Novel Nucleoside Analogues Containing Modified Sugars.

A thesis presented to The City University in part fulfilment of the requirements for the degree of Doctor of Philosophy.

By

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A. METE

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ABSTRACT

Synthetic routes to several novel series of nucleoside analogues, bearing modifications at the 2'- and 3'-positions, are presented. Attempts at preparing 2',3'-dihalogenated uridine nucleosides are described first, followed by the application of various organometallic reactions, on both purine and pyrimidine nucleosides, to afford a number of 3'- and 2'-alkylated derivatives.

The preparation of a number of 2',3'-dihalogenated-2',3'-dideoxy-uridines was attempted by several routes but without success. Two major side reactions were encountered which lead to 5'-O-trityl-3'-O-mesyl-O^2,2'-anhydro-uridine and 5'-O-trityl-2'-halo-2',3'-didehydro-2',3'-dideoxy-uridines. It proved possible to select the reaction conditions, so as to afford either one of the above two types of compounds as the sole product, in good overall yield. The 5'-O-trityl-2'-halo-2',3'-didehydro-2',3'-dideoxy-uridines are a class of compounds which have received limited attention in the literature, therefore a study of their reactions was undertaken (see below).

The application of some organometallic reactions on a uridine-2',3'-lyxo-epoxide, to give 3'-alkylated-3'-deoxy-ara-uridines, in good overall yields from uridine, is described. The use of three types of organometallic reagents was explored. These were the organolithiums, Grignards and lithium dialkylcuprates, which lead to the direct introduction of an alkynyl, alkenyl and alkyl side-chain respectively, at the 3'-position of the uridine molecule. Attack was shown to occur predominantly at the 3'-position by n.m.r. techniques. A limited amount of attack at the 2'-position was noted in most of the epoxide-opening reactions to yield a 2'-alkylated-2'-deoxy-xylo-uridine, although it proved possible to isolate this minor product in only one case. Attack at C-6 of the uridine molecule was observed only when the uridine-2',3'-lyxo-epoxide was treated with lithium 1,3-dithian-2-yl, in which case it was the sole course of reaction. With the most basic organometallic reagents (e.g. butyl lithium), abstraction of the 1'-proton occured to give a 1',2'-unsaturated nucleoside. Some of the 3'-alkylated-3'-deoxy-ara-uridines were converted to the corresponding ara-cytidine, ara-thymidine and 5-halo-ara-uridine derivatives by known methodology. A brief investigation of the reactions of the 3'-unsaturated side-chains (e.g. hydrogenation, hydroboration and epoxidation) is described. An attempt at inverting the configuration at C2', in 5'-O-trityl-3'-ethynyl-2'-O-mesyl-3'-deoxy-ara-uridine was made. However, the sole product of reaction, isolated in high yield, was 5'-O-trityl-3'-ethynyl-2',3'-didehydro-2',3'-dideoxy-uridine.

Some reactions of the 5'-O-trityl-2'-halo-2',3'-didehydro-2',3'-dideoxy-uridines (mentioned earlier) were explored. A variety of electrophilic reagents (e.g. MCPBA, BH_3, Br_2) did not react with the 2',3'-double bond. However, the 2'-bromo analogue reacted with lithium dimethylcuprate to undergo metal-halogen exchange and give a 2'-lithiated derivative. This could be protonated, to afford 5'-O-trityl-2',3'-didehydro-2',3'-dideoxy-uridine, as well as alkylated (with MeI) to give 5'-O-trityl-2'-C-methyl-2',3'-didehydro-2',3'-dideoxy-uridine; both obtained in good yield. Application of this reaction to the 2'-chloro analogue afforded the above mentioned 2'-C-methyl derivative, together with 5'-O-trityl-2'-keto- 3'-deoxy-uridine. A mechanism by which the latter two compounds might arise from a common intermediate is presented.

Finally, the application of some organometallic reactions on a 5'-protected adenosine-2',3'-lyxo-epoxide is described. This lead to a good synthetic route to 3'-methyl-3-deoxy-ara-adenosine from ara-adenosine.

ABBREVIATIONS

Ac=	Acetyl	ara=	Arabinosyl
Bu=	Butyl	9-BBN=	9-Borabicyclo[3.3.1]nonane
Bz=	Benzoyl	DBU=	1,5-Diazabicyclo[5.4.0]undec-2-ene.
DEAD=	Diethyl Azodicarboxylate	DMF=	Dimethylformamide
DMSO=	Dimethyl sulphoxide	DNA=	Deoxyribonucleic Acid
Et=	Ethyl	Hrs=	Hours
IPA=	Isopropyl Alcohol	HMPA=	Hexamethylphosphotriamide
lyxo=	Lyxofuranosyl	MCPBA=	m-Chloroperbenzoic Acid
Me=	Methyl	Mins=	Minutes
Ms=	Methanesulphonyl	NBS=	N-Bromosuccinimide
Ph=	Phenyl	Py=	Pyridine
Ribo=	Ribofuranosyl	RNA=	Ribonucleic Acid
Sat=	Saturated	TBDMS=	t-Butyldimethylsilyl
TFA=	Trifluoroacetic Acid	THF=	Tetrahydrofuran
T.M.S.=	Tetramethylsilane	Tr=	Trityl
Trf=	Trifluoromethanesulphonyl	Ts=	4-Toluenesulphonyl
xylo=	Xylofuranosyl		

b.p.=	Boiling Point	i.r.=	Infra-red
m.p.=	Melting Point	n.m.r.=	Nuclear Magnetic Resonance
R.T.=	Room Temperature	t.l.c.=	Thin Layer Chromatography
u.v.=	Ultra-violet	Δ=	Heat

Any other abbreviations as used in Journal of the Chemical Society.

1) INTRODUCTION.

1.1) THE STRUCTURE OF THE NUCLEIC ACIDS.[1]

The nucleic acids are large biomolecules with molecular weights of up to 10^9 Daltons and can be divided into two main types: the deoxyribonucleic acids (DNA) and the ribonucleic acids (RNA). These can be further subdivided into groups depending on their location in the cell (e.g. mitochondrial DNA) or specific function (e.g. messenger RNA, ribosomal RNA). The nucleic acids play a central role in life, their most important functions involving the storage and replication of a cell's genetic information (DNA) and its subsequent translation into cellular events (RNA).

A brief description of the structure of the nucleic acids and their subunits is given below.

1.1.1) The Subunits of Nucleic Acids.

The nucleic acid macromolecules can be broken down both chemically and formally into three subunits: a group of heterocyclic bases, two types of sugar and a phosphoric acid residue. These simple molecules can form more complex nucleic acid subunits known as nucleosides and nucleotides.

1.1.2) The Heterocyclic Bases.

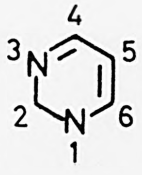
Two classes of heterocyclic bases are found in nucleic acids. One consists of derivatives of pyrimidine, the other of derivatives of purine (Diag. 1.1).

Of the pyrimidine bases, the most commonly found ones are uracil (in RNA), cytosine (in RNA and DNA) and thymine (in DNA). To a lesser extent 5-methylcytosine can be found in DNA while in some coliphages 5-hydroxymethylcytosine replaces cytosine in DNA. Other less common

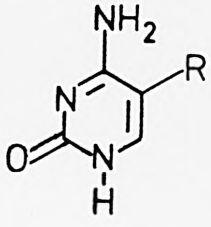
pyrimidine bases are also known and found in some transfer RNAs. Only two purine bases are commonly found in both DNA and RNA. These are adenine and guanine. Various methylated purines, as well as hypoxanthine and xanthine, are also found to a small extent in some RNAs.

DIAGRAM 1.1

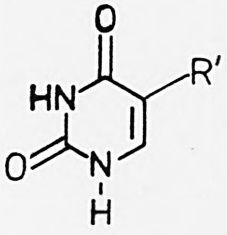
The Pyrimidine Bases.



Pyrimidine

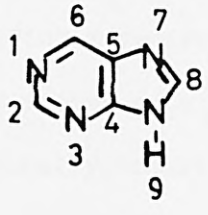


R= H Cytosine
R= Me 5-Methylcytosine
R= CH_2OH 5-Hydroxymethyl-Cytosine

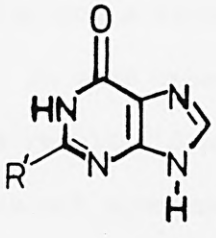


R'= H Uracil
R'= Me Thymine

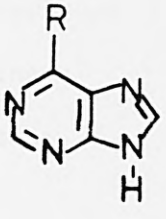
The Purine Bases.



Purine



R'= NH_2 Guanine
R'= OH Xanthine



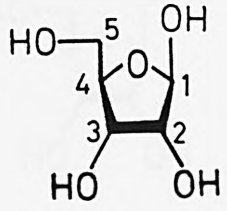
R= NH_2 Adenine
R= OH Hypoxanthine

1.1.3) The Sugar Ring.

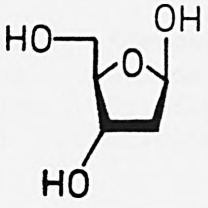
Two sugars are found in nucleic acids. In RNA the sugar is D-ribose present in its β-D-furanose form, while in DNA the sugar is 2-deoxyribose also present in its β-D-furanose form (Diag. 1.2). This small difference in the sugar ring has a profound effect on the relative physical

and chemical properties of RNA and DNA.

DIAGRAM 1.2 The Sugars.



β-D-Ribose



β-D-2-Deoxyribose

1.1.4) The Nucleosides.

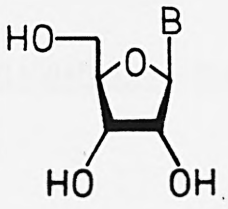
A nucleoside is formed when a purine or pyrimidine base is covalently bonded to a ribose or deoxyribose sugar molecule. Pyrimidines use their N-1 atom whereas purines use their N-9 atom to become attached to the C-1 atom of either ribose (giving a ribonucleoside) or deoxyribose (giving a deoxyribonucleoside). In most cases a nucleoside having a β-D-ribose or a β-D-deoxyribose results (Diag. 1.3), although some naturally-occuring α-nucleosides are also known.

1.1.5) The Nucleotides.

This type of compound is formed when a hydroxyl group of a nucleoside reacts with phosphoric acid to give a phosphate ester. Depending on which hydroxyl group is used we may get a 5'- or 3'- or 2'-monophosphate (the latter in ribonucleotides only). The ribonucleotide 5'-monophosphates (e.g. AMP) may be further phosphorylated at the 5'-position to give the 5'-di- and the 5'-triphosphates (e.g. ADP and ATP), (Diag. 1.4). Ribonucleotide-3',5'-diphosphates, 2',3'-cyclomonophosphates and 3',5'-cyclomonophosphates can also occur.

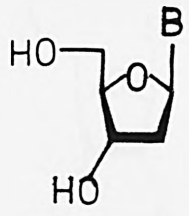
DIAGRAM 1.3

Ribonucleosides.



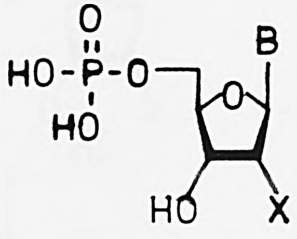
B	Nucleoside
Adenine	Adenosine (A)
Guanine	Guanosine (G)
Cytosine	Cytidine (G)
Uracil	Uridine (U)

Deoxyribonucleosides.



B	Nucleoside
Adenine	Deoxyadenosine (dA)
Guanine	Deoxyguanosine (dG)
Cytosine	Deoxycytidine (dC)
Thymine	Deoxythymidine (dT)

DIAGRAM 1.4 Some Nucleotides.

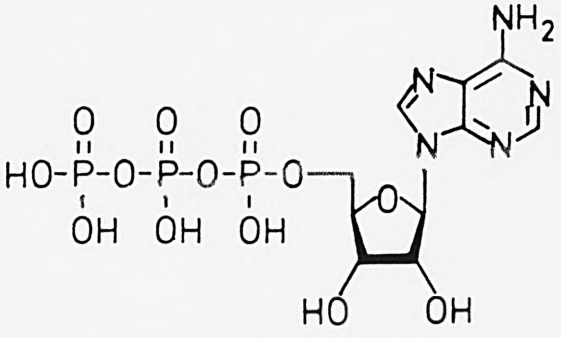


Ribonucleoside-5'-monophosphates

X= OH, B= Adenine, Guanine, Cytosine, Uracil.

Deoxyribonucleoside-5'-monophosphates

X= H, B= Adenine, Guanine, Cytosine, Thymine.

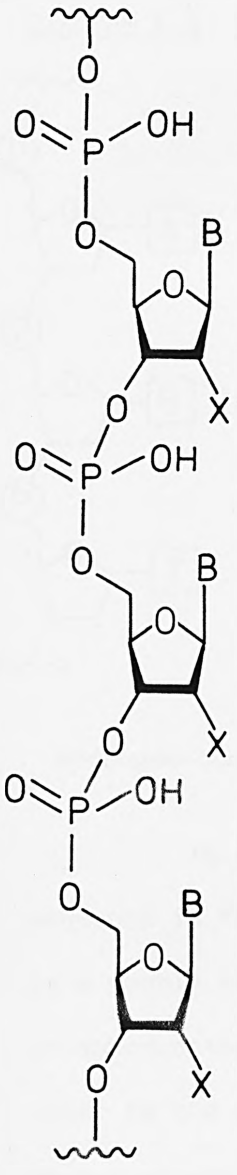


Adenosine-5'-triphosphate (ATP)

1.1.6) The Structure of DNA and RNA.

DNA and RNA consist of long chains of nucleotides held together by a phosphodiester bond between the 3'- and the 5'-positions of adjacent sugar rings (Diag. 1.5).

DIAGRAM 1.5 The Primary Structure of DNA and RNA.



DNA

X = H, B = Adenine, Guanine, Cytosine, Thymine

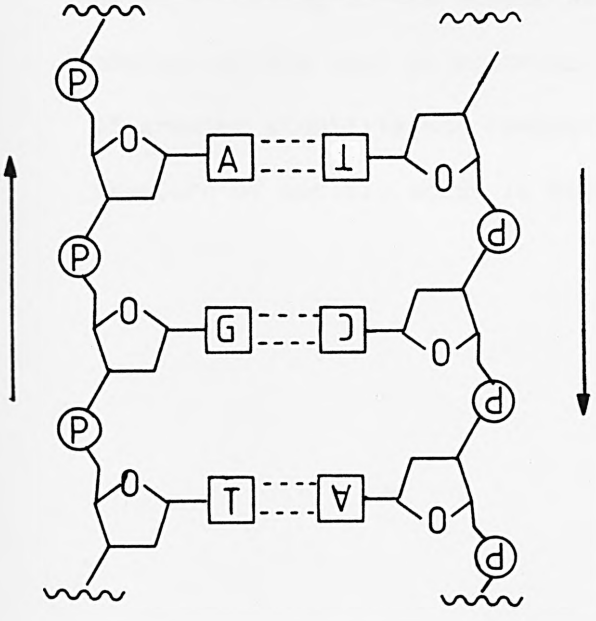
RNA

X = OH, B = Adenine, Guanine, Cytosine, Uracil

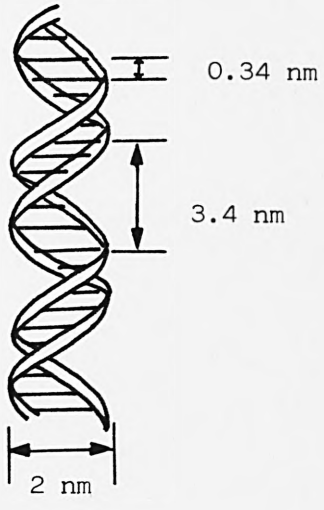
The heterocyclic bases thus play no part in the internucleotide bond. This is known as the primary structure of DNA and RNA.

In DNA, pairs of polydeoxynucleotide chains form a right-handed double helix held together by hydrogen bonding between pairs of heterocyclic bases. The spatial requirements within the helix means that only certain bases can hydrogen bond with each other. Thus adenine pairs with thymine while guanine pairs with cytosine (Diag. 1.6).

DIAGRAM 1.6 The Secondary Structure of DNA.



Hydrogen-bonding Interactions



The Double Helix

This means that the base sequence along one strand determines the sequence in the second strand which must be complementary. The two chains in a double helix have opposite polarity so that starting from one end the phosphodiester link of one runs in the 5'→3' direction while that of the other in the 3'→5' direction. Within the helix the base pairs lie stacked on top of each other, almost perpendicular to the axis of the spiral. The two strands cannot be separated without unwinding the helix. This description of DNA is known as its B structure, it is similar to the Watson-Crick model for DNA[2] and is the conformation most likely adopted in

solution. Other structures (A and C) are also known, which although double helices, differ in the pitch and number of bases per turn. In addition, in both the A and C structures the bases are not perpendicular to the axis of the helix. These two structures are encountered in some double stranded RNA and DNA-RNA hybrids (i.e. during transcription). In general DNA is double stranded whereas RNA in less uniform having many single and double stranded regions within a molecule. This is known as the secondary structure of the nucleic acids.

Further elaboration of the nucleic acid structure can occur such as supercoiling of the double helix and interactions with other macromolecules such as proteins (e.g. histones). These result in stuctures of greater stability and compactness. However, further description of the stucture of nucleic acids is beyond the scope of this work.

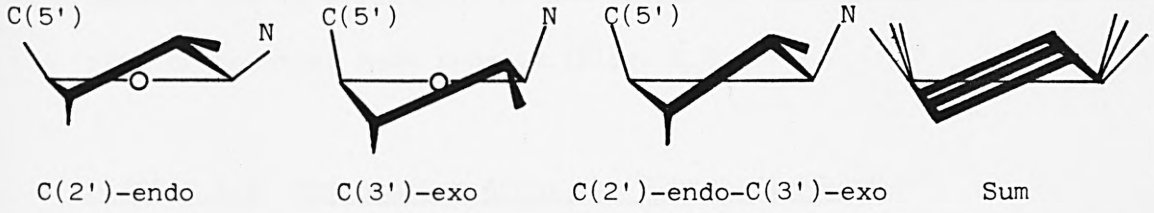
1.2) THE CONFORMATION OF NUCLEOSIDES AND NUCLEOTIDES.[3,4]

The structure of nucleosides and nucleotides allows them to adopt a number of conformations depending on (i) the sugar ring puckering, (ii) the orientation about the glycosidic bond and (iii) the position of the O(5') atom. Each of these aspects is considered in turn below.

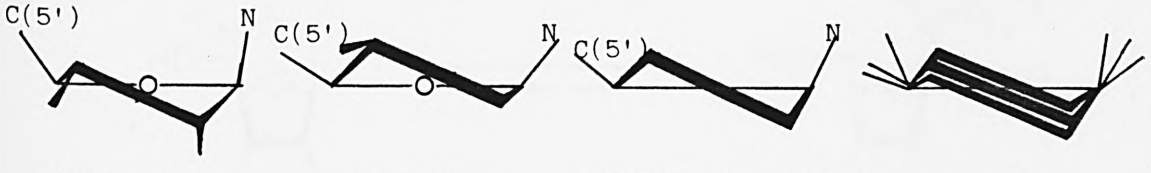
1.2.1) Sugar Ring Puckering.

