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SYNTHESIS OF NUCLEOSIDE ANALOGUES WITH POTENTIAL ANTIVIRAL ACTIVITY AGAINST NEGATIVE STRAND RNA VIRUS TARGETS

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The work described here is an attempt to synthesise nucleoside analogues which are targetted against the replicase of negative-strand viruses. Two lines of investigation have been attempted: (i) the synthesis of 5-vinyl substituted pyrimidine ribonucleosides and (ii) the synthesis of potential nucleotide pro-drugs.

A series of compounds of type (i) have been synthesised and submitted for biological evaluation. Strategies for the synthesis of compounds of type (ii) have been developed but we have not had time to prepare sufficient suitable material for testing purposes.

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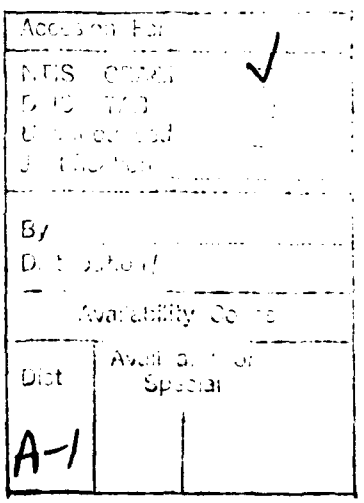

A thesis submitted by

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FOREWORD

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APPENDIX A

SYNOPSIS

A summary of the classification of viruses is presented with specific reference to three of the families of the negative-stranded RNA viruses, the Rhabdoviridae, the Bunyaviridae and the Arenaviridae.

An account of the replicative methods of the RNA viruses is given together with the specific details of the three RNA virus families mentioned above, and the possible methods of inhibition that can be used to target nucleoside analogues against them.

The ester (E)-5-(2-carbomethoxyvinyl)uridine had previously been shown to have slight antiviral activity against the Yellow Fever virus. In order to determine the structure/activity relationship of this ester and, in the hope of obtaining a more active compound, a series of 18 esters of (E)-5-(2-carboxyvinyl) uridine were synthesized.

Amides were obtained by treatment of the methyl ester with ammonia and amines and in the case of dimethylamine a Michael addition to the vinylic double bond followed by ester hydrolysis was observed.

The chemistry of substituted 5-vinyl-2,4-dimethoxypyrimidines was investigated starting with the preparation of the acid chloride from the acid (E)-5-(2-carboxyvinyl)-2,4-dimethoxypyrimidine. The reactions of the aldehyde 5-formyl-2,4-dimethoxypyrimidine with active methylene compounds in the Knoevenagel reaction was used to form compounds with a vinylic side-chain.

The nucleoside condensation product from (E)-5-(2-azidocarbonylvinyl)-2,4-dimethoxypyrimidine was found to cyclise upon treatment with ammonia, to give a known pyrrido-pyrimidine, and with methoxide to give the first example of a previously unknown class of nucleosides, the pyrano-pyrimidines.

The original preparation of 5-formyl-2',3'-isopropylideneuridine was improved and the reaction of this aldehyde with nitromethane with various catalysts was determined.

A preliminary investigation into the use of lithium diisopropyl amide (LDA)

gave deprotonation of the C-6 position of a blocked sugar uracil nucleoside and the first example of a 6-substituted-5-substituted vinyl uracil nucleoside is reported.

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The author also wishes to thank all his contemporaries in the Nucleic Acids Research Group, especially Mr. S. P. Wanklin. Special thanks to Dr. A. Kumar for all of his advice.

Finally, mention must be made of all the Research and Technical staff without whom this work could not be presented.

CONCLUSION

We have managed to synthesise a wide range of 5-substituted uridine analogues (see Appendix A) and have developed the methodology for the synthesis of nucleotide pro-drugs.

The project was started because of an initial report from USAMRIID on two compounds submitted to the virus screen, AVS 1116 and AVS 1117. Data obtained on 07-14-85 showed significant activity against yellow fever for both compounds. Thus we thought we had a lead compound and could do rational design by chemical modification of the lead compounds and biological evaluation of such derivatives.

The chemical synthesis proceeded very well and a large range of analogues was made over the three year period. Unfortunately, the biological evaluation by USAMRIID was totally unsatisfactory. We got no more results until 01-24-89 (which in fact did not reach us by sea mail until August 1989 as the contract was ending) so that we could not respond to an increase or decrease in activity caused by the chemical modifications. Thus all the chemical synthesis throughout the project had to be done blind. Even worse than this, was in July 1989 it was finally confirmed that the original lead compounds were not in fact active after all. Thus it is not too surprising that none of the analogues tested was active.

Thus, although we have done some interesting chemistry, the exercise as originally planned was based on a false premise and not rectified until too late because of the chaotic system of testing compounds by USAMRIID. There clearly is little point in funding chemical synthesis if the necessary biological assays are not available and cannot be relied upon to deliver an accurate and rapid result.

The assay system obviously still has problems. AVS 4172 received on 88-02-18 and not tested until 88-08-09 and not reported to us until 89-07-19 is meant to be significantly active against HIV. In the current climate, a compound with a TI $>$ 345 would normally generate incredible excitement, particularly as the compound is of a structure quite unlike any other HIV-active compounds. Yet it took 1½ years to communicate this information to us and we have far more reliable evidence to suggest that the compound is not active anyway.

Thus we are most disappointed with the support we got from USAMRIID which was crucial to the success of this project. Not only were the biological results delayed so long that they were useless, they also could not be relied upon as all the active compounds originally identified have subsequently been found to be inactive.

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INTRODUCTION

Progress in the development of antiviral chemotherapy, when compared with the advances made in antibacterial chemotherapy, has been very meagre and slow.[1] There are undoubtedly many reasons for this, but quite clearly it should be easier to develop compounds to prevent bacterial growth which have an entirely independent life style from their "host" than it is to interfere selectively with viral replication which necessarily involves the host's biochemistry. However, antiviral chemotherapy has suffered severely from the prejudice of clinicians[2] and senior management in pharmaceutical companies that it would be impossible to obtain a safe antiviral drug and so the effort expended has been much less than in the antibacterial field. Thus even a compound like Amantadine which has been known for many years and undoubtedly has a potential use against influenze virus which is one of the few commericially-viable viral diseases, has never been given the clinical investigation it deserves.[3]

Recently, the climate of opinion has begun to change. With the application of the modern techniques of Molecular Biology to the virus field, our previous, almost total ignorance, of the methods of replication used by the different virus classes has been gradually replaced by a realization that they are often not so totally dependent upon host metabolic processes as was once thought.[4,5] This has given rise to the realization that specific viral targets might indeed exist for antiviral agents and that a non-toxic antiviral compound might be a distinct possibility. Unfortunately, this realization has come at a time when the call for stringent toxicity controls using animals has increased the cost of developing a drug to the point where there remain very few commercially-viable virus targets for a pharmaceutical company to attack. The majority of viral infections are not lethal and the more dangerous infections usually occur in the third world tropical countries who could not afford chemotherapeutical treatment anyway.

One exception to this uninviting prospect for the future of chemotherapy has proved to be herpes virus infections. Incidence of various herpes virus infections is very high and one also has the associated problem of virus latency such that infections are likely to recur. In immunosuppressed and elderly patients, infection is often life-threatening so a need for an effective antiherpes drug is easily demonstrable. Despite this, until very recently, little other than 5-iodo-2'-deoxyuridine was available. In the last 10 years a whole range of second-generation drugs have been synthesised by the chemist which are far more effective and le ; toxic and which has resulted in the first of these, 9-(2-hydroxyethyoxymethyl)guanine (Acyclovir), reaching the clinic. Apart from demonstrating that antiviral activity and general toxicity could indeed be separated, the discovery of this generation of drugs has encouraged many scientists to determine their mode of action so that further advance in synthetic design can be made.

It is now realized that the herpes viruses code for several enzymes (notably a thymidine kinase and a DNA polymerase) which are specifically required for viral replication and which have different specificities from the corresponding host enzymes. It is these differences which Acyclovir, and other members of this new generation of drugs, exploit.

Antiviral chemotherapy research has also been hindered by the lack of suitable antiviral screens. Most antibacterial compounds have been produced from lead compounds turned up in general random screening procedures and very few such lead compounds themselves have so far been logically designed. However, the prevailing climate against antivirals has been such that until recently, very few such screens, where a meaningful assessment of the data was made available, were in existence. Viruses are obviously more difficult to grow than bacteria and the process is more costly. There were few precedents which suggested that anything useful would be found and few comercially-viable viruses to assay.

The availability of sophisticated antiviral screening procedures for several classes of negative strand RNA viruses which present a significant hazard to personnel in tropical regions, has stimulated us to try and capitalize on our experience over many years in the nucleoside field, culminating in the synthesis of E-5-(2-bromovinyl)-2'-deoxyuridine (BVDU)[7] as a potent anti-herpes virus drug, by designing compounds which might be active against these classes of viruses.

The viral-encoded transcriptase of negative strand RNA viruses which is responsible for producing mRNA and when modified, complete copies of positive strand RNA used as templates in replication, is a suitable target for chemotherapeutic attack from nucleoside analogues.[8]

The molecular biology of negative strand viruses is still at a very elementary state (we exclude ortho and paramyxo viruses). Indeed even the classification of some viruses (e.g. Korean Haemorrhagic Fever virus) is not clear. Hoever, it is quite clear that an enzyme crucial for virus replication is the virus-encoded transcriptase, molecules of which are actually contained in the virus particles. This enzyme requires nucleoside triphosphates as substrates and has a magnesium requirement. It clearly must have a different specificity from host cell polymerases and from experience with the virus-encoded DNA polymerase of herpes-virus, it seems possible that one ought to be able to exploit this difference with the use of analogues of nucleosides or their triphosphates.

The present work describes the synthesis of a range of 5-substituted uridine analogues as previous samples submitted to USAMRIID for testing, AVS 1116 and AVS 1117, had shown significant activity against yellow fever virus. A detailed rationale,

experimental and discussion of the results obtained are given in Appendix A (Ph.D. thesis, R.F. Whale, University of Birmingham, April 1989).

Attempts to synthesise suitable pro-drugs of 5'-nucleotides has proved to be more difficult. We now have a strategy for doing such synthesis but unfortunately we do not have the time available on the grant to make sufficient quantities for testing purposes. The experimental details and discussion of the results obtained are given in Appendix B (M.Sc. thesis, S.F. Wanklin, University of Birmingham, January 1990).

INTRODUCTION

There exists in the world today many diseases for which cures have been developed. The irradication of smallpox stands as a major achievement in this area although there still remain many diseases caused by viral agents for which there are no vaccines for prophlaxis and no effective drugs for therapy. Despite much intensive effort, a useful therapy for Acquired Immune Deficiency Syndrome (AIDS) caused by the virus Human Immune Deficiency Virus (HIV) is still elusive despite almost a decade of work.

Progress in the field of antiviral chemotherapy has been slow. The first nucleoside analogues with activity were used more than a quarter of a century ago and although 5-iodo-2'-deoxyuridine (IDU) and 5-trifluoromethylthymidine (TFT) are still widely used for the topical treatment of herpetic eye infections, very few other antiviral agents have found their way past their clinical trials. Indeed, the number of licenced compounds number less than a dozen.

The main area of success for antiviral compounds remains the herpes-viruses, a group of DNA viruses whose treatment in the Western world is economically feasible.

In the developing world, however, there are many viruses of a very hazardous nature where progress in their treatment with chemotherapeutic agents has been very slow. The only exception to this is the vaccine for the Yellow Fever virus. It must be realised, however, that the systematic development of such compounds is expensive and the infected areas do not represent a viable market, especially when the outbreak is very limited in extent compared to the occurence of other problem viruses such as the herpes-viruses.

These so called 'exotic' viruses include Rift Valley fever, Venezuelen Equine encephalitis, Lassa fever, Sandfly fever and Dengue. The threat from such recurrent viruses is always present. Australia, for example, can face outbreaks of Dengue fever.[1] Unknown causes of viral disease are regularly isolated and, as in the case of the agent responsible for non-A, non-B hepatitis, characterised

as a single-stranded RNA virus belonging to the family Togaviridae[2] to which, incidentally, Yellow fever and Dengue belong.

The basis for the effectiveness of such compounds as IDU rests on the interference of the compound in the normal cellular functions of nucleosides and nucleotides. These functions include precursors of DNA and RNA and biosynthetic intermediates such as energy donors and metabolic regulators. The chemical basis for the biological activity of nucleoside analogues relies on their ability to interact with the enzymes, such as the nucleotide reductases, polymerases and kinases, or their ability to causes malfunctions during the processes of replication and transcription if they become incorporated into the DNA or RNA.

The various interactions of each analogue in the cellular chemistry is specific. It is this specificity that the work described here has tried to use by targeting analogues against several of the families of the negative-stranded RNA viruses; namely the Rhabdoviridae, the Bunyaviridae and the Arenaviridae.

To understand the relationship between the active nucleoside analogues and the viral method of replication it is necessary to present an outline of the replicative methods of the four types of RNA viruses and also to describe the slightly different replicative methods of negative-stranded RNA viruses.

Firstly, however, a brief summary of viruses and virus classification will be presented.

Until 40 years ago, little was known about the viruses apart from their ability to cause disease. It is not surprising, therefore, that the classification of viruses is a relatively recent achievement.

Classification was originally based on the response of the host to the virus and occasionally the similarity of the symptoms did lead to a classification that has now been shown to be essentially correct. Also, it is now apparent that the vast majority of the major groups of viruses which affect man and the animals of importance to man, for example the domesticated animals, have been isolated

and described.

Viruses[3] are entities whose genome is nucleic acid, which can be either DNA or RNA, which reproduce inside a living cell and use the synthetic machinery of the cell to direct the synthesis of specialised particles, the virion.

Viruses are the most efficient of the parasites that self-reproduce, however, they are not able to synthesize proteins or to generate metabolic energy. Viruses differ from other intracellular parasites in several ways;

1. The virion (the virus particle) has one type of nucleic acid
2. Host ribosomes are used for the synthesis of virus-specified proteins.
3. Multiplication consists of assembling their constituent parts which are made independently.

Viral complexity varies greatly. The simplest virus is the bacteriophage $Q\beta$, a single-stranded RNA virus infecting bacteria which has only three genes. This can be compared to the double-stranded DNA viruses, the Poxviruses, which contain about 250 genes.

The virion is the completed extracellular product of virus multiplication. The virion consists of the nucleic acid which is surrounded by the capsid (or nucleocapsid), a layer of protein whose function is to protect the genome from the harsh extracellular enviroment and enzymatic attack. Some of the more complicated animal viruses also have a lipid and glycoprotein envelope. a bilayer membrane containing virus proteins and host cell lipids.

As the viral genome is not large enough to code for a capsid consisting of one large protein coat, the capsid is made up of a large number of one or a few types of protein subunit. The limited number of different types of subunit restricts the possible shapes and stability is achieved by having a symmetrical shape of which there are two types. The first is a cylindrical shape with helical symmetry, the second is a spherical shape with icosahedral (cubic) symmetry. It is the animal viruses with helical capsids which are enveloped, whereas many plant

viruses exist as a naked helical capsid.

Some virions also contain enzymes. The first to be discovered was a neuraminidase from influenza. It is now known that many viruses contain other enzymes collectively known as transcriptases. These can be either an RNA-dependent RNA polymerase (RNA transcriptase) or an RNA-dependent DNA polymerase (reverse transcriptase).They synthesize mRNA from viral nucleic acid or DNA from a single-stranded RNA template, respectively.

It is possibe to show that the transcriptases are essential for infection because the protein-free nucleic acid is not infectious whereas nucleic acid from virions that do not contain such enzymes eg. the Picornviridae, Togaviridae, Papovaviridae and the Adenoviridae, is infectious on its own.

The basic classification of viruses is concerned with the chemical nature of the genome, whether the viral nucleic acid is DNA or RNA, whether it is single- or double-stranded and if the virion has an envelope.

A pictorial representation of such an outline classification of the animal viruses is shown in Figure 1.[4]

Even in such a classification there are a few exceptions, the major one being that some members of the Iridoviridae, which includes the African swine fever group and the genus Ranavirus which infect frogs, contain an envelope.

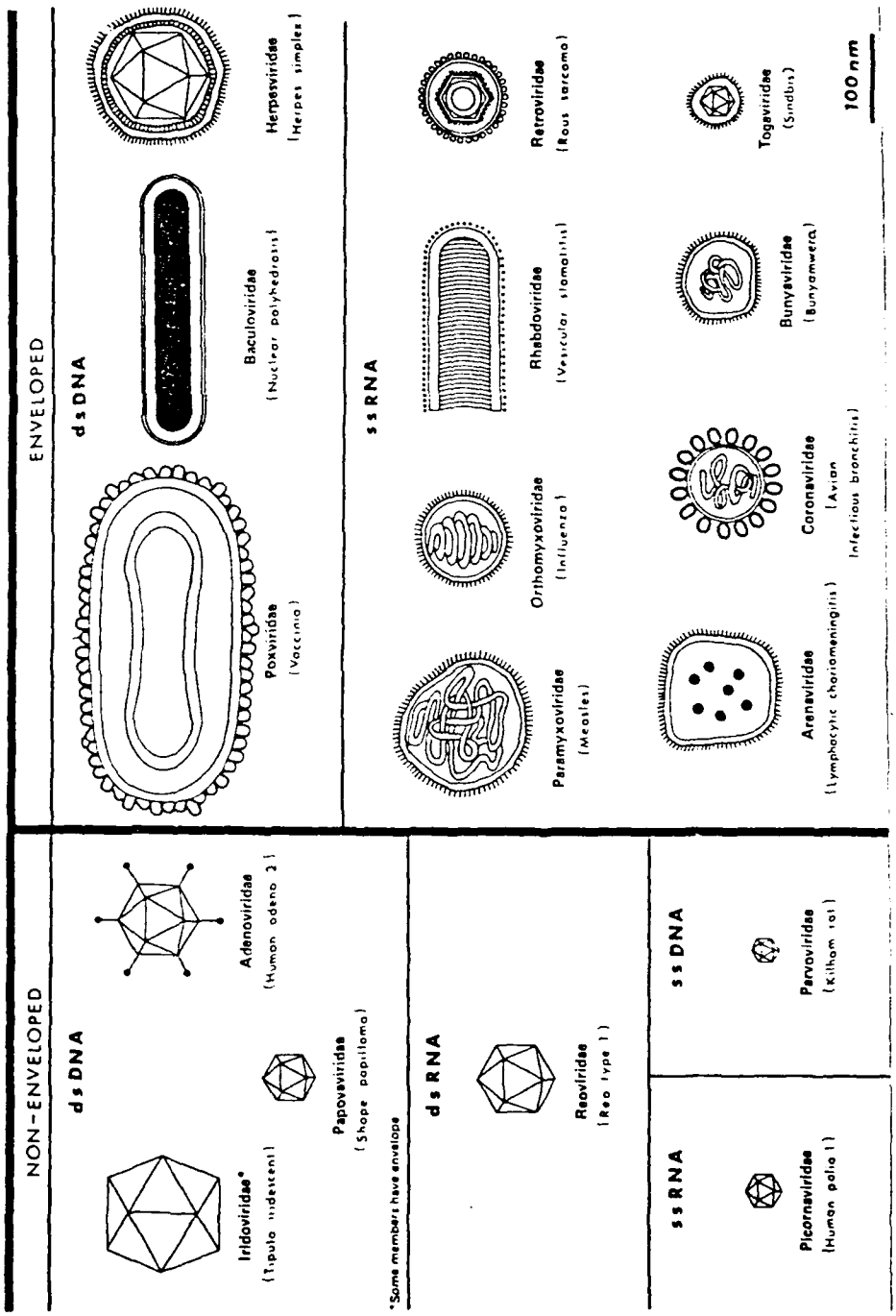
The detailed classification of viruses[4] is based on the following divisions;

1. Capsid symmetry, cubic, helical, unsymmetric or unknown
2. Virion, naked or enveloped.
3. Site of capsid assembly.
4. Site of nucleocapsid assembly.
5. Reaction to ether, resistant or sensitive.
6. Number of capsomeres
7. Diameter of virion.
8. Molecular weight of nucleic acid in the virion.

Viruses with a DNA genome have been classified into 7 families with varying

Figure 1

The Families of Animal Viruses



from: R. E. F. Matthews, Intervirology, 1979, 12, 129

numbers of genera and members. Representative members of the genera and their associated family are shown in Table 1.

Viruses with an RNA genome have been classified into 11 families. Representative members of the genera and their associated family are shown in Table 2.

Viruses can also be further classified by their mechanism of replication. In order that the virus be replicated, it is necessary that it become attached to the cell membrane and, once penetration has occurred, the viral nucleic acid is released into the cell. The nucleic acid serves two purposes:

1. Act directly as messenger RNA (mRNA) or, act as a template for transcription of mRNA which is then translated into viral proteins

and 2. To direct the synthesis of progeny nucleic acid (replication)

Viruses can be classified according to how they synthesize mRNA[5] , Figure 2, which depicts the various methods.

It is a matter of convention that the base sequence in the mRNA during replication is designated as having positive polarity, the positive sense strand (plus strand, message strand) is the strand that contains the coding triplets which are translated on the ribosome. Therefore by definition, in Figure 2, in the single-stranded RNA viruses which have a positive strand, the positive strand must be mRNA. In these viruses, such as the Picornviridae and Togaviridae families which include Poliovirus and Yellow Fever virus, genome transcription and replication cannot be differentiated on the basis of complementarity between the viral mRNA and the genome.

Also by convention, the negative strand (minus strand) is the strand with a base sequence complementary to the positive sense strand. Double-stranded viruses have strands of both polarities.

Double-stranded DNA viruses have what can be considered to be the 'normal' mode of transcription as this is the mode of replication that occurs in the transfer of genetic information in eukaryotic cells. The double-stranded DNA viruses thus serve as a good model.

Table 1 Viruses with a DNA genome

FAMILY	GENERA	EXAMPLES
Parvoviridae	Parvovirus	Rat virus (Kulham's)
	Densovirus	Densoviruses of insects
	Dependovirus	Adeno-associated virus
Papovaviridae	Papillomavirus	Human papilloma virus
	Polyomavirus	Simian virus 40 (SV40)
Adenoviridae	Mastadenovirus	Adenoviruses of man
	Aviadenovirus	certain fowl viruses
Herpesviridae		
Alphaherpesvirinae+	Simplexvirus	Human simplex viruses, 1 and 2 (HSV 1, HSV 2)
	Poikilovirus	Pseudorabies virus
	Varicellavirus	Varicella-zoster virus (VSV)
Betaherpesvirinae+	Cytomegalovirus	human cytomegaloviruses
	Muromegalovirus	murine cytomegaloviruses
Gammaherpesvirinae+	Lymphocryptovirus	Epstein-Barr virus
	Thetalymphocryptovirus	Turkey herpesvirus
	Rhadinovirus	Herpesvirus saimiri
Iridoviridae	African swine fever gp.	African swine fevers
	Ranavirus	various frog viruses
	Iridovirus	Tipula iridescent virus
	Chloridovirus	Mosquito iridescent virus
Poxviridae		
Chorodopoxvirinae+	Orthopoxvirus (vaccinia)*	vaccinia, smallpox
	Parapoxvirus (Orf)*	Orf virus
	Avipoxvirus (Fowlpox)*	Fowlpox virus
	Capripoxvirus (sheep pox)*	Sheep pox virus
	Leporipoxvirus (myxoma)*	Myxoma virus of hares

Entomopoxvirinae +	Suipoxvirus (swine pox) 3 genera: A,B and C	Swinepox virus poxviruses of insects
Hepadnaviridae	Hepadnavirus	Human hepatitis B (HBV) (hepadnavirus type 1)

footnotes

\+ subfamilies

* subgroups

Table 2 . Viruses with an RNA genome

Family	RNA	Genera	Examples
Picornaviridae	ss, +ve	Enterovirus	Polioviruses
		Cardiovirus	encephalomyocarditis (EMC)
		Rhinovirus	common cold viruses, 1A-114
		Aphthovirus	foot and mouth disease
Caliciviridae	ss, +ve	Calicivirus	Vesicular exanthema of swine
Reoviridae	ds	Reovirus	types 1-3
		Orbivirus	Colorado tick fever subgroup
		Rotavirus	Human rotavirus
Togaviridae	ss, +ve	Alphavirus	Sindbis virus, Venezuelen and Western equine encephalitis viruses
		Flavivirus	Yellow fever, Dengue and Japanese encephalitis
		Rubivirus	Rubella virus
		Pestivirus	Micosal disease virus
Orthomyxoviridae	ss, -ve	Influenzavirus	Influenzaviruses types A,B
Paramyxoviridae	ss -ve	Paramyxovirus	Human parainfluenza, mumps
		Morbillivirus	Measles, Rinderpest (cattle)

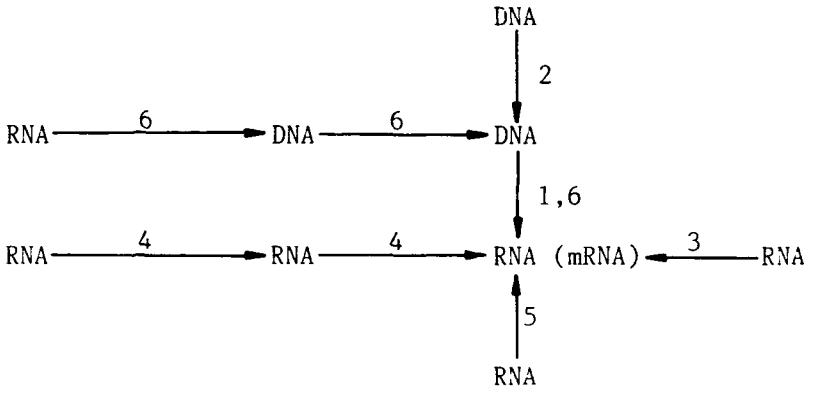
		Pneumovirus	canine distemper viruses Respiratory syncytial virus
Rhabdoviridae	ss, -ve	Vesiculovirus Lyssavirus	VSV of horses and cattle Rabies and Mokola viruses
Coronaviridae	ss, +ve	Coronavirus	Avian infectious bronchitis
Bunyaviridae	ss, -ve	Bunyavirus Phlebovirus Nairovirus Uukuvirus Hantaan virus	Bunyamwera virus Sandfly fever viruses Crimean-Congo haemorrhagic fever viruses Uukuniemi virus Korean haemorrhagic fever viruses
Retroviridae Oncovirinae* Spumavirinae* Lentivirinae*	ss, +ve	 type C oncovirus x type B oncovirus x	 Sarcoma/leukemia viruses mouse mammary tumour viruses Syncytial and foamy viruses Visna virus of sheep
Arenaviridae	ss, -ve	Arenavirus	Lymphocytic choriomeningitis, Lassa fever, Junin and Pichinde viruses

Footnotes

ss single-stranded
ds double-stranded
-ve negative sense strand of RNA
+ve positive sense strand of RNA
* subfamily
x group

Figure 2

Pathways of mRNA Synthesis



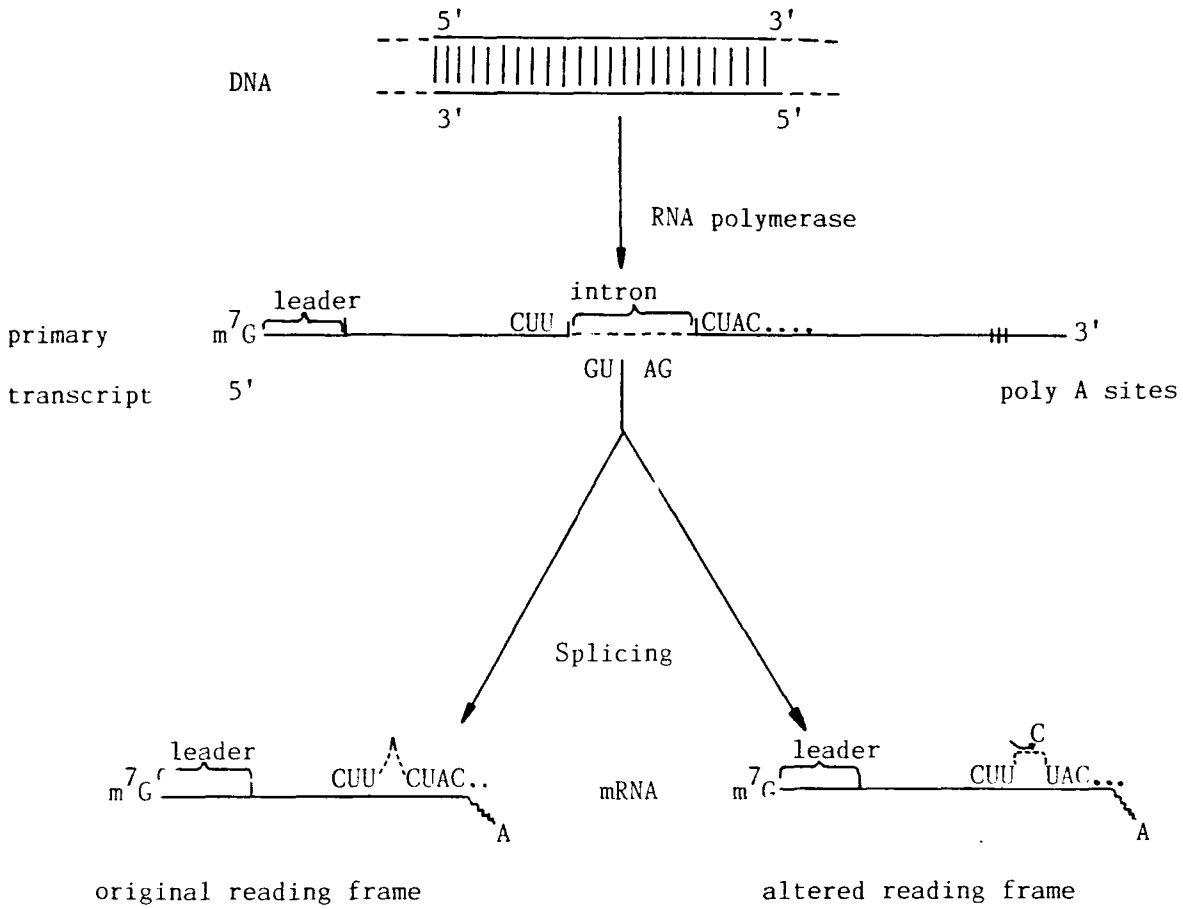
mRNA defined as RNA

1. ds DNA viruses
2. ss DNA viruses
3. ds RNA viruses eg. Reovirus
4. ss RNA viruses, mRNA identical in sequence to virion RNA (+sense RNA) eg. Poliovirus and Qβ phage
5. ss RNA viruses, genome complementary in sequence to mRNA (-sense RNA) eg. Rabies virus
6. ss viruses, DNA intermediate, Retroviruses eg. Rous sarcoma virus, HIV

In this model, Figure 3, one of the DNA strands is the template from which the viral equivalent of DNA-dependent RNA-polymerase II (transcriptase) copies a complementary RNA primary transcript. The primary transcript is capped at the 5'-end and polyadenylated at the 3'-end. By the process of splicing, the primary transcript is shortened in an exact manner by removing one or more internal segments (introns) and rejoining the remainder (exons). If the number of nucleotides removed in the intron is a multiple of three, the mRNA has the same reading frame as the primary transcript, but if one or two are removed then there is an altered reading frame in the mRNA.

The replication of RNA viruses therefore poses a problem, the host cells,

Figure 3



which replicate their genome according to the above system have a DNA-directed RNA polymerase and the viruses have an RNA genome from which the host enzyme is unable to take instructions. This is overcome by RNA viruses containing the genetic information for the synthesis of an RNA-directed RNA polymerase (RNA transcriptase, RNA synthetase) or an RNA-directed DNA polymerase (reverse transcriptase) and this enzyme must therefore also be packaged in the completed virion.

From Figure 2 it is seen that the double-stranded RNA viruses are exemplified by the family Reoviridae and the virus Reovirus. These particular viruses have segmented genomes, that is, the virion contains ten different ±RNA molecules

instead of being one continuous molecule of nucleic acid.

Upon entering the host, the virus loses its outer shell, this activates a viral RNA polymerase in the core of the virion which transcribes the ten (±)RNA to give $^{+}$RNA the same length at the genome. Each of the mRNA's is then translated and a complete set of the ten $^{+}$RNA molecules is assembled with the capsid proteins and ten (-) strands are then synthesized.

In contrast, the viruses with a genome which is a positive strand RNA, for example Poliovirus have a (+) strand which is translated immedietly it gets into the cytoplasm. Polio RNA polymerase then synthesises a complementary (-) strand which is then used as a template by RNA polymerase to make many $^{+}$RNA strands which bind capsid protein to form the new virions. The (+) RNA chain acts as a messenger for one continuous polypeptide which host proteases cleave to give six proteins which includes the RNA polymerase and coat proteins.

Rous sarcoma virus (RSV) is a single-stranded (+) RNA virus that replicates via -DNA and then ±DNA intermediates, Figure 2(6). The RNA tumour viruses (or leukoviruses) are the sole members of this group. The infection process of these viruses is inhibited by inhibiors of DNA synthesis such as 5-fluoro-2'-deoxyuridine and cytosine arabinoside and it was concluded that DNA synthesis is required for the growth of these viruses. Also, production of progeny virions is inhibited by inhibitors of RNA from DNA templates and it was therefore concluded that transcription of DNA is essential for the multiplication of these viruses. It was proposed that a DNA provirus is an intermediate in the replication and oncogenic properties of the tumour viruses. This proposal was not accepted until the discovery of a new enzyme, which copies DNA from an RNA template, called RNA-directed DNA polymerase or reverse transcriptase. In the replication of the tumour viruses the ±DNA intermediate is incorporated into the host genome and after cell division the provirus is transcribed. The infected cell is permanently transformed because its genome contains the provirus.

The Bunyaviridae, Rhabdoviridae and Arenaviridae are three families of

(-) stranded RNA viruses and as they are the area of interest in this work a more detailed description of them and their replication will be presented.

Upon infection with a (-) stranded RNA virus, Figure 2(5), an RNA polymerase synthesises several short (+)RNA strands each of which is then translated into one polypeptide chain. Then replication occurs so that a (+) strand the same length as the (-) strand but complementary in sequence is made and in turn many (-) strands are made from this.

The Rhabdoviridae[7,8,9] is a family of approximately 75 viruses that infect plants, invertebrates and vertebrates. The members of the Rhabdoviridae that infect vertebrates and have a significant effect on man include Rabies virus[10] and Vesicular Stomatitus virus (VSV) in the Americas and Bovine Ephemeral Fever virus in Australasia. Of the Rhabdoviridae, only Rabies has a world wide distribution. Other important viruses in this family include Chandipura virus (man), Marburg virus (man, a highly pathogenic virus from monkeys) and Ebola virus which occurs in Africa and is responsible for human haemorrhagic fever. The fish rhabdoviruses are also of economic importance because they cause recurrent epidemics in fish hatcheries.

Thus, the most important of this family of viruses to man and domestic animals is rabies and this has been known as a human pathogen for at least 4000 years. The results of rabies in humans is viral encephalitis and neuronal degeneration. Upon infection of a wound the virus goes into the peripheral tissues and replicates (often in myocytes) and gains access to the neurons and eventually becomes involved with the brain neurons. It then travels via the peripheral nerves to such sites as the salivary glands which results in the presence of the virus in the saliva and the potential for virus transmission. The symptoms of salivation and the fear of water are widely known.

Rabies is very widely distributed worldwide but areas where it does not occur include parts of the Caribbean, Scandinavia, Oceania and the United Kingdom and Japan.

The main characteristics of the Rhabdoviridae are:

1. Invertebrate and vertebrate viruses are bullet shaped
2. Width from 60-95nm, length 130-380nm
3. Have unit-membrane envelopes with 5-10nm spikes
4. Have coiled nucleocapsids, helical with 50nm diameter
5. Most contain 5 structural proteins

   L Large transcriptase component

   G glycoprotein

   N nucleocapsid protein

   NS non-structural

   M matrix protein

The RNA is transcribed to give mRNA whose size corresponds to the structural proteins.

The arrangement of the genetic information along the genome of VSV may be shown diagramatically[11] as:

(3') short non-coding region-N-NS-M-G-L-short non-coding region (5')

Often in viral nucleic acids the 5'-end has a modified base sequence called a cap. In the above sequence the 5'-end is not capped and the 3'-end is not polyadenylated although in the mRNA the 5'-ends are capped[12] and methylated. The general structure of a 5'-cap is shown in figure 4. There is a blocking guanosine bound to the penultimate mRNA residue via a 5'-5' inverted linkage. The cap is almost always N-7-methylguanosine (m^7G), while the penultimate (N_1) and adjacent (N_2) nucleosides can have purine or pyrimidine bases with O-methylated 2'-hydroxyls. As either or both these nucleosides can be methylated this gives rise to three distinct cap structures:

$$^{7}mG(5')ppp(5')N_1N_2p..... \qquad \text{(cap 0)}$$

$^{7}mG(5')ppp(5')N_{1m}pN_{2}p.....$ (cap 1)

$^{7}mG(5')ppp(5')N_{1m}pN_{2}^{m}p....$ (cap 2)

The first and usually second adenosine residues in the sequence are also 2'-O-methylated.[13]

Infections of the Rhabdoviridae involve adsorption, penetration, primary mRNA transcription, mRNA translation, RNA replication then secondary transcription, translation and replication. The replication of VSV is well known and characteristic of the family and is summarised in figure 5[14].

With nucleoside triphosphates from the host cell, primary transcription occurs sequentially and repetitively yielding several hundred copies of each 5'-capped and methylated mRNA species. The process is called primary transcription to differentiate it from later transcription. The 3'-ends are polyadenylated.

The genomic RNA is transcribed by the NS-L protein complex and gives a full length (+) strand which is cleaved to a leader RNA and five mRNA species. The leader transcript consists of 47 nucleotides which starts with the sequence pppApCpGp.

Various models have been proposed for mRNA synthesis. The best model postulates one entry site for the transcriptase at the 3'-end and start/restart transcription of the mRNA species.[15] Another model postulates that the polymerase attaches to the genome at the place necessary to start transcription.

After primary transcription there is an amplified rate of mRNA synthesis which is termed secondary transcription and is blocked by inhibitors of protein synthesis. Some one hundred thousand copies of each mRNA are made, secondary transcription is the result of RNA replication. The difference between transcription and replication is that an exact copy is made of the genome which is transcribed totally to RNA.

The Bunyaviridae[16,17] is a large family of viruses with some 250 members identified since the family was first described.[18] They are divided into some 15 serogroups and although individual members are geographically limited the

Figure 4

mRNA 5'-cap structure

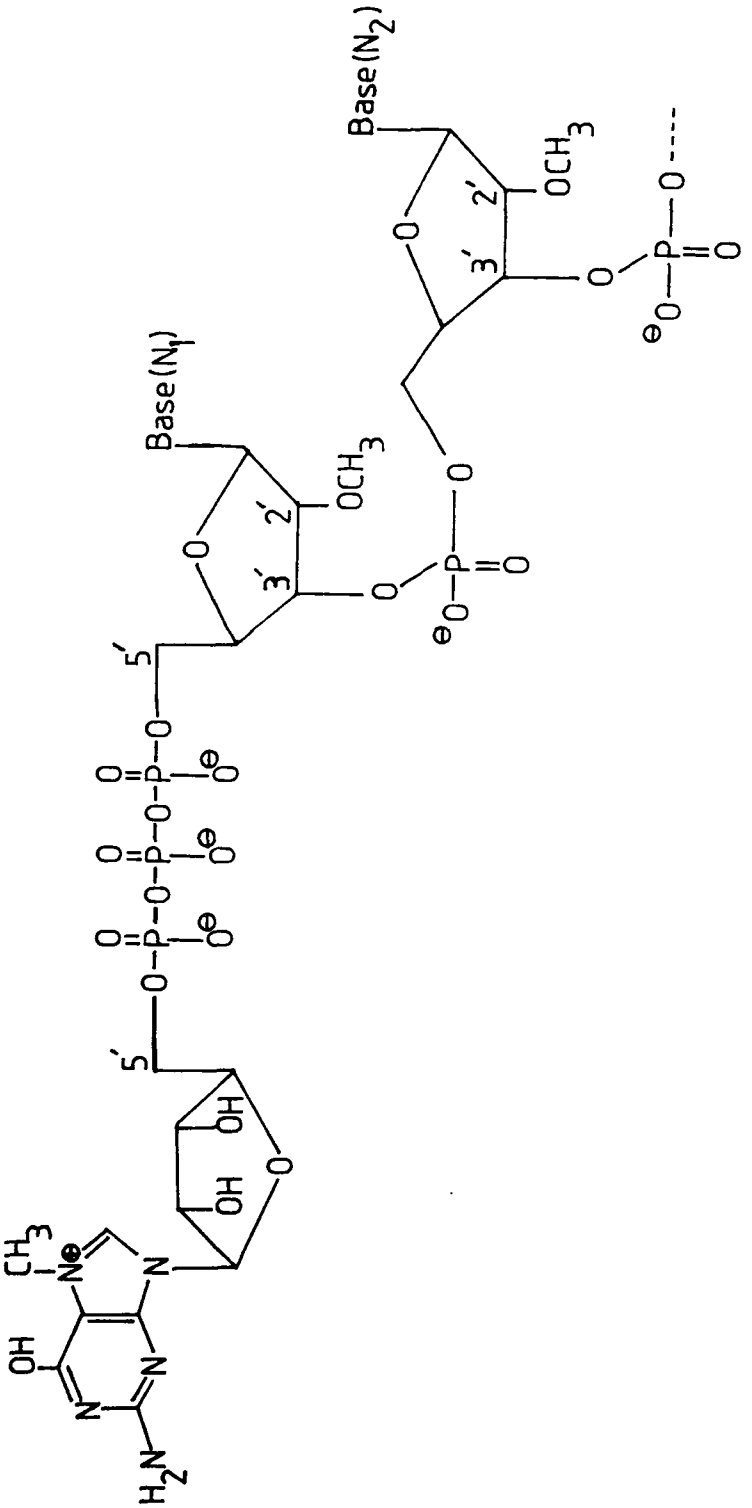
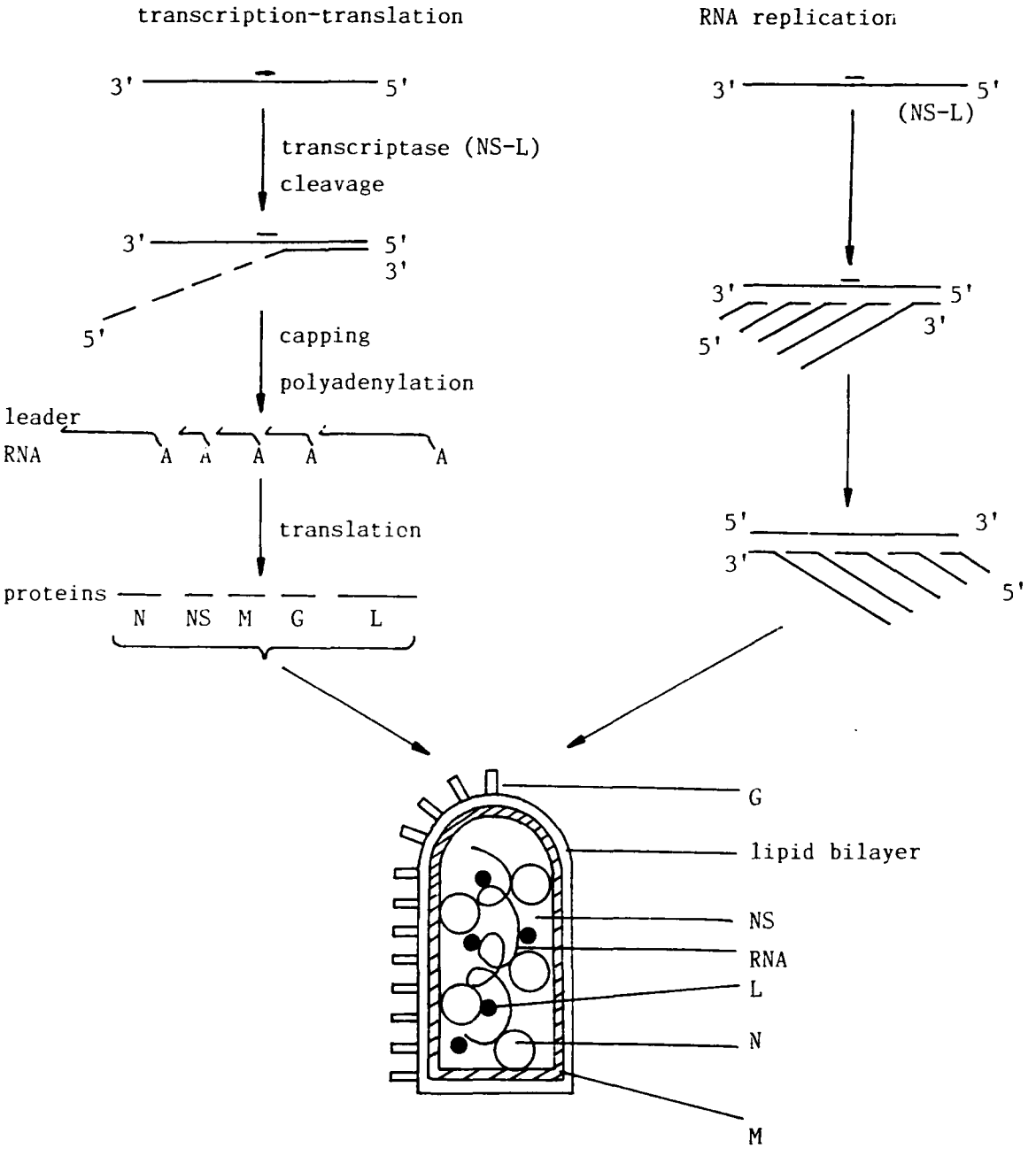


Figure 5 Replication of the Rhabdovirus VSV



Bunyaviridae are widely distributed worldwide with the exception of Antarctica.

The family consists of four defined genera. In general, members of the Bunyavirus genus are mosquito-transmitted although Nairovirus and Uukuviruses are tick-transmitted while the Phleboviruses are sand-fly transmitted. Some of the viruses cause fatal disease in humans, for example, La Crosse virus (viral

encephalitis), Akabane virus, the Sandfly fever viruses, Rift Valley fever virus and Crimean-Congo haemorrhagic fever virus. The majority of the members of the Bunyaviridae occur in the tropical areas of Africa and Latin America due to the ecological complexity of the areas. Temperate climates are also well represented with for example La Crosse virus and Sandfly fever. Some members occur in Arctic habitats, for example, Inkoo and Uukumieni viruses.

The viruses have complex cycles with the host and this implies a long co-evolution of the virus and vertebrate host. This has led quite often to local geographic distributions of the viruses. Rift Valley fever occurs in Africa and occasionally the virus extends out of its usual areas as exemplified by the Egyptian epidemic of 1977-1979. Man is not known as a natural host or amplifier for the Bunyaviridae but becomes infected when entering the ecological niche of the vector. Several of the viruses (Rift Valley fever, Akabane and Nairobi sheep disease viruses) are of economic importance as they are important animal pathogens.

The properties of the Bunyaviridae may be summarised as follows:

1. Viruses are enveloped, sensitive to lipid solvents, spherical and 90-100nm in diameter
2. Each virion contains three circular nucleocapsids, each consisting of nucleoprotein, a unique end hydrogen-bonded -ss RNA genome and a transcriptase. The transcriptase is coded by a large (L) RNA, nucleoprotein (N) by the small (S) RNA. S RNA also codes for a non-structural protein (NS), there is also a medium (M) RNA.
3. There is also an outer layer of glycoproteins.
4. Viral mRNA species have host-derived 5'-primer sequences.
5. The virus buds into the Golgi region of infected cells.
6. The virus can genetically be associated with closely related viruses and they are capable of genetic reassortment.

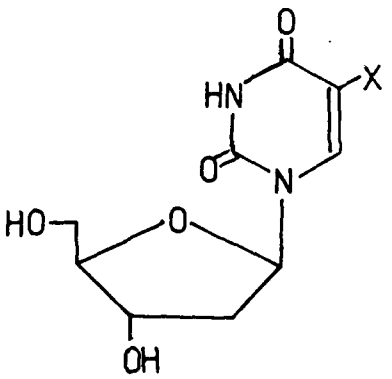
The replication of the Bunyaviridae has not been investigated in detail but each nucleocapsid is associated with a few molecules of a large protein which is

presumably a transcriptase. Following entry into the cell, the genome is transcribed in primary transcription which is necessary for RNA replication. A primer may be needed for mRNA synthesis.

There are few members of the Arenaviridae family[19,20,21] but four members regularly cause serious human disease; Lassa Fever virus, Junin virus (causes Argentine Haemorrhagic fever, AHF), Machupo virus (Bolivian Haemorrhagic fever, BHF) and Lymphocytic Choriomeningitis virus (LCMV). The natural hosts of the Arenaviridae are rodents, except for Tacaribe virus of bats, and they have little effect on the health of the infected animal. When they spread to man the consequences range from mild symptoms to fatal haemorrhagic fevers. The probability of human infection is directly dependent upon the extent of contact with rodents. One problem with the Arenaviridae is that animals develop persistant infection and are constantly shedding viruses.

The Arenaviridae[22] are enveloped viruses with 5-10nm surface projections. The genome is segmented and consists of two pieces of (-) RNA, large (L) and small (S), and each nucleocapsid contains a transcriptase.[23] The viruses are round to phleomorphic and from 50-300nm in diameter.[24] The virion contains 3 main structural proteins and structures thought to be ribosomes.

As with the Bunyaviridae, the infection processes of the Arenaviridae are only known in outline but the order of events is believed to be similar to the Rhabdoviridae. The replication of the Arenaviridae is not inhibited by antagonists of DNA synthesis such as 5-bromodeoxyuridine (1a) or 5-iododeoxyuridine (1b).[25]



1a X=Br

1b X=I

Work has also been done on the inhibition of Arenaviridae by common chemical reagents such as chloroform.[26]

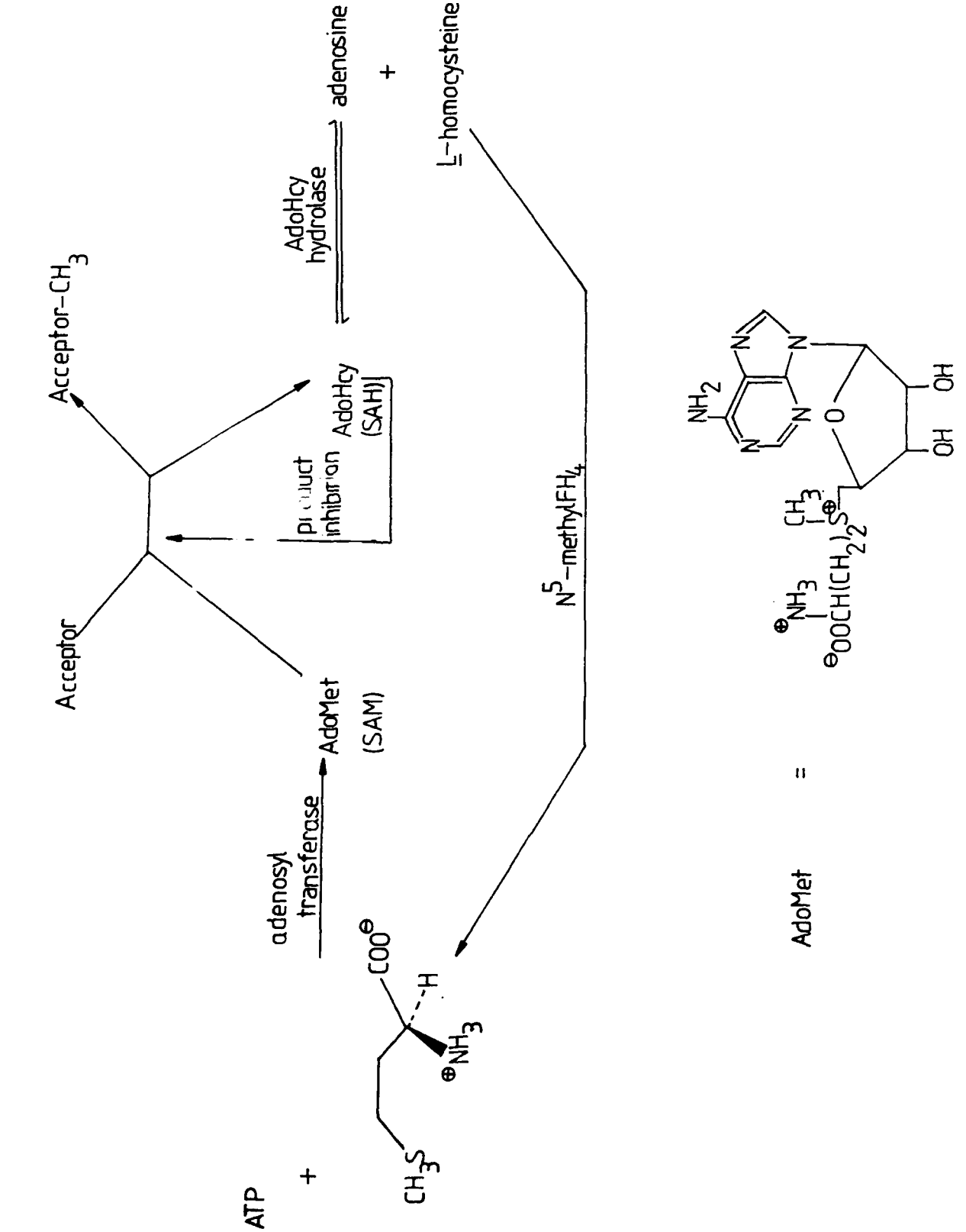
From the description of the replicative methods of the (-) RNA viruses, two possible routes to their inhibition and therefore control are seen. One route would be to inhibit the enzymes involved in transcription and translation. As a second approach, there is evidence that capping aids initiation of translation[27] and is a requirement for efficient translation. Another method of inhibition therefore, would be to prevent the capping of the mRNA species. It is towards this route, focussing on the post-transcriptional events, that most effort has been directed. One function of capping is thought to be to protect the mRNA against nuclease digestion from the 5'-end and hence increase mRNA stability in the cytoplasm.[27,28]

Since their discovery,[30] much has been determined about the structure, mechanism and synthesis of the 5'-caps.[31] For example, it is known that many animal viruses such as the DNA Poxviruses, double-stranded RNA and single-stranded (-) RNA viruses contain mRNA with a cap, the structure of which depends on the virion and whether the mRNA is studied in vitro or the cytoplasm.

