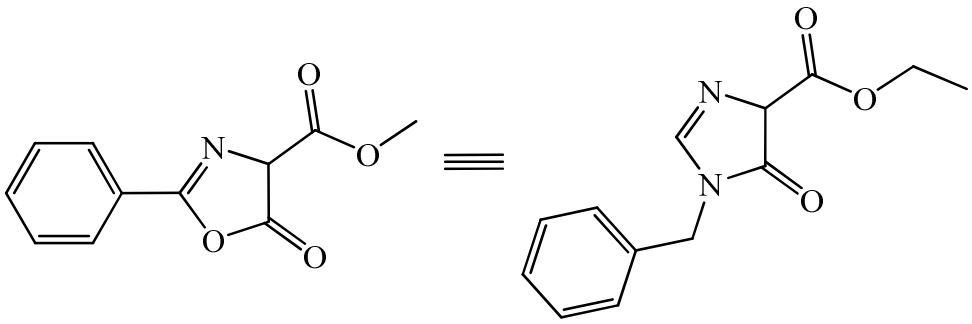
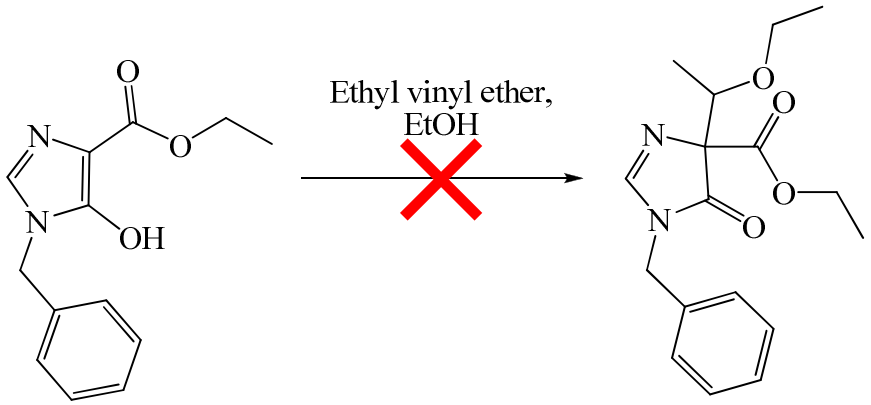


Figure 38 A demonstration of the close structural similarities between methyl ester oxazolones (left hand side) and imidazole-4-carboxylate ethyl esters (right hand side).

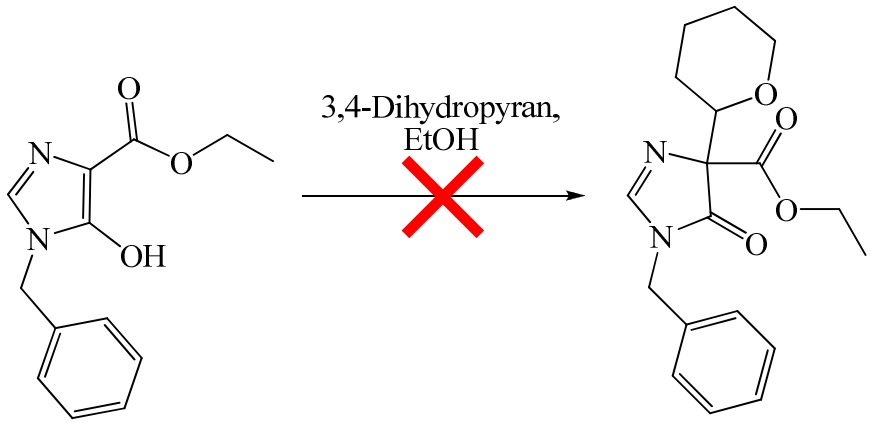
Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was suspended in ethanol and an excess of ethyl vinyl ether was added (scheme 60). The solution was stirred at room temperature for 24 h and the solvent was removed under reduced pressure to give an oil. TLC analysis indicated no reaction had occurred. The oil was dissolved in a minimal volume of ethanol and rendered turbid with diethyl ether to yield only starting material.



(39) (73)

Scheme 60 Reaction of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with ethyl vinyl ether.

Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was suspended in ethanol and an excess of 3,4-dihydro-(2*H*)-pyran (tetrahydropyranyl ether or THP ether) was added (scheme 61). Unfortunately the reaction was unsuccessful and only starting material was recovered.

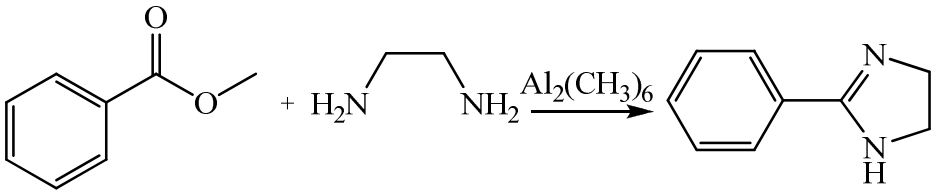


(39) (74)

Scheme 61 Reaction of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with 3,4-dihydropyran.

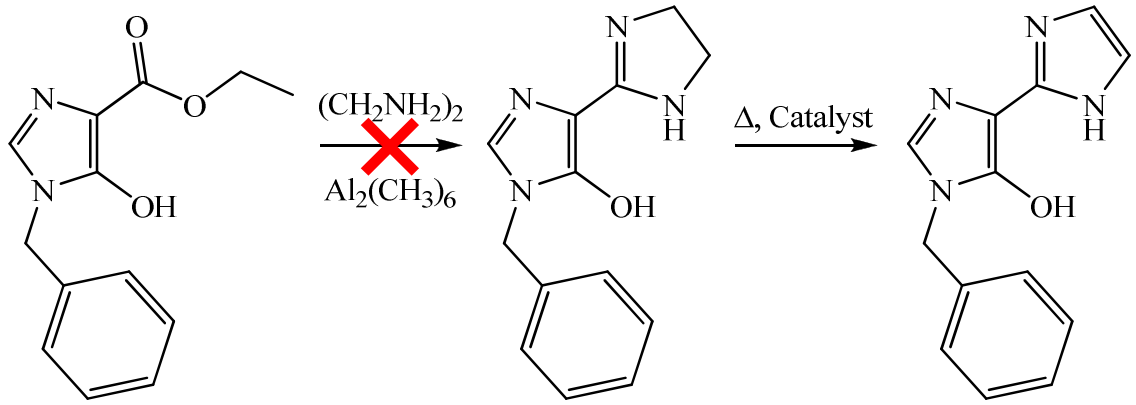
This may give some indication of the reactivity of the carbon at position 4 of the imidazole aglycone, the fact that the reactions both failed may indicate that the ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate would prefer to maintain the enol form rather than adopt the less favourable keto form.

Anastassiadou *et al.*[244] synthesised imidazolines for pharmacological evaluation by nucleophilic addition of ethylene diamine to aromatic esters in the presence of trimethylaluminium (scheme 62).



Scheme 62 Synthesis of imidazolines.

Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate bears some close structural similarities to the aromatic esters used by Anastassiadou *et al.*, so a similar series of reactions were investigated in an attempt to isolate the corresponding dihydroimidazolyl-imidazole (75). If this was successful the dihydroimidazolyl-imidazole product could have been further reduced by dehydrogenation to give the bis-imidazole (76) (scheme 63).



(39) (75) (76)

Scheme 63 Reaction of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate with ethylene diamine.

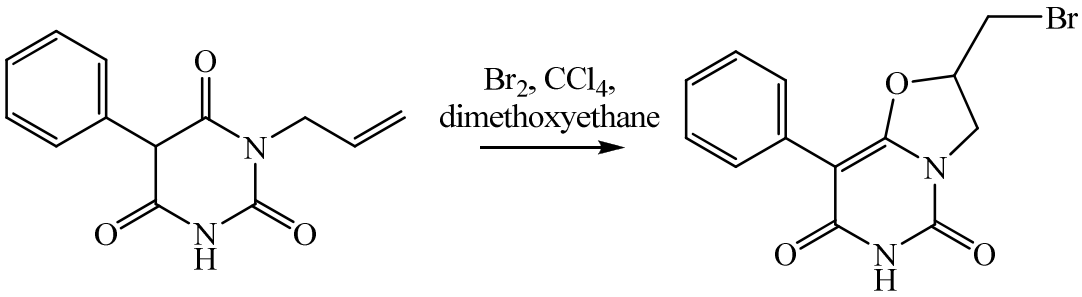
Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was refluxed, under a nitrogen atmosphere, with an excess of ethylene diamine in the presence of trimethylaluminium in toluene for 10 h. Methanol and dichloromethane were added to the solution which was refluxed for a further 15 min., then the aluminium hydroxide was filtered off and

the organic phase collected, dried and the solvent removed under reduced pressure. However this method only resulted in the breakdown of the imidazole to give *N*-benzyl formamide.

5.3 Reactivity of Ethyl 1-Allyl-5-hydroxyimidazole-4-carboxylate

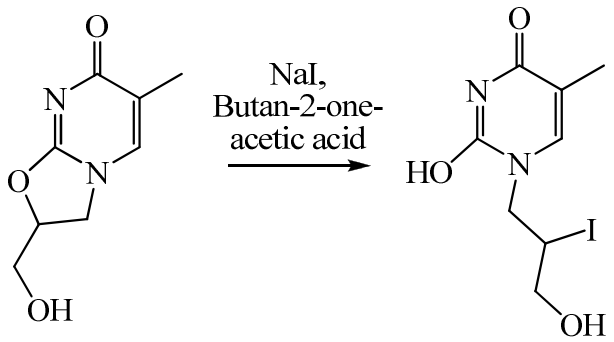
The synthesis of a variety of 1-substituted-5-hydroxyimidazoles have been explored previously in chapter four, section 4.4.3. However one particularly interesting imidazole synthesised, ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate (structure 46), allowed the opportunity for further development by manipulation of the allylic group present at position 1 of the imidazole ring.

An interesting publication[245] involved the bromine ether linkage of barbituric acid by reacting it with bromine in the presence of tetrachloromethane and dimethoxyethane (scheme 64). If this reaction could be mimicked using ethyl 1-allyl-5-hydroxy-1*H*-imidazole-4-carboxylate it would allow an alternative route to the manipulation of the ethyl ester carboxylate group without interference of the 5-hydroxyl group.



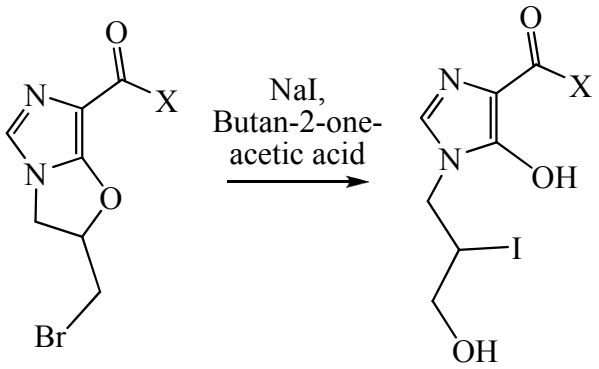
Scheme 64 Synthesis of a bromo-ether linked derivative of barbituric acid.

If a similar bromo-ether link could be successfully achieved using ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate, and manipulation of the ethyl ester carboxylate could also then be investigated, a possible method for the deprotection of the 5-hydroxyl could be using a method similar to Skaric *et al*.[246] Skaric took ether linked thymine derivatives and reacted them with sodium iodide in butan-2-one-acetic acid to give the hydroxylated derivatives (scheme 65).



Scheme 65 Reaction of ether linked thymine derivatives to give hydroxylated derivatives.

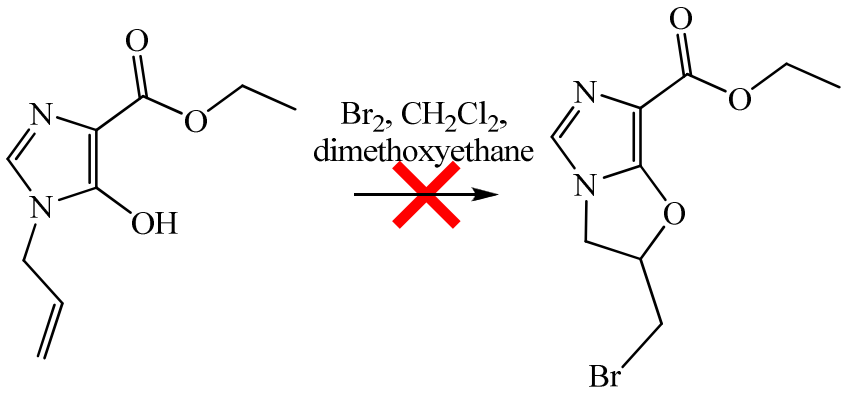
If the bromo-ether linked imidazole (77) could be successfully synthesised, and manipulation of the ethyl ester was achieved, then the ether linkage could be cleaved in a similar manner to that used by Skaric to give a product with an amino alcohol present at position 1 of the imidazole (78). This would not only allow the opportunity to attempt the synthesis of compounds with the desired functional groups at position 4 but also produce acyclic sugar analogues of bredinin.



(77) (78)

Scheme 66 Possible hydroxylation of bromo-ether linked ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate.

Reaction of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate (46) with bromine was attempted (scheme 67). Ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate was suspended in dichloromethane and a mol equivalent of bromine in dichloromethane was added to the reaction flask and the solution was stirred at room temperature for 24 h. The solvent was removed under reduced pressure to leave a pale brown oil.



(46) (79)

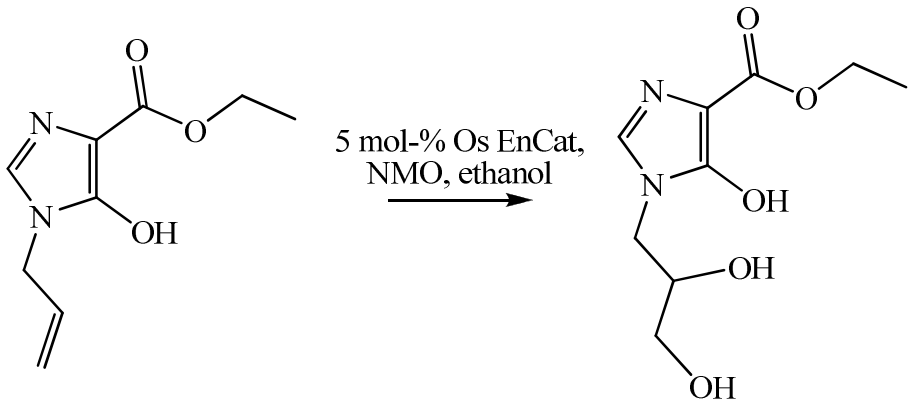
Scheme 67 Reaction of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate with bromine.

NMR analysis of the crude product revealed that the imidazole had degraded, from ^{1}H-NMR spectroscopy it could be seen that the product had no signals corresponding to the ethyl ester protons and the ^{13}C-NMR revealed that there were no corresponding carbonyl signals. The imidazole is believed to have broken down due to the strong nucleophilic properties of the bromide ion, which is a result of the formation of HBr.

If the reaction were to be repeated, it could be attempted using iodine instead of bromine to give the iodo-ether linked imidazole. Iodide is less nucleophilic than bromide so the imidazole would be subjected to slightly less harsh conditions, and additionally a base also needs to be added to mop up any HI that forms.

Additionally, ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate could also be simply mono- or di-hydroxylated across the allylic alkene. This would allow for the development of a selection of acyclic sugar analogues of the ethyl 5-hydroxyimidazole-4-carboxylates.

Choudary *et al.*[247] recently designed and developed a microencapsulation technique developed to envelop OsO_4 in a polymer capsule which affords a recoverable, non-toxic and reusable osmium catalyst for the dihydroxylation reaction of olefins employing *N*-methylmorpholine-*N*-oxide (NMO) as the co-oxidant. Dihydroxylation of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate (46) was attempted in a Sharpless asymmetric-type dihydroxylation reaction with polymer-bound osmium tetroxide (scheme 68), following the method developed by Choudary *et al.*[247,248]



(46) (80)

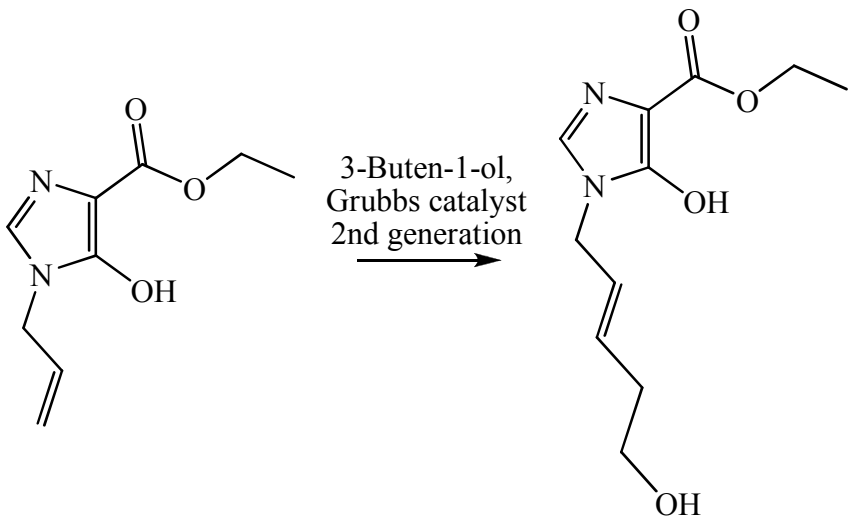
Scheme 68 Sharpless dihydroxylation of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate.

The dihydroxylation of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate with osmium tetroxide was attempted. Ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate was suspended in ethanol and encapsulated osmium tetroxide and *N*-methylmorpholine-*N*-oxide were added. The reaction mixture was allowed to stir for 48 h, then the mixture was heated to 50 ºC and a hot filtration was performed to remove the encapsulated osmium tetroxide catalyst. The solvent was removed under reduced pressure and the resulting oil was dissolved in a minimal amount of dimethylformamide and rendered turbid using diethyl ether.

Collection of the needle-like crystals was attempted, however the product appeared to decompose when it came into contact with moisture in the air, and when the crystals were isolated they immediately began to form a yellow gum. NMR analysis and mass spectrometry were carried out on the amorphous product obtained from the reaction mixture. The NMR spectrum obtained was inconclusive, the sample was very impure and the spectrum could not be used to ascertain whether or not the reaction had been successful. The mass spectrum collected didn't reveal a mass ion peak for the desired product but it did reveal some unreacted allylamine as well as some *N*-methylmorpholine-*N*-oxide. In addition to these peaks, the base peak was *m/z* 235 which could be due to either the di-hydroxylated product undergoing multiple ionisation (due to the high number of basic sites present) or it could correspond to the sodium salt of the product minus one hydroxyl group.

The viability of this reaction has been investigated, however it cannot be stated conclusively if it was successful or not. Further work needs to be dedicated into developing the reaction further to allow the subsequent purification and isolation of the product. The instability of these 5-hydroxyimidazole compounds means that the usual methods used for isolation and purification, such as silica gel flash column chromatography couldn't be used due to the acidic nature of the silica column.

In an extension of this work, the cross metathesis of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate (46) with 3-buten-1-ol was investigated (scheme 69). Cross metathesis involves the intermolecular reaction of two terminal alkenes (transalkylidenation) with the release of ethene. Blackwell *et al*.[249] reported several new approaches for the cross-metathesis of unhindered terminal olefins using ruthenium metathesis catalysts, also known as Grubbs catalysts.



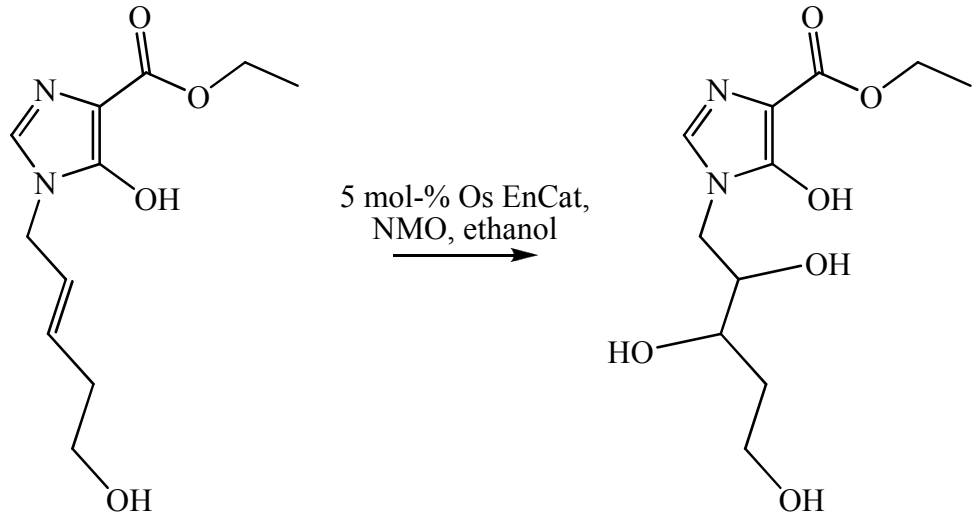
(46) (81)

Scheme 69 Cross metathesis of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate with 3-buten-1-ol.

Ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate was suspended in ethanol and reacted with 3-buten-1-ol in the presence of a 2nd generation Grubbs catalysts, the solution was left to stir for 24 h. A hot filtration of the reaction was performed to remove the Grubbs catalyst and then the solvent was removed under reduced pressure to give a dark brown gum, which wouldn't crystallise from bench solvents. NMR analysis of the crude gum obtained was inconclusive.

Mass spectrometry of the crude gum obtained revealed an *m/z* peak at 241 which relates to the corresponding product, however as previously discussed, the instability of these 5-hydroxyimidazole compounds means that the usual methods used for isolation and purification, such as silica gel column chromatography couldn't be used. Due to time restrictions optimisation of the reaction conditions required wasn't achieved, however further development of this reaction would be a useful next step in order to further increase the library of acyclic sugar analogues of the 5-hydroxyimidazole compounds.

If this optimisation of the Grubb's reaction could be successfully achieved and the product isolated, the next logical step would be to take the hydroxypent-2-enyl derivative (81) and providing the reaction had been optimised, subject the hydroxypent-2-enyl derivative to the same conditions as used previously for the Sharpless asymmetric-type dihydroxylation reaction (as used for ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate) to give the tri-hydroxypentyl derivative (82) as an acyclic sugar analogue (scheme 70).



(81) (82)

Scheme 70 Possible dihydroxylation of ethyl 5-hydroxy-1-(5-hydroxypent-2-enyl)-imidazole-4-carboxylate.

If successful, this type of reaction would allow accessibility to a whole host of acyclic sugar analogues of bredinin to be produced, further developing the library of analogues available for biological testing.

5.4 Summary

Bromination of ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate wasn't successfully achieved. This result means that currently the best method of incorporation of substituents at position 2 of the imidazole ring is to use different variants of the orthoimidates.

Both the ethyl ester and the carbon at position 4 of the imidazole ring proved to be stable to all attempted reactions targeted at these positions. No degradation of the imidazole was observed in any of the attempted reactions but the conditions used in all examples were relatively mild.

Although evidence exists that the 5-hydroxyimidazoles exhibit tautomeric forms, and indeed even in the present study this idea appears to be supported, attempted exploitation of the 1,3-dipole-type system that exists whilst the imidazole is in the keto form proved unsuccessful and only starting material was recovered from the reaction. This could be an indication of the additional stability the keto form experiences due to conjugation of the negative charge through the imidazole ring as well as the carbonyl bond.

Reactivity of the hydroxyl group at position 5 of the imidazole ring was highly substrate dependent. No suitable functional groups were found that could be reacted with the hydroxyl function which could additionally double up as an appropriate method of protecting this hydroxyl. Without this protection subsequent manipulation of the ethyl ester at position 4 in order to obtain the desired secondary amide derivatives of these 5-hydroxyimidazole systems couldn't be attempted.

Unexpected benzylation on the nitrogen at position 3 of the imidazole was seen when ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate was reacted with benzyl bromide. However, this leaves the carbon at position 2 of the ring more susceptible to nucleophilic attack and this would result in degradation of the imidazole ring. So length of reaction times could be an important factor that needs to be taken into account when trying to work with these types of imidazole systems.

It is still unclear if the dihydroxylation of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate was successful and therefore further investigation and development of the method is needed to confirm whether this is a viable route to acyclic sugar analogues of the 5-hydroxyimidazoles.

Cross metathesis of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate and 3-buten-1-ol is thought to have been successfully achieved, however once again further development is necessary to increase yields obtained as well as allowing more satisfactory isolation and purification of the product.

From the reactivity studies carried out it was clear that direct manipulation of the ethyl ester at position 4 wasn't going to be as easily achieved as first hoped, so parallel studies were also conducted in an attempt to develop synthetic routes to different 4-substituted analogues of the 5-hydroxyimidazole systems. These parallel synthetic studies are explored in greater detail in chapter six.

CHAPTER SIX

THE CHEMICAL SYNTHESIS OF

4-SUBSTITUTED-5-HYDROXYIMIDAZOLES

6.1 Introduction

As previously stated in the rationale for the present study (chapter four, section 4.2), the aim of the research programme was to design a synthetic strategy that would allow the production of a library of secondary amide analogues of bredinin (figure 39).

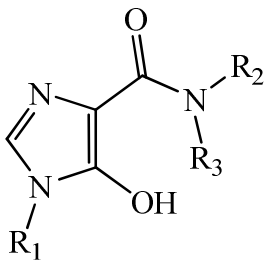


Figure 39 Amide analogue of bredinin.

It was evident from the reactivity studies carried out on the ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate that any desired protection of the hydroxyl function would be difficult. This meant that any subsequent modification of the ethyl ester function of the ethyl 1-subtituted-5-hydroxyimidazole-4-carboxylates couldn't be considered, hence an alternative route to other 4-substituted derivatives was investigated.

With this in mind, two parallel synthetic studies were conducted to assess the possibility of synthesising a different 5-hydroxyimidazole target with the hope for functional group manipulation at the 4-position to give the secondary amide derivatives of the 5-hydroxyimidazole-4-carboxamides.

6.2 Synthesis of 5-Hydroxyimidazole-4-carbonitriles

6.2.1 Synthetic Strategy

In a parallel study the synthesis of 5-hydroxyimidazole-4-carbonitriles was attempted (figure 40). If these 5-hydroxyimidazole-4-carbonitriles derivatives could be successfully synthesised it would allow for the nitrile function at position 4 on the imidazole ring to be hydrolysed under mild conditions directly to the primary amide derivatives of the desired 1-substituted-5-hydroxyimidazole-4-carboxamides.