The five membered sugar ring can adopt two puckered forms; a twist (half-chair), T, or an envelope, E, form. Although the pucker moves around the sugar ring, the prefered puckering modes involve the C(2') and the C(3') atoms and are shown below (Diag. 1.7).

DIAGRAM 1.7 Preferred Sugar Ring Puckering Modes.



C(2')-endo C(3')-exo C(2')-endo-C(3')-exo Sum



C(2')-exo C(3')-endo C(2')-exo-C(3')-endo Sum

C(2')-endo means that the C(2') atom is out of the plane described by the C(1'), C(3'), C(4') and O(1') atoms by about 0.5 Å and on the same side as C(5'). In C(2')-exo, the C(2') atom is on the opposite side of the plane with respect to the C(5') atom. It should be noted that for each ring pucker there is an associated orientation of all the

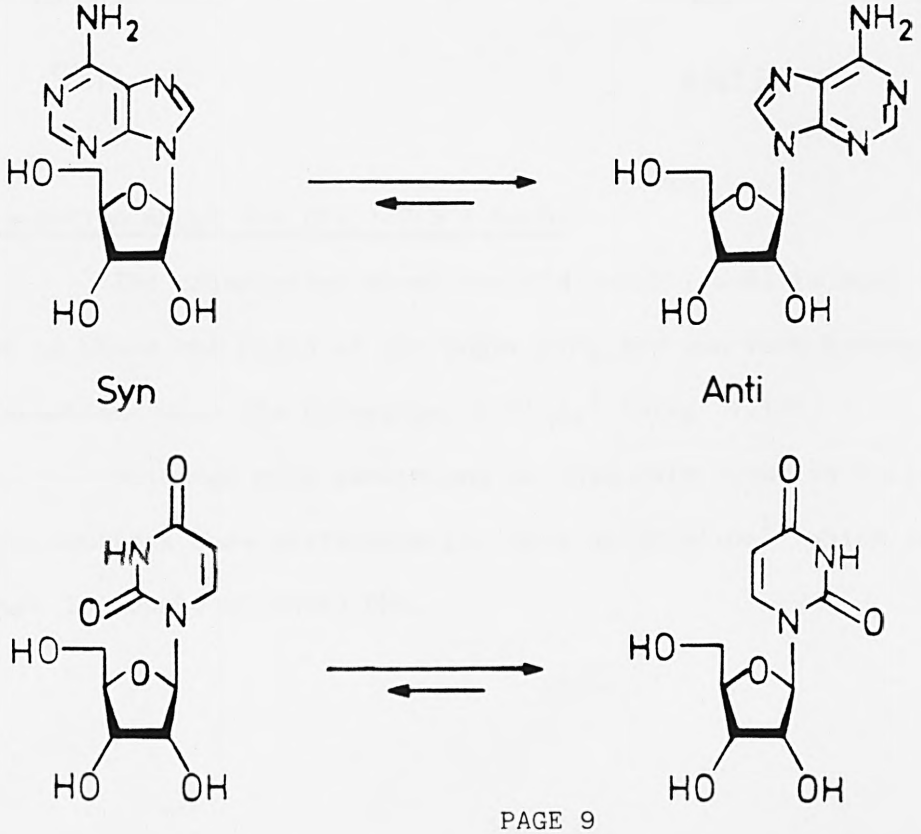
exocyclic bonds. Energetically, the conformations shown along a horizontal line (Diag. 1.7) are close, whereas there is an energy barrier of ca. 5 Kcal/mole separating the two sets of conformations. The whole range of sugar ring conformations has been described by Altona and Sundaralingam who make use of a pseudorotation cycle.[5]

In solution, nucleotides display a C(2')-endo ⇌ C(3')-endo equilibrium.[3] However, in DNA and RNA, the double-helical structure imposes constraints on the sugar conformations. Thus B-, and C-DNA display a C(3')-exo ring pucker.[6] In RNA however, the C(3')-endo ring pucker is the one most commonly found.[7]

1.2.2) Orientation About the Glycosidic Bond.

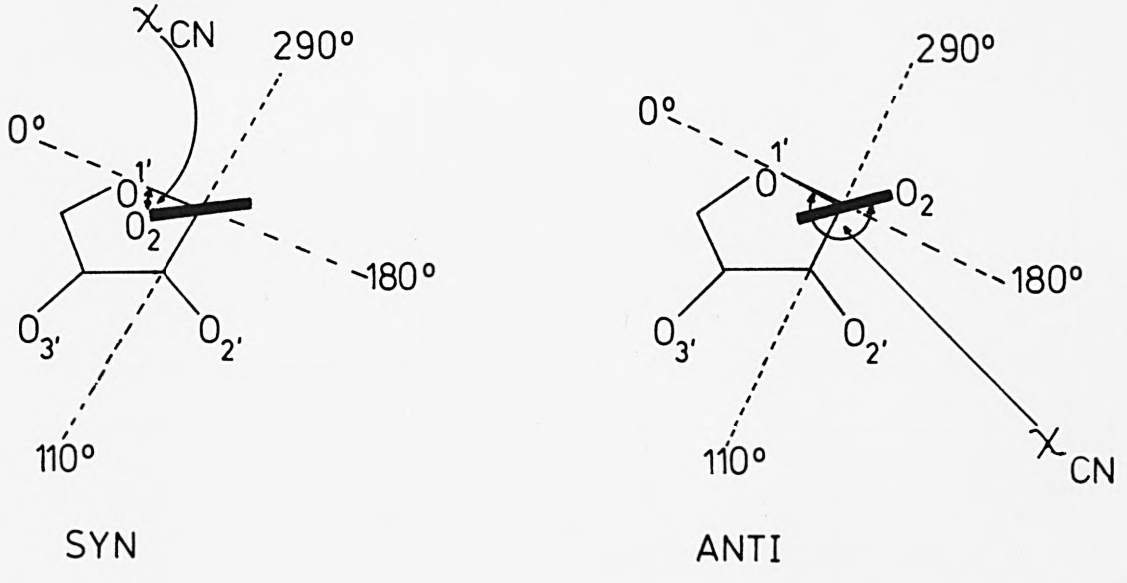
The base in a nucleotide generally adopts one of two conformations. These are known as syn and anti, roughly depending on whether O-2 in pyrimidine or N-3 in purine nucleotides is above the sugar ring or pointing away from it (Diag. 1.8).

DIAGRAM 1.8 Orientation About the Glycosidic Bond.



The position of the base is given by the dihedral angle χ_{C-N} as defined in DIAGRAM 1.9. Thus the syn and anti conformations are defined by values of χ_{C-N} between 290-110° and 110-290° respectively. Although the two conformations exist in dynamic equilibrium in solution, the anti conformation is preferred in both purine and pyrimidine nucleotides.[6] However, the syn orientation is preferred in some 6-substituted pyrimidine and some 8-substituted purine nucleotides.[8,9]

DIAGRAM 1.9 Syn and Anti Orientations in Pyrimidines.

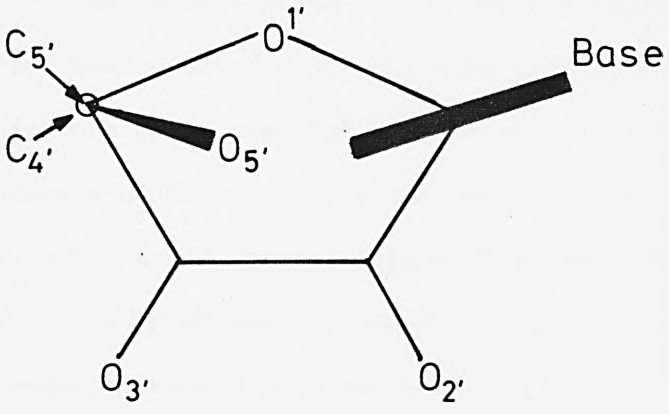


1.2.3) Orientation about the C(4')-C(5') Bond.

The orientation about the C(4')-C(5') bond is such that the O(5') atom is above the plane of the sugar ring and can form hydrogen bonding interactions with the heterocyclic ring,[3] (Diag. 1.10).

Although many exceptions to this rule occur in nucleosides, most 5'-nucleotides show preference for this orientation[10] which is the one that occurs in double stranded DNA.

DIAGRAM 1.10 Position of the O(5') Atom.



1.3) THE BIOLOGICAL BASIS FOR THE ANTIVIRAL AND ANTITUMOUR ACTIVITY OF NUCLEOSIDE ANALOGUES.[1,11,12,13]

The nucleic acids play a central role in the life of a cell, and synthetic analogues of their building blocks, nucleosides and nucleotides, can therefore exert powerful effects on a cell by interfering with the biosynthesis of the nucleic acids. The pathways from simple molecules (e.g. sugars, amino acids, bases), via nucleotides, to nucleic acids are very complex and a brief description of the salient features directly related to this research project is given below.

1.3.1) Biosynthesis of Nucleotides and DNA.[1]

In the biosynthesis of purine nucleotides the purine base is 'built on' to a phosphorylated ribose sugar, in a number of steps, to give inosine-5'-monophosphate (IMP). From this intermediate both guanosine-5'-monophosphate (GMP) and adenosine-5'-monophosphate (AMP) are formed. An alternative pathway uses preformed purine bases which are combined with 5-phosphoribosyl-1-pyrophosphate (PRPP) to give the purine monophosphates.

In pyrimidine biosynthesis, orotic acid (a derivative of uracil) is first formed which reacts with PRPP to give orotidine-5'-monophosphate (OMP). This, under the influence of orotidine monophosphate decarboxylase, loses carbon dioxide to give uridine-5'-monophosphate (UMP). Methylation of dUMP by thymidylate synthetase then gives thymidine-5'- monophosphate (TMP). However, UMP needs to be converted to the triphosphate (UTP), by the action of phosphorylating enzymes (i.e. kinases), before conversion to cytidine-5'-triphosphate (CTP) occurs. Enzymes are also present which can reverse this amination reaction in both pyrimidines and purines. All the above monophosphates are converted, via the diphosphates, to the triphosphates by enzymes known as kinases. An important member of this

group of enzymes is thymidine kinase (TK) which phosphorylates free nucleosides to the 5'-monophosphates.

The deoxyribonucleotides are derived from the above ribonucleotides by the action of the enzyme ribonucleotide reductase. This enzyme acts at the diphosphate level and reduces UDP, CDP, ADP and GDP to the deoxynucleotides dUDP, dCDP, dADP and dGDP. The latter three are again converted to the triphosphates by kinases prior to incorporation into DNA. However, dUDP is rapidly hydrolysed to dUMP which is converted to dTMP which as dTTP is subsequently incorporated into DNA.

Once the nucleoside triphosphates are formed, they can be used in DNA synthesis. For this to occur double stranded DNA having free 3'-OH groups is required to act as a template for the polymerisation enzymes. Some unwinding of the double helix takes place and chain elongation occurs by attack of the 3'-OH on the α-phosphate group of a nucleoside triphosphate. These processes are controlled by a number of enzymes but the ones most directly concerned with DNA synthesis are the DNA polymerases (usually three are found). These enzymes differ from each other in the type of DNA they require (i.e. double or single stranded), their rates of DNA synthesis and the additional activities that they may possess as nucleases. All these properties determine whether the main function of the enzyme is in DNA synthesis or in the repair of DNA once formed. The types of repair functions that these enzymes can carry out range from joining up breakages in DNA strands to identifying and replacing wrongly placed or unnatural nucleosides.

1.3.2) The Basis for Selective Antiviral and Antitumour Activity.[11,12,13]

Nucleoside analogues can, in principle, exert their antiviral and antitumour activities by affecting either the de novo synthesis of nucleotides or interfering with the later stages of DNA synthesis. In fact some analogues of bases and nucleosides can act at both these levels.

However, they must first be converted to the nucleotide. The phosphorylation is generally carried out by thymidine kinase (TK), which therefore plays an essential role in the activation of potentially active compounds.

When a cell is in the process of replication, many of the above mentioned enzymes (e.g. TK, TS, ribonucleotide reductase, DNA polymerases, see Section 1.3.1) are in a high state of activity. Thus in cancer or virus-infected cells, which are usually dividing rapidly, nucleoside analogues may show selective toxicity because of the high activity of these cells' DNA-synthesising enzymes. This is especially important in anticancer drugs.

Some added selectivity is seen in virus-infected cells. This is because viruses contain nucleic acids (DNA or RNA) which can code for their own DNA-synthesising enzymes once inside a host cell. These enzymes often differ from those of the host in kinetic properties and in generally having wider substrate specificity, the latter property making them more susceptible to unnatural nucleosides. Once a nucleoside is phosphorylated by the highly active TK enzyme (and hence potentially toxic), it is less able to cross the cell's lipophilic membrane and so is confined to the infected cell. The disruption caused to a cell's normal metabolism by a viral infection may affect some of its protective enzyme functions. Thus if its DNA repair mechanisms are affected then inclusion of unnatural nucleosides or mis-incorporation of natural ones will go uncorrected. Some reactions such as deamination also offer some protection for a healthy cell. Thus derivatives of the highly active (and toxic) ara-cytidine and ara-adenosine are deaminated to the less active ara-uridine and ara-inosine derivatives respectively.

Finally, many viruses which use RNA to carry their genetic information have been shown to possess an enzyme, necessary for their growth, which synthesises DNA from their RNA template. This enzyme is known

as reverse transcriptase and is a good target for selective inhibition of these viruses' growth.

The mode of action of some representative nucleoside analogues is now discussed.

1.4) THE BIOLOGICAL ACTIVITY OF NUCLEOSIDE ANALOGUES.[13,14,15]

1.4.1) 5-Substituted-2'-deoxy-ribofuranosyl-pyrimidine Nucleosides.

This class of derivatives has provided a wealth of biologically active compounds in both uridine and cytidine series (TABLE 1.1). In fact some of the first nucleoside analogues to be used clinically are in this class (e.g. 5-iodo-2'-deoxyuridine and 5-trifluoromethyl-2'-deoxyuridine).

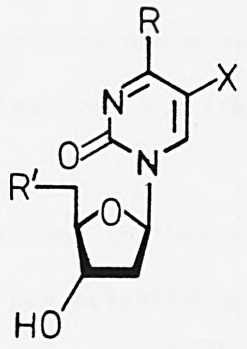
Although the mode of action of all these compounds will not be discussed here, indeed this is not always known, the cases that have been studied indicate that they can act at various stages of DNA synthesis. Thus several of the 2'-deoxyuridines (X= -F, $-NO_2$, -CN, -SCN, -CHO, -C≡CH) have been shown to exert an inhibitory effect on thymidylate synthetase.[16,17] Since this enzyme is involved in the de novo synthesis of deoxythymidine, these compounds will not be selective antiviral agents and more likely to find a use in anticancer therapy. Of the other derivatives, 5-(E)-bromovinyl-2'-deoxyuridine and its iodovinyl analogue are highly active antiviral agents.[17] The former is a candidate for clinical use and has been shown to be selectively phosphorylated by virally induced TK. Then, as the triphosphate, it is a strong inhibitor of viral polymerases under conditions in which cellular polymerases are only minimally affected.[18]

1.4.2) Arabinosyl Nucleosides.

This class of compounds contains arabinose as the sugar component and many such compounds, both in the pyrimidine and the purine series, have potent antiviral and antitumour activities. A number of the more important arabinosyl nucleoside analogues are shown in TABLE 1.2.

Many of these compounds seem to act at the DNA polymerase level. Thus ara-adenosine, which has been used clinically as an antiviral agent, is phosphorylated in both healthy and virus-infected cells but achieves

TABLE 1.1 5-Substituted-Pyrimidine Nucleosides.



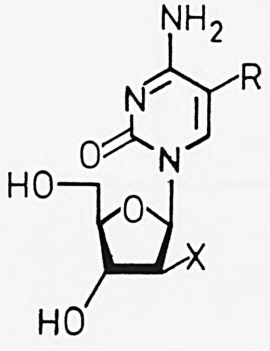
R= OH		R= NH_2	
X	R'	X	R'
I	OH, NH_2	I	OH
CF_3	OH, NH_2	Br	OH
NHR	OH	F	OH
Et	OH	NO_2	OH
Pr	OH	C≡CH	OH
$CH=CH_2$	OH	Et	OH
CH=CHBr	OH		
CH=CHI	OH		
CH_2OCH_3	OH		
CH_2SH	OH		
CH_2SCH_3	OH		
$OCH_2C\equiv CH$	OH		
F	OH, NH_2		
CHO	OH		
SH	OH		
NO_2	OH		
$CH_2CH=CH_2$	OH		
CH_2OH	OH		

selective antiviral activity because its triphosphate (ara-ATP), inhibits virus induced DNA polymerase to a greater extent than it does the cellular DNA polymerases α and β.[19] A similar mode of action applies to ara-thymidine. Ara-cytidine has a very potent antiherpes activity.[20] However it is not selective, and its high toxicity has limited its use to anticancer therapy.

Recent additions to this class of compounds which have shown high and selective antiherpes activity are a group in which the 2'-OH function has been replaced by fluorine.[21,21,23] In particular 5-iodo-2'-fluoro -2'-deoxy-ara-cytidine (FIAC) and 5-methyl-2'-fluoro-2'-deoxy-ara-uridine (FMAU) are strong candidates for clinical use.

TABLE 1.2 Purine and Pyrimidine Arabinosyl Nucleosides.

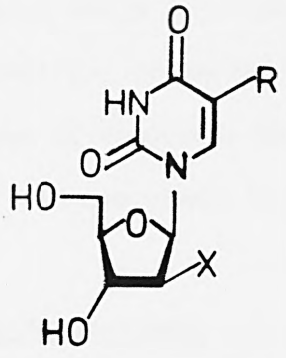
a) Derivatives of ara-Cytidine.



R	X
H	F, Cl, Br
Me	F, Cl, Br
H	OH, N_3
H	ONO_2
F	OH
Br	F
I	F, Cl, Br

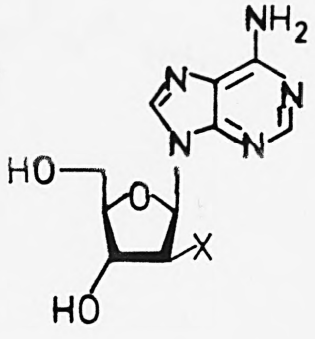
TABLE 1.2 (Contd).

b) Derivatives of ara-Thymidine and ara-Uridine.



R	X
H	OH
H	F
Me	F, Cl, Br, OH
Et	OH
F	OH
Br	F, OH
I	F, OH

c) Derivatives of ara-Adenosine.

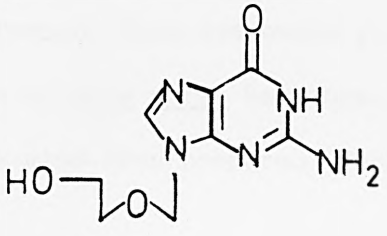


X= OH, N_3, NH_2

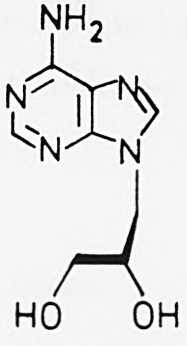
1.4.3) Acyclic Nucleosides.