Methylation of the cap occurs by an enzymatic transmethylation reaction from s-adenosylmethionine (AdoMet, SAM), as shown in Figure 6, which is brought about by virus specific AdoMet-dependent mRNA transferases which occur in the virion.

One of the products of this reaction is s-adenosyl-L-homocysteine (AdoHcy, SAH) which is a potent inhibitor of the AdoMet-dependent methyl transferases involved. The ratio AdoHcy/AdoMet controls the rate at which the mRNA can be methylated and AdoHcy must therefore be metabolised continuously and is removed by AdoHcy hydrolase.[32] This is a reversible reaction which lies in favour of synthesis but is driven to the right by elimination of the products, adenosine and L-homocysteine. The adenosine is further metabolised by phosphorylation or deamination while the L-homocysteine is further converted to methionine by N^5-methyltetrahydrofolate (N^5-methylFH_4) which donates the methyl group. Thus, when AdoHcy hydrolase is inhibited, AdoHcy accumulates and the methylation reactions are impaired. As the methylation of viral mRNA is necessary for

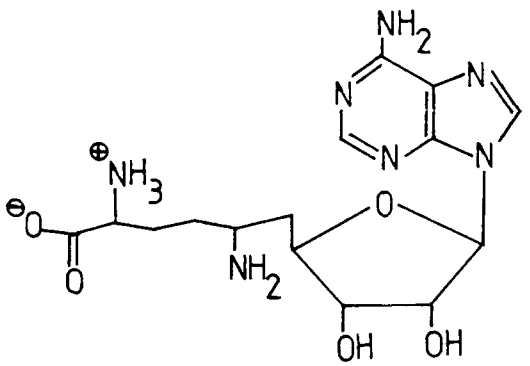
Figure 6. Adomet-dependent transmethylation



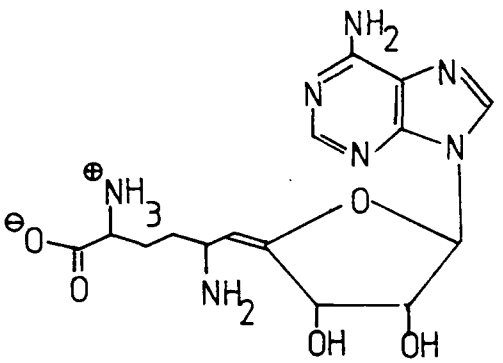
replication and translation, the 5'-capping seems a good target for nucleoside analogues.

There are three approaches for inhibitor design of viral capping. The first, which also reduces synthesis of polyamine and hence induces toxicity, is designed to reduce levels of AdoMet by inhibiting adenosyl transferase activity. Only a few analogues have been obtained and these have only low activity due to the high structural specificity of the enzymes.[33]

The second approach is to inhibit the various AdoMet-transferases using AdoMet or AdoHcy analogues as inhibitors [34] but only poor results have been obtained again due to specificity problems. The two antibiotics sinefungin (A9145) and A9145C have shown activity[35,36] against the transferases of viruses such as



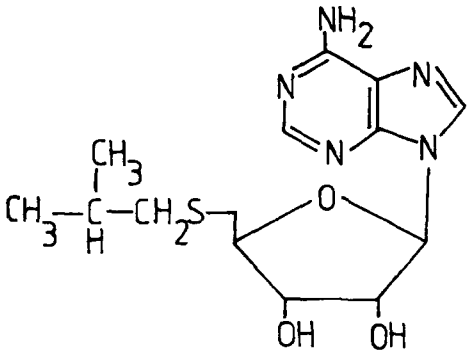
sinefungin



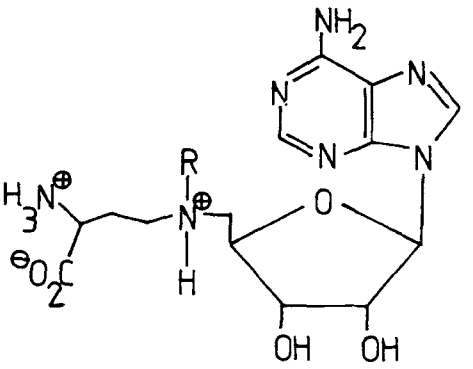
A9145C

Newcastle Disease virus and Vaccinia virus. Sinefungin is a potent competitive inhibitor of the virion mRNA (guanine-7-)methyltransferase. Data from <u>in vitro</u> studies shows that high concentrations of the drug are necessary due to low cellular permeability. 5'-S-Isobutyladenosine[34] (SIBA, 2) has different effects on different methylases.[37]

The main problem with developing analogues in this area of study is the inherent instability imparted by the sulphonium moiety leading to racemization.[38] An effort to overcome this has resulted in analogues where the S is replaced by a tertiary amine,[39] 3.



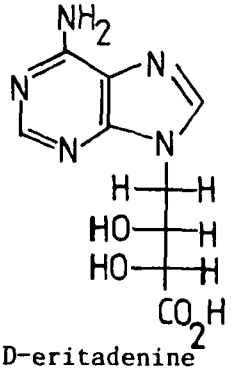
2 SIBA



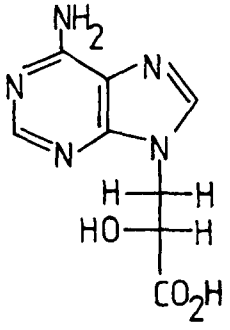
3

The main target area, however, has been AdoHcy hydrolase (SAH Hydrolase), as inhibition of this will result in a direct increase in cellular levels of the product inhibitor of AdoMet-dependent methyltransferase reactions.[40]

Initial studies found two inhibitors, 3-deazaadenosine and 5'-deoxy-5'-(iso butylthio)-3-deazaadenosine (3-deaza-SIBA) which inhibit Rous Sarcoma virus.[41] Over 70 alkyl derivatives have been tested for their effectiveness . There are several structural features that must be fulfilled for these derivatives to be active. These are that an intact adenine species and an alkyl chain at N-9 with a vicinal 2',3'-diol having S,S stereochemistry must be present.[42] Also, a series of similar compounds with a carboxyalkyl (C_3-C_5) chain at N-9 have been tested.[43] The best compounds were found to be D-eritadenine and the 3-(adenin-9-yl)-2-hydroxypropanoic acids which were active against VSV.



D-eritadenine

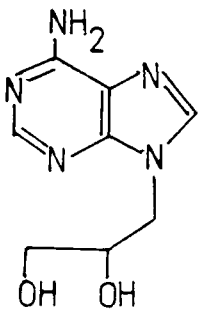


3-(adenin-9-yl)-2-hydroxypropanoic acid

The alkyl esters of these acids have also been tested and are inhibitors of

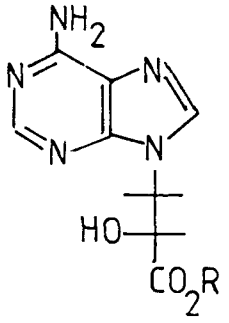
a broad range of viruses including VSV and reovirus.[44] Since then, many adenosine analogues have been synthesized and have shown significant activity, Figure 7.

Figure 7

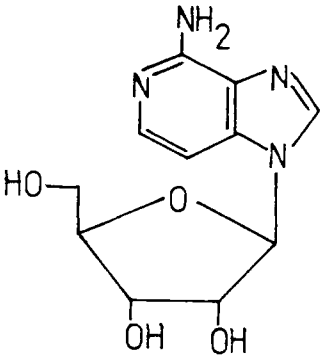


(S)-DHPA

(S)-9-(2,3-dihydroxy-propyl)adenine

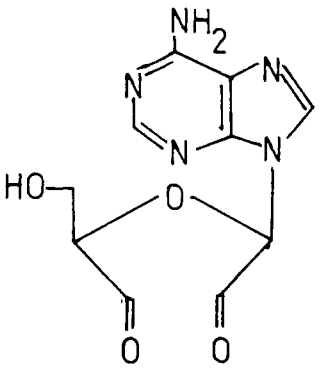


(R,S)-AHPA

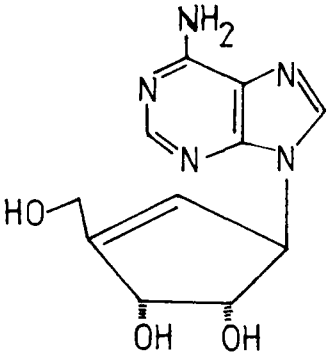


X=O 3-deazaadenosine (C^3 Ado)

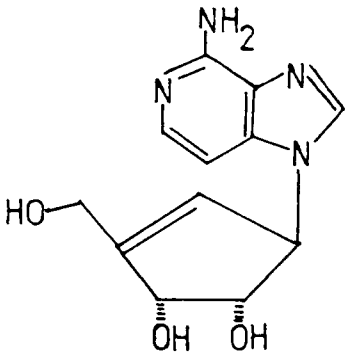
$X=CH_2$ carbocyclic 3-deaza-adenosine (C-c^3 Ado)



adenosine dialdehyde



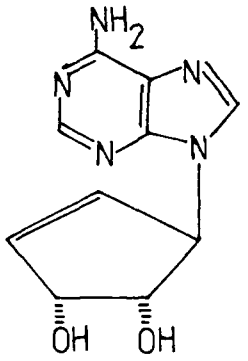
neplanocin A



3-deazaneplanocin A

Neplanocin A, [45,46] a naturally-occurring antibiotic, is a cyclopentenyl analogue of adenosine which exhibits antitumour activity and inhibits AdoHcy hydrolase irreversibly. [47] It is active against vaccinia virus and HSV-1 and HSV-2.[48]

3-Deazaadenosine has activity against the DNA poxviruses and negative- and double-stranded RNA viruses. Analogues of adenine and 3-deazaadenosine structurally related to neplanocin A, namely 9-(trans-2',trans-3'-dihydroxycyclopent-4'-enyl) derivatives, 4 and 5, are potent inhibitors of bovine liver AdoHcy hydrolases.[49]



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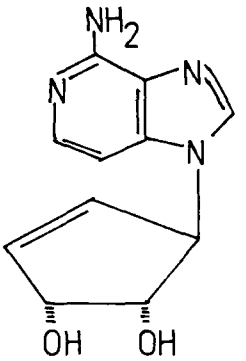


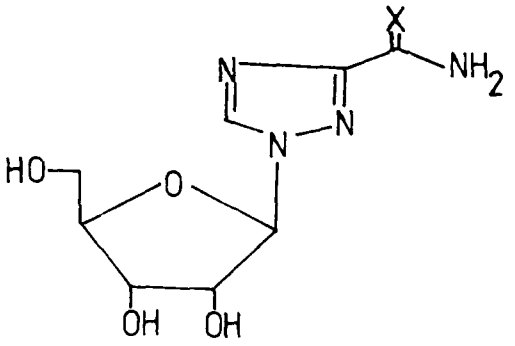
5

A report by Glazer et al[50] states that the inhibitory activity of 3-deazaneplanocin A against AdoHcy is higher than anything seen before.

The carbocyclic analogue of 3-deazaadenosine has been found[51] to have an activity 100 times greater than ribavirin and (S)-DHPA[52] against several viruses including VSV at concentrations of 0.2-1.0 g/ml. (S)-DHPA has broad spectrum activity against DNA viruses, rhabdoviruses and reoviruses.[53,44] Other analogues with useful activity include adenosine dialdehyde[54,55] which has structural similarities to the various 3'-keto intermediates during the enzymatic reaction.[56]

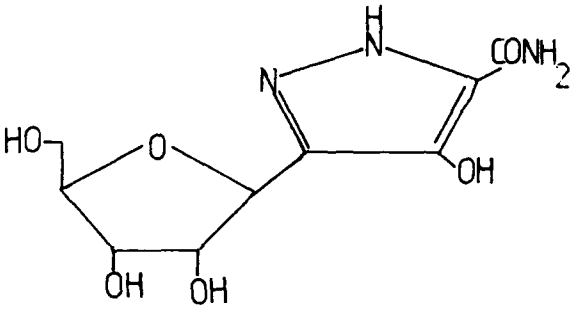
Ribavirin,[57] (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, virazole) an analogue some of the activity of which is thought to be associated with the inhibition of guanosine in the 5'-capping of the mRNA. The 5'-triphosphate has been shown to inhibit vaccinia virus mRNA guanyltransferase,[58] it is a potent competitive inhibitor of capping guanylation of viral mRNA. This would account for why it is inactive against polio virus which has uncapped mRNA. It has shown selective action against a number of DNA and RNA viruses[59,60,61] including a wide range of the Arenaviridae and Bunyaviridae[62,63] including lassa fever for which

favourable results were obtained.[64]



6 X=O ribavirin

7 X=NH amidine of 6



8 pyrazofurin

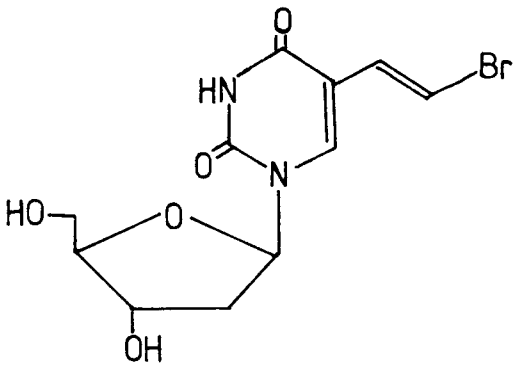
One of the mechanisms of action of ribavirin is concerned with pertubation of nucleotide pools as well as mRNA capping.[65] It is thought that the $CONH_2$ group rotates so that the nucleoside resembles either adenosine or guanosine.[66]

Ribavirin is phosphorylated by adenosine kinase to the 5'-monophosphate (RMP) then to the triphosphate (RTP). It seems that the inhibition results from inhibition of the capping enzymes, guanyl transferase and N-7-methyltransferase by RTP. RTP inhibition is competitive with respect to GTP and ATP demonstrating its structural similarities.[67]

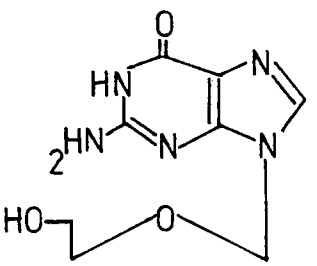
Other antivirals active against several negative-stranded RNA viruses have recently been found[68] one of the most promising of which is the amidine analogue of ribavirin, 7, which is of use in certain animal systems. Also, pyrazofurin, 8, inhibits the <u>in vitro</u> replication of Rift Valley fever and Pichinde.[69]

In addition to the AdoHcy hydrolase inhibitors there is the possibility of nucleoside analogues being active by their inhibition of the virus-coded polymerase and transcriptases and this would be another legitimate target.

Most of the anti-viral drugs in use act against enzymes and most of these drugs are effective against the herpes viruses, especially the compounds (<u>E</u>)-5-(2-bromovinyl)-2'-deoxyuridine (BVDUrd) and acyclovir[70,71] which are active against HSV-1, HSV-2 and VSV. The herpes viruses are known to code for several



BVDUrd



Acyclovir (ACV)

enzymes in their biosynthetic pathway,[72] for the nucleosides to be active it is necessary that they are firstly phosphorylated by the virus-specified enzyme dThd(dCyd) kinase which converts the nucleoside to the monophosphates; BVDUrdMP and acycloguanosine monophosphate (ACGMP).[74] ACV is further phosphorylated by a cellular GMP kinase while for BVDUrdMP the second phosphorylation step is carried out by the same enzyme responsible for the first phosphorylation.[75]

The primary target for their activity is the DNA polymerisation reaction against which the triphosphates act either as inhibitors or as substrates. ACGTP competes with dCTP and is incorporated into DNA where its lack of a 3'-hydroxyl causes chain termination.[76,77] As the initial phosphorylation by the viral kinase only occurs in virus-infected cells, the triphosphates are only found in these infected cells and not to any detectable amount in uninfected cells. It is, therefore, the preactivation by the kinase in infected cells which gives the specificity of BVDUrd and ACV

One member of the Retroviridae that has attracted much attention for anti-viral chemotherapy since its discovery has been HIV which is the causative agent of AIDS. These Retroviridae contain the enzyme reverse transcriptase, an RNA-directed DNA polymerase and this enzyme has been concidered as a target for anti-retrovirus compounds. Many active compounds have been synthesized including 3'-azido-2',3'-dideoxythymidine (AZT) and a series of 2',3'-dideoxynucleosides.[78]

Due to the high public profile of this virus, much work has gone into finding an anti-HIV compound. Much, therefore, has been elucidated about the

mechanism of action of this virus and the possible targets of attack. Not only has the reverse transcriptase been considered as a target for anti-retroviral compounds, other areas could include blocking the binding of the virus to the cell, inhibiting uncoating, inhibiting protein modification and inhibition of assembly of the virion.[79]

Cellular enzymes phosphorylate AZT to its 5'-mono-, 5'-di- and 5'-tri-phosphates. The 5'-triphosphate inhibits the reverse transcriptase or it can be another substrate which, if incorporated, results in chain termination due to the lack of the required 3'-hydroxyl group. It is the dependence upon phosphorylation by the cellular kinase which gives the reduced specificity.

The work to be discussed in this thesis consists of 5-substituted pyrimidine nucleosides with the emphasis on an unsaturated chain at the 5-position. The methods of functionalising the C-5 position of pyrimidine nucleosides are varied and can be grouped together into several areas, some of which appeared in a brief and now outdated review.[80] An expanded list of methods includes the following:

a. hydroxymethylation

b. Mannich reactions

c. halogenation

d. hydroxylation

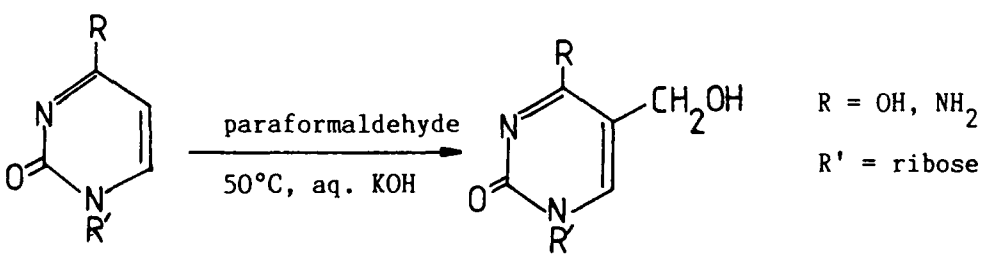
e. organometallic compounds (isolatable)

f. lithiation (transient)

g. nitration

and the chemistry of each will be discussed.

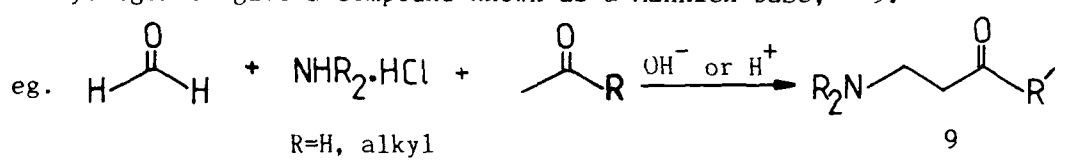
Hydroxylation is the acid- or base-catalysed addition of formaldehyde to the 5-position of a pyrimidine nucleoside. Base catalysis is the usual method:



The 5-hydroxymethyl compounds are known as constituents of nucleic acids. For example, in the DNA of T-even bacteriophages, cytosine is replaced by 5-hydroxymethylcytosine[81] and in some Bacillus subtilis bacteriophage DNA there is 5-hydroxymethyl-2'-deoxyuridine instead of thymidine.[82]

Uracil is hydroxymethylated in good yield by the base-catalysed addition of paraformaldehyde to uracil. Uridine reacts only under acidic conditions in moderate yield but 2',3'-isopropylideneuridine reacts in good yield under acid[83] or base[84] catalysis. The base-catalysed preparation of 5-hydroxymethylcytidine proceeds in low yield.[85] 2',3'-Isopropylidene-4-thiouridine does not react although 2',3'isopropylidene-2-thiouridine reacts well under basic catalysis,[86] possibly due to reduced polarisation of the 5,6-double bond which prevents the intramolecular nucleophilic attack on C-5. The reaction has also been done with several nucleotides, 5-Hydroxymethyl -2'-deoxyuridine 5'-monophosphate can be formed in low yields under acid[87] or base catalysis, however, the cytidine compound reacts with formaldehyde in the presence of base.[88] A similar reaction involving alkylation of the C-5 of uracil has been reported.[89] The acid-catalysed reaction between uracil and an aromatic aldehyde led to direct introduction of the -CH(OH)Ar side chain at C-5.

The Mannich reaction is a widely used reaction in organic synthesis which consists of the reaction between formaldehyde, an amine and a compound with an active hydrogen to give a compound known as a Mannich base,[90] 9.

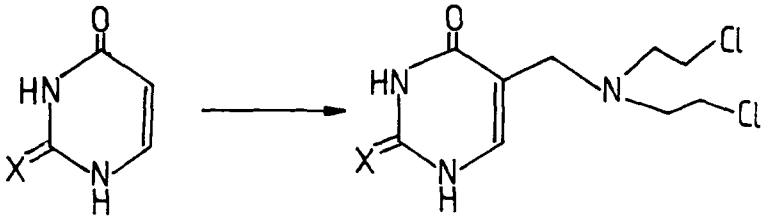


The Mannich bases are not particularly useful themselves but by alkylation and elimination of amine are especially useful for in situ preparation of α,β-unsaturated compounds which are usually stored as the Mannich bases . The reaction has found wide use in the synthesis of uracil derivatives. One of the first applications was the reaction of uracil and 6-methyluracil with β,β-dichlorodiethylamine in an effort to make compounds with an alkylating function that

could be incorporated into nucleic acids,[91,92] scheme 1, where X = O.

scheme 1.

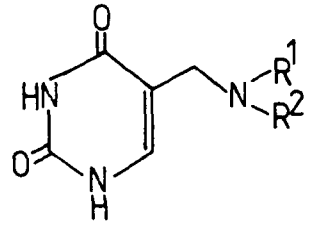
X = O,S



2-Thiouracil has also been aminomethylated in an effort to make nitrogen mustard derivatives as potential inhibitors of cancerous growths,[93] scheme 1, X=S. The best yields were obtained with paraformaldehyde and acidic media. 4-Methyl uracil, β-chloroethylamine and paraformaldehyde in dimethylformamide (DMF) gives 4-methyl-5-(2-chloromethylamino)methyluracil.[94]

Other studies on 5-(substitutedmethyl)uracils have used simple amines such as piperidine[95] and alkyl and aryl amines.[96] In the latter work, the aim was to synthesize analogues of thymine with cytotoxic activity. It was found that the products from primary amines,10, were hygroscopic solids while those from aromatic amines had low solubility and precipitated from solution. It was found,

10

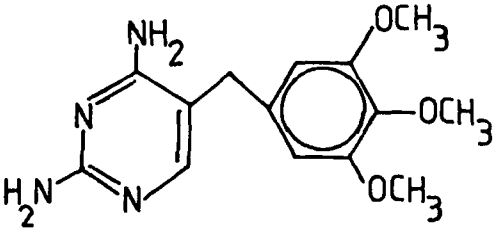


R^1	R^2
H	^nBu, hex, Ph, p-ClPh, p-NO_2Ph
Me	Me

however, that aromatic amines needed H or a ring activating group, hence SO_2NH_2, NO_2, CO_2H and CN do not react and the products from these came <u>via</u> 5-chloromethyl uracil.

An efficient synthesis of the antibacterial compound trimethoprin involves

trimethoprin



phenolic Mannich bases.[97]

An application of the reaction to nucleosides enables 5-methyluridine (ribothymidine) to be made by Pt/H_2 reduction of the adduct from diethylamine.[98,99] 2',3'-isopropylideneuridine (IPUrd), formaldehyde and pyrrolidone in boiling aqueous solution give Mannich bases useful for preparing 1-β-D-arabinofuranosylthymine.[100] The intermediate iodide salt formed from such reactions reacts with N-nucleophiles such as glycine ^{t}butyl ester to give 5-carboxymethylaminomethyl uridine, a constituent of some ^{t}RNA's.[101] Other compounds such as cyanomethyluridine can also be obtained,[102] Figure 8.

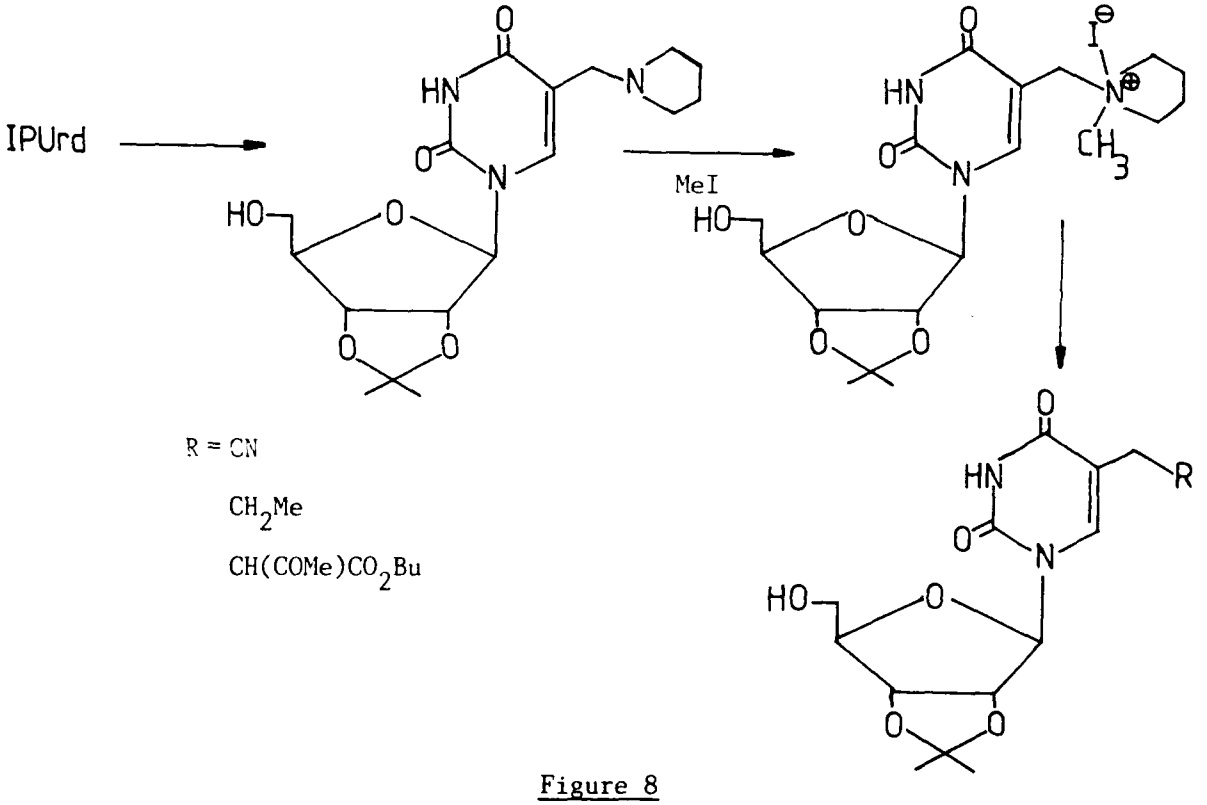
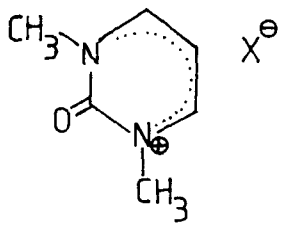


Figure 8

Much is known about the 5-halogenation of pyrimidines and pyrimidine nucleosides, however, the halogenation reactions of pyrimidines, such as 4-aminopyrimidine, are outside the scope of this discussion. The literature in the general area of pyrimidine chemistry has been thoroughly reviewed by Brown in the volume 'The Pyrimidines'[103] and its subsequent supplements.[104,105]

The first attempt to introduce iodine directly into uracil and cytosine was

in 1905 by Johnson and Johns.[106] It was known that uracil was attacked at C-5 by nitric acid or bromine[107] and so they used iodine in alkaline solution and got smooth conversion to 5-iodouracil in good yield. Oxidation of 1,2-dihydro-1,3-dimethyl-2-oxopyridinium salts, 11, gives a mixture of the base and the 5-iodo-



11

compound when X = I. When X = HSO_4 you get a mixture of the base, the iodocompound and the ring contracted product 1,3-dimethyl-2,4-oxazolidenedione.[108]

5-Iodouracil has slight anti-microbial activity.[109]

5-Halogeno-2-thiouracils and 6-methyl-5-halogeno-2-thiouracils have been synthesized[110] although it appears that 2-thiouracil is halogenated only when the sulphur is blocked, especially with 2-benzylthio. Uracil can also be halogenated with N-iodosuccinimide (NIS) in refluxing chloroform.[111]

There are many successful methods for the iodination of uracil nucleosides (see discussion for detail) but the iodination of cytosine nucleosides is more difficult. Iodination is not as easy as chlorination or bromination; iodination in dilute nitric acid gives 5-iodocytosine, the method of Johnson and Johns gives deamination. It is known that aromatic compounds can be iodinated in the presence of iodic acid[112] and this method applied to dCyd gives yields of IdCyd highly dependent upon solvent and temperature.[113]

Cytosine is easily iodinated in basic media by a suggested mechanism which

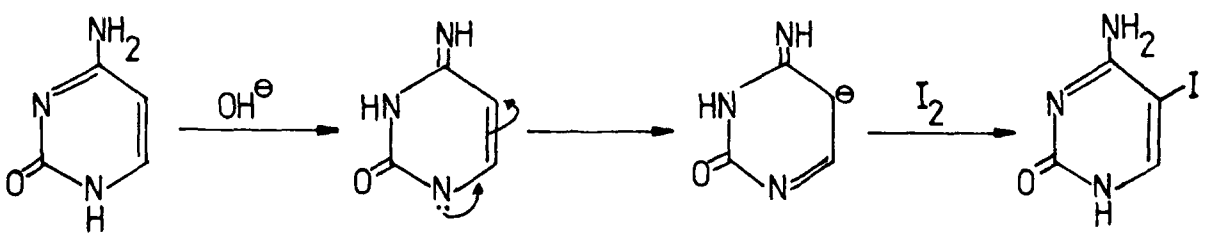
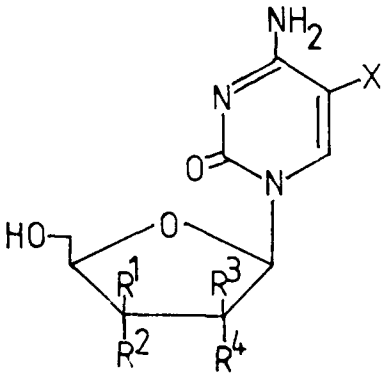


Figure 9

is not possible for the nucleoside, Figure 9,[114] where the increased lability of the NH_2 due to decreased aromaticity of the pyrimidine moiety is a factor also. In the presence of iodic acid a 68% yield of IdCyd together with some 5,6-dihydro-5,6-diiodo-2'-dUrd were obtained. The method of Chang and Welsh has been applied to 5-ICyd with modification and the method improved.[115]

Other nucleosides that have been halogenated include various cytosine nucleosides, 12a-c, [116] 3'-deoxy-5-halogeno-2'-arauridines, [117], various 3'-deoxynucleosides, 13a-d[118] and carbocyclic halocytidines, 14.[119]

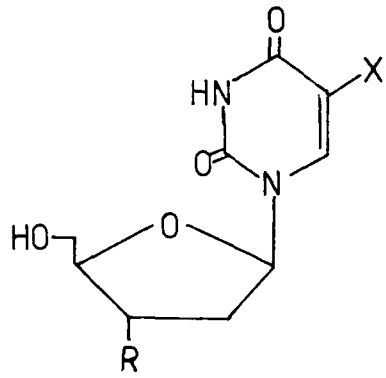


12

a. X=Cl,Br,I. $R^1=R^4=H$. $R^2=R^3=OH$

b. X=Cl,I $R^1=R^3=H$. $R^2=R^4=OH$

c. X=I $R^2=R^4=H$. $R^1=R^3=OH$



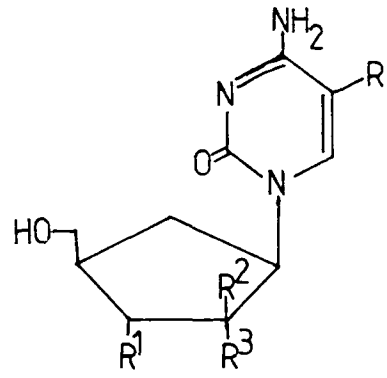
13

a. X=I,Br R=N_3

b. X=I,Br R NH_2

c. X I R Br

d. X R Br



14

R=Cl,Br,I $R^1=R^3=OH$

R=I $R^1=R^2=OH$ $R^3=H$

Pyrimidine nucleotides have also been iodinated. The reagents include NIS on UTP [120] and iodine/nitric acid on UCP,[121] UMP,[122] uridine 5'-dihydrogenphosphate[123] and cytidine 5'-phosphate. [124] Iodine/iodine trichloride in dioxan/dilute nitric acid has been used for cytidine polynucleotides. [124]

Uracil and cytosine are chlorinated readily. Uracil in water gives a mixture of 5-chlorouracil and 5,5-dichloro-6-hydroxyuracil, [125] and a small yield of 5-chlorouracil in acetic acid.[110] When treated with N-chlorosuccinimide (NCS) in glacial acetic acid 5-chlorouracil is obtained in 52% yield.[126] The use of

HCl/H_2O_2 gives addition of HCl to 2,4-diketopyrimidines.[127] 5-(Chloromethyl)-uracil is a reactive compound made in one step from uracil with HCl and paraformaldehyde in hydrochloric acid, it reacts readily with ROH to give ethers.[128]

5-Chlorouridine[129,130] and 5-chloro-2'-deoxyuridine[131] have been made by treatment of the nucleoside in acetic acid with Cl_2/CCl_4. NCS has also been used to prepare 5-chloro-2',3'-isopropylideneuridine[132] and 5-chlorouridine, 5-chloro cytidine and 5-chloroaracytidine.[133] 5-Chlorocytidine is a minor constituent in salmon sperm DNA.[134] Other reagents used have included iodobenzene dichloride for Urd[135] and MCPBA/HCl in aprotic solvents for Urd and Cyd.[136]

The most common reagent remains chlorine, for uridine UV irradiation is not required[137] although the difficulty in chlorinating cytidine may be due to salt formation with the AcOH solvent as the resulting $4\text{-}\overset{\oplus}{N}H_3$ would lower the acidity of position 5 and inhibit halogenation. Attack of chlorine at C-6 would not be expected because position 5 is meta to heteronitrogens and hence expected to be more susceptable to electrophilic attack due to internal mesomerism.

Chlorination of pyrimidine nucleotides has used tetrabutylammonium iodotetrachloride in DMF[138] and chlorine/CCl_4/AcOH.[121,123,139]

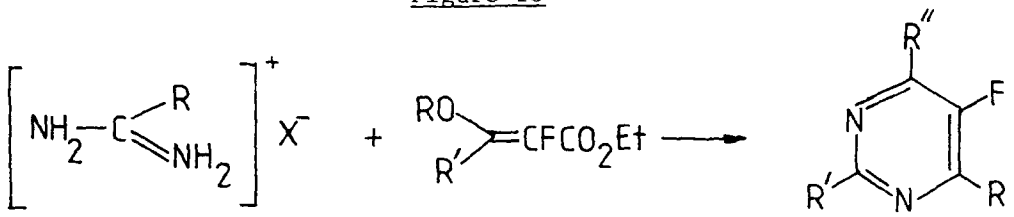
The standard method for bromination of pyrimidines is aqueous bromine.[140] 5-Bromouracil[141,142] and 5-bromocytosine[143] are made this way, the former is also obtained by bromination with dioxan dibromide.[130] 5-Bromouridine can be made with the sugar blocked[144,145,129] or unblocked[146,147] and the reaction proceeds <u>via</u> the 5-bromo-6-hydroxy-5,6-dihydrouridine adduct.

Bromine/DMF[148] and bromine/AcOH[149] have found limited use for uracil nucleosides as has Br_2/pyridine/AcOH for cytosine nucleosides.[150] Unlike Urd, Cyt requires UV irradiation when aqueous bromine is used.[151] Bromination of pyrimidine nucleotides has been accomplished using bromine[152,153,154] and NBS in formamide[155,156] or DMF.[157]

The original preparation of 5-fluorouracil and 5-fluorocytosine involved

de novo synthesis of the pyrimidine ring starting from the highly toxic ethyl fluoroacetate[158], Figure 10. Pseudourea and pseudothiourea salts are reacted

Figure 10



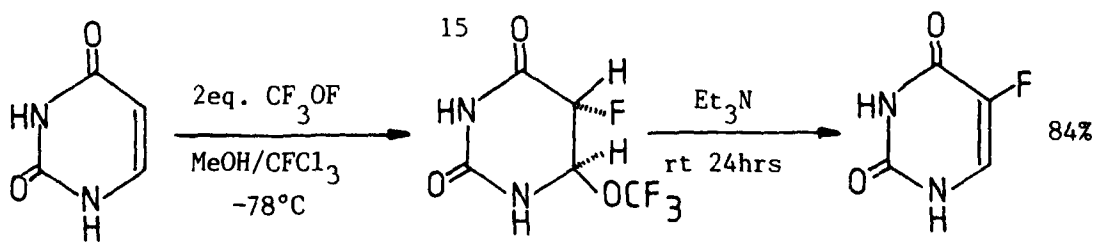
where

R=	OH	OH	SEt	SH
R'=	OH	NH_2	OH	OH
R''=	H	H	CO_2H	H

with α-fluoro-β-ketoester enolates. Nucleoside condensation methods were then required to form the nucleoside although 5-fluorocytidine is best made from 5-fluorouridine. Of the many routes to 5-fluorouracil nucleosides, the most common methods include condensation of the chlorosugar with 5-fluorouracil,[159] 2,4-dimethoxy-5-fluoropyrimidine[160] or 2,4-bis(trimethylsilyl)-5-fluoro-pyrimidine.[161]

In an attempt at direct fluorination, it was found that treating (±)-1-methyl-5-bromo-6-methoxy-5,6-dihydrouracil, the adduct from 1-methyluracil and methyl hypobromite, with silver fluoride gave 1-methyluracil via elimination of methanol.[162] This led to the use of the electrophilic reagent trifluoromethyl hypofluorite,[163,164] , CF_3OF, a powerful though toxic oxidising agent. The reaction with uracil goes via 5-fluoro-6-trifluoromethoxy-5,6-dihydrouracil, 15, which eliminates CF_3OH upon treatment with base,[165,166], Figure 11.

Figure 11



The reaction goes with complete stereoselectivity and regioselectivity.[167]

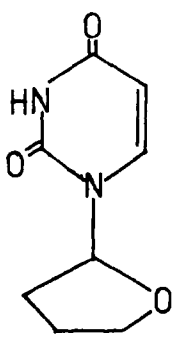
Uracil can also be fluorinated with $H_2O/CF_3COOH/CF_3OF$ systems,[168] electrolytically in HF,[169] in 10% yield by XeF_2/liquid HF[170] and by F_2/liquid HF *via* a 5,6-difluoro adduct.[171]. Uracil and 2,4-dimethoxypyrimidine are fluorinated by passing nitrogen diluted fluorine into their solutions in pyridine/HF/acetonitrile.[169] Work has also appeared on the fluorination of pyrimidines including 5-fluoroorotic acid from orotic acid and the isolation of dihydro-adducts from the fluorination of 5-substituted pyrimidines.[172,173]

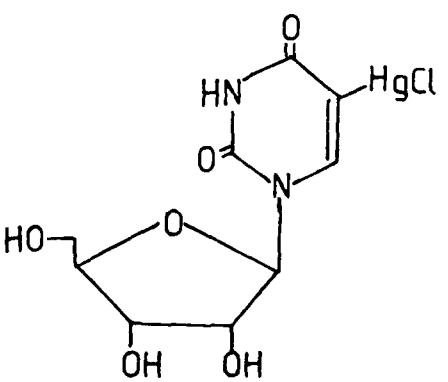
For cytosine, CF_3OF was used to effect the first direct synthesis of 5-fluorocytosine.[174] The fluorinating agent $FOSO_2F$ has found limited use.[175]

Uridine and 2'-deoxyuridine have been fluorinated with CF_3OF[165] as have the 2'-O-methyl-, 3'-O-methyl- and 2',3'-di-O-methyl ethers.[167] With the sugar protected[167,176,177] and unprotected[174], cytosine nucleosides have been fluorinated by CF_3OF and uracil[178] and protected uracil nucleosides by F_2/AcOH.[171] Uracil, uridine, 2'-deoxyuridine and 2',3'-dideoxy-3'-fluorouridine have been fluorinated by passing nitrogen diluted fluorine into their acetic acid solutions[180] as has a series of α-L-- and β-D-arabino-, lyxo- and xylo uracil nucleosides by F_2/AcOH.[179] Both cytosine in HF and cytidine in HF/AcOH are fluorinated using fluorine.[171]

The compound 1-(tetrahydro-2-furanyl)uracil, 16, has been fluorinated using

16





17

CF_3OF.[181,182] This rather unusual nucleoside analogue is obtained *via* addition of HCl to 2,3-dihydrofuran then condensation with a uracil moiety. The product of

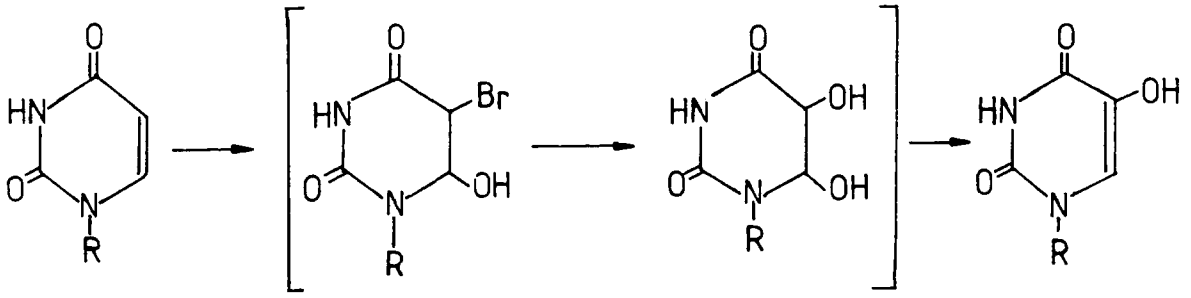
the fluorination, 1-(tetrahydro-2-furanyl)-5-fluorouracil (Ftorofur) is reported to be twice as active as 5-fluorouracil towards certain cancers. Fluorination of 2'amino-2'-deoxyuridine by CF_3OF proceeds only when the amino function is protected as the trifluoroacetamido group.[183]

The 5-position of an acylated nucleoside has been directly converted to a CF_3 group in moderate yield using CF_3I/Cu powder in HMPA.[184] The same transformation can be accomplished for uracil but not uridine by the use of bis(trifluoromethyl)mercury with irradiation[185] or using N-trifluoromethyl-N-nitrosotrifluoromethanesulphonamide, $CF_3SO_2N(NO)CF_3$, with irradiation.[186]

For nucleotides, CF_3OF has been used to fluorinate uridine 5'-phosphate salts.[187]

The hydroxylation of uridine [188,189,190] proceeds *via* addition of bromine water to the 5,6-double bond followed by hydrolysis and elimination of water,[146] Figure 12.

Figure 12



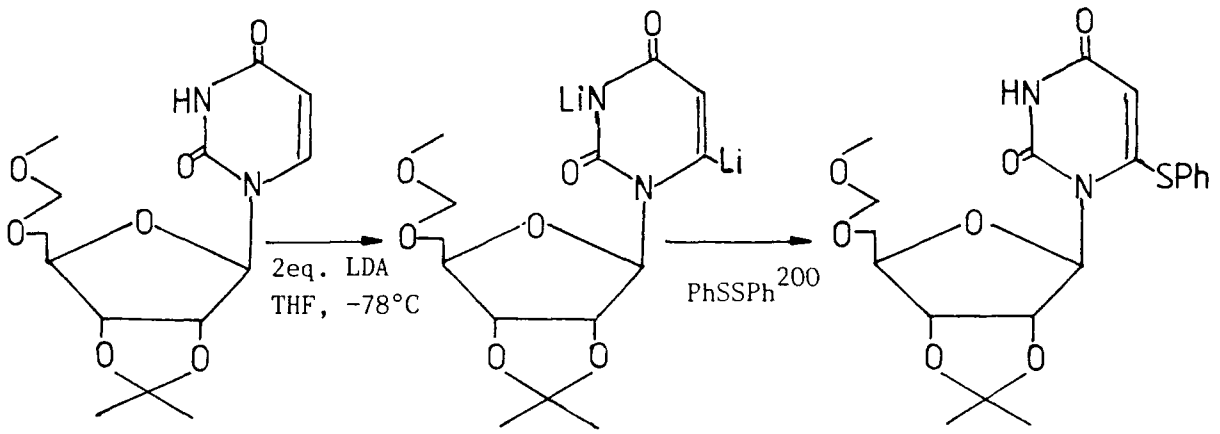
R=H, ribose, 2'-deoxyribose

Uracil[191] and uridine 5'-monophosphate[192] have also been hydroxylated.

5-Chloromercuriuridine, 17, is an example of a metallated nucleoside which can be isolated pure as a discrete compound. These compounds had synthetic use for coupling to vinylic compounds although they have now been superceded by better methods. Uracil can be mercurated with mercuric acetate or chloride to the 5-acetoxy or 5-chloromercuri compounds. These can be halodemercurated with

KI/I_2, KBr/Br_2 or NBS or S_2Cl_2 to the 5-halouracil compounds.[193,194] The nucleosides are mercurated with mercuric salts and are formed in good yield,[195, 196] Uracil and cytosine nucleotides are also readily mercurated.[197,198]

The generation and subsequent reactions of carbanions is now of important synthetic use.[199] The species used to generate these carbanions include methyl lithium, n-butyl lithium and lithium diisopropylamide (LDA). It has been found by Tanaka and coworkers in their extensive study on the lithiation of pyrimidine nucleosides, that it is possible to deprotonate selectively the H-5 and H-6 positions of the uracil ring. This forms the organolithium compounds *in situ* as reactive intermediates. The usual starting material was 2',3'-isopropylidene-5'-methoxymethyl uridine, 18.



18

Lithiation with LDA takes place at the C-6 position and many analogues have been synthesized. For the C-5 position there are several methods that can be used. These involve a 5,6-dihydrouridine derivative,[201] phenylthiolation of C-6 followed by reaction at C-5 using lithium tetramethylpiperide (LTMP) then stannylation to remove the group at C-6.[202,203] It has also been found that the C-5 position can be deprotonated selectively from 2',3',5'-tris-O-(*tert*-butyl-dimethylsilyl)uridine using *sec*-BuLi/tetramethylethylenediamine.[204]

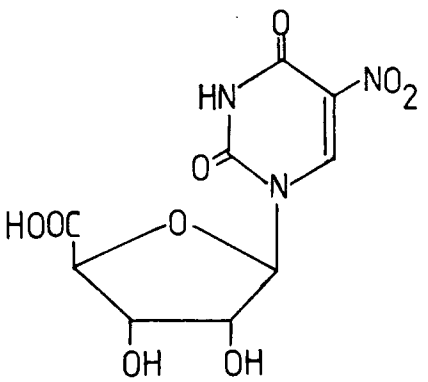
Another useful synthetic functionalisation of the C-5 position of pyrimidine nucleosides is nitration. Uracil itself is nitrated at C-5 without complication

in good yield[205] and 5-nitrouracil nucleosides have been made from the base by the $Hg(CN)_2/CH_3CN$ condensation procedure. This modification was introduced by Helferich et al[207,208] to improve the yields of glycosides in the Koenigs-Knorr condensation, it allows condensation in high yields without isolation of an intermediate mercury compound and proceeds in high yields even with pyrimidines possessing a strongly electron withdrawing group at the C-5 position.

The Hilbert-Johnson condensation method for 5-NO_2-2'-dUrd from 5-nitro-2,4-dimethoxypyrimidine in benzene and toluene fails while the condensation of 5-nitrouracylylmercury and chlorosugar gives a low yield of both isomers.[209] The condensation of bis(trimethylsilyl)-5-nitrouracil with the ribose chlorosugar, however, is almost quantitative when catalysed by $SnCl_4$.[210,211]

The first report of introducing a 5-NO_2 group into a nucleoside was reported by Levene and LaForge[146] who reported the preparation of 'nitro-uridin-carbosaure' from uridine and nitric acid where oxidation of the 4'-CH_2OH occurred.

19 nitro-uridin-carbosaure



5-Nitrouridine was first synthesized by the action of nitric acid on 2',3',5'-tris-O-(3,5-dinitrobenzoyl)uridine.[212] The 2'-deoxyuracil nucleoside has a potent inhibitory effect against Vaccinia virus[213,214] and was first synthesized enzymatically using deoxyribolase from Lactobacillus.[213] The 5'-monophosphate is an active-site directed irreversible inhibitor of thymidylate synthetase of Lactobacillus Caesi.[215,216]

The blocking of the sugar by the dinitrobenzoate ester resulted in a more

nitration resistant compound, the nitration was accomplished in 71% yield using fuming nitric acid/sulphuric acid. This nitration was unsuccessful for cytidine, the nitro compound of which has been made by a condensation method starting from di(5-nitrocytosine)mercury.[217] The dinitrobenzoate method does not work for 2'-dUrd but works in a reasonable yield for 5-nitro-araUrd.[218] There is a dramatic decrease in the antiviral activity of this compound when it is compared to AraUrd.