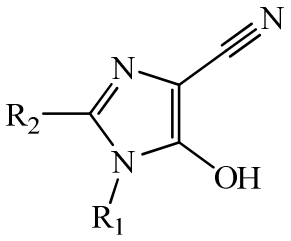


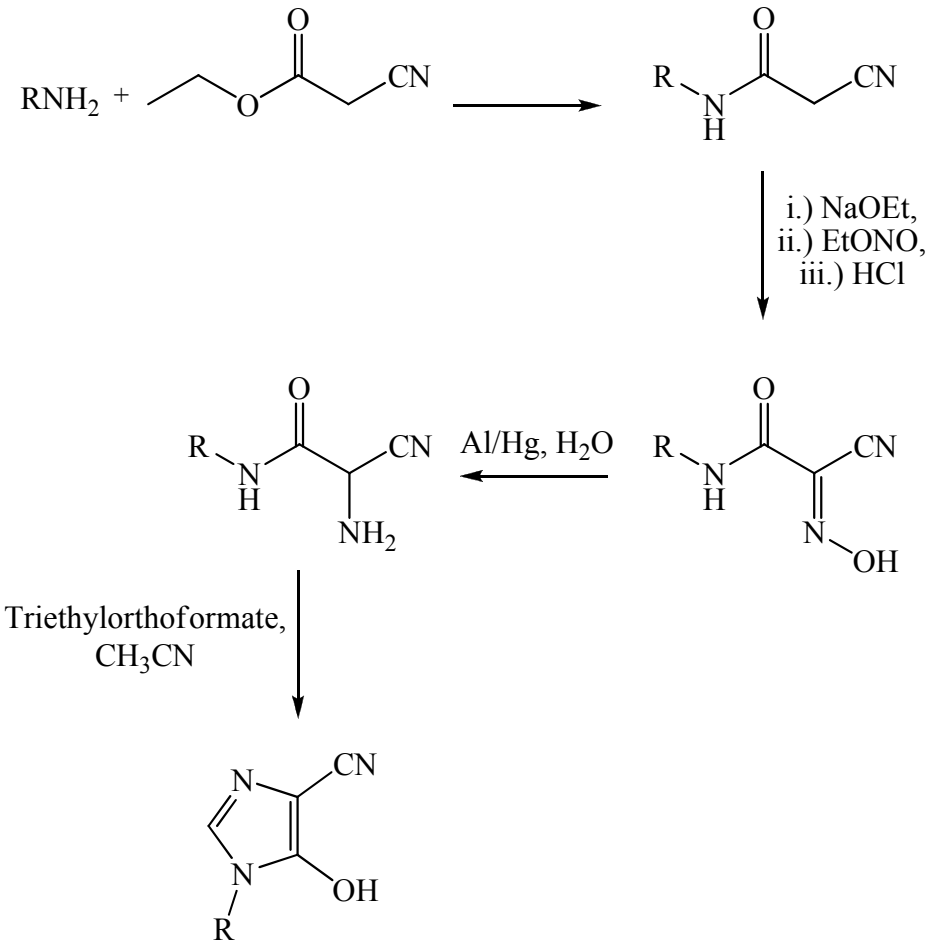
Figure 40 Carbonitrile analogue of bredinin.

Additionally, the nitrile function would allow access to a variety of other functional groups at position 4:

- Hydrolysis could provide access to amides or carboxylic acids,
- Reduction could provide access to amines,
- Nitriles can also undergo nucleophilic addition with alcohols in the presence of an acid catalyst to give imino esters or alkyl imidates.

In the case of the present study, the 5-hydroxyimidazole-4-carbonitriles (scheme 71) were the initial synthetic target. The final developed synthetic strategy involved the reaction of ethyl cyanoacetate with a primary amine to give *N*-substituted cyanoacetamide. The resulting *N*-substituted cyanoacetamide was then reacted with a solution of ethyl nitrite in ethanol in the presence of an equimolar amount of sodium ethoxide. This produced the sodium salt of the oxime, which can then be transformed into the oxime by acidification with hydrochloric acid.

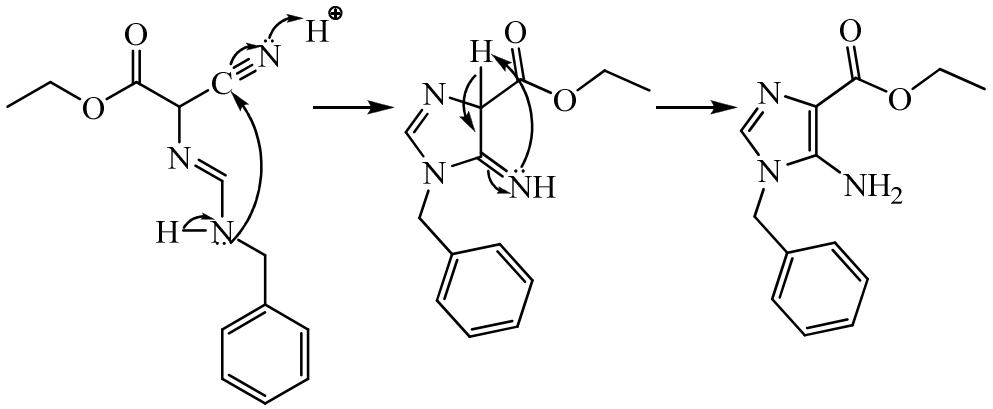
Reaction of the oxime with an aluminium amalgam gave the 2-amino-*N*-substituted-2-cyanoacetamide. The imidate, could then be prepared *in situ* by reaction of 2-amino-*N*-substituted-2-cyanoacetamide with triethyl orthoformate in refluxing acetonitrile. This should then undergo intramolecular cyclisation to give the 1-substituted-5-hydroxyimidazole-4-carbonitrile.



Scheme 71 Developed carbonitrile synthesis of bredinin analogues.

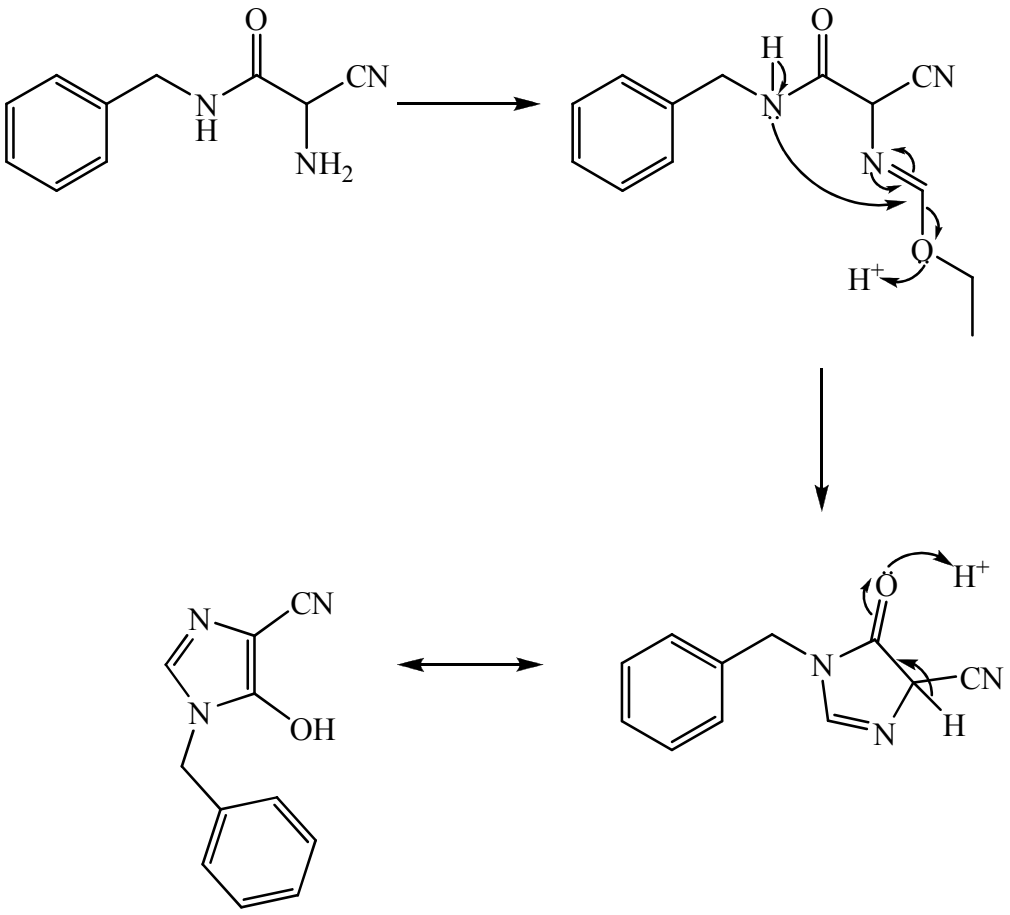
An important point to be noted with this particular synthetic strategy is that unlike the synthetic route devised for the ethyl 5-hydroxyimidazole-4-carboxylates, the modified Shaw synthesis couldn't be used. This is due to the fact that in the modified Shaw synthesis, the final step involves the amidine undergoing intramolecular cyclisation to form the imidazole. However, if this were to occur with the equivalent ethyl N-2-substituted-1-cyano-2-oxoethylformimidate, intramolecular attack would occur on the cyano carbon, as opposed to the ethyl ester carbonyl carbon resulting in the synthesis of

a 5-aminoimidazole instead (scheme 72). It is for this reason that an alternate synthetic route to the 5-hydroxyimidazole-4-carbonitriles was sought.



Scheme 72 Intramolecular cyclisation to form ethyl 1-substituted-5-aminoimidazole-4-carboxylate.

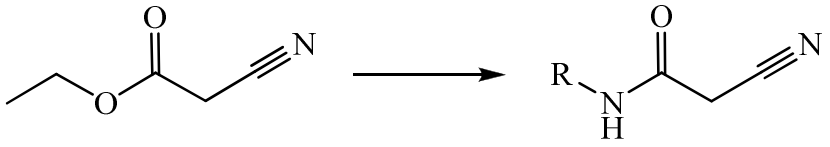
Contrary to the developed synthetic strategy for the synthesis of ethyl 5-hydroxyimidazole-4-carboxylate derivatives, ethyl cyanoacetate is reacted with the primary amine of choice first. This should then result in the formation of the desired 1-substituted-5-hydroxyimidazole-4-carbonitrile as opposed to the corresponding ethyl 1-substituted-5-aminoimidazole-4-carboxylate (scheme 73).



Scheme 73 Mechanism for the formation of 1-substituted-5-hydroxyimidazole-4-carbonitriles.

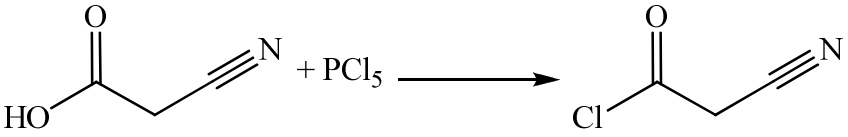
6.2.2 Development of Synthetic Strategy

As with the ethyl ester derivatives, the first objective of the research was to optimise the synthetic scheme for the synthesis of the 1-substituted-5-hydroxyimidazole-4-carbonitriles. To do this each step in the synthetic scheme was developed individually and optimised in order to keep the yields of the precursors synthesised as high as possible.



Scheme 74 Formation of *N*-substituted cyanoacetamides.

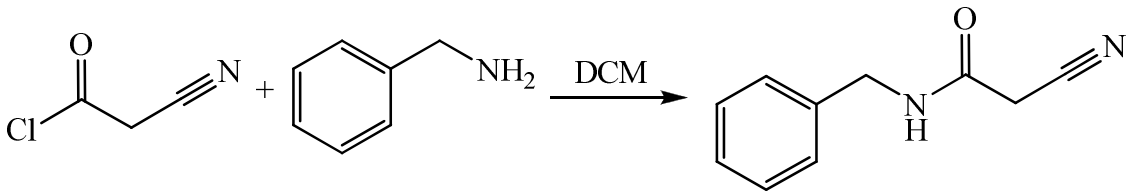
Of the two approaches that were designed, the first was developed following a method reported by Ireland *et al*.[250] in which cyanoacetic acid was suspended in anhydrous diethyl ether and then phosphorus pentachloride was added, in portions, to the solution. The mixture was cooled with an ice bath to prevent excessive refluxing, and after the addition was complete, stirred for a further 0.5 h. Ether was removed under reduced pressure, after which phosphoryl chloride was removed by evaporation with an oil pump and Cryo cool trap with a bath temperature of 35 °C, to give the 2-cyanoacetyl chloride as a red, oily residue (scheme 75).



Scheme 75 Formation of 2-cyanoacetyl chloride.

As benzylamine resulted in the synthesis of one of the most easily isolated and purified ethyl 5-hydroximidazole-4-carboxylates, due to it being suitably lipophilic, it seemed a logical choice for it to be the first amine tried in the carbonitrile synthetic strategy.

2-Cyanoacetyl chloride was then added drop-wise to a solution of dry benzylamine in dichloromethane in the presence of triethylamine, stirred for 1 h at 0 ºC, then left at 4 ºC overnight. The mixture was quenched by being poured into a rapidly stirring, ice cold solution of saturated sodium hydrogen carbonate. The organic phase was extracted, washed with water, dried and the solvent removed under reduced pressure to give a dark red oil (scheme 76).

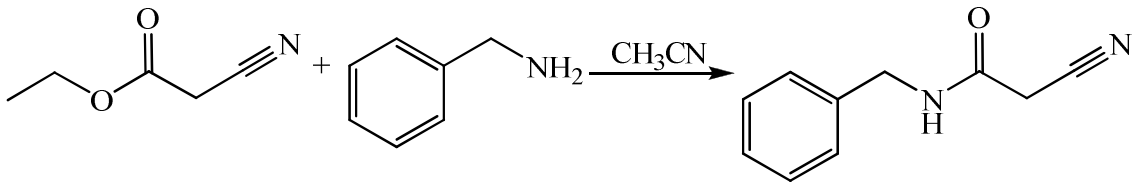


(83)

Scheme 76 Formation of *N*-benzyl cyanoacetamide.

NMR analysis of the oil obtained showed the presence of several impurities. In addition, as an alternative one step method to synthsise *N*-substituted cyanoacetamides had been found the above method was not pursued further.

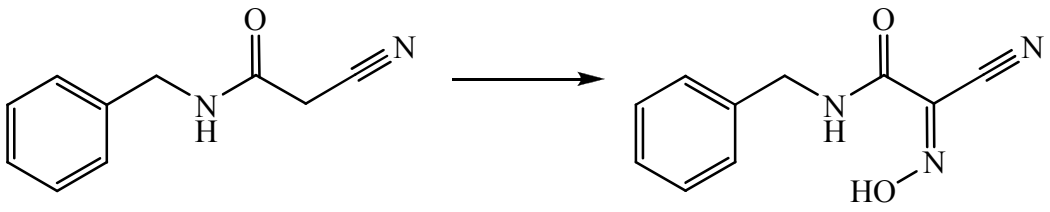
A more recent publication[251] reported the direct synthesis of *N*-substituted-cyanoacetamides by simply reacting ethyl cyanoacetate and benzylamine directly.



(83)

Scheme 77 Direct formation of *N*-benzyl cyanoacetamide.

Ethyl cyanoacetate and benzylamine were refluxed in acetonitrile overnight, the solution was cooled and the solvent removed under reduced pressure to give a pale yellow oil which was dissolved in ethyl acetate and left at 4 ºC overnight. The *N*-benzyl cyanoacetamide (83) was collected as pale yellow, needle-like crystals (81 %). IR examination of the gum showed ν C=O 1682 cm^{-1}, ν CN 2258 cm^{-1} and ν N-OH 3368 cm^{-1}. The structure of the *N*-benzyl cyanoacetamide was further confirmed by NMR spectroscopy and mass spectrometry.



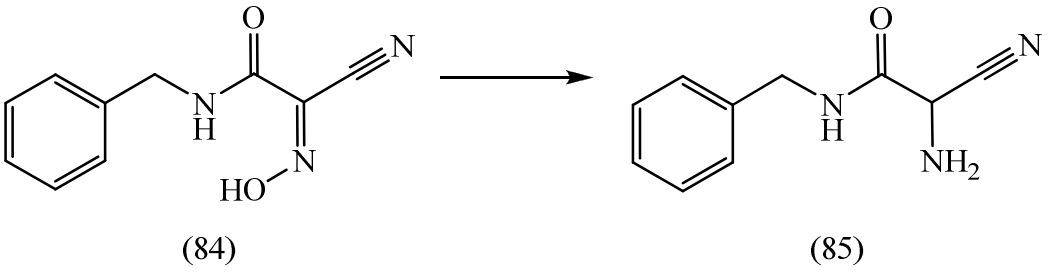
(83) (84)

Scheme 78 Formation of 2-(benzylamino)-*N*-hydroxy-2-oxoacetimidoyl cyanide.

Initially the same method of oxime synthesis as that used in the synthesis of the diethyl-2-hydroxyimino malonate[210] was used, however no reaction was observed by TLC and only the starting material was recovered. This particular method of nitrosation under acidic conditions ($NaNO_2$ and AcOH) relies on the malonate derivative forming the enol structure and then attack of the enol on the nitrosonium ion to give the oxime. It

should also be noted that this method is also believed to be inappropriate for *N*-substituted amides because these compounds are virtually insoluble in water which is the solvent system used in acidic nitrosation reactions.[252]

An alternative method[253,254] was found in which a solution of ethyl nitrite in ethanol was added to a suspension of the enolate of the desired *N*-substituted cyanoacetamide in ethanol, generated by reaction in the presence of an equimolar amount of sodium ethoxide. This rapidly produces the sodium salt of the respective oxime in high yields, which can then be transformed into the oxime (84) by acidification with hydrochloric acid. TLC examination of the pale green crystals obtained revealed a green spot when visualised with 5 % copper sulphate solution and IR examination of the gum showed ν C=NOH 1649 cm-1, ν C=O 1694 cm^{-1} ν CN 2252 cm^{-1} and ν N-OH 3325 cm^{-1}. The structure of this oxime was further confirmed by NMR spectroscopy and mass spectrometry.



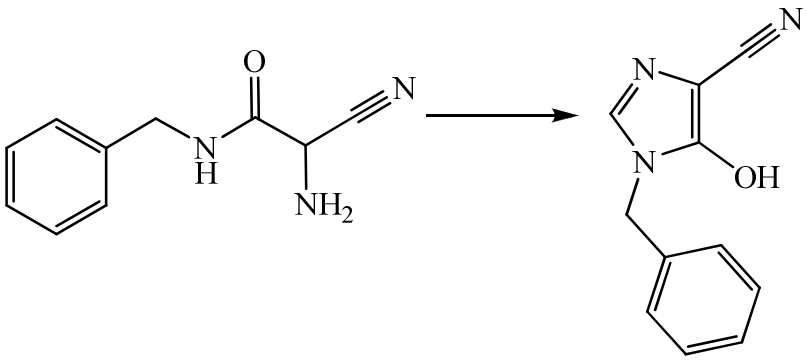
(84) (85)

Scheme 79 Formation of 2-amino-*N*-benzyl-2-cyanoacetamide.

Several different methods have been discussed previously for the reduction of this oxime to its corresponding amine (85). However, for the reduction of 2-(benzylamino)-*N*-hydroxy-2-oxoacetimidoyl cyanide problems were experienced with all of those attempted, except the aluminium amalgam.

Sodium dithionite was tried initially, however solubility issues were experienced with 2-(benzylamino)-*N*-hydroxy-2-oxoacetimidoyl cyanide in the solvent system necessary for the reduction to work successfully. Reduction was then attempted using ammonium formate and zinc dust, but no reaction was observed on TLC. Hydrogenation using 10 % palladium on carbon wasn't considered because it could result in cleavage of the benzyl group.

2-(Benzylamino)-*N*-hydroxy-2-oxoacetimidoyl cyanide was dissolved in methanol and added to a freshly prepared suspension of aluminium amalgam in methanol. Water was added at such a rate so as to maintain a gentle reflux and the solution was left stirring for 1 h. The resulting 'cake' was filtered, washed with methanol and the solvent removed under reduced pressure to give 2-amino-*N*-benzyl-2-cyanoacetamide as a pale orange gum which crystallised on cooling. TLC examination of the crystals obtained revealed a brown spot when visualised with 5 % copper sulphate solution. The structure of this amine was further confirmed by NMR spectroscopy and mass spectrometry.



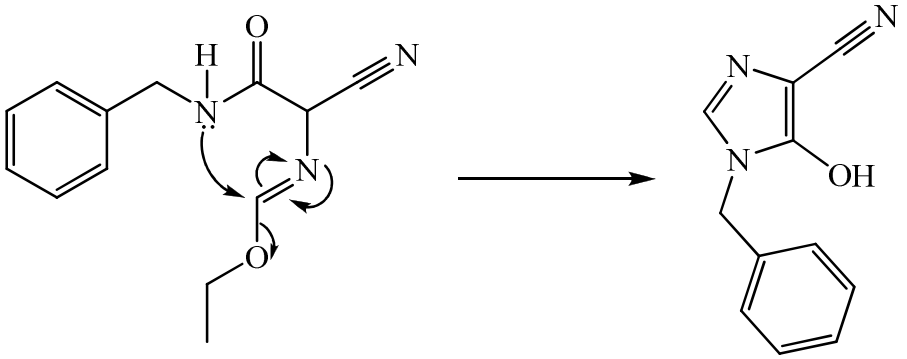
(85) (86)

Scheme 80 Formation of 1-benzyl-5-hydroxyimidazole-4-carbonitrile.

The cyclisation step of the synthetic strategy was again attempted using both triethyl orthoformate and ethyl formimidate hydrochloride. However isolation of the product proved difficult when using ethyl formimidate hydrochloride due to the by-product formed, ammonium chloride. In light of this, it was decided that in all future syntheses of this type, triethyl orthoformate would be used in preference to ethyl formimidate hydrochloride.

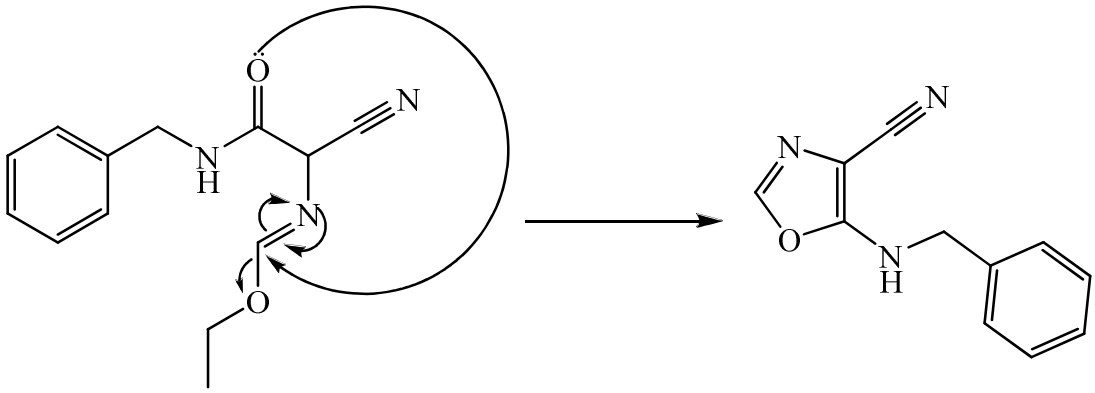
^{1}H-NMR analysis of the product formed appeared to indicate the imidazole had possibly formed, however the signal expected for the proton at position 2 of the imidazole ring was shifted slightly further downfield than expected and it appeared to show a splitting. Additionally, the benzylic -CH_2- was split into a doublet which was believed to be a result of coupling of these protons with an -NH-. Both of these observations suggesting that if cyclisation had occurred, it hadn't involved the amide nitrogen. This result led to the investigation of alternative methods of cyclisation. Two cyclisations were identified:

- The first one is the expected route, in which the lone pair on the nitrogen of the primary amine undergoes intramolecular cyclisation onto the carbon of the imidate.



Scheme 82 Expected cyclisation of ethyl *N*-2-(benzylamino)-1-cyano-2-oxoethylformimidate.

- The second was one in which the lone pair on the oxygen of the carbonyl undergoes intramolecular cyclisation onto the carbon of the imidate.



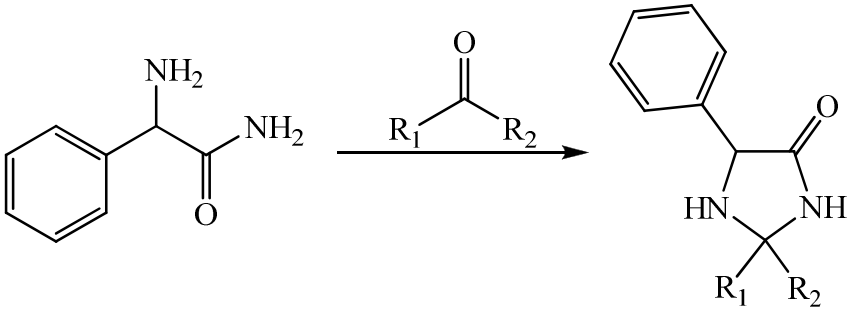
Scheme 83 Alternative cyclisation of ethyl *N*-2-(benzylamino)-1-cyano-2-oxoethylformimidate.

The alternative cyclisation proposed above appeared to support the ^{1}H-NMR data, however ^{13}C-NMR analysis of the product obtained showed that only nitrile and benzylic carbon signals were present, and no heterocycle signals were observed. Although even with the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylate derivatives, when the product had been successfully synthesised, the aromatic heterocycle peaks were sometimes fairly weak and difficult to see if an aromatic group

was present in the compound. Therefore to rule this out, a ^{13}C-NMR with more scans was also performed to see if any imidazole peaks were actually present but just being hidden in the noise of the original scan. This spectrum didn't reveal any extra peaks, indicating either the ethyl *N*-2-(benzylamino)-1-cyano-2-oxoethylformimidate hadn't formed or if it had that it hadn't cyclised as expected.

Mass spectrometry of the product obtained revealed a mass ion peak of 168.9, but no fragmentation pattern could be assigned to correspond to this peak. Therefore, it is believed from all of the analysis conducted that the formation of ethyl *N*-2-(benzylamino)-1-cyano-2-oxoethylformimidate wasn't occurring as expected. 2-Amino-*N*-benzyl-2-cyanoacetamide can be successfully synthesised and is fully characterised, therefore the reaction sequence must be failing at a point after this step in the reaction sequence, but the exact nature of the product obtained couldn't be resolved.