This class of compounds does not have a sugar ring attached to the base. In its place there is a short chain which in certain conformations is identical to parts of the sugar ring that it replaces. A number of these types of compounds have potent and selective antiviral properties [24,25,26] and are shown in TABLE 1.3

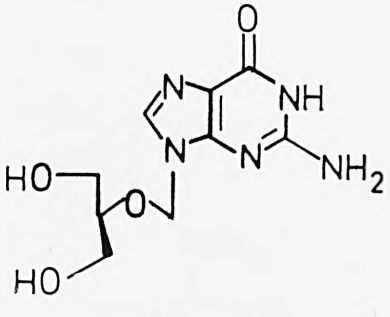
TABLE 1.3. Acyclic Nucleosides.



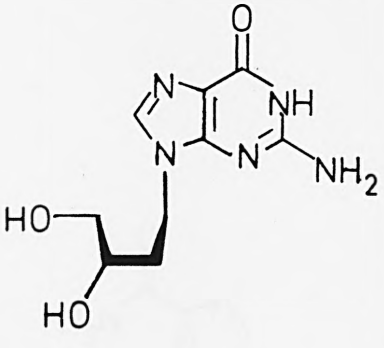
ACV



DHPA



DHPG



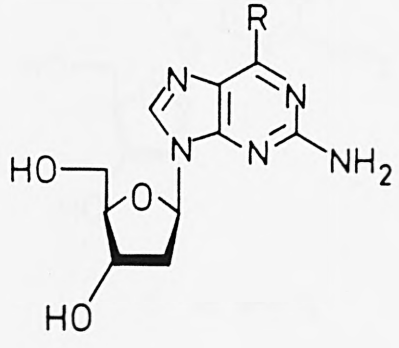
DHBG

Three of these compounds, (R)-9-(3,4-dihydroxybutyl) guanine (DHBG), 9-(2-hydroxyethoxymethyl) guanine (acyclovir, ACV) and 9-(1,3-dihydroxy-2-propoxymethyl) guanine (DHPG) seem to have a common mode of action. First, the compounds are phosphorylated by viral TK, and, after conversion to the triphosphates, inhibit DNA synthesis only in infected cells. This field is currently receiving much attention.[27,28,29]

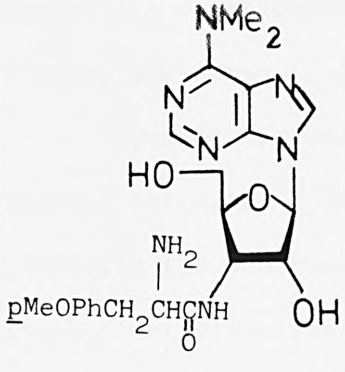
1.4.4) Nucleosides Containing Modified Purines.

A variety of modifications to the purine bases have been performed. Thus compounds in which a nitrogen atom is replaced by a carbon atom or *vice versa* have been prepared. Many derivatives of 6-thiopurines have also been prepared (TABLE 1.4).

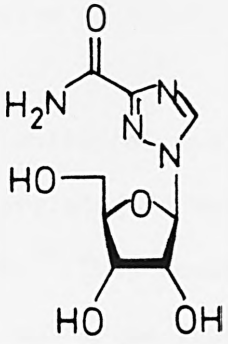
TABLE 1.4. Nucleosides with Modified Purine Bases.



R
SH
SMe
$SCH_2CH=CH_2$
NH_2NH_2



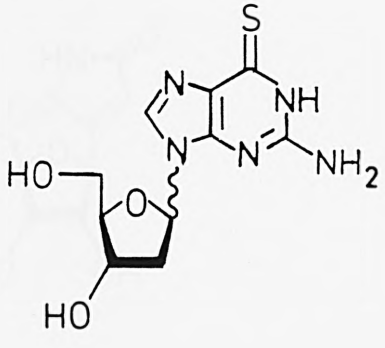
Puromycin



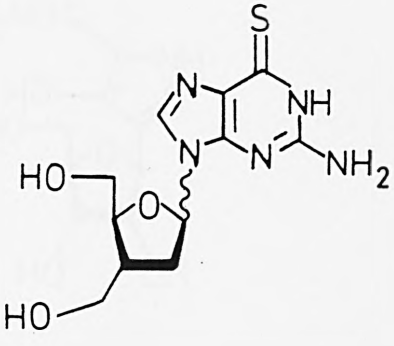
Virazole

These types of compounds generally affect the de novo synthesis of purine nucleotides and are therefore not selective antiviral agents but more useful in anticancer chemotherapy. For example, ribavirin has been shown to act at two levels. Firstly, it inhibits the formation of GMP from IMP.[30] Secondly, it can be phosphorylated, and its triphosphate inhibits influenza virus RNA polymerase.[31]

An interesting group of nucleosides from this class are the pairs of anomers I and II.[32] Compounds Iα and Iβ are both phosphorylated in cancer cells and incorporated into DNA where they stop further chain elongation. However, the more active anomer Iβ is the more toxic. By synthesising the 3'-hydroxymethyl pairs of anomers (IIα and IIβ), two compounds of similar activity to Iβ were obtained, but with lower toxicity.



I α and β



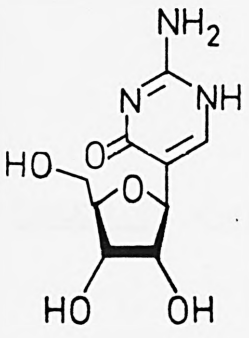
II α and β

1.4.5) C-Nucleosides.

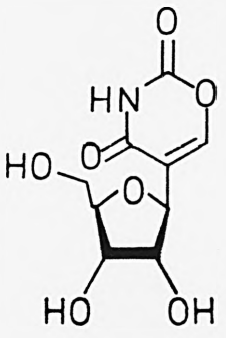
This class of nucleoside analogues has the base and sugar connected via a carbon-carbon bond. Some examples of active compounds in this class are shown in TABLE 1.5.

Pyrazofurin has a broad spectrum of antiviral activity as well as antitumour activity.[33] The compound is phosphorylated to the monophosphate and inhibits the decarboxylation of OMP to UMP.[34] This accounts for its antitumour activity. Its antiviral properties may arise from inhibition of nucleic acid synthesis at a higher level.

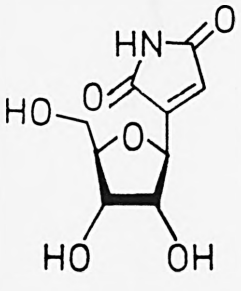
TABLE 1.5 C-Nucleosides.



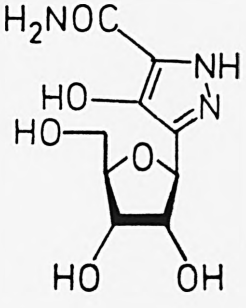
Pseudoisocytidine



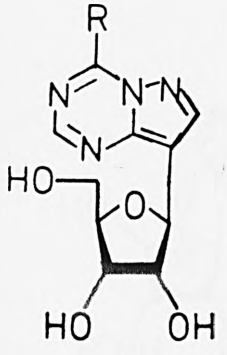
Oxazinomycin



Showdomycin



Pyrazomycin ∝ and β



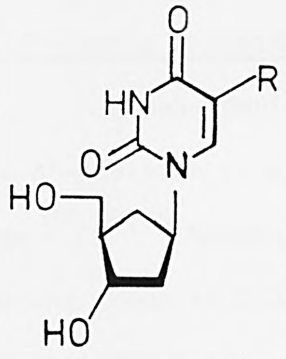
R = OH, NH_2

8-(β-D-Ribofuranosyl)pyrazolo[1,5-a]-1,3,5-triazines

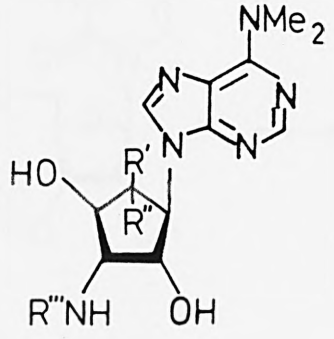
1.4.6) Carbocyclic Nucleoside Analogues.[13,15,35]

A number of such carbocyclic analogues are known both in the pyrimidine and purine series. Some of the active ones are shown below (TABLE 1.6).

TABLE 1.6 Carbocyclic Nucleosides.



R
Me
Br
I
NHMe



R' = OH, R'' = H
R' = H R'' = OH
} R''' = COCHCH$_2$Ph-p-OMe
|
NH$_2$

1.5) CHEMICAL MODIFICATIONS AT THE 2'- AND 3'-POSITIONS OF NUCLEOSIDES.

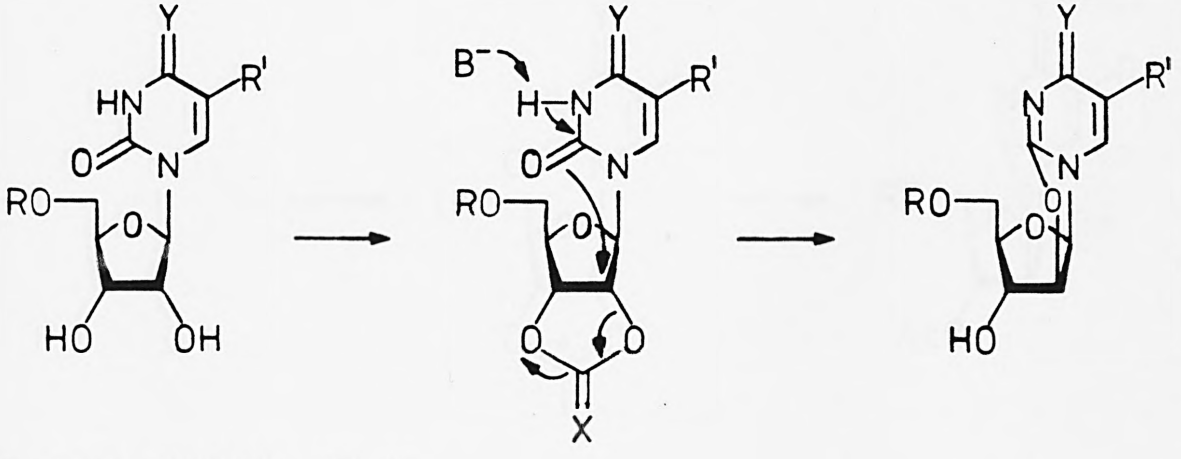
Nucleoside analogues containing modified sugars are well known and a wide variety of substituents have been introduced by many synthetic methods.[36] In this research work, interest was centred at the 2'- and 3'-positions of the nucleoside molecule and a survey of the major modifications performed at these positions is now given.

1.5.1) Modifications at the 2'-Position of Pyrimidine Nucleosides.

O^2,2'-cyclic Pyrimidine Nucleosides.

These derivatives have played a central role in many reactions at the 2'-position of pyrimidine nucleosides and have been known for a long time.[37,38,39] Generally, the compounds are arrived at by generating a leaving group at C-2' which is displaced, intramolecularly, by the O-2 of the pyrimidine ring under the influence of base (Diag 1.11).

DIAGRAM 1.11 Mechanism of Formation of O^2,2'-cyclic Pyrimidine Nucleosides.



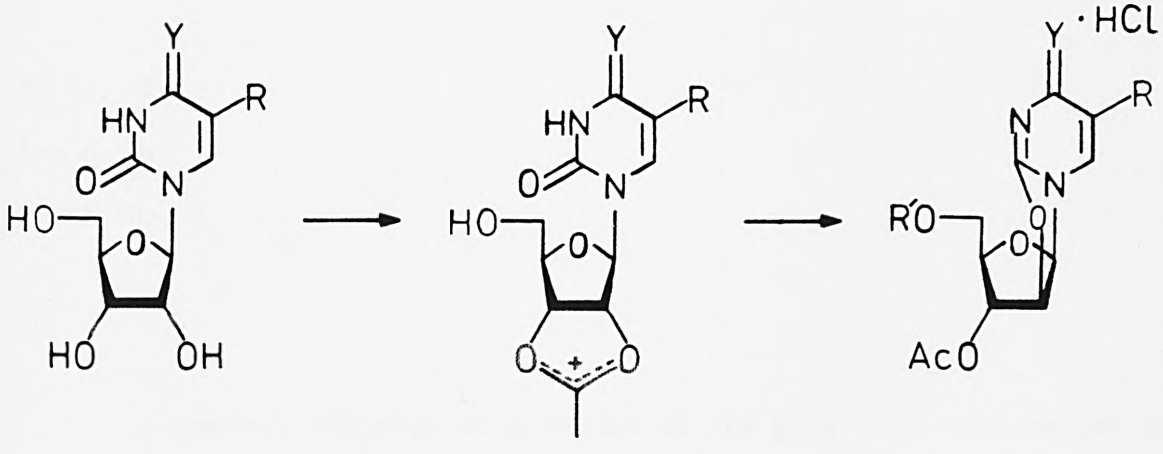
R= H or Protecting Group
Y= O, NH
R'= H, F, Me
X= O, S

Thus treatment of uridine with diphenylcarbonate in DMF[40] or HMPA,[41] followed by sodium bicarbonate gives O^2,2'-cyclouridine in high yield. This method proceeds via a 2',3'-cyclic carbonate and has found wide application in pyrimidine nucleoside analogue synthesis.[42,43]

Other methods which go through a similar cyclic intermediate have used carbonyl diimidazole[44] and thiocarbonyl diimidazole.[45] A related method using thionyl chloride has been applied to cytidine and gives O^2,2'-cyclocytidine hydrochloride in 73% yield.[46]

Another approach converts the cis diol function of ribonucleosides to a cyclic acetoxonium ion. The O-2 of the pyrimidine ring again attacks C-2' to give the O^2,2'-cyclonucleoside. Thus treatment of uridine[47] or cytidine and many of its derivatives[48] with 2-acetoxyisobutyryl chloride led to the isolation of high yields of the O^2,2'-cyclonucleoside hydrochlorides (Diag. 1.12).

DIAGRAM 1.12 O^2,2'-cyclic Pyrimidine Nucleosides via 2',3'-cyclic Acetoxonium Intermediates.



For Y= O, R= H, R'= H

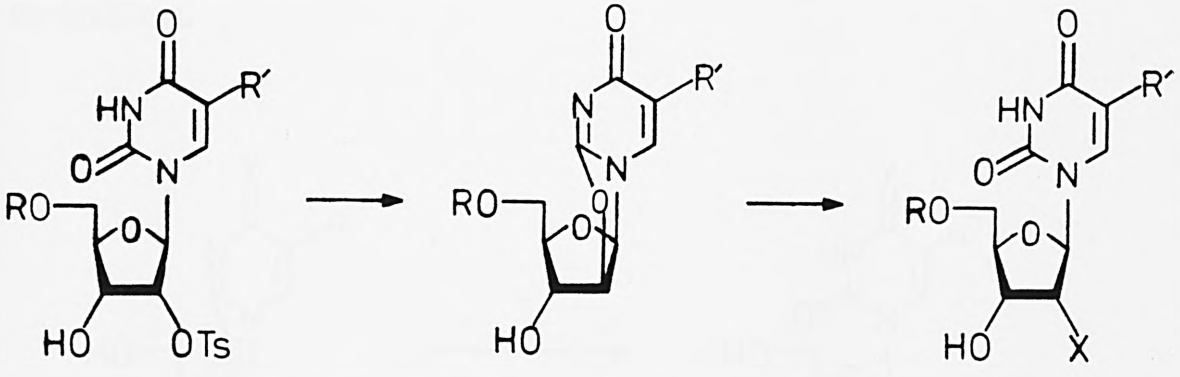
For Y= NH R= Hal, Me, H R'= H or -O-trimethyldioxolanone ether

These cyclonucleosides serve as versatile intermediates for preparing 2'-modified nucleosides (vide infra). For instance, hydrolysis at the oxygen-bridge leads to ara-uridines and ara-cytidines.[49-51]

2'-Halogenated Pyrimidine Nucleosides.

These can be prepared by first introducing a leaving group at the 2'-position. Treatment with a halide salt then gives a 2'-halo-2'-deoxy-ribonucleoside, evidently via a O^2,2'-cyclic intermediate. This method has been used for various uridines[52] (Diag. 1.13).

DIAGRAM 1.13 2'-Halogenated Pyrimidine Nucleosides from 2'-O-Sulphonate Derivatives.



R= Ac, Tr

R'= H, Me

X= Cl, Br, I

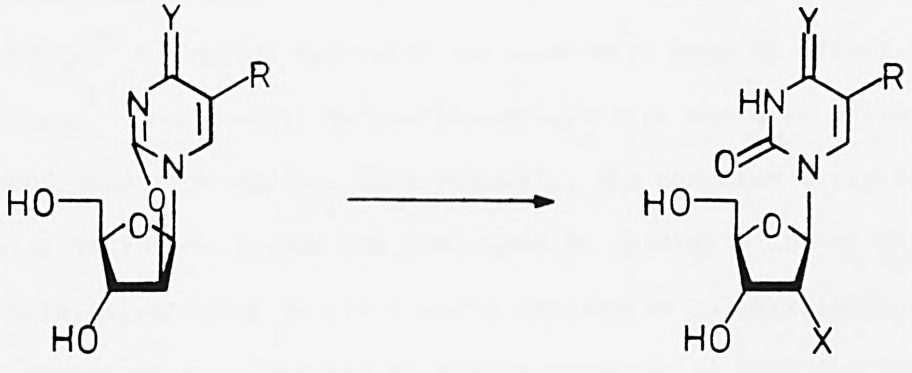
However, the ease of formation of the O^2,2'-cyclonucleosides has made them attractive starting points for introducing a 2'-substituent. This approach has led to many 2'-halo-2'-deoxyuridines and cytidines.[53,56,115] Protonation at N-3 of the pyrimidine ring is required before reaction can occur. Thus treatment of O^2,2'-cyclouridine with sodium iodide does not

lead to opening of the ring, whereas with hydrogen iodide ring-opening does occur.[53,56] The use of hydogen halides in DMF seems to give the best yields[54] (Diag. 1.14).

As mentioned earlier, treatment of uridine with 2-acetoxy-isobutyryl chloride gives the cyclic derivative. However, longer reaction times give 3'-O-acetyl-2'-chloro-2'-deoxyuridine.[47] This reaction has also been applied to 5'-halo-5'-deoxyuridines[55] and 6-azauridines.[36]

Finally, treatment of uridine[57], 6-azauridine[44] and 5-fluoro-uridine[59] with acetyl bromide or propionyl bromide in acetonitrile leads directly to the corresponding 3',5'-di-O-acyl-2'-bromo-2'-deoxyuridines, presumably via 2',3'-acyloxonium and O^2,2'-cyclonucleoside intermediates.

DIAGRAM 1.14 2'-Halogenated Pyrimidine Nucleosides from O^2,2'-cyclic Derivatives.



For Y= O R= H, Me X= F, Cl, Br, I

For Y= NH R= H X= F, Cl, Br, I

Other 2'-Substituted Pyrimidine Nucleosides.