A widely used nitrating agent introduced by Olah[219] is nitronium tetrafluoroborate, NO_2BF_4. With uracil in sulpholane it gives good yields of the nitro-compound but with Urd/2'-dUrd, even when blocked, 5-nitrouracil results.[220] With nucleotides a mixture was obtained consisting of the required product, 5-nitrouracil and 3'-O-nitro-5-nitro-2'-deoxyuridine 5'-monophosphate.[221]

Nitronium tetrafluoroborate has been used to nitrate 2',3'-di-O-acetyl-2'-deoxyuridine 5'-phosphate but is unsuccessful for CMP.[222]

Nitration of cytosine by nitronium tetrafluoroborate in sulpholane is almost quantitative but dCMP gives a mixture of 5-NO_2-2'-dCyd (1.5%), 5-NO_2-2'-dCyd-3'-ONO_2 (37%) and 2'-dCyd-3'-ONO_2 (1.7%).[224]

5-Nitro-2'-deoxycytidine is an antiviral agent active against VSV. The 5-substituded 2'deoxyuridines which are not HSV selective become selective when converted to the cytidine analogues. BVDUrd and the iodo compound IVDUrd follow this specificity change and this may be because they must be substrates for two enzymes, the virus induced dTHP/Cyd kinase and the deamination enzyme dCMP deaminase.[224]

It is the subtle interactions of nucleoside analogues with the enzymes involved in viral disease that are responsible for the viral activities of nucleoside analogues. Sometimes, as in the case of the herpes-viruses, the synthesis of many analogues allows a pattern of structural requirements for antiviral activity to be elucidated.[225] In the work to be described in this thesis it was necessary to start with, as is often the case, a lead

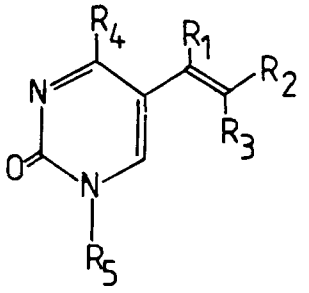
generated from the significant but not useful antiviral activity of one nucleoside analogue and to exploit this activity by synthesizing as broad a range as possible of similar analogues.

RESULTS AND DISCUSSION

The aim of this work was to synthesize new pyrimidine ribonucleoside analogues bearing a substituted vinylic side chain at the 5-position that would possess antiviral activity against several families of the negative-stranded RNA viruses, namely the Arenaviridae, Bunyaviridae and the Rhabdoviridae.

A review in 1984 covered the known 5-substituted vinyl pyrimidine nucleosides in the literature up to that time[226] although many more analogues have since been made. The variety of possible structures can be shown by 20, where R_4 can be hydroxyl or amino (uridine or cytidine nucleosides respectively) and R_1,R_2 and R_3 can be various substituents such as alkyl, aryl, substituted

20



aryl, H, halogen, carboalkoxy, alkoxy, nitrile and trifluoromethyl.

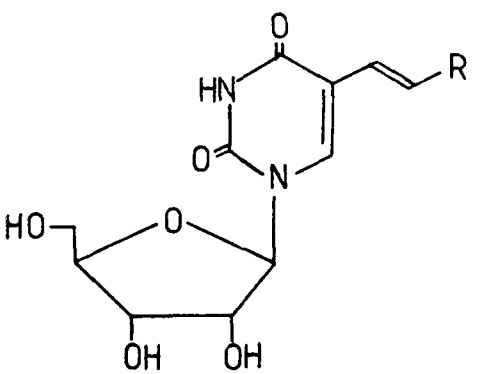
The variation present on the vinylic side chain is also seen in the sugar moiety, R_5, where the common species are α- and β-2'-deoxyribosyl, ribosyl, arabinosyl, 2'-fluoroarabinosyl and (2-hydroxyethoxy)methyl.

The 5-vinylpyrimidine bases were found to be biologically active in work, the purpose of which was to synthesize potentially active thymidine analogues where the methyl group of thymine is replaced by another group of similar size.[227] At the nucleoside level several non-vinylic 5-substituted 2'-deoxyuridines have shown useful biological activity. 5-Iodo-2'-deoxyuridine[228] was the first nucleoside analogue to have clinical usage[229] for the treatment of herpetic eye infections. It was closely followed by 5-trifluoromethylthymidine which has the same use, both compounds possess some toxicity. 5-Iodo-2'-deoxycytidine[230]

and 5-ethyl-2'-deoxyuridine[230] have found use in the topical treatment of herpetic keratitus and mucocutaneous herpes lesions.

The first example of thymine analogues with unsaturation such that the delocalised electrons were conjugated with the pyrimidine π-system were the 5-vinyl and 5-ethynyl-2'-deoxynucleosides. Although since then many substituted 5-vinylpyrimidine nucleosides have been made, most are 2'-deoxynucleosides. The initial work tended to concentrate on the herpes viruses; herpes simplex virus 1 (HSV-1) and 2 (HSV-2). As these are viruses with a DNA genome it was not expected that ribonucleosides would show any significant activity as one would not expect the ribonucleoside to be readily converted into the deoxynucleoside triphosphate. This is the form necessary for incorporation into DNA, it is known that thymine ribonucleosides are poor precursers for DNA thymine.[233]

5-Vinyluridine,[234,235] 5-vinylcytidine,[236] 5-ethynyluridine[237,238] and 5-ethynylcytidine[237,239,240] have been reported as have some substituted 5-vinyl pyrimidine ribonucleosides. The derivatives of uridine that are known are compounds 21-26.



	R
21	CO_2CH_3
22	$CO_2CH_2CH_3$[241]
23	COOH
24	Br
25	Ph[235]
26	CN

For cytidine, the methyl ester analogous to 21 has been made[242] although attempts to isolate the vinylic nitrile were unsuccessful and this compound was formed <u>in situ</u> and reduced directly to the cyanoethyl compound.

Although 5-allyluridine and 5-allylcytidine are not strictly vinylic pyrimidine nucleosides, the double bond can be isomerised to the vinylic compound

using Wilkinsons catalyst and both these compounds are known.[243]

A derivative of the bromovinyl compound, 24, has been obtained where the $-OCHF_2$ group is in the 4-position and is thus a derivative of the 4-methoxypyrimidinone.[244]

(E)-5-(2-bromovinyl)uridine (BVrUrd, 24) and (E)-5-(2-carbomethoxyvinyl) uridine, 21, were originally synthesized and, in a study that also included 5-vinyluridine, were tested against a number of viruses such as Venezuelen Equine encephalitis, Yellow fever, Rift Valley fever and Pichinde viruses.[231] 5-Vinyl uridine was found to be totally inactive although the former two compounds had a slight activity against the Yellow Fever virus.

The Inhibitory Dose 50 (ID_{50}), the concentration of the drug that causes a 50% reduction in virus replication, was found to be 60·1μg/ml for (E)-5-(2-bromo vinyl)uridine and 20·93μg/ml for (E)-5-(2-carbomethoxyvinyl)uridine. The therapeutic index is a measure of the antiviral potential of a drug and a figure above 50 is indicative of a useful compound. The therapeutic index for the bromovinyl compound and the ester were 8·32 and 23·89 respectively against the Yellow Fever virus.

The therapeutic index for the methyl ester is about half that which could be considered a useful value. It was initially decided to exploit this lead and synthesize a series of esters of the acid (E)-5-(2-carboxyvinyl)uridine in the hope that another ester would be more active.

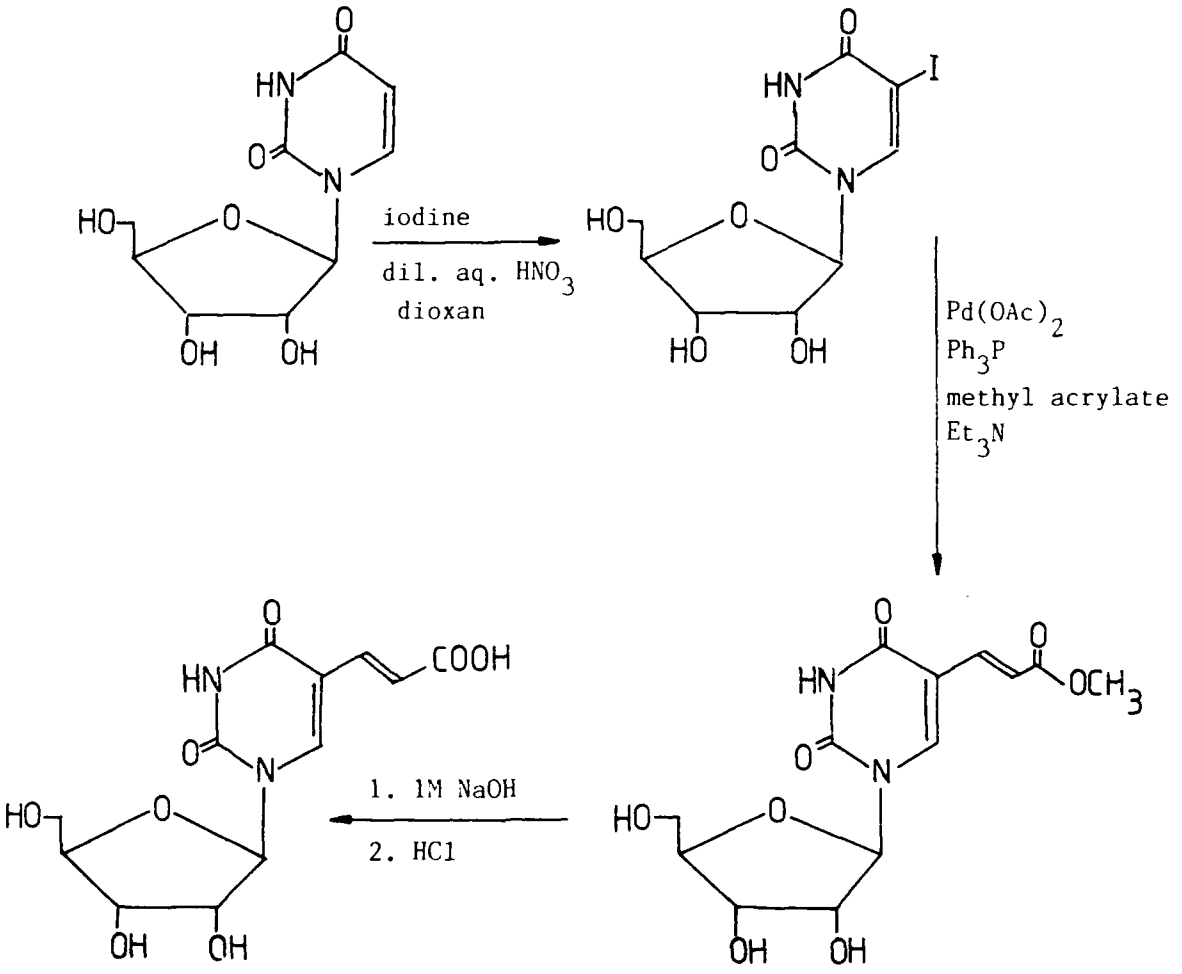
It is thought that the compounds made can be targeted against the unique transcriptase in the negative-stranded RNA viruses. The aim is to make nucleosides that will be substrates of the cellular kinase and the 5'-triphosphates then inhibit the transcriptase.

5-Vinylpyrimidine nucleosides can be made by modifying the pre-existing nucleoside but the majority of analogues are made from the nucleoside or 5-iodonucleoside *via* organopalladium reagents.

The initial approach to these esters was from the acid. The acid itself is

obtained in a three stage synthesis from uridine, Figure 13, the first stage of which requires the iodination of uridine to give 5-iodouridine. There are many

Figure 13



methods of iodinating pyrimidine nucleosides. The first iodination[232] used iodine, uridine, dilute nitric acid and a trace of chloroform. 5-Iodo-2'-deoxyuridine has been synthesized in an analogous reaction.[227,245] The π-electron density of the pyrimidine ring is greater at C-5 than C-6 and this facilitates electrophilic attack by the positive iodine species. Since then, other systems have been used. These include iodine chloride on 2'-deoxyuridine and uridine

which are protected as the p-toluoyl esters, this proceeds in high yield.[135] Iodine chloride has also been used to iodinate uridine in solution in N-ethyl acetamide or DMSO.[120]

Cerium (IV) ammonium nitrate (CAN) and iodine are used to iodinate benzeneoid aromatics[246] and when used with uridine and 2'-deoxyuridine protected as the acetate or benzoate esters gives good yields of the 5-iodocompound.[247] Ill defined 5-mercurinucleoside derivatives have been iodinated in aqueous alcohol[193] while the method of Johnson and Johns, namely iodine in dilute aqueous sodium hydroxide, works well for 2'-deoxyuridine.[227]

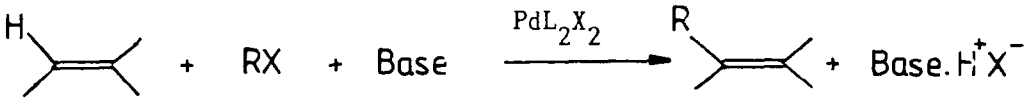
As the 5-iodouridine was required unblocked it was decided to use a slightly modified method of the original procedure[123] for 5-iodouridine 5'-phosphate. 5-Iodouridine was therefore prepared from uridine in 99% yield by refluxing a solution of uridine and iodine in dilute aqueous nitric acid/dioxan. If necessary the 5-iodouridine can be recrystallised from water but is sufficiently pure for most purposes to be used directly.

The reaction proceeds rapidly at an elevated temperature to give one nucleoside product. The NMR spectrum of the product showed the absence of the H-5 doublet, the H-6 proton now appears as a singlet at 68·40. The fast atom bombardment (FAB) Mass spectrum shows a molecular ion which is consistant with the replacement of the C-5 proton by iodine.

The next step involves the joining of the unsaturated side-chain to the C-5 position. This is accomplished using the well known Heck reaction in which an arylmercury salt or aryl halide reacts with alkenes to generate new carbon-carbon bonds. Several reviews have appeared covering the general synthetic applications of the reaction.[249,250,251]

Few reactive intermediates which enable carbon-carbon bond formation show the selectivity shown by the organopalladium intermediates in their reactions with alkenes, the Heck reaction is a valuable one as the transformation cannot

The Heck reaction



R= Ar, heterocyclic, benzyl, vinylic

X= Br, I (rarely Cl)

L= ligand

be carried out in one-step by any other method except in certain limited Meerwein arylation reactions.

In nucleoside chemistry, the Heck reaction was first utilised by Bergstrom and coworkers who published a series of papers. These included the coupling reactions of nucleosides with allylic halides,[243] olefinic compounds[196,235] and allylic chlorides, alcohols and acetates.[252] As in the Heck reaction in general, it is possible to use the aryl component, in this case a nucleoside, functionalised at the 5-position with halogen (usually iodine) or as the chloromercury compound.[195,253]

The two methods that can be used for such palladium reactions involve different catalyst systems for the generation of the palladium intermediate. The first method involves the coupling of the 5-chloromercurinucleoside with the olefin in methanol using equivalent proportions of expensive Li_2PdCl_4.[196,243] The mercurinucleoside undergoes metal exchange with the palladium complex when mercury is bound to an sp^2 hybridised carbon.[254] The second method involves coupling a 5-iodonucleoside with the olefin[235] using the preparation in situ of catalytic palladium complexes present to the extent of 0·05 molar equivelents. This[255] was in improvement on the previous procedure of using palladium (II) acetate with an amine[256] or the use of the organomercury compounds.[257,258,259]

The best catalytic palladium complexes were found to be formed using triphenyl phosphine which was more reactive than the amine used initially.

(E)-5-(2-carbomethoxyvinyl)uridine[260] was prepared using the palladium (II) acetate, triphenyl phosphine catalyst system. 5-Iodouridine and methyl acrylate were added to the catalyst, prepared *in situ*, which consisted of palladium (II) acetate (0·05 molar equivalents), triphenyl phosphine (0·1 molar equivalents) and an excess of dry triethylamine in dry dioxan. The catalyst solution was activated by heating the solution to 70°C until a deep red colour had developed, this forms the catalytic palladium complexes. After addition of the reactants, the solution was refluxed for 1 hour then filtered to remove the black suspension of palladium metal and the product obtained by crystallisation from the filtrate upon standing overnight. Washing the filtered crystals with ether removed triethylamine hydrochloride and triphenyl phosphine, then recrystallisation from ethanol gave the pure ester in 50% yield. The ester was characterised by its UV, NMR and FAB mass spectra. The configuration about the double bond was assigned as *trans* based on the coupling constant of J=16Hz.

An analogous reaction has been used to prepare the 2'-deoxynucleoside and the arauridine[261,262] esters, the latter in 53% yield. The ribonucleoside ester has also been made photochemically by coupling methyl acrylate to uridine in 38% yield.[263]

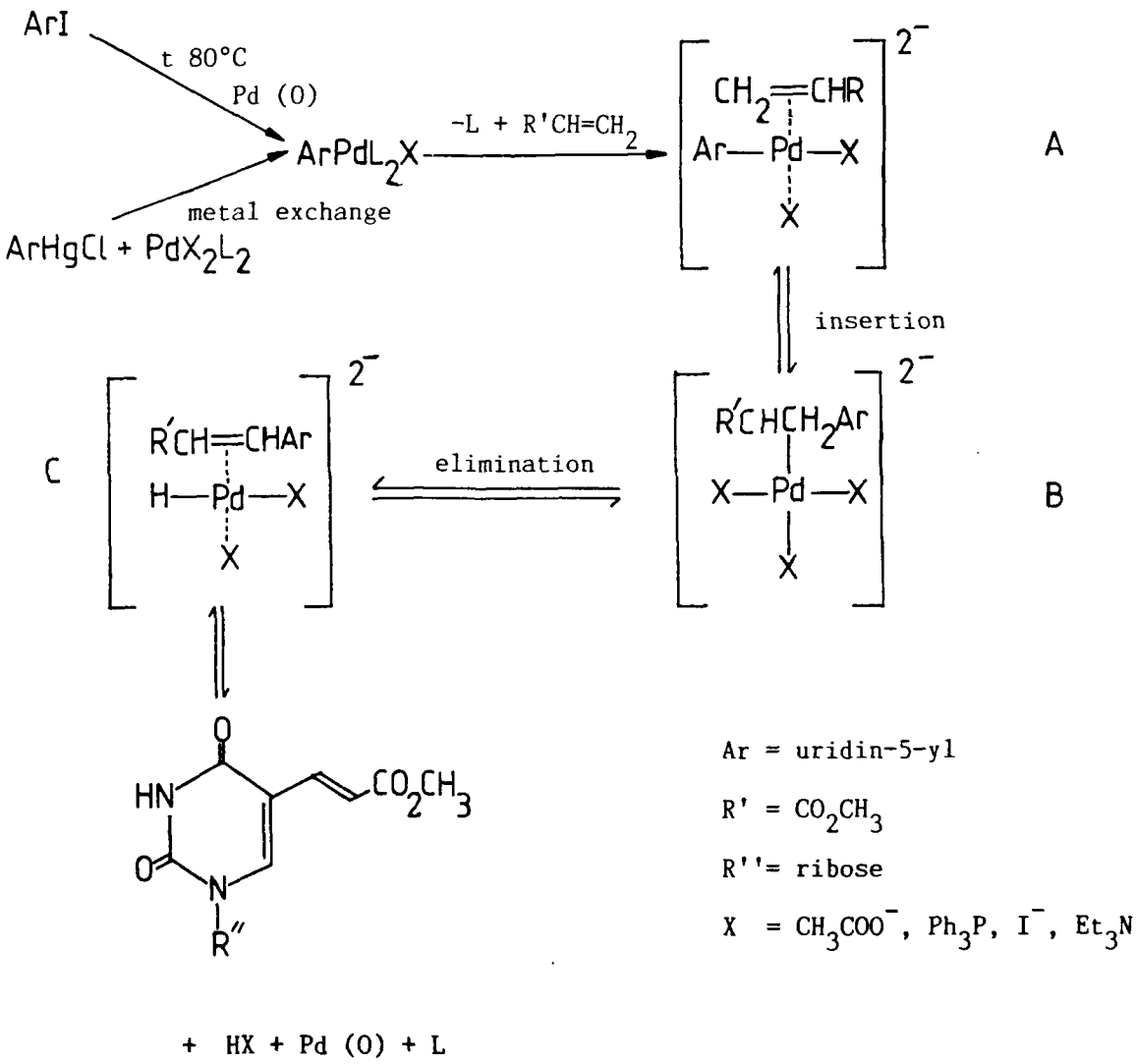
The reaction is compatable with many functional groups including alkyl, aryl, *m*-NO_2 aryl, *m*-NH_2 aryl, azido, hydroxyl, alkoxy, nitrile, halogens, amide, carbonyl carboxyl. Also, the conditions do not need to be anhydrous or anaerobic except under certain circumstances such as the use of tri-p-toluoylphosphine.

The mechanism of the reaction, Figure 14, is thought to resemble that proposed for the arylation of olefins with organometallic compounds and analogous to that of the coupling of 5-chloromercurated nucleosides.[264]

Palladium (0) is initially formed by reduction of the $Pd(OAc)_2$ /Ph_3P complex to a Pd (0)-phosphine complex by the alkene. Then, the organopalladium

intermediate $ArPdL_2X$ is formed and a weakly bound ligand can exchange for olefin to form complex A. The key step involves the π-bonded methyl acrylate insertion into the Ar-Pd σ-bond to form complex B with the loss of iodide ion. Complex B then undergoes cis-elimination of Pd-H to give the product as a π-bonded species C. This complex then dissociates to give the product together with HI and palladium (0).

Figure 14



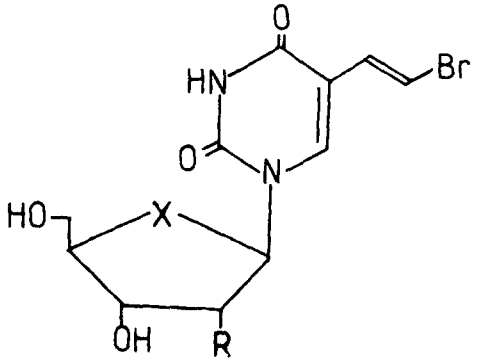
The reaction is catalytic overall in palladium as the Pd (0) is regenerated in the last step. When the mercurinucleosides are used the metal exchange

proceeds at room temperature whereas the oxidative addition of Pd (0) to aryl halides requires heating. The type of product depends on the olefin, conjugated olefins such as acrylate esters couple regioselectively at the terminal alkenyl carbon giving trans olefins.[236]

The acid (E)-5-(2-carboxyvinyl)uridine is prepared from the ester, [260] saponification of which using 1M aqueous sodium hydroxide proceeds rapidly at room temperature. The acid was isolated by cooling the solution of the sodium salt and acidifying. The precipitate that formed was filtered and washed carefully with ice-cold water to remove traces of sodium chloride. The NMR spectrum of the product showed the absence of the ester methyl group at δ3·7. The FAB mass spectrum showed the loss of 14 mass units, consistant with the change $-CO_2CH_3$ to -COOH.

It is possible to brominate the acid via a decarboxylative bromination to the bromovinyl compound, the palladium catalysed route to the acid was originally developed, in the 2'-deoxynucleoside series, as a synthesis of the selective and potent anti-herpes compound (E)-5-(2-bromovinyl)-2'-deoxyuridine, BVDUrd, 27.[265] The ribonucleoside analogue, (E)-5-(2-bromovinyl)uridine, BVUrd, 28, is prepared in an analogous manner to that for BVDUrd. Although the (E) isomer of BVDUrd has a high activity, the (Z) isomer is essentially inactive[266] whereas the carbocyclic analogue, 29, is almost as active as (E)-BVDUrd against HSV-1 and VSV in cell culture.[267] It is not susceptable to glycosidic bond cleavage by pyrimidine nucleoside phosphorylases which cleave the N^1-1' bond.[268]

The original synthesis of BVDUrd[269] was a condensation procedure from bis



27. X=O R=H

28. X=O R=OH

29. $X=CH_2$ R=H

(trimethylsilyl) (E)-5-(2-bromovinyl)uracil, a modification of the industrial preparation of BVDUrd, the same reactions as Figure 13, was used to prepare a sample of BVUrd.[270] This involved the reaction of the potassium salt of the acid with N-bromosuccinimide in DMF and the needles obtained after recrystallisation from water were characterised by NMR, UV and mass spectroscopy.

BVUrd has also been synthesized by a condensation reaction from 5-ethyl uracil then photobromination and deblocking,[271] the triphosphate has been made in a '1 pot' synthesis *via* a 5'-cyclophosphate.[272]

The other halovinyluridines are known. Contrary to what has been published [241], it has now been found that the best method for the synthesis of the chlorovinyl compound uses chlorine in dry DMF and not N-chlorosuccinimide. For the iodovinyl compound N-iodosuccinimide is used.

(E)-5-(2-fluorovinyl)-2'-deoxyuridine must be made *via* an indirect route which involves treating 3',5'-di-O-acetyl-5-bromomethyl-2'-deoxyuridine with the anion of ethyl fluoroacetate, saponification and bromination of the side chain. Dehydrobromination and decarboxylation then gave the fluorovinyl side-chain.[273]

The 1-β-D-arabinofuranosyl-(E)-5-(2-halovinyl)uracils are known where halogen is Cl, Br and I. They were made by halogenation/decarboxylation with the appropriate N-halosuccinimide.[274]

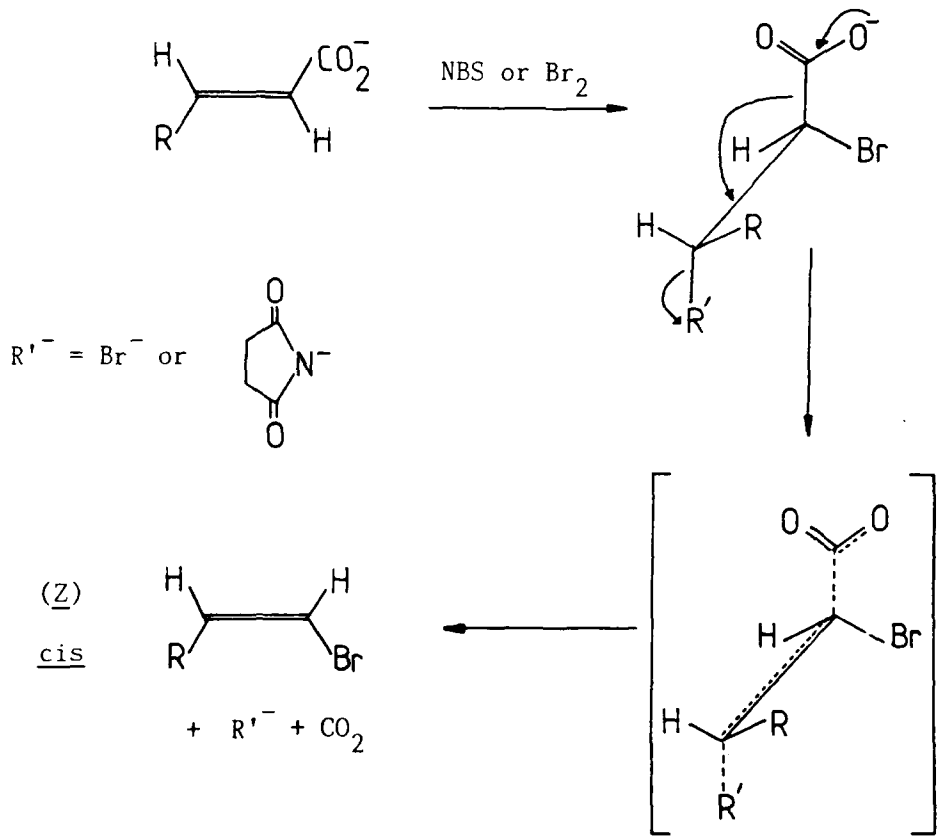
The treatment of the acid with NBS (or Br_2) is an example of the classic decarboxylative bromination reaction, a fragmentation reaction which involves the loss of bromide ion from the *erythro*-α,β-dibromoethylcarboxylic acid, if bromine was used; or the loss of succinimide from the *erythro*-α-bromo-β-succinimidoethyl carboxylic acid if N-bromosuccinimide was used. Carbon dioxide is also lost.

It is not surprising that when (E)-5-(2-bromovinyl)uridine and the 2'-deoxy nucleoside were first synthesized that no (Z)-isomer was formed. In other systems, for example, 2-(2-carboxyvinyl)-5-nitrofuran[275] and cinnamic acid,[276, 277, 278] such decarboxylative brominations gave either (Z)-isomers (in relatively

non-polar solvents) or both isomers (in more polar solvents).

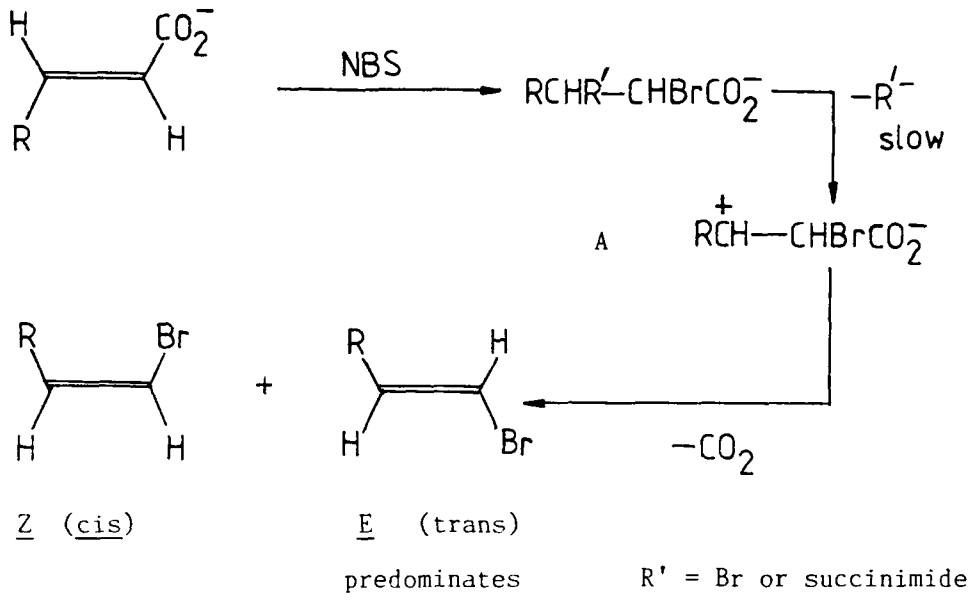
The mechanism of the bromination in non-polar solvents[279] is thought to involve a stereospecific trans-elimination of bromide or succinimide and carbon dioxide and is similar to an E2 elimination of alkyl halides. A postulated transition state is shown in Figure 15.

Figure 15 Non-polar bromination



In polar solvents, Figure 16, the mechanism has been compared to an E1 elimination where intermediate formation of a dipolar ionic compound A by loss of bromide or succinimide is followed by decomposition to the trans alkene. The polar solvent stabilises the ionic intermediate which allows rotation about the carbon-carbon single bond to give the trans-isomer as the major product. The participation in the mechanism of non-polar solvents as well is thought to be

Figure 16 Polar bromination



why a small amount of the (Z)-isomer is also seen in polar solvent brominations.

There are many possible methods for the synthesis of esters apart from the reactions of carboxylic acids. In most, however, the reaction conditions would be too harsh for nucleoside chemistry, even it the required nucleoside derivative were available.

Direct esterification of the acid with an alcohol is a possible route to the required esters. The reaction, however, is reversible and only proceeds in the required direction by either adding an excess of one of the reactants, removal of ester or water by distillation or by removing the water by the use of a dehydrating agent. Common catalysts include sulphuric acid, dicyclohexylcarbodiimide (DCC), trifluoroacetic anhydride and BF_3/ether. Other possibe routes would be alcoholysis of an anhydride or acyl halide or by transesterification.

It was the alcoholysis of an acyl halide that was used first to attempt the preparation of the esters of (E)-5-(2-carboxyvinyl)uridine. It was decided to use the more readily available (E)-5-(2-carboxyvinyl)-2'-deoxyuridine as a model

compound because large quantities were available as a result of the commercial scale preparation of BVDUrd.

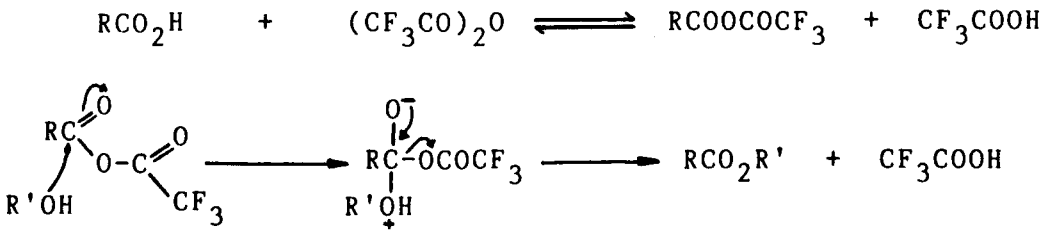
It was decided not to block the sugar of the acid in order to reduce the number of steps in the sequence and also to see whether it was possible to make an acid chloride in the presence of hydroxyl groups or whether polymerisation or other side reactions would predominate.

The acid chloride would be made *in situ* from an appropriate acid chloride such as thionyl chloride or oxalyl chloride then excess reagent together with any solvent if used removed by distillation under reduced pressure. The acid chloride would then be quenched immediately with an alcohol to form the ester. Propan-1-ol was used in order to make the n-propyl ester.

Firstly, the reaction was investigated using thionyl chloride. Minute amounts of non-nucleosidic material were obtained after repetitive column chromatography. Refluxing destroyed all starting material very rapidly. The results with oxalyl chloride were more encouraging but yields were less than 2%. The use of a solvent and either thionyl chloride or oxalyl chloride were then investigated. The best combination was found to be a 10 fold molar excess of oxalyl chloride in pyridine which gave a 6·9% yield of (E)-5-(2-carbopropoxy-vinyl)-2'-dUrd after quenching with propan-1-ol. The product was characterised by its UV and NMR spectra. When the ribonucleoside acid was used, however, the yield was only 2%.

Another method to the esters from the acid is by alcoholysis of the mixed anhydride with trifluoroacetic anhydride.[280] Bourne *et al* have shown that the unsymmetric anhydride can be made, Figure 17. For example, benzoic acid/TFAA

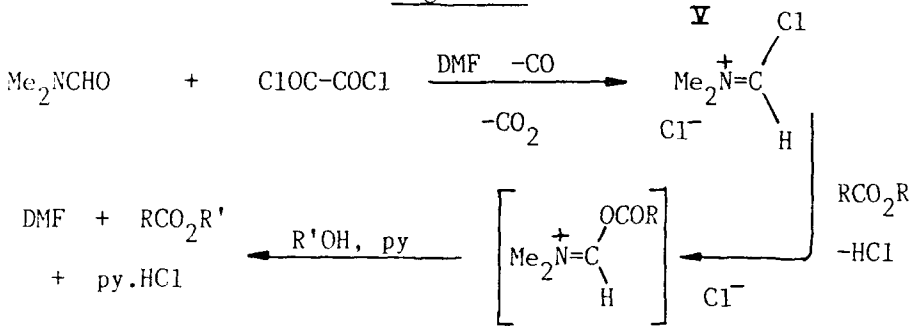
Figure 17



gave benzoyl trifluoroacetate upon distillation which drives the equilibrium of the above reaction to the right. Reaction of such mixed anhydrides with alcohols was found to give the esters in good yield. The reaction with the alcohol proceeds rapidly due to the trifluoromethyl group stabilising the trifluoroacetate anion as a leaving group. The reaction of trifluoroacetic anhydride with the 2'-deoxyacid, however, was not successful due to the complete lack of solubility of the acid in TFAA.

Another system that was investigated was the Vilsmeier reagent.[281,282] In this, DMF is reacted with thionyl chloride or oxalyl chloride to form the Vilsmeier reagent, dimethylchloroformininium chloride, V, Figure 18. This forms

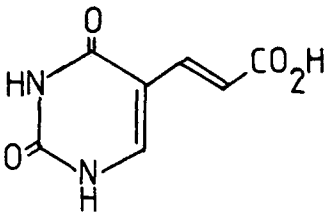
Figure 18



an activated complex with the acid which reacts rapidly with an alcohol in the presence of pyridine to from an ester, DMF and pyridinium hydrochloride.

The reaction of the Vilsmeier reagent with (E)-5-(2-carboxyvinyl)uridine and quenching with propan-1-ol gave only traces of the n-propyl ester. This was isolated by column chromatography and identified by its NMR spectrum.

The major problem with the acid chloride approach is the cleavage of the glycosidic bond between N^1 and the 1'-carbon of ribose. This was seen by isolation of the two expected possibilities upon removal of the ribose ring, 30 and 31. These reactions always went a very dark colour and this can be attributed to polymerisation reactions and the total decomposition of the ribose ring. Also formed as a by product in one reaction and present in just sufficient quantities



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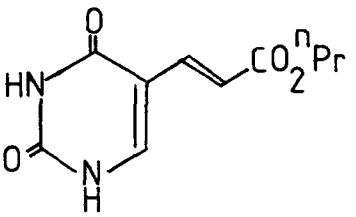


31

to be isolated was the 5'-chloro-5'-deoxy ester. As thionyl and oxalyl chlorides are common reagents for the conversion OH to Cl this is not really that surprising. In light of the poor results obtained, the attempts to make an acid chloride of an unblocked nucleoside were discontinued.

Instead, it was decided that a totally different approach to the required ribonucleoside esters would be tried. Previously, work at the nucleoside level had concentrated on making an acyl halide of the acid. The acid was itself obtained by alkaline hydrolysis of the methyl ester which was made by the palladium catalysed coupling of 5-iodouridine and methyl acrylate. It was therefore decided to make the required esters by coupling 5-iodouridine with the appropriate alkyl acrylate ester.

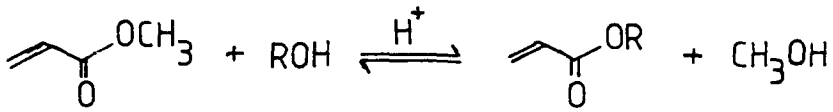
Of the acrylate esters required, only a few are commercially available and these were obtained from these sources.

The lower alkyl acrylates are liquids with strong odours, all the esters tend to polymerise unless kept refridgerated with traces of a polymerisation inhibitor present. There are many methods described in the literature for the synthesis of these compounds including the reaction of acryloyl chloride with the alcohol[283] and direct esterification methods.[284,285]

The most common method, however, is the acid- or base catalysed transesterification of methyl or ethyl acrylate. In a series of papers, Rehberg et al have described the synthesis of various classes of acrylic acid esters. These included esters of primary[286] and secondary[287] alcohols , substituted alcohols such as 2-bromoethyl and 2-cyanoethyl acrylates,[288] amino alcohols via basic

catalysis,[289,290] esters of ether alcohols,[291], cyclohexyl and substituted cyclohexyl acrylates[292] and olefinic esters from allyl, crotyl and methallyl alcohols.[293]

In the acid-catalysed transesterification the following equilibrium is obtained:



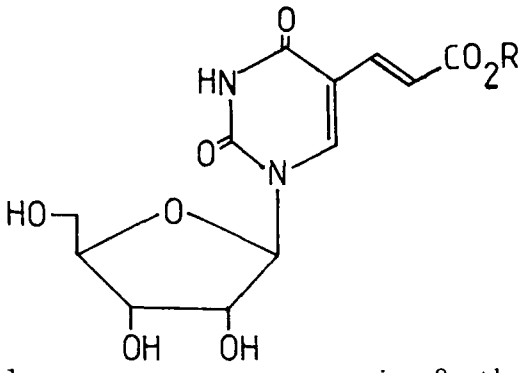
R= ^{n}Pr, ^{i}Pr, ^{n}Bu, $^{n}Pent$, ^{n}Oct, 2-methoxyethyl, 2-ethoxyethyl, 2-butoxyethyl, 2-cyanoethyl, 2-chloroethyl, 2-bromoethyl

The methanol is removed by distillation as its azeotrope with excess methyl acrylate and once production of the azeotrope had ceased, the excess methyl acrylate was removed by distillation. The acrylate ester was then distilled at reduced pressure, this to prevent polymerisation of the ester some of which are very high boili g. Hydroquinone was added as a polymerisation inhibitor.

The esters made were characterised by their NMR specta and, for the 2-monosubstituted ethyl esters, by their mass spectra also. The vinylic protons of the esters appear as a complex pattern at δ5·6-6·5 and the other protons appear in the region characteristic of the ester, the low resolution was not able to fully show the expected splitting pattern.

Of the nucleoside esters made by the palladium catalysed coupling of the acrylate esters to 5-iodouridine, only the ethyl ester is known.[241] The ester (E)-6-(2-carbomethoxyvinyl)uridine has been made from the Wittig reaction on 6-formyluridine, this was in turn obtained by oxidising 6-methyluridine with selenium dioxide.[294]

The yields of the 5-iodouridine/acrylate esters varied a great deal. Several of the esters had good yields although none was obtained in greater than 50% yield. Some of the reaction mixtures were easy to work up but many were difficult due to the low solubility of the nucleoside. As the length of the alkyl



32a-r

a. ethyl
b. n-propyl
c. iso-propyl
d. n-butyl
e. t-butyl
f. n-pentyl
g. n-octyl
h. benzyl
i. 2-methoxyethyl
j. 2-ethoxyethyl
k. 2-butoxyethyl
l. 2-hydroxyethyl
m. 4-hydroxybutyl
n. 2-cyanoethyl
o. 2-chloroethyl
p. 2-bromoethyl
q. 2,2,2-trifluoroethyl
r. tetrahydrofuryl

side chain of the nucleoside esters increased, the product became noticably less soluble in the usual solvents used for column chromatography of unblocked uracil nucleosides, solvents such as chloroform/methanol or ethanol and ethyl acetate/ethanol. Also the esters became more difficult to obtain crystalline as the length of the side chain increased, the n-pentyl ester, for example, crystallised from acetone but attempts to crystallise the n-octyl ester resulted in the precipitation of large amorphous masses of a 'soapy' appearance. It was also found that the removal of the final traces of colouration due to palladium complexes was best accomplished by recrystallisation.

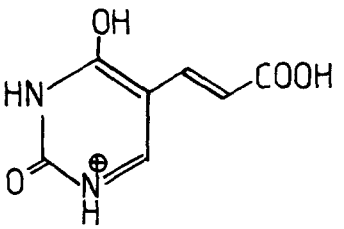
The 5-(carboalkoxyvinyl)uridine esters 32a-r were characterised by their UV, mass and NMR spectra and elemental analysis. In each case, the vinylic side chain was assigned as trans on account of the large coupling constants which

were obtained.

Some of the proton NMR spectra showed the characteristic splittings of the ester side chain. Thus, the spectrum of the iso-propyl ester, 32c, showed the OCH proton as a septuplet at δ4·95 while the t-butyl ester had a singlet at δ1·45 equivalent to 9 protons, the three methyl groups.

A common fragment in the FAB mass spectrum of the esters was protonated 5-(2-carboxyvinyl)uracil, 33, produced by cleavage of both the glycocidic and ester bonds.

33



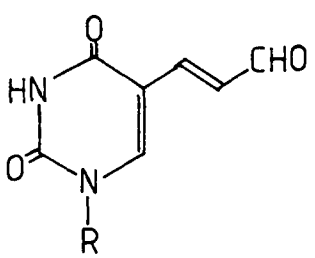
The coupling reactions of two other acrylate esters were attempted. 2-Nitroethyl acrylate is easily made by the acid catalysed transesterification of 2-nitroethanol.[295] In the Heck reaction with 5-iodouridine, however, the only nucleoside product as seen by TLC was uridine. Upon refluxing the reaction mixture a black tar was deposited and brown fumes of NO_2 were evolved. Similarly, commercially available glycidyl acrylate (2,3-epoxypropyl acrylate) also gave uridine and a black oil of the polymerised ester which is heat sensitive.

The synthesis of the unsaturated aldehyde 34 was then investigated using the Heck reaction. It is known that acrolein itself does not work very well in the Heck reaction as it polymerises rapidly, however, the methyl and ethyl acetals react normally. The reaction was therefore attempted with both these compounds but only uridine was formed, both acetals polymerised to a black tar under the reaction conditions.

It was also suggested that the Heck reaction should be attempted with an allene.[296] The allene 3-methylpenta-1,2-diene is easily made from 3-methyl-1-yn--3-ol by reaction with hydrochloric acid then rearrangement of the resulting

3-chloro-3-methylpent-1-yne to the allene using a zinc-copper couple.[297] The allene upon coupling would give 35 but a couple of seconds after adding the allene to the activated catalyst and 5-iodouridine a black oil was deposited. That the allene decomposed before it had time to react is shown by 5-iodouridine being the only nucleoside in the reaction mixture. Usually, the catalyst will deiodinate the 5-iodouridine to uridine if there is no alkene available to insert into the palladium complex, the lack of formation of uridine in this case can be attributed to decomposition of the complex by the allene.

R=ribose



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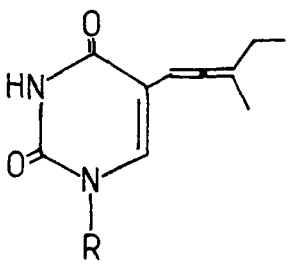

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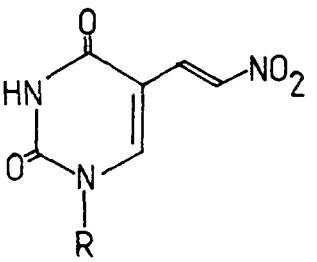
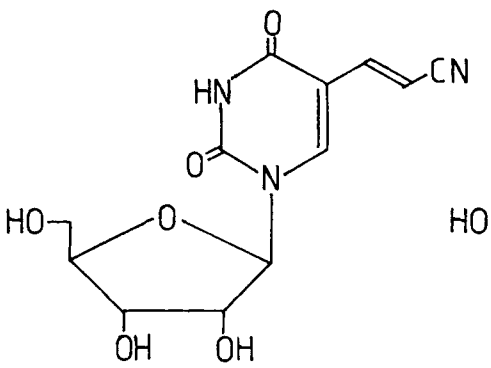


36

(E)-5-(2-nitrovinyl)uridine, 36, has not been reported in the literature and so the synthesis of this compound by the Heck reaction was attempted using the reaction of 5-iodouridine with nitroethylene. Nitroethylene is a pale green powerfully lachrymatory liquid which was prepared according to the method of Buckley and Scaife in which 2-nitroethanol is dehydrated with phthalic anhydride.[298] Upon addition of the nitroethylene to the activated catalyst at 70°C there was a violent reaction, the alkene polymerised instantly to give a black solid and nitrogen dioxide. The synthesis of the nitrovinyl nucleoside by this method was not pursued further.

It was decided to make (E)-5-(2-cyanovinyl)uridine, 37, and (E)-5-(2-m-nitrophenylvinyl)uridine, 38, using the Heck reaction. Although the former



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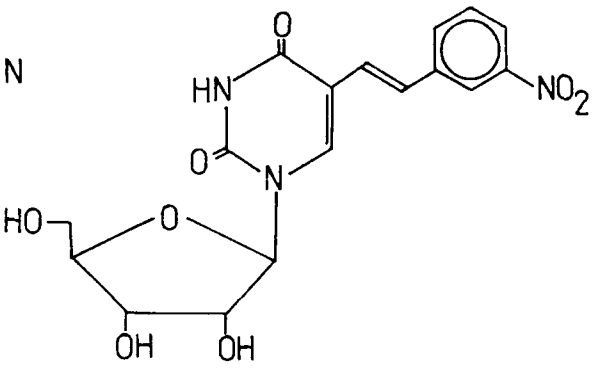


38

compound is known, it was prepared via a photochemical induced coupling between 5-iodouridine and acrylonitrile in 12% yield and no attempt was made to isolate the geometric isomers as the product was reduced to the cyanoethyl compound.[263] The ara[299] and 2'-deoxynucleosides[225] are also known.

Using the conditions of the Heck reaction it was found that acrylonitrile coupled readily to 5-iodouridine in 25% yield. The product was characterised by its NMR, UV and mass spectra and elemental analysis. The 2'-deoxynucleoside was prepared in 16% yield using the Li_2PdCl_4 method, the palladium/phosphine method is therefore of greater preparative value.

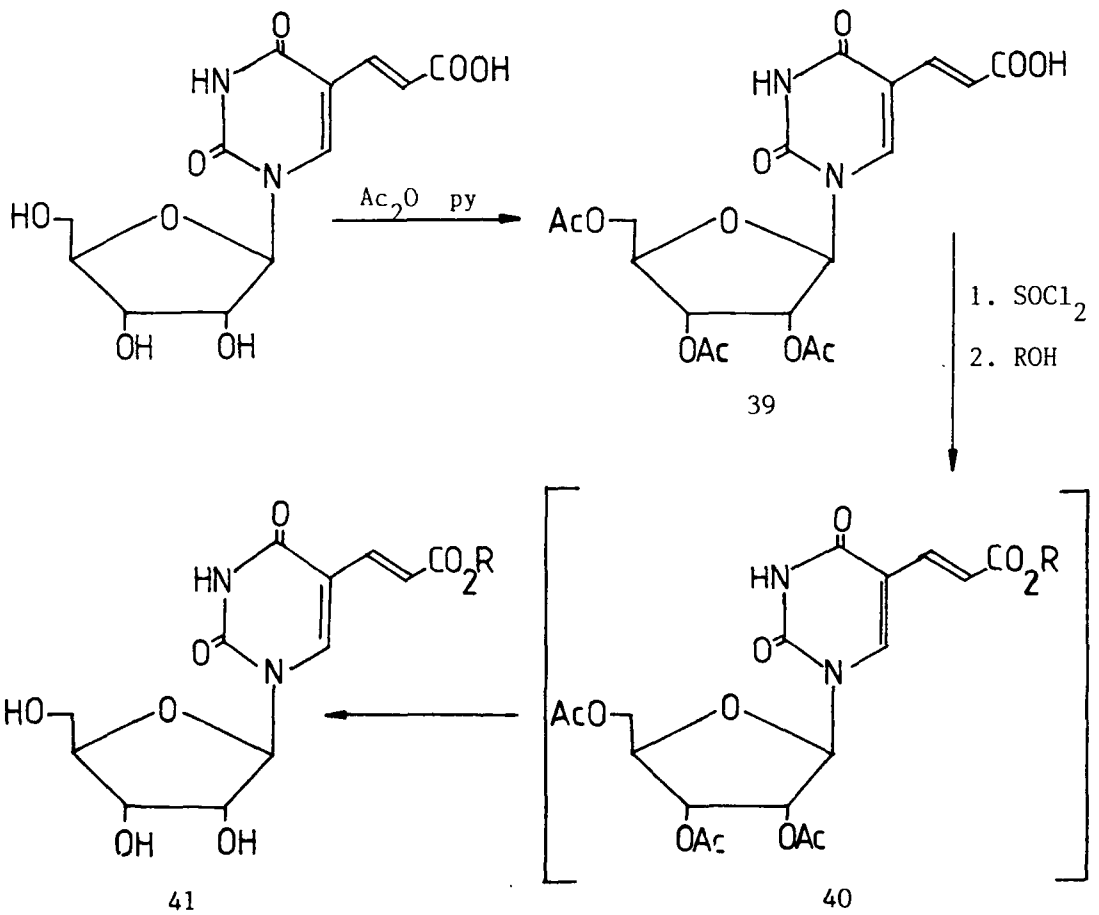
The palladium catalysed coupling of 3-nitrostyrene has been used previously to prepare the 2'-deoxy compound via 5-HgCldUrd and Li_2PdCl_4.[300] Although (E)-5-(2-m-nitrophenylvinyl)uridine has been reported, it was made by coupling the alkene with poly(5-acetoxymercuryuridylic) acid followed by hydrolysis to the nucleoside.[301] The reaction of 3-nitrostyrene and 5-iodouridine proceeded in 41% yield to give the product as fine yellow crystals. The compound was fully characterised in the usual way.

The palladium-catalysed acrylate coupling with 5-iodouridine was used to synthesize the required esters because the acid chloride of the unblocked acid (E)-5-(2-carboxyvinyl)uridine gave much decomposition. The yields of some of the esters by the palladium-catalysed reaction were not high and, although some

were not optimized, the yield is generally low and the product difficult to separate from residual coloured palladium compounds. It was therefore thought interesting to briefly investigate whether the blocking of the sugar could be made a useful synthetic alternative. The blocking group chosen was acetyl and therefore 2',3',5'-tri-O-acetyl-(E)-5-(2-carboxyvinyl)uridine, 39, was required, Figure 19. The acid was acetylated using an excess of acetic anhydride in pyridine.

Figure 19

a. ROH = $HOCH_2CH(CH_3)_2$ b. ROH = $HOCH_2CH_2CH(CH_3)_2$



The blocked acid was isolated by short silica-gel chromatography in 85% yield and was characterised by NMR, UV and mass spectroscopy and elemental analysis. The NMR spectrum showed the loss of the 3 hydroxyl peaks and the addition of the 3 acetate methyl groups. That the N-H was not acetylated was

shown by the presence of the N-H at δ11·75.