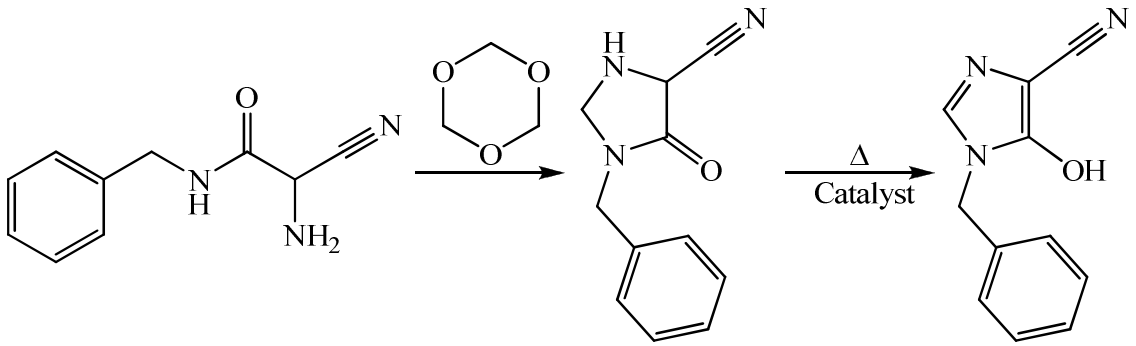
Since the reaction wasn't proving to be straightforward, an alternative approach considered was to react an amino amide with formaldehyde, or an equivalent derivative such as 1,3,5-trioxane (in acidic conditions, it decomposes to give three molecules of formaldehyde), to give an imidazolidinone.[255]



R_1	R_2
$-(CH_2)_4-$	
$-(CH_2)_5-$	
$-CH_3$	$-C_2H_5$
$-CH_3$	$-CH_3$
$-C_6H_5$	-H

Scheme 84 Synthesis of substituted imidazolidinones.

If a similar reaction could be carried out using 1,3,5-trioxane and 2-amino-*N*-benzyl-2-cyanoacetamide, dehydrogenation of the imidazolidine, 1-benzyl-5-oxoimidazolidine-4-carbonitrile, to the corresponding imidazole could be investigated.



Scheme 85 Possible alternative synthesis of the 5-hydroxyimidazole-4-carbonitriles.

Since 2-amino-*N*-benzyl-2-cyanoacetamide can be synthesised this route may offer a good method for the synthesis of 5-hydroxyimidazole-4-carbonitriles. Due to time restrictions however, this method was never attempted but it could be considered in the future if an approach to these 5-hydroxyimidazole-4-carbonitriles was required.

6.3 Synthesis of 5-hydroxyimidazole-4-carboxamides

6.3.1 Synthetic Strategy

As an extension of the previous investigations on the 5-hydroxyimidazole-4-carbonitrile synthesis, a direct route to the primary amide derivatives of the 5-hydroxyimidazole-4-carboxamides was also attempted (figure 41).

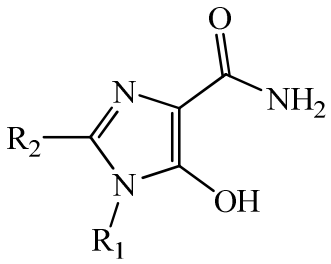


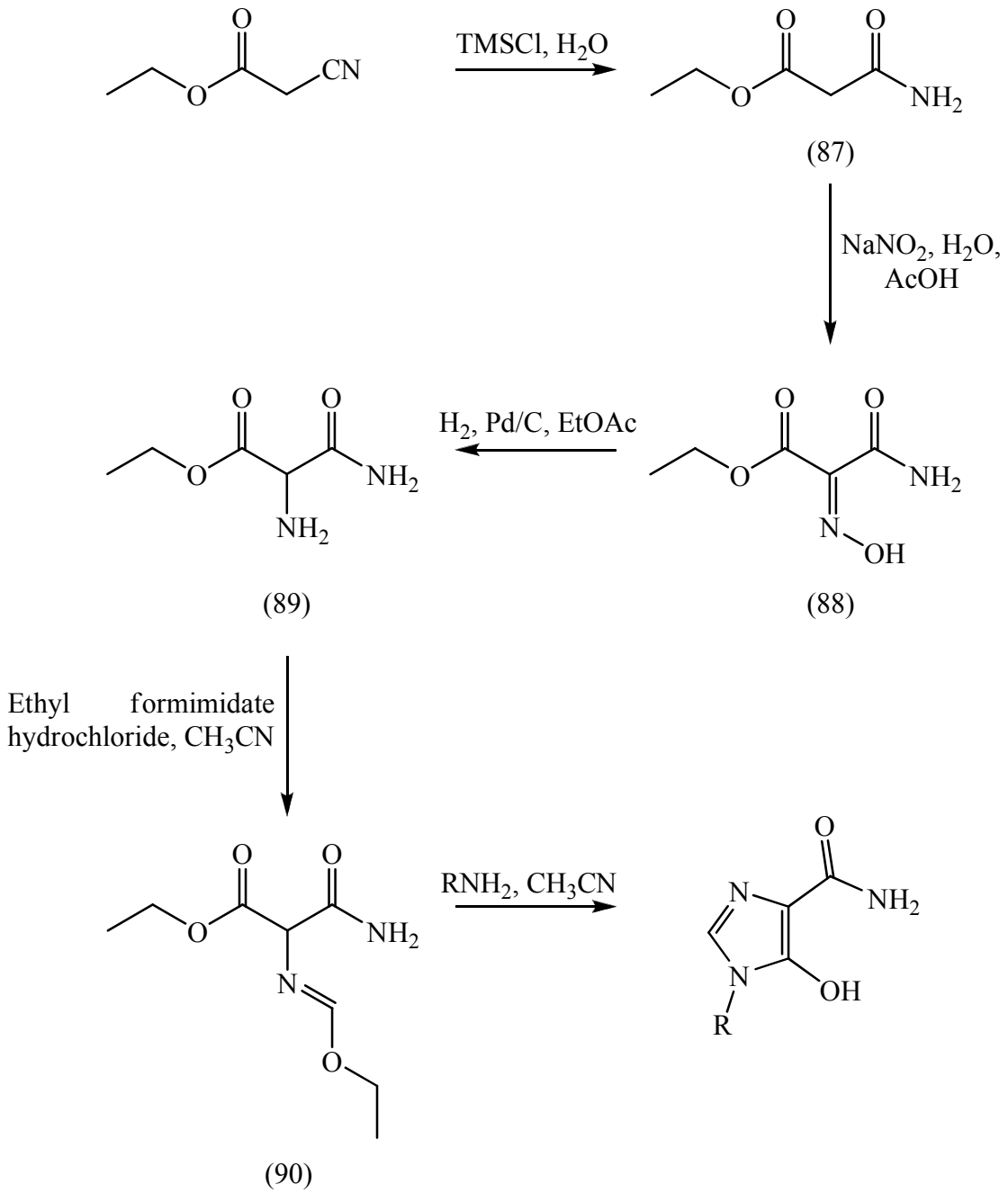
Figure 41 Primary amide analogue of bredinin.

The synthetic targets were the 5-hydroxyimidazole-4-carboxamides (scheme 86). The developed strategy involved the reaction of ethyl cyanoacetate with trimethylsilyl chloride and water to give ethyl 3-amino-3-oxopropanoate (87). Reaction of the resulting ethyl 3-amino-3-oxopropanoate with aqueous sodium nitrite in the presence of acetic acid to give ethyl 3-amino-2-(hydroxyimino)-3-oxopropanoate (88) as a yellow gum which would not crystallise from bench solvents.

Hydrogenation of 3-amino-2-(hydroxyimino)-3-oxopropanoate with 10 % palladium on carbon in ethyl acetate gave ethyl 2,3-diamino-3-oxopropanoate (89). The imidate, ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate (90), was then prepared by reaction of ethyl 2,3-diamino-3-oxopropanoate with ethyl formimidate hydrochloride in acetonitrile at room temperature for 1 h, followed by filtration of the ammonium chloride by-product.

Finally, as with the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates, addition of a 2 mol equivalent of a primary amine to ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate should hopefully result in the formation of an amidine which can undergo intramolecular cyclisation to furnish the desired 1-substituted-5-hydroxyimidazole-4-carboxamides as a salt of the primary amine added.

If successful this salt could then be purified to give the 'free' 5-hydroxyimidazole. It is believed that a recrystallisation from a solvent such as ethanol would be the best method for this purification. An acid-base workup, as used for the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates, could not be used for these derivatives due to the increased solubility of the 5-hydroxyimidazole-4-carboxamides in aqueous solutions.

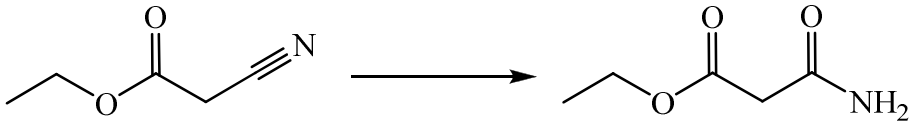


(87) (88) (89) (90)

Scheme 86 Developed direct amide synthesis of bredinin analogues.

6.3.2 Development of Synthetic Strategy

Once again the first objective of the research was to optimise the synthetic scheme for the synthesis of the 1-substituted-5-hydroxyimidazole-4-carboxamides.



Scheme 87 Formation of ethyl 3-amino-3-oxopropanoate.

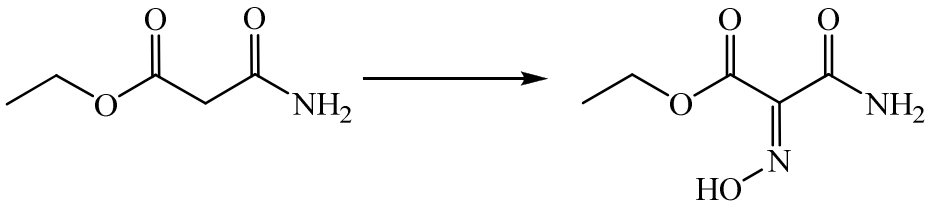
A recent publication[256] showed how to effectively transform a nitrile function into an amide under very mild reaction conditions. The direct synthesis of amide analogues of the 5-hydroxyimidazoles was attempted without the need of protection, hydrolysis and subsequent deprotection as would be required for the ethyl ester 5-hydroxyimidazole carboxylates (covered in more detail in chapter five), or the hydrolysis of the 5-hydroxyimidazole-4-carbonitriles, thus helping to keep yields higher.

Basu *et al*.[256] detailed the transformation of various nitriles into amides using mild conditions. Following this method ethyl cyanoacetate was reacted with trimethylsilyl chloride and water in dichloromethane to give ethyl 3-amino-3-oxopropanoate. The reaction proceeds via protonation of the nitrile group by HCl, which is generated *in situ* from one equivalent of trimethylsilyl chloride and half an equivalent of water:

$$TMSCl + ½\ H_2O \rightarrow HCl + ½\ (TMS)_2O$$

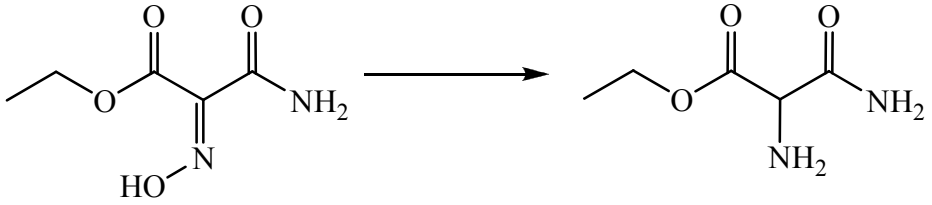
$$RCN + HCl + H_2O \rightarrow RCONH_2.HCl$$

An equivalent of water then reacts with the protonated nitrile group via nucleophilic attack. A proton is then lost to form the lactim tautomer. This then tautomerises by transfer of a proton from the hydroxyl group to the nitrogen to form the more stable lactam tautomer. The water was added drop-wise to ensure it reacted with trimethylsilyl chloride to form HCl rather than acting as a nucleophile and reacting with ethyl cyanoacetate first. IR examination of the clear oil showed ν N-H 3401 cm^{-1}, ν C=O (ester) 1747 cm^{-1}, ν C=O (amide) 1686 cm^{-1}. The structure of this amide ethyl ester was further confirmed by NMR spectroscopy and mass spectrometry.



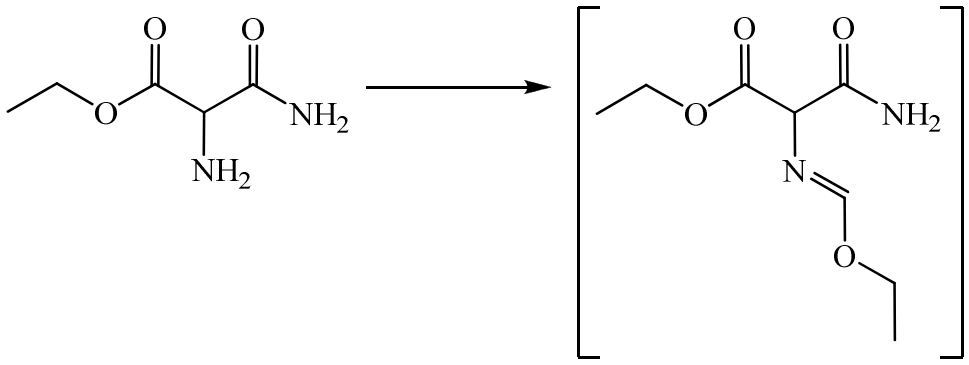
Scheme 88 Formation of 3-amino-2-(hydroxyimino)-3-oxopropanoate.

Reaction of the ethyl 3-amino-3-oxopropanoate with aqueous sodium nitrite in acetic acid gave 3-amino-2-(hydroxyimino)-3-oxopropanoate. The method involved the drop-wise addition of acetic acid to a stirring solution of ethyl 3-amino-3-oxopropanoate, water and sodium nitrite. Sodium nitrite was added as a solid instead of as an aqueous solution due to the instability of sodium nitrite solutions, and the crude 3-amino-2-(hydroxyimino)-3-oxopropanoate was extracted into diethyl ether. TLC examination of the gum revealed a green spot when visualised with 5 % copper sulphate solution and IR examination of the gum showed ν N-H (amide) 3367 cm^{-1}, ν N-H (amine) 3307 cm^{-1}, ν C=O (ester) 1745 cm^{-1}, ν C=O (amide) 1671 cm^{-1}. The structure of this oxime was further confirmed by NMR spectroscopy and mass spectrometry.



Scheme 89 Formation of ethyl 2,3-diamino-3-oxopropanoate.

3-Amino-2-(hydroxyimino)-3-oxopropanoate was reduced, via hydrogenation over 10 % palladium on carbon catalysts, to give ethyl 2,3-diamino-3-oxopropanoate. The reaction initially proceeds by binding of hydrogen to the palladium catalyst by oxidative addition. The oxime then binds to the resulting dihydride complex. Two hydrogen atoms are transferred from the metal to the oxime. Dissociation of the formed amine by reductive elimination then follows. TLC examination of the gum revealed a brown spot when visualised with 5 % copper sulphate solution. The structure of this amine was confirmed by NMR spectroscopy and mass spectrometry.

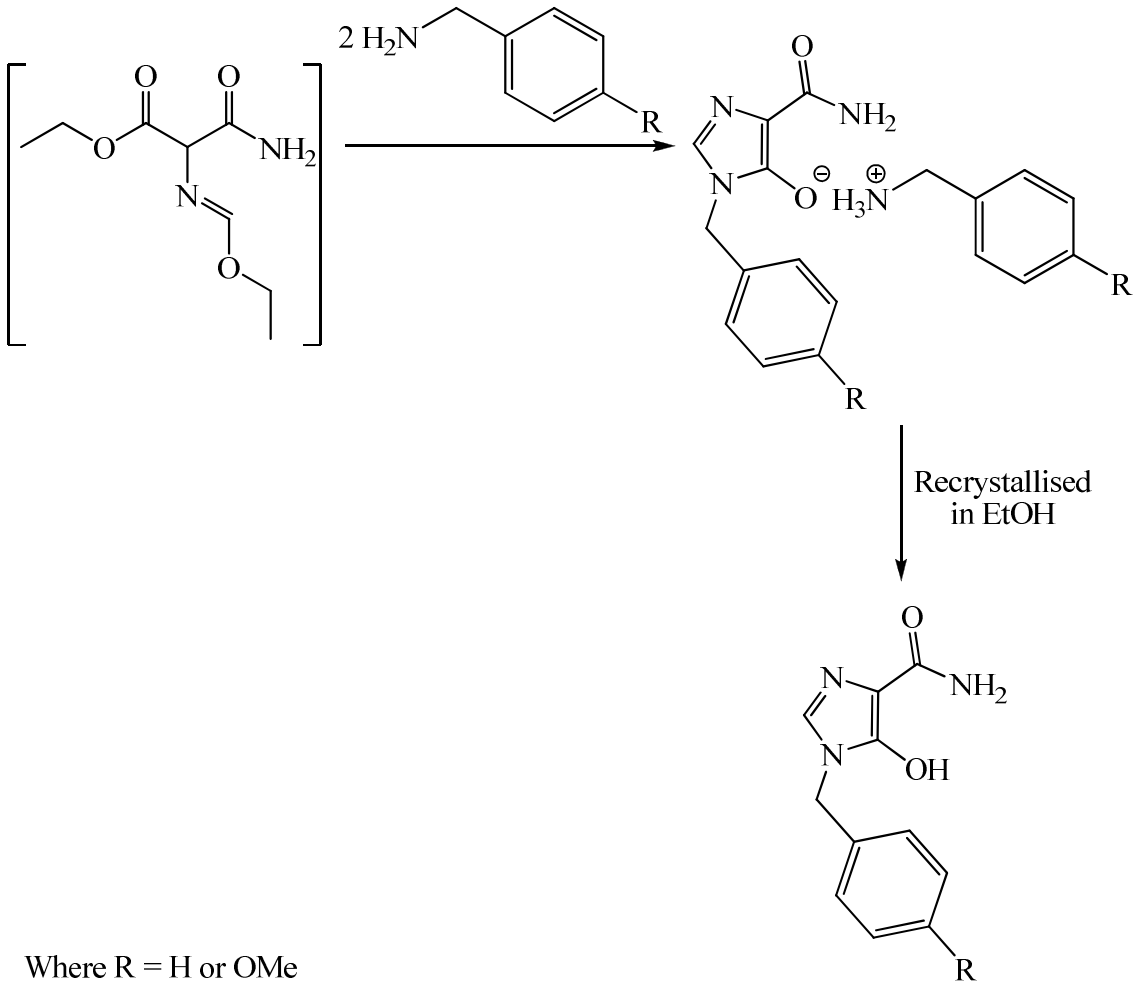


Scheme 90 Formation of ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate.

The imidate, ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate, was prepared following a similar method to that employed in the previously reported ethyl 1-substituted-5-hydroxyimidazole-4-carboxylate derivative synthesis. This involved the reaction of ethyl 2,3-diamino-3-oxopropanoate with ethyl formimidate hydrochloride in acetonitrile, followed by filtration of the ammonium chloride produced. Again as with the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates, ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate was not isolated.

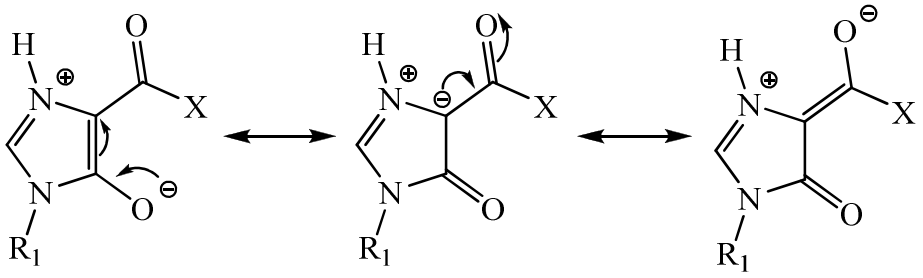
The filtrate containing ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate was then reacted with a 2 mol equivalent of the chosen primary amine with the hope of obtaining the primary amine salt of the 5-hydroxyimidazole-4-carbonitrile.

Again with this synthetic strategy, benzylamine was the first primary amine to be tested in the synthetic route in order to assess the validity of the proposed strategy for the synthesis of the 5-hydroximidazole-4-carboxamide derivatives. In addition to benzylamine, 4-methoxybenzylamine was also tested in the route. As before, this was because both primary amines resulted in the synthesis of the most easily isolated and purified imidazoles for the ethyl 5-hydroximidazole-4-carboxylates, so seemed a logical choice (scheme 91).



Scheme 91 Desired product from reaction of ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate with aromatic primary amines.

The reactions appeared to proceed smoothly and initially were thought to have given the desired products. The ^{13}C-NMR of the products obtained from each reaction each showed two carbonyl peaks. The anomaly of seeing two carbonyl signals in the ^{13}C-NMR was an observation that was sometimes also seen with the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylate derivatives. This is believed to be due to the fact that the imidazole can exist in two tautomeric forms (scheme 92), resulting in the carbonyl carbon giving a slightly different chemical shift depending on the state it is in.



Scheme 92 Tautomerisation of 5-hydroxyimidazoles.

In the case of the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates this tautomeric theory could not be investigated. This is because the investigation would involve adding a base, such as NaOH, to form the sodium salt of the imidazol-5-olate and subsequent analysis of this species. In the case of the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates, the addition of a base would result in the cleavage of the ethyl ester group at position 4 and then the subsequent degradation of the imidazole ring itself.

An investigation was conducted in order to test if this tautomerisation could be the reason that the product wasn't giving the expected ^{13}C-NMR signal pattern. A study was devised that involved repeating the ^{13}C-NMR under different conditions. The NMR sample was re- run in DMSO-d_6 as before, but this time a few drops of 2 M NaOH made up in D_2O were added. The ^{13}C-NMR analysis of the product in the presence of a base revealed no changes from the initial spectra obtained. These results couldn't be used with any confidence to indicate whether or not the 5-hydroxyimidazole systems did in fact switch between tautomeric forms.

Once again the ^{13}C-NMR of the products revealed a lack of imidazole signals. As before, a ^{13}C-NMR with more scans was collected on each sample. These new spectra didn't reveal any extra peaks, indicating that ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate either hadn't formed as expected or that the subsequent cyclisation hadn't occurred and the desired 1-substituted-5-hydroxyimidazole-4-carboxamides had not been successfully synthesised.

In addition, mass spectrometry of the product obtained revealed a mass ion peak at 210.8 for the 4-methoxybenzylamine derivative (expected 247), and one at 180.8 for the

benzylamine derivative (expected 217), but no fragmentation pattern could be assigned to correspond to these peaks. However, the results indicate that whatever the products obtained are, both of the derivatives have reacted in a similar way.

It is believed from all of the analysis conducted that the formation of ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate isn't occurring as expected. However, it is clear that ethyl 2,3-diamino-3-oxopropanoate can be successfully synthesised. Therefore as with the 5-hydroxyimidazole-4-carbonitrile synthetic sequence, the reaction sequence is thought to be failing at a point after this step. Unfortunately, again the exact nature of the product obtained couldn't be resolved.

A point that must be noted is that the final step in the reaction scheme involving the addition of a primary amine to the imidate, is similar to that used by Tarumi et al.[154] who successfully obtained 1-benzyl-5-hydroxyimidazole-4-carboxamide. This would imply that had ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate been successfully synthesised, the imidazole should have also been obtained. Indicating further still that it is the formation of ethyl 3-amino-2-(ethoxymethyleneamino)-3-oxopropanoate which is hindering this reaction sequence.

6.4 Summary

Both routes described above need further development if they were going to be of any use in the future. The final step of each reaction appears to be the one that's failing, so further investigation is needed in order to see if either route would be a viable access route to 4-substituted-5-hydroxyimidazoles.

With the 5-hydroximidazole-4-carbonitriles, it is unclear if it is the formation of the imidate or the subsequent cyclisation that is failing. However, it is clear with the 5-hydroxyimidazole-4-carboxamides that the formation of the imidate is the step that is failing. Therefore alternative methods for the formation of the imidate must be explored.

As yet neither route can be considered as feasible alternatives to the route developed for the ethyl 1-substituted-5-hydroximidazole-4-carboxylates, to obtain other 4-substituted-5-hydroxyimidazoles.

If the routes were to be developed and 4-substituted-5-hydroxyimidazoles were synthesised, the next most logical step would be to try and optimise the reaction sequences using acyclic sugar analogues.

CHAPTER SEVEN

CONCLUDING REMARKS

7.1 Conclusions

Several routes currently exist for the synthesis of 5-hydroxyimidazoles, however they are ambiguous and not easily adapted to the production of a library of compounds for biological analysis. In the present study, novel linear and convergent strategies for the synthesis of several potentially, biologically important 5-hydroxyimidazoles have been developed. Using these new devised routes it's possible to successfully synthesise a variety of 5-hydroxyimidazoles unambiguously with an assortment of functional groups present on the imidazole ring itself.

The convergent synthetic route devised involved the use of simple acyclic precursors to synthesise the desired ethyl 5-hydroxyimidazole-4-carboxylates. The benefit of this new strategy, compared with those already reported in the literature, is that it allowed for the addition of substituents in both position 1 and 2 of the imidazole ring unambiguously. However, the route is limited by the fact that the final step of the synthesis involves the intramolecular cyclisation of an amidine which cannot occur if the nitrogen attacking is not nucleophilic enough or is hindered if the side chain attached to this nitrogen is sterically demanding.

This route is useful in providing a library of compounds for initial biological testing, however if a comprehensive library is required then this route will fail to provide compounds which have several polar groups present making them particularly hydrophilic. This is due to the problems experienced with the isolation and purification of the compounds.

Purification of the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates obtained from the convergent synthesis proved difficult for two reasons; (a) their solubility varied depending on the functional groups present on the imidazole ring system, and (b) traditional methods of purification, such as silica gel column chromatography, proved unsuccessful. Therefore, a qualitative investigation was carried out which hoped to explore any possible relationship between lipophilicity of the primary amine used in the cyclisation step of the synthetic route, and the ease of isolation and purification of the resulting ethyl 1-substituted-5-hydroxyimidazole-4-carboxylate.

The study found that both lipophilicity and steric bulk played a crucial role, and that a compromise between the two must be achieved to give the optimal reaction conditions. The more lipophilic the amine was, the easier the resulting ethyl 1-substituted-5-hydroxyimidazole-4-carboxylate was to isolate and subsequently purify. However, those highly lipophilic amines, that were also sterically demanding, hindered the cyclisation step of the synthesis so the ethyl 1-substituted-5-hydroxyimidazole-4-carboxylate couldn't form. These observations must be taken into account when planning any new synthetic targets.

Additionally a novel, linear synthetic route to bredinin from acyclic precursors was also developed. This route is particularly advantageous over those already reported in the literature because it allows for the addition of substituents in positions 1, 2, 4 and 5 of the imidazole ring unambiguously.

Another advantage of this route is that it allows for the possibility of synthesising secondary amide derivatives of 5-hydroxyimidazole-4-carboxamides, which as stated in the rationale for the present work, are the ultimate target compounds. This can be achieved before cyclisation has occurred and therefore means that it wouldn't require the manipulation of any groups on the imidazole ring itself. This means that there would be no interference from other functional groups present; a particular issue that has to be addressed when using the convergent synthetic strategy for the synthesis of these ethyl 1-substituted-5-hydroxyimidazole-4-carboxylates. However, as yet the yields obtained are modest, therefore the route would need further development if it were to provide a reasonable access route to the much desired secondary amide derivatives of the 5-hydroxyimidazole-4-carboxamides.