Reaction of alkali metal azides with $\underline{O}^2$,2'-cyclonucleosides leads to the introduction of the azide group at C-2'.[41,60] Reduction of the azide group affords the 2'-amino derivatives.[41] The introduction of a 2'-thio group in uridine has been achieved by reaction of the $\underline{O}^2$,2'-cyclouridine with thioacetic acid in dioxane or DMF followed by mild hydrolysis.[61] Attack with thiolate anion has recently been reported.[62]

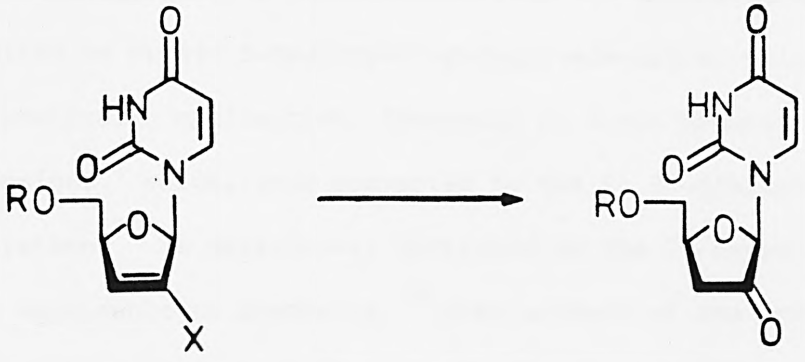
An important use of nucleosides bearing a halogen or sulphur atom at C-2' is in the preparation of 2'-deoxynucleosides. Thus, 2'-bromo and iodo groups have been reduced with hydrogen over a palladium catalyst[57,63] or with tributyltin hydride.[42,44] The 2'-thio group has been reduced with Raney nickel.[64]

Pyrimidine 2'-Ketonucleosides.

Oxidation of 3',5'-di-$\underline{O}$-trityl-uridine using DMSO/DCC in the presence of pyridinium trifluoroacetate affords the 2'-keto derivative in 63% yield.[65] Ruthenium tetroxide has also been used to effect this oxidation.[66] The 2'-keto derivative of cytidine was also obtained using the DMSO/DCC oxidation system. More recently, the chromium trioxide / pyridine / acetic anhydride system has been used to oxidise 3',5'-di-$\underline{O}$-t-butyl-dimethylsilyl-uridine to its 2'-keto derivative in excellent yield.[67] All these compounds were reduced by sodium borohydride from the less hindered α-side to give ara-nucleosides.

Some 2'-keto-3'-deoxynucleosides have also been reported. They have been obtained by hydrolysis of enol tosylates[68] and enol benzoates[69] and by the reduction of an enol azide, presumably via an imine intermediate[70] (Diag.1.15).

DIAGRAM 1.15 Pyrimidine 2'-Ketonucleosides from 2',3'-Unsaturated Derivatives.



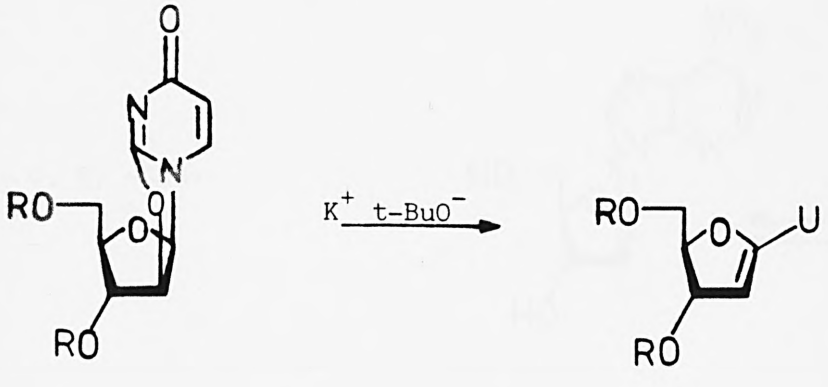
R= Benzoyl

X= OTs, OBz, N_3

1',2'-Unsaturated Pyrimidine Nucleosides.

Few examples of this type of compound are known. However, treatment of 3',5'-di-O-t-butyldimethylsilyl-O^2,2'-cyclouridine with potassium t-butoxide[71] led to the 1',2'-unsaturated derivative in DIAGRAM 1.16.

DIAGRAM 1.16 1',2'-Unsaturated Pyrimidine Nucleosides.



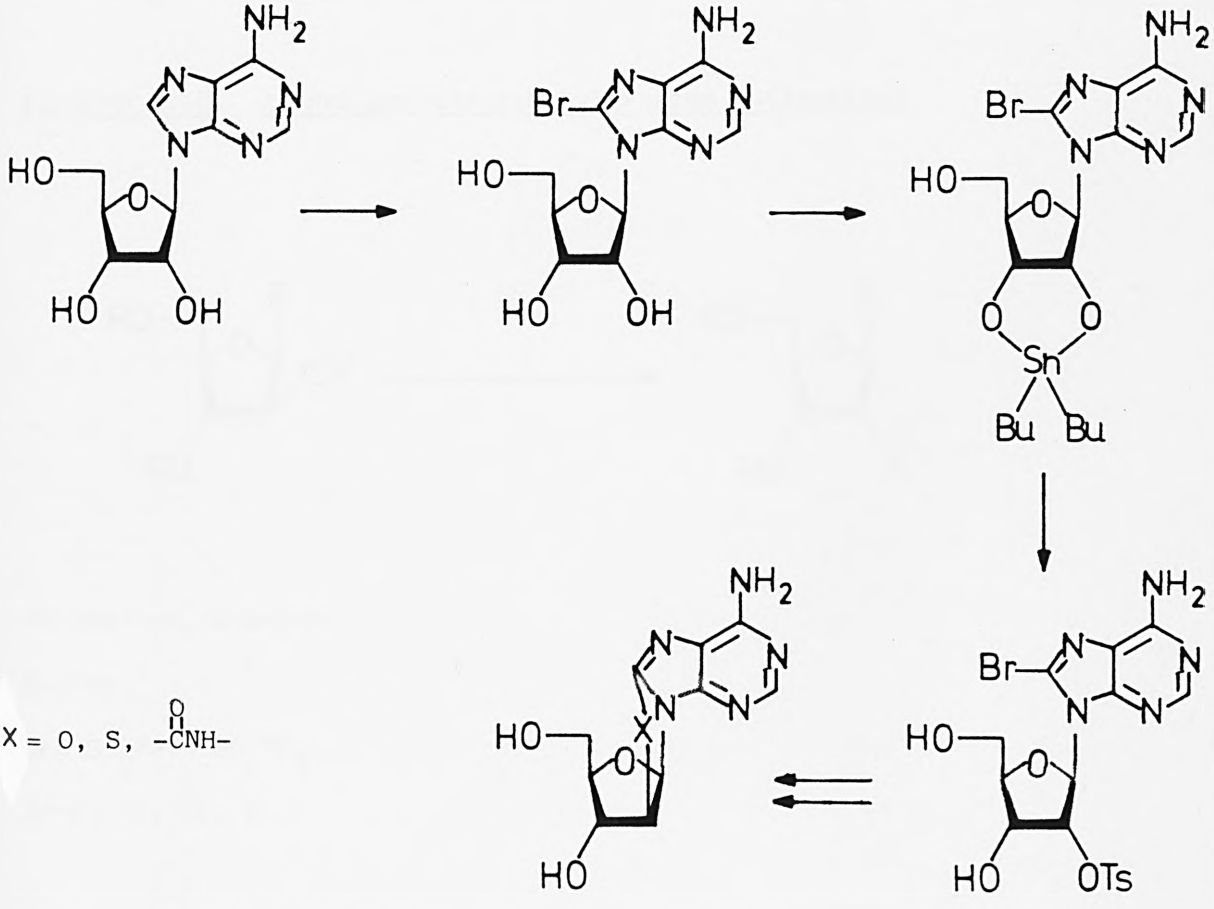
R= TBDMS

1.5.2) Modifications at the 2'-Position of Purine Nucleosides.

Purine 8,2'-Cyclonucleosides.

This type of compound is not as readily accessible as are the O^2,2'-cyclopyrimidine nucleosides. Thus for adenosine a number of steps are required to obtain 8-hydroxy-2'-O-tosyl-adenosine, which is able to undergo intramolecular cyclisation. Adenosine is first brominated to 8-bromo-adenosine,[72] which, once converted to the 2',3'-dibutylstannylene derivative,[73] is selectively tosylated on the 2'-oxygen.[74] This scheme is also applicable to guanosine.[74] Displacement of the bromine by a variety of nucleophiles leads to 8,2'-cyclic purine nucleosides with oxygen,[72] sulphur[75] and carboxamide bridges[76] (Diag. 1.17).

DIAGRAM 1.17 Preparation of 8,2'-Cyclic Purine Nucleosides.

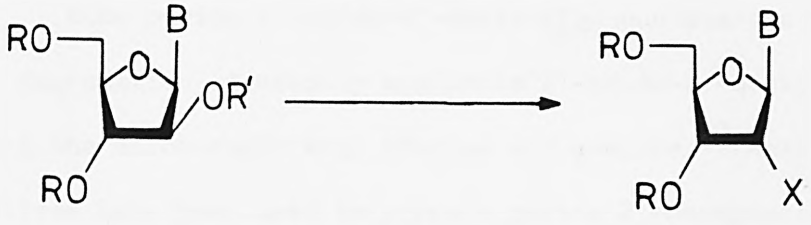


Opening of the 8,2'-bridge usually occurs by nucleophilic attack at C-8 leading to 8-substituted derivatives of 9-(β-D-arabinosyl) purines.[75,77-80] If hydrogen sulphide is used the resulting 8-mercapto-ara-adenosine can be desulphurised to give ara-adenosine.[81] Alternatively, 8-hydrazo-ara-adenosine can be converted to ara-adenosine in excellent yield upon treatment with yellow mercuric oxide.[77]

2'-Halogenated Purine Nucleosides.

Purine nucleosides bearing a halogen at the 2'-position in the ara-configuration can be obtained as minor products when the ribonucleoside is treated with 2-acetoxy-isobutyryl halides (see 1.5.4). This reaction has been applied to adenosine, guanosine and inosine.[82,83,84] Similarly, low yields of 2'-halo-2'-deoxy-arabinosyl purine nucleosides are obtained when 2',3'-O-methoxy ethylidene derivatives are treated with pivaloyl chloride (see 1.5.4).[85]

DIAGRAM 1.18 2'-Halogenated-ribosyl Purine Nucleosides.



B= Adenine, Guanine

R= THP

R'= SO_2Me, SO_2CF_3

X= F, Cl, Br, I

The 2'-halo-2'-deoxyribonucleosides cannot be obtained directly from natural purine nucleosides. However, displacement of a leaving group at the 2'-position from an ara-nucleoside leads to them. Thus, 2'-O-mesylate and 2'-O-triflate groups in ara-adenosines have been displaced with halides.[86,87] This approach has been extended to guanosine and neplanocin A[88,89] (Diag. 1.18).

Other 2'-Substituted Purine Nucleosides.

The azide anion attacks 8,2'-cycloadenosine at the 2'-position to give 8-oxo-2'-azido-2'-deoxyadenosine.[90] Similarly ethyl mercaptide gave the 2'-ethylthio analogue.[95] Displacement by azide ion of a 2'-O-triflate group in ara-adenosines[86] and of a 2'-O-mesylate group in ara-guanosine[91] has been used to afford the 2'-azido-2'-deoxy derivatives. These compounds have been reduced to the 2'-amino analogues. The 2'-thioacetyl derivative of adenosine was also obtained.[86] The direct displacement of a 2'-O-triflate or a 2'-O-tosylate group in purine ribonucleosides by azide, has been achieved to give 2'-azido-2'-deoxy-ara-adenosines[92] and -ara-guanosines[93] and subsequently the 2'-ara-amino analogues. This method also yielded the 2'-thioacetyl-ara-adenosine derivative.

Some purine 2'-azido-2'-deoxy-ribo-nucleosides have been prepared by the degradation of readily available 2'-azido-2'-deoxyuridine and then coupling the azido-sugar with adenine and guanine.[60] Many of these derivatives have been used to prepare purine 2'-deoxynucleosides by the methods already mentioned for pyrimidine nucleoside.[75,80,99]

Purine 2'-Ketonucleosides.

9-(3,5-O-Isopropylidene-β-D-xylofuranosyl) adenine has been oxidised in moderate yield to the 2'-keto derivative using ruthenium tetroxide.[66] More recently, the chromium trioxide/ pyridine/ acetic anhydride system has been used to obtain 2'-keto derivatives of adenosine

in high yields.[67] Extensions of this approach to prepare other 2'-keto derivatives have been reported.[96,97] Reduction of these 2'-keto derivatives with sodium borohydride was performed and shown to occur from the less hindered α-side to give the ara-nucleosides.

1',2'-Unsaturated Purine Nucleosides.

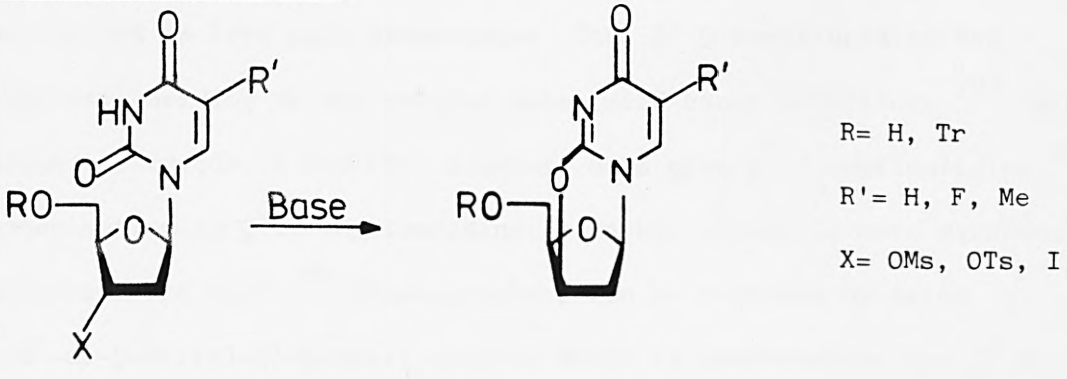
These have been obtained by treatment of 3',5'-protected-ara-nucleoside derivatives bearing a leaving group at the 2'-position with strong base. The leaving groups used have been iodide[98] and triflate.[86]

1.5.3) Modifications at the 3'-Position of Pyrimidine Nucleosides.

O^2,3'-Cyclic Pyrimidine Nucleosides.

The formation of O^2,3'-cyclonucleoside derivatives in 2'-deoxy-uridines and 2'-deoxythymidines is well established. The general conditions require introduction of a leaving group at the 3'-position followed by treatment with base. Thus in early methods, the 3'-iodo, 3'-O-mesylate and 3'-O-tosylate derivatives of 2'-deoxythymidines and 2'-deoxyuridines were treated with bases such as hydroxide, alkoxides and ammonia to give the corresponding O^2,3'-cyclic derivatives.[100] The use of bases such as DBU allow milder reaction conditions to be used.[101]

DIAGRAM 1.19 O^2,3'-Cyclic Pyrimidine Nucleosides by Displacement of a Stable 3'-Leaving Group.



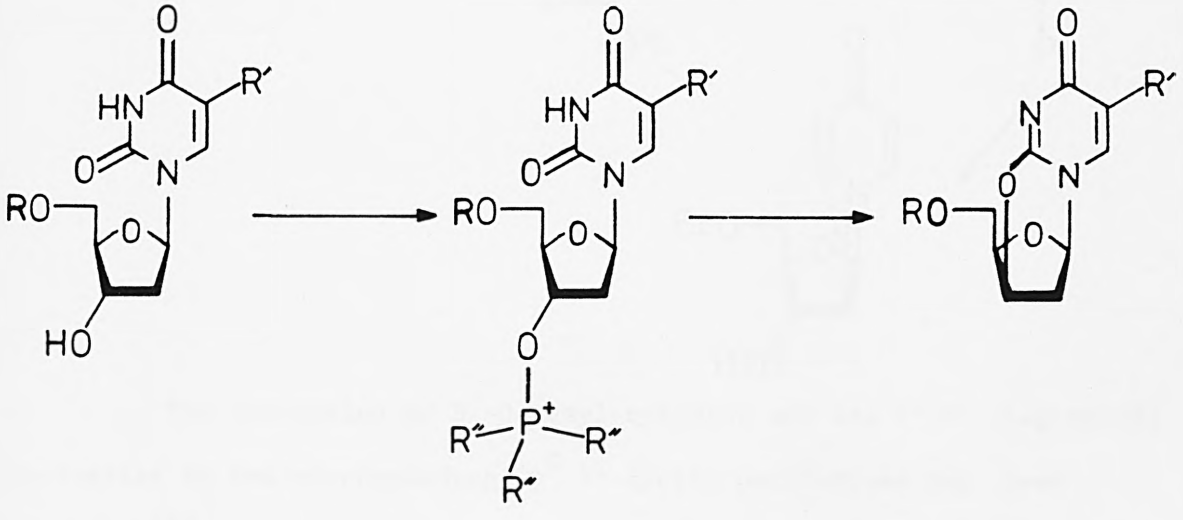
R= H, Tr

R'= H, F, Me

X= OMs, OTs, I

The introduction of transient leaving groups at the 3'-position has led to the formation of $\underline{O}^2$,3'-cyclic nucleosides. Thus treatment of 5'-$\underline{O}$-trityl-2'-deoxythymidine with methyltriphenoxyphosphonium iodide in pyridine gives the $\underline{O}^2$,3'-cyclic derivative in 70% yield.[102] A similar reaction occurs if 5'-$\underline{O}$-trityl-2'-deoxythymidine is treated with triphenyl phosphine and diethyl azodicarboxylate.[103] Both these reactions are thought to involve the intermediacy of an oxyphosphonium species at C-3' which is displaced by O-2 of the pyrimidine ring (Diag. 1.20).

DIAGRAM 1.20 $\underline{O}^2$,3'-Cyclic Pyrimidine Nucleosides by Displacement of a Transient 3'-Leaving Group.