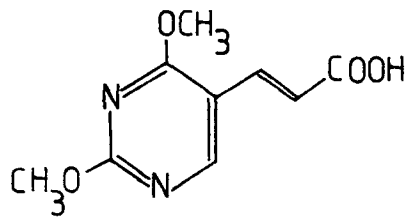
The acid chloride of 39 was made *in situ* without characterisation. The excess thionyl chloride used was removed under reduced pressure with rigorous exclusion of moisture then the alcohols isobutanol (2-methylpropan-1-ol) and isoamyl alcohol (3-methylpropan-1-ol) were added and the resulting solution heated under reflux until HCl was no longer evolved. Excess alcohol was removed under high vacuum and the product isolated by short column chromatography. It was expected that the products would be the tri-*O*-acetyl esters and that the sugar would need to be deblocked. Upon analysis of the NMR spectra, however, it was seen that there were no acetyl groups in either molecule although the expected ester of the side chain had indeed been formed. The FAB mass spectra also showed the loss of the acetyl groups, therefore compounds 40a,b were only formed as intermediates and were deblocked by the HCl produced in the reaction of the acid chloride with the alcohol. In retrospect, this was not really surprising and means that one step in the synthesis is eliminated. The yields of the esters 41a and 41b were 71% and 58% respectively and this is significantly higher than the palladium reaction, especially of the higher alkyl esters such as 41a,b. Both new esters were also characterised by elemental analysis.

A change of direction was then made with the reactions of 5-substituted-2,4-dimethoxypyrimidines explored to see whether 5-substituted vinylpyrimidines could have synthetic use. Previously, 5-vinyl-2,4-dimethoxypyrimidines have been studied with the preparation of polyhalogen compounds, especially those of fluorine.[302,303]

The initial work was centered around the chemistry of the vinyl acid (*E*)-5-(2-carboxyvinyl)-2,4-dimethoxypyrimidine, 42. The presence of the methoxy blocking groups tends to make these compounds readily soluble in commom organic solvents whereas the deblocked pyrimidines, ie uracils, are difficultly soluble.

The proposed route to 42 is analogous to that for the synthesis of the uracil acid, namely the reaction between the 5-formyl compound and the anion of malonic

acid.[241] The aldehyde 5-formyl-2,4-dimethoxypyrimidine, 43, was therefore required.



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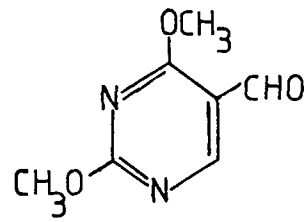
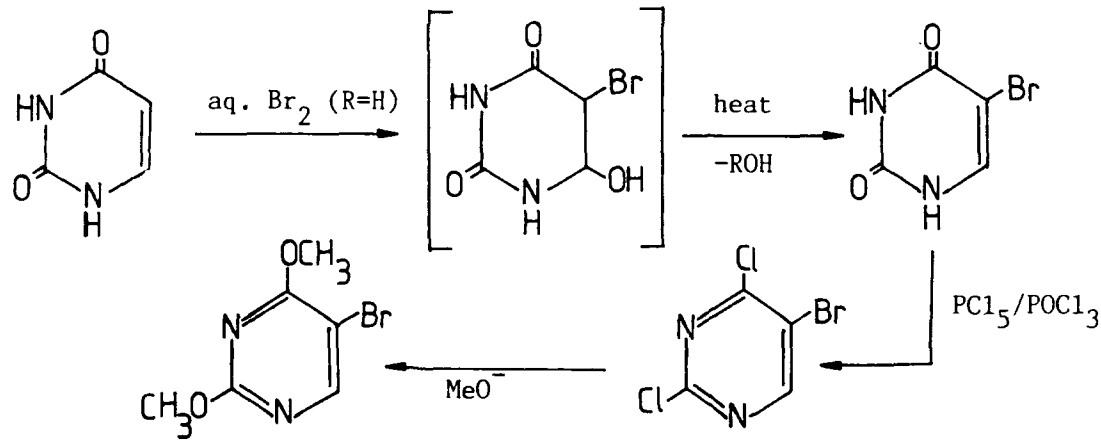


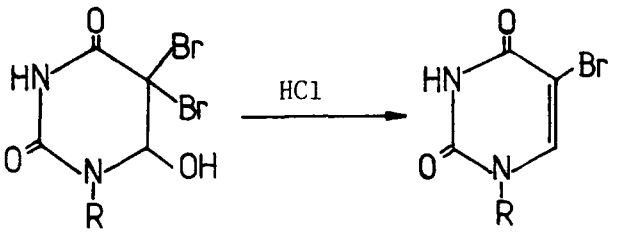
43

5-Formyl-2,4-dimethoxypyrimidine was synthesized from 5-bromo-2,4-dimethoxypyrimidine. A sample of the latter was used which had been made in a 3 step synthesis from uracil, Figure 20. Uracil is converted to the 5-bromo

Figure 20



compound by the method of Wang[141] in which uracil is treated with aqueous bromine to give the adduct 5-bromo-6-hydroxyuracil (R=H)[146,304] A similar compound is formed with 1,3-dimethyluracil. In ethanolic solution the adduct has R=Et.[305] The hydroxy compound loses water on standing or heating, the dehydration step is spontaneous and quantitative. Two equivalents of bromine could form 5,6-dibromo-6-hydroxyhydrouracil, 44 R=H. Initial reports of this



44 R = H,ribose, 2-deoxyribose

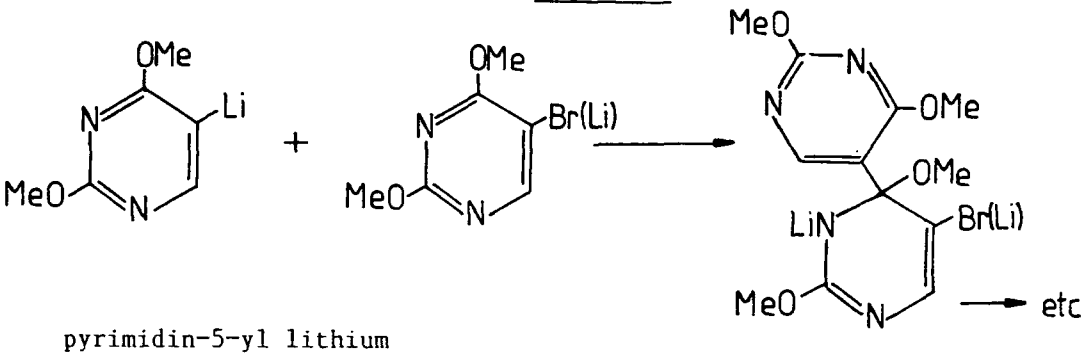
adduct thought it to be unstable but to transform it to 5-bromouracil requires refluxing in dilute HCl.[306]

Using a method developed by Mulvey et al[307] 5-bromo-2,4-dichloropyrimidine is then obtained by the action of phosphorus pentachloride and phosphorus oxychloride on 5-bromouracil. It was known that 2,4-dichloropyrimidines[308] and 2,4-dichloro-5-methylpyrimidines[309] could be converted to the 2,4-dialkoxy compounds by nucleophilic displacement of chloride ion with sodium alkoxides. This method applied to the 5-bromo compound gives good yields of 5-bromo-2,4-dimethoxypyrimidine.[310] These dialkoxy compounds are important intermediates in the Hilbert-Johnson procedure for the synthesis of pyrimidine nucleosides.[311]

5-Formyl-2,4-dimethoxypyrimidine, 43, has been made from the bromo compound by a halogen-metal exchange reaction using n-butyl lithium. The use of organolithium compounds is complicated by their reaction with azomethine bonds, -C=N-. These reactions are reduced at low temperatures[312] as are polymerisation reactions of lithiated pyrimidine derivatives,[313] however, 2,4-dimethoxypyrimidin-5-yl lithium still undergoes some self-condensation at -70°C.[314] In pyridines at room temperature, RLi adds rapidly to the azomethine linkage. This is much reduced at low temperature. In pyrimidines, the two azomethine linkages are meta and the adjacent carbons are particularly susceptable to attack. The principle side reaction is polymerisation and such species as 45, Figure 21, are formed.

The halogen-metal exchange is reversible even at low temperatures with the equilibrium dependent upon the relative electronegativities of the groups concerned, the lithium becomes attached to the more electronegative group. The

Figure 21



pyrimidin-5-yl lithium

45

reactions of pyrimidin-5-yl lithium compounds have not been investigated in detail but have been made and used for the preparation of carboxylic acids, by carbonation,[313,315] 5-arylhydroxymethyl-2,4-dimethoxypyrimidines[316] and aldehydes and polysulphides.[315]

5-Formyl-2,4-dimethoxypyrimidine was synthesized by a method similar to that of Strogryn[317] in which the yellow suspension of 2,4-dimethoxypyrimidin-5-yl lithium was reacted with a five molar excess of ethyl formate[302] instead of with DMF, at -70°C under positive pressure of dry nitrogen. N-Formylpiperidine as a formylating agent has also been used in pyrimidine chemistry.[318] Instead of n-BuLi, lithium tetramethylpiperide (LTMP) in conjunction with DMF can be used.[319]

Quenching of the reaction mixture with water, extraction into ether then purification by column chromatography on silica gel and recrystallisation from hexane gave the product in 58% yield. The proton NMR now shows the resonance characteristic of -CHO at 10·05, the mass spectrum gave a molecular ion at m/e of $(M+H)^+$. Elemental analysis confirmed the structure. It was also found that the reaction gives varying amounts of 2,4-dimethoxypyrimidine which is a pale coloured viscous oil with a peculiar odour. No matter how dry the reagents are some of this by-product is always formed by protonation of the lithium compound by traces of water or methanol in the ethyl formate. The formation of the by-product is kept to a minimum by refluxing over and then fractionating the ethyl

formate from calcium hydride.

An alternative to the n-butyl lithium method would be to go via the Grignard reagent. Attempts to make the Grignard reagent of 5-bromo-2,4-dimethoxy pyrimidine failed under normal conditions and also when 1,2-dibromoethane was used as an entrainer.[320,321]

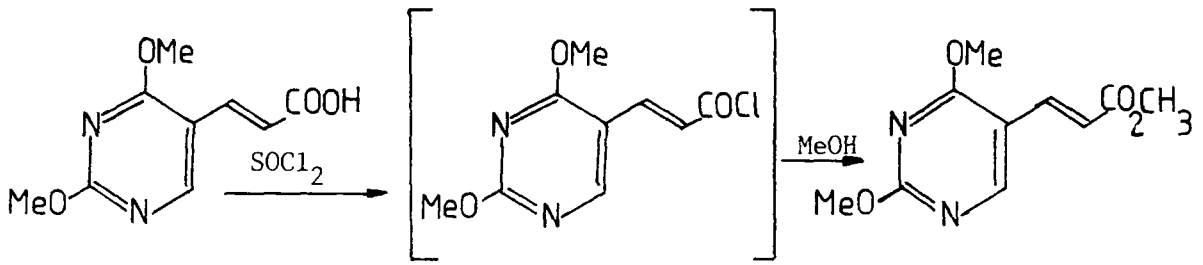
The preparation of (E)-5-(2-carboxyvinyl)-2,4-dimethoxypyrimidine involves the reaction of the aldehyde with malonic acid in pyridine at 100°C in the presence of piperidine. This reaction is the Verley-Doebner modification of the Knoevenagel reaction. The Knoevenagel reaction is a reaction of great utility in the production of carbon-carbon double bonds from aldehydes or ketones and a compound possessing an active methylene group, RCH_2R'. R,R' can be COOH, CN, CO_2R and which are usually contained in malonic acid derivatives such as malonic esters, malonamides, malononitrile, cyanoacetic acid and its esters and cyanoacetamides.[232] In most cases, two of the groups are required and the product is an unsaturated compound although in some cases these react further in a Michael type addition.

Very few reactions of ketones and malonic acid are known, aldehydes usually supply the carbonyl group. The preparation of acrylic or cinnamic type compounds is the most usual reaction and is achieved by heating the aldehyde and malonic acid on a steam bath until no more CO_2 is evolved. These conditions applied to 5-formyl-2,4-dimethoxypyrimidine gave rapid evolution of CO_2 when a 100% excess of malonic acid was used.

After evolution of CO_2 had stopped, the excess malonic acid was removed by recrystallisation of the reaction mixture from water then methanol. The product is much more soluble in water than the uracil acid. The product crystallised readily from methanol as fine white needles in 57% yield. The NMR spectrum showed the disappearance of the -CHO peak at δ10·05 and the appearance of two vinylic protons as doublets at δ7·50 and δ6·55. The trans-configuration was assigned on the basis of the coupling constant of 16Hz. The mass spectrum and

elemental analysis confirmed the structure.

Firstly, the acid chloride of the acid was made by the action of freshly redistilled thionyl chloride on the acid. The acid dissolved instantly the thionyl chloride was added and the acid chloride, 46, was obtained as a yellow coloured solid upon removal of excess $SOCl_2$.



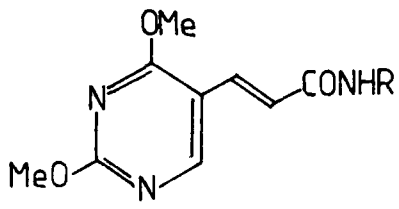
46

The acid chloride is highly reactive and readily hydrolysed and no attempt was made to isolate it in a pure form after it decomposed upon distillation in a Kugelruhr apparatus, charring began at 200°C at 0·3mmHg. The mass spectrum of the acid chloride showed a molecular ion at m/e 229/331 which corresponded to the isotopic abundence ratio for chlorine for $(M+H)^+$ for this compound.

The crude acid chloride was used immediately in further reactions, however, exposure to the acidic media for too long results in demethylation of the pyrimidine ring and subsequent reduced yields.

The methyl ester was obtained in 42% yield by the addition of dry methanol to the crude acid chloride. The ester was recrystallised from hexane. The 6-methyl analogue has been made by the coupling of methyl acrylate to 5-iodo-2,4-dimethoxy-6-methylpyrimidine.[333]

The amide 47 and the N-methylamide were obtained from the acid chloride by reaction of the appropriate amine in aqueous solution. The low yields suggests a better route would be to use the dry amine dissolved in solvent. Both compounds are analogoues to the nucleosides discussed later. Both 47 and 48 were obtained

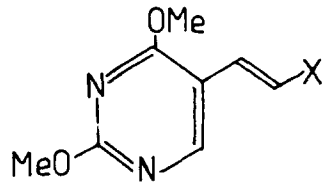


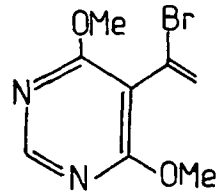
47 R=H (31%)

48 R=CH_3 (47%)

as highly crystalline solids upon recrystallisation from water. The $-NH_2$ protons of the amide show on the NMR as two peaks at δ7·55 and δ7·08, such splitting is characteristic. The amides were further fully characterised by UV and mass spectroscopy and analysis.

Of the (E)-5-(2-halovinyl)-2,4-dimethoxypyrimidines, only the fluorovinyl compound, 49, has been reported although a similar compound, 5-(α-bromovinyl)-4,6-dimethoxypyrimidine, 51, is known from a study of 5-vinylpyrimidines.[334]





49 X=F[318]

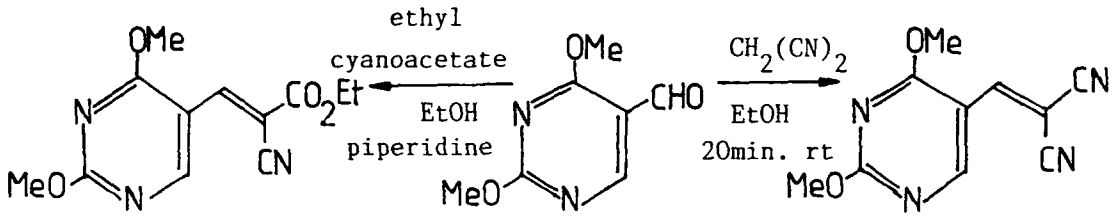
50 X=Br

51

The synthesis of 51 was achieved by the selective bromination of 5-ethyl-4,6-dimethoxypyrimidine to the 1,1-dibromoethyl compound by NBS and then elimination of HBr with KO^tBu.

NBS was also used for the synthesis of 50 by the decarboxylative bromination of the acid in an analogous manner to that for the synthesis of BVDUrd and BVUrd by reaction of the potassium salt of the acid in dry DMF. Analysis of the reaction mixture showed a new component which was isolated by column chromatography and recrystallisation in 45% yield. The NMR spectrum showed 2 vinylic doublets with J=14Hz which shows the double bond to be trans. The FAB mass spectrum had molecular ion peaks at 245/249, $(M+H)^+$

As previously outlined, the Knoevenagel reaction can be done with other active methylene compounds apart from malonic acid. The reaction of 5-formyl-2,4-dimethoxypyrimidine with malononitrile and ethyl cyanoacetate was investigated.



52 53

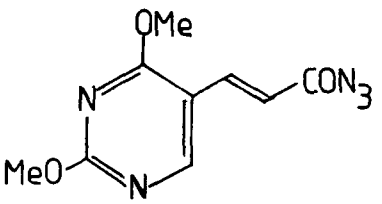
The reaction between the aldehyde and the cyanoacetic ester when catalysed by piperidine was rapid and gave the α,β-unsaturated cyanoacetate 52 in 84% yield as a pale yellow crystalline solid which gave an NMR with the expected resonances and the expected splitting for the ethyl ester group. The reaction of the aldehyde with malononitrile to give 53 was extremely rapid and this is not surprising as malononitrile is one of the most reactive methylene compounds used in the Knoevenagel reaction. The 2,2-dicyanovinyl pyrimidine was isolated in 89% yield as a bright yellow crystalline solid, the NMR of which shows the resonances of only 8 protons attributable to the H-6, the vinylic and the two methoxy groups.

It is known that the condensation of dialkoxypyrimidines with a strongly electron withdrawing substituent in the 5-position do not condense well with blocked halosugars in the Hilbert-Johnson reaction. Indeed, the 5-nitrocompound is so deactivated that it does not react at all. Prystas and Sorm showed that the more electronegative the 5-substituent the lower the yield of the condensation product.[160,335]

The 2,2-dicyanovinylgroup would be expected therefore to prevent reaction between 53 and a blocked halosugar. This was indeed found to be so with only an

intractable mixture of slow running nucleosides formed. A modification of this reaction using acetonitrile as solvent was also unsuccessful.[336]

The acyl azide (E)-5-(2-azidocarbonylvinyl)-2,4-dimethoxypyrimidine, 54,



54

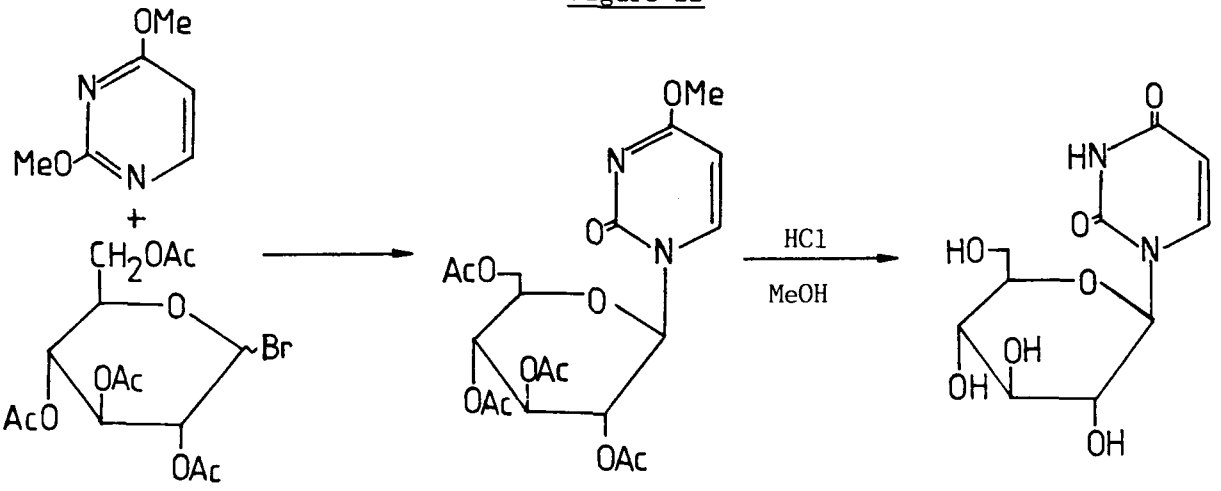
has not been reported in the literature and its synthesis was undertaken. The most common synthesis of acyl azides is the nucleophilic displacement by azide ion of halogen from an acyl halide.[337] This is accomplished by slowly adding a concentrated solution of the acyl halide in an inert water miscible solvent to a 50% aqueous acetone solution of sodium azide. The required acid chloride was prepared *in situ* as previously described then dissolved in dry THF and dropped slowly into the azide solution. Upon removal of the solvent the reaction mixture showed traces of starting material and a new, major component. This was isolated by column chromatography and recrystallised to give 54 as long fine white needles in 72% yield after recrystallisation from acetone. The NMR spectrum of the product showed no carboxyl proton at δ12·0 while the mass spectrum had a small peak at 235 corresponding to the molecular ion. The azide molecule readily loses nitrogen and the peak at 307 ($M-N_2$) is therefore the most intense, $M-N_2$ is very characteristic of acyl azides where the molecular ion is not often seen.

The degree of conjugation in this molecule is shown by the shift to higher wavelength in the UV spectrum where λmax is shifted to 311·5nm compared to the normal figure of the vinyl nucleoside of approximately 300nm.

Hilbert and Johnson undertook a comprehensive study of the reactions of

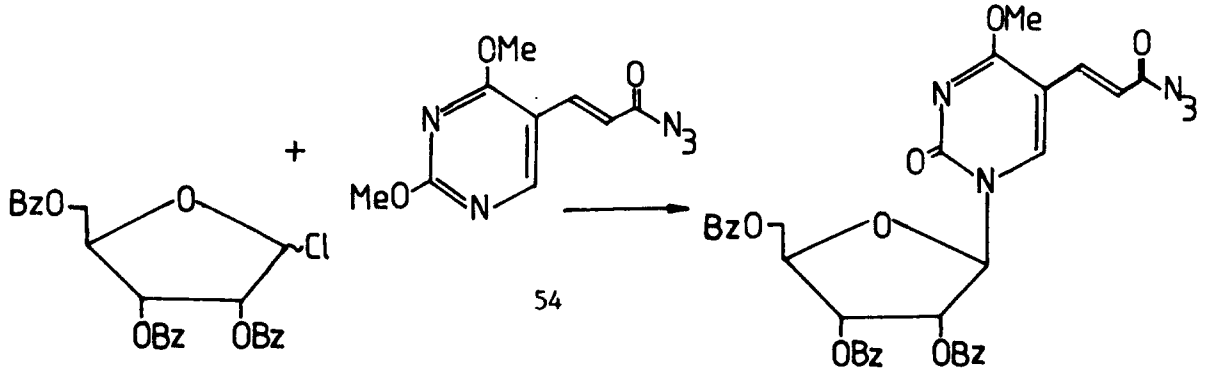
2,4-dialkoxy- and 2-thio- or 2-alkylmercaptopyrimidines towards alkyl halides [338] but obtained attack at N-1, N-3 or both. Other work then found that it was possible to alkylate selectively at N-1 and so condensed the dialkoxypyrimidines with an acetobromoglucose to give 2-oxo-4-methoxy-3-tetraacetylglucoso-pyrimidines which were deblocked with methanolic HCl, Figure 22.

Figure 22



It is also possible to dealkylate the dialkoxypyrimidines to the corresponding uracils by sodium iodide in AcOH[339] and EtOH/HCl.[338]

The Hilbert-Johnson reaction of (E)-5-(2-azidocarbonylvinyl)-2,4-dimethoxy-pyrimidine was done with the chlorosugar prepared *in situ* from 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose and stannic chloride. The condensation product, 55, was obtained in 52% yield and was characterised fully.



54

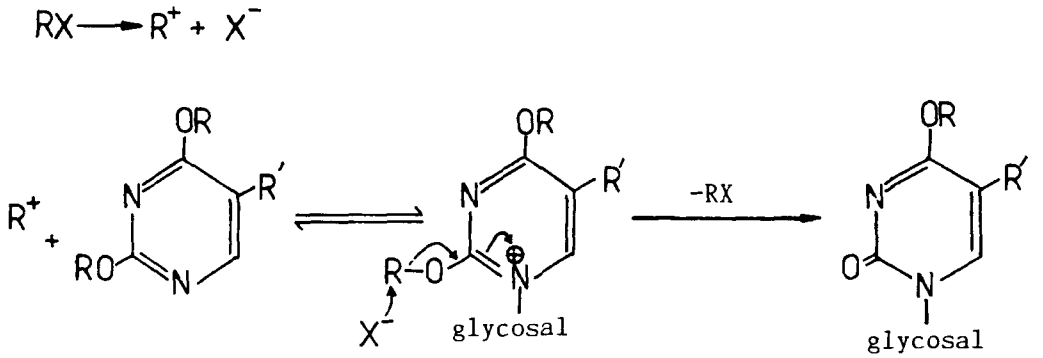
55

Bz=benzoyl

The Hilbert-Johnson reaction is now widely used although the recently introduced silyl-modifications of the reaction could be used. These involve condensation of the silylated base with the halogenose in the presence of perchlorate,[342] mercuric bromide[343] or by the action of heat.[344]

The mechanism of the Hilbert-Johnson reaction is shown in Figure 23. The

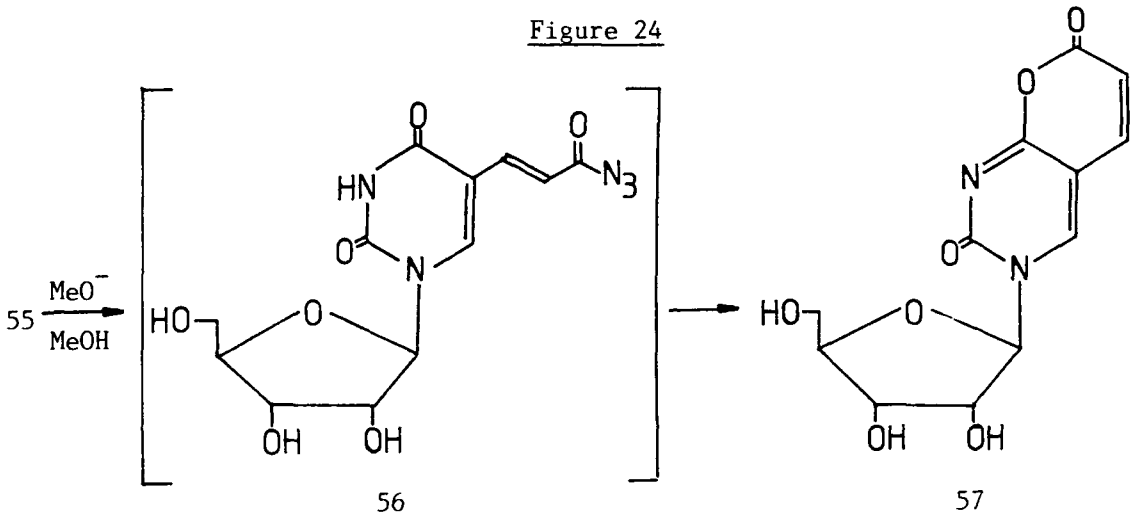
Figure 23



presence of a strongly electron withdrawing group R' at C-5 of the pyrimidine would lower the nucleophilicity of N-1 in the attack on the anomeric centre of the halosugar thus preventing production of the intermediate quaternary salt. This would therefore explain the known lack of reactivity of 5-nitro-2,4-dimethoxypyrimidine and the found lack of reactivity of the 5,5-dicyanovinyl compound.

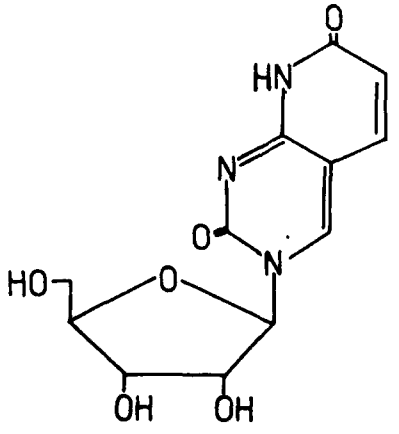
The standard method of deblocking such 4-methoxypyrimidinones as 55 to the uracil nucleoside involves treatment with methoxide in methanol. The expected product, (E)-5-(2-azidocarbonylvinyl)uridine, 56, was not formed, Figure 24. Instead a product was obtained whose NMR spectrum showed the presence of an H-6 proton as a singlet at δ8·94 and two more vinylic resonances at δ7·41 and δ6·43 which look most unlike the usual side-chain vinylic resonances and more closely resemble the two vinylic protons of unsubstituted uridines. The mass spectrum has m/e at 343 and 165. This data can be assigned by an intramolecular cyclisation reaction with elimination of azide ion to give 3-β-D-ribofuranosyl-

Figure 24



2,7-dioxo-5,6-dihydro-2*H*-pyrano[2,3-d]pyrimidine, 57, a previously unknown pyrano-pyrimidine. The m/e 343 is thus $(M+H+2Na)^+$ while m/e 165 can be assigned to $(M-ribose+2H)^+$. Elemental analysis has not been obtained correct although the data obtained clearly showed the loss of the azide group.

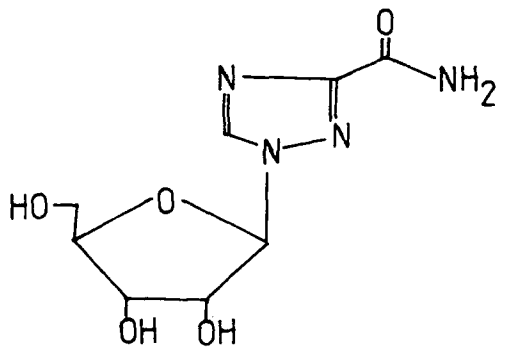
2,4-Dimethoxypyrimidine condensation products are deblocked to the cytosine nucleoside by methanol/ammonia. Treatment of the blocked azide with a saturated solution of dry ammonia gas in methanol at room temperature for 2 days gave, on TLC, 2 nucleoside products. The faster running component possessed a blue fluorescence when irradiated under UV light at 254nm. This compound was characterised as the known compound 3-β-D-ribofuranosyl-2,7-dioxopyrido[2,3-d] pyrimidine, 58. The two olefinic protons are retained but the coupling constant J=11Hz shows a cis relationship. The second nucleoside could not be characterised



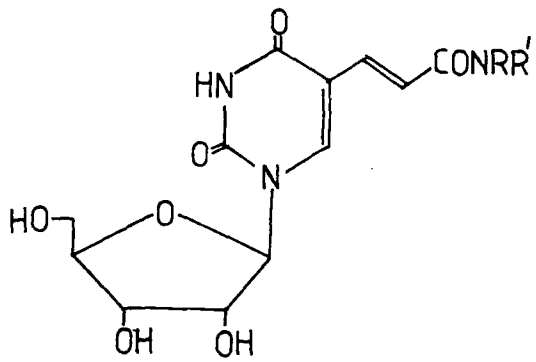
58

as it decomposes to 58 upon standing, this decomposition was rapid upon heating. The total yield of the fluorescent nucleoside was 70% and this is comparable to the yield obtained by Bergstrom and coworkers[242] who made this compound when attempting a photochemical trans to cis isomerism of (E)-5-(2-carbomethoxyvinyl) cytidine. Compound 58 has been used as a fluorescent probe in polynucleotide studies.[345,346]

Ribavirin is a nucleoside analogue with an amide side chain. This nucleoside, as previously discussed, has useful antiviral properties. As no 5-vinylamide nucleosides are known their synthesis was undertaken, the three target molecules were the amide, N-methylamide and N,N-dimethylamide of the acid (E)-5-(2-carboxy-vinyl)uridine, 59-61 respectively. The unsubstituted amide 59 was obtained in



ribavirin



59 R=R'=H

60 R=H R'=CH_3

61 R=R'=CH_3

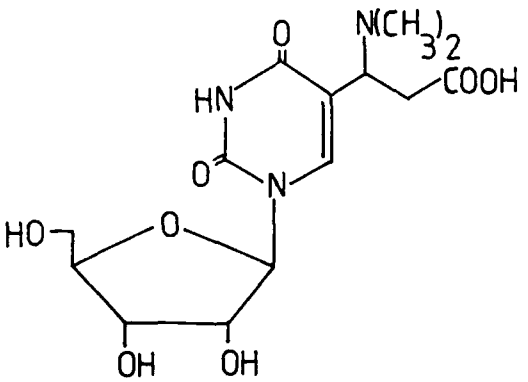
58% yield by treating the methyl ester with a large excess of concentrated aqueous ammonia at room temperature for 24 hours. It was isolated by crystallisation from water from which it separated upon cooling as fine needles. The NMR spectrum showed the $-NH_2$ as two singlets which is characteristic of amides. The analysis shows the molecule to contain water.

The N-methylamide 60 was obtained in 41% yield as fine white crystals with

1 mole of water of crystallisation. The NMR spectrum shows the presence of the amide NH at δ7·96 as a badly resolved quartet (D_2O exchangeable) while the NCH_3 is at δ2·63 as a doublet which collapses to a singlet upon addition of D_2O.

The reaction between the ester and 25/30% aqueous dimethylamine, when analysed after 48 hours by TLC, showed the presence of two new nucleoside products of rf 0·45 and 0 (baseline). The two nucleosides were separated by crystallisation and column chromatography. The nucleoside with rf 0·45 had an NMR which showed it to have one $N(CH_3)$ group and the vinylic double bond while the mass spectrum had the molecular ion consistant with the amide, 61. This was confirmed by analysis. The yield of the required product, however, was only 7%.

The NMR of the baseline compound showed the loss of the vinylic side chain and the presence of 2 new methyl groups which is consistant with the incorporation of one dimethylamino group. The mass spectrum had a molecular ion at 359 which is data consistant with structure 62. It would be expected that the CH_2COOH protons

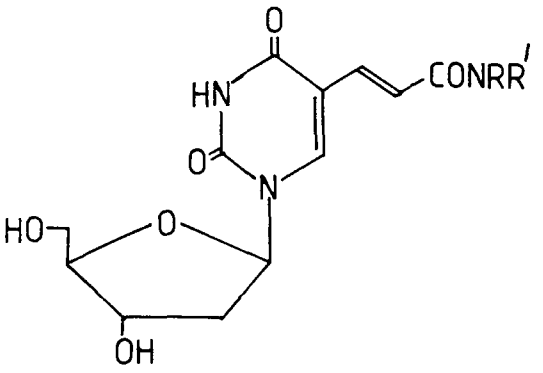


62

in the NMR spectrum would appear at δ2·34, however, these 2 protons are next to a chiral centre and thus are diasteriotopic and therefore non-equivalent. They appear as multuplets at δ2·76 and δ2·36. The CH proton is shifted downfield by the pyrimidine ring. The UV spectrum has a maximum at 269·5nm and not at the expected figure of around 300nm for the unsaturated side chain. The structure was confirmed by elemental analysis. The yield based on this structure is 16%.

Although low, the yield of neither compound was optimized. The low yield of the required product, the dimethlamino amide, showed that the ammonolysis of the ester does occur to some extent but that a Michael addition to the vinylic side-chain followed by an hydrolysis reaction to the acid is prefered.

As large quantities of the ester (E)-5-(2-carbomethoxyvinyl)-2'-deoxy-uridine were available from the commercial preparation of BVDUrd the two amides 63 and 64 were obtained by the action of the amine on the ester in an analogous manner to that used for the ribonucleoside amides. Both compounds were fully



63 R=R'=H

64 R=R'=CH_3

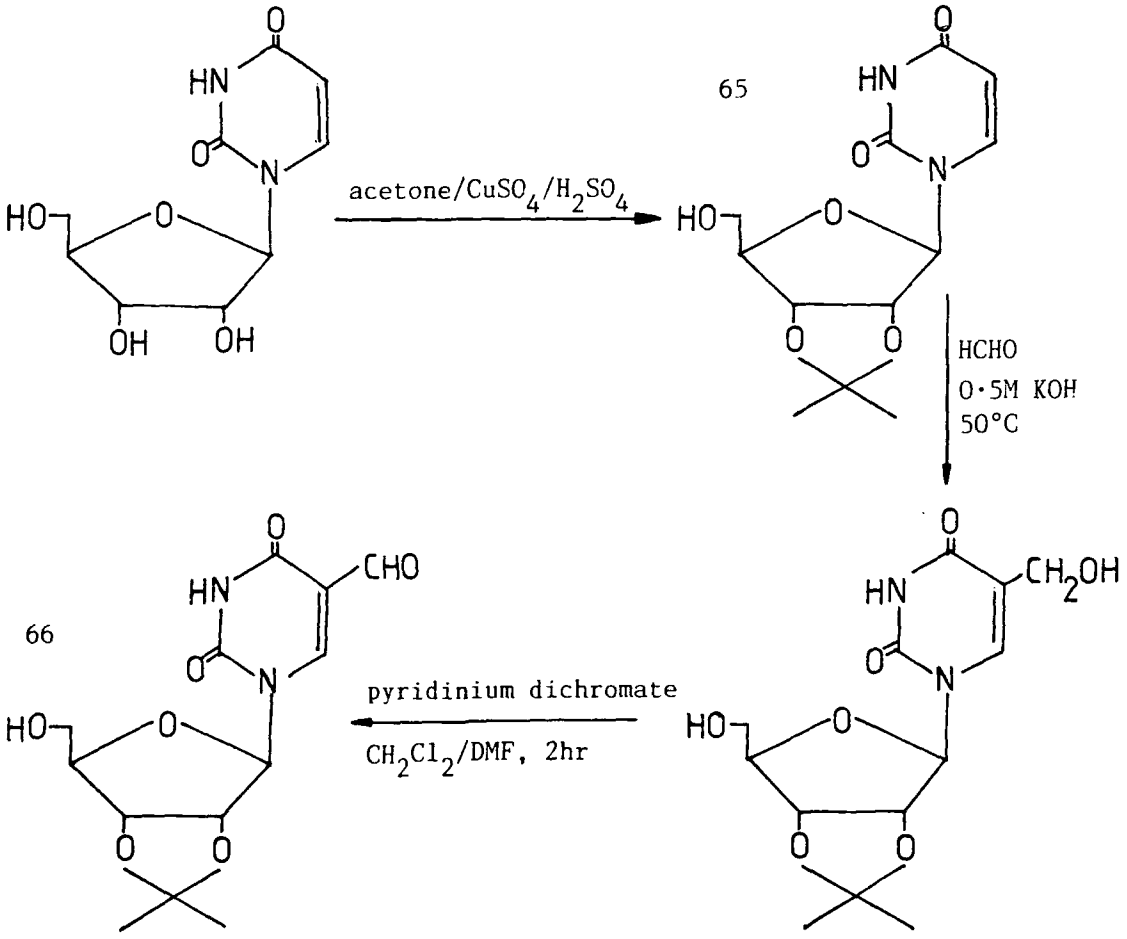
characterised. The amide 63 was obtained in 79% yield and the dimethylamide 64 in 9% yield, the by-product from the latter was not isolated.

The reactions of the aldehyde 5-formyl-2,4-dimethoxypyrimidine with activated methylene compounds in the Knoevenagel reaction to give substituted 5-vinyl-2,4-dimethoxypyrimidines was shown to proceed in good yield. The problems associated with the condensation of the 2,2-dicyanovinyl compound may be expected from other vinyl pyrimidines and thus this route to substituted 5-vinyl-pyrimidine nucleosides would be expected to be of little preparative value especially of the more reactive side chains. The preparation of such compounds at the nucleoside level therefore requires investigation.

The route to the required aldehyde, 5-formyl-2',3'-isopropylideneuridine is a 3 step reaction from uridine, Figure 25. The aldehyde was used in its iso-

propylidene form as this is required in order for the reaction to the hydroxymethyl compound to proceed. Also it confers greater solubility and hence ease of separation in later stages.

Figure 25



The first step involves the isopropylidenation of uridine. There are many methods for the synthesis of 2',3'-isopropylideneuridine including the use of 2,2-dimethoxypropane and acid catalysis,[348,349] ethyl orthoformate in DMF[350] and acetone with ferric chloride as catalyst.[351] The method used, acetone, anhydrous copper (II) sulphate and sulphuric acid[352] proceeded in quantitative yield. The NMR spectrum showed the loss of the cis diol system and replacement with the two

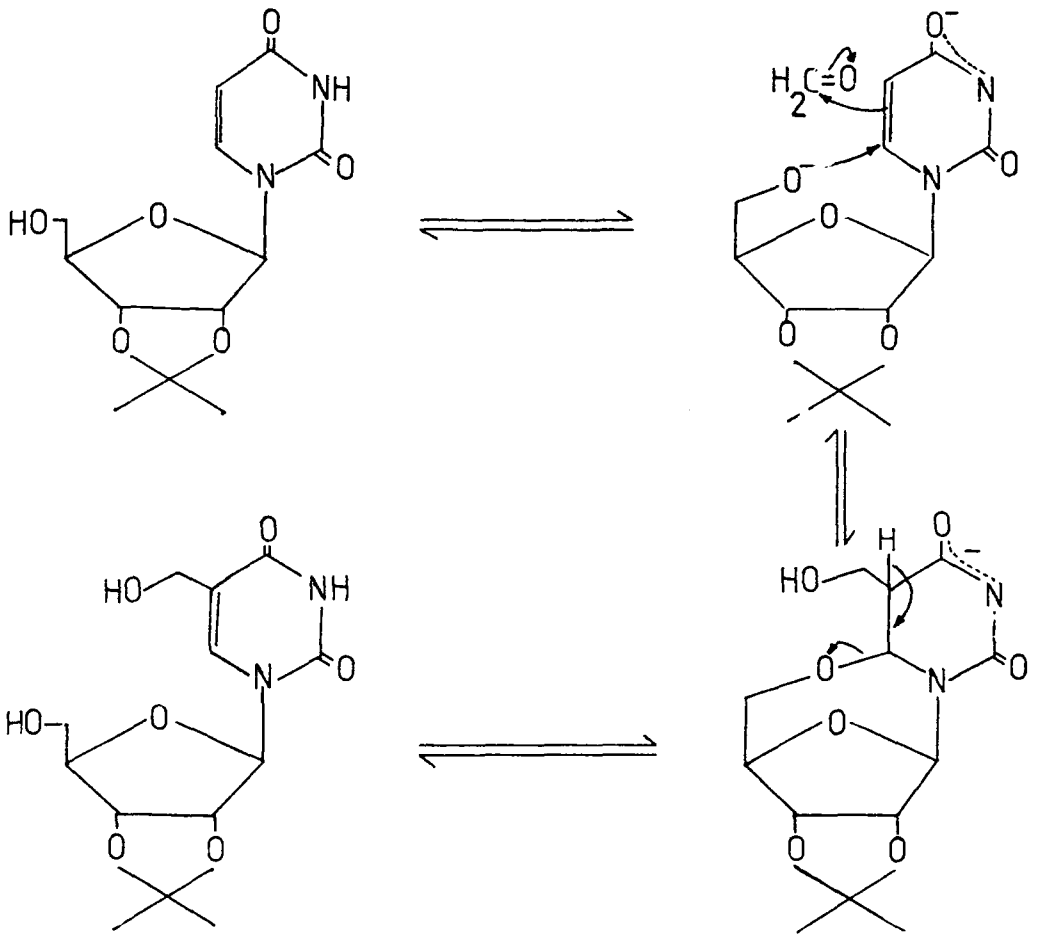
methyl resonances at δ1·46 and δ1·28.

The hydroxymethylation of uridine under acid catalysis gives a 20% yield,[83] however, the reaction of 2',3'-isopropylideneuridine and paraformaldehyde with base catalysis has been reported to proceed in 85% yield.[84] The latter method of Scheit was adopted, IPUrd, 2 equivalents of paraformaldehyde in dilute aqueous potassium hydroxide were treated at 50°C for 2 days. Neutralisation of the solution with glacial acetic acid and removal of solvent gave a thick viscous oil which upon short column chromatography to remove excess formaldehyde gave the product in 86% yield. The H-6 proton now appears as a singlet at δ7·65 and the 2 hydroxy groups are on top of the H-2' and H-3' protons at δ5·15-4·65, D_2O causes this region to be reduced to 2 protons. The incorporation of the 5-CH_2 is shown at δ4·10 along with the H-4'. The FAB Mass spectrum confirms the incorporation of the 5-hydroxymethyl group.

The reaction with formaldehyde solution has been reported to proceed in 72% yield.[353] When this method was followed the product was obtained in the same yield as that published. Facile reaction of uridine and paraformaldehyde only occurs under basic catalysis when the vicinal 2'- and 3'-hydroxyls are blocked as acetals or ketals and when 5'-OH is unblocked. The mechanism of the reaction[86], Figure 26, involves the alteration of the conformation of the sugar by the isopropylidene group such that the 5'-OH can attack the C-6 position allowing reaction with $H_2C=O$ in a Michael addition type reaction.

The oxidation of 5-hydroxymethyl-2',3'-isopropylideneuridine has been reported in 37% yield using manganese dioxide.[353] The reaction is done in dry dichloromethane but attempts to repeat this failed, probably due to poorly activated manganese dioxide. Since the advent of manganese dioxide many new oxidising agents have been introduced, many of which are soluble in the reaction medium and therefore a homogenous reaction system is possible. The recently made available 4-methylmorpholine N-oxide/tetrapropylammonium perruthenate system in dichloromethane[354] was tried but gave no reaction.

Figure 26



Pyridinium dichromate (PDC)[355] is a recently developed oxidising agent for the oxidation of primary and secondary alcohols to carbonyl compounds, it is especially useful for acid sensitive molecules for which pyridinium chlorochromate is not suitable.[356] The usual method is to suspend the PDC in dichloromethane and add the alcohol in dichloromethane in one go and stir the suspension vigorously until reaction is complete. The reaction with the nucleoside was monitored by TLC and after 2 days no further increase was seen in the proportion of the new faster running moving nucleoside. This product was separated from starting material by short column chromatography and recrystallisation from ethanol and was obtained in 20% yield. The NMR spectrum

of the product showed 1 proton at δ9·73 which is the CHO proton. That the 5-CH_2OH has been oxidised and not the 4'-CH_2OH is shown by the product possessing the H-5' protons at δ3·57 while the resonance due to the 5-CH_2OH methylene protons has disappeared. The mass spectra shows the loss of 2 protons and the UV spectrum has λmaximum shifted from 264·0nm (starting material) to 288·0nm due to the increased conjugation of the carbonyl group with the 5,6-double bond. The product gave a correct elemental analysis.

The yield of the aldehyde was surprisingly low concidering the usual high efficiency of PDC. As this was thought to be due to non-homogeneity of the reaction mixture DMF was added to dissolve the PDC. The reaction was monitored by TLC and was complete in 0·5 hours. The product was isolated by removal of solvent under high vacuum at room temperature followed by short column chromatography to give 5-formyl-2',3'-isopropylideneuridine in 63% yield. The product was shown to be identical to that previously prepared.

(E)-5-(2-nitrovinyl)uridine, 36, is not known and as nitrovinyl side chains are generated from aldehydes it was decided to attempt the preparation this way. It had previously been shown that it was unobtainable via the palladium catalysed coupling of 5-iodouridine and nitroethylene. From the aldehyde, the usual method is to condense the anion of nitromethane in an aldol-type reaction with an aldehyde. β-Nitrostyrene, $PhCH=CHNO_2$, for example, is readily generated from benzaldehyde, nitromethane and aqueous sodium hydroxide.[357] Initially, the nucleoside aldehyde was reacted with ethoxide in ethanol but the nucleoside precipitated rapidly from solution before much reaction had taken place. TLC, however, did reveal a small amount of a slower moving nucleoside which was isolated and identified by NMR, UV and mass spectroscoy and elememtal analysis as 5-(1-hydroxy-2-nitroethyl)-2',3'-isopropylideneuridine, 67. It was obtained in only 9% yield. The NMR shows the presence of the hydroxy side chain, this is confirmed by the UV spectrum which does not possess a vinylic side chain absorbance at about 300nm.

The formation of α-hydroxy-β-nitro compounds is called the Henry reaction [358] and is an example of the more general Knoevenagel reaction. Many catalysts have been used to promote reaction such as alkoxides and organic bases. In some cases the reaction proceeds spontaneously to the nitro-olefin with elimination of water and this is especially prevalent for β-nitro-α-hydroxyphenylalkanes such as that obtained as an intermediate for β-nitrostyrene. For example, cyclohexanone with ethoxide gives the hydroxynitro compound[359] while ammonium acetate and 2,3,5-trimethoxy-3-methylbenzaldehyde gives the nitro-olefin.[360]

The tendency for such spontaneous dehydration when aromatics are used is quite general and the hydroxycompounds are rarely isolatable. This is due to conjugation of the double-bond with the aromatic system, hence as the C-5 position of uracil nucleosides is 'pseudo-aromatic' there is less incentive for spontaneous dehydration.

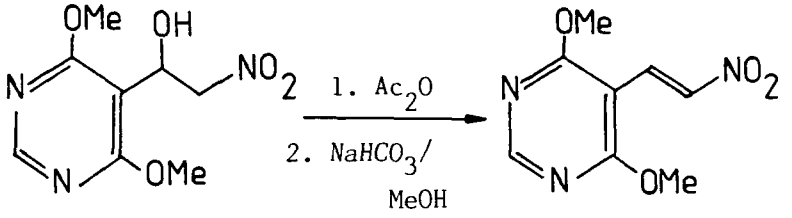
In order to increase the yield, the reaction of the nucleoside aldehyde with nitromethane was performed using sodium hydride to generate the anion of the nitromethane. The reaction proceeded in 64% yield and gave a product identical in all respects with that described above.

The use of triethylamine as a catalyst is known to give β-hydroxynitro-compounds and this was confirmed using the nucleoside.[361] The reaction when catalysed by triethylamine was incomplete and the residual starting material was difficult to separate, the sodium hydride method is therefore more efficient.

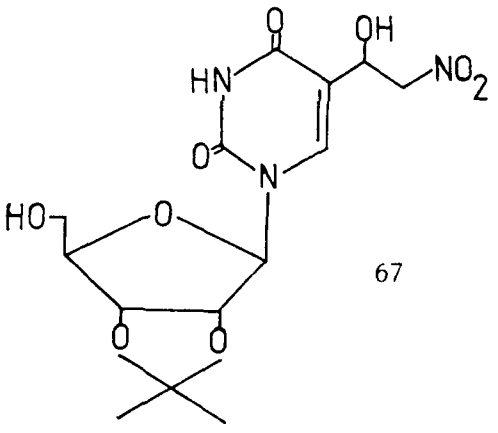
It is possible to generate the nitro-olefin from the hydroxycompound by treatment with acetic anhydride and base. This has been done in the case of 5-(1-hydroxy-2-nitroethyl)-4,6-dimethoxypyrmidine, Figure 27. Acetic anhydride/DMSO have been used for a 2'-arabinohydroxy nucleoside where the nitromethyl group is in the ribo-configuration.[363]

Another method for generation of the nitro-olefin from the hydroxy compound involves heating the reaction mixture to eliminate the water. Thus, refluxing 5-formyl-2',3'-isopropylideneuridine with nitromethane in the presence of

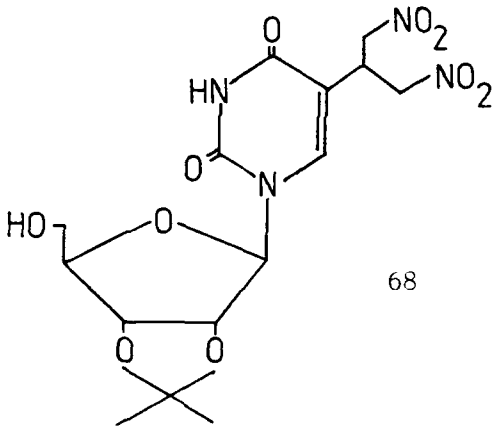
Figure 27



ethoxide catalysis gave a compound faster running on TLC than the hydroxy-compound. Isolation by column chromatography then recrystallisation from ethanol gave a compound whose NMR showed the incorporation of 2 nitromethyl groups. This was confirmed by the mass spectrum which had a m/e 417 and the structure 68 was



67



68

tentatively postulated. The UV spectrum shows no absorbance at around 300nm and hence the side chain is saturated. Elemental analysis confirmed the structure as 68. This compound therefore arises from attack of excess of the nitromethane anion on the initially formed nitrovinyl compound in a Michael addition. Presedence for this is seen by the Michael addition of nitrostyrenes.[364] The nitro group greatley activates the double bond and β-nitrostyrene, for example, is readily condensed with malonic esters and ethyl nitroacetate with diethylamine catalysis.

Compound 68 was deprotected using 50% aqueous trifluoroacetic acid to give

the fully characterised compound in 70% yield.