An interesting difference between the two synthetic routes is that the sugar analogues of the 5-hydroxyimidazoles were successfully synthesised using the linear synthetic strategy. By contrast, the sugar analogues could not be synthesised using the convergent strategy. This is thought to be because the nitrogen on the nucleoside was not nucleophilic enough to be able to attack and undergo intramolecular cyclisation to give the resulting 5-hydroxyimidazole nucleoside. This result indicates that nucleophilicity of the amine in the final step of the synthesis could play a role in

determining whether or not the cyclisation in the convergent synthesis is successful, a factor which might be worth exploring further in the future.

The reactivity of the 5-hydroxyimidazole system was also assessed using ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate as a model. The investigation revealed several key intricacies about the 5-hydroxyimidazole system;

- The nitrogen at position 3 of the imidazole ring is the most basic atom in the ring,[231] after attempts to benzylate the oxygen of the 5-hydroxyl group resulted in benzylation of this nitrogen instead.
- The carbon at position 4 of the imidazole ring was stable to all attempts of intermolecular attack.
- Although these 5-hydroxyimidazole systems are believed to exhibit both keto and enol tautomeric structures, and bear close structural similarity to 1,3-dipole systems, they do not react in a similar way.
- The reactivity of the hydroxyl group at position 5 of the imidazole ring is substrate dependent.

As already mentioned, the reactivity of the 5-hydroxyl group was assessed, this was to see if any suitable protecting groups could be introduced at this position to allow the manipulation of the ethyl ester group at position 4 of the imidazole. In the initial stages of the present study it was hoped that if these ethyl 5-hydroxyimidazole-4-carboxylates could be synthesised that they would provided a relatively easy route to the synthesis of a variety of other 5-hydroxyimidazoles. It was hoped that by protecting the hydroxyl oxygen with an orthogonal group, that the ethyl ester group could be relatively easily manipulated to give the desired secondary amide derivatives of the 5-hydroxyimidazoles. Unfortunately no suitable groups were found that could be reacted successfully to allow subsequent manipulation of the ethyl ester at position 4.

With this in mind, a route to the benzyl 5-hydroxyimidazole-4-carboxylates was also investigated. A route was developed which could successfully synthesise benzyl 1-benzyl-5-hydroxyimidazole-4-carboxylate. This provides an alternative avenue to pursue in the future if no suitable hydroxyl protecting group can be found for the ethyl

5-hydroxyimidazole-4-carboxylates. Unlike the ethyl ester which is cleaved under basic conditions, the benzyl ester is cleaved under neutral conditions. The only groups that could be successfully reacted at the hydroxyl position were the acid chloride derivatives which are cleaved under basic conditions. Protection using the acid chlorides would not have been suitable for protecting the hydroxyl position of the ethyl ester imidazoles, however they are suitable for protecting the hydroxyl position of the benzyl ester imidazoles. These benzyl 5-hydroxyimidazole-4-carboxylates may also prove to be easier to isolate and purify than the ethyl 5-hydroxyimidazole-4-carboxylates due to the lipophilicity added by having a benzyl ester group present at position 4 of the imidazole ring.

One thing that should be noted from the present study is that these 5-hydroxyimidazole systems proved incredibly difficult to work with, a point that is illustrated in the present study. This is thought to be because they can exhibit zwitterionic properties, which means that the reactivity of the 5-hydroxyimidazole system can't be easily predicted and their purification and solubility causes problems.

The reactivity of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate was also assessed. Dihydroxylation was attempted and is believed to have been successful, however the product was difficult again to isolate and purify. Further development of the method is necessary to correct this and also in order to increase the yield of the product obtained.

Cross metathesis of ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate was also attempted and again is believed to have been successfully achieved, however as before the product was difficult again to isolate and purify. Further development of the method is necessary to correct this and also in order to increase the yield of the product obtained. This could then be used to provide access to a variety of saturated, unsaturated and polyhydroxylated long chain acyclic sugar analogues of the 1-substituted-5-hydroxyimidazoles.

7.2 Future Directions

For the convergent synthetic strategy, the route to benzyl 1-substituted-5-hydroxyimidazole-4-carboxylates currently appears to be the most promising for the exercise of producing compounds which could be manipulated into a variety of secondary amide derivatives of bredinin. Using the benzyl 1-substituted-5-hydroxyimidazole-4-carboxylate derivatives and then reacting the hydroxyl function at position 5 of the imidazole, effectively protecting this position, would allow for the opportunity to manipulate the benzyl ester to a variety of other functional groups without the interference of the hydroxyl or cleavage of the protecting group at this position. This would hopefully allow for the synthesis of the much desired secondary amide derivatives, which could then be sent for testing to assess their potential as biologically active compounds.

If any of the compounds synthesised proved to be useful drugs targets then further development would be required to develop an alternative method for the reduction of dibenzyl 2-(hydroxyimino)malonate to give dibenzyl 2-aminomalonate because currently the aluminium mercury reduction is used and this would not be acceptable for the synthesis of compounds which could potentially be used in human clinical trials.

Should a suitably optimised method be devised for both the dihydroxylation and the cross metathesis of the allylic imidazole derivatives, then it would be a good idea to take the hydroxypent-2-enyl product of the cross metathesis reaction and subject it to the same conditions as used in the dihydroxylation reaction, to give the tri-hydroxypentyl derivative as an acyclic sugar analogue.

The linear strategy is promising in terms of the number of substituents that can be added throughout the strategy, however yields are modest and further development to increase the yields of the products would be a good idea.

EXPERIMENTAL

General Methods

All solvents and reagents were purchased from Sigma Aldrich, Fisher Scientific, or Lancaster Chemicals (Alfa-Asear), and were used as supplied unless otherwise stated. Drying of organic solvent extracts used magnesium sulphate. Bulk solvent removal was by rotary evaporation under reduced pressure. Flash chromatography was carried out using Fluorochem silica gel 60 Å (35-70 μ). All procedures and chemicals were assessed according to COSHH guidelines, prior to commencement of experimental procedures.

Analytical Techniques

Reactions were monitored by thin layer chromatography using Merck aluminium-backed TLC plates coated with silica gel 60 F_{254}. Plates were visualised using either UV radiation (240 nm or 354 nm), 5 % copper (II) sulphate solution, 5 % iron (III) chloride solution, or iodine vapour. All products were homogenous by TLC in various solvents systems unless otherwise stated.

The ^{1}H NMR and proton decoupled ^{13}C NMR spectra were collected on a JEOL JNM-LA400 spectrometer at 400 and 100 MHz respectively. Chemical shifts are given in ppm relative to the residual solvent peaks at 7.27 (^{1}H) and 77.2 (^{13}C) for $CDCl_3$ and 2.50 (^{1}H) and 39.5 (^{13}C) for DMSO-d_6 as internal standards. Assignments were accomplished by coupling constants and integrals. Splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; br, broadened. Hydroxyl signals often weren't visible in the ^{1}H-NMR spectra due to their rapid exchange with either the solvent or water present in the sample. Additionally the amide signal was rarely seen in the ^{1}H-NMR spectra. Some imidazole carbon signals were hidden in the noise and weren't visible on ^{13}C-NMR spectra unless a large number of scans was performed with a zero signal to noise ratio, for these particular compounds it was assumed, from other characterisation data collected, that they had been successfully synthesised.

Mass spectral analysis was carried out by electrospray ionisation (ESI), or electron impact ionisation (EI). Low resolution ESI spectra were collected on a Finnigan LCQ

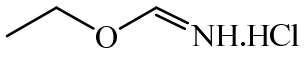
spectrometer, or by the EPSRC National Mass Spectrometry Service at the University of Swansea. Low resolution EI spectra were collected on a Shimadzu QP5050A GC/MS spectrometer. High resolution ESI spectra were collected by the EPSRC National Mass Spectrometry Service at the University of Swansea, on a Finnigan MAT 900 XLT spectrometer with polyethyleneimine as the reference compound. The mass spectrometer was coupled to an HPLC machine on which all samples gave a chromatogram with a single peak unless otherwise stated. All calculated models for accurate mass analysis are based on predominant natural isotopes of C, H, N and O.

UV-Vis spectra were obtained using a Perkin Elmer Lambda Bio 10 UV/VIS Spectrometer.

Infrared spectra were obtained using a Perkin Elmer Spectrum RXI FT-IR Spectrometer.

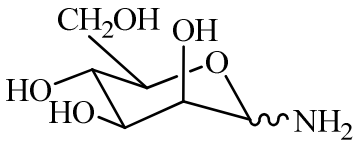
Melting points were determined in capillary tubes, using a Sanyo GallenKamp melting point apparatus and are uncorrected.

Ethyl formimidate hydrochloride[257]



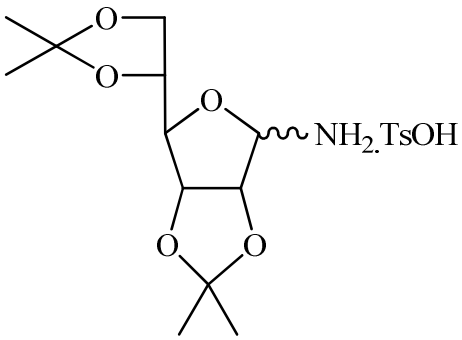
A solution of formamide (45.0 g, 1.0 mol) and ethanol (46.0 g, 1.0 mol) were added drop-wise, with stirring, to a solution of benzoyl chloride (140.6 g, 1.0 mol) in anhydrous diethyl ether (700 ml). The solution was left at 4 ºC for 24 h and the crystalline product was collected, washed well with diethyl ether, and used without any further purification (103.7 g, 0.9 mol, 95 %); δ**H** (400 MHz; DMSO) 8.83 (1H, CH=N, s), 4.57 (2H, CH_2, q, J = 7.1), 1.28 (3H, CH_3, t, J = 7.1); δ**C** (100 MHz; DMSO) 163.3 (CH=N), 59.6 (CH_2), 14.6 (CH_3).

D-Mannopyranosylamine monohydrate[258]



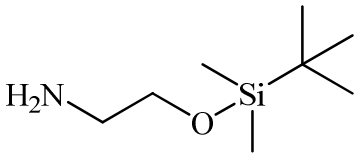
D-Mannose (100.0 g, 0.6 mol) was added in portions to a stirred, ice-cold solution of ammonium chloride (0.5.0 g, 10.0 mmol) and ammonia saturated methanol (100 ml) contained within a screw cap polythene container. During the addition, dry ammonia gas was bubbled through the mixture until all of the D-mannose had dissolved. The treatment with ammonia was continued for a further 15 min. and the reaction mixture seeded, sealed and stored at 4 ºC for 4 days. The product was broken up, filtered, washed with methanol and dried in vacuo (97.0 g, 0.50 mol, 98 %); mp 92 °C (dec), lit. value[258] 93-94 ºC (dec); IR (KBr) cm^{-1} 3470-3175 (O-H, str), 2934 (N-H, str), 1599 (N-H, def).

2,3;5,6-Di-*O*-isopropylidene-D-mannofuranosylamine toluene-p-sulphonate[217]



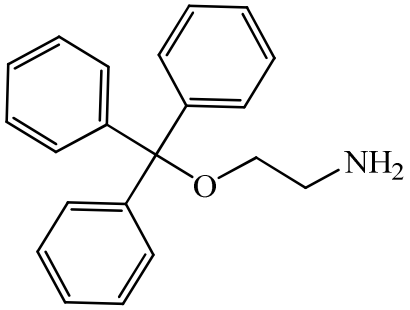
A solution of toluene-p-sulphonic acid monohydrate (126.0 g, 0.7 mol) in 2,2-dimethoxypropane (270 ml, 2.1 mol) and dry acetone (1.2 L) was stirred at room temperature for 15 min. Dry, finely powdered D-mannopyranosylamine monohydrate (66.0 g, 0.3 mol) was added to the reaction mixture and stirred for 2.5 h. The resulting pale brown solution was filtered to remove any solid, and the filtrate evaporated to about one half its original volume. Dry diethyl ether was added to render the solution slightly turbid (~ 500 ml) and the solution set aside at 4 ºC for 24 h. The colourless needle-like crystals were collected, washed with acetone and then diethyl ether and dried *in vacuo* (67.3 g, 0.2 mol, 70 %); mp 131-134 °C (dec), lit. value[216] 132-134 ºC (dec); IR (KBr) cm^{-1} 1382 (gem dimethyl), 1370 (gem dimethyl).

2-(*tert*-Butyldimethylsilyloxy)ethanamine[212]



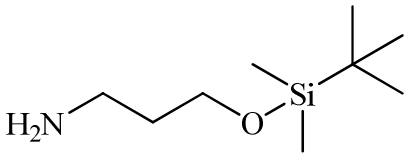
To a solution of ethanolamine (6.7 g, 110.0 mmol) and *tert*-butyl-dimethyl-silylchloride (18.2 g, 120.0 mmol) in dichloromethane (200 ml), was added triethylamine (16.7 g, 165.0 mmol). The cloudy mixture was stirred vigorously at ambient temperature for 18 h. The resulting solution was washed with water and the organic phase collected and dried ($MgSO_4$). The solvent was removed under reduced pressure and the crude oil purified by distillation (bp 65-70 ºC at 3.5 mmHg) to give the title compound as a colourless oil (15.6 g, 89.0 mmol, 81 %); δ**H** (400 MHz; $CDCl_3$) 3.98 (2H, CH_2-O, t, J = 6.2), 3.03 (2H, CH_2-N, t, J = 6.2), 0.84 (3H, CH_3-C, s), -0.01 (3H, CH_3-Si, s); δ**C** (100 MHz; DMSO) 66.1 (CH_2-O), 42.8 (CH_2-N), 30.6 ($C(CH_3)_3$), 25.8 (CH_3-C), -5.4 (CH_3-Si); LRMS (ESI) $[M]^+$ requires 175, found $[M + H]^+$ 176.

2-(Trityloxy)ethanamine[213]



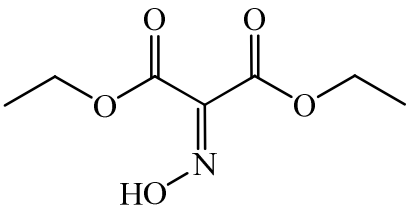
Ethanolamine hydrochloride (6.0 g, 61.5 mmol) was added to a solution of triphenylmethyl chloride (6.0 g, 21.5 mmol) in anhydrous pyridine (20 ml). The mixture was stirred for 3 days and then poured into vigorously stirring water (250 ml). The resulting suspension was filtered and the solid was washed with water and dissolved in ether (50 ml). The solution was transferred to a separating funnel and washed rapidly with 3M HCl (2 x 50 ml). The organic layer was extracted and the solvent removed under reduced pressure to give the *O*-trityl protected amine hydrochloride salt as a white precipitate. The free amine was obtained by stirring the salt in a solution of ether and concentrated potassium hydroxide, the organic phase was collected, washed with water, dried ($MgSO_4$) and the solvent was removed under reduced pressure to give the free *O*-trityl protected amine as a white precipitate (5.3 g, 17.4 mmol, 81 %); δ**H** (400 MHz; $CDCl_3$) 7.21 (15H, Ph, m), 3.76 (2H, CH_2-O, t, J = 7.0), 3.09 (2H, CH_2-N, t, J = 7.0); δ**C** (100 MHz; $CDCl_3$) 135.7 (Ph), 128.40 (Ph), 127.4 (Ph), 126.2 (Ph), 94.5 ($C(Ph)_3$), 68.3 (CH_2-O), 38.9 (CH_2-N); LRMS (ESI) $[M]^+$ requires 303, found $[M + Na]^+$ 326.

3-(*tert*-Butyldimethylsilyloxy)propan-1-amine[212]



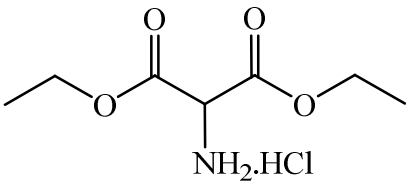
To a solution of 3-amino-1-propanol (8.3 g, 110.0 mmol) and *tert*-butyl-dimethyl-silylchloride (18.2 g, 120.0 mmol) in dichloromethane (200 ml), was added triethylamine (16.7 g, 165.0 mmol). The cloudy mixture was stirred vigorously at ambient temperature for 18 h. The resulting solution was washed with water and the organic phase collected and dried ($MgSO_4$). The solvent was removed under reduced pressure and the crude oil purified by distillation (bp 65-70 ºC at 3.5 mmHg) to give the title compound as a colourless oil (16.9 g, 90.0 mmol, 81 %); δ**H** (400 MHz; $CDCl_3$) 3.65 (2H, CH_2-O, t, J = 6.2), 2.74 (2H, CH_2-N, t, J = 6.2), 1.62 (2H, CH_2, p, J = 6.2), 0.84 (3H, CH_3-C, s), -0.01 (3H, CH_3-Si, s); δ**C** (100 MHz; $CDCl_3$) 60.1 (CH_2-O), 39.4 (CH_2), 35.8 (CH_2-N), 30.6 ($C(CH_3)_3$), 25.8 (CH_3-C), -5.4 (CH_3-Si); LRMS (ESI) $[M]^+$ requires 189, found $[M + Na]^+$ 212.

Diethyl 2-(hydroxyimino)malonate[210]



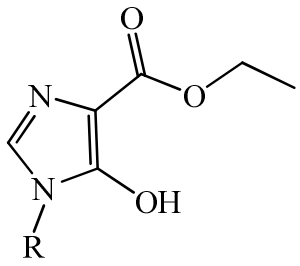
To a mixture of diethylmalonate (50.0 g, 0.3 mol) stirred and cooled to 0 °C, were added glacial acetic acid (57 ml, 0.9 mol) and water (81 ml). Sodium nitrite (65.0 g, 0.9 mol) was added in portions over a period of 1 h. The reaction mixture was stirred at ambient temperature for a further 24 h until all starting materials had been consumed according to TLC. The resulting mixture was extracted with ether (50 ml x 2), the organic phase collected, washed with sodium bicarbonate (80 ml x 6), dried ($MgSO_4$) and evaporated under reduced pressure to give a faint yellow gum as the crude compound (50.6 g, 0.3 mol, 86 %). R_f (chloroform-methanol, 9:1) 0.71 green spot when revealed with $CuCl_2$; δ**H** (400 MHz; $CDCl_3$) 11.34 (1H, OH, s), 4.28 (4H, CH_2, q, J = 7.1), 1.14 (6H, CH_3, t, J = 7.1); δ**C** (100 MHz, $CDCl_3$) 160.8 (C=O), 144.0 (C=N), 41.5 (CH_2), 13.8 (CH_3); IR cm^{-1} 3500-3350 (O-H, str), 2976-2920 (CH_3 and CH_2, str), 1752 (C=O, str), 1452-1437 (C-CH_3, def), 1345 (C-N, str), 1174, 1098, 1029 (C-O); LRMS (ESI) [M^+] requires 189, found [M + Na]$^+$ 212.

Diethyl 2-aminomalonate hydrochloride



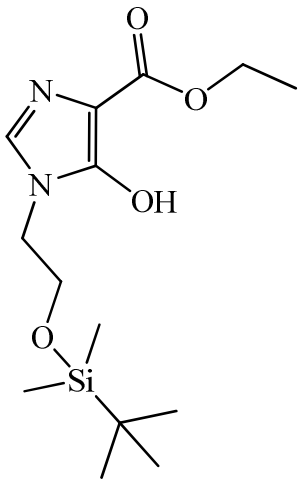
To a stirred solution of diethyl 2-(hydroxyimino)malonate (50.0 g, 0.3 mol), water (161 ml) and saturated sodium hydrogen carbonate (133 ml), was added sodium dithionite (56.6 g, 0.3 mol) in portions. The reaction mixture was heated to 35 ºC and after 5 h, all starting materials were consumed according to TLC. The resulting mixture was extracted with ether (50 ml x 2), the organic layer was collected, dried ($MgSO_4$) and evaporated under reduced pressure to give the crude amine as a faint yellow gum (32.1 g, 0.2 mol, 68 %), R_f (chloroform-methanol, 9:1) 0.36 brown spot when revealed with $CuCl_2$. The crude diethyl aminomalonate was diluted with ether (150 ml), stirred and cooled in an ice bath and dry hydrogen chloride was passed over the solution. The white crystalline precipitate was filtered off and washed with ether to give the title compound (33.5 g, 0.2 mol, 59 %,). The ether washings were combined and the process was repeated until no more precipitate was obtained. Recrystallisation from alcohol-ether affords a more pure product. R_f (chloroform-methanol, 9:1) 0.47 white spot when revealed with $CuCl_2$; mp 140 °C; δ**H** (400 MHz; DMSO) 8.18 (2H, NH_2, s), 4.97 (1H, CH, s), 4.21 (4H, CH_2, q, J = 7.1), 1.04 (6H, CH_3, t, J = 7.1); δ**C** (100 MHz, $CDCl_3$) 163.9 (C=O), 70.0 (C-N), 54.8 (CH_2), 13.9 (CH_3); IR (KBr) cm^{-1} 3349 (NH_3^+, str), 2985-2860 (CH_3 and CH_2, str), 1758 (C=O, str), 1454-1433 (C-CH_3, def), 1368 (C-N, str), 1169, 1084, 1013 (C-O); LRMS (ESI) $[M]^+$ requires 175, found $[M + H]^+$ 176.

General Procedure for the Synthesis of Ethyl 1-Substituted-5-Hydroxyimidazole-4-Carboxylates (unless otherwise stated)



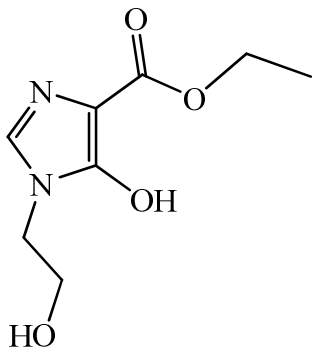
Diethyl 2-aminomalonate hydrochloride (5.0 g, 23.6 mmol) was suspended in dichloromethane (50 ml) and saturated sodium bicarbonate was added (2 x 50 ml). The mixture was shaken in the separating funnel and the organic layer was collected, dried ($MgSO_4$) and evaporated to give the free amine as a faint yellow gum. R_f (chloroform-methanol, 9:1) 0.36 brown spot when revealed with $CuCl_2$; δ**H** (400 MHz; $CDCl_3$) 8.39 (2H, NH_2, s), 5.15 (1H, CH, s), 4.02 (4H, CH_2, q, J = 7.2), 1.08 (6H, CH_3, t, J = 7.2); δ**C** (100 MHz, $CDCl_3$) 168.9 (C=O), 61.7 (C-N), 57.9 (CH_2), 13.6 (CH_3); IR cm^{-1} 3393 and 3326 (N-H, str), 1738 (C=O, str), 1597 (N-H, def). The amine was dissolved in acetonitrile (50 ml), ethyl formimidate hydrochloride (2.7 g, 24.6 mmol) was added and the solution was stirred at room temperature for 1 h until the reaction had gone to completion according to TLC, R_f (chloroform-methanol, 9:1) 0.68 revealed with UV light or iodine vapour, the imidate was not isolated. The solution was filtered to remove the ammonium chloride and to the filtrate was added the desired amine (47.2 mmol) and the solution was left at 4 ºC overnight. The solvent was removed under reduced pressure and the resulting gum was dissolved in a minimal volume of ethanol and rendered turbid with the addition of diethyl ether. The precipitate was collected, washed with acetone and purified accordingly (as detailed below).

Ethyl 1-(2-(*tert*-butyldimethylsilyloxy)ethyl)-5-hydroxyimidazole-4-carboxylate (47)



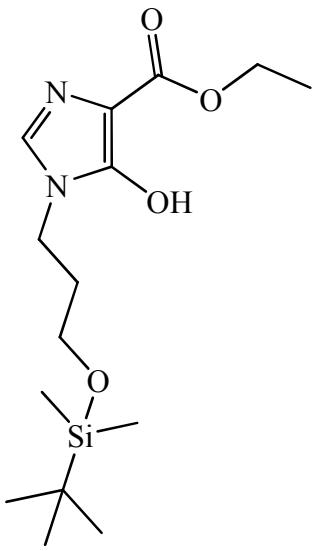
Using 2-(*tert*-butyldimethylsilyloxy)ethanamine. Purified by recrystallisation from acetone to give pale yellow crystals (1.6 g, 5.0 mmol, 21 %); mp 91-92 °C; R_f (chloroform-methanol, 9:1) 0.41 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 7.22 (1H, CH imidazole, s), 4.10 (2H, CH_2, q, J = 7.1), 3.69 (2H, CH_2, t, J = 5.7), 3.35 (2H, CH_2, t, J = 5.7), 1.26 (3H, CH_3, t, J = 7.1), 0.83 (9H, CH_3-C, s), 0.00 (6H, CH_3-Si, s); δ**C** (100 MHz; DMSO) 158.4 (C=O), 136.1 (CH imidazole), 128.6 (CH imidazole), 61.9 (CH_2-O), 49.2 (CH_2 ester), 43.0 (CH_2-N), 42.2 ($C(CH_3)_3$), 25.7 (CH_3-C), 17.9 (CH_3), -5.6 (CH_3-Si); IR (KBr) cm^{-1} 3451 (O-H, str), 1742(C=O, str); LRMS (ESI) $[M]^+$ requires 314, found $[M + H]^+$ 315.

Ethyl 5-hydroxy-1-(2-hydroxyethyl)imidazole-4-carboxylate (44)



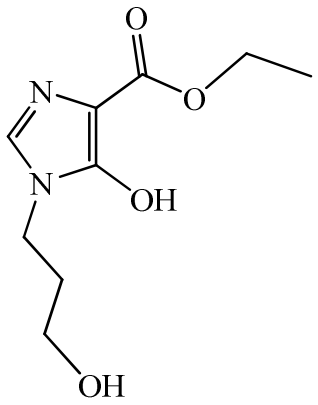
Using ethanolamine (2.0 g, 9.8 mmol, 41 %). Crude imidazole washed with ethanol and acetone successively to give bright white crystals (72 %); mp 85-86 °C; R_f (chloroform-methanol, 9:1) 0.29 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 8.15 (1H, CH imidazole, s), 4.10 (2H, CH_2, q, J = 7.0), 3.69 (2H, CH_2-N, t, J = 7.1), 3.35 (2H, CH_2-O, t, J = 7.1), 1.18 (3H, CH_3, t, J = 7.0); δ**C** (100 MHz; DMSO) 161.08 (C=O), 155.43 (CH imidazole), 128.40 (CH imidazole), 104.52 (CH imidazole), 58.19 (CH_2 ethyl), 57.63 (CH_2-OH), 38.91 (CH_2-N), 14.79 (CH_3); IR (KBr) cm^{-1} 3453-3119 (O-H, str), 1698 (C=O, str); LRMS (ESI) $[M]^+$ requires 200, found $[M + H]^+$ 201; HRMS (ESI) calculated for $[M + H]^+$ requires 201.0870, found 201.0872.