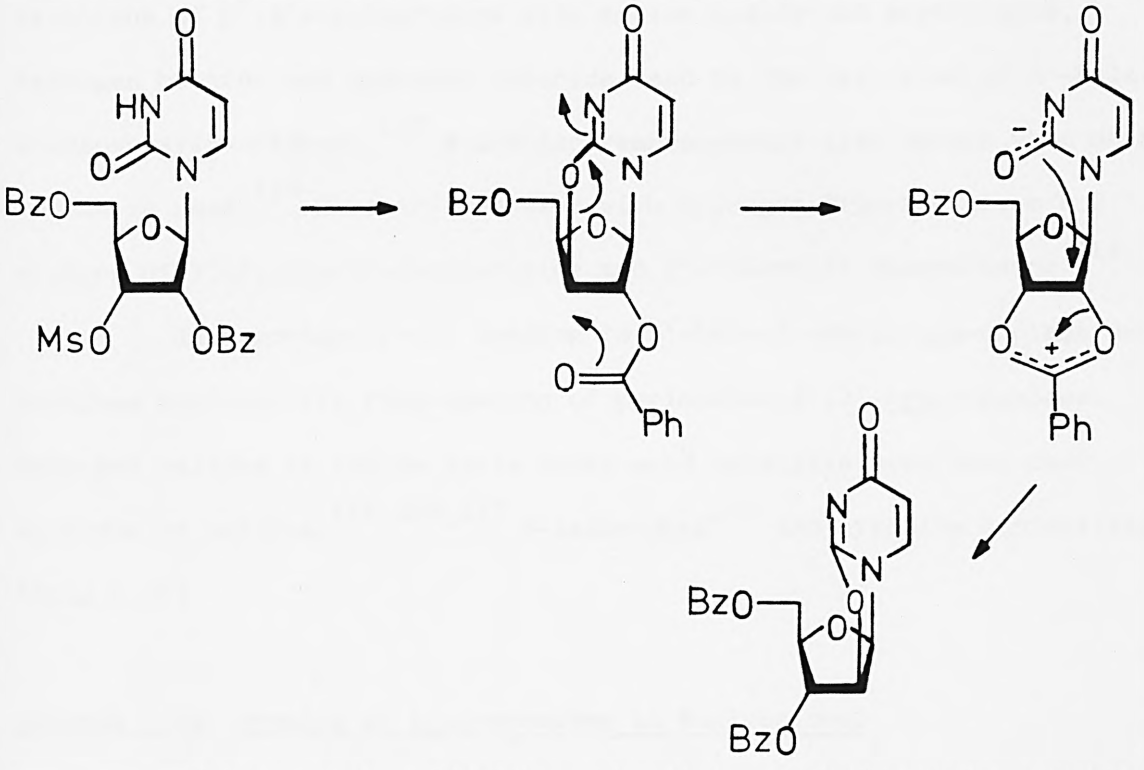


R= Tr R'= Me R''= Ph, OPh

In pyrimidine ribonucleosides formation of the $\underline{O}^2$,3'-cyclic derivatives is less well established. Thus 3'-$\underline{O}$-mesyl-uridine and 3'-$\underline{O}$-tosyl-uridine do not cyclise under mild basic conditions.[104] Using sodium t-butoxide in hot DMF, however, does give $\underline{O}^2$,3'-cyclouridine.[104] Isomerisation to $\underline{O}^2$,2'-cyclouridine, however, occurs if more strenuous conditions are used.[105] These problems can be overcome by using 2',5'-di-$\underline{O}$-trityl-3'-$\underline{O}$-mesyl-uridine which is converted to the $\underline{O}^2$,3'-cyclic

derivative on prolonged heating with sodium benzoate in DMF, albeit in low yield.[106] The analogous reaction using the 2',5'-di-O-benzoyl analogue, however, gives 3',5'-di-O-benzoyl-O^2,2'-cyclouridine (presumably via a 2',3'-benzoxonium cation intermediate)[106] (Diag.1.21).

DIAGRAM 1.21 Rearrangement of 2',5'-Di-O-Benzoyl-O^2,3'-cyclouridine.



The conversion of 3'-O-mesyl-cytidine and its 2',5'-di-O-trityl derivative to the corresponding O^2,3'-cyclic derivatives has been reported.[107]

3'-Halogenated Pyrimidine Nucleosides.

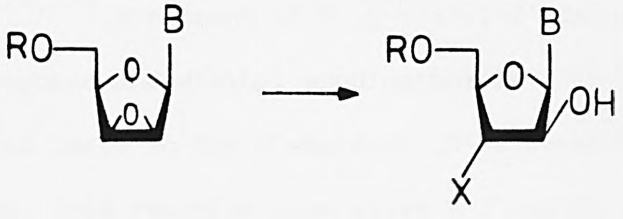
There are several important routes to 3'-halogenated pyrimidine nucleosides. Opening of O^2,3'-cyclic nucleosides has found application in the preparation of 3'-halo-2',3'-dideoxythymidines.[108,109] Reactions in which 3'-O-mesyl-2'-deoxynucleosides are treated with halide, to give the 3'-halo derivative with retention of configuration at C-3', also occur via a O^2,3'-cyclic intermediate produced under the reaction conditions. This method has been applied to the 2'-deoxythymidine and 2'-deoxyuridine

series.[110]

In the ribonucleosides, reactions of this type generally take a different course. Thus treatment of 5'-O-acetyl-3'-O-tosyl-6-azauridine with sodium iodide gave the 3'-deoxy-3'-iodo derivative with the D-xylo configuration.[111] Rearrangements often occur in intended ring-opening reactions of O^2,3'-cyclic derivatives of ribonucleosides. Thus the reactions of O^2,3'-cyclouridine with sodium iodide and acetic acid, hydrogen bromide and hydrogen chloride lead to the isolation of 5'-halo-5'-deoxy-xylo-uridines.[112] A similar rearrangement also occurs when methyl iodide is used.[113] However, reaction with hydrogen fluoride gives a 2:3 mixture of 3'-fluoro-3'-deoxyuridine and 2'-fluoro-2'-deoxyuridine.[114]

An important route leading to 3'-halo-3'-deoxy-ara-nucleosides involves nucleophilic ring-opening of pyrimidine-2',3'-lyxo-epoxides. Hydrogen halides or halide salts under acid catalysis have been used with epoxides of uridine,[115,116,117] 6-azauridine[118] and cytosine derivatives[119] (Diag.1.22).

DIAGRAM 1.22 Opening of Lyxo-epoxides in Nucleosides.



R= H, Ac, Tr

B= Uracil, Cytosine
Adenine, 6-Aza-uracil

X= F, Cl, Br, I,
N_3, NH_2, SAc

Recently, the opening of uridine-2',3'-lyxo-epoxide with hydrogen fluoride has been reported to give a 1:1 mixture of 3'-fluoro-3'-deoxy-ara-uridine and 3'-fluoro-3'-deoxyuridine.[120]

Direct halogenation using complex reagents such as methyl-triphenoxyphosphonium iodide has been achieved. Thus, in pyrimidine 5'-protected-2'-deoxynucleosides, a 3'-iodo group is introduced with retention of configuration at C-3'. The initially formed oxophosphonium cation at C-3' is displaced by O-2 to give a O^2,3'-cyclic derivative which is attacked by iodide.[102] Application to 2',5'-di-O-trityl-uridine leads to the 3'-iodo derivative with inversion of configuration at C-3'.[102,121] The mixed reagent, triphenylphosphine / tetrahalomethane, has also been used in halogenation reactions on pyrimidine nucleosides. With 2'-deoxythymidine the tetrachloromethane reagent results in chlorination at both the 3'- and the 5'-positions with inversion at C-3'.[122] This method has also been applied to 5-substituted-2'-deoxyuridines.[123]

Other 3'-Substituted Pyrimidine Nucleosides.

O^2,3'-Anhydro-2'-deoxythymidine and its 5'-O-trityl derivative are attacked by azide salts in DMF to give 3'-azido-2',3'-dideoxy-thymidines.[124,125] Treatment of 5'-O-trityl-2'-deoxythymidine with triphenylphosphine and diethyl azodicarboxylate in the presence of hydrazoic acid leads to the 3'-azido-2',3'-dideoxy derivative with the xylo configuration. This reaction also gives a 3'-azido compound with 2',5'-di-O-trityl-uridine, with concomitant inversion of the configuration at C-3'.[103]

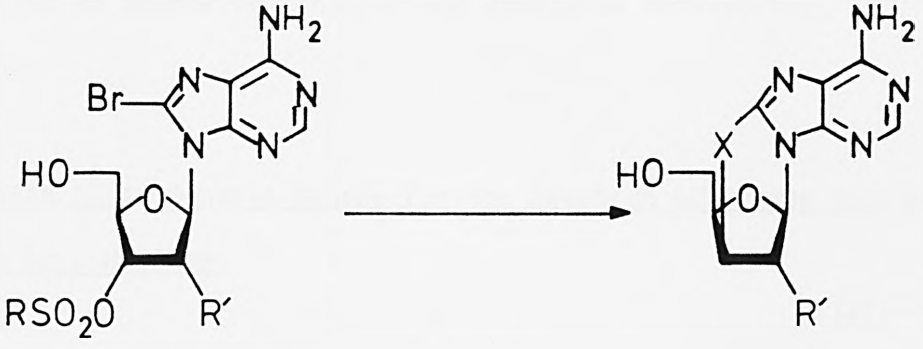
Opening of 2',3'-lyxo-epoxides of uridine[126,127] and cytidine[119] with sodium azide leads to 3'-azido-3'-deoxy-ara-nucleosides. Ammonia reacts similarly to give the 3'-amino-3'-deoxy-ara derivatives of some uridines.[128,129] Ring opening of lyxo-epoxides has also proved useful in the preparation of the analogous 3'-thio derivatives of some uridines and

cytidines.[130,119] Many of these analogues have been used as a route to 3'-deoxy derivatives of pyrimidine nucleosides by the general methods already mentioned (see Section 1.5.1).[110,121,131]

Pyrimidine 3'-Ketonucleosides.

The instability of these compounds in basic conditions has meant that they have received limited attention. However, 2',5'-di-O-trityl-uridine can be oxidised with DMSO/DCC to give the 3'-keto derivative in 46% yield.[65] A similar oxidation was performed on 2',5'-di-O-trityl-cytidine and its N-4 acetyl derivative.[133] A mild oxidation procedure involving the photolysis of 5'-O-trityl-3'-O-pyruvyl-2'-deoxythymidine and its 5'-O-benzoyl derivative also yields 3'-keto nucleosides.[134] Recently the chromium trioxide / pyridine / acetic anhydride system has been applied to prepare 3'-keto-derivatives of 2'-deoxythymidine.[67]

DIAGRAM 1.23 Preparation of Some Derivatives of 8,3'-Cyclo-adenosine.



R= pMe-C_6H_4-

R'= OH, H

X= O, S

1.5.4) Modifications at the 3'-Position of Purine Nucleosides.

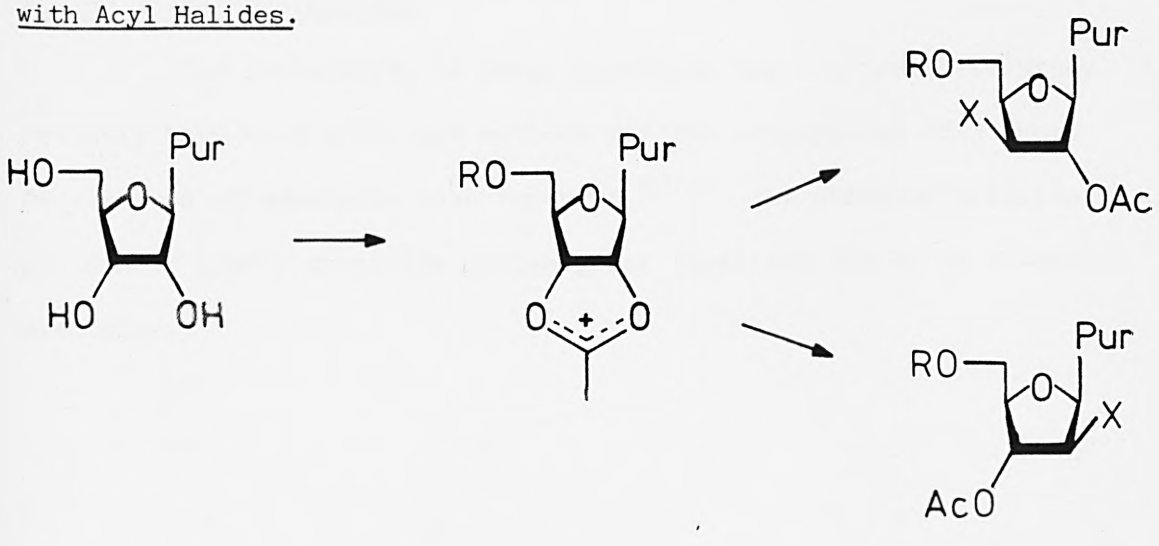
Purine 8,3'-Cyclonucleosides.

The 8,3'-cyclonucleosides are readily available by treatment of 3'-O-alkyl- or 3'-O-arylsulphonated 8-bromo-purine nucleosides with oxygen or sulphur nucleophiles in a reaction analogous to the preparation of the 8,2'-cyclo-nucleosides already described (see Section 1.5.2).[135] This type of reaction has been applied in adenosines and 2'-deoxyadenosines[135,136] and has been extended to the preparation of the 8,3'-thio-cyclic derivatives of adenosine, 2'-deoxyadenosines and guanosine [135,137,138] (Diag. 1.23).

3'-Halogenated Purine Nucleosides.

A major route to this type of compound consists in treatment of purine ribonucleosides with 2-acetoxyisobutyryl halides. The major product is 9-(2-O-acetyl-3-halo-3-deoxy-β-D-xylofuranosyl) purines together with a small amount of the 2'-halogenated ara-nucleoside. This reaction has been applied to adenosine, inosine and guanosine derivatives[82-84,148] (Diag. 1.24).

DIAGRAM 1.24 General Scheme for the Reaction of Purine Ribonucleosides with Acyl Halides.



An analogous reaction, leading to similar mixtures of halogenated products, is that of 2',3'-O-methoxyethylidene purine nucleosides with pivaloyl chloride which has been applied to adenosine and guanosine species[99,139,140,141] (Diag. 1.24).

The opening of purinyl sugar 2',3'-epoxides with hydrogen halides has also led to 3'-halogenated nucleosides. Thus, treatment of the 2',3'-lyxo-epoxide of adenosine with various halides leads to 3'-halo-3'-deoxy-ara-adenosines.[142] Similarly the 2',3'-ribo-epoxide of adenosine also reacts at C-3' to give, in this case, 3'-halo-3'-deoxy-xylo-adenosines.[143,144]

Other heteroatoms have been introduced at C-3' by extensions of the above methods. Thus, the 2',3'-lyxo-epoxide of adenosine reacts with metal azides to give 3'-azido-3'-deoxy-ara-adenosine in high yields.[140,145] Also, opening of the 2',3'-ribo-epoxide of adenosine with azide gives the 3'-azido-3'-deoxy-xylo derivative.[99,144] Virtually all the azido compounds have been reduced to the amino derivatives. Sulphur has also been introduced at the 3'-position of purine nucleosides by attack of various sulphur nucleophiles on lyxo-epoxides.[145,146,147] Reduction of many of these 3'-thiated derivatives has been performed to give purine 3'-deoxy-nucleosides.[82,83,99,148]

Purine 3'-Ketonucleosides.

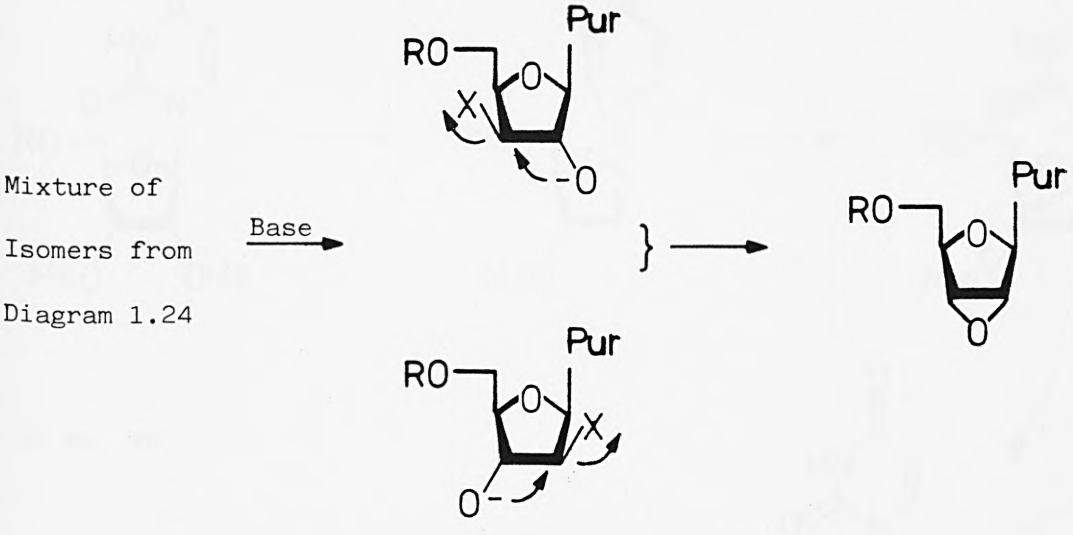
The instability of these compounds has limited their study. Only recently have mild efficient methods for the preparation of 3'-keto derivatives of adenosine been reported.[67,97] The chromium trioxide / pyridine / acetic anhydride system gives excellent yields of 3'-keto-adenosine.

1.5.5) Nucleoside Analogues Modified at both 2'- and 3'-Positions.

2',3'-Epoxy Nucleosides.

Purine ribo-epoxides are readily available from the acetylated trans-halohydrins described in section 1.5.4. Treatment of either of the halohydrin isomers with base leads to good yields of the ribo-epoxide in adenosine[82,99,135,150] and guanosine[83,136,137,151] systems (Diag. 1.25).

DIAGRAM 1.25 Formation of Purine Ribo-epoxides.



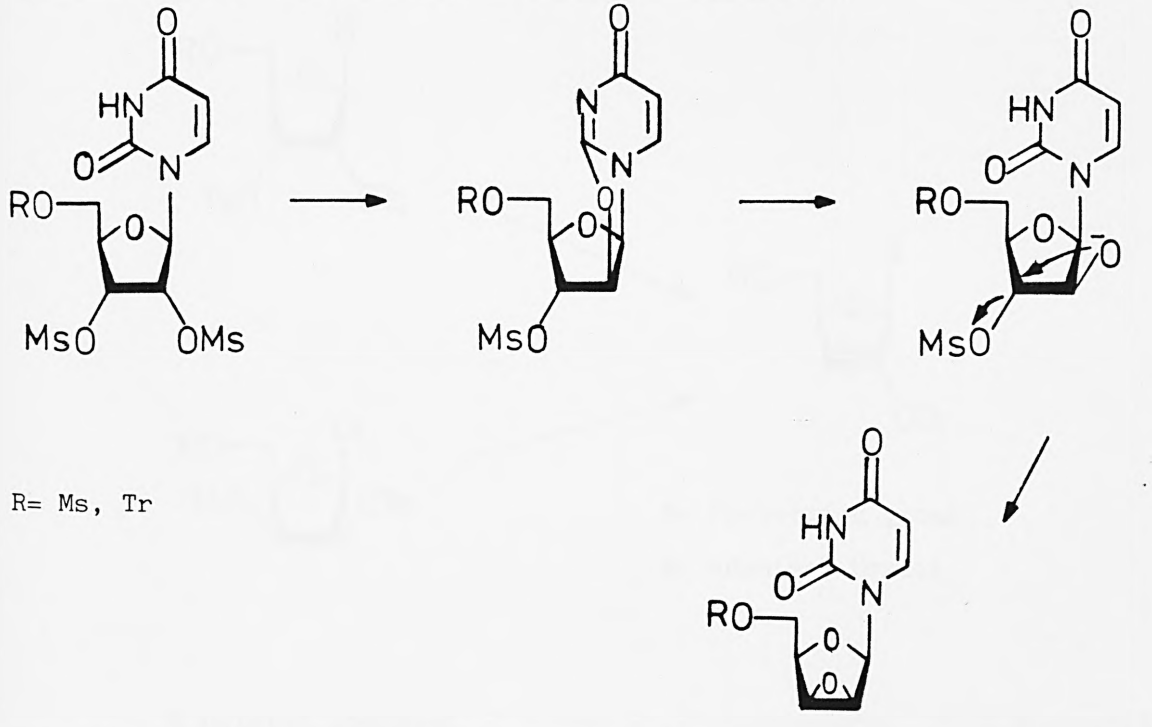
The ribo-epoxides of pyrimidine nucleosides cannot usually be isolated as they undergo rapid intramolecular attack by O-2 to give the O^2,2'-cyclo-nucleosides.[127] However, this intramolecular cyclisation reaction can be suppressed by methylating the N-3 atom, leading to isolation of the ribo-epoxide.[154]

Lyxo-epoxides are available in both purine and pyrimidine nucleosides. In the former, treatment of 9-(2-O-mesyl-β-D-xylofuranosyl) adenine with base leads to the 2',3'-lyxo-epoxide[155] and this procedure has been extended to guanosines.[156] Another method involves treatment of ara-adenosine or xylo-adenosine with triphenylphosphine and diethyl

azodicarboxylate to give the adenosine 2',3'-lyxo-epoxide by way of a triphenylphosphonium intermediate.[157]

In pyrimidine nucleosides, 2',3'-lyxo-epoxides are available by treating the 2',3'-di-O-sulphonate derivatives with aqueous base. This method has yielded the lyxo-epoxide of uridine[158] and 6-azauridine.[129] Treatment of 2',3',5'-tri-O-mesyl-uridine with aqueous base has also given good yields of uridine lyxo-epoxide[128] (Diag. 1.26).