Many 5,6-disudstituted- and 6-substituted uridine derivatives nave been made although no substituted 5-vinyl-6-substituted uridines or 2'-deoxyuridines are known. As the ester (E)-5-(2-carbomethoxyvinyl)uridine possessed some activity against the Yellow frver virus it was decided to functionalise this compound at the C-6 position.

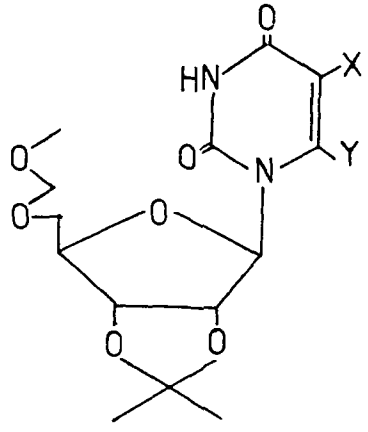
Until quite recently, few 6-substituted pyrimidine nucleosides had been made,[365,366] most probably due to the difficulties encountered in their synthesis. This lack of progress was despite the reported anti-viral activities of 6-methyl-uridine and 6-methylcytidine.[366]

6-Substituted pyrimidine bases are readily made by the primary synthesis routes but attempts to condense these with sugar derivatives leads mainly to the N^3-ribosylated product.[367,368]

The most common synthesis of 6-substituted pyrimidine nucleosides involves the deprotonation of the H-6 position using organolithium bases. One of the earliest uses was to make $^{14}CH_3$ thymidine although only in 14% yield due to the low solubility of the tetralithium compound in the solvent.[314] The trimethylsilyl group (TMS) has been used to derivatise the sugar to increase the solubility of the nucleoside[319] but it was found that 3',5'-bis-(TMS)-5-bromo-2'-deoxyuridine when lithiated with n-BuLi then treated with ethyl bromide gave 5-Et-2'-dUrd and 6-Et-2'-dUrd in 2% and 4% yields respectively after deprotection.[370] Lithiation of tris-(TMS)uridine occurs at both C-5 and C-6 positions in 28% and 14% yields respectively,[371] while lithiation of a 2',3',5'-tri-O-protected-5-bromouridine with butyl lithium[372] and 2',3'-isopropylidene-5'-methoxymethyluridine with butyl lithium results in lithiation at C-5 and C-6.

In the work of Tanaka et al, the lithiation of a protected sugar nucleoside gave 6-substituted uridines in good yields. Many analogues were made including 6-alkyluridines,[373,374] 6-alkylthiouridines,[375] 6-aroyluridines[200,376] and

6-hydroxymethyl-, 6-hydroxyethyl-, 6-formyl- and 6-carboethoxyuridines.[377] 6-Aryluridines have also been made photochemically.[378] In all the examples involving lithiation, it was found that lithiation with lithium diisopropyl amide (LDA) resulted in regiospecific deprotonation of the C-6 position. LDA is not effective to generate the C-5 anion and this can be accomplished with lithium tetramethylpiperide (LTMP).[202] When the C-5 is functionalised, the reaction with LDA can be utilised for the synthesis of 5,6-disubstituted compounds, 69.



69

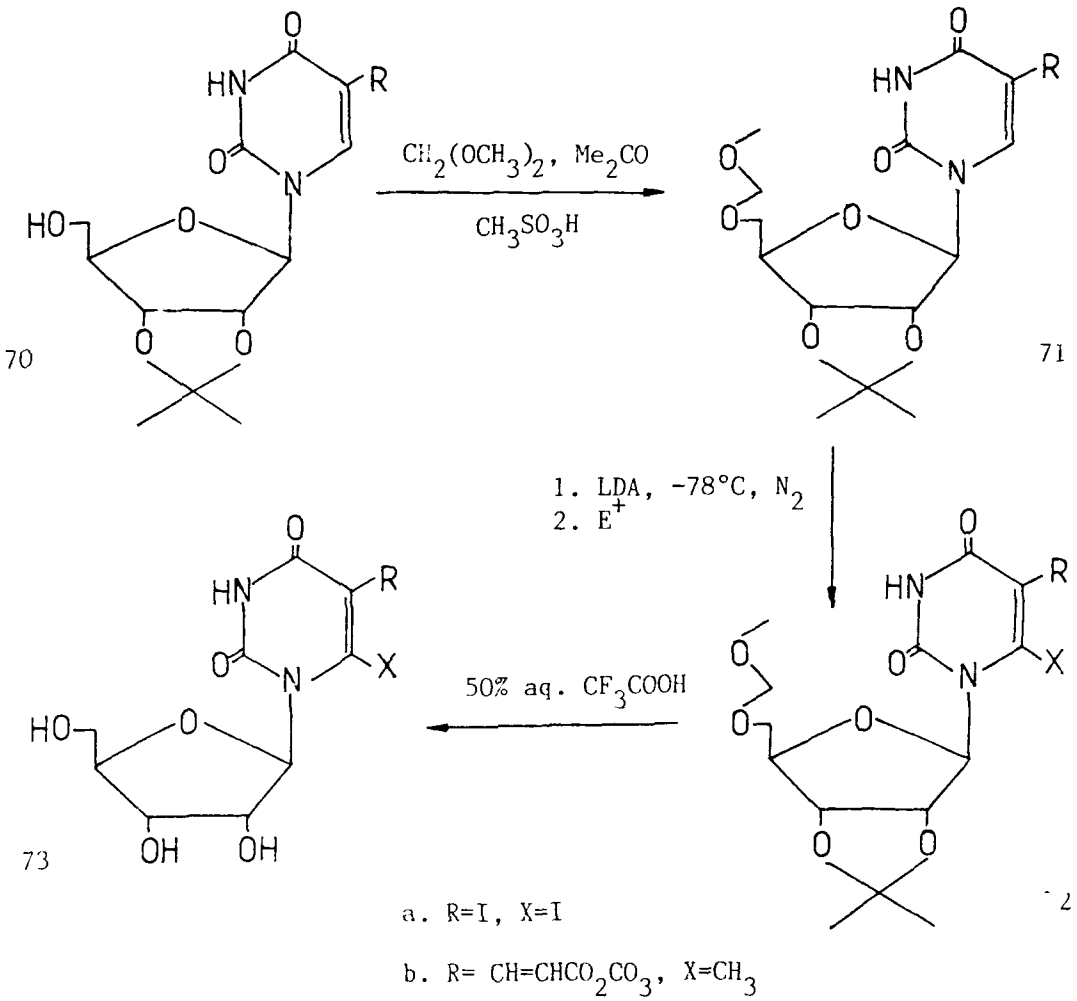
X	Y
SPh	SPh[377]
F,Cl,Br,I	I,SPh
CO_2Et	CO_2Et[377]

Sec-BuLi/tetramethylethylenediamine[379] has been used to selectively remove the C-5 proton to form 5-substituted uridines[380] which can also be made by protecting the C-6 with phenylthio and lithiating with LTMP.

As 5-iodouridine had already been made as part of this work it was decided to synthesize the known 5,6-diiodouridine as a model compound.[381]

The route to 5,6-diiodouridine is outlined in Figure 28 and involves the protection of the ribose by conversion to 5-iodo-2',3'-iospropylidene-5'-methoxymethyluridine, 71. Firstly, 5-iodouridine was isopropylidenated by the acetone/anhydrous copper (II) sulphate/sulphuric acid method and the product, 70, was obtained in 80% yield after crystallisation from ethanol. The NMR spectrum clearly showed the loss of the cis-diol system and replacement by the two isopropylidene methyl groups.

Figure 28



Methoxymethylation of the 5'-hydroxyl was accomplished in an 87% conversion using the published method for the uridine compound.[379] This involved the transetherification reaction between a large excess of dimethoxymethane and the nucleoside in acetone with methane sulphonic acid catalysis. Initially, only moderate yields were obtained, these were improved to 87% by drying then fractionating the dimethoxymethane over sodium wire. That the methoxymethylation occurs at the 5'-OH and not on N-3 is shown by the presence of the N-H at δ11·72. The product was fully characterised.

In the original preparation of the 5,6-diiodocompound, the 5-iodouridine was

lithiated using 2·5 equivalents of LDA. These reaction conditions could not be repeated despite obtaining the full experimental procedure.[382] It was found, however, that the blocked 5,6-diiodo compound could be obtained by the use of 5 equivalents of LDA in 40% yield which is comparable to that originally published. The NMR of the product clearly showed the absence of the H-6 proton at the expected δ8·0-8·5. The incorporation of the iodine at C-6 is clearly demonstrated by the mass spectrum with an m/e 581 of $(M+H)^+$ and m/e 365 $(M-ribose+2H)^+$.

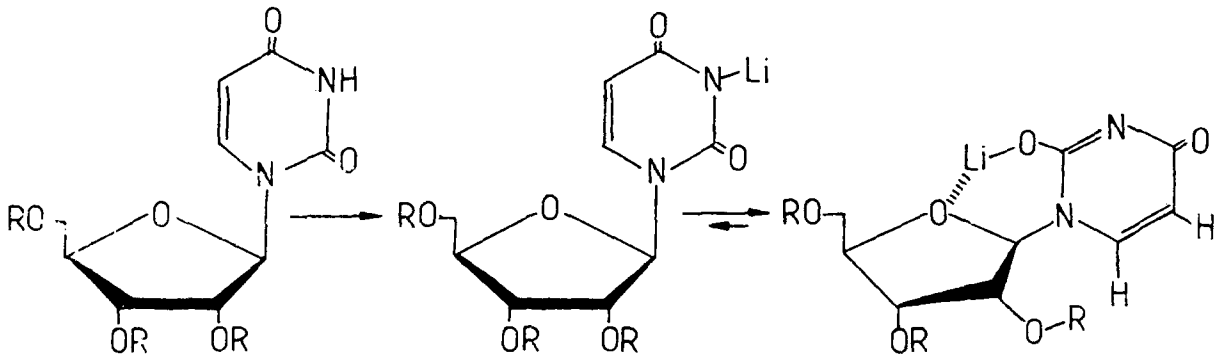
According to the published procedure for this preparation, the deblocking of the sugar with 50% aqueous trifluoroacetic acid to give 5,6-diiodouridine proceeded in 46% yield. When repeated, two nucleoside products were obtained, the first of which the NMR shows to be the 5'-methoxymethyl-5,6-diiodouridine and the second, by NMR, which appears to be a mixture of the required product and 5-iodouridine. It may be postulated that firstly the isopropylidene group is removed and then the methoxymethyl group. The 5,6-diiodouridine then lost the 6-iodo group to give the NMR which shows the H-6 proton in the mixture of what appeared on TLC to be one component.

The steric factors affecting the lithiation reaction have been determined experimentally.[383] The normal conformation of pyrimidine nucleosides is <u>anti</u> in which the 5,6-double bond of the base sits over the sugar. Experiments with various blocking groups on the sugar, especially those in which the 2'-OH protecting group is bulky, reduce or prevent reaction at C-6 and so it was concluded that the initially formed N^3-lithiated species exists in the <u>syn</u>-conformation by intramolecular chelation,[380,384] Figure 29.

The approach of the lithiating agent to the C-6 position is interfered with by the 2'-substituent which rotates about the C(2)-O bond.

Lithium dialkylamides are thought to act in an 'acid-base' mechanism[385] and it was found that LDA is not basic enough to deprotonate the less acidic H-5.

Figure 29



anti-conformer syn-conformer

Despite the unexpected difficulty in deblocking the model compound it was thought worthwhile to derivatise the methyl ester in the 6-position and to investigate the incorporation of a 6-methyl group as it was hoped that methyl would be easier to introduce than iodine due to steric reasons.

The route to (E)-5-(2-carbomethoxyvinyl)-6-methyluridine, 73b, is analogous to that used for the diiodo compound. The ester was isopropylideneated using acetone and acid catalysis. The isopropylidene ester, 70b, was obtained in 70% yield. The previous reported synthesis was via the palladium catalysed oxidative coupling of 2',3'-isopropylideneuridine with methyl acrylate in 74% yield although this method used 1·2 equivalents of palladium. [386] The spectroscopic data obtained was in full agreement with the published data.

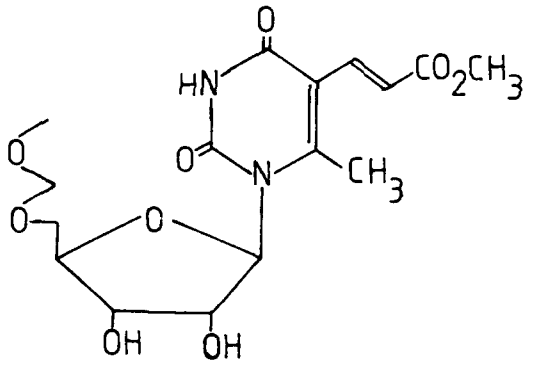
The next step, the methoxymethylation, was preformed in 92% conversion. The NMR showed the absence of the 5'-OH and the gain of the CH_2OCH_3 group and this compound was fully characterised.

Lithiation of the protected methyl ester by 5 equivalents of LDA at -78°C under an inert atmosphere proceeded rapidly as shown by the rapid formation of the orange solution of the dianion. This was reacted with an excess of methyl

iodide and TLC of the reaction mixture showed total conversion to one nucleoside product which was isolated by short column chromatography and crystallisation from acetone/hexane.

The NMR of the product lacked the H-6 proton of the starting material and had a resonance corresponding to 3 protons at δ3·24 as a singlet. The mass spectrum had m/e at 427 $(M+H)^+$. The replacement of H by CH_3 was confirmed by elemental analysis.

The blocked compound was deblocked using 25% aqueous trifluoroacetic acid. The product isolated has an NMR spectrum which shows the expected signals for the 2'-OH and 3'-OH and therefore the isopropylidene group has been removed. The 5'-OH is not seen and instead the signals of the methoxymethyl group are still present. The product of this reaction is therefore compound 74 and this



74

was confirmed by mass spectroscopy with an m/e of 387 which is $(M+H)^+$. This compound needs to be deprotected and once the conditions have been determined, other analogues substituted in the 6-position made.

The methylation of the dianion of the blocked vinyl methyl ester, however, has been found to proceed easily, especially when compared to the iodination of the 5-iodo compound. This poor reaction of the latter must invariably be due to steric effects resulting from putting two iodines in such close proximity. The iodination of the 5-iodo compound always resulted in the presence of starting

material, even when 10 equivalents of LDA were used.

The original aim of this project was to synthesize a range of compounds for their potential anti-viral activity against several of the (-)-stranded RNA viruses and to have any activity evaluated by anti-viral screening. Any positive leads, like that originally obtained for (E)-5-(2-carbomethoxyvinyl)uridine, could then be exploited and further derivatives then made if a lead was obtained or a change of direction made if no lead was obtained. As no anti-viral testing results were obtained in time to influence this work, a change of emphasis to a broad range of analogues resulted and it is hoped that this has been accomplished.

EXPERIMENTAL

Thin Layer Chromatography (TLC)

The plates used were precoated silica gel supplied by E. Merck A. G., Darmstadt, Germany. Developement was by the ascending method. After evaporation of the solvent, pyrimidine bases and nucleosides were detected by quenching of the fluorescence at 254nm on irradiation with a UV lamp.

Column Chromatography

Columns were prepared from a known weight of silica gel powder (Kieselgel 60, 70-250 mesh ASTM, type 7734) supplied by E. Merck A. G., Darmstadt, Germany). A slurry of this material in the eluent to be used was packed under gravity. The material to be separated was applied either as a solution in the solvent to be used or absorbed onto silica gel. Fractions were monitored by TLC.

UV Spectroscopy

UV spectra were recorded on a Perkin Elmer 552 spectrophotometer which employed a digital readout of both absorbance and wavelength. Samples were dissolved in either distilled water or spectroscopic grade ethanol.

NMR Spectroscopy

NMR spectra were recorded on a Jeol FX90Q (90MHz) and a Jeol GX270 (270MHz) spectrometer.

Mass Spectroscopy

Mass spectra were obtained on a Kratos MS80 mass spectrometer with a DS 55 data system employing automatic digital readout of data. Electron impact or Fast Atom Bombardment (FAB) ionization were used.

Elemental Analysis

Elemental analysis were obtained in the micro-analytical laboratory of this department.

Solvents and reagents

Acetonitrile

Acetonitrile was dried by refluxing over phosphorus pentoxide and then distilled. This was repeated, the product was stored over molecular sieve.

Methanol and ethanol

The alcohol (50ml) was warmed with dry magnesium turnings and a few crystals of iodine. After complete formation of the magnesium alkoxide a further portion of the alcohol (800ml) was added and the mixture refluxed for two hours prior to distillation.

Diethyl ether

This was dried overnight over calcium chloride then filtered and stored over sodium wire.

Dimethylformamide (DMF)

This was dried overnight with phosphorus pentoxide then filtered and stirred overnight with fresh P_2O_5. The solvent was then fractionated under high vacuum and stored over molecular sieve.

Tetrahydrofuran (THF)

THF was heated under reflux over potassium in the presence of benzophenone until a purple solution had developed then distilled and used immediately.

1,4-Dioxan

Dioxan was passed down an alumina column and stored over potassium hydroxide pellets. It was then heated under reflux over sodium in the presence of benzophenone until a purple solution had developed then distilled and used immediately.

Pyridine and Triethylamine

These were heated under reflux over calcium hydride then distilled onto molecular sieve prior to use.

Thionyl chloride

Thionyl chloride was twice distilled from linseed oil under an atmosphere

of nitrogen through an efficient fractionating column, the fraction boiling between 76-78°C being collected.

Alkyl Acrylates

Methyl, ethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl and 2-hydroxybutyl acrylates were obtained from Aldrich Chemical Co Ltd, t-butyl acrylate from Fluka Chemicals and benzyl acrylate from Phase Separations Ltd.

Palladium (II) acetate

This was obtained from Aldrich Chemical Co Ltd and was stored desiccated from chemical vapours to prevent inactivation.

Preparation of 5-iodouridine

To a solution of uridine (24.4g, 100mmole) in a mixture of 0.5M nitric acid (200ml) and dioxan (800ml) was added iodine (51.0g, 200mmole) and the mixture gently refluxed for 6 hours after which time TLC in 70/30 chloroform/methanol showed the absence of starting material. The mixture was then evaporated under reduced pressure to give a dark solid which was then coevaporated several times with ethanol until a light pink solid was obtained. This was then suspended in ether, filtered, and dried under vacuum to give 5-iodouridine as a white solid, 36.69g, (99.2%).

UV Spectrum pH 6 in ethanol

λ max. 286.1nm ϵ =8590

λ min. 245.0nm ϵ =2050

NMR Spectrum δ(d_6-DMSO) 11.4(1-H,s,N-H), 8.4(1-H,s,H-6), 5.65(1-H,d,H-1'), 5.2-4.5(3-H,bd,2'-OH,3'-OH,5'-OH), 4.1-3.4(5-H,m,H-2',H-3',H-4',H-5').

FAB Mass spectrum m/e 371 $(M+H)^+$

Preparation of (E)-5-(2-carbomethoxyvinyl)uridine

Palladium (II) acetate (0.60g, 2.6mmole), triphenylphosphine (1.44g, 5.4 mmole) and redistilled triethylamine (10ml) were combined in dry dioxan (80ml) and stirred at 70°C until a deep red colouration had developed. Then, 5-iodo-uridine (20.00g, 54mmole) and methyl acrylate (9.30g, 108mmole) were added and the mixture was stirred under reflux for 1 hour and filtered while hot. The filtrate was stored at room temperature overnight and filtered under suction. The brown solid was washed twice with ether then recrystallised twice from ethanol to give the product as white crystals (8.05g, 50%).

UV Spectrum pH 6 in ethanol

λ max. 303.0nm ϵ =17740

shoulder 270.0nm ϵ =10630

λ min. 230.0nm ϵ = 3090

NMR Spectrum $\delta(d_6$-DMSO) 11.65(1-H,bs,N-H), 8.50(1-H,s,H-6), 7.35(1-H,d,vinylic H,J=16Hz), 6.50(1-H,d,vinylic H,J=16Hz), 5.75(1-H,d,H-1'), 5.40(1-H,d,2'-OH), 5.25(1-H,t,5'-OH), 5.00(1-H,d,3'-OH), 4.05(2-H,m,H-2' and H-3'), 3.80(1-H,m,H-4'), 3.7-3.5(5-H,m,H-5' and $-CO_2CH_3$)

FAB Mass spectrum m/e 329 $(M+H)^+$

Preparation of (E)-5-(2-carboxyvinyl)uridine

A solution of (E)-5-(2-carbomethoxyvinyl)uridine (6.75g, 20.56mmole) in aqueous sodium hydroxide (1M, 525ml) was stirred for two hours at room temperature temperature. The solution was then cooled to 0°C and brought to pH 2 by the addition of 4M HCl. The mixture was left to stand in ice for 30 minutes then filtered. The filtrate was evaporated to dryness under reduced pressure and water (100ml) added. The resulting precipitate was filtered and washed with a little ice water. The precipitates were combined and dried under vacuum to give (E)-5-(2-carboxyvinyl)uridine as a white solid (4.30g, 67%).

UV Spectrum pH 6 in ethanol

λ max.	310.0nm	ε =20610
shoulder	266.0nm	ε =13050
λ min.	225.0nm	ε = 3910

NMR Spectrum $\delta(d_6$-DMSO) 11.65(1-H,s,N-H), 8.50(1-H,s,H-6), 7.30(1-H,d,vinylic H,J=16Hz), 6.75(1-H,d,vinylic H,J=16Hz), 5.78(1-H,d,H-1'), 5.60-4.70(3-H,m,2'-OH, 3'-OH,5'-OH), 4.20-3.40(5-H,m,H-2',H-3',H-4',H-5')

FAB Mass spectrum m/e 315 $(M+H)^+$

Preparation of (E)-5-(2-bromovinyl)uridine

To a solution of (E)-5-(2-carboxyvinyl)uridine (4.20g, 13.75mmole) in dry DMF (20ml) was added potassium carbonate (4.10g,32.0mmole) and the solution stirred at room temperature for 15 minutes. A solution of N-bromosuccinimide (2.50g, 13.75mmole) in dry DMF (20ml) was added dropwise over 30 minutes at room temperature. The resulting suspension was filtered at once under suction and the precipitate washed carefully with dry DMF. This solution was evaporated under

high vacuum at 40°C with the receiving flask cooled in liquid air. The resulting oil was dissolved in methanol and silica gel added. Removal of solvent under reduced pressure gave a free flowing powder which was applied to the top of a silica gel column prepared in chloroform/ethanol 90:10. Elution with the same solvent and the combination of the appropriate fractions as determined by TLC followed by removal of the solvent under reduced pressure gave a solid which upon recrystallisation from water gave the title compound as colourless needles, (1.20g 33%).

UV Spectrum pH 6 in ethanol

λ max. 295.0nm ε =10250

λ min. 271.0nm ε = 4100

λ max. 251.0nm ε =13150

NMR Spectrum $\delta(d_6$-DMSO) 11.50(1-H,s,N-H), 8.15(1-H,s,H-6), 7.25(1-H,d,vinylic H,J=14.7Hz), 6.80(1-H,d,vinylic H,J=14.7Hz), 5.75(1-H,d,H-1'), 5.50-4.80(3-H,bd, 2'-OH,3'-OH,5'-OH), 4.20-4.00(2-H,m,H-2',H-3'). 3.95-3.75(1-H,m,H-4'), 3.70-3.55(2-H,m,5'-CH_2).

FAB Mass spectrum m/e 350$(M+H)^+$

Preparation of alkyl acrylates

In a 250ml flask were placed the alcohol (0.25mmole), methyl acrylate (43g, 0.5mole), hydroquinone (1g) and toluene-4-sulphonic acid (0.5g).

The flask was attached to a fractionating column and the solution heated to reflux on an oil bath. The column was operated under total reflux until the temperature of the vapours at the still-head fell to 62-63°C, this is the boiling point of the methanol/methyl acrylate azeotrope. This is then distilled as rapidly as it is formed. The temperature of the still-head did not exceed 65°C. When production of the azeotrope had become very slow the excess methyl acrylate was distilled off and the alkyl acrylate was fractionally distilled at reduced pressure. The main fraction obtained was then redistilled at reduced pressure.

Preparation of n-propyl acrylate

Boiling point 36.0°C at 20.1mmHg

NMR Spectrum $\delta(CCl_4)$ 6.5-5.6(3-H,m,vinylics), 4.1(2-H,t, $-OCH_2$), 1.7(2-H,m, $-OCH_2\underline{CH_2}CH_3$), 1.0(3-H,t,$-CH_3$)

Preparation of iso-propyl acrylate

Boiling point 21.0°C at 16mmHg

NMR Spectrum $\delta(CCl_4)$ 6.5-5.6(3-H,m,vinylics), 5.0(1-H,m,-OCH), 1.2(6-H,d, 2 methyl groups).

Preparation of n-butyl acrylate

Boiling point 62.0°C at 15mmHg

NMR Spectrum $\delta(CCl_4)$ 6.5-5.6(3-H,m,vinylics), 4.1(2-H,t,$-OCH_2$), 1.9-1.7(7-H, m,$-CH_2CH_2CH_3$).

Preparation of n-pentyl acrylate

Boilin point 59.5-59.9°C

NMR Spectrum $\delta(CCl_4)$ 6.5-5.6(3-H,m,vinylics), 4.1(2-H,t,$-OCH_2$), 1.8-0.8(9-H, m,$-CH_2CH_2CH_2CH_3$).

Preparation of n-octyl acrylate

Boiling point 77-78°C at 0.5mmHg

NMR Spectrum $\delta(CCl_4)$ 6.5-5.6(3-H,m,vinylics), 4.1(2-H,t,$-OCH_2$), 1.9-0.7(15-H, m, $-CH_2CH_2CH_2CH_2CH_2CH_2CH_3$).

Preparation of 2-methoxyethyl acrylate

Boiling point 57.0°C at 18mmHg

NMR Spectrum $\delta(CCl_4)$ 6.6-5.6(3-H,m,vinylics), 4.3-4.1(2-H,m,$-CO_2CH_2-$), 3.6-3.4 (2-H,t,$\underline{CH_2}OCH_3$), 3.3(3-H,s,$-OCH_3$).

Preparation of 2-ethoxyethyl acrylate

Boiling point 60.5°C at 15.5mmHg

NMR Spectrum $\delta(CCl_4)$ 6.5-5.6(3-H,m,vinylics), 4.3-4.1(2-H,t,$-CO_2CH_2$), 3.7-3.0 (4-H,m, 2X-OCH_2), 1.15(3-H,t,CH_3)

EI Mass spectrum m/e 99(M-ethoxy)

Preparation of 2-butoxyethyl acrylate

Boiling point 58°C at 1mmHg

NMR Spectrum $\delta(CCl_4)$ 6.6-5.6(3-H,m,vinylics), 4.2(2-H,t,$-CO_2CH_2-$), 3.7-3.2 (4-H,m,$2XOCH_2$), 1.5(4-H,bs,$2XCH_2$), 0.9(3-H,s,$-CH_3$).

EI Mass spectrum m/e $173(M+H)^+$, 99(M-butoxy)

Preparation of 2-cyanoethyl acrylate

The fraction boiling at 54.4°C at 0.4mmHg was purified by silica gel chromatography with elution in chloroform.

NMR Spectrum $\delta(CCl_4)$ 6.6-5.6(3-H,m,vinylics), 4.35(2-H.t.$-OCH_2$), 2.7(2-H,t,$-CH_2CN$).

EI Mass spectrum m/e 99 ($M-CH_2CN$)

Preparation of 2-chloroethyl acrylate

Boiling point 59.0-60.5°C at 18.5mmHg

NMR Spectrum $\delta(CCl_4)$ 6.7-5.8(3-H,m,vinylics), 4.4(2-H,t,$-OCH_2$), 3.7(2-H,t, $-CH_2Cl$).

EI Mass spectrum m/e $135/137(M+H)^+$

Preparation of 2-bromoethyl acrylate

The fraction boiling at 57.0-58°C at 4mmHg was purified by silica gel chromatography with elution in chloroform.

NMR Spectrum $\delta(CCl_4)$ 6.7-5.7(3-H,m,vinylics), 4.45(2-H,t,$-OCH_2$), 3.55(2-H,t, $-CH_2Br$).

EI Mass spectrum m/e 179/181 $(M+H)^+$

Preparation of (E)-5-(2-carboethoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate were added 5-iodouridine (2.00g, 5.4mmole) and ethyl acrylate (1.10g, 1.2ml, 10.8 mmole). The mixture was stirred under reflux for 2 hours, after which time TLC showed the absence of starting material. The reaction mixture was filtered while

hot, the crystals that separated on standing overnight were filtered off, dissolved in methanol and silica gel added. The solvent was removed to give a free flowing powder which was applied to the top of a silica gel column prepared in 90:10 chloroform/methanol with which it was eluted. The appropriate fractions were combined, the solvent removed under reduced pressure and the resulting white solid recrystallised from ethanol to give the title compound as white crystals, (0.438g, 24%).

UV Spectrum pH 6 in ethanol

λ max.	302.0nm	ε =18530
shoulder	262.0nm	ε =10900
λ min.	227.0nm	ε = 3510

NMR Spectrum $\delta(d_6$-DMSO) 11.65(1-H,s,N-H), 8.50(1-H,s,H-6), 7.35(1-H,d, vinylic H,J=15.6Hz), 6.80(1-H,d,vinylic H,J=15.6Hz), 65.80(1-H,d,H-1'), 5.45(1-H, d,2'-OH), 5.30(1-H,t,5'-OH), 5.10(1-H,d,3'-OH), 4.30-3.80(5-H,m,H-3',H-4',-OCH_2), 3.7-3.5(2-H,m,5'-H), 1.20(3-H,t,CH_3).

FAB Mass spectrum m/e 345 $(M+H)^+$, 365 $(M+Na)^+$, 685 $(2M+H)^+$

Analysis $C_{14}H_{18}N_2O_8$ requires

C=49·12% H=5·30% N=8·18%

found C=48·8% H=5.3% N=7·9%

Preparation of (E)-5-(2-carbopropoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate were added 5-iodouridine (2·00g, 5·4mmole) and n-propyl acrylate (1·23g, 10·8mmole). Then, the mixture was stirred under reflux for 1 hour after which time TLC in 90:10 chloroform/ethanol showed the absence of 5-iodouridine. The reaction mixture was filtered while hot and then allowed to cool overnight. The crystals were filtered, extracted with ether (3X100ml) to remove triphenylphosphine and applied as a dry pack to a column prepared in 70:30 chloroform/ethanol with which the column was eluted. This was repeated until the appropriate fractions showed 1 spot by TLC. The fractions were combined, the solvent removed under

reduced pressure and the white powder recrystallised from ethanol to give the product (0·391g, 21%).

UV Spectrum pH 6 in ethanol

λ max.	303.0nm	ε =16330
shoulder	265·0nm	ε =10500
λ min.	230·0nm	ε = 3660

NMR Spectrum δ(d_6-DMSO) 11.65(1-H,bs,N-H), 8.50(1-H,s,H-6), 7.35(1-H,d, vinylic H,J=15·8Hz), 6·85(1-H,d,vinylic H,J=15·8Hz), 5.75(1-H,d,H-1'), 5.45(1-H, d,2'-OH), 5·30(1-H,t,5'-OH), 5·10(1-H,d,3'-OH), 4·10-3.50(7-H,m, H-5',H-4',H-3', H-2',-OCH_2-), 1·63(2-H,m,-$\underline{CH_2}CH_3$), 0·90(3-H,m,-CH_3).

FAB Mass spectrum m/e 357 $(M+H)^+$

Analysis $C_{15}H_{20}N_2O_8$ requires

C=50·56% H=5.65% N=7·86%

found C=50.85% H=5.5% N=7.5%

Preparation of (E)-5-(2-carboisopropoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate were added 5-iodouridine (2·00g, 5·4mmole) and iso-propyl acrylate (1·23g, 10·8mmole) and the mixture stirred under reflux for 2 hours after which time TLC in 80:20 chloroform/ethanol showed the absence of 5-iodouridine. The reaction mixture was filtered while hot and then allowed to cool overnight. The crystals were filtered, extracted with ether (3X60ml) to remove triphenylphosphine and then repeatedly columned in 77·5:22·5 chloroform/ethanol until TLC of the appropriate fractions showed one component. The relevant fractions were combined, the solvent removed under reduced pressure and the white powder recrystallised from acetone to give the product as colourless crystals, (0·116g, 6%).

UV Spectrum pH 6 in ethanol

λ max.	301.0nm	ε =19790
shoulder	270.0nm	ε =12315
λ min.	229·0nm	ε = 4360

NMR Spectrum δ(d_6-DMSO) 11·65(1-H,s,H-6), 8·50(1-H,s,H-6), 7·30(1-H,d,vinylic H,J=16·0Hz), 6·80(1-H,d,vinylic H,J=16·0Hz), 5·75(1-H,d,H-1'), 5.45(1-H,d,2'-OH), 5.25(1-H,t,5'-OH), 5·08(1-H,d,3'-OH), 4·95(1-H,septuplet,-OCH), 4·05(2-H,m,H-2', H-3'), 3·85(1-H,m,H-4'), 3.80-3.50(2-H,m,H-5'), 1·23(6-H,d,2XCH_3).

FAB Mass spectrum m/e 357 $(M+H)^+$

Analysis $C_{15}H_{20}N_2O_8$ requires

C=50·56% H=5.65% N=7·86%

found C=50·3% H=5·65% N=7·6%

Preparation of (E)-5-(2-carbobutoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate were added 5-iodouridine (2·00g, 5·4mmole) and n-butyl acrylate (1·38g, 10·8mmole) and the mixture stirred under reflux for 2 hours after which time TLC in 90:10 chloroform/ethanol showed the absence of 5-iodouridine. The mixture was filtered while hot and the crystals that separated on standing overnight were filtered off and extracted with ether (3X75ml) to remove triphenylphosphine and repeatedly columned in 90:10 chloroform/ethanol until TLC of the appropriate fractions showed 1 component. Recrystallisation from acetone gave the product as white crystals (0·170g, 9%).

UV Spectrum pH 6 in ethanol

λ max. 301·0nm ε=17430

shoulder 270·0nm ε=11040

λ min. 230·0nm ε= 4120

NMR Spectrum δ(d_6-DMSO) 11·65(1-H,s,N-H), 8·50(1-H,s,H-6), 7·35(1-H,d,vinylic H,J=15·8Hz), 6·80(1-H,d,vinylic H,J=15·8Hz), 5·75(1-H,d,H-1'), 5·45(1-H,d,2'-OH), 5·30(1-H,t,5'-OH), 5·10(1-H,d,3'-OH), 4·3-3.5(7-H,m,H-2',H-3',H-4',-OCH_2-), 1·80-0.75(7-H,m,-$OCH_2CH_2CH_3$).

FAB Mass spectrum m/e 371 $(M+H)^+$, 741 $(2M+H)^+$, 393 $(M+Na)^+$, 763 $(2M+Na)^+$.

Analysis $C_{16}H_{22}N_2O_8$ requires

	C=51·89%	H=5·99%	N=7·56%
found	C=51·6%	H=6·0%	N=7·3%

Preparation of (E)-5-(2-carbotertbutoxtvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate were added 5-iodouridine (2·00g, 5·4mmole) and t-butyl acrylate (1·38g, 10·8mmole) and the mixture stirred under reflux for 1 hour after which time TLC in 90:10 chloroform/ethanol showed the absence of 5-iodouridine. The reaction mixture was filtered while hot and allowed to cool overnight. The crystals were filtered off and columned in 75:25 chloroform/ethanol to give the product as a white powder (0·73g, 37%).

UV Spectrum pH 6 in ethanol

λ max.	302·0nm	ε =23570
shoulder	267·0nm	ε =11450
λ min.	228·0nm	ε = 4190

NMR Spectrum δ(d_6-DMSO) 11·65(1-H,s,N-H), 8·45(1-H,s,H-6), 7·25(1-H,d,vinylic H,J=15·0Hz), 6·70(1-H,d,vinylic H,J=15·0Hz), 5·75(1-H,d,H-1'), 5·45(1-H,d,2'-OH), 5·30(1-H,t,5'-OH), 5·05(1-H,d,3'-OH), 4·20-3·50(5-H,m,H-5',H-4',H-3',H-2'), 1·45 (9-H,s,methyls).

FAB Mass spectrum m/e 315 $(acid+H)^+$, 183 (acid-ribose)

Analysis $C_{16}H_{22}N_2O_8$ requires

	C=51·89%	H=5·99%	N=7·56%
found	C=51·6%	H=6·2%	N=7·3%

Preparation of (E)-5-(2-carbopentoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate were added 5-iodouridine (2·00g, 5·4mmole) and n-pentyl acrylate (1·53g, 10·8mmole) and the mixture stirred under reflux for 2 hours after which time TLC in 90:10 chloroform/ethanol showed the absence of 5-iodouridine. The reaction mixture was filtered while hot and allowed to cool overnight. The crystals were filtered off and extracted with ether (3X50ml) then columned in 72·5:27·5 chloroform/ethanol until

the appropriate fractions showed one component. The fractions were combined and the solvent removed under reduced pressure to give a white solid which upon recrystallisation from acetone gave the product as colourless crystals, (0·25g, 12%).

UV Spectrum pH 6 in ethanol

λ max.	300·5nm	ε =19180
shoulder	268·0nm	ε =11360
λ min.	230·0nm	ε = 3680

NMR Spectrum δ(d_6-DMSO) 11·68(1-H,s,N-H), 8·40(1-H,s,H-6), 7.32(1-H,d, vinylic H,J=15·8Hz), 6·85(1-H,d,vinylic H,J=15·8Hz), 5.75(1-H,d,H-1'), 5·45(1-H, d,2'-OH), 5·30(1-H,t,5'-OH), 5·10(1-H,d,3'-OH), 4·08(2-H,m,-OCH_2-), 4·02(2-H,m, H-2',H-3'), 3·85(1-H,m,H-4'), 3·80-3·50(2-H,m,H-5'), 1·70-1·20(6-H,m,-$CH_2CH_2CH_2$-), 0·90(3-H,m,CH_3).

FAB Mass spectrum m/e 385 $(M+H)^+$, 407 $(M+Na)^+$

Analysis $C_{17}H_{24}N_2O_8$ requires

C=53·1% H=6·3% N=7·3%

found C=52·8% H=6·2% N=7·5%

Preparation of (E)-5-(2-carbooctoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate were added 5-iodouridine (2·00g,5·4mmole) and n-octyl acrylate (1·98g, 10·8mmole) and the mixture stirred under reflux for 2 hours after which time TLC in 90:10 chloroform /ethanol showed the absence of 5-iodouridine. The reaction mixture was filtered while hot an allowed to cool overnight. The crystals were filtered off, extracted with ether (2X80ml) to remove triphenylphosphine then repeatedly columned in 70:30 chloroform/ethanol until the appropriate fractions showed 1 spot by TLC. The fractions were combined, solvent removed by distillation under reduced pressure to give the title compound as a white powder, (0·258g, 11%).

UV Spectrum pH 6 in ethanol

λ max.	302·0nm	ε =14130

shoulder 268·0nm ε = 9330

λ min. 235·0nm ε = 3900

NMR Spectrum δ(d_6-DMSO) 11·67(1-H,s,N-H), 8·50(1-H,s,H-6), 7·35(1-H,d, vinylic H,J=15·8Hz), 6·85(1-H,d,vinylic H,J=15·8Hz), 5·75(1-H,d,H-1'), 5·45(1-H, d,2'-OH), 5·30(1-H,t,5'-OH), 5·10(1-H,d,(3'-OH), 4·08(2-H,m, $-OCH_2$), 4·02(2-H,m, H-2',H-3'), 3·88(1-H,m, H-4'), 3·80-3·55(2-H,m,H-5'), 1·60(2-H,m,$-OCH_2\underline{CH_2}-$),1·25 (10-H,m,5XCH_2), 0·86(3-H,t,$-CH_3$).

FAB Mass spectrum m/e 427 $(M+H)^+$, 295 (ester-ribose)

Analysis $C_{20}H_{30}N_2O_8$ requires

C=56·32% H=7·0% N=6·57%

found C=56·3% H=6·7% N=6·3%

Preparation of (E)-5-(2-carboxybenzyloxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate were added 5-iodouridine (2·00g, 5·4mmole) and benzyl acrylate (1·75g, 10·8mmole) and the mixture stirred under reflux for 1·5 hours. Then the solution was filtered, solvent removed by distillation in vacuo and the resulting darkly coloured solid applied to the top of a silica gel column and eluted with 80:20 chloroform/ ethanol . The appropriate fractions were pooled, the solvent removed under reduced pressure to give a pink solid which was recrystallised from ethanol to give the product as a white solid, (1·00g, 46%).

UV Spectrum pH 6 in ethanol

λ max. 304.4nm ε =16070

shoulder 267·4nm ε = 8550

NMR Spectrum δ(d_6-DMSO) 11·70(1-H,s,N-H), 8·50(1-H,s,H-6), 7·36(1-H,d, vinylic H,J=16·1Hz), 7·36(5-H,s,aromatics), 6·88(1-H,d,vinylic H,J=16·1Hz), 5·77 (1-H,d,H-1'), 5·46(1-H,d,2'-OH), 5.28(1-H,t,5'-OH), 5·18(2-H,s,benzylic CH_2), 5·10(1-H,d,3'-OH), 4·05(2-H,d,H-2',H-3'), 3·90(1-H,m,H-4'), 3·66(2-H,m,H-5').

FAB Mass spectrum m/e 405 $(M+H)^+$

Analysis $C_{19}H_{20}N_2O_8$ requires

C=56·43% H=5·00% N=6·93%

found C=56·2% H=5·0% N=6·8%

Preparation of (E)-5-(2-carbomethoxyethoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (10ml) were added 5-iodouridine (2·00g, 5·4mmole) and 2-methoxyethyl acrylate (1·40g,10·8mmole) and the mixture stirred under reflux for 1 hour after which time TLC in 80:20 chloroform/methanol showed the absence of starting maretial and the appearance of a new nucleoside with rf. 0·28.

The solvent was removed under reduced pressure and the resulting oil columned in 80:20 chloroform/methanol then recrystallised from methanol to give the product as a white solid (0·83g, 41%).

UV Spectrum pH 6 in ethanol

λ max.	300·0nm	ε =20560
shoulder	270·0nm	ε =11740
λ min.	225·0nm	ε = 2480

NMR Spectrum δ(d_6-DMSO) 11·68(1-H,s,N-H), 8·50(1-H,s,H-6), 7·35(1-H,d, vinylic H,J=17·0Hz), 6·84(1-H,d,vinylic H,J=17·0Hz), 5·77(1-H,d,H-1'), 5·45(1-H, d,2'-OH), 5·30(1-H,t,5'-OH), 5·05(1-H,d,3'-OH), 4·30-3·50(12-H,m,H-2',H-3',CO_2CH_3, OCH_2,H-5',H-4').

FAB Mass spectrum m/e 373 $(M+H)^+$

Analysis $C_{15}H_{20}N_2O_9$ requires

C=48·38% H=5·41% N=7·52%

found C=48·4% H=5·5% N=7·6%

Preparation of (E)-5-(2-carboethoxyethoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (15ml) were added 5-iodouridine (2·00g, 5·4mmole) and 2-ethoxyethyl acrylate (1·55g,10·8mmole) and the mixture stirred under reflux for 1 hour. the solvent was then removed by distillation in vacuo and the product obtained by

column chromatography in 80:20 chloroform/methanol followed by recrystallisation from ethanol, (0·87g, 42%)·

UV Spectrum pH 6 in ethanol

λ max.	302·0nm	ε =22540
shoulder	273·0nm	ε =12130
λ min.	227·0nm	ε = 1395

NMR Spectrum δ(d_6-DMSO) 11·68(1-H,s,N-H), 8·50(1-H,s,H-6), 7·35(1-H,d,vinylic H,J=17·0Hz), 6·84(1-H,d,vinylic H,J=17·0Hz), 5·77(1-H,d,H-1'), 5·45(1-H,d,2'-OH), 5·30(1-H,t,5'-OH), 5·05(1-H,d,3'-OH), 4·30-3·20(11-H,m,H?2',H-3',-CO_2CO_3, 2 X -OCH_2,H-5',H-4'), 1·10(3-H,t,CH_3).

FAB Mass spectrum m/e 387 $(M+H)^+$

Analysis $C_{16}H_{22}N_2O_9$ requires

C=49·74% H=5·7% N=7·3%

found C=50·0% H=5·4% N=7·6%

Preparation of (E)-5-(2-carbobutoxyethoxyvinyl)uridine

To the activated catalyst based on 60mg palladium (II) acetate in dry dioxan (15ml) were added 5-iodouridine (2·00g, 5·4mmole) and 2-butoxyethyl acrylate (1·86g, 10·8mmole) and the mixture stirred under reflux for 2 hours. After the removal of the solvent by distillation under reduced pressure, TLC in 80:20 chloroform/methanol showed the absence of starting material. Short column chromatography in 80:20 chloroform/methanol, treatment with celite then recrystallisation from ethanol gave the title compound as a white solid, (0·75g, 33%).

UV Spectrum pH 6 in ethanol

λ max.	303·0nm	ε =20620
shoulder	271·1nm	ε =11025
λ min.	228·0nm	ε = 925

NMR Spectrum δ(d_6-DMSO) 11·68(1-H,s,N-H), 8·50(1-H,s,H-6), 7·37(1-H,d, vinylic H,J=15·8Hz), 6·85(1-H,d,vinylic H,J=15·8Hz), 5·45(1-H,d,2'-OH), 5·30

(1-H,t,5'-OH), 5·10(1-H,d,3'-OH), 4·30-3·30(11-H,m,H-2',H-3',H-4',H-5',-CO_2CH_2, 2X-OCH_2), 1·60-1·10(4-H,m,2ZCH_2), 0·85(3-H,t,CH_3).

FAB Mass spectrum m/e 415 $(M+H)^+$

Analysis $C_{18}N_{26}N_2O_9$ requires

C=52·17% H=6·32% N=6·76%

found C=52·2% H=6·3% N=6·9%

Preparation of (E)-5-(2-carbohydroxyethoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (15ml) were added 5-iodouridine (2·00g, 5·4mmole) and 2-hydroxyethyl acrylate (1·25g, 10·8mmole) and the mixture stirred under reflux for 1 hour. The solution was filtered while hot and allowed to cool overnight then filtered. The resulting crystals were applied to the top of a silica gel column and eluted with 70:30 chloroform/methanol then 50: 0 ethyl acetate/ethanol. The solution of the appropriate pooled fractions were treated with celite, solvent removed under reduced pressure and the resulting solid recrystallised from ethanol to give the product as a white crystalline solid (0·62g 32%).

UV Spectrum pH 6 in ethanol

λ max.	303·0nm	ε =17180
shoulder	276nm	ε = 9000
λ min.	227·5nm	ε = 1030

NMR Spectrum δ(d_6-DMSO) 11·67(1-H,s,N-H), 8·50(1-H,s,H-6), 7·35(1-H,d, vinylic H,J=17·0Hz), 6·85(1-H,d,vinylic H,J=17·0Hz), 5·75(1-H,d,H-1'), 5·45(1-H, d,2'-OH), 5·30(1-H,t,5'-OH), 5·10(1-H,d,H-3'), 4·85(1-H,t,-CH_2OH), 4·20-3·40(9-H, H-2',H-3',H-4',H-5',-$CO_2CH_2CH_2$-).

FAB Mass spectrum m/e 359 $(M+H)^+$

Analysis $C_{14}H_{18}N_2O_9$ requires

C=46·93% H=5·06% N=7·82%

C=46·9% H=4·8% N=7·9%

Preparation of (E)-5-(2-carbohydroxybutoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (15ml) were added 5-iodouridine (2·00g. 5·4mmole) and 4-hydroxybutyl acrylate (1·56g, 10·8mmole) and the mixture heated under reflux for 1 hour. The solvent was then removed under reduced pressure and the resulting darkly coloured oil was applied to the top of a silica gel column prepared in ethyl acetate/ethanol 50:50 with which it was eluted. The appropriate fractions were pooled and treated with celite and the product then obtained by recrystallisation from methanol, (0·48g, 23%).

UV Spectrum pH 6 in ethanol

λ max. 301·2nm ε =20330

shoulder 271·4nm ε =11980

λ min. 230·1nm ε = 3840

NMR Spectrum $\delta(d_6\text{-DMSO})$ 11·68(1-H,s,N-H), 8·50(1-H,s,H-6), 7·35(1-H,d, vinylic H,J=17·0Hz), 6·80(1-H,d,vinylic H,J=17·0Hz), 5·75(1-H,d,H-1'), 5·60-4·25(4-H,broad,D_2O exchangeable, 4XOH), 4·30-3·30(9-H,m,H-2',H-3',H-4',H-5',2X OCH_2), 1·58(4-H,m,$-CH_2CH_2$).

FAB Mass spectrum m/e 387 $(M+H)^+$

Analysis $C_{16}H_{22}N_2O_9$ requires

C=49·74% H=5·74% N=7·25%

found C=49·4% H=5·9% N=7·0%

Preparation of (E)-5-(2-carbocyanoethoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (15ml) were added 5-iodouridine (2·00g, 5·4mmole) and 2-cyanoethyl acrylate (1·35g, 10·8mmole) and the solution stirred under reflux for 1·5 hours. The solvent was then removed by distillation in vacou and the resulting oil then applied to the top of a silica gel column which was eluted with 70:30 chloroform /methanol. The appropriate fractions were combined then treated with celite and the solvent removed by distillation under reduced pressure to give a pink solid

which was recrystallised from ethanol to give the product as white crystals (0·72g, 36%).

UV Spectrum pH 6 in ethanol

λ max.	302·0nm	ε =17540
shoulder	272·0nm	ε = 8660
λ min.	228·0nm	ε = 1945

NMR Spectrum δ (d_6-DMSO) 11·70(1-H,s,N-H), 8·55(1-H,s,H-6), 7·40(1-H,d, vinylic H,J=17·0Hz), 6·89(1-H,d,vinylic H,J=17·0Hz), 5·75(1-H,d,H-1'), 5·46(1-H, d,2'-OH), 5·30(1-H,t,5'-OH), 5·10(1-H,d,3'-OH), 4·30(2-H,t,$-OCH_2$), 4·20-4·00(2-H, m,H-2',H-3'), 3·95-3·80(1-H,m,H-4'), 3·80-3·60(2-H,m,H-5'), 2·90(2-H,t,$-CH_2CN$).

FAB Mass spectrum m/e 368 $(M+H)^+$, 236 $(M+H\text{-}ribose)^+$

Analysis $C_{15}H_{17}N_3O_8$ requires

C=49·05% H=4·66% N=11·4%

found C=48·9% H=4·4% N=11·1%

Preparation of (E)-5-(2-carbochloroethoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (15ml) were added 5-iodouridine (2·00g, 5·4mmole) and 2-chloroethyl acrylate (1·45g, 10·8mmole) and the solution stirred under reflux for 1·5 hours. Solvent was then removed by distillation under reduced pressure and the resulting oil applied to the top of a silica gel column prepared in 70:30 chloroform/ methanol with which it was eluted. The appropriate fraction were pooled and then treated with celite, the solvent removed under reduced pressure and the pink solid recrystallised from ethanol to give the product as a white solid, (0·76g, 37%).

UV Spectrum pH 6 in ethanol

λ max.	302·5nm	ε =19080
shoulder	270·0nm	ε =10160
λ min.	224.0nm	ε = 1340

NMR Spectrum $\delta(d_6$-DMSO) 11·65(1-H.s.N-H), 8·50(1-H,sH-6), 7·35(1-H,d, vinylic H,J=15·7Hz), 6·85(1-H,d,vinylic H,J=15·7Hz), 5·75(1-H,d,H-1'), 5·45(1-H, d,2'-OH), 5·30(1-H,t,5'-OH), 5·05(1-H,d,3'-OH), 4·35(2-H,t,$-CO_2CO_2$), 4·20-3·50 (7-H,m,m,H-2',H-3',H-4',H-5',$-CH_2Cl$).

FAB Mass spectrum m/e 377 $M+H)^+$

Analysis $C_{14}H_{17}ClN_2O_8$ requires

C=44·63% H=4·55% N=7·46%

found C=44·3% H=4.8% N=7.3%

Preparation of (E)-5-(2-carbobromoethoxyvinyl)uridine

To the activated catalyst based on 150mg of palladium (II) acetate in dry dioxan (30ml) were added 5-iodouridine (5·00g, 13·5mmole) and 2-bromoethyl acrylate (4·35g, 24mmole) and the solution stirred under reflux for 2 hours. The solvent was removed in vacuo and the resulting oil columned twice on a silica gel column eluted with 70:30 chloroform/methanol. After treatment with celite, the brownish solid was recrystallised from ethanol to give the product as a light brown coloured solid, (0·53g, 23%).