Ethyl 1-(3-(*tert*-butyldimethylsilyloxy)propyl)-5-hydroxyimidazole-4-carboxylate



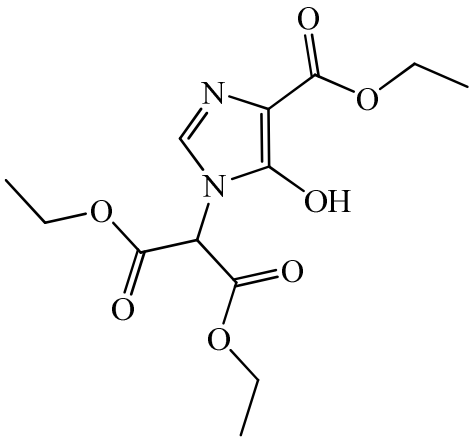
Using 3-(*tert*-butyldimethylsilyloxy)propan-1-amine. Purified by recrystallisation from acetone to give pale yellow crystals (1.3 g, 4.1 mmol, 17 %); mp 99-101 °C; R_f (chloroform-methanol, 9:1) 0.45 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 7.21 (1H, CH imidazole, s), 4.11 (2H, CH_2, q, J = 7.0), 3.69 (2H, CH_2, m), 3.64 (2H, CH_2-N, t, J = 5.7), 2.85 (2H, CH_2-O, t, J = 5.7), 1.26 (3H, CH_3, t, J = 7.0), 0.83 (9H, CH_3-C, s), -0.01 (6H, CH_3-Si, s); δ**C** (100 MHz; DMSO) 163.8 (C=O), 128.6 (CH imidazole), 116.3 (CH imidazole), 62.7 (CH_2-O), 59.4 (CH_2), 49.2 (CH_2 ester), 43.0 (CH_2-N), 42.2 ($C(CH_3)_3$), 25.8 (CH_3-C), 14.7 (CH_3), -5.6 (CH_3-Si); IR (KBr) cm^{-1} 3239 (O-H, str), 1765 (C=O, str); LRMS (ESI) $[M]^+$ requires 328, found $[M + K]^+$ 367.

Ethyl 5-hydroxy-1-(3-hydroxypropyl)imidazole-4-carboxylate (43)



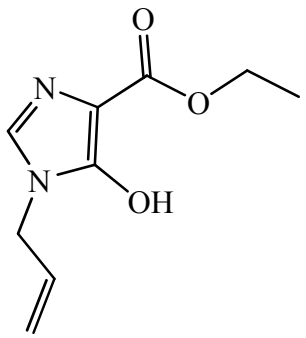
Using 3-aminopropan-1-ol (1.2 g, 5.5 mmol, 23 %). Purified by recrystallisation from acetone to give pale yellow crystals (69 %); mp 89-90 °C; R_f (chloroform-methanol, 9:1) 0.31 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 8.15 (1H, CH imidazole, s), 4.10 (2H, CH_2, q, J = 7.0), 3.69 (2H, CH_2, t, J = 7.1), 3.35 (2H, CH_2, t, J = 7.1), 1.71 (2H, CH_2, quin, J = 7.1), 1.18 (3H, CH_3, t, J = 7.0); δ**C** (100 MHz; DMSO) 161.1 (C=O), 155.4 (CH imidazole), 128.4 (CH imidazole), 104.5 CH imidazole), 58.2 (CH_2 ethyl), 57.6 (CH_2-OH), 38.9 (CH_2-N), 31.9 (CH_2), 14.8 (CH_3); IR (KBr) cm^{-1} 3347 (O-H, str), 1798 (C=O, str); LRMS (ESI) $[M]^+$ requires 214, found $[M + Na]^+$ 237.

Diethyl 2-(4-(ethoxycarbonyl)-5-hydroxyimidazol-1-yl)malonate (45)



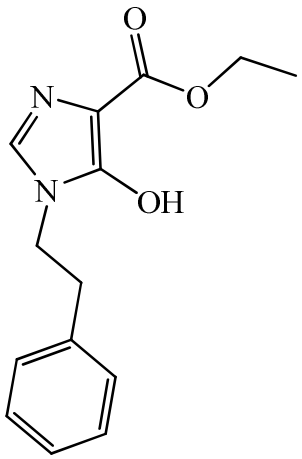
Using diethyl-2-amino malonate (3.5 g, 11.0 mmol, 46 %). Purified by recrystallisation from ethanol to give pale yellow crystals (58 %); mp 139-140 °C (dec); R_f (chloroform-methanol, 9:1) 0.26 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 8.36 (1H, CH imidazole, s), 5.75 (1H, CH, s), 4.19 (4H, CH_2, m), 4.09 (2H, CH_2, q, J = 7.1), 1.18 (6H, CH_3, t, J = 7.1),1.16 (3H, CH_3, t, J = 7.1); δ**C** (100 MHz; DMSO) 164.5 (C=O), 160.3 (C=O), 154.2 (CH imidazole), 128.5 (CH imidazole), 93.4 (CH imidazole), 62.7 (CH), 58.3 (CH_2), 55.7 (CH_2), 14.7 (CH_3), 13.8 (CH_3); IR (KBr) cm^{-1} 3391 (O-H, str), 1758 (C=O, str); LRMS (ESI) $[M]^+$ requires 314, found $[M + Na]^+$ 337; HRMS (ESI) calculated for $[M + H]^+$ requires 315.1187, found 315.1188.

Ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate (46)



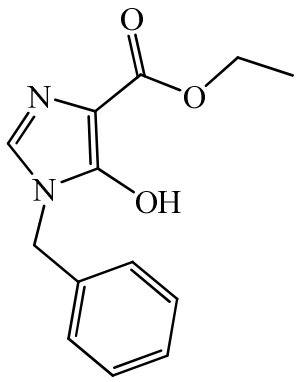
Using allylamine (2.6 g, 13.4 mmol, 56 %). Purified using an acid base wash, the pure imidazole was collected as a bright white precipitate (71 %); mp 93-95 °C (dec); R_f (chloroform-methanol, 9:1) 0.38 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 7.00 (1H, CH imidazole, s), 5.86 (1H, CH, m), 5.26 (1H, CH, dd, *J* = 12.1), 5.03 (1H, CH, dd, *J* = 12.1), 4.11 (2H, CH_2, d, *J* = 5.7), 4.01 (2H, CH_2, q, *J* = 7.1), 1.18 (3H, CH_3, t, *J* = 7.1); δ**C** (100 MHz; DMSO) 161.9 (C=O), 134.0 (CH imidazole), 131.3 (CH allylic), 127.4 (CH imidazole), 119.6 (CH imidazole), 116.7 (CH allylic), 57.7 (CH_2 ethyl), 42.7 (CH_2-N), 14.9 (CH_3); IR (KBr) cm^{-1} 3391 (O-H, str), 1743 (C=O, str), 1670 (vinyl, str); LRMS (ESI) $[M]^+$ requires 196, found $[M + Na]^+$ 219; HRMS (ESI) calculated for $[M + H]^+$ requires 197.0921, found 197.0921.

Ethyl 5-hydroxy-1-phenethylimidazole-4-carboxylate (40)



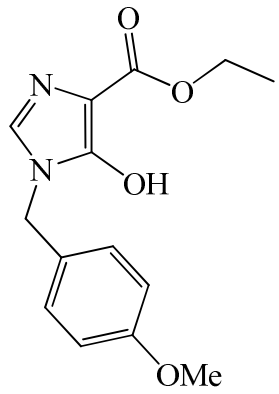
Using phenethylamine (5.12 g, 20.0 mmol, 82 %). Purified using an acid base wash, the pure imidazole was collected as a pale yellow precipitate (64 %); mp 157-158 °C (dec); R_f (chloroform-methanol, 9:1) 0.35 blue/brown spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 8.00 (1H, -CH= imidazole, s), 7.25 (5H, Ph, m), 4.09 (2H, CH_2, q, J = 7.1), 3.87 (2H, CH_2, t, J = 7.3), 2.93 (2H, CH_2, t, J = 7.3), 1.18 (3H, CH_3, t, J = 7.1); δ**C** (100 MHz; DMSO) 162.9 (C=O), 129.3 (CH imidazole), 128.6 (Ph), 128.6 (CH imidazole), 128.4 (Ph), 128.1 (Ph), 126.5 (Ph), 58.1 (CH_2), 41.8 (CH_2-N), 35.3 (CH_2-Ph), 15.0 (CH_3); IR (KBr) cm^{-1} 3335 (O-H, str), 1739 (C=O, str); LRMS (ESI) $[M]^+$ requires 260, found $[M + Na]^+$ 283; HRMS (ESI) calculated for $[M + H]^+$ requires 261.1234, found 261.1235.

Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate (39)



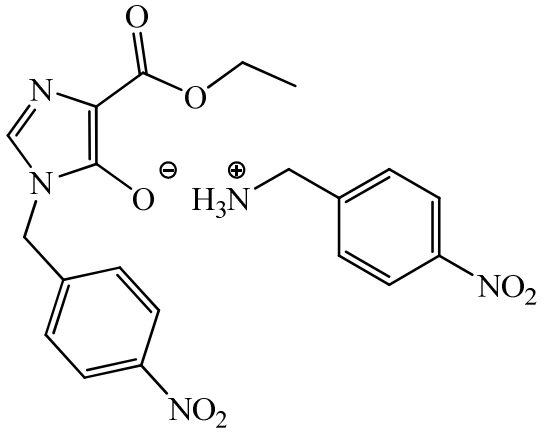
Using benzylamine (4.7 g, 19.0 mmol, 79 %). Purified using an acid base wash, the pure imidazole was collected as a bright white precipitate (61 %); mp 178-180 °C (dec), lit. value 180 ºC (dec); R_f (chloroform-methanol, 9:1) 0.39 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 7.30 (1H, CH, s), 7.23 (5H, Ph, m), 4.78 (2H, CH_2, s), 4.06 (2H, CH_2, q, J = 7.1), 1.17 (3H, CH_3, t, J = 7.1); δ**C** (100 MHz; DMSO) 160.1 (C=O), 138.4 (CH imidazole), 128.4 (Ph), 128.0 (CH imidazole), 127.3 (Ph), 127.2 (Ph), 119.4 (CH imidazole), 57.5 (CH_2-N), 43.7 (CH_2), 14.9 (CH_3); IR (KBr) cm^{-1} 3354 (O-H, str), 1745 (C=O, str); LRMS (ESI) $[M]^+$ requires 246, found $[M + Na]^+$ 269; HRMS (ESI) calculated for $[M + H]^+$ requires 247.1077, found 247.1077.

Ethyl 5-hydroxy-1-(4-methoxybenzyl)imidazole-4-carboxylate (41)



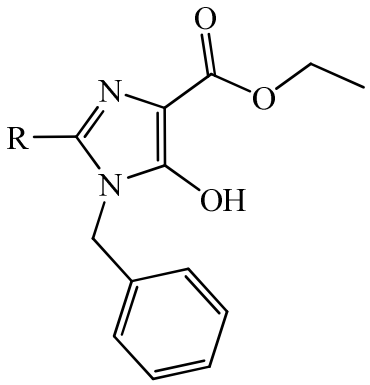
Using 4-methoxybenzylamine (5.6 g, 20.2 mmol, 84 %). Purified using an acid base wash, the pure imidazole was collected as a white precipitate (63 %); mp 167-168 °C (dec); R_f (chloroform-methanol, 9:1) 0.33 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 8.32 (1H, CH imidazole, s), 7.24 (2H, Ph, d, J = 8.5), 6.88 (2H, Ph, d, J = 8.5), 4.77 (2H, CH_2, s), 4.10 (2H, CH_2, q, J = 7.0), 3.71 (1H, CH_3, s), 1.19 (3H, CH_3, t, J = 7.0); δ**C** (100 MHz; DMSO) 158.8 (C=O), 129.3 (Ph), 129.1 (Ph), 128.2 (Ph), 113.9 (Ph), 58.2 (CH_2), 55.1 (O-CH_3), 43.7 (CH_2-N), 14.7 (CH_3); IR (KBr) cm^{-1} 3350 (O-H, str), 1758 (C=O, str); LRMS (ESI) $[M]^+$ requires 276, found $[M + Na]^+$ 299; HRMS (ESI) calculated for $[M + H]^+$ requires 277.1183, found 277.1184.

(4-Nitrophenyl)methanaminium 4-(ethoxycarbonyl)-1-(4-nitrobenzyl)imidazole-5-olate (42)



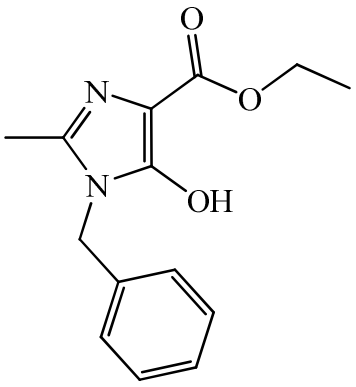
Using 4-nitrobenzylamine, salt couldn't be purified further to give the 5-hydroxyimidazole (7.1 g, 16.1 mmol, 67 %); R_f (chloroform-methanol, 9:1) 0.27 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 8.40 (1H, CH imidazole, s), 8.24 (4H, Ph, m), 7.65 (4H, Ph, m), 4.85 (2H, CH_2, s), 4.76 (2H, CH_2, s), 3.03 (2H, CH_2, q, J = 7.0), 1.19 (3H, CH_3, t, J = 7.0); δ**C** (100 MHz; DMSO) 155.7 (C=O), 147.1 (Ph), 144.8 (Ph), 143.4 (Ph), 128.9 (Ph), 123.7 (Ph), 123.6 (Ph), 49.6 (CH_2-N), 45.3 (CH_2-N salt), 44.5 (CH_2), 8.4 (CH_3); IR (KBr) cm^{-1} 3236-3114 (O-H, str), 1724 (C=O, str); LRMS (ESI) $[M]^+$ requires 443, found $[M]^+$ 443.

General Procedure for attempted Synthesis of 2-Substituted Imidazole-4-Craboxylate Ethyl Esters



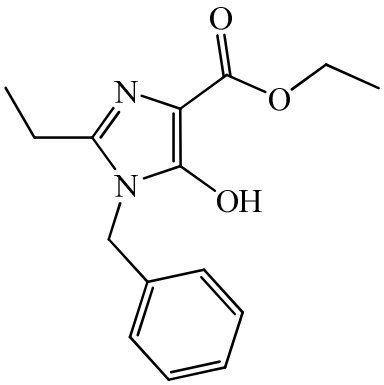
Diethyl 2-aminomalonate hydrochloride (5.0 g, 23.6 mmol) was suspended in dichloromethane (50 ml) and saturated sodium bicarbonate was added (50 ml). The mixture was shaken in the separating funnel and the organic layer was collected, dried ($MgSO_4$) and evaporated to give the free amine as a faint yellow gum. R_f (chloroform-methanol, 9:1) 0.36 brown spot when revealed with $CuCl_2$; δ**H** (400 MHz; $CDCl_3$) 8.39 (2H, NH_2, s), 5.15 (1H, CH, s), 4.02 (4H, CH_2, q, J = 7.2), 1.08 (6H, CH_3, t, J = 7.2); δ**C** (100 MHz, $CDCl_3$) 168.9 (C=O), 61.7 (C-N), 57.9 (CH_2), 13.6 (CH_3); IR cm^{-1} 3393 and 3326 (N-H, str), 1738 (C=O, str), 1597 (N-H, def). The amine was dissolved in acetonitrile (50 ml) and added drop-wise to a refluxing solution of the desired triethyl orthoimidate (47.2 mmol) and a catalytic amount of trifluoroacetic acid (5 mmol). The solution was refluxed until the reaction had gone to completion according to TLC (approximately 3 h), R_f (chloroform-methanol, 9:1) 0.68 revealed with UV light or iodine vapour, the resulting imidate was not isolated. The solvent was removed under reduced pressure and the imidate re-dissolved in acetonitrile (50 ml). Benzylamine (5.1 g, 47.2 mmol) was added to the forgoing solution which was refluxed for 15 min. The solution was allowed to cool and was then left at 4 ºC overnight. The solvent was removed under reduced pressure and the resulting gum was dissolved in a minimal volume of ethanol and rendered turbid with the addition of diethyl ether. The precipitate was collected, washed with acetone and purified accordingly (as detailed below).

Ethyl 1-benzyl-5-hydroxy-2-methylimidazole-4-carboxylate (48)



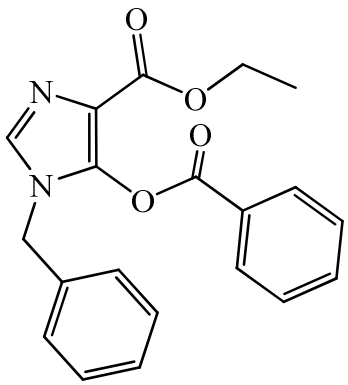
Using benzylamine (2.1 g, 8.2 mmol, 34 %). Purified by recrystallisation from ethanol to give colourless crystals (64 %); mp 166-168 °C (dec); R_f (chloroform-methanol, 9:1) 0.41 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 7.31 (5H, Ph, m), 4.87 (2H, CH_2, s), 4.11 (2H, CH_2, q, J = 7.0), 2.23 (3H, CH_3, s), 1.17 (3H, CH_3, t, J = 7.0); δ**C** (100 MHz; DMSO) 160.6 (C=O), 136.8 (CH imidazole), 128.6 (Ph), 128.5 (Ph), 127.9 (Ph), 127.4 (CH imidazole), 127.3 (Ph), 57.9 (CH_2), 42.2 (CH_2-N), 14.8 (CH_3), 11.4 (CH_3); IR (KBr) cm^{-1} 3216-3114 (O-H, str), 1680 (C=O, str); LRMS (ESI) $[M]^+$ requires 260, found $[M + H]^+$ 261; HRMS (ESI) calculated for $[M + H]^+$ requires 261.1234, found 261.1230.

Ethyl 1-benzyl-2-ethyl-5-hydroxyimidazole-4-carboxylate (49)



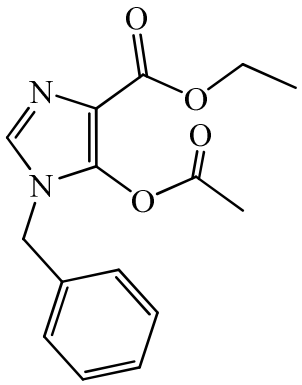
Using benzylamine (59 mg, 2.16 mmol, 9 %). Purified by recrystallisation from ethanol to give colourless crystals (59 %); mp 168-170 °C (dec); R_f (chloroform-methanol, 9:1) 0.45 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 7.23 (5H, Ph, m), 4.61 (2H, CH_2-N, s), 4.13 (2H, CH_2 ester, q, J = 7.1), 2.30 (2H, CH_2, q, J = 7.2), 1.15 (3H, CH_3 ester, t, J = 7.1), 1.01 (3H, CH_3, t, J = 7.2); δ**C** (100 MHz; DMSO) 167.44 (C=O), 143.9 (CH imidazole), 141.1 (CH imidazole), 136.36 (Ph), 128.71 (Ph), 127.55 (Ph), 126.42 (Ph), 61.63 (CH_2 ester), 42.89 (CH_2-N), 21.64 (CH_2), 13.84 (CH_3 ester), 8.61 (CH_3); IR (KBr) cm^{-1} 3234-3058 (O-H, str), 1749 (C=O, str); LRMS (ESI) $[M]^+$ requires 274, found $[M + H]^+$ 275.

Ethyl 5-(benzoyloxy)-1-benzylimidazole-4-carboxylate (69)



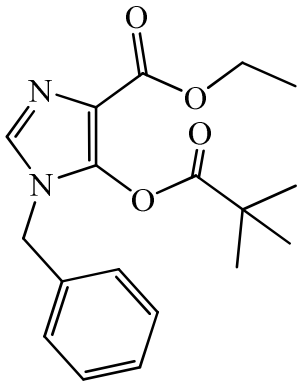
Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate (200.0 mg, 0.8 mmol) was suspended in chloroform. Triethylamine (90.0 mg, 0.9 mmol) and dimethylaminopyridine (DMAP) (10.0 mg, 0.1 mmol) were added, the solution was stirred and cooled to 0 ºC. Benzoyl chloride (130.0 mg, 0.9 mmol) was added to the stirring solution and the reaction mixture was allowed to warm up to room temperature. Stirring was continued for a further 24 h. The solution was washed with ice cold 0.1 M HCl, saturated sodium hydrogen carbonate, water and dried ($MgSO_4$). The solvent was removed under reduced pressure and dissolved in hexane-ethyl acetate, 1:1, and left at 4 ºC over night. The product was collected by filtration as bright, white crystals (176.0 mg, 0.5 mmol, 62 %); R_f (chloroform-methanol, 9:1) 0.57 revealed with UV light or iodine vapour; mp 116 °C; δ**H** (400 MHz; DMSO) 8.08 (1H, CH imidazole, s), 7.77 (5H, Ph, m), 7.28 (5H, Ph, m), 5.23 (2H, CH_2, s), 4.02 (2H, CH_2, q, J = 7.1), 0.91 (3H, CH_3, t, J = 7.1); δ**C** (100 MHz; DMSO) 162.2 (C=O), 160.8 (C=O), 137.8 (CH imidazole), 132.9 (Ph), 130.2 (Ph), 129.2 (Ph), 128.7 (Ph), 128.6 (Ph), 128.1 (Ph), 127.7 (CH imidazole), 127.1 (Ph), 118.3 (CH imidazole), 59.7 (CH_2), 47.3 (CH_2-N), 13.8 (CH_3); IR (KBr) cm^{-1} 1781 (C=O, str), 1727 (C=O, str); LRMS (ESI) $[M]^+$ requires 350, found $[M + H]^+$ 351.

Ethyl 5-acetoxy-1-benzylimidazole-4-carboxylate (70)



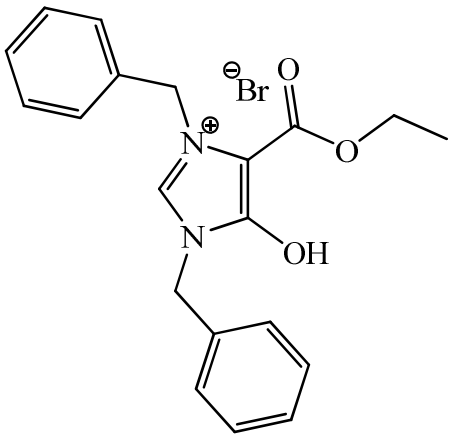
Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate (200.0 mg, 0.8 mmol) was suspended in chloroform. Triethylamine (90.0 mg, 0.9 mmol) and dimethylaminopyridine (DMAP) (10.0 mg, 0.1 mmol) were added, the solution was stirred and cooled to 0 ºC. Acetyl chloride (70.0 mg, 0.9 mmol) was added to the stirring solution and the reaction mixture was allowed to warm up to room temperature. Stirring was continued for a further 24 h. The solution was washed with ice cold 0.1 M HCl, saturated sodium hydrogen carbonate, water and dried ($MgSO_4$). The solvent was removed under reduced pressure and dissolved in hexane-ethyl acetate, 1:1, and left at 4 ºC over night. The product was collected by filtration as pale green crystals (136.0 mg, 0.5 mmol, 58 %); R_f (chloroform-methanol, 9:1) 0.48 revealed with UV light or iodine vapour; mp 105-107 °C; δ**H** (400 MHz; DMSO) 8.01 (1H, CH imidazole, s), 7.28 (5H, Ph, m), 4.86 (2H, CH_2, s), 4.09 (2H, CH_2, q, J = 7.1), 3.16 (1H, CH_3, s), 1.18 (3H, -CH_3, t, J = 7.1); δ**C** (100 MHz; DMSO) 170.8 (C=O), 162.6 (C=O ester), 137.7 (Ph), 129.2 (Ph), 128.2 (Ph), 127.6 (Ph), 58.8 (CH_2), 48.4 (CH_3), 15.4 (CH_3 ester); IR (KBr) cm^{-1} 1735 (C=O, str), 1723 (C=O, str); LRMS (ESI) $[M]^+$ requires 288, found $[M + H]^+$ 289.

Ethyl 1-benzyl-5-(pivaloyloxy)imidazole-4-carboxylate (71)



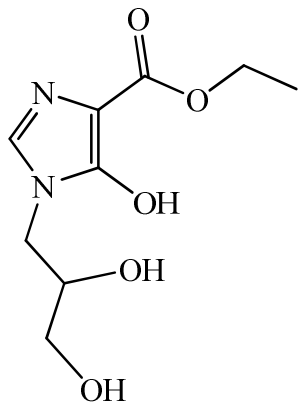
Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate (200.0 mg, 0.8 mmol) was suspended in chloroform. Triethylamine (90.0 mg, 0.9 mmol) and dimethylaminopyridine (DMAP) (10.0 mg, 0.1 mmol) were added, the solution was stirred and cooled to 0 ºC. Pivaloyl chloride (70.0 mg, 0.9 mmol) was added to the stirring solution and the reaction mixture was allowed to warm up to room temperature. Stirring was continued for a further 24 h. The solution was washed with ice cold 0.1 M HCl, saturated sodium hydrogen carbonate, water and dried ($MgSO_4$). The solvent was removed under reduced pressure and dissolved in hexane-ethyl acetate, 1:1, and left at 4 ºC over night. The product was collected by filtration as green crystals; (86.0 mg, 0.3 mmol, 32 %); R_f (chloroform-methanol, 9:1) 0.51 revealed with UV light or iodine vapour; mp 109 °C; δ**H** (400 MHz; DMSO) 8.12 (1H, CH imidazole, s), 7.33 (5H, Ph, m), 5.46 (1H, CH_2, s), 4.29 (2H, CH_2, q, J = 6.9), 1.25 (9H, CH_3, s), 1.18 (3H, CH_3, t, J = 6.9); δ**C** (100 MHz; DMSO) 173.8 (C=O), 161.9 (C=O ester), 137.7 (CH imidazole), 130.2 (Ph), 128.7 (Ph), 128.6 (Ph), 127.1 (Ph), 25.4 (CH_3), 14.3 (CH_3 ester); IR (KBr) cm^{-1} 1777 (C=O, str), 1733 (C=O, str); LRMS (ESI) $[M]^+$ requires 330, found $[M + Na]^+$ 353.