DIAGRAM 1.26 Formation of Pyrimidine Lyxo-epoxides.



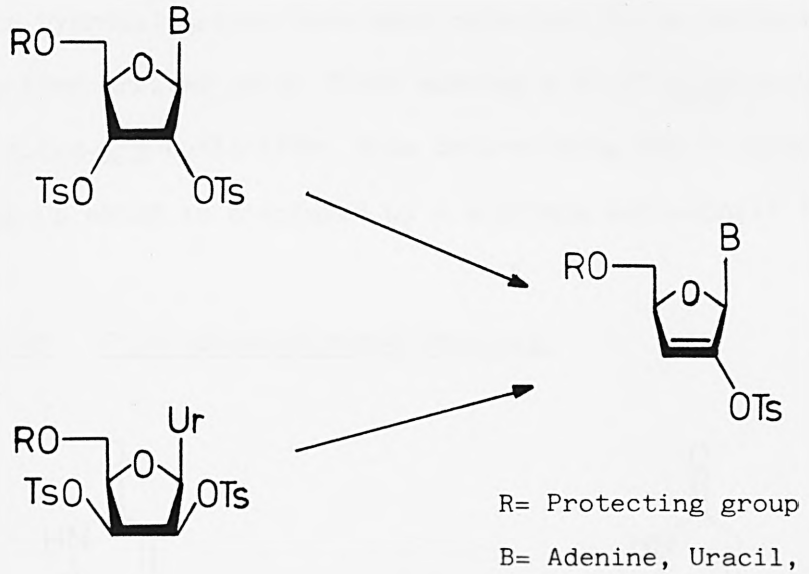
2',3'-Unsaturated Nucleosides.

A number of methods are available to prepare these compounds. In 5'-protected-2'-deoxynucleosides, placing a leaving group at the 3'-position and treatment with a strong base (e.g. alkoxides) has led to 2',3'-unsaturated nucleosides in uridines[160,161] and adenosines.[162] Treatment of 5'-O-trityl-O^2,3'-anhydro-2'-deoxyuridine with potassium t-butoxide also leads to the 2',3'-unsaturated derivative.[116,160] Mesylated trans-iodohydrins, upon treatment with sodium iodide, have given the 2',3'-unsaturated nucleosides in both uridines[116] and adenosines.[139]

Some purine nucleoside 2',3'-di-O-tosylates give enol tosylates

when treated with sodium methoxide at 100°C. In these reactions the 2'-proton is abstracted preferentially.[68] An analogous reaction occurs with $\underline{N}^3$-benzyl-2',3'-di-$\underline{O}$-mesyl-uridine, with the 2'-proton again being abstracted preferentially. The same enol sulphonate is obtained if the 2',3'-di-$\underline{O}$-tosylate of lyxo-uridine is subjected to the same reaction conditions[163-165] (Diag. 1.27).

DIAGRAM 1.27 Selective Eliminations in 2',3'-Di-$\underline{O}$-sulphonated Nucleosides.



R= Protecting group

B= Adenine, Uracil,

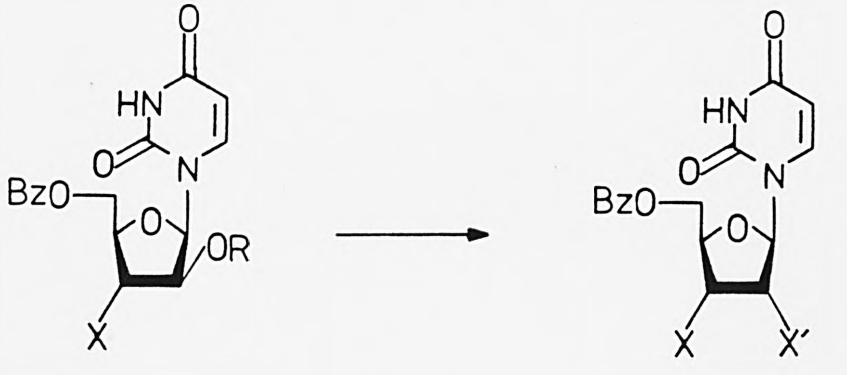
A related compound, 2'-bromo-2',3'-didehydro-2',3'-dideoxyuridine, was obtained by a similar elimination reaction.[166]

Direct use of the purine nucleoside trans-halohydrin acetates, described in section 1.5.4,has been made by Moffatt who has treated them with chromous acetate and ethylene diamine in ethanol at -78°C to induce elimination of the halo acetate function and give good yields of the 2',3'-unsaturated derivative.[147] A similar transformation of these trans-halohydrin acetates can also be achieved using a zinc:copper couple.[167] In a similar method, 3'-$\underline{O}$-acetyl-2'-bromo-2'-deoxyuridine was converted to the 2',3'-unsaturated derivative by zinc powder in ethanol.[168]

2',3'-Disubstituted Nucleosides.

A variety of nucleoside analogues are known bearing modifications at both the 2'- and the 3'-positions. Most of these have already been described in the sections on 2',3'-epoxy nucleosides, 2',3'-unsaturated nucleosides, 3'-substituted ara-nucleosides and 2'-substituted xylo-nucleosides. However, in none of these cases have both the 2'- and the 3'-hydroxyl groups been replaced by other substituents. A few nucleoside derivatives in which both the 2'- and the 3'-positions bear substituents other than hydroxyl groups have been reported. These derivatives have generally been arrived at by first opening a 2',3'-lyxo-epoxide to give a 3'-substituted-ara-nucleoside, then derivatising the 2'-hydroxyl to form a leaving group which is displaced by a suitable nucleophile (Diag. 1.28).

DIAGRAM 1.28 2',3'-Disubstituted Uridines.



For X= Cl R= H X= X'= Cl

For X= N_3 R= Ms X= X'= N_3, NH_2

Thus in the pyrimidine nucleosides, our area of interest, Sasaki et al.[70] treated 5'-O-benzoyl-3'-azido-2'-O-mesyl-3'-deoxy-ara-uridine with excess lithium azide and obtained 5'-O-benzoyl-2',3'-diazido-2',3'-dideoxyuridine, which was subsequently reduced to the 2',3'-diamino analogue. David and De Sennyey[169] treated 5'-O-benzoyl-3'-chloro-3'-deoxy-ara-uridine with the tetrachloromethane / triethyl phosphate / triphenylphosphine reagent to give 5'-O-benzoyl-2',3'-dichloro-2',3'-dideoxyuridine. In a slightly different approach, the same reagent converted 5'-O-benzoyl-uridine to 5'-O-benzoyl-2',3'-dichloro-2',3'-dideoxy-xylo-uridine.[169] However, these routes have not been applied to prepare other 2',3'-disubstituted nucleosides.

1.6) NUCLEOSIDE ANALOGUES CONTAINING BRANCHED SUGARS.

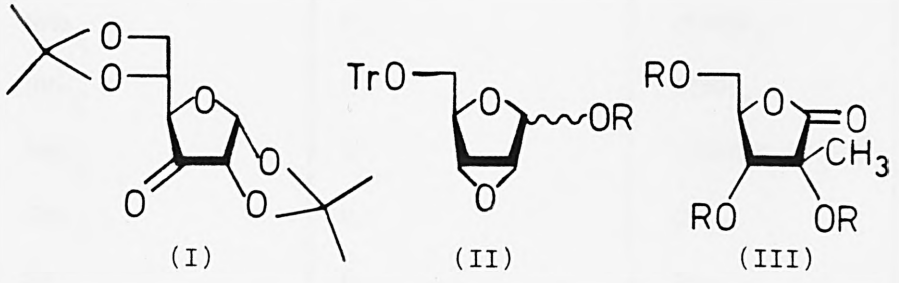
Nucleoside analogues containing branched sugars are far less common than those containing other modifications on the sugar ring.[36] One reason for this can possibly be attributed to the lack of short and efficient general methods for their synthesis. Thus, the main methods available involve introducing the required modification on a sugar which is then condensed with a suitable base.[171-190] These methods, although in principle of general applicability, are often lengthy, of low overall yields, and can give rise to anomeric mixtures of the final nucleoside products.

The approach in which the carbon-carbon bond-forming reaction is performed directly onto the sugar ring of a nucleoside is less common, having received some limited attention only recently.[191-199]

1.6.1) Preparation of Nucleoside Analogues from Branched-sugar Precursors.

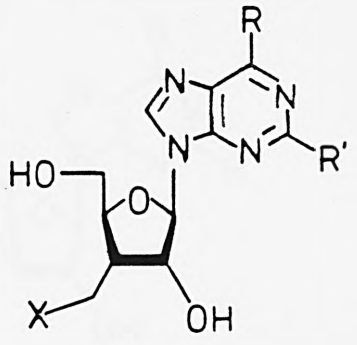
This approach has yielded most of the branched-sugar nucleosides known (Tables 1.7 - 1.12) and, in general, a few sugar derivatives have served as common starting materials to many of the more useful schemes (Diag. 1.29).

DIAGRAM 1.29 Some Useful Sugar Derivatives.



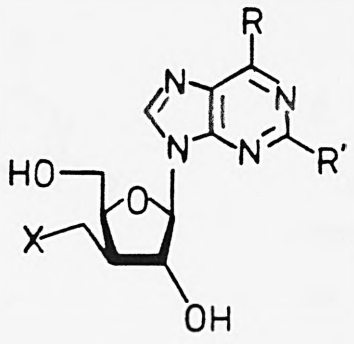
(I) (II) (III)

TABLE 1.7 3'-Branched-3'-Deoxyribofuranosyl Purine Nucleosides.



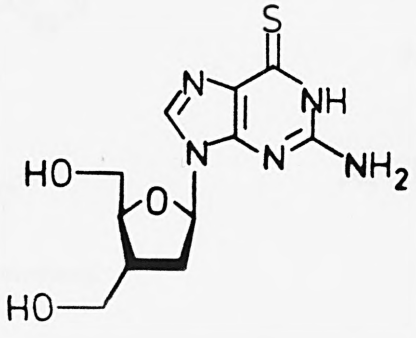
R	R'	X
NH_2	H	H
NH_2	H	CH_3
NH_2	H	$CH_2CH_2CH_3$
NH_2	H	$(CH_2)_4CH_3$
NH_2	H	CH_2OH
NH_2	H	OH
Cl	H	NO_2
NMe_2	H	NO_2
NMe_2	H	NH_2
OH	OH	PO_3H_2
SH	H	PO_3H_2
SMe	H	PO_3H_2
NH_2	H	PO_2H_2
NMe_2	H	CH_2NHAc
NMe_2	H	$CONMe_2$
NMe_2	H	$CONH_2$
NMe_2	H	$CONHCH_2CO_2Et$
NMe_2	H	C≡N
SH	H	NH_2
SMe	H	NH_2
NHOH	H	NH_2

TABLE 1.8 3'-Branched-3'-Deoxyxylofuranosyl Purine Nucleosides.



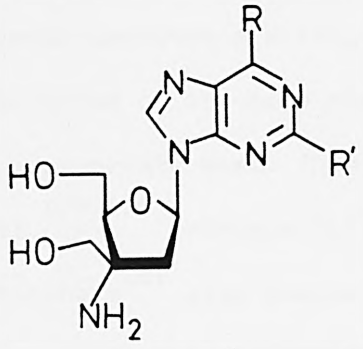
R	R'	X
NH_2	H	OH
NH_2	H	H
OH	NH_2	OH
NH_2	F	H
Me	H	H
Cl	H	H
SH	H	H
SMe	H	H
NHMe	H	H

TABLE 1.9 Purine 3'-Branched-2',3'-dideoxynucleosides.

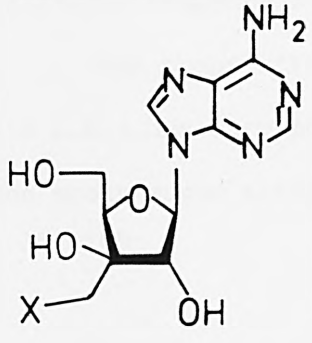


∝ and β Anomers

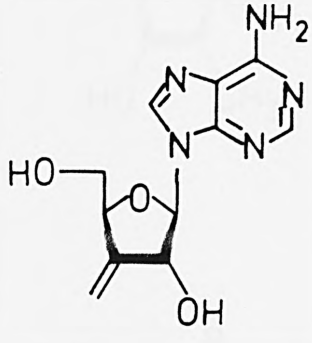
TABLE 1.10 Miscellaneous Purine and Pyrimidine 3'-Branched Nucleosides.



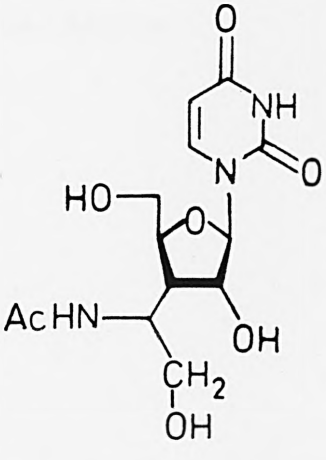
R	R'
NH_2	H
NMe_2	H
OH	NH_2



X = H, OH



∝ and β anomers



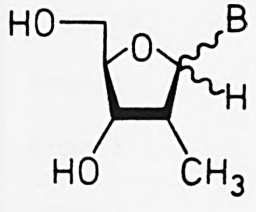
In the preparation of 3'-C-substituted nucleosides containing a D-ribose sugar, the readily available 1,2:5,6-di-O-isopropylidene-3-keto-α-D-glucofuranose[171,172] (I) has often been employed. The most common approach involves a Wittig-type reaction at the 3-keto group and, after elaboration of the side chain so introduced, the sugar is condensed with the appropriate base. This type of approach has led to nucleosides bearing alkyl,[175,176] hydroxyalkyl,[177,178,183,184] aminoalkyl[179,180] and alkyl-phosphonate[181] side chains at the 3'-position (Tables 1.7, 1.9, 1.10).

An alternative approach which leads to alkylamino side chains at the 3'-position is available and involves the base-mediated condensation of nitromethane with the keto-sugar (I).[182]

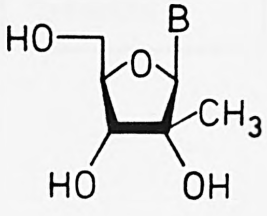
Another approach involves reaction of sugar (I) with Grignard or alkyllithium reagents to afford 3'-C-alkyl-uridines.[173,174]

The preparation of various 3'-C-alkyl-3'-deoxy-xylo-purines (Table 1.8) has been achieved by the initial reaction of Grignard reagents on the epoxy sugar methyl 5-O-trityl-2,3-anhydro-β-D-ribofuranoside (II).[187,188]

TABLE 1.11 2'-Branched Purine and Pyrimidine Nucleosides.



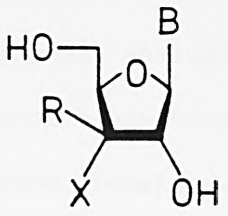
B= Thymine, Adenine



B= Adenine, Cytosine, 5-Fluorocytosine
5-Fluorouracil

Relatively few 2'-C-substituted nucleosides are known and those shown in Table 1.11 are derived from the common sugar derivative 2-C-methyl-D-ribono-γ-lactone (III). Thus use of (III) has led to the 2'-C-methyl-ribonucleosides.[189,190] However if (III) is dehydrated and then hydrogenated prior to the coupling sequence, the 2'-C-methyl-2'-deoxy-nucleosides are obtained.[189,190]

TABLE 1.12 3'-Branched Pyrimidine Nucleosides.



B	R	X
Cytosine	Me	H
Uracil	Me	H
Uracil	F	CH_2OH
Uracil	H	$CH_2PO_3H_2$
Cytosine	H	$CH_2PO_3H_2$
Thymine	H	$CH_2PO_3H_2$
Uracil	Et	OH
Uracil	Bu	OH
Uracil	Me	OH
Cytosine	Me	OH
Uracil	CH_2OH	NH_2
Cytosine	CH_2OH	NH_2

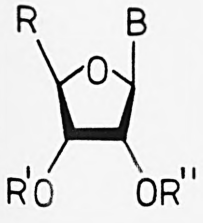
1.6.2) Carbon-Carbon Bond-forming Reactions on Nucleosides.

Some of the first reactions of this type were performed at the C-5' position of various nucleosides. Generally the C-5' hydroxyl group is oxidised to the aldehyde or acid and these functions are subjected to C-C bond forming reactions.[191]

Thus Harper and Hampton[192] oxidized 2',3'-O-isopropylidene adenosine to the 5'-carboxyl derivative which, after esterification, was treated with excess methylmagnesium chloride to afford the 5,5'-di-C-methyl derivative (Table 1.13). Moffatt[193] has used the 5'-aldehyde in Wittig reactions to give 5'-phosphonate analogues of uridine- and adenosine-5'-monophosphate. This approach has been extended to prepare various 5'-C-substituted purine ribonucleosides.[194]

A different approach has been used by Meyer[195] who displaced a 5'-O-tosyl group with the cyanide anion. The resulting cyano group was converted to a carboxamide and a carboxyl group (Table 1.13).

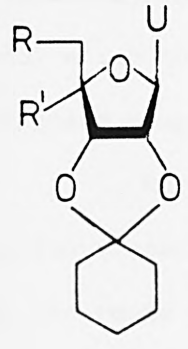
TABLE 1.13 Nucleosides with 5'-C-Substituents.



B	R', R''	R
Ade	H	$CH_2CH_2PO_3H_2$
Ura	H	$CH_2CH_2PO_3H_2$
Ade		$C(CH_3)_2OH$
Ade	H	$(CH_2)_2CONH_2$
Ade	H	$(CH_2)_2CO_2Et$
6-Methylthio-purine	H	$(CH_2)Ph$
"	H	$(CH_2)CO_2Et$
"	H	$(CH_2)CONH_2$
"	H	$(CH_2)C\equiv N$

Few methods are available for introducing a C-C bond at the C-4' position. However, Secrist[196] has described the preparation of a uridine 4',5'-enamine which after alkylation with allyl halides can be made to undergo a Claisen-type rearrangement to afford 4'-C-allyl uridine derivatives (Table 1.14). The terminal vinyl group in these compounds was subjected to catalytic hydrogenation, epoxidation and OsO_4/IO_4^- cleavage to add various functional groups at the 4'-position.