UV Spectrum pH 6 in ethanol

λ max. 301·6nm ε=22540

shoulder 271·0nm ε=11840

λ min. 223·0nm ε= 570

NMR Spectrum $\delta(d_6$-DMSO) 11·65(1-H,s,N-H), 8·50(1-H,s,H-6), 7·35(1-H, vinylic H,J=15·5Hz), 6·85(1-H,d,vinylic H,J=15·5Hz), 5·75(1-H,d,H-1'), 5·00-3·30(12-H, m, $-OCH_2CH_2Br$,H-2',H-3',H-4',H-5',2'-OH,3'-OH,5'-OH).

FAB Mas spectrum m/e 421 (M)

Analysis $C_{14}H_{17}BrN_2O_8$ requires

C=39·9% N=4·1% N=6·6%

found C=39·6% N=4·1% N=6·6%

Preparation of (E)-5-(2-carbotrifluoroethoxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (10ml) were added 5-iodouridine (2·00g, 5·4mmole) and 2,2,2-trifluoroethyl acrylate (1·66g, 10·8mmole) and the solution stirred under reflux for 1 hour. The solvent was removed by distillation under reduced pressure and the resulting oil applied to the top of a silica gel column and eluted with 70:30 chloroform/ethanol. The appropriate fractions were treated with celite, solvent removed in vacuo and the white solid recrystallised from ethanol to give the product as white crystals, (0·52g, 24%).

UV Spectrum pH 6 in ethanol

λ max. 305·0nm ε =18370

shoulder 270·0nm ε = 7385

NMR Spectrum $\delta(d_6$-DMSO) 11·60(1-H,s,N-H), 8·55(1-H,s,H-6), 7·45(1-H,d, vinylic H,J=16·0Hz), 6·90(10H,d,vinylic H,J=16·0Hz), 5·75(1-H,d,H-1'), 5·70-5·10(3-H,bs,2'-OH,3'-OH,5'-OH), 4·80(2-H,q,CH_2CF_3), 4·20-3·50(5-H,m,H-2',H-3', H-4',H-5').

^{19}F NMR Spectrum -72·00ppm, triplet, J=8·80Hz

FAB Mass spectrum m/e 397 $(M+H)^+$

Analysis $C_{14}H_{15}F_3N_2O_8.H_2O$ requires

C=40·6% H=4·1% N=6·76%

found C=40·9% H=3·9% N=6·5%

Preparation of (E)-5-(2-carbo-2-tetrahydrofuryloxyvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (10ml) were added 5-iodouridine (2·00g, 5·4mmole) and tetrahydrofuryl acrylate (1·67g, 10·8mmole) and the mixture stirred under reflux for 1 hour. The solution was then evaporated in vacuo to an oil and applied to the top of a silica gel column poepared in 80:20 chloroform/methanol with which it was eluted. The appropriate fractions were treated with celite and the product then obtained by recrystallisation from ethanol (0·54g, 26%).

UV Spectrum pH 6 in ethanol

λ max.	302·5nm	ε =19520
shoulder	267·4nm	ε =10650
λ min.	226·0nm	ε = 3080

NMR Spectrum δ(d_6-DMSO) 11·65(1-H,s,N-H), 8·50(1-H,s,H-6), 7·35(1-H,d,vinylic H,J=16·1Hz), 7·83(1-H,d,vinylic H,J=16·1Hz), 5·75(1-H,d,H-1'), 5·42(1-H,d,2'-OH), 5·25(1-H,t,5'-OH), 5·05(1-H,d,3'-OH), 4·30-3·50(10-H,m,H-2',H-3',H-4',H-5', $-CO_2CH_2-$, $-CHOCH_2-$, -CH-), 2·10-1·50(4-H,m,CH_2CH_2 of side chain).

FAB Mass spectrum m/e 399 $(M+H)^+$

Analysis $C_{17}H_{22}N_2O_9$ requires

C=51·25% H=5·56% N=7·03%

found C=51·0% H=5.4% N=6·9%

Preparation of (E)-5-(2-cyanovinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (8ml) were added 5-iodouridine (2·00g, 5·4mmole) and acrylonitrile (0·57g, 0·49ml,10·8mmole) and the solution stirred under reflux for 2 hours after which time TLC in 80:20 chloroform/methanol showed the absence of starting material.

The solvent was removed under reduced pressure and the reaction mixture applied to the top of a silica gel column prepared in 80:20 chloroform/methanol with which it was eluted followed by elution in 70:30 chloroform/methanol. The combined fractions containing the new nucleoside were treated with celite then recrystallised from ethanol to give the product as a white crystalline solid (0·40g, 25%.

UV Spectrum pH 6 in ethanol

λ max.	301·0nm	ε =19730
shoulder	270·0nm	ε =11530

NMR Spectrum δ(d_6-DMSO) 11·74(1-H,bs,N-H), 8·64(1-H,s,H-6), 7·16(1-H,d, vinylic H,J=16·2Hz), 6·50(1-H,d,vinylic H,J=16·2Hz), 5·73(1-H,d,H-1'), 5·49

(1-H,bs,2'-OH), 5·26(1-H,bs,5'-OH), 5·10(1-H,bs,3'-OH), 4·03(2-H,m,H-2',H-3'), 3·89(1-H,m,H-4'), 3·70(2-H,dd,H-5').

FAB Mass spectrum m/e 296 $(M+H)^+$

Analysis $C_{12}H_{13}N_3O_6$ requires

C=48·82% H=4·44% N=14·23%

found C=48·7% H=4·5% N=14·1%

Preparation of (E)-5-(2-m-nitrophenylvinyl)uridine

To the activated catalyst based on 60mg of palladium (II) acetate in dry dioxan (10ml) were added 5-iodouridine (2·00g,5·4mmole) and 3-nitrostyrene (10·8mmole) and the mixture stirred under reflux for 1·5 hours. The solvent was removed by distillation under reduced pressure and the resulting oil applied to the top of a silica gel column prepared in 70:30 chloroform/methanol with which it was eluted initially. Then it was eluted with 80:20 ethyl acetate/ethanol. The yellow solution containing the new nucleoside was then treated with celite and the product obtained after recrystallisation from ethanol as fine yellow needles, (0·86g, 41%).

UV Spectrum pH 6 in ethanol

λ max. 315·0nm ε=20640

shoulder 276·2nm ε=15750

NMR Spectrum δ(d_6-DMSO) 11·60(1-H,bs,N-H), 8·50-6·75(6-H,m,vinylics and 4 aromatics), 4·85(1-H,d,H-1'), 5·70-5·00(3-H,bs,2'-OH,3'-OH,5'-OH), 4·10(1-H,s, H-2'), 3·90(1-H,s,H-3'), 3·70(2-H,s,H-5').

FAB Mass spectrum m/e 392 $(M+H)^+$

Analysis $C_{17}H_{17}N_3O_8$ requires

C=52·2% H=4·4% N=10·7%

found C=51·9% H=4·7% N=10·4%

Preparation of 2',3',5'-tri-O-acetyl-(E)-5-(2-carboxyvinyl)uridine

(E)-5-(2-carboxyvinyl)uridine (5·00g,15·9mmole) was added to dry pyridine

(80ml) and after dissolution (5 minutes), acetic anhydride (80ml) was added. The clear solution was stirred at room temperature for 24 hours and then the solvent was removed by distillation under reduced pressure. The white solid was then coevaporated with toluene (5X25ml) and ethanol (2X20ml). After short column chromatography in 60:10 ethyl acetate/ethanol the fractions were pooled and the solvent removed in vacuo to give the title compound as a white powder, (5·97g, 85%).

UV Spectra pH 6 in ethanol

λ max.	296·0nm	ε =12620
shoulder	268·0nm	ε =10130
λ min.	228·0nm	ε = 3580

NMR Spectrum δ(d_6-DMSO) 11·75(1-H,bs,N-H), 8·25(1-H,s,H-6), 7·35(1-H,d, vinylic H,J=16·5Hz), 6·76(1-H,d,vinylic H,J=16·5Hz), 5·95(1-H,d,H-1'), 5·45(1-H, m,H-5'), 4·32(3-H,m,H-2',H-3',H-4'), 2·09(9-H,s, $3CO_2CH_3$).

FAB Mass spectrum m/e 441 $(M+H)^+$

Analysis $C_{18}H_{20}N_2O_{11}$ requires

C=49·09% H=4·57% N=6·36%

found C=49·2% H=4·7% N=6·1%

Preparation of (E)-5-(2-carboisopropoxyvinyl)uridine

2',3',5'-Tri-O-acetyl-(E)-5-(2-carboxyvinyl)uridine (0·720g,1·63mmole) was added to freshly redistilled thionyl chloride (20ml) and the resulting solution refluxed for 20 minutes. The excess thionyl chloride was then removed in vacuo, care being taken to ensure the exclusion of moisture. Then, dry isobutanol (2-methylpropan-1-ol, 3ml) was added and the solution refluxed for 10 minutes. The excess alcohol was removed under high vacuum and the product isolated by column chromatography with elution in 80:20 chloroform/methanol followed by recrystallisation from ethanol (0·43g, 71%).

UV Spectrum pH 6 in ethanol

λ max.	302·1nm	ε =18330

shoulder 265·2nm ε=9790

λ min. 225·0nm ε= 360

NMR Spectrum δ(d_6-DMSO) 11·70(1-H,bs,N-H), 8·51(1-H,s,H-6), 7·35(1-H,d, vinylic H,J=16·2Hz), 6·86(1-H,d,vinylic H,J=16·2Hz), 5·76(1-H,d,H-1'), 5·48(1-H, bs,2'-OH), 5·32(1-H,t,5'-OH), 5·11(1-H,bs,3'-OH), 4·09(1-H,m,H-2'), 4·02(1-H,m, H-3'), 3·90(3-H,m,H-4', $-CO_2CH_2$),3·70-3·50(2-H,dd,H-5'), 1·92(1-H,septuplet, -CH-), 0·93(3-H,s,CH_3), 0·90(3-H,s,CH_3).

FAB Mass spectrum m/e 371 $(M+H)^+$

Analysis $C_{16}H_{22}N_2O_8$ requires

C=51·89% H=5·98% N=7·56%

found C=51·6% H=6.0% N=7·3%

Preparation of (E)-5-(2-carboisobutoxyvinyl)uridine

2',3',5'-Tri-O-acetyl-(E)-5-(2-carboxyvinyl)uridine (1·00g, 2·3mmole) was added to freshly redistilled thionyl chloride (15ml) and the resulting solution refluxed for 30 minutes. The excess thionyl chloride was then removed in vacuo, care being taken to exclude moisture. Then, dry isoamyl alcohol (3-methylpropan-2-ol, 10ml) was added and the mixture refluxed for 30 minutes. The excess alcohol was removed under high vacuum and the product isolated by short column chromatography with elution in 80:20 chloroform/methanol followed by recrystallisation from ethanol, (0·38g). A second crop was obtained by another recrystallisation of the filtrate from acetone/hexane (0·13g), total yield 0·51g. (58%).

UV Spectrum pH 6 in ethanol

λ max. 302·5nm ε =19710

shoulder 267·9nm ε =11060

λ min. 228·0nm ε = 2155

NMR Spectrum δ(d_6-DMSO) 11·66(1-H,s,N-H), 8·48(1-H,s,H-6), 7·33(1-H,d, vinylic H,J=15·67Hz), 6·81(1-H,d,vinylic H,J=15·67Hz), 5·75(1-H,d,H-1'), 5·60-

4·90(3-H,bs,2'-OH,3'-OH,5'-OH), 4·25-3·75(5-H,m,H-2',H-3', H-4',-OCH_2-), 3·75-3·50(2-H,bs,H-5'), 1·50(1-H,m,-CH-), 1·00-0·80(6-H,2s,$2CH_3$).

FAB Mass spectrum m/e 385 $(M+H)^+$

Analysis $C_{17}H_{24}N_2O_8$ requires

C=53·1% H=6·3% N=7·2%

found C=52·8% H=6·3% N=6·9%

Preparation of 5-formyl-2,4-dimethoxypyrimidine

A solution of n-butyl lithium (48ml,1·6M,73·6mmole) in n-hexane was added dropwise over 5 minutes to a stirred suspension in dry ether (240ml) of 5-bromo-2,4-dimethoxypyrimidine (16·0g, 72·9mmole) at -70°C under an atmosphere of dry nitrogen. Ethyl formate (28·0g, 377mmole) previously dried and fractionated from calcium hydride, was then added to the yellow suspension of 2,4-dimethoxy-pyrimidin-5-yl lithium and the resulting orange solution was stirred at -70°C for one hour then allowed to warm slowly to room temperture. The reaction mixture was quenched with water (400ml), the aqueous layer separated and then extracted with ether (3X200ml). The extracts were combined with the ether layer and dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The residue was analysed by TLC in 90:10 chloroform/ethanol to show only a trace of starting material.

The residue was dissolved in methanol, silica gel added and the solvent removed by distillation under reduced pressure to give a free-flowing solid which was applied to the top of a silica gel columnprepared in 70:30 hexane/ethyl acetate, with which the column was eluted. The fractions containing the product were combined, solvent removed under reduced pressure and the white solid recrystallised from hexane/ethyl acetate to give 5-formyl-2,4-dimethoxy-pyrimidine as fine white needles (6·89g, 56%).

UV Spectrum pH 6 in ethanol

λ max. 279·9nm ε = 9340

λ max. 245·0nm ε =10645

λ min. 258·0nm ε = 6120

NMR Spectrum δ(d_6-DMSO) 10·05(1-H,s,-CHO), 8·75(1-H,s,H-6), 4·08(3-H,s, methyl), 4·02(3-H,s,methyl).

EI Mass spectrum m/e 169 $(M+H)^+$

Analysis $C_7H_8N_2O_3$ requires

C=50·00% H=4·79% N=16·66%

found C=50·1% H=4·5% N=16·9%

Preparation of (E)-5-(2-carboxyvinyl)-2,4-dimethoxypyrimidine

5-Formyl-2,4-dimethoxypyrimidine (10·52g, 62·6mmole) was dissolved in dry pyridine (60ml) and malonic acid (13·02g, 126·2mmole) and freshly redistilled piperidine (2ml) were added. The mixture was heated on a steam bath for 10 hours then the solvent was removed by distillation under reduced pressure.

The resulting oil was coevaporated with water (3x25ml) and the solid thus obtained recrystallised firstly from water then dry methanol to give the title compound as white needles, 6·45g. A second crop was obtained from the filtrate by removal of the solvent and recrystallisation from methanol to give 1·08g. Total yield 7·53g (57%).

UV Spectrum pH 6 in ethanol

λ max. 298·0nm ε =19290

shoulder 270·0nm ε =17650

λ min. 227·0nm ε = 2390

NMR spectrum δ(d_6-DMSO) 12·00(1-H,bs,-COOH), 8·68(1-H,s,H-6), 7·50(1-H,d, vinylic H,J=16·0Hz), 6·55(1-H,d,vinylic H,J=16·0Hz), 4·02(3-H,s,methyl), 3·95 (3-H,s,methyl).

EI Mass spectrum m/e 210 (M)

Analysis $C_9H_{10}N_2O_4$ requires

C=52·43% H=4·79% N=13·33%

found C=52·1% H=4·8% N=13·1%

Preparation of crude (E)-5-(2-chlorocarbonylvinyl)-2,4-dimethoxypyrimidine

To (E)-5-(2-carboxyvinyl)-2,4-dimethoxypyrimidine (0·100g) was added freshly redistilled thionyl chloride (40ml), care being taken to ensure the exclusion of moisture. The resulting solution was left at room temperature for 20 minutes then slowly heated until refluxing gently. After 10 minutes the excess thionyl chloride was removed under reduced pressure to give the crude acid chloride as a yellowish solid. The acid chloride (0·1087g) was then used directly in subsequent steps.

EI Mass spectrum m/e 228/230 (M)

Preparation of (E)-5-(2-carbomethoxyvinyl)-2,4-dimethoxypyrimidine

To freshly prepared (E)-5-(2-chlorocarbonylvinyl)-2,4-dimethoxypyrimidine (0·108g, 0·47mmole) suspended in dry ether (20ml) was added dry methanol (5ml). After 20 minutes at room temperature the solvent was removed by distillation under reduced pressure to give a yellowish solid which was dissolved in methanol and silica gel added. The methanol was then removed by distillation to give a free flowing powder which was applied to the top of a silica gel column prepared in 70:30 hexane/ethyl acetate with which the column was eluted. The appropriate fractions were combined, solvent removed and the white powder then recrystallised from hexane to give the title compound as fine white needles (0·0446g, 42%).

UV Spectrum pH 6 in ethanol

λ max. 293·0nm ε=18430

λ min. 228·0nm ε= 1960

NMR Spectrum $\delta(d_6$-DMSO) 8·73(1-H,s,H-6), 7·58(1-H,d,vinylic H,J=16·0Hz), 6·65 (1-H,d,vinylic H,J=16·0Hz), 4·03(3-H,s,methyl), 3·95(1-H,s,methyl), 3·71(3-H,s, ester methyl).

EI Mass spectrum m/e 224 (M)

Analysis $C_{10}H_{12}N_2O_4$ requires

C=53·59% H=5·39% N=12·49%

found C=53·4% H=5·2% N=12·6%

Preparation of (E)-5-(2-aminocarbonylvinyl)-2,4-dimethoxypyrimidine

To freshly prepared (E)-5-(2-chlorocarbonylvinyl)-2,4-dimethoxypyrimidine (0·815g, 3·56mmole) suspended in dry ether (20ml) was cautiously added ammonia solution (d=0·880, 2ml) with vigorous stirring. After standing at room temperature for 10 minutes the reaction mixture was evaporated to dryness under reduced pressure and the resulting solid was recrystallised from water to give the title compound as fine white needles, (0·23g, 31%).

UV Spectrum pH 6 in ethanol

λ max. 288·0nm ε =18580

shoulder 272·0nm ε =18520

λ min. 223·0nm ε = 3740

NMR Spectrum $\delta(d_6$-DMSO) 8·55(1-H,s,H-6), 7·55(1-H,bs,N-H), 7·38(1-H,d, vinylic h,J=16·0Hz), 7·08(1-H,bs,N-H), 6·71(1-H,d,vinylic H,J=16·0Hz), 4·02(3-H, s,methyl), 3·40(3-H,s,methyl).

EI Mass spectrum m/e 229 (M)

Analysis $C_9H_{11}N_3O_3$ requires

C=52·67% H=5·29% N=20·08%

found C=52·4% H=5·2% N=19·9%

Preparation of (E)-5-(2-methylaminocarbonylvinyl)-2,4-dimethoxypyrimidine

To freshly prepared (E)-5-(2-chlorocarbonylvinyl)-2,4-dimethoxypyrimidine (0·217g, 0·949g) suspended in dry ether (20ml) was cautiously added methylamine (40% aqueous solution, 2ml) with vigorous stirring. After 10 minutes at room temperature the solvent was removed by distillation under reduced pressure and the resulting yellowish solid recrystallised from water to give the title compound as fine white needles (0·1004g, 47%).

UV Spectrum pH 6 in ethanol

λ max. 273·0nm ε =20840

λ max. 287·0nm ε =20520

λ min. 222·0nm ε = 3630

NMR Spectrum δ(d_6-DMSO) 8·53(1-H,s,H-6), 8·07(1-H,bd,N-H), 7·50(1-H,d, vinylic H,J=16·1Hz), 6·72(1-H,d,vinylic H,J=16·1Hz), 4·02(3-H,s,$-OCH_3$), 3·95(3-H, s,$-OCH_3$), 2·70(3-H,s,$-NCH_3$).

EI Mass spectrum m/e 222 (M)

Analysis $C_{10}H_{13}N_3O_3$ requires

C=53·80% H=5·87% N=18·82%

found C=53·7% H=6·0% N=18·5%

Preparation of (E)-5-(2-bromovinyl)-2,4-dimethoxypyrimidine

To a solution of (E)-5-(2-carboxyvinyl)-2,4-dimethoxypyrimidine (0·300g, 1·427mmole) in dry DMF (5ml) was added potassium carbonate (0·45g, 5·25mmole). After stirring at room temperature for 15 minutes a solution of N-bromosuccinimide (0·258g, 1·45mmole) in dry DMF (4ml) was added dropwise over 10 minutes. The resulting suspension was filtered immedietly, the precipitate washed with dry DMF (2X4ml) and the filtrate evaporated under high vacuum. The resulting solid was dissolved in methanol, TLC in 70:30 hexane/ethyl acetate showed 1 new component with rf. 0·45. To the reaction mixture in methanol was added silica gel, the solvent removed and the resulting free flowing powder applied to the top of a silica gel column prepared in 70:30 hexane/ethyl acetate with which the column was eluted. The appropriate fractions were pooled and the solvent removed to give the title compound as fine white crystals after recrystallisation from hexane, (0·1561g, 45%).

UV Spectrum pH 6 in ethanol

λ max. 260·0nm ε =19560

λ min. 211·0nm ε = 3950

NMR Spectrum δ(d_6-DMSO) 8·45(1-H,s,H-6), 7·24(1-H,d,vinylic H,J=14·2Hz), 7·02

(1-H,d,vinylic H,J=14·2Hz), 3·98(3-H,s,methyl), 3·89(3-H,s,methyl).

FAB Mass spectrum m/e 245/247 $(M+H)^+$

Analysis $C_8H_9BrN_2O_2$ requires

C=40·20% H=3·70% N=11·43%

found C=39·9% H=3·6% N=11·5%

Preparation of 5-(2-carboethoxy-2-cyanovinyl)-2,4-dimethoxypyrimidine

To a suspension of 5-formyl-2,4-dimethoxypyrimidine (0·500g, 2·97mmole) in ethanol (10ml) was added ethyl cyanoacetate (0·336g, 2·97mmole) and piperidine (0·1ml). The resulting yellow solution was stirred at room temperature for 1·5 hours after which time the solvent was removed in vacuo and the product isolated by column chromatography with elution in 70:30 hexane/ethyl acetate followed by recrystallisation from ethanol to give the title compound as fine pale yellow needles (0·62g, 84%).

UV Spectrum pH 6 in ethanol

λ max. 350·5nm ε =20360

λ min. 284·0nm ε = 4155

λ max. 268·0nm ε = 8050

λ min. 240·0nm ε = 2580

NMR Spectrum $\delta(d_6$-DMSO) 9·08(1-H,s,vinylic H), 8·21(1-H,s,H-6), 4·30(2-H,q, $-OCH_2$), 4·05(3-H,s,$-OCH_3$), 4·00(3-H,s,$-OCH_3$), 1·32(3-H,t,CH_3).

FAB Mass spectrum m/e 264 $(M+H)^+$

Analysis $C_{12}H_{13}N_3O_4$ requires

C=54·75% N=4·97% N=15·96%

found C=54·8% N=5·0% N=16·0%

Preparation of 5-(2,2-dicyanovinyl)-2,4-dimethoxypyrimidine

To a suspension of 5-formyl-2,4-dimethoxypyrimidine (1·50g, 8·92mmole) in ethanol (30ml) was added malononitrile (0·588g, 8·9mmole) then piperidine (0·1 ml). The yellow solution was stirred at room temperature for 20 minutes after

which time the product started to crystallise from solution. TLC showed the reaction to be complete. The solvent was removed and the product isolated by column chromatography with elution in chloroform. Recrystallisation from ethanol gave the title compound as fine lemon yellow coloured crystals (1·72g, 89%).

UV Spectrum pH 6 in ethanol

λ max. 334·0nm ε =2590

λ min. 303·6nm ε =1680

λ max. 273·0nm ε =7315

λ min. 253·0nm ε =7440

NMR Spectrum $\delta(d_6\text{-DMSO})$ 8·95(1-H,s,vinylic H), 8·32(1-H,s,H-6), 4·01(6-H, s,2XCH_3).

EI Mass spectrum m/e 216 (M)

Analysis $C_{10}H_8N_4O_2$ requires

C=55·56% H=3·70% N=25·9%

found C=55·6% H=3·5% N=25·9%

Preparation of (E)-5-(2-azidocarbonylvinyl)-2,4-dimethoxypyrimidine

(E)-5-(2-carboxyvinyl)-2,4-dimethoxypyrimidine (0·400g, 1·90mmole) was gently refluxed in freshly redistilled thionyl chloride (8ml) for 10 minutes. The excess thionyl chloride was removed by distillation under reduced pressure. The resulting crude yellow acid chloride was dissolved in dry THF (10ml) and added dropwise to a stirred solution of sodium azide (0·18g) in 50:50 water/ acetone. The solution was stirred for 10 minutes then the solvent removed under high vacuum. The yellow oil obtained was applied to a silica gel column and eluted with 80:20 toluene/acetone, the appropriate fractions were applied and evaporated to give a white solid which was coevaporated with ethanol then dissolved in the minimum quantity of acetone at 50°C. The solution was filtered and stored overnight at 4°C. The resulting crystals were filtered off to give

the title compound as fine long white needles (0·32g, 72%).

UV Spectrum pH 6 in ethanol

λ max. 311·5nm ε =19720

λ min. 235·0nm ε = 2400

NMR Spectrum δ(d_6-DMSO) 8·75(1-H,s,H-6), 7·64(1-H,d,vinylic H,J=16·12Hz), 6·63(1-H.d.vinylics,J=16·12Hz), 4·02(3-H,s,OCH_3), 3.95(3-H,s,OCH_3).

EI Mass spectrum m/e 235 (M), 207 ($M-N_2$), 177($M-N_2-OCH_3$)

Analysis $C_9H_9N_5O_3$ requires

C=45·96% H=3·86% N=29·77%

found C=46·2% H=3·8% N=29·6%

Preparation of 1-(2,3,5-tri-O-benzoyl-α-D-ribofuranosyl)-4-methoxy-5-[(E)-azidocarbonylvinyl]-2(1*H*)pyrimidinone

To a solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (1·73g, 3·4mmole) in dry dichloromethane (5ml) was added stannic chloride (0·897g, 0·40 ml, 3·4mmole) and the solution was stirred at 0°C while a solution of (E)-5-(2-azidocarbonylvinyl)-2,4-dimethoxypyrimidine (0·81g, 3·4mmole) in dry dichloromethane (4ml) was added in one portion.

The reaction mixture was stirred at 0°C for 30 minutes then at room temperature for 7 hours after which time TLC analysis in 90:10 toluene/acetone showed the absence of the sugar and azide. The organic solution was poured onto ice/water (30ml) and separated and extracted sequentially with hydrochloric acid (2M, 5X15ml), aqueous sodium bicarbonate (1M,3X15ml) and water (15ml). The organic layer was dried with magnesium sulphate, filtered, and the solvent then removed by distillation under reduced pressure to give a white foam.

The product was purified by silica gel chromatography in 90:10 toluene/acetone then 50:50 ethyl acetate/ethanol to give the title compound as a white foam (1·19%, 52%).

UV Spectrum pH 6 in ethanol

λ max. 310·0nm ε =21560

λ min 230·0nm ε = 3050

NMR Spectrum δ(d_6-DMSO) 8·76(1-H,s,H-6), 8·03-7·40(16-H,complex m, aromatic protons, 1 vinylic), 6·51(1-H,d,vinylic H,J=16·2Hz), 6·30(1-H,d,H-1'), 4·87-4·74 (4-H,m,H-2',H-3',H-5'), 4·04-3·93(1-H,m,H-4'), 3·99(3-H,s,OCH_3).

FAB Mass spectrum m/e 666 $(M+H)^+$

Analysis $C_{34}H_{27}N_5O_{10}.H_2O$ requires

C=59·7% H=4·3% N=10·2%

found C=60·0% H=4·5% N=9·9%

Preparation of 3-β-D-ribofuranosyl-2,7-dioxo-5,6-dihydro-2*H*-pyrano-[2,3-d] pyrimidine

To a solution of 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4-methoxy-5-[(E)-5-azidocarbonylvinyl]-2(1*H*)pyrimidinone (0·448g, 0·673mmole) in dry methanol (150ml) was added sodium methoxide (0·036g, 0·66mmole) in dry methanol (30ml). The solution was stirred at room temperature for 3 hours then quenched with glacial acetic acid (0·5ml) and taken to dryness in vacuo. TLC in 90:10 chloroform/methanol shows a new slower moving nucleoside which was isolated by short column chromatography with elution in 90:10 chloroform/ethanol to give the title compound as a white powder (0·16g, 80%).

UV Spectrum pH 6 in ethanol

λ max. 277·0nm ε =15875

λ min. 235·0nm ε = 3420

NMR Spectrum δ(d_6-DMSO) 8·94(1-H,s,H-4), 7·41(1-H,d,H-5), 6·43(1-H,d,H-6), 5·75(1-H,d,H-1'), 5·59(1-H,bs,2'-OH), 5·45(1-H,bs,5'-OH), 5·05(1-H,bs,3'-OH), 4·01-3·64(5-H,m,H-2',H-3',H-4',H-5').

FAB Mass spectrum m/e 343 $(M+H+2Na)^+$, 165 $(M-ribose+2H)^+$

Preparation of 3-β-D-ribofuranosyl-2,7-dioxopyrido[2,3-d]pyrimidine

To 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4-methoxy-5-[(E)-azidocarbonyl-

vinyl]-2(1)pyrimidinone (0·3219g, 0·483mmole) was added methanol saturated at 0°C with dry ammonia gas (25ml) and the yellow solution stirred at room temperature for 48 hours. The solution was then evaporated to dryness and co-evaporated with methanol (2X40ml) to give a solid, TLC analysis of which in 70:30 chloroform/methanol showed a fast moving fluorescent nucleoside and a slow moving nucleoside.

The fluorescent nucleoside was obtained by column chromatography in 70:30 chloroform/methanol and recrystallisation from acetone/hexane to give the title compound, 0·01g (7%). The slower moving nucleoside was not identified as it decomposed on standing to give the title compound, decomposition was rapid on heating, to give a further yield, 0·09g. Total yield (0·1g,70%).

UV Spectrum pH 6 in ethanol

λ max.	331·0nm	ε =12200
λ min.	290·7nm	ε = 2605
λ max.	254·0nm	ε = 9810

NMR Spectrum $\delta(d_6$-DMSO) 11·75(1-H,bs,N-H), 9·05(1-H,s,H-4), 7·60(1-H,d,H-5, J=10·8Hz), 6·19(1-H,d,H-6,J=10·8Hz), 5·81(1-H,s,H-1'), 5·60(1-H,d,2'-OH), 5·38 (1-H,t,5'-OH), 5·10(1-H,d,3'-OH), 4·30-3·60(5-H,m,H-2',H-3',H-4',H-5').

FAB Mass spectrum m/e 296 $(M+H)^+$, 164 $(M+2H-ribose)^+$

Analysis $C_{12}H_{13}N_3O_6$ requires

C=48·82% H=4·44% N=14·23%

found C=48·5% H=4·1% N=13·9%

Preparation of (E)-5-(2-aminocarbonylvinyl)uridine

To (E)-5-(2-carbomethoxyvinyl)uridine (0·15g, 0·46mmole) was added concentrated aqueous ammonia solution (5ml). The resulting solution was stirred at room temperature for 24 hours after which time TLC in 90:10 chloroform/ethanol showed the disappearence of starting material. The excess ammonia solution was removed by distillation under reduced pressure and the resulting colourless solid was recrystallised from water to give long fine white needles of the

title compound (0·083g, 58%).

UV Spectrum pH 6 in ethanol

λ max.	301·0nm	ε=12380
shoulder	270·0nm	ε = 6050
λ min.	228·5nm	ε = 895

NMR Spectrum δ(d_6-DMSO) 11·50(1-H,s,N-H), 8·35(1-H,s,H-6), 7·50(1-H,s,H of NH_2), 7·15(H,d,vinylic H,J=15·6Hz), 6·90(1-H,d,vinylic H,J=15·6Hz), 6·90(1-H,s, H of NH_2), 5·76(1-H,d,H-1'), 5·40(1-H,m,2'-OH), 5·25(1-H,t,5'-OH), 5·05(1-H,m, 3'-OH), 4·20-3·80(3-H,m,H-2',H-3',H-4'), 3·65(2-H,m,H-5').

FAB Mass spectrum m/e 314 $(M+H)^+$, 627 $(2M+H)^+$.

Analysis $C_{11}H_{15}N_3O_7.H_2O$ requires

C=43·5% H=51·7% N=12·69%

found C=43.6% H=5·2% N=12·4%

Preparation of (E)-5-(2-methylaminocarbonylvinyl)uridine

To (E)-5-(2-carbomethoxyvinyl)uridine (1·50g, 4·57mmole) was added a 40% aqueous solution of methyl amine (40ml). After stirring for 2 hours, TLC analysis in 70:30 chloroform/methanol showed the absence of starting naterial. The reaction mixture was taken to dryness and applied to the top of a silica gel column and eluted with 50:50 ethyl acetate/ethanol. The appropriate fractions were combined, treated with celite and the solvent removed. Recrystallisation from methanol gave the title compound as fine white crystals (0·63g, 41%).

UV Spectrum pH 6 in ethanol

λ max.	310·0nm	ε=14280
λ min.	276·0nm	ε=10060
λ max.	265·0nm	ε=10650

NMR Spectrum δ(d_6-DMSO) 11·53(1-H,s,N-H), 8·34(1-H,s,H-6), 7·96(1-H,q,N<u>H</u>CH$_3$), 7·14(1-H,d,vinylic H,J=15·6Hz), 6·91(1-H,d,vinylic H,J=15·6Hz), 5·75(1-H,d,H-1'), 5·50-4·90(3-H,bs,2'-OH,3'-OH,5'-OH), 4·05(2-H,m,H-2',H-3'), 3·85(1-H,m,H-4'), 3·65(2-H,bs,H-5'), 2·63(3-H,d,NHC<u>H</u>$_3$).

FAB Mass spectrum m/e 328 $(M+H)^+$

Analysis $C_{13}H_{17}N_3O_7.H_2O$ requires

C=45·21% H=5·55% N=12·16%

found C=45.5% H=5.6% N=12·0%

Preparation of (E)-5-(2-dimethylaminocarbonylvinyl)uridine

To (E)-5-(2-carbomethoxyvinyl)uridine (0·5g, 1·52mmole) was added 25/30% aqueous solution of dimethylamine (25ml). After 48 hours stirring at room temperature TLC analysis in 80:20 chloroform/methanol showed the presence of two new nucleosides, one of rf. 0·45, the other baseline. The solvent was removed by distillation under reduced pressure and traces of amine removed by coevaporation with water (5X20ml).

Recrystallisation from water gave crystals with an rf. 0·45 which were further purified by short column chromatography with elution in 80:20 chloroform /methanol then recrystallisation from water. They were identified as the title compound (0·0348g, 7%).

UV Spectrum pH 6 in ethanol

λ max.	302·7nm	ε =16670
shoulder	270·4nm	ε =10500
λ min.	225·0nm	ε = 1180

NMR Spectrum δ(d_6-DMSO) 11·61(1-H,s,N-H), 8·45(1-H,s,H-6), 7·48(1-H,d, vinylic H,J=15·32Hz), 7·19(1-H,d,vinylic H,J=15·32Hz), 5·77(1-H,d,H-1'), 5·48 (1-H,d,2'-OH), 5·35(1-H,t,5'-OH), 5·11(1-H,d,3'-OH), 4·08(1-H,m,H-2'), 4·01(1-H, m,H-3'), 3·87(1-H,m,H-48), 3·80-3·50(2-H,m,H-5'), 3·05(3-H,s,NCH_3), 2·90(3-H, s,NCH_3).

FAB Mass spectrum m/e 342 $(M+H)^+$, 210 $(M+2H-ribose)^+$

Analysis $C_{14}H_{19}N_3O_7.H_2O$ requires

C=46·79% H=5·89% N=11·69%

found C=47·0% H=5·6% N=11·4%

The filtrate contained mainly the baseline component. The solvent was removed by distillation in vacuo and the white solid recrystallised from methanol to give crystals identified as 5-(1-dimethylamino-2-carboxyethyl)-uridine (0·0852g, 16%).

UV Spectrum pH 6 in ethanol

λ max. 269.5nm ε =7470

λ min. 229·0nm ε = 320

NMR spectrum $\delta(d_6$-DMSO) 11·37(1-H,s,N-H), 8·01(1-H,s,H-6), 5·78(1-H,d,H-1'), 5·50-5·00(3-H,vbs,2'-OH,3'-OH,5'-OH), 4·23-4·10(1-H,m,-CH-), 4·10-4·00(2-H,m, H-2',H-3'), 3·89(1-H,bs,H-4'), 3·72-3·57(2-H,m,H-5'), 2·76(1-H,m,1 of CH_2COOH), 2·36(1-H,m,1 of CH_2COOH), 2·14(2-H,s,NCH_3), 2·13(3-H,s,NCH_3).

FAB mass spectrum m/e 359 (M)

Analysis $C_{14}H_{21}N_3O_8$ requires

C=46·79% H=5·89% N=11·69%

found C=46·6% H=5·9% N=11·4%

Preparation of (E)-5-(2-aminocarbonylvinyl)-2'-deoxyuridine

To (E)-5-(2-carbomethoxyvinyl)2'-deoxyuridine (1·00g, 3·2mmole) was added concentrated aqueous ammonia solution (35ml). After 24 hours, TLC in 80:20 chloroform/methanol showed the absence of starting material. The solvent was removed under reduced pressure and the resulting white solid applied to the top of a silica gel column eluted with 80:20 chloroform/methanol. The fractions were combined, solvent removed in vacuo and the white solid recrystallised from water to give the title compound as fine white crystals (0·75g, 79%).

UV Spectrum pH 6 in ethanol

λ max. 300·0nm ε =10540

shoulder 270·0nm ε = 1990

NMR Spectrum $\delta(d_6$-DMSO) 11·55(1-H,s,N-H), 8·38(1-H,s,H-6), 7·50(1-H,s,1 of NH_2), 7·15(1-H,d,vinylic H,J=15·67Hz), 6·94(1-H,d,vinylic H,J=15·67Hz), 6·94(1-H,

s,1 of NH_2), 6·15(1-H,t,H-1'), 5·40-5·00(2-H,m,3'-OH,5'-OH), 4·28(1-H,t,H-3'), 3·80(1-H,m,H-4'), 3·63(2-H,m,H-5'), 2·15(2-H,t,H-2').

FAB mass spectrum m/e 298 $(M+H)^+$

Analysis $C_{12}H_{15}N_3O_6$ requires

C=48·5% H=5·08% N=14·13%

found C=48·8% H=5·3% N=14·4%

Preparation of (E)-5-(2-dimethylaminocarbonylvinyl)-2'-deoxyuridine

To (E)-5-(2-carbomethoxyvinyl)-2'-deoxyuridine (1·00g, 3·2mmole) was added an aqueous solution of dimethylamine (25ml). After 24 hours, TLC analysis in 75:25 chloroform/ethanol showed the absence of starting material. The reaction mixture was taken to dryness and the white solid applied to the top of a silica gel column eluted with 75:25 chloroform/ethanol. The solvent was then removed in vacuo to give a white solid which was recrystallised from methanol (0·145g, 9%).

UV Spectrum pH 6 in ethanol

λ max.	303·0nm	ε =19160
shoulder	270·0nm	ε =12420
λ min.	224.0nm	ε = 3250

NMR Spectrum δ(d_6-DMSO) 11·59(1-H,s,N-H), 8·37(1-H,s,H-6), 7·40(1-H,d, vinylic H,J=16·2Hz), 7·20(1-H,d,vinylic H,J=16·2Hz), 6·15(1-H,t,H-1'), 5·26(1-H, d,3'-OH), 5·20(1-H,t,5'-OH), 4·27(1-H,s,H-3'), 4·09(1-H,q,$CH_3\underline{OH}$), 3·79(1-H,d,4'-OH), 3·70-3·50(2-H,m,H-5'), 3·17(3-H,d,$C\underline{H}_3OH$), 3·05(3-H,s,NCH_3), 2·90(3-H,s, NCH_3), 2·18(2-H,m,H-2').

FAB Mass spectrum m/e 326 $(M+H^+$, 210 $(M+2H-sugar)^+$

Analysis $C_{14}H_{19}N_3O_6.CH_3OH$ requires

C=50·4% H=6·48% N=11·76%

found C=50·1% H=6·3% N=11·6%

Preparation of 5-hydroxymethyl-2',3'-isopropylideneuridine

A solution of 2',3'-iospropylideneuridine (10·00g, 35·0mmole) and para-formaldehyde (2·20g) in 0·5M aqueous potassium hydroxide (70ml) was heated to 50°C for 2 days after which time TLC in 90:10 chloroform/ethnaol showed the absence of starting material. The solution was adjusted to pH 7 with glacial acetic acid and then the solvent removed by distillation under reduced pressure. The resulting viscous oil was dissolved in chloroform (100ml), dried with $MgSO_4$, filtered, and after concentration to a small volume was applied to the top of a silica gel column and eluted with 90:10 chloroform/ethanol. The appropriate fractions were pooled and concentreated in vacuo to give the product as a white foam (9·50g, 86%).

UV Spectrum pH 6 in ethanol

λ max. 264·0nm ε =10255

λ min. 233·0nm ε = 3075

NMR Spectrum (d_6-DMSO) 11·40(1-H,s,N-H), 7·65(1-H,s,H-6), 5·85(1-H,d,H-1'), 5·15-4·65(4-H,m,H-2',H-3',2XOH), 4·10(3-H,m,H-4',CH_2OH), 3·55(2-H,t,H-5'), 1·46(3-H,s,CH_3), 1·28(3-H,s,CH_3).

FAB Mass spectrum m/e 315 $(M+H)^+$

Preparation of 5-formyl-2',3'-isopropylideneuridine

To a solution of pyridinium dichromate (33·21g, 88·3mmole) in dry DMF (40ml) was added 5-hydroxymethyl-2',3'-isopropylideneuridine (18·50g, 58·0mmole) in dry dichloromethane/DMF (7:1, 230ml) and the resulting solution stirred at room temperature for one hour or until the black solution shows total conversion to a faster running nucleoside product by TLC in 95:5 chloroform/ethanol. The solvent was removed under high vacuum and the resulting black oil applied as a dry pack to a silica gel column prepared in 95:5 chloroform/ethanol. The appropriate fraction were pooled, solvent removed and the product obtained as white crystals upon recrystallisation from ethanol (11·53g, 63%).

UV Spectrum pH 6 in ethanol

λ max. 288·4nm ε =10330

λ min. 246·6nm ε = 1770

NMR Spectrum $\delta(d_6$-DMSO) 11·88(1-H,s,N-H), 9·73(1-H,s,CHO), 8·68(1-H,s,H-6), 5·85(1-H,d,H-1'), 5·15(1-H,t,5'-OH), 4·94(1-H,m,H-2'), 4·73(1-H,m,H-3'), 4·23 (1-H,m,H-4'), 3·57(2-H,t,H-5'), 1·45(3-H,s,CH_3), 1·25(3-H,s,CH_3).

FAB Mass spectrum m/e 313 $(M+H)^+$

Analysis $C_{13}H_{16}N_2O_7$ requires

C=50·00% H=5·16% N=8·97%

found C=50·0% H=5·1% N=9·2%

Preparation of 5-(1-hydroxy-2-nitroethyl)-2',3'-isopropylideneuridine

To a stirred suspension of 5-formyl-2',3'-isopropylideneuridine (0·300g, 0·96mmole) in dry THF (5ml) and dry nitromethane (5ml) was added sodium hydride (0·182g, 3.0mmole) and the resulting yellow solution stirred at room temperature for 30 minutes. After acidification with glacial acetic acid (0·2ml), TLC showed the presence of a new slower running product. The solvent was removed in vacuo and the new component isolated by short column chromatography in 95:5 chloroform/methanol to give the product (0·230g, 64%).

UV Spectrum pH 6 in ethanol

λ max. 262·8nm ε =9820

λ min. 229·0nm ε =3240

NMR Spectrum $\delta(d_6$-DMSO) 11·61(1-H,s,N-H), 7·83(1-H,d,H-1',J=8·1Hz), 5·98 (1-H,t,-CH(OH)-), 5·85(1-H,d,H-1'), 5·07(2-H,m,H-2',5'-OH), 4·90(1-H,m,H-3'), 4·77(2-H,m,CH_2NO_2), 4·51(1-H,t,CH), 4·08(1-H,m,H-4'), 3·57(2-H,t,H-5'), 1·49 (3-H,s,CH_3), 1·29(3-H,s,CH_3).

FAB Mass spectrum m/e 374 $(M+H)^+$

Analysis $C_{14}H_{19}N_3O_9$ requires

C=45·04% H=5·13% N=11·25%

found C=45·0% H=5·1% H=11·2%

Preparation of 5-(1-nitromethyl-2-nitroethyl)-2',3'-isopropylideneuridine

Sodium (0·116g, 5·0mmole) was dissolved in dry ethanol (15ml) then nitromethane (0·30g, 0·27ml, 5·04mmole) and 5-formyl-2',3'-isopropylideneuridine (0·315g, 1·00mmole) were added. The solution was gently refluxed for 1·5 hours then adjusted to pH 7 with glacial acetic acid. The solvent was removed in vacuo and the product isolated by column chromatography in 95:5 chloroform/ethanol to give a yellow coloured solid which was then recrystallised from ethanol to give the product as colourless crystals (0·27g, 57%).

UV Spectrum pH 6 in ethanol

λ max. 265·5nm ε =8470

λ min. 230·0nm ε =2260

NMR Spectrum δ(d_6-DMSO) 11·70(1-H,s,N-H), 7·95(1-H,s,H-6), 5·76(1-H,s,H-1') 5·14(1-H,t,5'-OH), 4·87(5-H,m,CH_2NO_2,H-4'), 4·75(1-H,m,CH), 4·10-4·00(1-H,m,H-2', H-3'), 3·57(2-H,m,H-5'), 1·48(3-H,s,CH_3), 1·29(3-H,s,CH_3).

FAB Mass spectrum m/e 417 $(M+H)^+$, 245 $(M+2H\text{-ribose})^+$

Analysis $C_{15}H_{20}N_4O_{10}$ requires

C=43·27% H=4·84% N=13·46%

found C=43·3% H=4·7% N=13·2%

Preparation of 5-(1-nitromethyl-2-nitroethyl)uridine

5-(1-Nitromethyl-2-nitroethyl)-2',3'-isopropylideneuridine (0·0986g, 0·236 mmole) was stirred in solution in 50% aqueous trifluoroacetic acid (5ml) for 30 minutes after which time TLC in 80:20 chloroform/methanol showed the absence of starting material. The solution was evaporated to dryness under high vacuum and the product then obtained by short column chromatography in 80:20 chloroform/methanol then recrystallisation from ethanol (0·062g, 70%).

UV Spectrum pH 6 in ethanol

λ max. 268·0nm ε =8850

λ min. 232·6nm ε=2560

NMR Spectrum δ(d_6-DMSO) 11·60(1-H,bs,N-H), 8·05(1-H,s,H-6), 5·74(1-H,d, H-1'), 5·42(1-H,d,2'-OH), 5·18(1-H,t,5'-OH), 4·95(2-H,s,CH_2NO_2),4·87(2-H,s, CH_2NO_2), 4·35(1-H,t,3'-OH), 4·25-3·50(5-H,m,H-2',H-3',H-4',H-5',CH).

FAB Mass spectrum m/e 377 $(M+H)^+$

Analysis $C_{12}H_{16}N_4O_{10}$ requires

C=38·3% H=4·3% N=14·9%

found C=38·2% H=4·6% N=14·6%

Preparation of 5-iodo-2',3'-isopropylideneuridine

5-Iodouridine (8·00g, 21·6mmole), dry acetone (150ml), anhydrous copper (II) sulphate (15·0g) and sulphuric acid (0·1ml) were stirred vigorously for 4 hours when TLC in 92:8 chloroform/ethanol showed no starting material. Short column chromatography in 98:2 chloroform/ethanol followed by recrystallisation from ethanol afforded the title compound (in two crops, 7·12g, 80%).

UV Spectrum pH 6 in ethanol

λ max. 275·0nm ε =10575

λ min. 240·0nm ε = 2950

NMR Spectrum δ(d_6-DMSO) 11·70(1-H,s,N-H), 8·30(1-H,s,H-6), 5·80(1-H,s, H-1'), 5·15(1-H,t,5'-OH), 4·94(1-H,m,H-2'), 4·75(1-H,m,H-3'), 4·10(1-H,m,H-4'), 3·60(2-H,d,H-5'), 1·46(3-H,s,CH_3), 1·25(3-H,s,CH_3).

FAB Mass spectrum m/e 411 $(M+H)^+$

Preparation of 5-iodo-2',3'-isopropylidene-5'-methoxymethyluridine

5-Iodo-2',3'-isopropylideneuridine (5·90g, 14·3mmole), dry acetone (75ml), dry dimethoxymethane (dried then fractionated from sodium wire, 260ml) and methane sulphonic acid (2ml) were stirred at room temperature for 24 hours then poured into ammonia solution (d=0·880, 100ml) then evaporated to dryness, dissolved in chloroform (100ml), dried with magnesium sulphate then applied to

the top of a silica gel column and eluted with chloroform/ethanol 95:5. the appropriate fractions were pooled and evaporated under reduced pressure to give the product as a white foam (5·68g, 87%).

UV Spectrum pH 6 in ethanol

λ max. 284·6nm ε =5020

NMR Spectrum $\delta(d_6\text{-DMSO})$ 11·72(1-H,s,N-H), 8·16(1-H,s,H-6), 5·80(1-H,d, H-1'), 4·98(1-H,m,H-2'), 4·75(1-H,m.H-3'). 4·60(2-H,s,$-OCH_2OCH_3$), 4·20(1-H,q, H-4'), 3·64(2-H,t,H-5'), 3·30(3-H,s,OCH_3), 1·45(3-H,s,CH_3), 1·28(3-H,s,CH_3).

FAB Mass spectrum m/e 455 $(M+H)^+$

Analysis $C_{14}H_{19}IN_2O_7$ requires

C=37·02% H=4·2% N=6·17%

found C=36·9% H=4·0% N=5·9%

Preparation of 5,6-diiodo-2',3'-isopropylidene-5'-methoxymethyluridine

Into a dry 30ml sample tube was weighed diisopropylamine (0·56g, 5·00mmole) then dry THF (20ml) was added. After thorough mixing, the solution was injected into a dry 3 necked flask (250ml) fitted with a rubber septum and through which dry nitrogen was flowing. The flask was then immersed in an acetone/dry ice bath at -78°C and, with the contents of the flask stirring, n-butyl lithium solution in n-hexane (1·6M, 3·2ml, 5·00mmole) was added dropwise keeping the temperature of the contents of the flask below -70°C.

After stirring for 1 hour, a solution of 5-iodo-2',3'-isopropylidene-5'-methoxymethyluridine (0·454g, 1·00mmole) in dry THF (10ml) was added dropwise via syringe then the dianion solution was stirred for 2 hours. Then, iodine (0·577g, 2.3mmole) in dry THF (15ml) was added dropwise and the reaction mixture stirred for a further 3 hours.

After quenching with glacial acetic acid (0·3ml) and warming to room temperature, the solvent was removed in vacuo and the resulting dark solid then applied to the top of a silica gel column prepared in 80:20 toluene/acetone with which it was eluted. After recrystallisation of the appropriate fractions from

acetone/hexane the product was obtained as white crystals (0·230g, 40%.

UV Spectrum pH 6 in ethanol

λ max. 289·5nm ε =6410

λ min. 247·0nm ε =2060

NMR Spectrum δ(d_6-DMSO) 11·99(1-H,s,N-H), 6·28(1-H,s,H-1'), 5·20(1-H,d, H-2'), 4·74(1-H,m,H-3'), 4·54(2-H,s,OCH_2OCH_3), 4·12(1-H,m,H-4'), 3·61(2-H,m, H-5'), 3·23(3-H,s,OCH_3), 1·49(3-H,s,CH_3), 1·28(3-H,s,CH_3).

FAB mass spectrum m/e 581 $(M+H)^+$

Preparation of 5,6-diiodo-5'-methoxymethyluridine

A solution of 5,6-diiodo-2',3'-isopropylidene-5'-methoxymethyluridine (0·190g, 0·327mmole) in aqueous trifluoroacetic acid (30%, 20ml) was kept at 4°C for 12 hours then at room temperature for 4 hours after which time TLC in 95:5 chloroform/ethanol showed the absence of two slower running nucleoside products, the faster running being the major component.