1,3-Dibenzyl-4-(ethoxycarbonyl)-5-hydroxyimidazol-3-ium bromide (68)



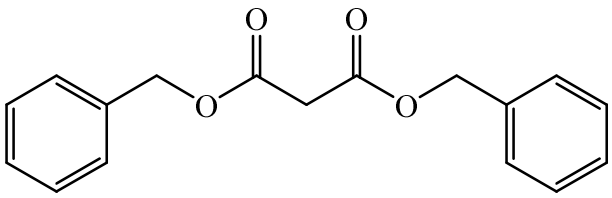
Ethyl 1-benzyl-5-hydroxyimidazole-4-carboxylate (500.0 mg, 2.0 mmol) was suspended in DMF (10 ml). Benzyl bromide (417.0 mg, 2.4 mmol) and potassium fluoride (295.0 mg, 5.1 mmol) were added and the solution refluxed for 24 h. Then solution was allowed to cool and water (10 ml) was added. The precipitate was collected as pale yellow crystals (669.0 mg, 1.6 mmol, 79 %); δ**H** (400 MHz; DMSO) 8.57 (1H, CH, s), 7.25 (10H, Ph, m), 5.36 (2H, CH_2, s), 4.83 (2H, CH_2, s), 3.96 (2H, CH_2, q, J = 6.9), 1.03 (3H, CH_3, t, J = 6.9); δ**C** (100 MHz; DMSO) 160.6 (C=O), 155.3 (CH imidazole), 136.8 (CH imidazole), 136.4 (Ph), 131.1 (Ph), 129.9 (Ph), 128.7 (Ph), 128.6 (Ph), 127.8 (Ph), 127.1 (CH imidazole), 58.0 (CH_2), 51.6 (CH_2-N), 44.1 (CH_2-N salt), 14.5 (CH_3); LRMS (ESI) $[M]^+$ requires 337, found $[M + H]^+$ 338.

Attempted synthesis of ethyl 1-(2,3-dihyroxypropyl)-5-hydroxyimidazole-4-carboxylate (80)



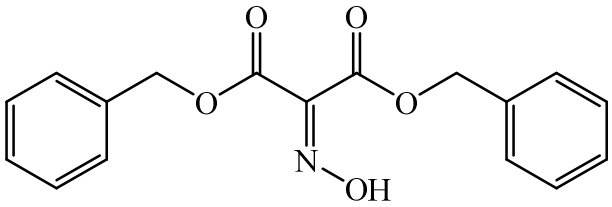
Ethyl 1-allyl-5-hydroxyimidazole-4-carboxylate (500 mg, 2.2 mmol) was suspended in ethanol (10 ml) and encapsulated osmium tetroxide (56 mg, 0.2 mmol) and *N*-methylmorpholine-*N*-oxide (335 mg, 2.9 mmol) were added. The reaction mixture was allowed to stir for 48 h, then the mixture was heated to 50 ºC and a hot filtration was performed to remove the encapsulated osmium tetroxide catalyst. The solvent was removed under reduced pressure and the resulting oil was dissolved in a minimal amount of dimethylformamide and rendered turbid using diethyl ether. Collection of the needle-like crystals was attempted, however the product appeared to decompose when it came into contact with moisture in the air, and when the crystals were isolated they immediately began to form a yellow gum. Analysis of crude gum obtained; δ**H** (400 MHz; DMSO) 3.95 (2H, CH_2, q, J = 6.9), 3.82 (d, J = 12.0), 3.60 (t, J = 12.0), 3.24 (d, J = 12.0), 1.21 (3H, CH_3, t, J = 6.9); LRMS (ESI) $[M]^+$ requires 230, found $[M + H]^+$ 235.

Dibenzylmalonate (50)



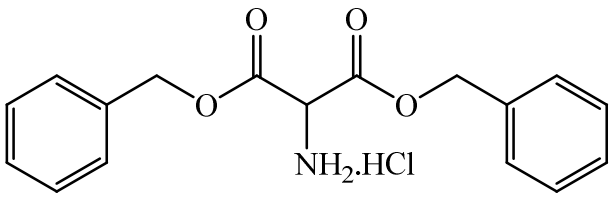
A mixture of malonic acid (100.0 g, 1.0 mol), benzyl alcohol (170 ml, 2.9 mol), p-toluene sulphonic acid (0.5 g, 2.6 mmol) and toluene (200 ml) was refluxed using Dean-Stark apparatus. After approximately 35 ml of water was removed, the reaction mixture was washed with a saturated solution of sodium hydrogen carbonate. The resulting solution was evaporated under reduced pressure to give the crude compound as a yellow gum which was then dissolved in toluene, washed with saturated sodium bicarbonate, water, dried ($MgSO_4$) and distilled to give a clear oil (188-190 ºC, 193.3 g, 0.7 mol, 68 %). R_f (chloroform : methanol, 9:1) 0.73 revealed with UV light or iodine vapour; δ**H** (400 MHz; DMSO) 7.34 (10H, Ph, m), 5.14 (4H, CH_2, s), 3.65 (2H, CH_2, s); δ**C** (100 MHz, DMSO) 166.4 (C=O), 128.2 (CH_2), 66.4 (CH_2), 41.2 (CH_3); IR cm^{-1} 3034 (aromatic C-H, str), 1735 (C=O, str), 1558, 1498, 1455 (aromatic C=C, str), 1147, 1081, 1004 (C-O); LRMS (ESI) $[M]^+$ requires 284, found $[M + Na]^+$ 307.

Dibenzyl 2-(hydroxyimino)malonate (51)



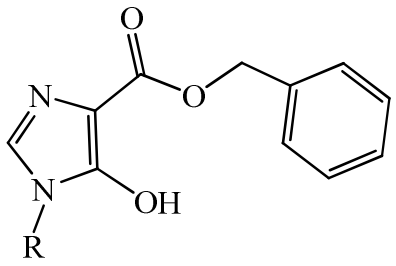
To a mixture of dibenzylmalonate (89.4 g, 0.3 mol) stirred and cooled to 0 °C, was added glacial acetic acid (57 ml, 0.9 mol) and water (81 ml). Sodium nitrite (65.0 g, 0.9 mol) was added in portions over a period of 1 h. The reaction mixture was stirred at ambient temperature for a further 24 h until all starting materials had been consumed according to TLC. The resulting mixture was extracted with ether (50 ml x 2), the organic phase collected, washed with sodium bicarbonate (80 ml x 6), dried ($MgSO_4$) and evaporated under reduced pressure to give the crude compound as a yellow semi-crystalline material (95.0 g, 0.3 mol, 96 %). R_f (chloroform-methanol, 9:1) 0.43 green spot when revealed with $CuCl_2$; δ**H** (400 MHz; DMSO) 10.73 (1H, OH, s), 7.33 (10H, Ph, m), 5.18 (4H, CH_2, s); δ**C** (100 MHz, DMSO) 166.6 (C=O), 143.97 (C=N), 134.3 (Ph), 128.3 (Ph), 127.9 (Ph), 127.0 (Ph), 67.8 (CH_2); IR cm^{-1} 3260 (O-H, str), 1744 (C=O, str); LRMS (ESI) $[M]^+$ requires 313, found $[M + Na]^+$ 336.

Dibenzyl 2-aminomalonate hydrochloride (52)



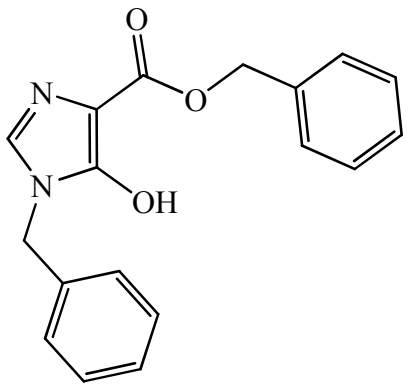
Aluminium foil (2.5 g, 96.0 mmol, torn into 1cm^2 pieces) was placed in a 3-neck round bottom flask. The foil was covered with a solution of mercuric chloride (200 ml, 1 %) and after 1 min the turbid solution was decanted off. The residue was washed successively with water (100 ml) and methanol (4 x 100 ml) and suspended in diethyl ether (200 ml). The flask was placed in an ice-bath and fitted with two condensers, one of which had a dropping funnel fitted, and a thermometer. A solution of the oxime (25.0 g, 79.8 mmol) in diethyl ether (50 ml) was added to the amalgam and water (10 ml) was added at such a rate as to maintain a reaction temperature of 35-40 ºC. After the addition was complete, stirring was continued at room temperature for a further 3 h upon which all starting materials were consumed according to TLC. The reaction mixture was filtered through a pad of 'Celite' filter aid and the residue was washed with diethyl ether. The combined filtrates were dried and evaporated to a yellow gum (17.5 g, 55.9 mmol, 73 %), R_f (chloroform-methanol, 9:1) 0.68 brown spot when revealed with $CuCl_2$. The crude dibenzyl aminomalonate was diluted with ether (100 ml), stirred and cooled in an ice bath and dry hydrogen chloride was passed over the solution. The pale yellow crystalline precipitate was filtered off and washed with ether to give the title compound (15.3 g, 45.5 mmol, 57 %, mp 124 °C). The ether washings were combined and treated again with hydrogen chloride, any precipitate was again filtered, washed and the process was repeated until no more precipitate was obtained. Recrystallisation from alcohol-hexane affords a more pure product (145 °C). R_f (chloroform-methanol, 9:1) 0.16 white spot when revealed with $CuCl_2$; δ**H** (400 MHz; DMSO) 9.18 (2H, NH_2, br s), 7.34 (10H, Ph, m), 5.25 (4H, CH_2, t, J = 11.7), 3.76 (1H, CH, s); δ**C** (100 MHz, DMSO) 163.8 (C=O), 133.9 (Ph), 128.5 (Ph), 127.5 (Ph), 126.9 (Ph), 68.12 (CH_2); IR (KBr) cm^{-1} 3347 (NH_3^+, str), 1747 (C=O, str); LRMS (ESI) $[M]^+$ requires 299, found $[M + H]^+$ 300.

General Procedure for the Synthesis of Benzyl 1-Substituted-5-Hydroxyimidazole-4-Carboxylates (unless otherwise stated)



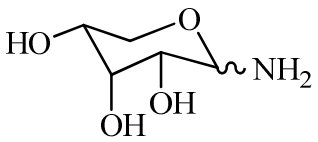
Dibenzyl 2-aminomalonate hydrochloride (10.0 g, 29.8 mmol) was suspended in dichloromethane (50 ml) and saturated sodium bicarbonate was added (2 x 50 ml). The mixture was shaken in the separating funnel and the organic layer was collected, dried ($MgSO_4$) and evaporated to give the free amine as a faint yellow gum. R_f (chloroform-methanol, 9:1) 0.36 brown spot when revealed with $CuCl_2$; δ**H** (400 MHz; $CDCl_3$) 8.73 (2H, NH_2, br s), 7.38 (10H, Ph, m), 5.15 (1H, CH, s), 4.29 (1H, CH, s); δ**C** (100 MHz, $CDCl_3$) 168.9 (C=O), 61.7 (C-N), 57.9 (CH_2), 13.6 (CH_3); IR cm^{-1} 3393 and 3326 (N-H, str), 1738 (C=O, str), 1597 (N-H, def). The amine was dissolved in acetonitrile (50 ml), ethyl formimidate hydrochloride (3.3 g, 30.0 mmol) was added and the solution was stirred at room temperature for 1 h until the reaction had gone to completion according to TLC, R_f (chloroform-methanol, 9:1) 0.68 revealed with UV light or iodine vapour, the imidate was not isolated. The solution was filtered to remove the ammonium chloride and to the filtrate was added the desired amine (59.6 mmol) and the solution was left at 4 ºC overnight. The solvent was removed under reduced pressure and the resulting gum was dissolved in a minimal volume of ethanol and rendered turbid with the addition of diethyl ether. The precipitate was collected, washed with acetone and purified accordingly (as detailed below).

Benzyl 1-benzyl-5-hydroxyimidazole-4-carboxylate (53)



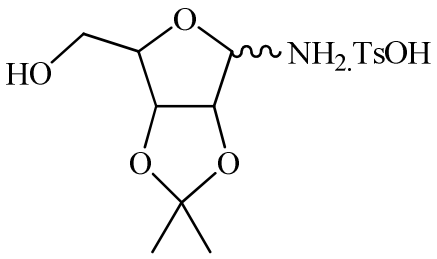
Using benzylamine. Purified by recrystallisation using ethanol to produce white crystals (2.1 g, 6.9 mmol, 23 %); mp 186 °C (dec); R_f (chloroform-methanol, 9:1) 0.46 blue spot when revealed with $FeCl_3$; δ**H** (400 MHz; DMSO) 8.07 (1H, -OH, s), 7.28 (10H, Ph, m), 5.06 (2H, -CH_2-, s), 4.71 (1H, -CH-, s), 4.44 (2H, -CH_2-, s); δ**C** (100 MHz; DMSO) 161.83 (C=O), 154.19 (Ph), 138.94 (Ph), 128.9 (Ph), 127.56 (Ph), 62.98 (CH_2), 44.57 (CH_2); IR (KBr) cm^{-1} 3206 (O-H, str), 1710 (C=O, str); LRMS (ESI) $[M]^+$ requires 308, found $[M + H]^+$ 309.

D-Ribopyranosylamine[258]



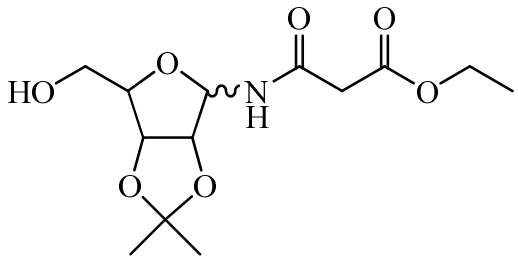
D-Ribose (100.0 g, 0.6 mol) was added in portions to a stirred, ice-cold solution of ammonium chloride (0.50 g, 10.0 mmol) and ammonia saturated methanol (100 ml) contained within a screw cap polythene container. During the addition, dry ammonia gas was bubbled through the mixture until all of the D-ribose has dissolved. The treatment with ammonia was continued for a further 15 min. and the reaction mixture seeded, sealed and stored at 4 ºC for 4 days. The product was broken up, filtered, washed with methanol and dried in vacuo (97.0 g, 0.7 mol, 98 %); mp 127-128 °C (dec), lit. value[259] 128-129 ºC (dec); IR (KBr) cm^{-1} 3378-3293 (O-H, str), 2937 (N-H, str), 1638 (N-H, def).

2,3-*O*-Isopropylidene-D-ribofuranosylamine toluene-p-sulphonate[217] (54)



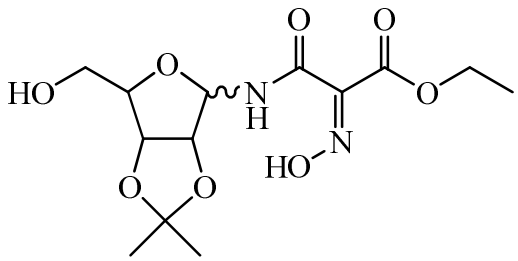
A solution of toluene-p-sulphonic acid monohydrate (105.0 g, 0.6 mol) in 2,2-dimethoxypropane (286 ml, 2.2 mol) and dry acetone (1 L) was stirred at room temperature for 15 min. Dry, finely powdered D-ribopyranosylamine (40.0 g, 0.3 mol) was added to the reaction mixture and stirred for 16 h. The resulting pale brown solution was filtered to remove any solid, and the filtrate evaporated to about one half its original volume. Dry diethyl ether was added to render the solution slightly turbid (~ 500 ml) and the solution set aside at 4 ºC for 24 h. The colourless needle-like crystals were collected, washed with acetone and then diethyl ether and dried *in vacuo* (67.3 g, 0.2 mol, 70 %); mp 136-138 °C (dec), lit. value[260] 138-139 ºC (dec); R_f (chloroform-methanol, 9:1) 0.72 a charring spot after development with ethanolic sulphuric acid (20 % v/v); IR (KBr) cm^{-1} 1372 (gem dimethyl); for NMR data see tables 4 and 5.

Ethyl *N*-(2,3-*O*-isopropylidene-α and β-D-ribofuranosyl)-malonate (55)



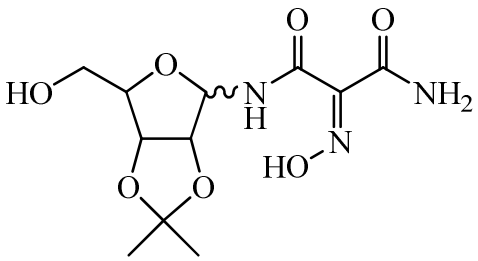
Ethyl malonyl chloride (10.0 g, 66.0 mmol) was added drop-wise with stirring to 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-p-sulphonate (24.0 g, 66.0 mmol) and dry triethylamine (13.5 g, 133.0 mmol) in dry dichloromethane (200 ml) at 0 ºC. The mixture was stirred for 1 h. and left overnight at 4 ºC. The crude reaction mixture was washed with 2 M sulphuric acid (3 x 50 ml), saturated sodium hydrogen carbonate (3 x 50 ml) and finally water (50 ml). The organic phase was collected, dried ($MgSO_4$) and evaporated to give an orange gum which was purified on a silica gel column (chloroform-methanol, 97:3) (11.4 g, 37.0 mmol, 59 %); R_f (chloroform-methanol, 9:1) 0.56 a charring spot after development with ethanolic sulphuric acid (20 % v/v); IR (KBr) cm^{-1} 1735 (C=O), 1682 (C=O), 1372 (gem dimethyl) ; for NMR data see tables 4 and 5.

Ethyl 2-hydroxyimino-*N*-(2,3-*O*-isopropylidene-α and β-D-ribofuranosyl)-malonate (56)



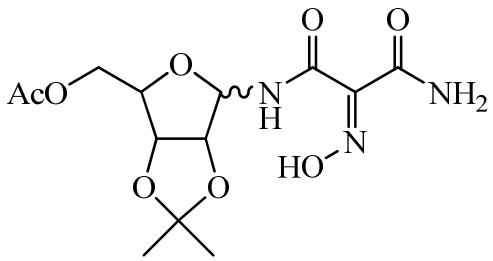
To ethyl *N*-(2',3'-*O*-isopropylidene-α and β-D-ribofuranosyl)-malonate (4.0 g, 13.0 mmol) stirred and cooled to 0 °C, were added glacial acetic acid (1.7 g, 40.0 mmol) and water (50 ml). Sodium nitrite (2.7 g, 40.0 mmol) was added in portions over a period of 1 h. The reaction mixture was stirred at ambient temperature for a further 24 h until all starting materials had been consumed according to TLC. The resulting mixture was extracted with ether (50 ml x 2), the organic phase collected, washed with sodium bicarbonate (20 ml x 6), dried ($MgSO_4$) and evaporated under reduced pressure to give a faint yellow gum as the crude compound (2.4 g, 7.0 mmol, 56 %); R_f (chloroform-methanol, 9:1) 0.86 green spot when revealed with $CuCl_2$; IR (KBr) cm^{-1} 3341 (O-H, oxime), 1731 (C=O), 1690 (C=O), 1666 (C=N), 1374 (gem dimethyl), 976 (N-O); LRMS (ESI) requires $[M]^+$ 332, found $[M + H]^+$ 333; HRMS (ESI) calculated for $[M + H]^+$ requires 333.1292, found 333.1191; for NMR data see tables 4 and 5.

2-Hydroxyimino-*N*-(2',3'-*O*-isopropylidene-α and β-D-ribofuranosyl)-malondiamide (57)



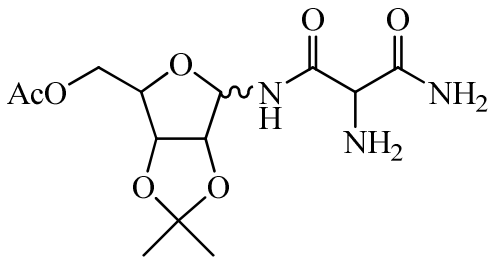
A solution of ethyl 2-hydroxyimino-*N*-(2,3-*O*-isopropylidene-α and β-D-ribofuranosyl)-malonate (10.0 g, 30.0 mmol) in dry ethanol (100 ml) at 0 ºC, contained within a screw cap polythene container, was saturated with dry ammonia. Treatment with ammonia was continued for 30 min., after which the reaction was sealed and stored at 4 ºC for 24 h. The solution was evaporated under reduced pressure to give a foam. TLC examination showed the disappearance of the starting material and the emergence of two new UV active spots (R_f 0.17, 0.20), which reacted positively with copper chloride (5 % w/v) and also showed charring when treated with ethanolic sulphuric acid (20 % v/v). The crude gum was purified by flash chromatography on silica gel (chloroform-methanol, 97:3), to give an unresolved mixture of the two anomers of the title compound as a white foam (7.6 g, 25.2 mmol, 93 %); LRMS (ESI) requires M^+ 303, found $[M + H]^+$ 304; HRMS (ESI) calculated for $[M + H]^+$ requires 304.1139, found 304.1120; for NMR data see tables 4 and 5.

2-Hydroxyimino-*N*-(5'-*O*-acetyl-2',3'-*O*-isopropylidene-α and β-D-ribofuranosyl)-malondiamide (58)



A solution of 2-hydroxyimino-*N*-(2',3'-*O*-isopropylidene-α and β-D-ribofuranosyl)-malondiamide, (5.0 g, 16.0 mmol) in dry pyridine (50 ml) was cooled to 0 ºC. Acetic anhydride (3.4 g, 33.0 mmol) was added drop-wise with stirring over a period of 15 min. The solution was stirred for a further hour at 0 ºC before being allowed to warm up to ambient temperature, where stirring was continued for a further 24 h. The solution was stirred into a solution of rapidly stirring ice-water (100 ml). The mixture was stirred until all the ice had melted and then extracted into dichloromethane (2 x 100 ml). The organic phase was washed successively with 2 M sulphuric acid (2 x 50 ml), saturated sodium hydrogen carbonate (2 x 50 ml) and water (50 ml). The organic phase was dried ($MgSO_4$) and evaporated to a pale yellow foam. TLC examination showed the presence of two new UV active spots (R_f 0.48, 0.51), which reacted positively with copper chloride (5 % w/v) and also showed charring when treated with ethanolic sulphuric acid (20 % v/v). The crude foam was purified by flash chromatography on silica gel (chloroform-methanol, 97:3), to give an unresolved mixture of the two anomers of the title compound as a yellow foam (3.6 g, 10.4 mmol, 67 %); LRMS (ESI) requires $[M]^+$ 345, found $[M + H]^+$ 346; HRMS (ESI) calculated for $[M + H]^+$ requires 346.1245, found 346.1245; for NMR data see tables 4 and 5.

2-Amino-*N*-(5'-*O*-acetyl-2',3'-*O*-isopropylidene-α and β-D-ribofuranosyl)-malondiamide (59)



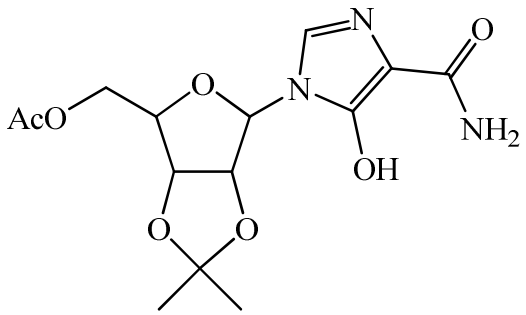
Aluminium foil (0.6 g, 24.0 mmol) torn into 1 cm^2 pieces was placed into a 100 ml conical flask and covered with aqueous mercuric chloride (50 ml, 1 % w/v). After 1 min. the turbid solution was decanted and the residue washed successively with water (50 ml), methanol (4 x 50 ml) and finally re-suspended in methanol (50 ml). The flask was fitted with a mechanical stirrer and cooled in ice. A solution of 2-hydroxyimino-*N*-(5'-*O*-acetyl-2',3'-*O*-isopropylidene-α and β-D-ribofuranosyl)-malondiamide (2.0 g, 6.1 mmol) in methanol (10 ml) was added to the amalgam. Water (1 ml) was added at such a rate as to maintain a reaction temperature of 35-40 ºC. After the addition was complete (~30 min.), stirring was continued for a further 30 min. The reaction mixture was filtered through a pad of 'Celite' filter aid and the residue washed with warm methanol (3 x 50 ml). The combined filtrates were evaporated under reduced pressure to a gum, dissolved in dichloromethane (50 ml) and passed through phase separating paper. TLC examination showed the disappearance of the starting material and the presence of two non-UV active spots (R_f 0.37, 0.46), which were revealed by treatment with ethanolic sulphuric acid (20 % v/v). The solution was evaporated under reduced pressure to a gum (0.6 g), which was purified by flash chromatography on silica gel (chloroform-methanol, 98:2), to give an unresolved mixture of the two anomers of the title compound as a foam (0.4 g, 1.3 mmol, 21 %); LRMS (ESI) requires $[M]^+$ 331, found $[M + H]^+$ 332; HRMS (ESI) calculated for $[M + H]^+$ requires 332.1452, found 332.1229.

Separation of anomers (-α and -β)

A solution of the gum (0.1 g) was chromatographed on a silica gel column (chloroform-methanol, 98:2). The α-anomer was eluted first as a white foam (17.0 mg, 0.05 mmol, 17 %); for NMR data see tables 4 and 5.

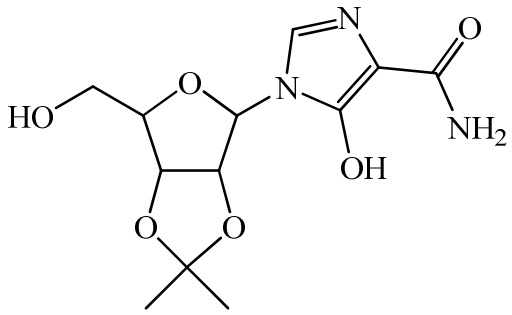
Followed by the β-anomer as a white foam (30.0 mg, 0.1 mmol, 30 %); for NMR data see tables 4 and 5.

4-Carbamoyl-(5'-*O*-acetyl-2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-5-hydroxyimidazole-4-carboxylate (60)



To dry 2-amino-*N*-(5'-*O*-acetyl-2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-malondiamide (410.0 mg, 1.2 mmol) in anhydrous dimethylformamide (5 ml) was added ethyl formimidate hydrochloride (136.0 mg, 1.2 mmol), under a nitrogen atmosphere. The reaction mixture was heated in an oil bath at 110 ºC for 5 min. The flask was allowed to cool, ammonium chloride was removed by filtration through a cotton wool plug in a Pasteur pipette. The solvent was removed using an oil pump vacuum (20 ºC). The pale green gum was dissolved in the minimal volume of dichloromethane and chromatographed by radial chromatography (Chromatotron, 4 mm silica disc). The silica gel disc was equilibrated with dichloromethane, followed by a stepwise methanol gradient (3-20 %). Fractions containing the desired nucleoside were colourless. The solvent was removed under reduced pressure to give a white foam (290.0 mg, 0.8 mmol, 69 %); for NMR see table 6.