TABLE 1.14 4'-Branched Uridine Nucleosides.



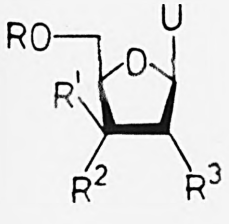
R	R'
OH	$CH_2CH=CH_2$
$CH_2CH=CH_2$	OH
OH	$CH_2C(Me)=CH_2$
$CH_2C(Me)=CH_2$	OH
$CH(Me)CH=CH_2$	OH
OH	$CH(Me)CH=CH_2$
OH	Pr
OH	CH_2CH_2OH

Ueda et al.[197] have employed a deamination reaction of 1-(3-amino-3-deoxy-β-D-glucopyranosyl) uracil to give 3'-deoxy-3'-formyl-xylo-uridine, which was subsequently reduced to the 3'-hydroxymethyl analogue. Similar deamination of 1-(3-amino-2,3-dideoxy-β-D-glucopyranosyl) uracil gave 3'-formyl-2',3'-dideoxy-xylo-uridine which was epimerised and reduced to afford the 2',3'-dideoxy-3'-(R and S)-hydroxymethyl-uridines (Table 1.15).

An interesting reaction was recently described by Grouiller et al.[198] who treated 5'-O-trityl-2'-O-tosyl-uridine with excess methyl magnesium chloride and obtained 5'-O-trityl-3'-C-methyl-2'-deoxy-uridine. Presumably initial elimination of the 2'-O-tosyl group occurs to give an enol which on tautomerising to the 3'-keto compound is attacked by the Grignard reagent (Table 1.15).

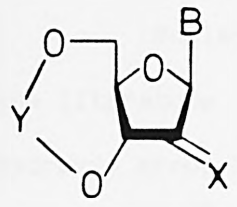
More recently, Ueda[199] has described preparations of various cyclonucleosides in which the sugar and base are bridged by carbon atoms. On the route towards such cyclonucleosides, C-C bond-forming reactions at the 2'-position of various nucleosides were performed. Thus, a 2'-keto group was first introduced in a suitably protected uridine or adenosine species and this was subjected to Wittig reactions and condensation reactions with nitromethane (Table 1.16).

TABLE 1.15 Some 3'-Branched Uridine Nucleosides.



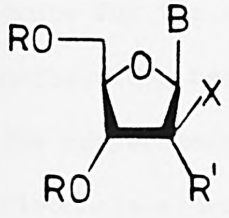
R	R'	R^2	R^3
H	CH_2OH	H	OH
H	CH_2OH	H	H
H	H	CH_2OH	H
Tr	Me	OH	H

TABLE 1.16 2'-Branched Purine and Pyrimidine Nucleosides.



Y= TIPS

B	X
Adenine	O
Adenine	$CHCO_2Et$
Uracil	O
Uracil	CH_2
Uracil	$CH\ CO_2Et$



B	X	R'	R
Adenine	CH_2CH_2OH	H	TIPS
N^6Benzoyl-Ade.	CH_2CH_2I	H	TIPS
Uracil	CH_2OH	OH	TIPS
Uracil	CO_2Et	H	TIPS
Uracil	CH_2CH_2OH	H	TIPS
5-Br-Ura.	CH_2CH_2I	H	TIPS
Uracil	OH	CH_2NO_2	H

2) RESULTS AND DISCUSSION

2.1) Studies Towards 2',3'-Dihalogenated Pyrimidine Nucleosides.

Nucleoside analogues containing modified sugars are well known in the literature, the most common ones bearing a sugar ring in which a single hydroxyl group has been replaced by a new substituent at the 2'-, 3'- or 5'-position.[36] The methods available for introducing such modifications are quite varied (see Sections 1.5 and 1.6) and have been adapted to the synthesis of some 2',5'-disubstituted-[200-202] and some 3',5'-disubstituted-nucleosides.[203-205] However, few routes are available for the synthesis of 2',3'-disubstituted nucleosides and such analogues are very scarce. In fact, as already mentioned, (Section 1.5.5), the few examples extant of nucleosides of this type are some 2',3'-dichloro-,[169] 2',3'-diazido- and 2',3'-diamino-2',3'-dideoxyuridines.[70]

We were attracted to the possibility of developing a general route for the synthesis of 2',3'-dihalogenated-2',3'-dideoxy-ribo-nucleosides because they might exhibit interesting biological properties. The replacement of both the 2'- and the 3'-hydroxyl groups of natural ribonucleosides by halogen atoms would afford analogues with substituents at C2' and C3' varying in size and electronegativity with respect to the natural hydroxyl groups. Other differences include the relative abilities to form hydrogen bonds and the inability of the halo-substituents to form phosphodiester bonds. These changes would give analogues which, it was envisaged, might interfere with nucleic acid metabolism in a number of ways. Thus, if these compounds were phosphorylated and incorporated into a nucleic acid chain, the 3'-hydroxyl group would not be available to form a phosphodiester bond and thus result in termination of nucleic acid synthesis. Also, the conversion of ribonucleoside diphosphates to their 2'-deoxy-derivatives might be affected by the presence of a halogen atom at

C2' in place of a hydroxyl group. Of course other less obvious processes in nucleic acid metabolism might be open to interference by these analogues. Furthermore, if any biological activity were to be found, the possibility of being able to use fluorine, chlorine, bromine and iodine substituents would give up to sixteen 2',3'-dihalogenated 2',3'-dideoxy-ribonucleoside analogues in each of the pyrimidine and purine series, so that many analogues would be available to give us some insight into how they were achieving their biological activity. For instance, differences in the size and electronegativity of the halo substituents could affect the conformation of the sugar ring, the overall steric requirements of the nucleoside and the strength of its bonding interactions with an enzyme molecule.

With these aims in mind, a general synthetic route to 2',3'-dihalogenated 2',3'-dideoxy-ribonucleosides was sought. As mentioned above, some 2',3'-dichloro-2',3'-dideoxyuridines have recently been prepared.[169] However, the route involved, as the final step, chlorination of a 5'-O-benzoyl-3'-chloro-3'-deoxy-ara-uridine using the carbon-tetrachloride / triphenylphosphine / triethylphosphate reagent. This type of reagent is not available for all the halogens and thus cannot serve as a general route, and indeed this scheme was not applied to afford other dihalogenated analogues.

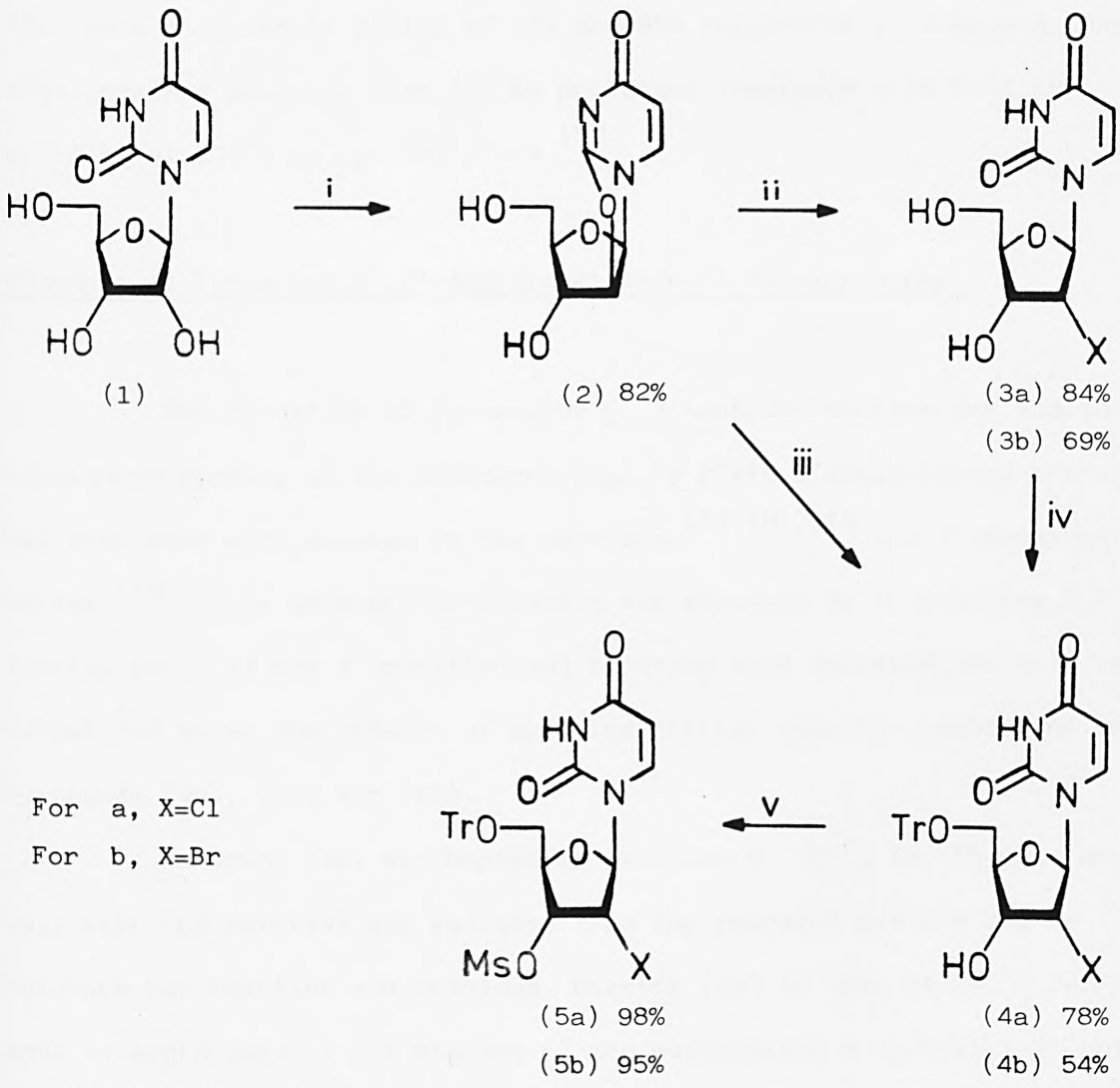
Our attempts at preparing some 2',3'-dihalogenated derivatives of uridine are detailed below.

2.1.1) Preparation of 5'-O-Trityl-3'-O-mesyl-2'-halo-2'-deoxyuridines.

The introduction of a variety of substituents at the 2'-position[41,52,53,56] and the 3'-position[100,101,110] of the sugar ring in pyrimidine nucleosides has often been achieved by the nucleophilic opening of the appropriate anhydro-bridge. By combining these two strategies to involve the formation and opening, first of an O^2,2'-anhydro- and then an

O^2,3'-anhydro bridge, we hoped to arrive at a short route to pyrimidine 2',3'-disubstituted-2',3'-dideoxy-ribonucleosides. Scheme 2.1 outlines the preparation of some 5'-O-trityl-3'-O-mesyl-2'-halo-2'-deoxyuridines (5), intermediates with the potential to cyclise to give O^2,3'-anhydro-nucleosides.

SCHEME 2.1 Routes to 5'-O-Trityl-3'-O-mesyl-2'-halo-2'-deoxyuridines.



For a, X=Cl
For b, X=Br

i) $(PhO)_2CO$/HMPA/145°C/$NaHCO_3$

ii) HX/DMF/100°C

iii) TrCl/Pyridine/105°C (For X = Cl)

iv) TrCl/Pyridine/80°C

v) MsCl/Pyridine/0°C

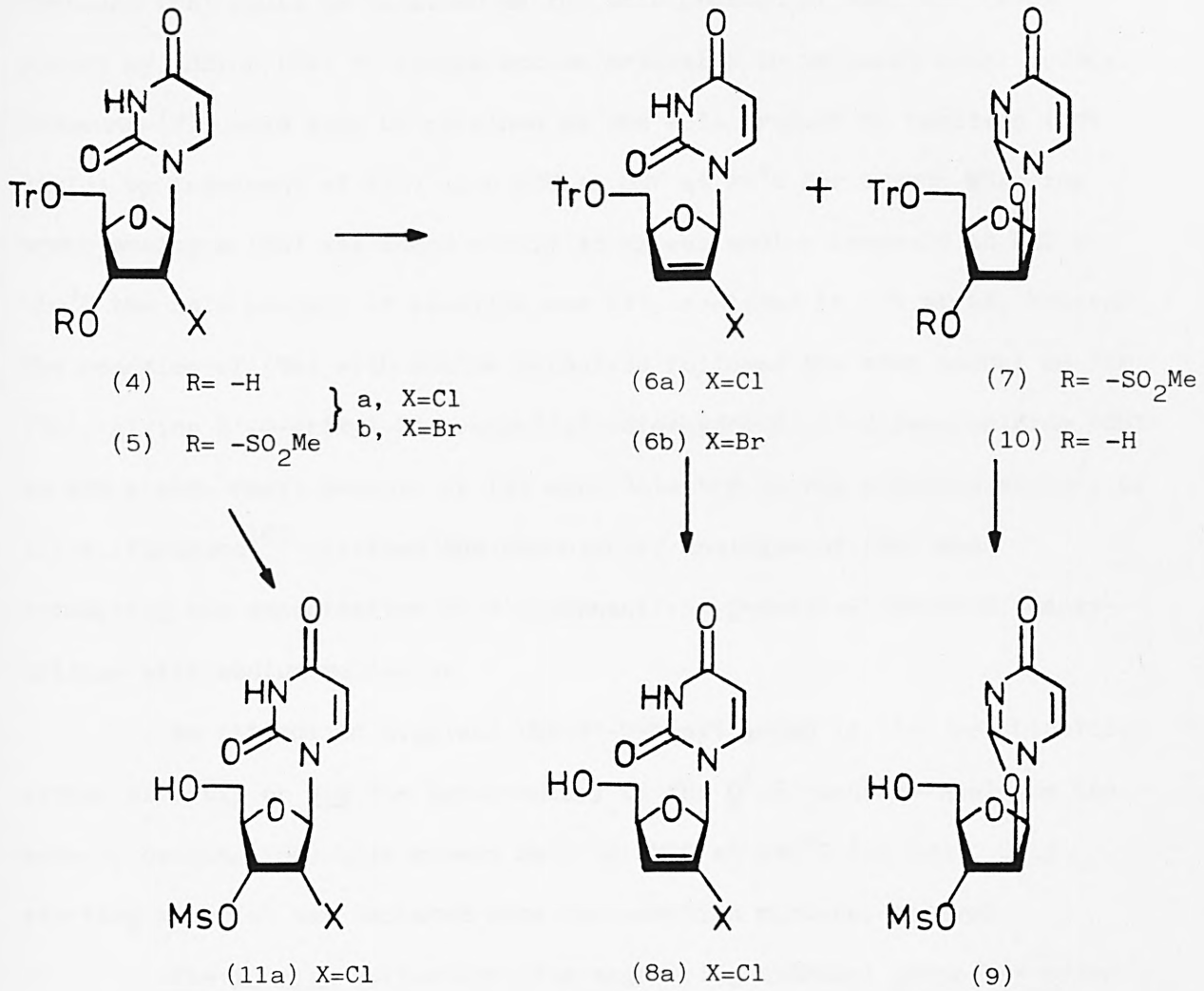
Uridine (1) was converted to O^2,2'-cyclo-uridine (2) in 82% yield by the known method.[40,41] At this stage of the synthesis a variety of substituents may be introduced at the 2'-position.[53,60] Thus treatment of (2) with HCl in DMF at 100°C gave 2'-chloro-2'-deoxyuridine (3a) in 84% yield.[42] The analogous reaction with HBr gave 2'-bromo-2'-deoxyuridine (3b) in 69% yield. Treatment of (3a) with triphenylchloromethane (TrCl) in pyridine at 80°C gave 5'-O-trityl-2'-chloro-2'-deoxyuridine (4a) in 78% yield, which reacted with methane sulphonylchloride in pyridine at 0°C to give (5a) in quantitative yield. Similarly, the bromo-analogues (4b) and (5b) were obtained in yields of 55% and 95% respectively. Compound (4a) was also obtained directly from (2) by prolonged treatment with TrCl in pyridine at 105°C in ca. 38% yield.[152]

2.1.2) Attempts at Preparing O^2,3'-Anhydro-2'-halo-2'-deoxyuridines.

The formation of pyrimidine O^2,3'-anhydro-nucleosides and the subsequent opening of the anhydro-bridge to yield 3'-substituted products has been used with success in the thymidine[100,101,110] and 2'-deoxyuridine series.[110,206] In general, cyclisation was achieved by introducing a leaving group at the 3'-position and treating this intermediate with base. Scheme 2.2 shows the results of applying similar reaction conditions to compounds (4a), (5a) and (5b).

Compound (5a) was heated in pyridine at 100°C for 18 hrs. However only starting material was isolated from the reaction mixture and no evidence for reaction was obtained. Heating (5a) in HMPA at 140°C for 9 hrs gave an approximately 1:1 mixture of the nucleosides 5'-O-trityl-2'-chloro-2',3'-didehydro-2',3'-dideoxyuridine (6a) and 5'-O-trityl-3'-O-mesyl-O^2,2'-anhydro-uridine (7). A similar mixture was obtained when (5a) was added in one portion to excess sodium benzoate in DMF at 100-105°C. When compound (5a) was added slowly to excess sodium benzoate in DMF at 110°C,

SCHEME 2.2 Attempts at Preparing O^2,3'-Anhydro-2'-halo-2'-deoxyuridines.



Starting Material	Reaction Conditions	Products (Yield)
4a	$(PhO)_2CO/NaHCO_3$/HMPA	10 (65%)
"	Ph_3P/DEAD/THF	6a (not pure)
5a	Δ/Pyridine/100°C	No Reaction
"	NaCl/DMSO/140°C	"
"	Δ/HMPA/140°C	6a & 7 (1:1 mixture)
"	DBU/DMF/RT	7 (64%)
"	NaO_2CPh/DMF/105-110°C	6a (70%) & 7 (Variable)
5b	"	7 (50%)
5a	NaOMe/MeOH/Reflux	6a (70%)
5b	"	6b (66%)
5a	HCl/MeOH/RT	11a (71%)
6a	TFA/BuOH/RT	8a (Incomplete)
6a	HCl/MeOH/RT	8a (80%)
7	TFA/BuOH/RT	9 (41%)

compound (6a) was obtained as the major product in ca. 70% isolated yield. Compound (6a) could be obtained as the sole product of reaction (>70% yield) by adding (5a) to excess sodium methoxide in methanol under reflux. Compound (7) could also be obtained as the sole product of reaction (64% yield) by treatment of (5a) with DBU in DMF at 20°C for 18 hrs. When the bromo analogue (5b) was added slowly to excess sodium benzoate in DMF at 110°C the only product of reaction was (7), isolated in 46% yield. However, the reaction of (5b) with sodium methoxide followed the same course as for (5a), giving 5'-O-trityl-2'-bromo-2',3'-didehydro-2',3'-dideoxyuridine (6b) in 69% yield. Small amounts of (7) were detected in the reaction mixture by t.l.c. Furukawa[166] obtained the deprotected analogue of (6b) when attempting the deprotection of 5'-O-benzoyl-3'-O-mesyl-2'-bromo-2'-deoxyuridine with sodium methoxide.