Column chromatography in 95:5 chloroform/ethanol gave the first component from which the solvent was removed by distillation in vacuo to give the title compound as a white powder (0·055g, 31%), which slowly decomposes with the evolution of iodine upon exposure to air and light.

UV Spectrum pH 6 in ethanol

λ max. 288·0nm ε =7380

λ min. 245·0nm ε =1685

NMR Spectrum δ(d_6-DMSO) 11·84(1-H,s,N-H), 5·85(1-H,d,H-1'), 5·25(1-H,d, 3'-OH), 5·04(1-H,d,2'-OH), 4·55(2-H,s,OCH_2OCH_3), 4·10(1-H,m,H-4'), 3·60(2-H,m, H-5'), 3·25(3-H,s,OCH_3).

FAB Mass spectrum m/e 541 $(M+H)^+$

Preparation of (E)-5-(2-carbomethoxyvinyl)-2',3'-isopropylideneuridine

To a suspension of (E)-5-(2-carbomethoxyvinyl)uridine (4·00g, 12·18mmole) in dry acetone (500ml) was added anhydrous copper (II) sulphate (17·0g) and concentrated sulphuric acid (0·30ml). After vigorous stirring for 3 hours, TLC in 85:15 chloroform/methanol showed the absence of starting material. The suspension was filtered, the solid washed with dry acetone (3X100ml) and the combined filtrates stirred with potassium carbonate (5·00g) for 1 hour, then filtered, concentrated under reduced pressure and the residue applied to the top of a silica gel column and eluted with 85:15 chloroform/methanol. the appropriate fractions were pooled, the solvent evaporated in vacuo and the resulting solid recrystallised from acetone/hexane to give the product (3·22g, 70%).

UV Spectrum pH 6 in ethanol

λ max.	301·0nm	ε =23920
shoulder	267·0nm	ε =13870
λ min.	228·4nm	ε = 4120

NMR spectrum $\delta(d_6$-DMSO) 11·73(1-H,s,N-H), 8·35(1-H,s,H-6), 7·35(1-H,d, vinylic H,J= 16 Hz), 6·83(1-H,d,vinylic H, 16 Hz), 5·85(1-H,d,H-1'), 5·22(1-H,t, 5'-OH), 4·95(1-H,m,H-2'), 4·76(1-H,m,H-3'), 4·14(1-H,m,H-4'), 3·65(5-H,m,H-5', OCH_3), 1·47(3-H,s,CH_3), 1·30(3-H,s,CH_3).

FAB Mass spectrum m/e 369 $(M+H)^+$

Analysis $C_{16}H_{20}N_2O_8$ requires

C=52·17% H=5·47% N=7·6%

found C=52·35% H=5·5% N=7·8%

Preparation of (E)-5-(2-carbomethoxyvinyl)-2',3'-isopropylidene-5'-methoxymethyl-uridine

To (E)-5-(2-carbomethoxyvinyl)-2',3'-isopropylideneuridine (1·30g, 3·53 mmole) in dry acetone (100ml) was added dimethoxymethane (200ml, refluxed over then fractionated from sodium) and methane sulphonic acid (0·2ml). The suspension

was stirred for 2 days at room temperature after which time dissolution had occured. Then, triethylamine (2ml) was added and the solvent removed by distillation under reduced pressure. After column chromatography with elution in 95:5 chloroform/ethanol, the product was obtained by recrystallisation from ethanol to give fine white needles (1·34g, 92%).

UV Spectrum pH 6 in ethanol

λ max.	299·0nm	ε =18480
shoulder	268·5nm	ε =12870
λ min.	229·0nm	ε = 3120

NMR Spectrum δ (d_6-DMSO) 11·76(1-H,s,N-H), 8·24(1-H,s,H-6), 7·35(1-H,d, vinylic H,J= 16 Hz), 5·84(1-H,d,H-1'), 5·03(1-H,m,H-2'), 4·80(1-H,m,H-3'), 4·58 (2-H,m,$C\underline{H}_2OCH_3$), 4·23(1-H,m,H-4'), 3·67(5-H,m,H-5',$CO_2C\underline{H}_3$), 3·28(3-H,s,OCH_3), 1·50(3-H,s,CH_3), 1·30(3-H,s,CH_3).

FAB Mass spectrum m/e 413 $(M+H)^+$

Analysis $C_{18}H_{24}N_2O_9$ requires

C=52·42% H=5·86% N=6·79%

found C=52·2% H=6·0% N=7·1%

Preparation of (E)-5-(2-carbomethoxyvinyl)-2',3'-isopropylidene-5'-methoxymethyl-6-methyluridine

To a stirred solution of LDA (5·0mmole) in dry THF (20ml) under a nitrogen atmosphere was added (E)-5-(2-carbomethoxyvinyl)-2',3'-isopropylidene-5'-methoxymethyluridine (0·412g, 1·0mmole) in dry THF (15ml) dropwise keeping the temperature below -70°C. After 1 hour, dry methyl iodide (0·710g, 5·0mmole) was added to the yellow solution of the dianion and the resulting suspension then stirred below -70°C for 2 hours then quenched with glacial acetic acid (0·4ml). After warming to room temperature, the solvent was removed by distillation under reduced pressure and the product isolated by short column chromatography with elution in 80:20 toluene/acetone. Recrystallisation from acetone/hexane afforded

the title compound (0·280g, 66%)

UV Spectrum pH 6 in ethanol

λ max. 301·4nm ε =20945

λ min. 224·0nm ε = 2410

NMR spectrum $\delta(d_6$-DMSO) 11·74(1-H,s,N-H), 7·48(1-H,d,vinylic H, J=16 Hz), 6·88(1-H,d,vinylic H,J=16 Hz), 5·85(1-H,s,H-1'), 5·24(1-H,d,H-3'), 4·78(1-H,t, H-2'), 4·54(2-H,s,CH_2OCH_3), 4·13(1-H,q,H-4'), 3·80-3.45(5-H,m,CO_2CH_3,H-5'), 3·24 (3-H,s,CH_3), 1·48(3-H,s,CH_3), 1·38(3-H,s,CH_3).

FAB Mass spectrum m/e 427 $(M+H)^+$

Analysis $C_{19}H_{26}N_2O_9$ requires

C=53·51% H=6·14% N=6·57%

found C=53·5% H=6·0% N=6·5%

Preparation of (E)-5-(2-carbomethoxyvinyl)-5'-methoxymethyl-6-methyluridine

(E)-5-(2-carbomethoxyvinyl)-2',3'-isopropylidene-5'-methoxymethyl-6-methyl uridine (0·168g, 0·39mmole) in an aqueous solution of trifluoroacetic acid (25%, 15ml) was stirred at room temperature for 4 hours after which time TLC in 80:20 chloroform/methanol showed total conversion to a slower running nucleoside. This was isolated by evaporation of the reaction mixture to dryness under high vacuum and column chromatography of the resulting white solid with elution in 80:20 chloroform/methanol. This gave the title compound as a white powder (0·10g, 66%).

UV Spectrum pH 6 in ethanol

λ max. 300·5nm ε =18965

λ min. 225·0nm ε = 2100

NMR Spectrum $\delta(d_6$-DMSO) 11·55(1-H,bs,N-H), 7·50(1-H,d,vinylic H, J=16Hz), 6·89(1-H,d,vinylic H,J=16Hz), 5·52(1-H,d,H-1'), 5·29(1-H,bs, 2'-OH), 5·08(1-H, bs,3'-OH), 4·55(2-H,s,CH_2OCH_3), 4·15(1-H,m,H-4'), 3·95-3·50(7-H,m,CO_2CH_3,H-2',

H-3',H-5'), 3·25(6-H,s,CH_3,OCH_3).

FAB Mass spectrum m/e 387 $(M+H)^+$

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THE SYNTHESIS OF MASKED NUCLEOSIDE MONOPHOSPHATES

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SYNOPSIS

A review is presented of the development of anti-virals and the main targets towards which they have been aimed. Emphasis is placed upon nucleoside analogues to reflect their importance in chemotherapy.

The work described in this thesis has been orientated towards the synthesis of masked nucleoside monophosphates which should be metabolised to their parent molecule *in vivo*.

The mechanism of the *in vivo* activation of the anti-cancer agent "cyclophosphamide" is described and the possible application of a similar transport system for the production of masked nucleoside monophosphates is discussed. The synthesis of 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide is described and the condensation of this molecule onto the 5'-position of several preformed nucleosides is presented. The synthesis of several new nucleosides has been achieved and the results of preliminary biological testing is presented

TO MY MOTHER FOR HER CONTINUOUS SUPPORT AND ENCOURAGEMENT AND Dr.A.GREEN FOR HIS IRREPLACEBLE FRIENDSHIP. FINALLY, TO THE BOYS OF 'B' BLOCK.

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TABLE OF CONTENTS

INTRODUCTION

Advances in the practice of medicine over the past two decades have been immense, particularly in the application of biomedical technology to the diagnosis and treatment of human disease. In contrast to this, human viral infections have proved particularly difficult to diagnose and treat.

The first effective viral vaccine was reported by Jenner in 1798 for the control of smallpox. It was another, 150 years before the first chemical antiviral agent was discovered.

After the discovery of the sulphonamides as potent antibacterials it occurred to Domagk to enhance their activity against the tubercle bacillus by cyclizing the sulphur-containing side chain, using thiosemicarbazones of benzaldehyde as a starting material. These were tested against *Mycobacterium tuberculosis* but all but one were inactive.

At the same time progress was being made in the handling of viruses in the laboratory that would lead to a practical way of drug screening. Brownlee and Hamre[1] discovered that when vaccinia virus was injected into the yolk sac of fertile eggs, the survival time was inversely proportional to the amount of virus injected. This gave a means of detecting antiviral activity, since any compound that reduced the multiplication of the virus would reveal its effect by prolonging the survival time of the embryo.

In the climate of opinion at that time, the probability of finding a clinically useful antiviral agent seemed remote.

Nevertheless, Brownlee and Hamre decided to use their system to test for antiviral drugs. The thiosemicarbazones were very much in the news at that time and, whether for that reason or another, they conducted their first test with p-amino-benzaldehyde thiosemicarbazone. They immediately found it to produce a reduction in virus replication. This work led Bauer[2] to test 1-methylindole-2,3-dione 3- thiosemicarbazone (1) against variola virus and by 1960 it was in clinical use as a prophylactic against smallpox under the name methisazone.

(1)

Antiviral chemotherapy had meanwhile been progressing in a different direction. Whereas the discovery of the thiosemicarbazones was a chance affair, a programme of directed research was developing synthetic nucleosides as antineoplastic agents due to their inhibition of DNA synthesis. One compound chosen for investigation was 5-iodo-2'-deoxyuridine (IdU,(2)). Initial work with IdU as an antineoplastic was disappointing. However, Kaufman[3] decided to look at its effect on double stranded DNA viruses, and was able to show that repeated application of a solution of IdU would cure herpetic keratitis in rabbits and man.

(2)

It is not clear from the literature whether methisazone or IdU was the first antiviral used in practice but it is certain that by 1962 antiviral chemotherapy had become a clinical reality.

Before we look in more detail at how strategies against viruses have developed, it is important to understand the need for effective antivirals in the world today.

Mortality from viral infections, unlike death from coronary artery diseases, hypertension and accidents, remains relatively insignificant for the mass population of the western world but it is a problem in the Third World.

In Africa, Asia and Latin America, an alarming cause of mortality is measles which results in many dying each year. It is estimated that virus-related diarrhoea is responsible for a further 5-10 million deaths and another 4-5 million are due to virus-related respiratory illness. Thus measles virus, influenza virus, poliovirus, rotavirus and coronavirus are responsible for the clinical symptomology of these countries. Excellent vaccines already exist for measles and polio but the lack of proper

refrigeration reduces the uselfulness of these in the third world. There clearly remains a need in the Third World for the control by chemotherapy of viruses which are already controlled in the Developed World. We may ask who will pay for research into diseases which we can already treat here?

Developed countries have concentrated their efforts on the control of respiratory viruses, rotaviruses, herpesviruses and more recently human immunodeficiency virus (HIV).

The development of specific antiviral drugs that are minimally toxic to normal cells has become more important with the threat to the lives of immunocompromised patients from cytomegalovirus infections[4], the increasing morbidity of recurrent genital herpes simplex infections[5] and most notably the rising epidemic of acquired immunodefficiency syndrome (AIDS) caused by human immunodeficiency virus[6].

In theory, antiviral drugs can work by interacting directly with the virus, with a viral coded enzyme or other protein, or with a cellular receptor or factor required for viral replication or pathogenesis. To date, the viral enzymes and proteins that affect assembly of the virus have been the effective molecular targets of antiviral treatment. An understanding of how antiviral drugs interact with viral or cellular function is an important factor in the prelude to the development of a safe and effective antiviral drug. By knowing how antivirals act at a molecular level we can create better and safer drugs, advise on protocols to produce synergistic effects[7] and avoid the antagonistic action of drugs[8].

Table 1.Viral Targets

1. Extracellular
a. Antibody
b. Chemicals
2. Adsorption of virus to cell membrane
3. Transport of virus across the cell membrane
4. Uncoating of virus
5. Intracellular events
6. Transcription (RNA formation)
7. Methylation of RNA
8a. Protein synthesis or processing
8b. Glycoprotein sysnthesis
9. Enzymes

Table 1[9] indicates various targets for which antiviral drugs have been demonstrated to exert an effect, or are potential targets for future development.

1 Extracellular Target

Before penetration of the virus into the cell it is possible to target the free virion with a variety of biological and physical agents and some of these have been of use in producing vaccines[10]:

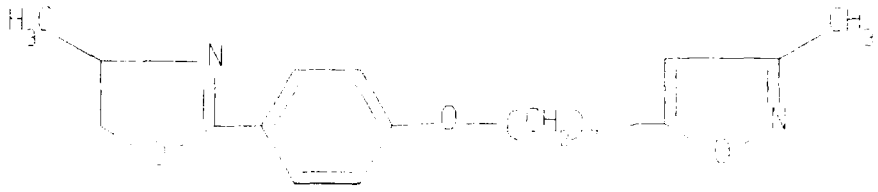
1a Antibodies

HIV can be prevented from attaching itself to cells by blocking its surface receptors. The receptor on the cell surface is known as CD4 and the gene for this has been cloned. This receptor,

expressed in a soluble form, binds to a glycoprotein spike (gp120) on the HIV envelope and stops the virus from entering the cells which express the CD4 receptor[11]. The CD4 molecule can also be linked to a Pseudomonas exotoxin that attaches itself to the cells which express the gp120 glycoprotein on their surface. This toxin might be taken up selectively by HIV-infected cells killing them and sparing uninfected cells[12]. However, recent work has shown that the CD4 receptor on T-cells interacts with MHC II (major histocompatibility complex) cells on target cells and gp120 on HIV. After mixing MHC II with T(CD4+)-cells, internalization of CD4 is seen as the cell membrane undergoes endocytosis. By using mutant cells with CD4 molecules incapable of undergoing endocytosis, it was shown that HIV can still enter the cell.[13]

1b Chemical Agents

There is a range of chemical agents which inactivate the virus outside the cell. Studies on Rhinovirus 14, one cause of the common cold, first described the succesful interaction of an antiviral drug with a virion at the atomic level[14]. The three dimensional structure of Rhinovirus 14 has been described. The icosahedral protein shell has a 250nm "canyon" around each of its 12 vertices which is thought to be the site that interacts with the cellular receptor. The protein sequence of the canyon floor is conserved among picornaviruses, suggesting that this region has structural importance. Drugs which fit into this site increase the viral stability considerably and prevent uncoating (3).



(3)

A mixture of neutral glycerides, phosphatidylcholine and phosphatidylethanolamine (7:2:1) affects HIV stability and it is thought that this is due to reduction of the cholesterol content of the viral membrane causing changes in the membrane fluidity which interferes with viral attachment[15].

Sodium dodecyl sulphate and non-ionic detergents (Triton-X-100) (4) inactivate viruses by solubilizing the viral envelope. Compounds such as guanidine (5), urea or phenol dissociate the polypeptides into their individual components. Formaldehyde interacts with amino groups of nucleic acid bases as well as the free amino groups of proteins.

(4)

(5)

2/3Viral Attachment and/or Penetration of Virus as Targets

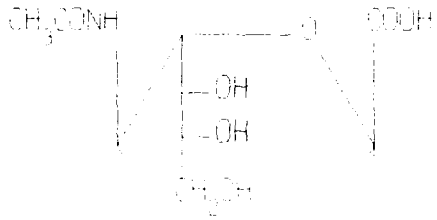
In most cases of virus infection of mammalian cells, there exist specific high-affinity receptors located on the cell surface which appear to mediate viral adsorption and entry into the cell[16]

Without these sites, it is likely that effects such as charge, dilution and particle motion would minimise collisions and virus uptake into the cell. These sites may be glycoprotein (as in HIV), glycolipid (as in vesicular stomatitis virus) or contain functional sulphur groups (as in picornaviruses).

Orthomyxovirus (influenza) and Paramyxovirus (mumps, parainfluenza) bind to an erythrocytic receptor which consists of N-acetylneuraminic acid (NANA) (6). The protein on the influenza virus responsible for binding to NANA is haemagglutinin (HA). Before penetration, HA must be activated by a cellular protease which cleaves HA into HA_1 and HA_2. 2-Deoxy-2,3-dihydro-N-trifluroacetyl-neuraminic acid (FANA) (7) is an analogue of NANA and has a strong in vitro action on orthomyxoviruses. It acts by inhibiting neuraminidase and causes an accumulation of neuraminic acid in the virus envelope which produces self-aggregation of the virus

Paramyxovirus has a protein spike which is cleaved to form F_1 and F_2 which are bonded by a disulphide link. A new N-terminus on the F_1-polypeptide is created which has a highly hydrophobic amino acid sequence and it is believed that this interacts with the cell membrane[17]. Richardson et al.[18] have synthesised various

oligopeptides resembling this region and carbobenzoxy-D-Phe-L-Phe-Gly-D-Ala-D-Val-D-Ile-Gly and carbobenzoxy-D-Phe-L-Phe-Gly produced potent inhibition of the measles virus.



(6)

(7)

Dubovi et al.[19] found that several aromatic mono-and di-amidines retarded the penetration of respiratory syncytial virus into the host cell. Two mechanisms were suggested. One was that there was competitive inhibition of the active site on the cell membrane; the other was that these compounds act as protease inhibitors.

In the field of HIV research, dextran sulphate and other sulphated polysaccharides, heparin (8), pentosan polysulphate (9) have been shown to inhibit HIV <u>in vitro</u> by inhibition of binding to T cells. However in a pilot study <u>in vivo</u>, no effect on HIV levels was seen and the test was halted[20].

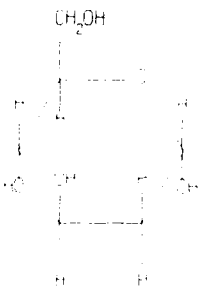
(8)

(9)

<u>3/4 Penetration and/or Uncoating of Virus as a Target</u>

Some drugs which affect the penetration or uncoating phases of the virus lifecycle have already been mentioned there remain a few other important ones which should be covered. Herpes Simplex Virus (HSV) grown in the presence of 2-deoxyglucose (10) was found to

have decreased infectivity as a result of a defect in its ability to penetrate into the cell or to uncoat[21]. When measured against control systems, there was only a small difference in its ability to attach itself to the cell membrane. DNA removed from both systems was found to be equally infective so subsequent steps following uncoating seem to be unaffected.



(10)

To understand how antivirals work during this stage, it is important to understand how cells internalise macromolecules. Electron microscopic studies have shown that the main route of entry into the cell is <u>via</u> endocytosis. The process involves coalescence of receptor-attached proteins in regions of the cell surface known as clathrin coated pits and the subsequent appearence of internal clathrin coated vesicles[22] or non-coated vesicles named receptosomes[23] . This route of entry is followed by a large number of physiologically important ligands including α_2-macroglobulin, epidermal growth factor, insulin and low density lipoprotein. The receptosome containing its protein cargo is transported to the edge of the nucleus where the Golgi endoplasmic reticulum lysosome (GERL) complexes further process the protein. The ultimate

bioavailability of the internalised protein probably requires fusion of the receptosome with the newly forming lysososmes in the GERL system.

To initiate cellular infection viruses need not only to penetrate the cell, they need to disassemble the genome from its protein capsid, a process called uncoating. This results in the naked genome being present in the cytoplasm in such a way that it can undergo transcription.

Amantadine (1-amino adamantane) (11) and its analogue rimantadine (α-methyl-1-adamantanamine) (12) are both potent anti-influenza A drugs for use both symptomatically and prophylactically. Amantadine got its clinical license in 1966 but for a number of reasons never achieved widespread use. In cell culture, amantidine exhibits two concentration-dependent inhibitory actions against viral replication[24]. A non-specific action of concentrations >0.1mM indirectly inhibits activation of the membrane fusion activity of the virus haemagluttinin involved in endocytosis. This action, which results from an elevation in the pH of endosomes, is not peculiar to amantadine but is effected by a variety of amines. Nor does it reflect the clinical spectrum of antiviral activity, since the replication of all influenza viruses, including influenza B strains as well as a number of other enveloped RNA viruses, e.g., paramyxovirus, togavirus and retroviruses, are also inhibited at this concentration.

In contrast, lower concentrations of 0.1-5μM exert a selective, strain-specific inhibition of stages involved either in initiating

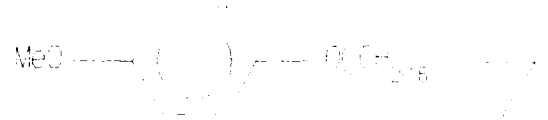
infection or in virus assembly. The basis of the sensitivity of influenza viruses to amantadine is associated with the two genes encoding the haemagglutinin and matrix and M_2 proteins respectively. The M_2 protein has a role in virus replication during assembly of virus particles and at a stage involved in initiating virus infection. Amantadine-resistant influenza virus mutants have been observed to have an altered M_2[25] protein. There is little precise information regarding either the way in which amantadine might block virus assembly or the role of the M_2 protein. To inhibit the participation of virus proteins in the assembly process the drug must be present prior to their synthesis suggesting that it interferes with interactions between virus components rather than causing dissociation of structures already formed. Not only does amantadine effect the expression of haemagglutinin on the cell surface but it also affects its reaction with antibody, indicating a significant structural alteration in this molecule. Therefore, the available data suggests that amantadine does not affect directly the haemagglutinin molecule but rather may interfere with interactions between the M_2 protein and this virus component.

A third mechanism relates to the ability of amines to act as potent inhibitors of transglutaminases, which mediate crosslinking of the receptor ligand complex to another component in the cell membrane, and hence blocking receptor-mediated internalisation. As mentioned before mono- and di-amidines have been shown to block cell fusion induced by RSV. Diarylamidines are potent inhibitors

of trypsin-like proteases. It is suggested that the diarylamidines exert their effect by specific inhibition of arginine- or lysine-directed esteroproteases which play a key part in RSV[19] fusion to cell membranes.

(11) (12)

An important addition to this area of antiviral development is a lipophilic diketone called Arildone (4-[6-(2-chloro-4-methoxy)phenoxy] hexyl-3,5-heptanedione) (13). This has no effect on virus adsorption or penetration but stops uncoating by changing the nature of the capsid structure. Drugs of this structure are currently being evaluated for use as topical anti-herpetics in humans.



(13)

5 Intracellular Events

After the virion has entered the cell and become uncoated, the nucleic acid can either stay in the cytoplasm or be transported into the nucleus. This provides several sites for antiviral action. Synthetic oligonucleotides (antisense) which bind to specific sites of viral nucleic acid could either block the formation of viral mRNA or alternatively they could inhibit translation of the mRNA directly or indirectly by inducing the activity of RNAase H to hydrolyse the mRNA strand of a RNA/DNA hybrid. Normal synthetic oligonucleotides are soon degraded in the cell but polymers which have different phosphate links, thiophosphate for example, are more stable[26]. Obviously site-specific targeting will be necessary and problems can arise with entry into the cell and degradation by cellular enzymes. Round and Stebbing found that a single-stranded copolymer of cytidine and 4-thiouridine inhibits the influenza virus transcriptase and had anti-influenza properties in vivo.

6 RNA Synthesis as a Target

There are a number of events that one could target to prevent the formation of RNA, or to increase its degradation. It is important to get selectivity of inhibition, since one would not want to interfere with normal cellular RNA formation. However, in a life-threatening situation, a certain level of toxicity is acceptable.

Mismatched double-stranded RNAs have an important role in recovery from viral infections, due to the induction of interferon and other lymphokines. Mismatching, i.e. introduction of an unpaired base (U) in the poly(C) strand (poly(I).poly($C_{12}U$) (Ampligen)), allows the double-stranded RNA to retain its interferon-inducing activity while losing part of its toxic potential.

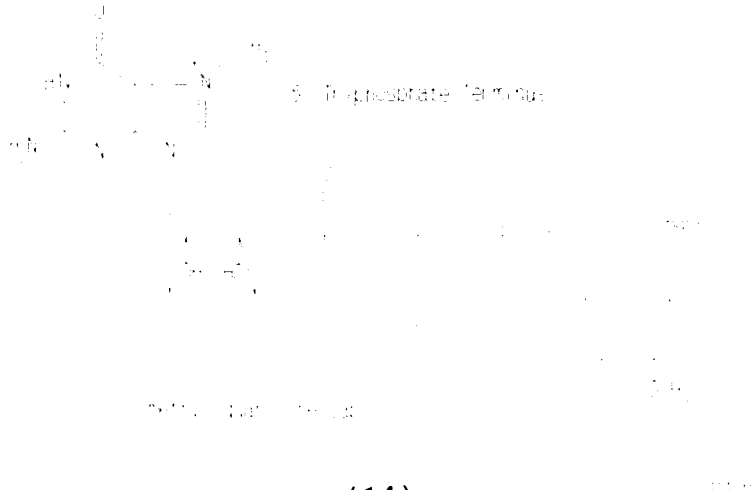
Another approach is to decrease the availability of viral mRNA this can be achieved by increasing the rate at which the mRNA is degraded. This can be bought about by interferon (IFN) or interferon inducers. Interferon refers to a group of proteins termed alpha(leucocyte), beta(fibroblast) and gamma(immune) which are naturally present in the cell. Interferons are not directly antiviral but cause biochemical changes in exposed cells that lead to viral resistance. The initial step invoves IFN binding to specific cell surface receptors, which are shared between IFN-α and -β, but are different for IFN-γ[9]. The onset of IFN antiviral action is rapid, and alterations in cellular mRNA occur within minutes. Depending on the virus and cell type, IFN's antiviral

effects are mediated through the inhibition of viral penetration and uncoating, synthesis or methylation of mRNA, translation of viral proteins, or viral assembly and release. For most viruses the principle replicative step inhibited by IFN is viral protein synthesis. After IFN exposure, cells produce a series of proteins, usually including a unique 2',5'-oligoadenylate synthetase and a protein kinase, either of which can inhibit protein synthesis in the presence of double stranded RNA. The 2',5'-oligoadenylate synthetase produces adenylate oligomers that activate a latent cellular ribonuclease (RNAase L) in the presence of double-stranded RNA to cleave both cellular and viral RNAs. The protein kinase selectively phosphorylates two proteins involved in protein synthesis, eukaryotic initiation factor 2 (eIF-2) and a ribosome-associated one called P_1. Interferon may also block mRNA capping by inhibiting transmethylation reactions. Interferon induction of a phosphodiesterase, which cleaves a portion of transfer RNA and thus prevents peptide elongation, also contributes to the inhibition of protein synthesis.

7 Methylation of RNA as a Target

Most viral and cellular mRNAs contain a guanylate at their 5'-terminus linked in a unique 5'-5' inverted linkage (14). The guanylate is methylated at its N-7 position by the enzyme guanine-7-methyltransferase . The function of this "cap" is to protect the molecule from nuclease digestion[27] as well as facilitating its binding to the ribosome for translation initiation. The methyl group is derived from S-adenosyl methionine (SAM) (15) which is

converted into S-adenosyl homocysteine (SAH) (16). S-Adenosyl homocysteine is hydrolysed to L-homocysteine (17) and adenine by the enzyme S-adenosyl homocysteine hydrolase[28].



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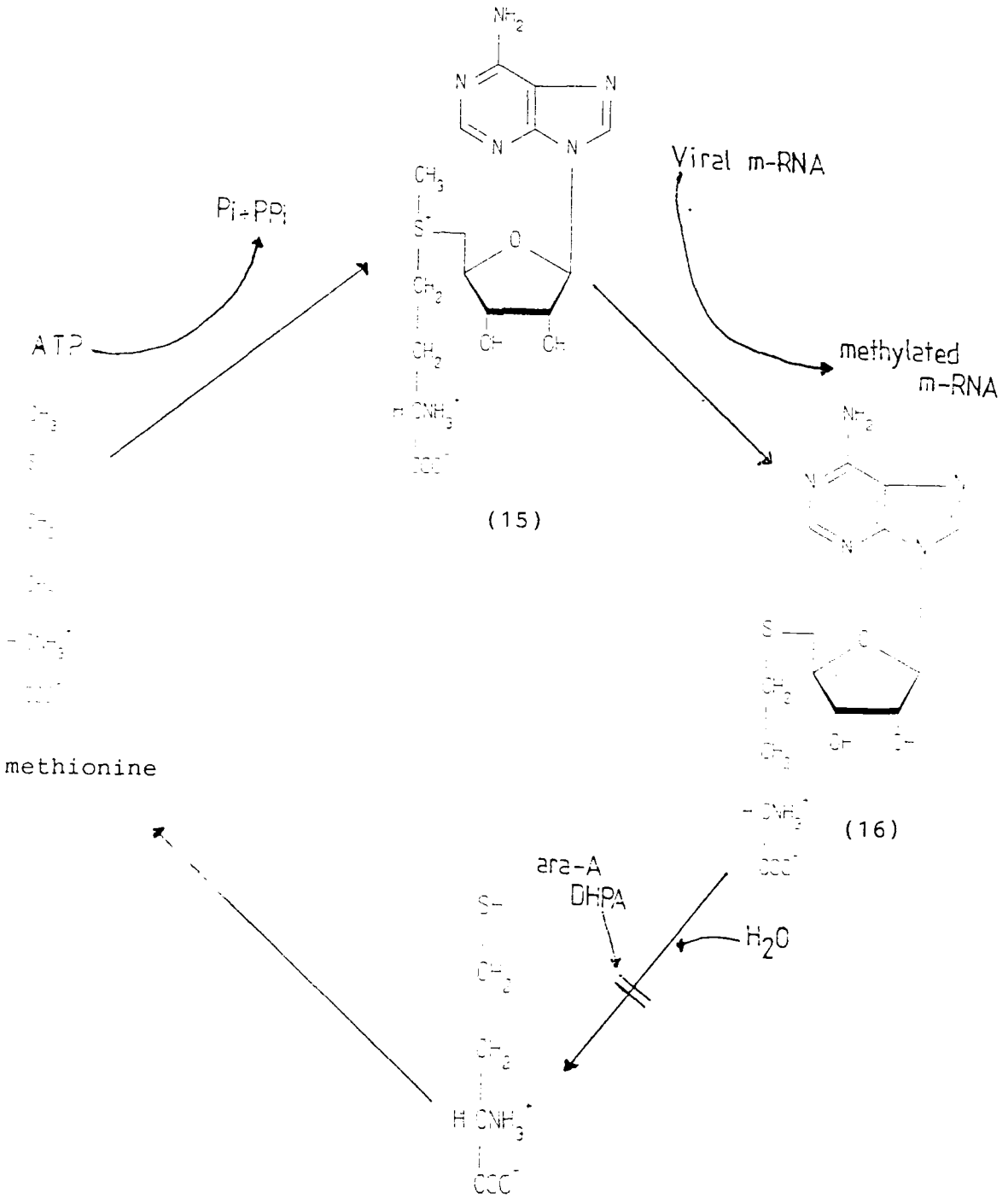
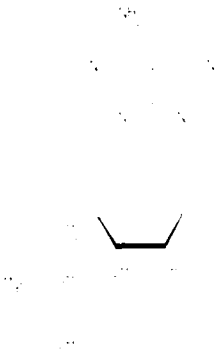

Pi+PPi
ATP
Viral m-RNA
methylated
m-RNA
(15)
(16)
methionine
ara-A
DHPA
H_2O
(17)

Many compounds have been found that inhibit the methyl transferase. When looked at these all bear a resemblance to SAM (18). 5′-Isobutyladenosine (19), sinefungin (20), ribavirin triphosphate and A9145C (21) all show inhibition of the methyl transferase[29].

(18)



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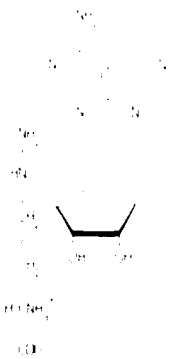

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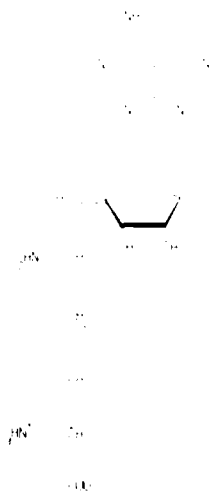
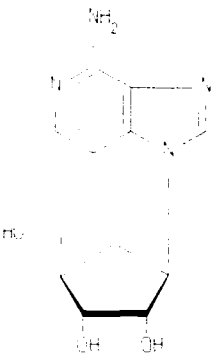


(21)

Several compounds such as 9-β-D-arabinosyl adenine (ara-A), 3-deazaadenosine[30](22) and its carbocyclic analogue 3-deazaaristeromycin (23), neplanocin A[31] (24) and 3-deazaneplanocin A (25) all have an effect on S-adenosyl homocysteine (SAH) hydrolase[30]. S-Adenosyl homocysteine is an inhibitor of the methyl transferase so the end result is a build up of SAM. 3-Deazaneplanocin A is the most potent inhibitor and also the least cytotoxic[32]. For neplanocin A and the other adenosine analogues to act preferentially at the SAH hydrolase level they should not be phosphorylated (by adenosine kinase) or deaminated (by adenosine deaminase). Phosphorylation would make the compounds cytotoxic due to inhibition of cellular RNA synthesis and deamination would render them biologically inert. Both phosphorylation and deamination depend on the presence of the 5'-hydroxyl group. In attempts to avert both these actions and still retain an inhibition of SAH hydrolase Keller and Borchardt[33] synthesised the cyclopentenyl derivatives of neplanocin A (26) and 3-deazaneplanocin A (27), DHCA and DHCDA. The results confirmed their predictions. DHCA and DHCDA both gained selectivity as SAH hydrolase inhibitors and as antiviral agents.

(22)



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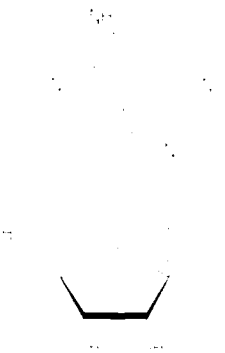

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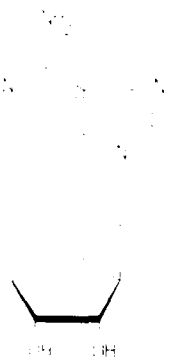


(27)

8a Protein Synthesis or Processing as a Target

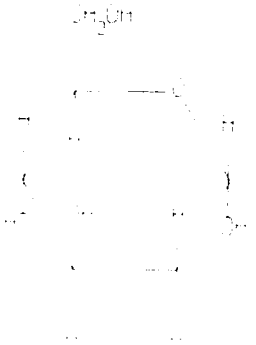
Viral protein synthesis usually occurs in two stages. Early translation takes place using parental viral mRNA. Late translation occurs using mRNA derived from new progeny nucleic acid which may have changed from the original. As mentioned before, Methisazone was one of the first antivirals ever used against poxviruses. Its mode of action depends on inhibition of an enzyme responsible for cleavage of a precursor required for one of the main structural polypeptides concerned with viral maturation.[34]

Carrasco and Smith[35] have shown that the permeability of cells changes after viral infection thus allowing compounds to penetrate which cannot normally gain entry. An example of this is the guanosine triphosphate analogue ($GppCH_2p$) which inhibits protein synthesis in virally infected cells but not in normal cells.

8b Glycoprotein Synthesis as a Target

Enveloped viruses contain one or more glycoproteins as integral membrane proteins with the carbohydrate portion exposed to the surface. These glycoproteins have 10-15 monosaccharides in an N- or O-glycosidic linkage to a polypeptide asparagine , serine or threonine residue[27]. Several compounds are known that inhibit this glycosylation. These include 2-deoxy-D-glucose (28), D-glucosamine (29) and tunicamycin (30). Deoxyglucose is utilized instead of glucose in the formation of guanosine diphosphate glucose and the GDP deoxyglucose binds to the lipid carrier dolichol pyrophosphate. This interferes with the synthesis of intermediates that participate in the formation of N-glycosidically linked

glycoproteins[36].



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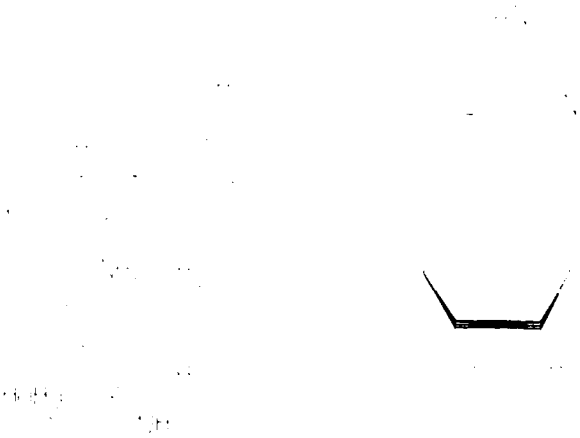


(30)

Tunicamycin blocks the transfer of N-acetylglucosamine-1 phosphate from uridine diphosphate N-acetylglucosamine to dolichol monophosphate and hence inhibits glycoprotein synthesis.

9 Enzymes as Targets

By far the biggest target of antiviral chemotherapy has been the design of enzyme inhibitors. Virally encoded enzymes are either brought in with the virus or induced during infection.

All negative-stranded viruses, such as the parainfluenza virus contain a RNA-dependent RNA polymerase (transcriptase) which is required for the synthesis of early viral mRNA.

Retroviruses contain a RNA dependent-DNA polymerase (reverse transcriptase) which synthesises a minus strand of DNA from the RNA genome. The RNA is then removed by a ribonuclease and a plus strand of DNA is formed to make a linear duplex. This duplex then migrates to the nucleus where it is converted into a circular form prior to integration into cellular DNA.

Table 2[37] indicates the enzymes encoded by members of the herpesvirus family, all of which can be considered as targets.

During the replication cycle of HIV, gene products are translated as polyproteins. These are subsequently processed by a viral proteinase to yield the structural proteins of the virus core and essential enzymes including the proteinase. On the basis of its primary amino acid sequence[38] and its crystal structure[39], HIV-1 proteinase has been classified as an aspartic proteinase. Several potent selective inhibitors have been produced[40]

Table 2.

	HSV-1	HSV-2	VZV	CMV	EBV
Thymidine kinase	+	+	+	-	-
DNA polymerase	+	+	+	+	+
DNase	+	+	+	+	+
Uracil-DNA-glycosylase	+	+	?	?	?
DUTPase	+	+	?	?	?
Ribonucleoside diphosphate reductase	+	+	?	?	+

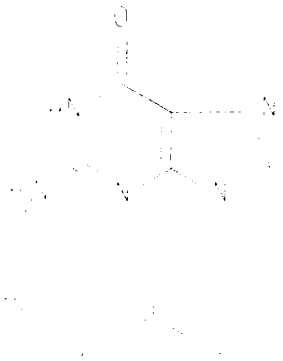
Thymidine kinase

Virally encoded thymidine kinase is one of the most important enzymes in the area of antiherpetics. The virally induced enzyme differs in molecular weight, substrate specificity, isoelectric point and immunolgical properties[41]. Unlike the cellular kinase it can phosphorylase compounds with a diverse structure.

The analogues that depend upon viral thymidine kinase can be split into two relative groups:

(i) acyclic guanosine analogues like ACV (30), DHPG (31) and DHBG (32)

(ii) 5-substituted 2'-deoxyuridines like BVDU (33), C-BVDU (34), BVaraU (35).



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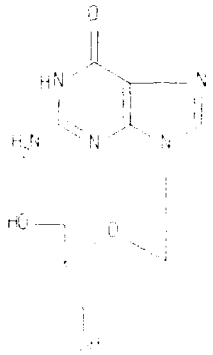

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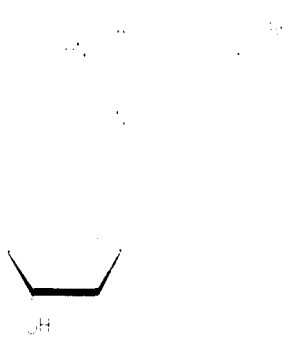

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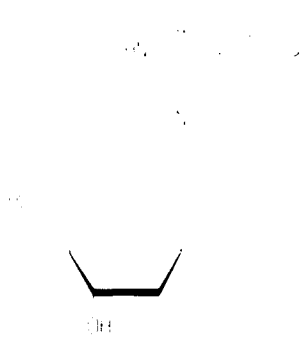

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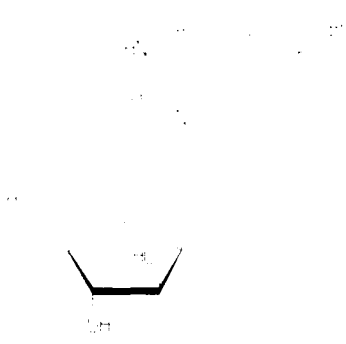


(35)

Acyclovir (ACV) has aquired an established position in the chemotherapy of HSV infections. It is the treatment of choice for first stage genital herpes[40] it is also used prophylactically against HSV in immunocompromised patients. Acyclovir suffers a number of drawbacks such as:

(1) a poor solubility in water (0.2% at 25°C)

(2) a low oral absorption (20% after 200 mg dose)

(3) it is limited to HSV and VZV

Because of its low solubility it cannot be given as eye drops in the treatment of herpetic keratitis or as intramuscular injections.

As could be expected, all these compounds are only active against viruses that encode their own thymidine kinase. Viruses like CMV and EBV are thus relatively insensitive to ACV, and BVDU and its analogues. However, CMV does show inhibition by DHPG[42] and it seems that DHPG is readily converted to its triphosphate in CMV-infected cells.

Once phosphorylated to their 5'-monophosphates, the acyclic analogues are converted to their corresponding di- and tri-phosphates by cellular enzymes. BVDU may be converted into its diphosphate by the same thymidylate kinase in some viruses. HSV-1 and VZV have thymidine kinases with dual enzymatic activity so that they can act as a dThd kinase as well as dTMP kinase. The thymidine kinase of HSV-2 and EHV-1 are only capable of the initial phosphorylation, this explains the drop in activity of BVDU against HSV-2 compared with HSV-1.

All TK-dependent nucleoside analogues probably rely on the presence of their triphosphates for their activity. They act as competitive inhibitors of the natural substrate for the viral DNA polymerase. In the case of the acyclic analogues, when they are incorporated into the DNA strand they act as chain terminators because they do not have a 3'-hydroxyl in the right configuration for chain growth. The 5-substituted-2'-deoxy nucleosides EDU, BVDU and C-BVDU are incorporated into the DNA strands to a greater extent than the acyclic nucleosides but here their mode of action seems to rely on an enhanced susceptibility of DNA strand breakage.

Thymidylate Synthetase

Some 5-substituted-2'-deoxyuridines having a small electronegative substituent at the 5-position such as a fluorine, nitro, acetylene or cyano group, have been seen to be potent inhibitors of thymidylate (dTMP) synthase.

This was shown by 5-nitro-2'-deoxyuridine-inhibition of vaccinia virus[43]. It has been shown that this mode of inhibition extends to TK^- virus strains. These mutant strains cannot rely on the dThd salvage pathway to ensure sufficent supply of dTMP, dTDP and dTTP for DNA synthesis (36), which makes them dependent on the de novo biosynthetic route of dTTP production starting from N-carbamoyl-aspartate and going through the dTMP synthase step. At this point there is competition between the nucleoside analogue and dUMP.

It appears that inhibition of TS for any cells is not beneficial and hence all these analogues are toxic.

DNA Polymerase

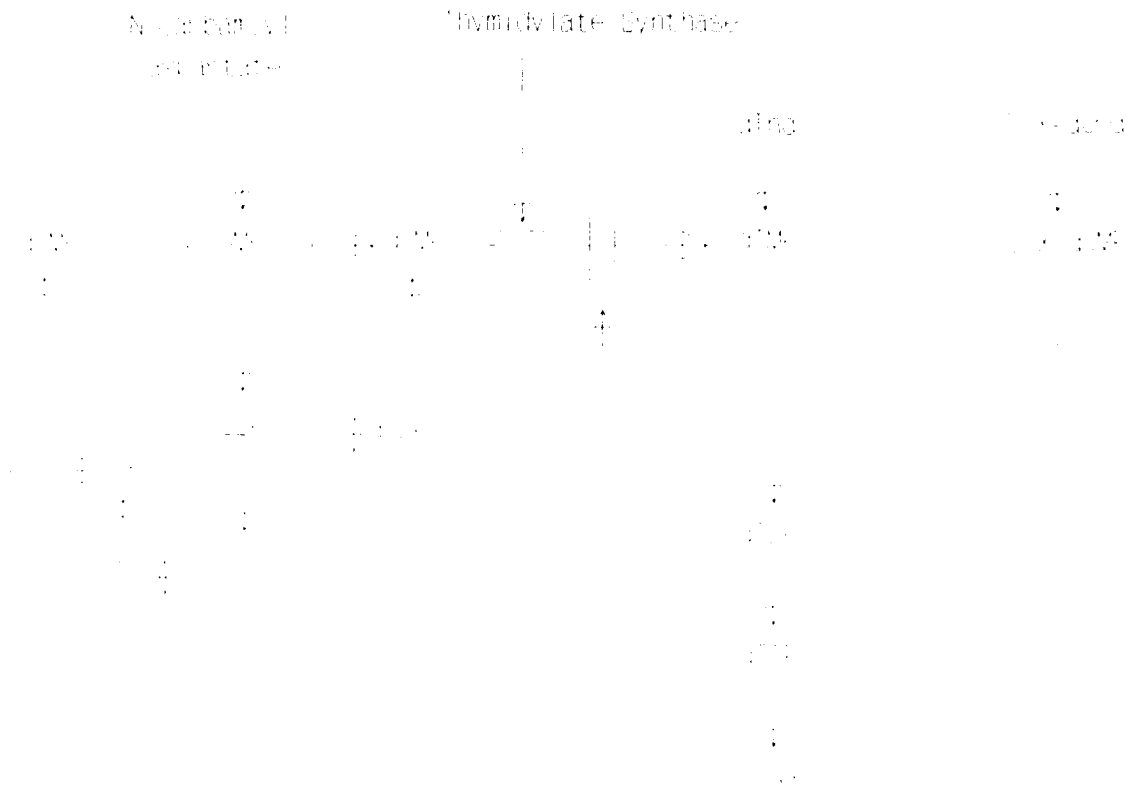


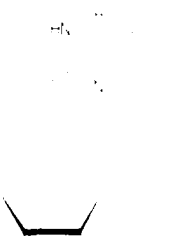
Fig 36

All the herpesvirus family encode a unique DNA polymerase which is a perfect target because it is an absolute requirement for replication. A large number of compounds are thought to inhibit DNA polymerase and they can be grouped into analogues of pyrophosphate and analogues of natural nucleosides.

During DNA or RNA synthesis (37), the nucleic acid is built in a 3′-5′ direction. The 3′-hydroxyl of the terminal nucleoside attacks the first phosphorus in the next nucleoside triphosphate to make the phosphate linkage, in doing this pyrophosphate is lost. A number of pyrophosphate analogues[34] have been synthesized and tested. Two analogues are phosphonoformate (PFA, foscarnet) and

phosphonoacetate (PAA). These are two of the most active compounds inhibiting HSV-1, HSV-2 and CMV polymerase to a much greater extent than cellular polymerases. They act by binding competitively onto the active site of the DNA polymerase amd stopping the formation of the 3′-5′ phosphodiester bond of the nucleic acid.

As already mentioned antiviral nucleoside triphosphates inhibit DNA polymerase by competing with the normal substrates.

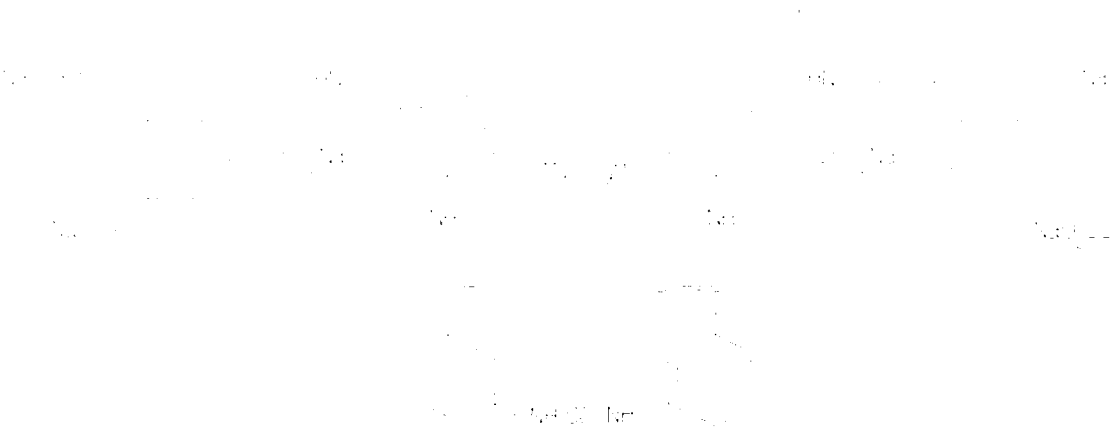


(37)

Reverse Transcriptase as a Target

Over the past 10 years antiviral drug therapy has been drawn towards a new target which is the RNA-dependent DNA-polymerase (reverse transcriptase, RT) which is encoded by the human immunodeficiency virus responsible for Aquired Immune Defficiency Syndrome (AIDS). Indeed most of the compounds described in the past few years as inhibitors of HIV are presumed to work at the RT level. This is certainly the case for suramin (38), aurin tricarboxylic acid (39) and rifabutin (40), as well as the 2′,3′-

dideoxynucleoside analogues with 3'-azido-3'-deoxythymidine (41) and 2',3'-dideoxycytidine (42). The nucleoside analogues are phosphorylated to their triphosphates and then they may either act as inhibitors , by direct competition with the natural substrate for the active site on the polymerase or be incorporated into DNA at the 3'-terminus, thereby preventing further chain growth.



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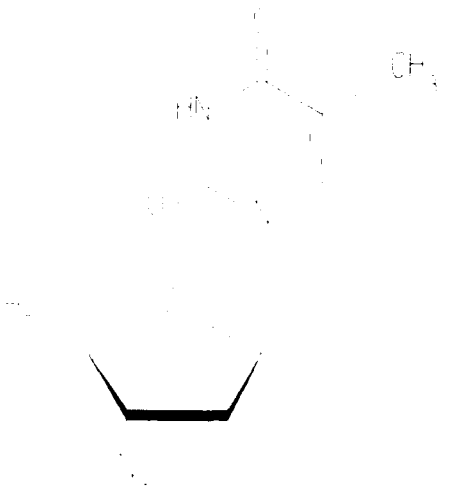

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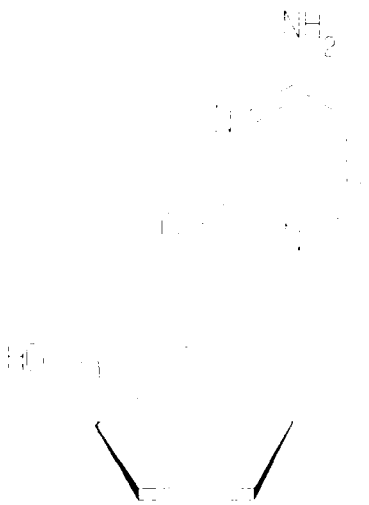


(42)

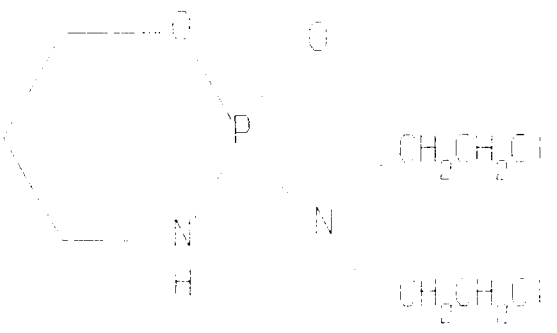
The limiting function in the last two sections has been the selectivity (herpes) or lack (HIV) of a viral thymidine kinase. To overcome this constraint particularly in the anti-HIV field one would have to introduce a nucleotide, nucleoside diphosphate or triphosphate into the cell. This poses a major problem in that charged species do not pass easily through the cell membrane. In this thesis is presented a masked nucleotide monophosphate system that requires cellular enzymes to breakdown it down to the monophosphate. It is hoped that this will enhance the activity of already active drugs and also enable a broader range of compounds to be synthesised which might be polymerase inhibitors.