Bredinin (7)



To 4-carbamoyl-(5'-*O*-acetyl-2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-5-hydroxyimidazole-4-carboxylate (17.0 mg, 0.5 x 10^{-5} mol) in water (0.1 ml) was added trifluoroacetic acid (0.088 ml, 1.15 mmol). After 3 h at room temperature, the solution was evaporated under reduced pressure (20 ºC) to a foam, dissolved in water (0.5 ml) and chromatographed by preparative HPLC on an O.D.S. column. The column was equilibrated with water and developed with a methanol gradient (0-20 %). Evaporation of the solvent gave a foam (8 mg, 2.7 x 10^{-5} mol, 61 %); for NMR data see tables 6 and 7.

The physical constants were identical to those of an authentic specimen obtained from Toyo Jozo (Japan).

^{1}H-NMR of intermediates

	Intermediate						
	54 $CDCl_3$	55 $CDCl_3$	56 $CDCl_3$	57 DMSO	58 DMSO	59α $CDCl_3$	59β $CDCl_3$
H-1	5.25	5.89, 5.73	5.90, 5.94	5.57	5.58, 5.73	5.77	5.70
H-2	4.69	4.75, 4.66	4.67	4.40 - 4.80	4.70 - 4.80	4.74	4.66
H-3	4.46	4.80, 4.87	4.36			4.71	4.70
H-4	4.18	4.15, 4.29	4.13	3.90	4.00 - 4.20	4.30	4.39
H-5	3.56	3.66, 3.78	3.30	3.30 - 3.35		4.18	4.27
H-5'	3.71	3.80, 3.78	3.46			4.15	4.15
NH_2					2.36	2.20	2.15
CMe_2	1.44, 1.26	1.61, 1.39 1.53, 1.33	1.48, 1.32 1.63, 1.55	1.43, 1.27 1.37, 1.25	1.47, 1.30 1.44, 1.28	1.60, 1.39	1.34, 1.54
CONH		8.30, 8.27	9.68, 9.53	8.60	7.39, 7.82	7.57	8.58
Ac					2.05, 2.07	2.10	2.18

Table 4 ^{1}H-NMR of intermediates.

^{13}C-NMR of intermediates

	Intermediate					
	54 $CDCl_3$	55 $CDCl_3$	58α $CDCl_3$	58β $CDCl_3$	59α DMSO	59β DMSO
C-1	82.9	81.5	81.4	85.8	82.0	81.3
C-2	71.8				80.8	85.6
C-3	76.0				79.6	83.4
C-4	64.2				79.5	81.7
C-5		62.2	64.0	64.1	65.0	65.0
C			170.1	170.2		171.1
CONH		164.9	162.4	162.7	171.0	171.3
$CONH_2$		162.6	160.3	161.3		170.7
C=N			147.9	148.0	57.2	57.1
CO_2		109.2	112.3	112.6	113.8	113.9
$C(Me)_2$	27.6, 26.5	22.3, 21.0	26.0, 24.6	26.7, 25.1	26.3, 25.0	26.9, 25.3

Table 5 ^{13}C-NMR of intermediates.

^{1}H-NMR of imidazoles

	Imidazole		
	60β $CDCl_3$	7β (Hull) $CDCl_3$	7β (Japan) $CDCl_3$
H-1	5.62	5.50	5.52
H-2	5.09	4.38	4.38
H-3	4.88	4.02	4.04
H-4	4.20	3.86	3.88
H-5	4.15	3.60	3.61
H-5'	4.10		3.49
H-2"	7.05	8.23	8.30

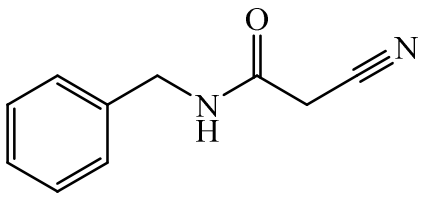
Table 6 ^{1}H-NMR data for imidazoles.

^{13}C-NMR of imidazoles

	Imidazole	
	60β (Hull) $CDCl_3$	7β (Japan) $CDCl_3$
C-1	86.6	87.0
C-2	81.4	70.1
C-3	83.1	73.0
C-4	83.3	85.4
C-5	64.4	61.6
C-2"	125.8	124.7
C-4"	107.6	99.0
C-5"	158.8	155.1
CO	167.9	161.7

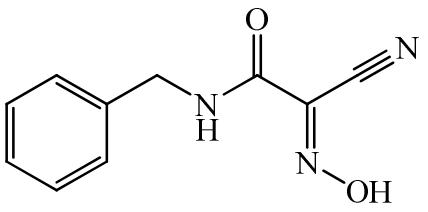
Table 7 ^{13}C-NMR data for imidazoles.

***N*-Benzyl-2-cyanoacetamide**[251] (83)



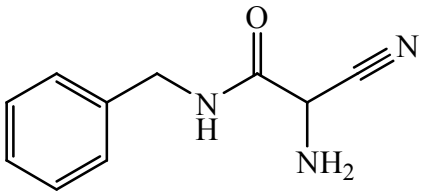
Ethylcyanoacetate (5.70 g, 50.0 mmol) and benzylamine (5.36 g, 50.0 mmol) in acetonitrile (100 ml) were refluxed with stirring for 24 h. The solution was allowed to cool and the solvent removed under reduced pressure to give a pale yellow oil. The oil was dissolved in ethanol (20 ml), rendered turbid with petroleum ether 40-60 º and left at room temperature for 24 h. The product was collect by filtration as bright, white, needle-like crystals (7.10 g, 40.0 mol, 81 %); R_f (chloroform-methanol, 9:1) 0.36 revealed with UV light or iodine vapour; δ**H** (400 MHz; DMSO) 8.69 (1H, NH, t, J = 5.6), 7.25 (5H, Ph, m), 4.25 (2H, CH_2, d, J = 5.6), 3.66 (2H, CH_2, s); δ**C** (100 MHz; DMSO) 162.2 (C=O), 138.6 (Ph), 128.4 (Ph), 127.4 (Ph), 127.0 (Ph), 116.2 (CN), 42.7 (CH_2-N), 25.3 (CH_2); IR (KBr) cm^{-1} 2245 (CN, str), 1634 (C=O, str),; LRMS (ESI) $[M]^+$ requires 174, found $[M + H]^+$ 175.

2-(Benzylamino)-*N*-hydroxy-2-oxoacetimidoyl cyanoanide[253,254] (84)



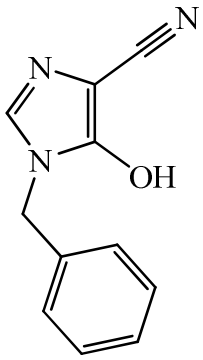
A mixture of concentrated H_2SO_4 (3 ml, d = 1.84), ethanol (3.5 ml), and water (28 ml) was slowly added drop-wise to a solution of sodium nitrite (7.1 g, 0.1 mol) in a mixture of ethanol (31 ml) and water (28 ml) with gradual heating (30-45 °C) and absorption of the released gas (ethyl nitrite) with ethanol (50 ml). To N-benzyl 2-cyanoacetamide (17.4 g, 0.1 mol) in ethanol (150 ml), a solution of sodium ethoxide (0.7 g, 0.1 mol) in ethanol (100 ml) was added. The mixture was stirred for 30 min, the obtained solution was cooled to 0 °C ethyl nitrite (10 ml, 12 mmol) was added, and the mixture was kept for 1 h. The solvent was removed under reduced pressure and the resulting oil dissolved in water (100 ml). The reaction mass was acidified with concentrated hydrochloric acid (~10 ml) to pH 5-6, and the precipitate was filtered off and washed with petroleum ether (40-60 º) to give a pale green precipitate (15.4 g, 76.0 mmol, 76 %); R_f (chloroform-methanol, 9:1) 0.51 green spot when revealed with $CuCl_2$; δ**H** (400 MHz; DMSO) 9.08 (1H, NH, t, J = 5.8), 7.27 (5H, Ph, m), 4.37 (2H, CH_2, d, J = 5.8); δ**C** (100 MHz; DMSO) 158.2 (C=O), 138.7 (Ph), 128.3 (Ph), 127.2 (Ph), 126.9 (Ph), 109.0 (CN), 42.4 (CH_2-N); IR (KBr) cm^{-1} 3509 (O-H, oxime), 2256 (CN, str), 1610 (C=O, str); LRMS (ESI) $[M]^+$ requires 203, found $[M + Na]^+$ 226.

2-Amino-*N*-benzyl-2-cyanoacetamide (85)



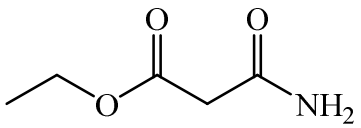
Aluminium foil (2.50 g, 96.0 mmol, torn into 1cm^2 pieces) was placed in a 3-neck round bottom flask. The foil was covered with a solution of mercuric chloride (200 ml, 1 %) and after 1 min the turbid solution was decanted off. The residue was washed successively with water (100 ml) and methanol (4 x 100 ml) and suspended in methanol (200 ml). The flask was placed in an ice-bath and fitted with two condensers, one of which had a dropping funnel fitted, and a thermometer. A solution of the oxime (12.5 g, 62.0 mmol) in methanol (50 ml) was added to the amalgam and water (10 ml) was added at such a rate as to maintain a reaction temperature of 35-40 ºC. After the addition was complete, stirring was continued at room temperature for a further 0.5 h upon which all starting materials were consumed according to TLC. The reaction mixture was filtered through a pad of 'Celite' filter aid and the residue was washed with methanol. The combined filtrates were dried and evaporated to a yellow gum. The crude 2-amino-*N*-benzyl-2-cyanoacetamide was used without any further purification (7.8 g, 41.0 mmol, 67 %); R_f (chloroform-methanol, 9:1) 0.62 brown spot when revealed with $CuCl_2$; δ**H** (400 MHz; DMSO) 8.70 (1H, NH, t, J = 6.1), 7.30 (5H, Ph, m), 4.49 (1H, CH_2, s), 4.22 (2H, CH_2, d, J = 6.1); δ**C** (100 MHz; DMSO) 161.8 (C=O), 137.5 (Ph), 128.5 (Ph), 127.0 (Ph), 126.8 (Ph), 116.1 (CN), 49.1 (CH), 43.5 (CH_2-N); IR (KBr) cm^{-1} 2213 (CN, str), 1645 (C=O, str); LRMS (ESI) $[M]^+$ requires 189, found $[M + K]^+$ 228.

Attempted synthesis of 1-benzyl-5-hydroxyimidazole-4-carbonitrile (86)



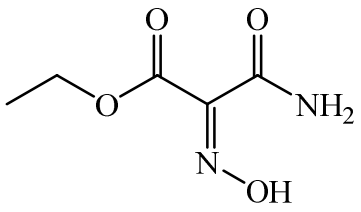
To 2-amino-*N*-benzyl-2-cyanoacetamide (1.90 g, 10.0 mmol) in acetonitrile (25 ml) was added triethyl orthoformate (1.49 g, 10.0 mmol), under a nitrogen atmosphere. The reaction mixture was refluxed for 3 h. The solvent was removed under reduced pressure to give a dark red oil. The oil was dissolved in ethanol (10 ml), rendered turbid with petroleum ether 40-60 º and left at room temperature for 24 h. The product was collected by filtration as pale brown crystals (1.23 g); δ**H** (400 MHz; DMSO) 9.32 (t, *J* = 6.3), 7.26 (5H, Ph, m), 4.31 (1H, CH_2, d, *J* = 6.3); δ**C** (100 MHz; DMSO) 160.1 (C=O/C=N), 138.7 (Ph), 128.3 (Ph), 127.4 (Ph), 126.9 (Ph), 39.9 (CH_2-N); LRMS (ESI) $[M]^+$ requires 199, found $[M + K]^+$ 169.

Ethyl 3-amino-3-oxopropanoate[256] (87)



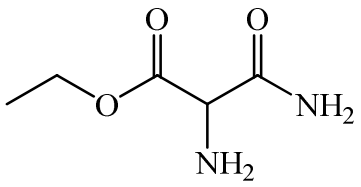
Trimethylsilyl chloride (24.2 g, 0.2 mol) was added to an ice-cold ethyl cyanoacetate (12.5 g, 0.1 mol). Water (4.0 g, 0.2 mol) was added drop-wise and once the addition was complete the solution was allowed to warm up to room temperature slowly over 4 h. The reaction mixture was neutralised with saturated sodium hydrogen carbonate (5 x 100 ml) at 0 ºC, and extracted in dichloromethane (2 x 250 ml). The organic phase was dried ($MgSO_4$) and the solvent removed under reduced pressure to give a colourless oil (11.7 g, 90.0 mmol, 40 %); δ**H** (400 MHz; $CDCl_3$) 4.16 (2H, CH_2, q, J = 7.4), 3.29 (2H, CH_2, s), 1.22 (3H, CH_3, t, J = 7.0); δ**C** (100 MHz; $CDCl_3$) 164.7 (C=O amide), 163.1 (C=O ester), 61.5 (CH_2 ester), 41.7 (CH_2), 14.0 (CH_3); IR (KBr) cm^{-1} 1735 (C=O, str), 1685 (C=O, str); MS (ESI) found $[M + H]^+$ 132, requires M^+ 131.

Ethyl 3-amino-2-(hydroxyimino)-3-oxopropanoate (88)



To a mixture of malonamic acid ethyl ester (15.0 g, 0.1 mol) stirred and cooled to 0 °C, were added glacial acetic acid (20.6 g, 0.3 mol) and water (100 ml). Sodium nitrite (23.7 g, 0.3 mol) was added in portions over a period of 1 h. The reaction mixture was stirred at ambient temperature for a further 24 h until all starting materials had been consumed according to TLC. The resulting mixture was extracted with ether (50 ml x 2), the organic phase collected, washed with sodium hydrogen carbonate (20 ml x 6), dried ($MgSO_4$) and evaporated under reduced pressure to give a faint yellow gum as the crude compound (5.5 g, 0.3 mol, 30 %); R_f (chloroform-methanol, 9:1) 0.75 green spot when revealed with $CuCl_2$; δ**H** (400 MHz; $CDCl_3$) 10.61 (1H, OH, s), 4.31 (2H, CH_2, q, J = 7.1), 1.29 (3H, CH_3, t, J = 7.1); δ**C** (100 MHz; $CDCl_3$) 163.2 (C=O amide), 161.8 (C=O ester), 62.3 (CH_2 ester), 45.7 (C=N), 13.0 (CH_3); IR cm^{-1} 3419 (O-H, oxime), 1732 (C=O, str), 1695 (C=O, str), 1676 (C=N); LRMS (ESI) $[M]^+$ requires 160, found $[M + H]^+$ 161.

Ethyl 2,3-diamino-3-oxopropanoate (89)



Aluminium foil (2.5 g, 96.0 mmol, torn into 1cm^2 pieces) was placed in a 3-neck round bottom flask. The foil was covered with a solution of mercuric chloride (200 ml, 1 %) and after 1 min the turbid solution was decanted off. The residue was washed successively with water (100 ml) and methanol (4 x 100 ml) and suspended in methanol (200 ml). The flask was placed in an ice-bath and fitted with two condensers, one of which had a dropping funnel fitted, and a thermometer. A solution of the oxime (12.5 g, 78.0 mmol) in methanol (50 ml) was added to the amalgam and water (10 ml) was added at such a rate as to maintain a reaction temperature of 35-40 ºC. After the addition was complete, stirring was continued at room temperature for a further 0.5 h upon which all starting materials were consumed according to TLC. The reaction mixture was filtered through a pad of 'Celite' filter aid and the residue was washed with methanol. The combined filtrates were dried and evaporated to an orange gum. The crude 2-amino-malonamic acid ethyl ester was used without any further purification (7.3 g, 50.0 mol, 64 %); R_f (chloroform-methanol, 9:1) 0.63 brown spot when revealed with $CuCl_2$; δ**H** (400 MHz; $CDCl_3$) 4.54 (1H, CH, s), 4.25 (4H, CH_2, q, J = 7.1), 1.17 (3H, CH_3, t, J = 7.1); δ**C** (100 MHz, $CDCl_3$) 168.8 (C=O), 163.5 (C=O), 68.1 (CH_2), 54.9 (C-N), 14.9 (CH_3); IR (KBr) cm^{-1} 1745 (C=O, str), 1671 (C=O, str); LRMS (ESI) $[M]^+$ requires 146, found $[M + H]^+$ 147.

REFERENCES

1. K. Mizuno, M. Tsujino, M. Takada, M. Hayashi, K. Atsumi, K. Asano, and T. Matsuda, *J. Antibiot.*, 1974, **27**, 775.

2. H. K. Webster and J. M. Whaun, *J. Clin. Invest.*, 1982, **70**, 461.

3. K. Sakaguchi, M. Tsujino, M. Yoshizawa, K. Mizuno, and K. Hayano, *Cancer Res.*, 1975, **35**, 1643.

4. K. Sakaguchi, M. Tsujino, M. Hayashi, K. Kawai, K. Mizuno, and K. Hayano, *J. Antibiot.*, 1976, **29**, 1320.

5. H. Koyama and M. Tsuji, *Biochem. Pharmacol.*, 1983, **32**, 3547.

6. Prevention & control of priority communicable diseases, World Health Organisation, http://www.searo.who.int/en/Section10/Section21/Section334_4008.htm, 2007.

7. S. R. Krungkrai, S. Aoki, N. M. Q. Palacpac, D. Sato, T. Mitamura, J. Krungkrai, and T. Horii, *Mol. Biol. Parasitol.*, 2004, **134**, 245.

8. D. A. Casteel, in *Burger's Medicinal Chemistry and Drug Discovery*, John Wiley and Sons Inc., 1997.

9. Malaria and Medicines, Medicines for Malaria Venture, http://www.mmv.org/, 2006.

10. Roll Back Malaria, World Health Organisation, http://w3.whosea.org/index.htm, 2006.

11. Plasmodium Life Cycle, Sanger Institute, http://www.sanger.ac.uk/PostGenomics/plasmodium/presentations/plasmodium_lifecycle.shtml, 2006.

12. I. W. Sherman, *Microbiol. Rev.*, 1979, **43**, 453.

13. Y. A. Abdi, L. L. Gustafsson, O. Ericsson, and U. Hellgren, in *Handbook of Drugs for Tropical Parasitic Infections*, Taylor & Francis, Second edn., 1995, chapter 2, pp. 39-46.

14. *WHO Guidlines for the Treatment of Malaria*, World Health Organization, 2006.

15. Y. A. Abdi, L. L. Gustafsson, O. Ericsson, and U. Hellgren, in *Handbook of Drugs for Tropical Parasitic Infections*, Taylor & Francis, Second edn., 1995, chapter 9, pp. 39-46.

16. A. F. G. Slater and A. Cerami, *Nature*, 1992, **355**, 167.

17. R. H. Black, C. J. Canfield, D. F. Clyde, W. Peters, and W. H. Wernsdorfer, in *Chemotherapy of Malaria*, Second edn., 1986.

18. P. Brasseur, R. Guiguemde, S. Diallo, V. Guiyedi, M. Kombila, P. Ringwald, and P. Olliaro, *Trans. Roy. Soc. Trop. Med. Hyg.*, 1999, **93**, 645.

19. G. Fadat, J. LeBras, C. Hengy, J. P. Louis, M. M. Gimou, and F. Verdier, *Lancet*, 1991, **338**, 1092.

20. P. Olliaro, C. Nevill, J. LeBras, P. Ringwald, P. Mussano, P. Garner, and P. Na Brasseur, *Lancet*, 1996, **348**, 1196.

21. Y. A. Abdi, L. L. Gustafsson, O. Ericsson, and U. Hellgren, in *Handbook of Drugs for Tropical Parasitic Infections*, Taylor & Francis, Second edn., 1995, chapter 33, pp. 39-46.

22. H. M. Bryson and K. L. Goa, *Drugs*, 1992, **43**, 236.

23. J. M. Karle, R. Olmeda, L. Gerena, and W. K. Milhous, *Exp. Parasitol.*, 1994, **76**, 345.

24. Y. A. Abdi, L. L. Gustafsson, O. Ericsson, and U. Hellgren, in *Handbook of Drugs for Tropical Parasitic Infections*, Taylor & Francis, Second edn., 1995, chapter 14, pp. 39-46.

25. X. D. Luo and C. C. Shen, *Med. Res. Rev.*, 1987, **7**, 29.

26. Y. A. Abdi, L. L. Gustafsson, O. Ericsson, and U. Hellgren, in *Handbook of Drugs for Tropical Parasitic Infections*, Taylor & Francis, Second edn., 1995, chapter 6, pp. 39-46.

27. D. L. Klayman, *Science*, 1985, **228**, 1049.

28. J. N. Cumming, P. Ploypradith, and G. H. Posner, *Adv. Pharmacol.*, 1997, **37**, 253.

29. A. V. Pandey, B. L. Tekwani, R. L. Singh, and V. S. Chauhan, *J. Biol. Chem.*, 1999, **274**, 19383.

30. G. Lefevre, S. Looareesuwan, S. Treeprasertsuk, S. Krudsood, U. Silachamroon, I. Gathmann, R. Mull, and R. Bakshi, *Am. J. Trop. Med. Hyg.*, 2001, **64**, 247.

31. T. K. Mutabingwa, D. Anthony, A. Heller, R. Hallett, J. Ahmed, C. Drakeley, B. M. Greenwood, and C. J. M. Whitty, *Lancet*, 2005, **365**, 1474.

32. Anon., in *Who Model Prescribing Information: Drugs Used in Parasitic Diseases*, World Health Organisation, Second ed., 1995.

33. S. R. Krungkrai, S. Aoki, N. M. Q. Palacpac, D. Sato, T. Mitamura, J. Krungkrai, and T. Horii, *Mol. Biol. Parasitol.*, 2004, **134**, 245.

34. W. Büngener and G. Nielsen, *Z. Tropenmed. Parasitol.*, 1968, **19**, 185.

35. W. Büngener and G. Nielsen, *Z. Tropenmed. Parasitol.*, 1967, **18**, 456.

36. W. E. Gutteridge and P. I. Trigg, *J. Protozool.*, 1970, **17**, 89.

37. K. van Dyke, G. C. Tremblay, C. H. Lantz, and C. Szustkiewicz, *Am. J. Trop. Med. Hyg.*, 1970, **19**, 203.

38. C. J. Walsh and I. W. Sherman, *J. Protozool.*, 1968, **15**, 503.

39. C. J. Walsh and I. W. Sherman, *J. Protozool.*, 1968, **15**, 763.

40. A. M. Gero and W. J. O'Sullivan, *Blood Cells*, 1990, **16**, 467.

41. I. W. Sherman, *Microbiol. Rev.*, 1979, **43**, 453.

42. V. Asawamahasakda and Y. Yuthavong, *Parasitology*, 1993, **107**, 1.

43. H. F. Hassan and G. H. Coombs, *FEMS Microbiol. Rev.*, 1988, **4**, 47.

44. J. Krungkrai, H. K. Webster, and Y. Yuthavong, *Mol. Biochem. Parasitol.*, 1989, **32**, 25.

45. M. S. P. Manandhar and K. van Dyke, *Exp. Parasitol.*, 1975, **37**, 138.

46. L. W. Scheibel and I. W. Sherman, in *Malaria: Principles and Practice of Malariology*, Wernsdorfer, W. H. & McGregor, I. Eds., 1988, vol. 1, pp. 234.

47. R. G. Ridley, *Nature*, 2002, **415**, 686.

48. M. J. Gardner, N. Hall, E. Fung, O. White, M. Berriman, R. W. Hyman, J. M. Carlton, A. Pain, K. E. Nelson, S. Bowman, I. T. Paulsen, K. James, J. A. Eisen, K. Rutherford, S. L. Salzberg, A. Craig, S. Kyes, M. S. Chan, V. Nene, S. J. Shallom, B. Suh, J. Peterson, S. Angiuoli, M. Pertea, J. Allen, J. Selengut, D. Haft, M. W. Mather, A. B. Vaidya, D. M. A. Martin, A. H. Fairlamb, M. J. Fraunholz, D. S. Roos, S. A. Ralph, G. I. McFadden, L. M. Cummings, G. M. Subramanian, C. Mungall, J. C. Venter, D. J. Carucci, S. L. Hoffman, C. Newbold, R. W. Davis, C. M. Fraser, and B. Barrell, *Nature*, 2002, **419**, 498.

49. P. Reyes, P. K. Rathod, D. J. Sanchez, J. E. K. Mrema, K. H. Reickmann, and H. G. Heidrich, *Mol. Biol. Parasitol.*, 1982, **5**, 279.

50. P. K. Rathod and P. Reyes, *J. Biol. Chem.*, 1983, **258**, 2852.

51. H. V. Scott, A. M. Gero, and W. J. O'Sullivan, *Mol. Biol. Parasitol.*, 1986, **18**, 3.

52. A. M. Gero and W. J. O'Sullivan, *Blood Cells*, 1990, **16**, 467.

53. J. Krungkrai, A. Cerami, and G. B. Henderson, *Biochemistry*, 1991, **30**, 1934.

54. J. Krungkrai, S. R. Krungkrai, and K. Phakanont, *Biochem. Pharmacol.*, 1992, **43**, 1295.

55. J. Krungkrai, *Drugs Fut.*, 1993, **18**, 441.

56. K. K. Seymour, S. D. Lyons, L. Phillips, K. H. Rieckmann, and R. I. Christopherson, *Biochemistry*, 1994, **33**, 5268.

57. J. Baldwin, A. M. Farajallah, N. A. Malmquist, P. K. Rathod, and M. A. Phillips, *J. Biol. Chem.*, 2002, **277**, 41827.

58. J. Krungkrai, *Biochim. Biophys. Acta*, 1995, **1243**, 351.

59. R. I. Christopherson, S. D. Lyons, and P. K. Wilson, *Acc. Chem. Res.*, 2002, **35**, 961.

60. A. B. G. Laing, *B. M. J.*, 1964, **2**, 1439.

61. Y. A. Abdi, L. L. Gustafsson, O. Ericsson, and U. Hellgren, in *Handbook of Drugs for Tropical Parasitic Infections*, Taylor & Francis, Second edn., 1995, chapter 34, pp. 39-46.

62. http://www.mmv.org/, http://www.mmv.org/, 2006.

63. Y. A. Abdi, L. L. Gustafsson, O. Ericsson, and U. Hellgren, in *Handbook of Drugs for Tropical Parasitic Infections*, Taylor & Francis, Second edn., 1995, chapter 31, pp. 39-46.

64. W. Trager and J. B. Jensen, *Science*, 1976, **193**, 673.

65. J. D. Haynes, C. L. Diggs, F. A. Hilnes, and R. E. Desjardins, *Nature*, 1976, **263**, 767.

66. H. K. Webster and J. M. Whaun, *J. Clin. Invest.*, 1982, **70**, 461.

67. D. G. Batt, *Exp. Opin. Ther. Patents*, 1999, **9**, 41.

68. P. R. Hirst, Ph. D, University of Hull, 2001.

69. R. L. P. Adams, J. T. Knowler, and D. P. Leader, in *The Biochemistry of Nucleic Acids*, Chapman and Hall, 1986, pp. 124.

70. A. White, P. Handler, E. L. Smith, R. L. Hill, and J. R. Hehman, in *Principles of Biochemistry*, McGraw-Hill, London, 1978, pp. 761.

71. D. T. Hurst, in *An Introduction to the Chemistry and Biochemistry of Pyrimidines, Purines and Pteridines*, John Wiley & Sons, 1980.

72. W. I. P. Mainwaring, J. H. Parish, J. D. Pickering, and N. H. Mann, in *Nucleic Acid Biochemistry and Molecular Biology*, Blackwell Scientific Publications, 1982.

73. I. N. Sujay Subbayya, S. S. Ray, and H. Balaram, *Indian J. Med. Res.*, 1997, **106**, 79.

74. G. Thomas, in *Medicinal Chemistry, An Introduction to*, John Wiley and Sons Inc., 2004, pp. 404.

75. K. M. Kerr, J. A. Digits, N. Kuperwasser, and L. Hedstrom, *Biochemistry*, 2000, **39**, 9804.

76. K. Mizuno, M. Tsujino, M. Takada, M. Hayashi, K. Atsumi, K. Asano, and T. Matsuda, *J. Antibiot.*, 1974, **27**, 775.

77. K. Sakaguchi, M. Tsujino, M. Yoshizawa, K. Mizuno, and K. Hayano, *Cancer Res.*, 1975, **35**, 1643.

78. K. Sakaguchi, M. Tsujino, M. Hayashi, K. Kawai, K. Mizuno, and K. Hayano, *J. Antibiot.*, 1976, **29**, 1320.

79. H. Iwata, H. Iwaki, T. Masukawa, S. Kasamatsu, and K. Okamoto, *Experientia*, 1976, **33**, 502.

80. H. Koyama and M. Tsuji, *Biochem. Pharmacol.*, 1983, **32**, 3547.

81. T. Inou, R. Kusaba, I. Takahashi, H. Sugimoto, K. Kuzuhara, Y. Yamada, J. Yamauchi, and O. Otsubo, *Transplantation Proc.*, 1981, **13**, 315.

82. J. Jain, S. J. Almquist, P. J. Ford, D. Shlyakhter, Y. Wang, E. Nimmesgern, and U. A. Germann, *Biochem. Pharmacol.*, 2004, **67**, 767.

83. R. C. Jackson and G. Weber, *Nature*, 1975, **256**, 331.

84. R. I. Christopherson, S. D. Lyons, and P. K. Wilson, *Acc. Chem. Res.*, 2002, **35**, 961.

85. J. McMurry and T. Begley, in *The Organic Chemistry of Biological Pathways*, Roberts Publishers, 2004, pp. 305.

86. B. S. Mitchell, J. S. Dayton, L. A. Turka, and C. B. Thompson, *Ann. N. Y. Acad. Sci.*, 1993, **685**, 217.

87. J. Balzarini, A. Karlsson, L. Wang, C. Bohman, K. Horska, I. Votruba, A. Fridland, A. Van Aerschot, P. Herdewijn, and E. J. De Clercq, *J. Biol. Chem.*, 1993, **268**, 24591.

88. Y. Yamada, Y. Natsumeda, and G. Weber, *Biochemistry*, 1988, **27**, 2193.

89. R. I. Christopherson, S. D. Lyons, and P. K. Wilson, *Acc. Chem. Res.*, 2002, **35**, 961.

90. Y. Yamada, Y. Natsumeda, and G. Weber, *Biochemistry*, 1988, **27**, 2193.

91. H. N. Jayaram, G. S. Ahluwalia, R. L. Dion, G. Gebeyehu, V. E. Marquez, J. A. Kelley, R. K. Robins, D. A. Cooney, and D. G. Johns, *Biochem. Pharmacol.*, 1983, **32**, 2633.

92. E. E. Conn, P. K. Stumpf, G. Bruening, and R. H. Doi, in *Outlines of Biochemistry*, John Wiley and Sons Inc., 1987.

93. T. P. Begley, T. C. Appleby, and S. E. Ealick, *Curr. Opin. Struct. Biol.*, 2000, **10**, 711.

94. T. C. Appleby, C. Kinsland, T. P. Begley, and S. E. Ealick, *Proc. Natl. Acad. Sci.*, 2000, **97**, 2005.

95. P. J. O'Brian and D. Herschlag, *Chem. Biol.*, 1999, **6**, R91.

96. A. Radzicka and R. Wolfenden, *Science*, 1995, **267**, 90.

97. M. J. Snider and R. Wolfenden, *J. Am. Chem. Soc.*, 2000, **122**, 11507.

98. A. Warshel and J. Florian, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 5950.

99. B. G. Miller and R. Wolfenden, *Annu. Rev. Biochem.*, 2002, **71**, 847.

100. A. Sievers and R. Wolfenden, *J. Am. Chem. Soc.*, 2002, **124**, 13986.

101. N. Wu, Y. Mo, J. Gao, and E. F. Pai, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 2017.

102. N. Wu, W. Gillon, and E. F. Pai, *Biochemistry*, 2002, **41**, 4002.

103. B. P. Callahan and R. Wolfenden, *J. Am. Chem. Soc.*, 2004, **126**, 14698.

104. A. Warshel, M. Strajbl, J. Villa, and J. Florian, *Biochemistry*, 2000, **39**, 14728.

105. P. Beak and B. Siegel, *J. Am. Chem. Soc.*, 1976, **98**, 3601.

106. J. I. Ehrlich, C.-C. Hwang, P. F. Cook, and J. S. Blanchard, *J. Am. Chem. Soc.*, 1999, **121**, 6966.

107. B. G. Miller, A. M. Hassell, R. Wolfenden, M. V. Milburn, and S. A. Short, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 2011.

108. T. C. Appleby, C. Kinsland, T. P. Begley, and S. E. Ealick, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 2000.

109. K. Toth, T. L. Amyes, B. M. Wood, K. Chan, J. A. Gerlt, and J. P. Richard, *J. Am. Chem. Soc.*, 2007, **129**, 12946.

110. T. L. Aymes, B. M. Wood, K. Chan, J. A. Gerlt, and J. P. Richard, *J. Am. Chem. Soc.*, 2008, **130**, 1574.

111. F. M. Wong, C. C. Capule, and W. Wu, *Org. Lett.*, 2006, **8**, 6019.

112. F. Y. Yeoh, R. R. Cuasito, C. C. Capule, F. M. Wong, and W. Wu, *Bioorg. Chem.*, 2007, **35**, 338.

113. M. A. Rishavy and W. W. Cleland, *Biochemistry*, 2000, **39**, 4569.

114. J. K. Lee and K. N. Houk, *Science*, 1997, **276**, 942.

115. S. Raugei, M. Cascella, and P. Carloni, *J. Am. Chem. Soc.*, 2004, **126**, 15730.

116. D. E. Dix, C. P. Lehman, A. Jakubowski, J. D. Moyer, and R. E. Handschumacher, *Cancer Res.*, 1979, **39**, 4485.

117. H. L. Levine, R. S. Brody, and F. H. Westheimer, *Biochemistry*, 1980, **19**, 4993.

118. R. I. Christopherson, S. D. Lyons, and P. K. Wilson, *Acc. Chem. Res.*, 2002, **35**, 961.

119. G. K. Brown and W. J. O'Sullivan, *Biochem. Pharmacol.*, 1977, **26**, 1947.

120. P. Pattanaik, J. Raman, and H. Balaram, *Curr. Top. Med. Chem.*, 2002, **2**, 483.

121. U. Sandbhor, P. Kulkarni, S. Padhye, G. Kundu, G. Mackenzie, and R. Pritchard, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 2877.

122. G. Shaw, *J. Chem. Soc.*, 1981, 299.

123. S. Ruhemann and A. V. Cunnington, *J. Chem. Soc. , Trans.*, 1899, 954.

124. T. Brown, Ph.D., University of Hull, 1979.

125. H. Yoshioka, K. Nakatsu, M. Hayashi, and K. Mizuno, *Tetrahedron Lett.*, 1975, **16**, 4031.

126. M. Shionoya, E. Kimura, and M. J. Shiro, *J. Am. Chem. Soc.*, 1993, **115**, 6730.

127. H. Yoshioka, K. Nakatsu, M. Hayashi, and K. Mizuno, *Tet. Lett.*, 1975, 4031.

128. K. Sakaguchi, M. Tsujino, M. Yoshizawa, K. Mizuno, and K. Hayano, *Cancer*, 1975, **35**, 1643.

129. T. Inou, R. Kusaba, I. Takahashi, H. Sugimoto, K. Kuzuhara, Y. Yamada, J. Yamauchi, and O. Otsubo, *Transplant. Proc.*, 1981, **8**, 315.

130. K. Okamoto, M. Ito, and Y. Suzuki, *Jpn. J. Pharmacol.*, 1983, **33**, 514.

131. K. Okamoto, M. Ito, and Y. Suzuki, *Jpn. J. Pharmacol.*, 1984, **34**, 33.

132. H. Ishikawa and K. W. Pankiewicz, *Curr. Med. Chem.*, 1999, **6**, 575.

133. B. S. Mitchell, J. S. Dayton, L. A. Turka, and C. B. Thompson, *Ann. N. Y. Acad. Sci.*, 1993, **685**, 217.

134. S. Shuto, H. Itoh, E. Endo, K. Fukukawa, M. Tsujino, and T. Ueda, *Chem. Pharm. Bull.*, 1987, **35**, 3523.

135. N. Yoshida, M. Nakamura, M. Fukui, S. Morisada, S. Ogino, M. Inaba, S. Tsukagoshi, and Y. Sakurai, *Cancer Res.*, 1983, **43**, 5851.

136. M. Fukuri, M. Inaba, S. Tsukagoshi, and Y. Sakurai, *Cancer Res.*, 1982, **42**, 1098.

137. Y. Kosugi, Y. Saito, S. Mori, J. Watanabe, M. Baba, and S. Shigeta, *Antiviral Chem. Chemother.*, 1994, **5**, 366.

138. H. Iwata, H. Iwaki, T. Masukawa, S. Kasamatsu, and K. Okamoto, *Experientia*, 1976, **33**, 502.

139. H. K. Webster and J. M. Whaun, *J. Clin. Invest.*, 1982, **70**, 461.

140. R. W. Humble, Ph. D, University of Hull, 1990.

141. D. F. Ewing, R. W. Humble, and G. Mackenzie, *Nucleosides & Nucleotides*, 1995, **14**, 369.

142. M. Hayashi, T. Hirano, M. Yaso, K. Mizuno, and T. Ueda, *Chem. Pharm. Bull.*, 1975, **23**, 245.

143. K. Fukukawa, S. Shuto, T. Hirano, and T. Ueda, *Chem. Pharm. Bull.*, 1984, **32**, 1644.

144. K. Kamata, M. Okubo, E. Ishigamori, Y. Masaki, H. Uchida, K. Watanabe, and N. Kashiwagi, *Transplantation*, 1983, **35**, 144.

145. T. Inou, *Jpn. J. Transplant.*, 1982, **17**, 547.

146. F. G. Germuth and E. Rodriguez, in *Immunopathology of the renal glomerulus: Immune complex deposit and anti basement membrane disease*, Little Brown, Boston, 1973, pp. 15.

147. K. Okamoto, M. Ito, and Y. Suzuki, *Jap. J. Pharmacol.*, 1983, **33**, 541.

148. K. Okamoto, M. Ito, and Y. Suzuki, *Jap. J. Pharmacol.*, 1984, **34**, 33.

149. Y. Kobayashi, H. Shigematsu, and Y. Masaki, *Virchows Arch. [Cell Pathol.]*, 1983, **43**, 103.

150. K. Kamata, M. Okubo, Y. Uchiyama, Y. Masaki, Y. Kobayashi, and T. Tanaka, *Clin. Immunol. Immunopathol.*, 1984, **33**, 31.

151. K. Mizuno and T. Miyazaki, *Chem. Pharm. Bull.*, 1976, **24**, 2248.

152. M. Inaba, M. Fukui, N. Yoshida, S. Tsukagoshi, and Y. Sakurai, *Cancer Res.*, 1982, **42**, 1103.

153. M. Fukuri, M. Inaba, S. Tsukagoshi, and Y. Sakurai, *Cancer Res.*, 1982, **42**, 1098.

154. Y. Tarumi, Y. Takebayashi, and T. Atsumi, *J. Heterocycl. Chem.*, 1984, **21**, 849.

155. H. Iwata, H. Iwaki, T. Masukawa, S. Kasamatsu, and K. Okamoto, *Experientia*, 1977, **33**, 502.

156. M. J. Gardner, N. Hall, E. Fung, O. White, M. Berriman, R. W. Hyman, J. M. Carlton, A. Pain, K. E. Nelson, S. Bowman, I. T. Paulsen, K. James, J. A. Eisen, K. Rutherford, S. L. Salzberg, A. Craig, S. Kyes, M. S. Chan, V. Nene, S. J. Shallom, B. Suh, J. Peterson, S. Angiuoli, M. Pertea, J. Allen, J. Selengut, D. Haft, M. W. Mather, A. B. Vaidya, D. M. A. Martin, A. H. Fairlamb, M. J. Fraunholz, D. S. Roos, S. A. Ralph, G. I. McFadden, L. M. Cummings, G. M.

Subramanian, C. Mungall, J. C. Venter, D. J. Carucci, S. L. Hoffman, C. I. Newbold, R. W. Davis, C. M. Fraser, and B. Barrell, *Nature*, 2002, **419**, 498.

157. H. K. Webster and J. M. Whaun, *J. Clin. Invest.*, 1982, **70**, 461.

158. G. A. Rutter, G. Da Silva Xavier, and I. Leclerc, *Biochem. J.*, 2003, **375**, 1.

159. D. R. Lara, O. P. Dall'Igna, E. S. Ghisolfi, and M. G. Brunstein, *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, 2006, **30**, 617.

160. R. A. Smith and W. Kirkpatrick, in *Ribavirin: A Broad Spectrum Antiviral Agent*, Academic Press Inc., 1980.

161. Y. F. Gong, R. V. Srinivas, and A. Fridland, *Mol. Pharmacol.*, 1993, **44**, 30.

162. N. Kohyama, T. Katashima, and Y. Yamamoto, *Synthesis*, 2004, **17**, 2799.

163. S. M. Ulrich, N. A. Sallee, and K. M. Shokat, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 3223.

164. M. Wall, J. H. Shim, and S. J. Benkovic, *Biochemistry*, 2000, **39**, 11303.

165. N. Minakawa, T. Takeda, T. Sasaki, A. Matsuda, and T. Ueda, *J. Med. Chem.*, 1991, **34**, 778.

166. N. Minakawa, N. Kojima, T. Sasaki, and A. Matsuda, *Nucleosides & Nucleotides*, 1996, **15**, 251.

167. R. Guezguez, K. Bougrin, K. El Akri, and R. Benhida, *Tetrahedron Lett.*, 2006, **47**, 4807.

168. R. W. Sidwell, J. H. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, and R. K. Robins, *Science*, 1972, **177**, 705.

169. T. I. Kalman and D. M. Houston, *Nucleosides & Nucleotides*, 1989, **8**, 899.

170. S. Crotty, C. E. Cameron, and R. Andino, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 6895.

171. G. M. Keating and G. L. Plosker, *Drugs*, 2005, **65**, 521.

172. P. G. Canonico, M. D. Kastello, C. T. Spears, J. R. Brown, E. A. Jackson, and D. E. Jenkins, *Toxicol. Appl. Pharmacol.*, 1984, **30**, 155.

173. J. Watson, *Curr. Opin. Investig. Drugs*, 2002, **3**, 680.

174. C. Simons, Q. Wu, and T. T. Htar, *Curr. Top. Med. Chem.*, 2005, **5**, 1191.

175. M. P. Walker, T. C. Appleby, W. Zhong, J. Y. N. Lau, and Z. Hong, *Antiviral Chem. Chemother.*, 2003, **14**, 1.

176. E. De Clercq, M. Cools, J. Balzarini, R. Snoeck, G. Andrei, M. Hosoya, S. Shigeta, T. Ueda, N. Minakawa, and A. Matsuda, *Antimicrob. Agents Chemother.*, 1991, **35**, 679.

177. D. G. Streeter, J. T. Witkowski, G. P. Khare, R. W. Sidwell, R. J. Bauer, R. K. Robins, and L. N. Simon, *Proc. Natl. Acad. Sci.*, 1973, **70**, 1174.

178. K. Gerzon, R. H. Williams, M. Hoehn, M. Gorman, and D. C. DeLong, 2nd International Congress of Heterocyclic Chemistry, 1969.

179. H. V. Scott, A. M. Gero, and W. J. O'Sullivan, *Mol. Biol. Parasitol.*, 1986, **18**, 3.

180. J. F. Worzalla and M. J. Sweeney, *Cancer Res.*, 1980, **40**, 1482.

181. K. K. Seymour, S. D. Lyons, L. Phillips, K. H. Rieckmann, and R. I. Christopherson, *Biochemistry*, 1994, **33**, 5268.

182. H. Yoshioka, K. Nakatsu, M. Hayashi, and K. Mizuno, *Tet. Lett.*, 1975, 4031.

183. R. Gu, W. Tang, and J. Pu, *Pharm. Indust.*, 1986, **17**, 129.

184. K. Fukukawa, S. Shuto, T. Hirano, and T. Ueda, *Chem. Pharm. Bull.*, 1986, **34**, 3653.

185. http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/08220, http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/08220, 2008.

186. J. K. Horton and M. F. G. Steven, *J. Pharm. Pharmacol.*, 1981, **33**, 308.

187. S. Shuto, K. Haramuishi, M. Fukuoka, and A. Matsuda, *J. Chem. Soc. , Perkin Trans. I*, 2000, 3603.

188. K. Kawana, G. A. Ivanovics, R. J. Rousseau, and R. K. Robins, *J. Med. Chem.*, 1980, **45**, 4020.

189. P. C. Srivastava, R. J. Rousseau, and R. K. Robins, *Chem. Commun.*, 1977, 151.

190. A. F. Cook, R. T. Bartlett, R. P. Greggson, and R. J. Quinn, *J. Org. Chem*, 1980, **45**, 4020.

191. C. S. Miller, S. Gurin, and D. Wright Wilson, *J. Am. Chem. Soc.*, 1952, **74**, 2892.

192. H. Finger, *J. Prakt. Chem.*, 1907, **76**, 93.

193. Y. Tarumi, K. Moriguchi, and T. Atsumi, *J. Heterocycl. Chem.*, 1984, **21**, 529.

194. Y. Tarumi and T. Atsumi, *J. Heterocycl. Chem.*, 1983, **20**, 875.

195. C. Simons, in *Nucleoside Mimetics: Their Chemistry and Biological Properties*, Taylor & Francis, First ed., 2000.

196. E. J. Corey, *Angew. Chem. Int. Ed. Engl.*, 1991, **30**, 455.

197. M. R. Grimmett, in *Imidazole and Benzimidazole Synthesis*, Harcourt Brace and Company, 1997.

198. G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J. Chem. Soc.*, 1959, 1648.

199. N. J. Cusack, P. W. Rugg, and G. Shaw, *Chem. Commun.*, 1971, 190.

200. N. J. Cusack, P. W. Rugg, and G. Shaw, *J. Chem. Soc. , Perkin 1*, 1974, 73.

201. N. J. Cusack, B. J. Hildick, D. H. Robinson, P. W. Rugg, and G. Shaw, *Chem. Commun.*, 1970, 1114.

202. N. J. Cusack, B. J. Hildick, D. H. Robinson, P. W. Rugg, and G. Shaw, *J. Chem. Soc. , Perkin 1*, 1973, 1720.

203. G. Mackenzie, G. Shaw, and S. Thomas, *Chem. Commun.*, 1976, **12**, 453.

204. R. N. Naylor, G. Shaw, D. V. Wilson, and D. N. Butler, *J. Chem. Soc.*, 1961, 4845.

205. D. H. Robinson and G. Shaw, *J. Chem. Soc. , Perkin 1*, 1972, 1715.

206. D. H. Robinson and G. Shaw, *J. Chem. Soc. , Perkin 1*, 1974, 774.

207. G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J. Chem. Soc.*, 1959, 1648.

208. G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 1962, 2937.

209. G. Shaw and D. N. Butler, *J. Chem. Soc.*, 1959, 4040.

210. A. J. Zambito and E. E. Howe, *Org. Syn.*, 1973, **5**, 373.

211. Y. Tarumi, K. Moriguchi, and T. Atsumi, *J. Heterocycl. Chem.*, 1984, **21**, 529.

212. A. Padwa, P. Rashatasakhon, A. D. Ozdemir, and J. Willis, *J. Org. Chem.*, 2005, **70**, 519.

213. M. Maltese, *J. Org. Chem.*, 2001, **66**, 7165.

214. P. R. Birkett, C. B. Chapleo, and G. Mackenzie, *Synthesis*, 1991, **2**, 157.

215. N. J. Cusack and G. Shaw, *Chem. Commun.*, 1970, 1114.

216. N. J. Cusack, P. W. Rugg, and G. Shaw, *J. Chem. Soc. D*, 1971, 190.

217. N. J. Cusack, D. H. Robinson, P. W. Rugg, G. Shaw, and R. Lofthouse, *J. Chem. Soc. , Perkin Trans. 1*, 1974, 73.

218. G. Mackenzie, G. Shaw, and S. E. Thomas, *J. Chem. Soc. , Chem. Commun.*, 1976, 453.

219. G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 1962, 2937.

220. K. Fukukawa, S. Shuto, T. Hirano, and T. Ueda, *Chem. Pharm. Bull.*, 1986, **34**, 3653.

221. J. K. Horton and M. F. G. Stevens, *J. Pharm. Pharmacol.*, 1981, **33**, 808.

222. G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J. Chem. Soc.*, 1959, 1648.

223. G. Mackenzie and G. Shaw, *J. Chem. Res. (S)*, 1977, 184.

224. G. Mackenzie and G. Shaw, *J. Chem. Res. (M)*, 1977, 2137.

225. M. E. Jung and S. M. Kaas, *Tetrahedron Lett.*, 1989, **30**, 641.

226. K. F. Bernady, M. Brawner Floyd, J. F. Poletto, and M. J. Weiss, *J. Org. Chem.*, 1979, **44**, 1438.

227. K. G. Liu, J. S. Smith, A. H. Ayscue, B. R. Henke, M. H. Lambert, L. M. Leesnitzer, K. D. Plunket, T. M. Willson, and D. D. Sternbach, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2385.

228. H. Stampe Jensen, G. Limberg, and C. Pedersen, *Carbohyd. Res.*, 1997, **302**, 109.

229. K. Kanai, I. Sakamoto, S. Ogawa, and T. Suami, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 1529.

230. T. Brown, K. Kadir, G. Mackenzie, and G. Shaw, *J. Chem. Soc. Perkin Trans. I*, 1979, 3107.

231. G. J. Litchfield and G. Shaw, *J. Chem. Soc. (C)*, 1971, 817.

232. J. Kraut and L. H. Jensen, *Acta. Cryst.*, 1963, **16**, 79.

233. S. Neidle, W. Kuhlbrandt, and A. Achari, *Acta. Cryst.*, 1976, **B32**, 1850.

234. M. Sunaralingam, *Acta. Cryst.*, 1966, **21**, 495.

235. C. E. Bugg and R. E. Marsh, *J. Mol. Biol.*, 1967, **25**, 67.

236. M. Sunaralingam and L. H. Jensen, *J. Mol. Biol.*, 1965, **13**, 914.

237. M. A. Viswamitra, B. S. Reddy, H.-Y. Lin, and M. Sunaralingam, *J. Am. Chem. Soc.*, 1971, **93**, 4565.

238. M. Sunaralingam and P. Prusiner, *Nucl. Acids Res.*, 1978, **5**, 4375.

239. R. Huisgen, *Angew. Chem. Int. Ed. Engl.*, 1963, **2**, 633.

240. R. Huisgen, *Angew. Chem. Int. Ed. Engl.*, 1963, **2**, 565.

241. T. W. Greene and P. G. M. Wuts, in *Protective Groups in Organic Synthesis*, John Wiley & Sons, Inc., 3rd edn., 1999, chapter 2, pp. 17-245.

242. A. H. Haines, *Adv. Carbohyd. Chem. Biochem.*, 1976, **33**, 11.

243. J. S. Fisk and J. J. Tepe, *J. Am. Chem. Soc.*, 2007, **129**, 3058.

244. M. Anastassiadou, S. Danoun, L. Crane, G. Baziard-Mouysset, M. Payard, D.-H. Caignard, M.-C. Rettori, and P. Renard, *Bioorg. Med. Chem.*, 2001, **9**, 585.

245. E. E. Smissman and J. W. Ayres, *J. Org. Chem.*, 1971, **36**, 2407.

246. V. Skaric, Z. Raza, and D. Skaric, *J. Chem. Soc. , Perkin Trans. 1*, 1982, 223.

247. B. M. Choudary, N. S. Chowdari, M. L. Kantam, and K. G. Raghavan, *J. Am. Chem. Soc.*, 2001, **123**, 9220.

248. B. M. Choudary, N. S. Chodari, K. Jyothi, and M. L. Kantam, *J. Am. Chem. Soc.*, 2002, **124**, 5341.

249. H. E. Blackwell, D. J. O'Leary, A. K. Chatterjee, R. A. Washenfelder, D. A. Bussmann, and R. H. Grubbs, *J. Am. Chem. Soc.*, 2000, **122**, 58.

250. R. E. Ireland and M. Chaykovsky, *Org. Syn.*, 1961, **41**, 5.

251. A. P. Tamiz, S. X. Cai, Z.-L. Zhou, P.-W. Yuen, R. M. Schelkun, E. R. Whittemore, E. Weber, R. M. Woodward, and J. F. W. Keana, *J. Med. Chem.*, 1999, **42**, 3412.

252. G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J. Chem. Soc.*, 1959, 1648.

253. V. P. Kislyi, E. B. Danilova, E. P. Zakharov, and V. V. Semenov, *Russ. Chem. Bull. , Int. Ed.*, 2004, **53**, 622.

254. Y. Y. Morzherm, M. Y. Kolobov, V. S. Mokrushm, M. Brauer, E. Anders, and V. A. Bakulev, *Chem. Heterocycl. Comp.*, 2000, **36**, 22.

255. A. Khalaj, R. D. Bazaz, and M. Shekarchi, *Monatsh. Chem.*, 1997, **128**, 395.

256. M. K. Basu and F.-T. Luo, *Tetrahedron Lett.*, 1998, **39**, 3005.

257. R. Ohme and E. Schmitz, *Angew. Chem. Int. Ed. Engl.*, 2003, **6**, 566.

258. H. S. Isbell and H. L. Frush, *J. Org. Chem.*, 1958, **23**, 1309.

259. R. S. Tipson, *J. Am. Chem. Soc.*, 1961, **26**, 2462.

260. N. J. Cusack and G. Shaw, *J. Chem. Soc. D*, 1970, 1114.