RESULTS AND DISCUSSION

The procedure of drug derivatisation has long been established. In 1899 Bayer synthesised the drug aspirin in an attempt to improve the efficacy of salicylic acid[44]. In this case it is nucleosides of proven activity which are being modified in order to improve them. As discussed earlier a major problem in the use of nucleosides is the specificity of the viral thymidine kinase for the nucleoside. In order to circumvent this problem it is desirable to get a nucleoside monophosphate into the cell. However, this is not practical because charged species do not pass through the cell membrane easily. It was therefore decided to synthesise a masked phosphate, that would under cellular conditions return to the parent molecule. Following work in this laboratory by Hunston[45] the moiety chosen was 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide(43).

(43)

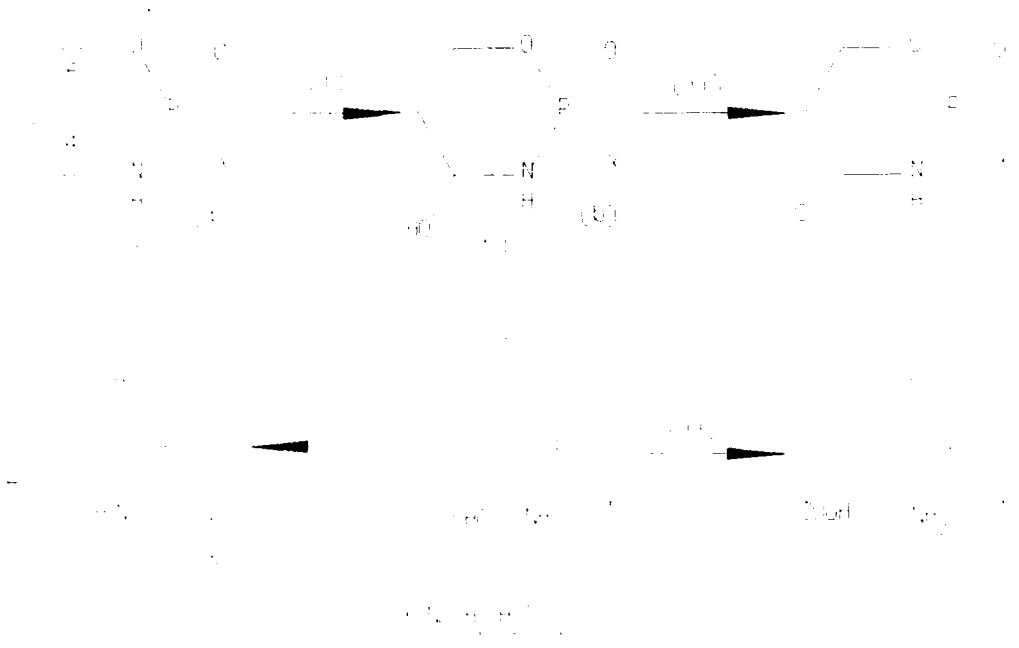
This molecule is a modification of a known anti-cancer agent cyclophosphamide, which is active against tumour cells only *in vivo*[46](44).



(44)

The original basis for the design of cylophosphamide[47] was the hope that tumour cells, known to be rich in the enzyme phosphoramidase, might mediate the hydrolysis of the P-N linkage, with the liberation of a reactive alkylating species and in particular 2'-dichloroethylamine, a known anti-tumour agent[48]. The use of cyclophosphamide as a transport system for the alkylating agent was found to considerably reduce the general toxicity of the drug while its chemotherapeutic potency was preserved. Studies with (^{14}C)-labelled cyclophosphamide in mouse liver microsomal systems showed N,N-bis(2-chloroethyl)-phosphorodiamidic acid to be an active cytotoxic agent generated in this system[49]

In vivo this ring system is broken down to yield a phosphate and acrolein. The initial activation step is hydroxylation, by liver microsomal oxidase, at the C-4 position to give 4-hydroxy-cyclophosphamide as the first metabolite[50], which is active both in vivo and in vitro (45). 4-Hydroxycyclophosphamide is in equilibrium with aldophosphamide and these tautomers have only weak activity.



(45)

(a) cyclophphosphamide

(b) 4-hydroxycyclophosphamide

(c) aldophosphamide

(d) N,N-bis(2-chloroethyl)-
phosphorodiamidic acid

(e) acrolein

(i) microsomal oxidase

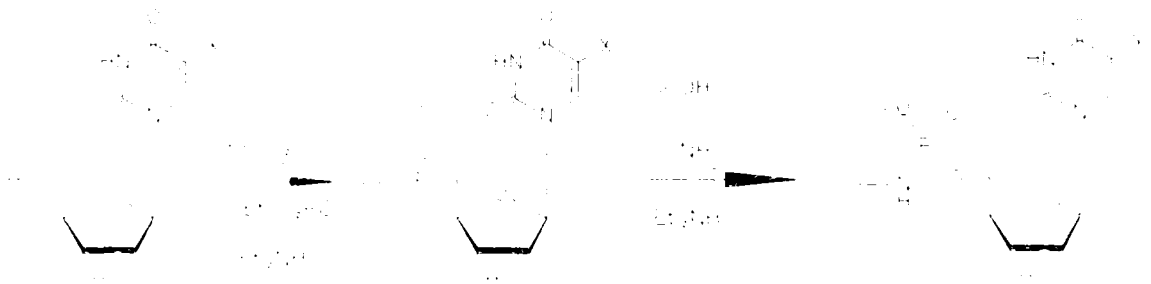
(ii) aldehyde dehydrogenase

(iii) chemical elimination

Therefore with the mechanism of activation understood it was thought that placing the oxazophosphorine ring system on the 5' position of a nucleoside would produce the monophosphate in the cell. There are two methods of producing 5'-oxazophosphorine derivatives of nucleosides. Firstly, we can phosphorylate the 5'-

hydroxyl selectively using phosphoryl trichloride. Yoshikawa[51,52] has noted that inclusion of trialkylphosphates in the reaction of phosphoryl trichloride with ribonucleosides gave 5'-selectivity. The mechanism is not known but it was thought that there maybe some interaction between the trialkylphosphate and the 2' and 3' hydroxyl groups. However, the reaction works just as well with 2'-deoxynucleosides. Phosphoryl chloride and triethylphopsphate are reacted with the nucleoside in the presence of triethylamine to give the intermediate phosphodichloridate, this is reacted directly with 3-aminopropan-1-ol and the product is purified on a silica column. However a typical yield is only between 10-20%. A side reaction has been observed to run much faster on TLC . The faster product gives NMR absorptions very similar to starting material and it is thought that this compound may be the 5'-chloro derivative produced by chlorination as opposed to phosphorylation by phosphoryl chloride. There is also a large percentage of baseline material. The alternative method of synthesis is the transferance of the preformed ring onto the nucleoside. Hunston[45] described the preparation of the six membered cyclic phosphochloridate 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide. Cyclisation is acheived by the initial reaction of the hydroxyl of 3-aminopropan-1-ol in the presence of one molar equivalent of base at -15°C in chloroform, and then the reaction of the amino function at 0°C after the addition of a further one molar equivalent of base (46). Filtration and evaporation of the solvent gave a solid which was extracted with several portions of dry acetone. The extracts are

evaporated to give a viscous oil. The oil is dissolved in chloroform, and carbon tetrachloride is added (in the ratio 1:3 respectively), on standing a white solid forms.



(46)

The NMR spectrum gave three areas of absorption, due to the hydrogens present at positions 4,5 and 6. Each region gave a complex pattern which can be attributed to coupling of the axial and equatorial hydrogens on each carbon to: (a) the adjacent hydrogens in the ring, (b) phosphorus and (c) each other. The compound is quite stable and can be stored for a long time at room temperature and pressure.

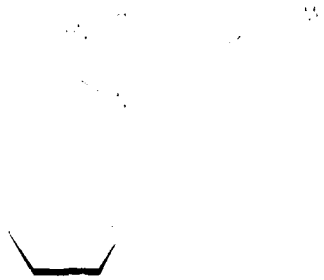
(47)

Many conditions were tried to condense the compound onto the 5'-hydroxyl of the nucleoside, the best was found to be a straight forward reaction between the nucleoside and one molar equivalent of the cyclic phosphochloridate in pyridine. The reaction is seen to go in yields of 25-40%. If the reaction is repeated using two molar equivalents of the cyclic compound the yield is no greater, but more importantly no product is seen to form from disubstitution of the nucleoside at the unblocked 3'-hydroxyl. The addition of catalytic amounts of dimethylaminopyridine (DMAP) made no appreciable difference to the overall yield. A series of simultaneous reactions were carried out to try and optimise the conditions for condensation. Four reactions were done with variations in the amount phosphorylating reagent, (one or two molar) and overall reaction time, (24-72 hours). The yield was seen to vary very little compared to a control, (1 mole phosphorylating reagent stirred for 24 hours). The NMR of the thymidine analogue 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-yl) thymidine (47) shows that the electron withdrawing effect of the phosphate at the 5'-carbon is sufficient to shift the 5'-CH_2 and the 4'-CH absorptions from their normal positions of δ3.55 an 3.75 respectively to the lower field region normaly occupied solely by the 3'-CH resonance at δ4.25. Because of this and also the resonance from the 6"-CH_2 protons, absorptions due to 6 hydrogens appear in the δ4.6-4.0 region of the spectra.

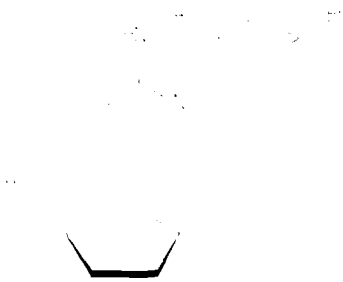
Now that an effective method of producing the 5'-substituted nucleosides was available a range of nucleoside starting materials

had to be made. It was decided to concentrate on a range of 5-vinyl nucleosides (48) since these all showed some activity already.

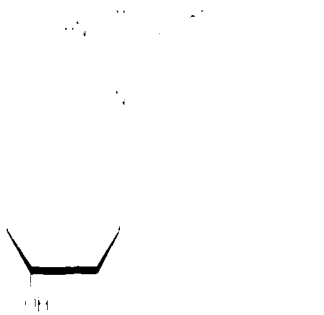
The first step involves the joining of the unsaturated side-chain to the C-5 position. This is accomplished using the well known and highly versatile Heck reaction in which an arylmercury salt or aryl halide reacts with alkenes to generate new carbon-carbon bonds. Several reviews have appeared which cover the reaction.[53,54,55]



MCVdU

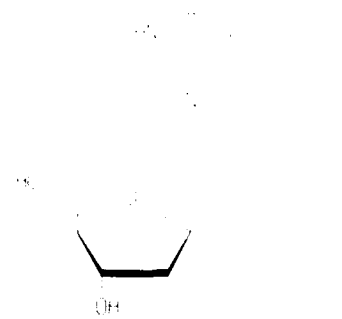


BVdU



EndU

(48)



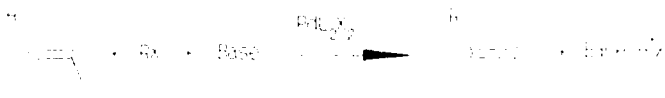
VdU

In nucleoside chemistry, the Heck reaction was first used by Bergstrom and co-workers who published a series of papers. These included the coupling reactions of nucleosides with allylic halides,[56] olefinic compounds[57] and allylic alcohols and acetates[58].

The reaction involves the coupling of 5-iodonucleoside with the olefin using the preparation in situ of catalytic palladium complexes present to a concentration of 0.05 molar equivalents.

(E)-5-(2-Carbomethoxyvinyl)-2'-deoxyuridine was prepared using the palladium (II) acetate, triphenyl phosphine catalyst system. 5-Iodo-2'-deoxyuridine and methyl acrylate were added to the catalyst, prepared in situ, which consisted of palladium (II) acetate (0.05 molar equivalents), triphenyl phosphine (0.1 molar equivalents) and an excess of triethylamine in dioxan. The catalyst was activated by heating the solution to 70°C until a deep red colour had developed, this forms the catalytic palladium complexes. After addition of the reactants, the solution was refluxed for 1 hour then filtered through Celite to remove the palladium salts. The solution is then evaporated to dryness and recrystallised from ethanol to give the pure ester in 60% yield. The ester was characterised by its NMR, FAB mass spectra and UV. The double bond was seen to have a coupling constant of J=16Hz indicating trans stereochemistry.

The mechanism of the reaction (49), is thought to resemble that proposed for the arylation of olefins with organometallic compound and analogous to that of the coupling of 5-chloromercurated nucleosides.[59]



(49)

Palladium(0) is initially formed by the reduction of the $Pd(OAc)_2/Ph_3P$ complex to a Pd (0)-phosphine complex by the alkene. Then, the organopalladium intermediate $ArPdL_2X$ is formed and a weakly bound ligand can exchange for olefin to form complex A. The key step involves the π-bonded methyl acrylate insertion into the Ar-Pd-σ-bond to form complex B with the loss of iodide ion. Complex B then undergoes cis-elimination of the Pd-H to give the prod+uct as a π-bonded species C. This complex then dissociates to give the product together with HI and palladium (0).
The reaction is catalytic overall in palladium as the Pd (0) is regenerated in the last step (50).

The acid (E)-5-(2-carboxyvinyl)-2'-deoxyuridine is prepared from the ester, saponification of which using 1M aqueous sodium hydroxide proceeds rapidly at room temperature. The acid was isolated by cooling the solution of the sodium salt and acidifying. The precipitate that formed was filtered and washed carefully with ice-cold water to remove traces of sodium chloride. The NMR spectrum of the product showed the absence of the ester methyl signal at δ3.7. The FAB mass spectrum showed the loss of 14 mass units, consistant with the change $-CO_2CH_3$ to -COOH.

(50)

The acid (E)-5-(2-carboxyvinyl)-2'-deoxyuridine is prepared from the ester, saponification of which using 1M aqueous sodium hydroxide proceeds rapidly at room temperature. The acid was isolated by cooling the solution of the sodium salt and acidifying. The precipitate that formed was filtered and washed carefully with ice-cold water to remove traces of sodium chloride. The NMR spectrum of the product showed the absence of the ester methyl

signal at δ3.7. The FAB mass spectrum showed the loss of 14 mass units, consistant with the change $-CO_2CH_3$ to -COOH.

It is possible to brominate the acid to the selective and potent anti-herpes compound (E)-5-(2-bromovinyl)-2'-deoxyuridine via a decarboxylative bromination. The potassium salt of the acid was treated with N-bromsuccinimide in DMF and the needles obtained after recrystallisation from water were characterised by NMR, mass spectroscopy and UV.

The treatment of the acid with NBS is an example of the classic decarboxylative bromination reaction (51), a fragmentation reaction which involves the loss of succinimide from the erythro-α-bromo-β-succinimidoethyl carboxylic acid. Carbon dioxide is also lost.

During the original synthesis of BVdU[60], none of the Z isomer was obtained, which is surprising as in similar systems eg. cinnamic acid[61], brominative decarboxylation gave either exclusive (Z)-isomer product (in a non-polar media) or a mixture of the (E)- and (Z)-isomers (in more polar media). The mechanism for this decarboxylation, in aqueous media, has been likened to an E1 elimination, invoving a dipolar ionic species. This intermediate, stabilised by the reaction media, may undergo rotation about the carbon bond to give the trans-isomer as the major product.

In 1982, three syntheses of 5-vinyl-2'-deoxyuridine from nucleoside precursors were reported. Pichat and co-workers[62] treated chloro-di-cyclopentadienyl zirconium hydride with acetylene to give the corresponding alkenylzirconium compound which could be reacted with 3'-5'-di-O-trimethylsilyloxy-5-iodo-2'-deoxyuridine

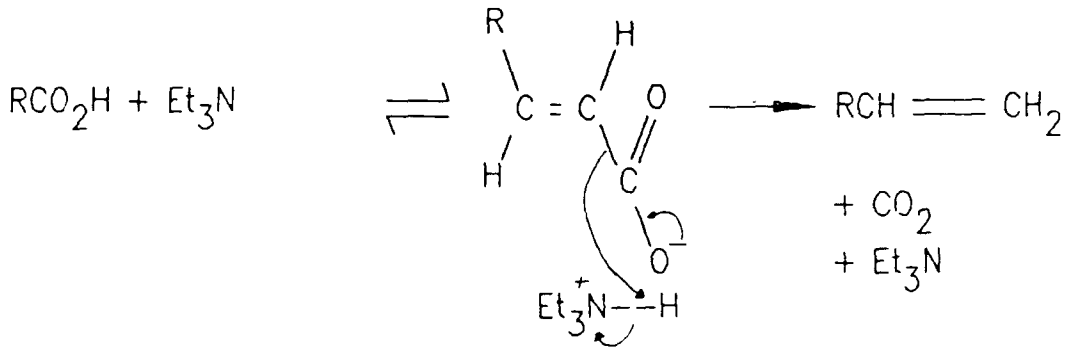
in the presence of palladium catalyst in THF to give the corresponding C-5 vinyl-substituted nucleoside in good yield.

(51)

Rahim et al[63] reported two syntheses, the palladium catalysed vinylation of 5-iodo-2'-deoxyuridine and the decarboxylation of (E)-5-(2-carboxyvinyl)-2'-deoxyuridine. The former reaction is reported to give a yield of 37%, under the conditions quoted by Rahim (totally anhydrous) the latter reaction gives yields of 20-25%. However, Slater[64] in the same laboratory has shown that by inclusion of water (ca 3%) the reaction can give yields of 55-60%.

The mechanism of the decarboxylation reaction is believed to be a concerted loss of carbon dioxide from the carboxylate anion and protonation of the terminal carbon of the vinyl group(52). In anhydrous conditions protonated triethylamine (Et_3NH^+) is the most

likely proton source and this species is reduced in concentration as the reaction proceeds. The inclusion of water traps releases carbon dioxide as triethylammonium bicarbonate and provides a higher and more constant proton source in the form of (Et_3NH^+) or even H_2O itself.



(52)

The final preparation is that of 5-ethynyl-2'-deoxyuridine. 5-ethynyl-2'-deoxyuridine is a potent inhibitor of the enzyme thymidylate synthetase _in vitro_[65], and is also inhibitory towards the replication of HSV-1, HSV-2 and vaccinia virus _in vitro_[66]. 5-Ethynyl-2'-deoxyuridine was originally synthesized simultaneously by two groups of workers[67,68,69,70], both groups utilizing a condensation procedure which necessitates the separation of α and β anomers.

Robbins and Barr[71], reported the efficient conversion of derivatives of 5-iodouracil to the corresponding 5-alkynyl-uracil derivatives _via_ a palladium catalysed coupling reaction. This

method can also be applied to the preformed β-nucleoside superseding the condensation method.

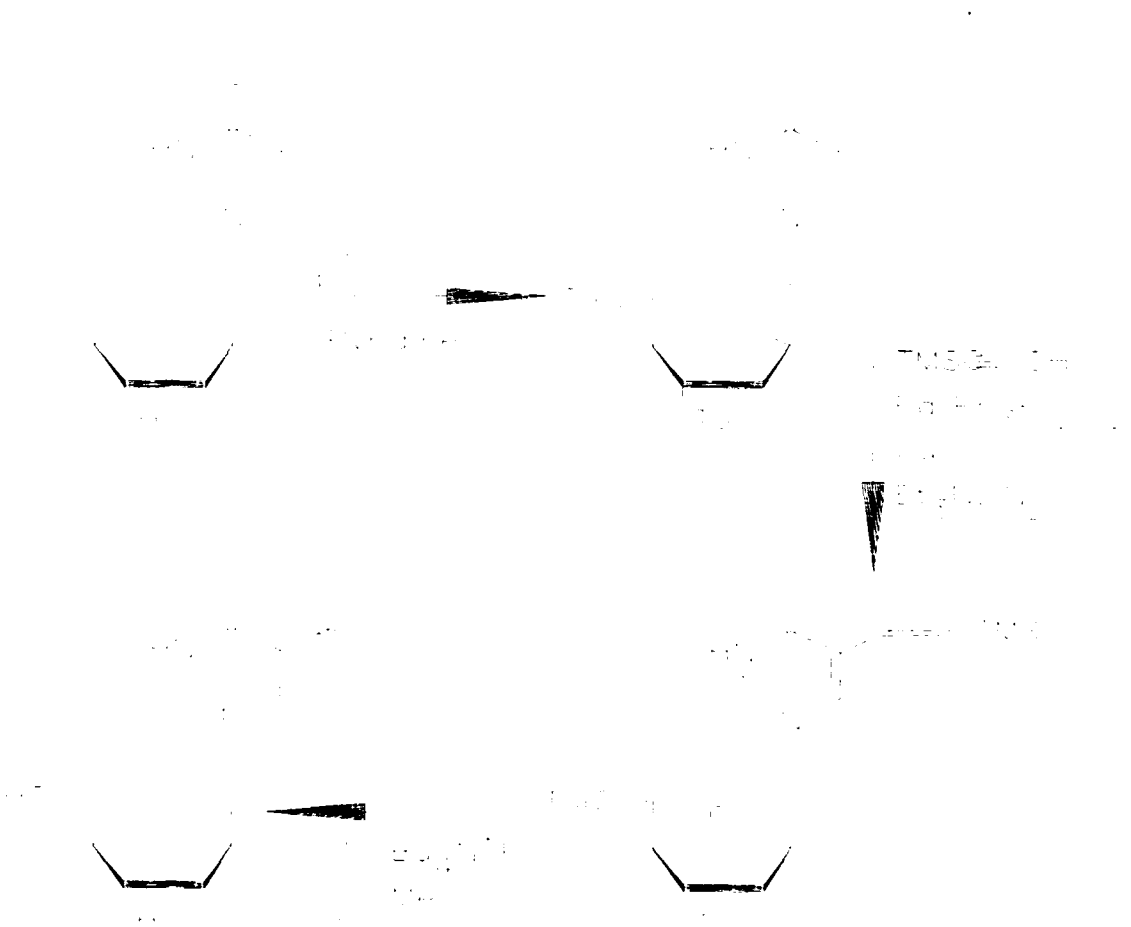
5-Ethynyl-2'-deoxyuridine was thus prepared in four steps from 5-iodo-2'-deoxyuridine (53). The first step involves the blocking of the hydroxyl groups as their p-toluoyl esters by treatment of 5-iodo-2'-deoxyuridine in dry pyridine with an excess of p-toluoyl chloride. This afforded 3',5'-di-O-p-toluoyl-5-iodo-2'-deoxyuridine in good yield after removal of p-toluic acid by column chromatography.

The palladium catalysed coupling reaction involved the treatment of 3',5'-di-O-p-toluoyl-5-iodo-2'-deoxyuridine with an excess of trimethylsilylacetylene, in the presence of catalytic amounts of bis(triphenylphosphine)palladium (II) chloride and copper (I) iodide in warm triethylamine under a nitrogen atmosphere. Filtration through Celite and evaporation gave a brown solid. This solid was dissolved in chloroform and extracted 3 times with a solution of EDTA (5%) to remove the copper salts, washed with water and dried. Evaporation and crystallization gave 3',5'-di-O-p-toluoyl-5-(2-trimethylsilylethynyl)-2'-deoxyuridine in high yield. The yellow palladium (II) catalyst was prepared in high yield by the method of Burmeister and Basolo[72].

Reaction of 3',5'-di-O-p-toluoyl-5-(2-trimethylsilylethynyl)-2'-deoxyuridine with tertiary butyl ammonium fluoride ($NBu^{+}F^{-}$) in dry THF and purification by column chromatography afforded 3',5'-di-O-p-toluoyl-5-ethynyl-2'-deoxyuridine in good yield.

5-Ethynyl-2'-deoxyuridine was formed quantitatively by the reaction of 3',5'-di-O-p-toluoyl-5-ethynyl-2'-deoxyuridine with sodium methoxide in methanol followed by column chromatography and crystallization.

(53)



This provided all the starting materials for production of the oxazophosphorine analogues. They were all formed by simple addition of nucleoside to dry pyridine containing a slight molar excess of cyclic phosphochloridate, left stirring overnight and purified by column chromatography. One other analogue was produced this was the 3'-azido-3'-deoxythymidine. The starting material here was provided by the Wellcome Company.

Biological Results

In vitro testing against a range of viruses are being performed by the Wellcome company. Preliminary results have shown that 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-yl)-(E)-5-(2-bromovinyl)-2'-deoxyuridine has an activity of 100% at 10 M against varicella zoster virus. It is planned that further in vitro testing and some in vivo testing will be undertaken.

Summary and Conclusion

In conclusion it is noted that all the compounds which were planned have been made. The activity of two of the compounds is interesting, without the presence of microsomal oxidase it was not envisaged that any activity would be seen. This raises two questions (a) is the cyclic nucleoside active in its own right or (b) is the base being cleaved by cellular glycosidases and then being reformed into the parent nucleoside?.

A hydrolysis experiment with 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-yl) thymidine in a buffered pH 7

solution at 37°C showed no breakdown of starting material after 72 hours.

Further *in vivo* testing is being carried out and this should give more conclusive results.

EXPERIMENTAL

Thin Layer Chromatography.

Precoated, aluminium-backed silica-gel plates were supplied by E.Merck A.G., Darmstadt, Germany. (Silica-gel 60 F_{254}, 0.2mm thickness). Development was by the ascending method. Detection was achieved by quenching of the fluorescence at 254nm, and by spraying with 10% sulphuric acid in ethanol followed by heating to 100°C to produce charring.

Column Chromatography.

Columns were slurry-packed in the chosen eluent under gravity with silica-gel (Kieselgel 60, 70-250 mesh ASTM, type 7734, or 230-400 mesh ASTM, type 9385, supplied by E.Merck A.G.). Samples were applied either as a solution in the same eluent, or absorbed onto silica-gel.

UV Spectroscopy.

Samples were dissolved in spectroscopic grade ethanol and spectra recorded on a Perkin-Elmer 552 spectrometer giving automatic digital- and pen-recorded-read-out of wavelength and absorbance.

NMR Spectroscopy.

^{1}H NMR spectra were recorded on Jeol FX90Q (90MHz) and Jeol GX270 (270MHz) spectrometers, relative to an internal tetramethylsilane reference.

Mass Spectrometry.

Spectra were obtained on a Kratos MS80 mass spectrometer with a DS 55 data system with automatic digital readout. Electron-impact or Fast Atom Bombardment (FAB) ionisation were used. Samples were dissolved in DMSO and 3-nitrobenzyl alcohol was used

as matrix. Sodium ion doping, to give enhanced peaks, was employed as necessary.

Elemental Analysis.

Analyses were obtained in the micro-analytical laboratory of this department.

Solvents and Reagents

Acetone

Acetone was refluxed with potassium permanganate for 2 hours then it was collected in a pressure equalizing funnel and dried over anhydrous potassium carbonate. This was then filtered and the acetone was distilled at room temperature.

Chloroform

Chloroform was heated under reflux over calcium hydride, distilled and stored over type-4A molecular sieves.

N,N-Dimethylformamide

N,N-Dimethylformamide was stirred overnight with phosphorus pentoxide, filtered and distilled from fresh phosphorus pentoxide at reduced pressure.

1,4-Dioxane

1,4-Dioxane was refluxed over calcium hydride, distilled and stored over molecular sieves.

Pyridine and triethylamine

Pyridine and triethylamine were heated under reflux over calcium hydride, then distilled and stored over potassium hydroxide pellets.

Tetrahydrofuran

Tetrahydrofuran was heated under reflux over sodium in the

presence of benzophenone until a deep blue colour had developed. Solvent was then distilled and stored over molecular sieve.

Palladium compounds

Palladium salts were obtained from Aldrich Chemical Co. Ltd. and stored desiccated from chemical vapours to prevent inactivation.

Nucleosides

Nucleosides were dried under high vaccuum over phosphorus pentoxide overnight.

Preparation of 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide

Phosphoryl chloride (46.5ml, 0.5m) was dissolved in dry chloroform (400ml), stirred and cooled to -15°. A solution of 3-aminopropan-1-ol (37ml, 0.5m) in dry chloroform containing triethylamine (70ml) was added dropwise with stirring at -10°. After addition a solution of triethylamine (70ml) in dry chloroform was added dropwise with stirring, the temperature was not allowed to rise above 0°. The mixture was allowed to warm to room temperature overnight. The solvent was evaporated under reduced pressure and the solid remaining was extracted with dry acetone (4x200ml). The acetone extracts were evaporated and the residue was treated with dry chloroform (100ml) and carbon tetrachloride was added (300ml). After trituration and standing crystals appeared which were collected and dried to give 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide (45g, 58% yield) m.p. 81, (lit: 80-83)[45].

Elemental Analysis: Found: C, 23.3; H, 4.7; N, 8.9; $C_3H_7NO_2PCl$ requires C,23.17; H, 4.54; N, 9.01%.

N.M.R. Spectrum ^1H, $\delta(d_6\text{-DMSO})$: 4.85-4.16 (3-H, m, H-6, NH), 3.7-3.0 (2H, m, H-4), 2.6-1.4 (2H, m, H-5).

FAB Mass Spectrum: m/e 157, 155 (M^+).

Preparation of (E)-5-(2-carbomethoxyvinyl)-2'-deoxyuridine

Palladium acetate (0.6g, 2.6mmole), triphenylphosphine (1.44g, 5.4mmole) and redistilled triethylamine (10ml) were combined in dry dioxane (80ml) and stirred at 70° until an intense red colouration had developed. Then, 5-iodo-2'-deoxyuridine (20g, 56.5mmole) and methylacrylate (9.30g, 108mmole) were added and the mixture stirred under reflux for 1 hour and filtered through Celite while hot. The filtrate was evaporated to dryness under reduced pressure and the solid was washed twice with ether then recrystallised from ethanol to give the product as white crystals (9.8g, 58% yield). M.pt. 172° (lit 170-173°)[73]

UV Spectrum: pH 7 in water

λmax=301nm	ε=16700
λmax=269nm	ε=10900
λmin=271nm	ε=10800

N.M.R. Spectrum ^{1}H, δ(d_6-DMSO): 11.0 (1-H, bs, N-H), 8.41 (1-H, s, H-6), 7.37 (1-H, d, vinylic J=16Hz), 6.38 (1-H, d, vinylic J=16Hz), 6.12 (1-H, t, H-1'), 5.2 (2-H, bs, OH-3' and 5'), 4.31 (1-H, m,H-3'), 3.8 (1-H, m, H-4'), 3.7 (3-H, s, $-CH_3$), 3.6 (2-H, m, H-5'), 2.2 (2-H, m, H-2').

Preparation of (E)-5-(2-carboxyvinyl)-2'-deoxyuridine

(E)-5-(2-carbomethoxyvinyl)-2'-deoxyuridine (2g, 6.4mmole) was dissolved in aqueous sodium hydroxide solution (100ml, 1M) and stirred for 1 hour at room temperature. The solution was then filtered and the clear filtrate was carefully acidified to pH2 with hydrochloric acid (4M). On cooling to 4° a white precipitate

formed which was washed with water (2x50ml) and acetone (2x50ml) and dried to give the required product (1.25g, 63% yield). M.pt. 265° (lit 267°)[73].

UV Spectrum:	pH 7 in water	
	λmax=297nm	ε=14600
	λmax=263nm	ε=11800
	λmin=275nm	ε=10900

N.M.R. Spectrum ^1H, δ(d_6-DMSO): 11.4 (1-H, bs, N-H), 10.9 (1-H, bs, COOH), 8.35 (1-H, s, H-6) 7.25 (1-H, d vinylic=16Hz), 6.74 (1-H. d vinylic=16Hz), 6.11 (1-H, t, H-1'), 5.15 (2-H, bs, OH-3' and 5'), 4.22 (1-H, m, H-3'), 3.8 (1-H, m, H-4'), 3.62 (2-H, m H-5'), 2.18 (2-H, m, H-2')

Preparation (E)-5-(2-bromovinyl)-2'-deoxyuridine

To a solution of (E)-5-(2-carboxyvinyl)-2'-deoxyuridine (3g, 10mmole) in DMF (50ml) was added potassium carbonate (2.2g, 17mmole) and the solution was stirred at room temperature for 15 minutes. A solution of N-bromosuccinimde (1.8g, 10.5mmole) in dry DMF (50ml) was added. This was accompanied by the immediate evolution of carbon dioxide. The resulting suspension was immediately filtered under suction and the precipitate was washed with DMF. This solution was evaporated under reduced pressure to give an oil. This oil was dissolved in methanol and silica gel added. Removal of the solvent under reduced pressure gave a free flowing powder which was applied to a silica gel column prepared with chloroform and methanol (9:1). Elution with the same solvent

and combination of the appropriate fractions followed by removal of solvent under reduced pressure gave a white powder. This powder was recrystallised from water to give the product as white crystals (1.1g ,33% yield). M.pt. 140-142° (lit 140°)[73].

UV Spectrum: pH 7 in ethanol

λmax=294nm	ε=10250
λmax=250nm	ε=13100
λmin=273nm	ε=6400

N.M.R. Spectrum ^1H, δ(d_6-DMSO): 11.57 (1-H, bs, NH), 8.08 (1-H, s, H-6), 7.26 (1-H, d vinylic J=14Hz), 6.82 (1-H, d vinylic J=14Hz), 6.13 (1-H, t, H-1'), 5.23 (2-H, d, OH-3'), 5.07(1-H, t, OH-5'), 4.25 (1-H, m, H-3'), 3.8 (1-H, m, H-4'), 3.60 (2-H, m, H-5'), 2.15 (2-H, m, H-2').

Preparation of 5-vinyl-2'-deoxyuridine

(E)-5-(2-carboxyvinyl)-2'-deoxyuridine (8g, 29mmole), triethylamine (30ml) and water (4ml) were combined in purified DMF (130ml) and the mixture kept at 105° overnight. The orange solution was then evaporated to dryness under reduced pressure and applied to a silica gel column prepared with chloroform and methanol (4:1). Elution with the same solvent and combination of the appropriate fractions (R.f. 0.41) followed by removal of the solvent gave a white powder which was recrystallised from chloroform:methanol (1:1) to give the product as colourless needles (4.0g, 60% yield).

UV Spectrum: pH 7 in ethanol

λmax=290nm	ε=8200

λmax=237nm ε=11000

λmin=262nm ε=4600

N.M.R. Spectrum ^1H, δ(d_6-DMSO): 11.53 (1-H, bs, NH), 8.15 (1-H, s, H-6), 6.4 (1-H, dd vinylic J= Hz H-1"), 6.13 (1-H, t, H-1'), 5.86 (1-H, dd vinylic J= Hz H-2"), 5.13 (3-H, m, vinylic H-2" and OH-3' and 5'), 4.24 (1-H, m, H-3'), 3.77 (1-H, m, H-4'), 3.57 (2-H, m, H-5'), 2.14 (2-H, m, H-2').

Preparation of 3',5'-di-O-p-toluoyl-5-iodo-2'-deoxyuridine

To a stirred solution of 5-iodo-2'-deoxyuridine (5.1g, 14.4mmole) in dry pyridine (70ml) at 0° was added a cold solution of p-toluoyl chloride (4.5ml, 35mmole) dropwise over 30 minutes. The mixture was allowed to slowly attain room temperature, after which water (100ml) was added followed by dichloromethane (80ml). The organic layer was separated, evaporated under reduced pressure and coevaporated with toluene, ethanol and acetone. The resulting white solid was applied to a silica column in chloroform and eluted with the same solvent initially, followed by chloroform-ethanol (20:1). Evaporation of the appropriate fractions (R.f. 0.63) gave a white solid. The solid was dissolved in the minimum volume of boiling chloroform. The addition of 5 volumes of methanol gave the product as white crystals (6.5g, 76% yield). M.pt. 194-196° (lit 195-196°)[74]

N.M.R. Spectrum ^1H, δ(d_6-DMSO): 11.8 (1-H, s, NH), 8.1 (1-H, s, H-6), 7.9 (4-H, m, phenyl H), 7.34 (4-H, m, phenyl H), 6.24 (1-H, t, H-1'), 5.38 (1-H, m, H-3'), 4.6 (2-H, m, H-5'), 4.53 (1-H, m,

H-4'), 2.6 (2-H, m, H-2'), 2.4 (6-H, s, toluoyl CH_3).

Preparation of bistriphenylphosphine palladium chloride

To a solution of $K_2(PdCl_4)$ (1 mmole of $PdCl_2$ and 2 mmole of KCl) in water (5ml) was added a solution of triphenylphosphine (2mmole) in ethanol (5ml) with stirring. Precipitation of $PdCl_2(PPh_3)_2$ as a bright yellow solid was instant. After stirring for 10 minutes the complex was filtered off, washed with water, ethanol and ether and dried (505mg, 72% yield).

UV Spectrum: pH 13 in water

λmax=272nm	ε=10070
λmax=232nm	ε=28100
λmin=261nm	ε=9560

Preparation of 3',5'-di-O-p-toluoyl-5-(2-(trimethylsilyl)ethynyl)-2'-deoxyuridine

To dry triethylamine (200ml) at 50° under nitrogen was added 3',5'-di-O-p-toluoyl-5-iodo-2'-deoxyuridine (3.3g 5.6mmole), bistriphenylphosphine palladium chloride (280mg), copper (I) iodide (280mg) and trimethylsilylacetylene (1.1g, 11mmole) and the mixture was maintained at 50° with stirring for 4 hours. The grey mixture was filtered hot through Celite and the filtrate was evaporated to dryness under reduced pressure. The solid was dissolved in chloroform (200ml) and extracted with 5% EDTA solution (2x120ml) and water (100ml). After drying with $MgSO_4$ and evaporation, the off white solid was dissolved in the minimum volume of boiling chloroform. The addition of 5 volumes of methanol gave the product

as white crystals (2.7g, 86% yield). M.pt. 255-256° (lit 255-256°)[71]

UV Spectrum: pH 13 in water

λmax=282nm	ε=7800
λmax=234nm	ε=27900
λmin=261nm	ε=5150

N.M.R. Spectrum ^1H, δ(d_6-DMSO): 11.75 (1-H, s, NH), 8.04 (1-H, s, H-6), 7.9 (4-H, m, phenyl H), 7.34 (4-H, m, phenyl H), 6.24 (1-H, t, H-1'), 5.58 (1-H, m, H-3'), 4.61 (2-H, m, H-5'), 4.53 (1-H, m, H-4'), 2.65 (2-H, m, H-2'), 2.39 (6-H, s, toluoyl CH_3), 0.13 (9-H, s, $-Si(CH_3)_3$).

Preparation of 3',5'-di-O-p-toluoyl-5-ethynyl-2'-deoxyuridine

To a suspension of 3',5'-di-O-p-toluoyl-5-(2-(trimethylsilyl) ethynyl)-2'-deoxyuridine (5.6g, 10mmole) in dry THF was added a solution of tetrabutylammonium fluoride in THF (1M, 10ml, 10mmole). After stirring for 1 hour the solution was evaporated to drynes and dissolved in chloroform-methanol (5:5) and applied to a silica column eluted with the same solvent. Evaporation of the appropriate fractions gave a white solid. The solid was dissolved in the minimum volume of boiling chloroform. The addition of 5 volumes of methanol gave the product as a white solid (3.5g, 71% yield). M.pt. 200-201° (lit 202-203°)[70]

N.M.R. Spectrum ^1H, δ(d_6-DMSO): 11.75 (1-H, s, NH), 8.04 (1-H, s, H-6), 7.9 (4-H, m, phenyl H), 7.34 (4-H, m, phenyl H), 6.24 (1-H, t, H-1'), 5.58 (1-H, m, H-3'), 4.61 (2-H, m, H-5'), 4.53 (1-H, m,

H-4'), 4.05 (1-H, s, acetylene H), 2.65 (2-H, m, H-2'), 2.39 (6-H, s, toluoyl CH_3),

Preparation of 5-ethynyl-2'-deoxyuridine

To a suspension of 3',5'-di-O-p-toluoyl-5-ethynyl-2'-deoxyuridine (3.0g, 6.1mmole) in dry methanol was added sodium methoxide (0.75g, 13.5mmole) and the mixture was stirred for 1 hour at room temperature whereupon tlc showed complete reaction. Neutralisation was achieved with glacial acetic acid the solution was then evaporated to dryness under reduced pressure. The solid was dissolved in chloroform and applied to a silica column eluted first with the same solvent and then with chlorform-methanol (4:1). Evaporation of the appropriate fractions (R.f. 4.1)gave a white solid which was recrystallised from chlorform-methanol to give the product as white needles (1.45g, 94% yield). M.pt. 207-209° (lit 207-209°)[70]

UV Spectrum: pH 6 in ethanol

λmax=286nm ε=11480

N.M.R. Spectrum ^1H, $\delta(d_6$-DMSO): 11.5 (1-H, bs, NH), 8.28 (1-H, s, H-6), 6.1 (1-H, t, H-1'), 5.2 (2-H, bs, OH-3' and OH-5'), 4.24 (1-H, m, H-3'), 4.05 (1-H, s, acetylene H), 3.8 (1-H, m, H-4'), 3.62 (2-H, m, H-5'), 2.15 (2-H, m, H-2').

Preparation of 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-2"-yl)thymidine

To a solution of thymidine (2.42g, 10mmole) in dry pyridine (20ml) was added 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide (1.56g, 10mmole) at room temperature. The mixture was left

to stir overnight and then evaporated to dryness, coevaporated with toluene, ethanol and acetone. This was then applied to a silica column and eluted in chloroform-methanol (4:1). Evaporation of the appropriate fractions gave the product (1.2g, 33% yield). M.pt 189°.

Elemental Analysis: Found: C,43.17; H, 5.66; N, 11.43;

$C_{13}H_{20}N_3O_7P$ requires C, 43.21; H, 5.54; N, 11.63%.

N.M.R. Spectrum ^1H, $\delta(d_6$-DMSO): 11.25 (1-H, bs, NH), 7.5 (1-H, d, H-6), 6.2 (1-H, t, H-1'), 5.4 (1-H, 2 singlets, N"H enantiomeric), 5.15 (1-H, bs, OH-3'), 4.4-3.8 (6-H, m, H-3', H-4', H-5', H-6"), 3.3-2.9 (2-H, m, H-4"), 2.2 (2-H, m, H-2'), 1.8 (3-H, s, CH_3), 1.7 (2-H, m, H-5").

Mass Spectrum: m/e 361 (m+)

Preparation of 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-2"-yl)thymidine

To a solution of phosphoryl chloride (1 ml, 10.8 mmol) and N-methyl morpholine (1 ml) in 1,2-dimethoxyethane (20 ml) was added at 0° under dry nitrogen a solution of thymidine (8 mmol) in 1,2-dimethoxyethane (40 ml). The mixture was allowed to reach room temperature and stirred overnight. The solution was recooled to 0° and 3-aminopropan-1-ol (1g, 14 mmol) was added in 1,2-dimethoxyethane (25 ml) and left to stir overnight. The solution was then evaporated to dryness and applied to a silica column with ethyl acetate/ethanol (3:1) as an eluant. Evaporation of the appropriate fractions (R.f. 0.64) gave a pale yellow oil.

Coevaporation with acetone gave the product as a white foam (204 mg, 6% yield).

N.M.R. Spectrum ^1H, $\delta(d_6\text{-DMSO})$: 11.25 (1-H, bs, NH), 7.5 (1-H, d, H-6), 6.2 (1-H, t, H-1'), 5.4 (1-H, 2 singlets, N"H enantiomeric), 5.15 (1-H, bs, OH-3'), 4.4-3.8 (6-H, m, H-3', H-4', H-5', H-6"), 3.3-2.9 (2-H, m, H-4"), 2.2 (2-H, m, H-2'), 1.8 (3-H, s, CH_3), 1.7 (2-H, m, H-5").

Attempts to Optimise the preparation of 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-2"-yl)thymidine

To a solution of thymidine (2.40g, 10mmole) in dry pyridine (20ml) was added 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide (1.55g, 10mmole) and a catalytic amount of dimethylaminopyridine at room temperature. The mixture was left to stir overnight and then evaporated to dryness, coevaporated with toluene, ethanol and acetone. This was then applied to a silica column and eluted in chloroform-methanol (4:1). Evaporation of the appropriate fractions gave the product (1.05g, 29% yield). M.pt 189°.

N.M.R. Spectrum ^1H, $\delta(d_6\text{-DMSO})$: 11.25 (1-H, bs, NH), 7.5 (1-H, d, H-6), 6.2 (1-H, t, H-1'), 5.4 (1-H, 2 singlets, N"H enantiomeric), 5.15 (1-H, bs, OH-3'), 4.4-3.8 (6-H, m, H-3', H-4', H-5', H-6"), 3.3-2.9 (2-H, m, H-4"), 2.2 (2-H, m, H-2'), 1.8 (3-H, s, CH_3), 1.7 (2-H, m, H-5").

Attempts to optimise the preparation of 5'-O-(tetrahydro-2H-

1",3",2"-oxazophosphorine 2"-oxide-2"-2"-yl)thymidine

Thymidine was added to four 50ml flasks (A=1.2g, 0.5 mmol; B=1.0g 0.4 mmol; C=1.13g 0.47 mmol and D=1.2g 0.5 mmol) . Pyridine (25 ml) was added to each flask and then 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide was added (A=0.78g 0.5 mmol; B=0.77g 0.5 mmol; C=1.55g 10 mmol and D=1.58g 10.2 mmol). Flasks A and C were left to stir at room temperature for 24 hours and the worked up as in the previous reaction. Flasks B and D were allowed to stir for 72 hours and worked in the same way.

Yield: A= 0.51 g 28%

B= 0.55 g 30%

C= 0.53 g 29%

D= 0.47 g 26%

Preparation of 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-yl)-5-(2-carbomethoxyvinyl)-2'-deoxyuridine

To a solution of (E)-5-(2-carbomethoxyvinyl)-2'-deoxyuridine (3.12g, 10mmole) in dry pyridine (20ml) was added 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide (1.56g, 10mmole) at room temperature. The mixture was left to stir overnight and then evaporated to dryness, coevaporated with toluene, ethanol and acetone. This was then applied to a silica column and eluted in chloroform-methanol (4:1). Evaporation of the appropriate fractions (R.f. 0.37) gave the product as a white foam (1.42g, 33% yield).

Elemental Analysis: Found: C, 43.2; H, 5.1; N, 9.2;

$C^{16}H^{22}N^{3}O^{9}P+H^{2}O$ requires C, 44.9; H, 5.3; N, 9.3%.

N.M.R. Spectrum ^1H, $\delta(d_6$-DMSO): 11.7 (1-H, bs, NH), 8.2 (1-H, s, H-6), 7.5 (1-H, d, vinylic J=16Hz), 6.9 (1-H, d, vinylic J=16Hz), 6.2 (1-H, t, H-1'), 5.4 (1-H, m, N"H), 5.15 (1-H, bs, OH-3'), 4.5-3.9 (6-H, m, H-3', H-4', H-5', H-6"), 3.7 (3-H, s, CH_3), 3.2-2.8 (2-H, m, H-4"), 2.2 (2-H, m, H-2'), 1.6 (2-H, m, H-5").

Preparation of 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-yl)-(E)-5-(2-bromovinyl)-2'-deoxyuridine

To a solution of (E)-5-(2-bromovinyl)-2'-deoxyuridine (3.3g, 10mmole) in dry pyridine (20ml) was added 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide (1.56g, 10mmole) at room temperature. The mixture was left to stir overnight and then evaporated to dryness, coevaporated with toluene, ethanol and acetone. This was then applied to a silica column and eluted in chloroform-methanol (4:1). Evaporation of the appropriate fractions (R.f. 0.43) gave the product foam (1.12g, 25% yield).

Elemental Analysis: Found: C,37.20 ; H,4.21 ; N,9.27 ; $C_{14}H_{19}N_3O_7PBr$ requires C,37.17 ; H,4.2 ; N,9.29 %.

N.M.R. Spectrum ^1H, $\delta(d_6$-DMSO): 11.62 (1-H, bs, NH), 7.9 (1-H, d, H-6), 7.3 (1-H, d, vinylic J=14Hz), 6.94 (1-H, d, vinylic J=14Hz), 6.2 (1-H, t, H-1'), 5.44 (1-H, m, N"H), 5.2 (1-H, m, OH-3'), 4.4-3.8 (6-H, m, H-3', H-4', H-5', H-6"), 3.2-2.7 (2-H, m, H-4"), 2.2 (2-H, m, H-2'), 1.57 (2-H, m, H-5").

Mass Spectrum: m/e 453 (M^+)

Preparation of 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-yl)-5-vinyl-2'-deoxyuridine

To a solution of (E)-5-vinyl-2'-deoxyuridine (2.54g, 10mmole) in dry pyridine (20ml) was added 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide (1.56g, 10mmole) at room temperature. The mixture was left to stir overnight and then evaporated to dryness, coevaporated with toluene, ethanol and acetone. This was then applied to a silica column and eluted in chloroform-methanol (4:1). Evaporation of the appropriate fractions (R.f. 0.48) gave the product as a foam (1.04g, 27% yield).

Elemental Analysis: Found: C, 45.21; H, 5.62; N,10.98;

$C_{14}H_{20}N_3O_7P$ requires C, 45.04; H, 5.36; N, 11.26%.

N.M.R. Spectrum ^1H, $\delta(d_6\text{-DMSO})$: 11.15 (1-H, bs, NH), 7.7 (1-H, d, H-6), 6.35 (1-H, dd, vinyl J=11Hz, Ha trans), 6.15 (1-H, t, H-1'), 6.0 (1-H, dd, vinyl J=5Hz, Hb cis), 5.4 (1-H, m N"H), 5.2 (1-H, bs, OH-3'), 5.15 (1-H, d, vinyl, Hx), 4.8-3.9 (6-H, m, H-3', H-4', H-5', H-6"), 3.2-2.9 (2-H, m, H-4"), 2.2 (2-H, m, H-2'), 1.6 (2-H, m, H-5").

Preparation of 5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-yl)-5-ethynyl-2'-deoxyuridine

To a solution of (E)-5-ethynyl-2'-deoxyuridine (2.52g, 10mmole) in dry pyridine (20ml) was added 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide (1.56g, 10mmole) at room temperature. The mixture was left to stir overnight and then evaporated to dryness, coevaporated with toluene, ethanol and acetone. This was then

applied to a silica column and eluted in chloroform-methanol (4:1). Evaporation of the appropriate fractions (R.f. 0.39) gave the product as a white foam (1.07g, 29% yield).

N.M.R. Spectrum ^1H, $\delta(d_6\text{-DMSO})$: 11.5 (1-H, bs, NH), 8.28 (1-H, s, H-6), 6.1 (1-H, t, H-1'), 5.4 (1-H, m N"H), 5.2 (2-H, bs, OH-3' and OH-5'), 4.8-3.9 (7-H, m, H-3', H-4', H-5', acetylene H, H-6"), 3.2-2.9 (2-H, m, H-4"), 2.2 (2-H, m, H-2'), 1.6 (2-H, m, H-5").

Preparation of 3'-azido-3'-deoxy-5'-O-(tetrahydro-2H-1",3",2"-oxazophosphorine 2"-oxide-2"-yl)thymidine

To a solution of 3'-azido-3'-deoxythymidne (2.67g, 10mmole) in dry pyridine (20ml) was added 2-chloro-tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide (1.56g, 10mmole) at room temperature. The mixture was left to stir overnight and then evaporated to dryness, coevaporated with toluene, ethanol and acetone. This was then applied to a silica column and eluted in chloroform-methanol (4:1). Evaporation of the appropriate fractions (R.f. 0.52) gave the product as a white/cream foam (1.46g, 38% yield)

N.M.R. Spectrum ^1H, $\delta(d_6\text{-DMSO})$: 11.33 (1-H, bs, N-H), 7.68 (1-H, s, H-6), 6.1 (1-H, t, H-1'), 5.4 (1-H, bs, N"-H), 4.84-3.8 (6-H, m, H-3',H-4',H-5',H-6"), 3.2-2.8 (2-H, m, H-4"), 2.4 (2-H, m, H-2') 1.78 (3-H, s, CH_3), 1.6 (2-H, m, H-5")

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