An attempt to displace the 3'-O-mesyl group in (5a) by chloride, either directly or via the intermediacy of the O^2,3'-anhydro-analogue was made by heating (5a) with excess NaCl in DMSO at 140°C for 5 hrs. Only starting material was isolated from the reaction mixture, however.

The in situ activation of a sugar ring hydroxyl group has often been used to effect cyclisation to give anhydro-nucleosides. Thus the conditions used to prepare compound (2) have been applied to 3',5'-di-O-trityluridine to give 3',5'-di-O-trityl-O^2,2'-cyclouridine.[207] However, subjecting compound (4a) to these conditions did not result in cyclisation to the 3'-position. In fact displacement of the chlorine atom occurred giving 5'-O-trityl-O^2,2'-anhydro-uridine (10).

Treatment of (4a) with the triphenylphosphine / diethyl azodicarboxylate reagent[248] also failed to give the desired O^2,3'-anhydro product, the sole nucleoside product of reaction being compound (6a). Presumably, the requisite 3'-arylphosphonium intermediate had formed, but β-elimination, rather than a nucleophilic displacement of triphenylphosphine oxide by the O2 atom, had occurred.

The compounds (5a), (6a) and (7) were deprotected using methanolic HCl to give the corresponding free nucleosides. Deprotection using TFA/butanol was less efficient giving incomplete reaction under the published conditions.[249]

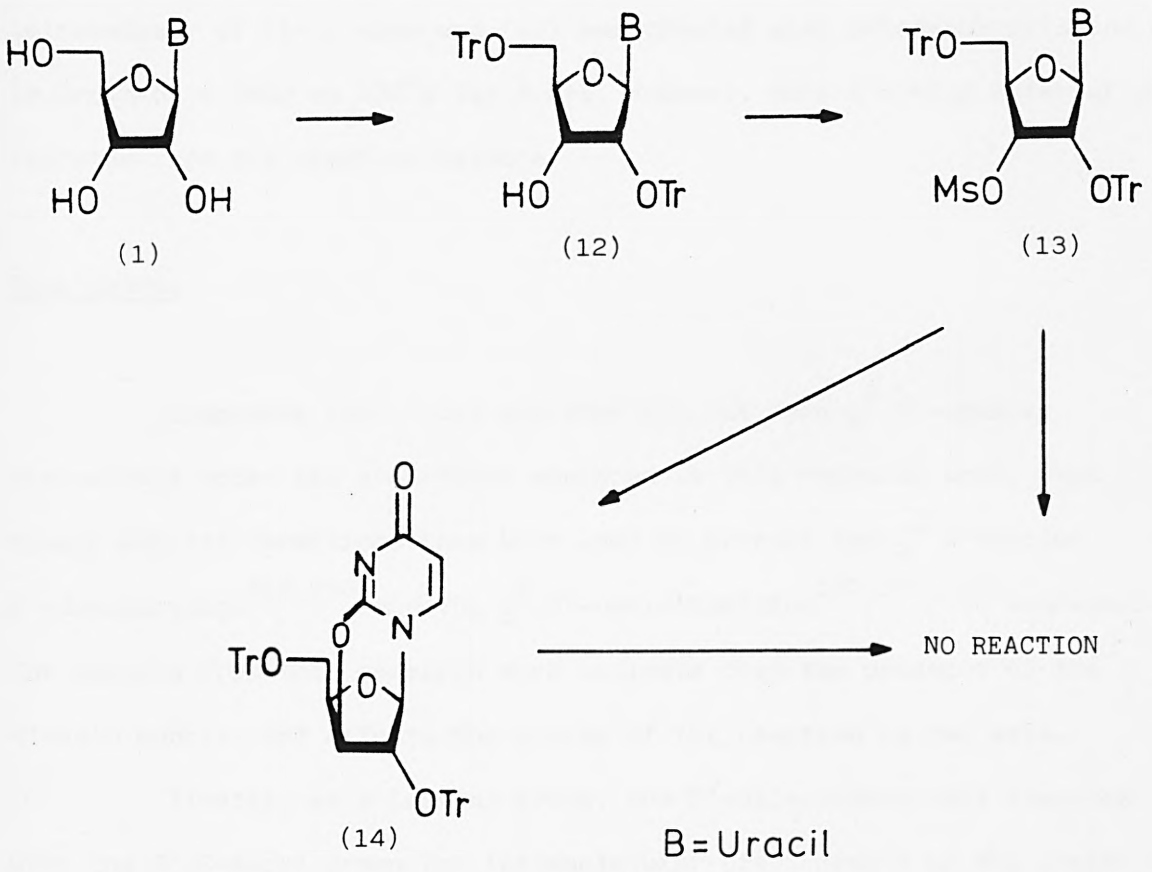
2.1.3) Approaches Towards 3'-Halogenated Uridines.

The failure of compounds (4a), (5a) and (5b) to undergo intramolecular cyclisation to give the O^2,3'-anhydro-analogues led us to consider reversing the order of events and introducing the 3'-substituent prior to the 2'-substituent. The feasibility of using 3'-substituted-3'-deoxy-ara-uridine derivatives as precursors for the preparation of 2',3'-disubstituted-2',3'-dideoxy-uridines has already been demonstated, albeit to a limited extent.[70,169] We therefore looked for an alternative approach.

Very few examples of 3'-substituted-3'-deoxyuridines exist in the literature[112-114] and we decided to reinvestigate the use of 2',5'-di-O-trityl-O^2,3'-anhydro-uridine (14) as an intermediate for introducing a substituent at the 3'-position.[106] The choice of the ditritylated compound (14) was based on the reports that the unprotected O^2,3'-cyclouridine[112-114] and the 2',5'-di-O-benzoyl derivative[106] undergo rearrangement reactions leading to undesired products. Furthermore, 2',5'-di-O-trityl-uridine (12) has been used to prepare some 3'-substituted xylo-uridine derivatives[103] indicating that the reactivity at the 3'-position is not entirely hindered by the adjacent bulky trityl groups. Scheme 2.3 shows the reactions attempted.

Preparation of 2',5'-di-O-trityl-O^2,3'-cyclo-uridine (14) from uridine (1) in 6% overall yield was carried out by the literature method.[106] An attempt to prepare (14) by treating (13) with DBU in DMF at 20^{o}C for 18 hrs failed (cf. reaction of (5a) with DBU/DMF).

SCHEME 2.3 Attempts at Preparing 3'-Halo-3'-deoxyuridines.



Starting Material	Reaction Conditions	Products (Yield)
1	TrCl/Pyridine/110°C	12 (26%)
12	MsCl/Pyridine/0°C	13 (80%)
13	DBU/DMF/RT	No Reaction
13	NaO_2CPh/DMF/130°C	14 (30%)
13	KCl/18-crown-6/DMSO/130°C	No Reaction
14	LiCl/DMF/$PhCO_2H$/105°C	No Reaction

Compound (14) was treated with excess lithium chloride and one equivalent of benzoic acid in DMF at 100°C for 3.5 hrs. However, no reaction occurred. Some similar failures have been reported in the literature.[121] In order to displace the 3'-O-mesyl group in (13) either directly or through the intermediacy of (14), compound (13) was treated with potassium chloride and 18-crown-6 in DMSO at 130°C for 4 hrs. However, only starting material was isolated from the reaction mixture.

2.1.4) Conclusions

Compounds (4a), (5a) and (5b) did not form O^2,3'-cyclic derivatives under the conditions explored in this research work, even though similar conditions have been used to prepare the O^2,3'-cyclo-2'-deoxyuridine[110,206] and the O^2,3'-cyclothymidine[100,101,110] analogues. The results from this research work indicate that the presence of the 2'-halo substituent affects the course of the reaction in two ways.

Firstly, as a leaving group, the 2'-halo substituent competes with the 3'-O-mesyl group for intramolecular displacement by the oxygen at C-2 of the pyrimidine ring. Thus under mildly basic conditions, or with sterically bulky bases (i.e. when the N-3 proton only is abstracted), the 2'-halo substituent is preferentially displaced leading to 5'-O-trityl-3'-O-mesyl-O^2,2'-cyclo-uridines such as (7).

Secondly, as an electron-withdrawing atom, it renders the 2'-proton more acidic than in 2'-unsubstituted-2'-deoxynucleosides. Thus strong, sterically compact bases will abstract the 2'-proton and cause β-elimination of the 3'-O-mesyl group, leading to 5'-O-trityl-2'-halo-2',3'-didehydro-2',3'-dideoxyuridines such as (6a) and (6b).

These two reaction courses have been observed for some adenosine-2',3'-di-O-tosylates,[163] uridine-2',3'-di-O-tosylates[52] and uridine-2',3'-di-O-mesylates.[164] In all these cases cyclisation to the 2'-position

or abstraction of the 2'-proton were the only reactions observed.

Preferential cyclisation to the 2'-position can be rationalised on the basis that a 5-membered ring is formed, and this is expected to be thermodynamically more stable than the 6-membered ring which would arise if cyclisation to the 3'-position occurred. The abstraction of the 2'-proton in preference to the 3'-proton is probably a result of the electron-withdrawing effect of the nitrogen atom at the C1' carbon being felt at C2', so that even in nucleosides having identical substituents at the 2'- and 3'-positions the 2'-proton is more acidic.

Finally, the conformation of the sugar ring may play an important part in determining which of the above two reaction pathways occurs. Studies in various nucleosides have shown that an equilibrium exists at ambient temperatures between the C2'-endo (S-type) and the C3'-endo (N-type) conformation of the sugar ring.[208] Studies on 2'-substituted 2'-deoxyadenosines have shown the S-type conformation of the sugar ring is preferred.[209] Thus, in 2'-chloro- and 2'-bromo-2'-deoxyadenosine at 25°C the percentage of the S-type conformer is in the region of ca. 75-80%. However, the energy barrier for interconversion is generally small (ca. 5 Kcal/Mol) making both conformations readily accessible.[3] Inspection of molecular models of compounds (4a), (5a) and (5b) shows that a C2'-endo ring pucker (i.e. S-type) not only places the C2'-proton and the 3'-O-substituent in an almost trans-periplanar arrangement, necessary for β-elimination, but it also brings the C2' atom in close proximity to the O2 oxygen of the pyrimidine ring and hence favours cyclisation to the 2'-position. Thus the formation of compounds of the type (6a), (6b) and (7) probably involves a transition state in which the sugar ring has a conformation close to a C2'-endo pucker (i.e.the preferred S-type conformation). The orientation of the pyrimidine ring must be close to a syn-type arrangement when (7) is formed but can be in any orientation when compounds (6a) and (6b) are formed.

The short investigation of the use of 2',5'-di-O-trityl-O^2,3'-cyclouridine (14) as an intermediate for arriving at 3'-halo-3'-deoxyuridines was unsuccessful and confirmed similar results in the literature.[112,113,121] The direct displacement of the 3'-O-mesyl group also failed. These disappointing results, coupled with the reported failures in the past and the poor overall yields in arriving at compound (14) made this scheme unattractive and it was not pursued further.

An approach described by Hirata et al.[52b] in which 3'-O-tosyl-O^2,2'-cyclo-uridines were converted to 3'-substituted-3'-deoxy-O^2,2'-cyclouridines, which might then be used to introduce the second substituent at C2', also suffered from low overall yields and was not explored as a route to 2',3'-disubstituted nucleosides.

Thus so far the only approach available for 2',3'-dihalogenated uridines is the one described by David.[169] However, as has been pointed out earlier, the second halogen substituent is introduced at C2' of a 3'-chloro-3'-deoxy-ara-uridine by the use of the carbontetrachloride / triphenylphosphine / triethylphosphate reagent. This type of reagent is not available for all the halogens and therefore this approach would not yield all the possible dihalo-derivatives. The method described by Sasaki[70] to arrive at the 2',3'-diazido and 2',3'-diamino-2',3'-dideoxyuridines could be adapted to arrive at 2',3'-dihalo-2',3'-dideoxyuridines.

Thus, preparation of a suitably protected uridine-2',3'-lyxo-epoxide is easily achieved.[129,70,119] Opening of this epoxide with a halide nucleophile is well established[119] and was infact used by David[169] to arrive at the 3'-halo-ara-uridine. Now Sasaki[70] formed a 2'-O-mesyl derivative of a 3'-azido-ara-uridine, which on reaction with azide gave the 2',3'-diazido-2',3'-dideoxyuridine. However, in order to displace the 2'-O-mesyl group, quite forcing conditions had to be used (i.e. NaN_3 / DMF / 115-120°C / 4.5 hrs). These conditions might cause the displacement of the 3'-halo as well as the 2'-O-mesyl group. Ranganathan[86,92] has displaced

the 2'-O-triflate group in some ara-adenosines with halides as well as other nucleophiles under very mild conditions (MX / HMPA / RT / 10 mins). Thus, preparing the 2'-O-triflate derivative of suitably protected 3'-halogenated-3'-deoxy-ara-uridines and subjecting them to the latter conditions could afford a good route to 2',3'-dihalogenated-uridine analogues. However the pressure of time did not allow the testing of this proposal, and it is put forward as a possible area for further study.

Although this part of the research work failed to give the required nucleoside analogues, it proved possible to to select the reaction conditions so as to arrive at either compounds of type (6) or type (7). The former class of compounds bear a relatively uncommon sugar ring modification[166] and it proved possible to make use of the halovinyl function in the next part of the research project aimed at the synthesis of nucleosides containing branched sugars.

2.2) SYNTHETIC ROUTES TO BRANCHED SUGAR NUCLEOSIDE ANALOGUES.

Introduction.

The survey on nucleosides containing branched-chain sugars (see Section 1.6) shows that compared to other nucleosides containing modified sugars (Sections 1.3 to 1.5), relatively few of the former are known. Furthermore, most of them have been arrived at by lengthy procedures involving preparation of the required sugar which was then coupled with the appropriate base. Of the few methods available for introducing a carbon chain directly onto a sugar of a nucleoside, the two approaches which may be of general applicability have been recently reported and as yet have not been widely applied.[198,199] The work of Moffatt[193] and of Montgomery[194] has demonstrated that application of C-C bond-forming reactions at C-5' of various nucleosides is possible. However, apart from the two recent reports already mentioned,[198,199] direct C-C bond-forming reactions at C-2' and C-3' have received limited attention. We therefore set out to explore possible synthetic routes to nucleosides bearing a chain branch at C-2' and C-3'. The requirements of the synthetic schemes that we were looking for are now outlined.

The initial requirement of our synthetic schemes was that they should start from intermediates readily prepared from natural or commercially-available nucleosides. The schemes should be applicable to both the purine and pyrimidine nucleosides. The reactions employed should give good stereo- and regio-control and allow a variey of side chains to be introduced. The side chains should be amenable to further elaboration once present on the nucleoside. Finally, the schemes should be shorter and afford better overall yields than those already reported starting from sugar analogues.

The approaches used to arrive at 3'- and 2'-branched nucleosides are now discussed in turn.

2.2.1) Preparation of 3'-Branched Pyrimidine Nucleosides.

Sections 1.5.3 and 1.5.4 outline the general approaches available for introducing a substituent at the 3'-position of nucleosides. Thus the general strategy has involved attack by a nucleophilic reagent on an electrophilic 3'-carbon atom. The nucleophiles so far used have been the halides as well as a variety of sulphur, oxygen and nitrogen-containing reagents. We wanted to extend this strategy by using carbon nucleophiles.

A variety of organometallic compounds are available as sources of carbon nucleophiles, the most commonly-used and studied being the Grignard, alkyllithium and lithium alkylcuprate reagents.[210,211] Between them, these reagents would allow a range of carbon nucleophiles to be used. They can attack a range of electrophilic carbon atoms. Thus they can displace halogen atoms from alkyl halides, attack carbonyl groups, open epoxides and undergo Michael addition with α-β-unsaturated carbonyl compounds. Thus a number of nucleoside derivatives with an electrophilic C-3' atom such as 3'-halo, 3'-O-sulphonated, 3'-keto, 2',3'-epoxy, O^2,3'-cyclo and O^8,3'-cyclo-nucleosides could in principle react with these reagents. However, not all of them fit the requirements set out above for our intended synthetic scheme. Thus the 3'-halo-nucleosides are not always readily available in good yields. This is true in the pyrimidine ribonucleosides.[112,113,114,121] Also, being secondary halides, they are relatively less reactive towards organometallic reagents than say a carbonyl group or an epoxide. Furthermore, depending on the configuration at C-3', they might undergo β-elimination under the basic conditions of organometallic reactions by abstraction of the C-2' or the C-4' proton. Intramolecular displacement by the O2 of the pyrimidine base might also pose a problem in some cases unless protection of the N^3 atom was performed. These considerations also apply to the 3'-O-sulphonates.

Of the remaining two options, the 3'-keto-nucleoside

derivatives have recently become available in both the purine and the pyrimidine series in good yields.[67] However, they are reputed to be very sensitive to basic conditions leading to rapid glycosidic bond cleavage.[212,213] Such basic conditions are inescapable when using organometallic reagents. The presence of a carbonyl group at C-3' would be expected to affect the acidity of the 4'-proton making it more labile to base so that epimerisation at C4' might occur under the reaction conditions via a 3',4'-enolate anion. Attack on the carbonyl group might occur from either side of the sugar ring, although studies in which 3'-keto groups have been reduced by sodium borohydride have shown a strong preference for attack occurring from the α-face of the sugar ring.[67] Finally, Moffatt[65] has reported that 2',5'-di-O-trityl-3'-keto-uridine does not react with methylmagnesium chloride. This lack of reactivity may be a result of steric hindrance exerted by the bulky trityl groups.

Considerations of the properties of epoxides show that such intermediates might be good candidates for attempting these organometallic reactions. Firstly, 2',3'-anhydro-lyxo-nucleosides are available in both the purine and pyrimidine series. Furthermore, the 2',3'-ribo-epoxides are also readily available in the purine series and recently it has been shown that such epoxides can be isolated in the pyrimidine series as long as the N^3 atom is suitably protected to prevent isomerisation to the O^2,2'-cyclo-nucleoside.[154] The 2',3'-lyxo-epoxides of both purine[139-142] and pyrimidine[115-119] nucleosides have been shown to react almost exclusively at C-3' with a variety of nucleophiles to give 3'-substituted-ara-nucleosides. Thus both regio- and stereocontrol are afforded in this reaction. The 2',3'-ribo-epoxides in purine nucleosides are also attacked predominantly at C-3' to yield 3'-substituted-xylo-nucleosides.[143,144] Thus by the appropriate choice of the starting epoxide, entry into the above two classes of nucleoside analogues is possible in one step. Finally, the 2'-hydroxyl group resulting from the above epoxide opening reactions could

be reduced[214] to afford the 2'-deoxy series of analogues, or its configuration could be inverted[86,92] to give 3'-C-substituted-ribo- and -lyxo-nucleosides. Thus by appropriate choice of the starting epoxide followed by deoxygenation or inversion reactions at C-2', several series of nucleoside analogues containing a carbon substituent at C-3' would be accessible.

2.2.2) Preparation of Some Pyrimidine 2',3'-Lyxo-epoxides.

The initial exploratory reactions were to be performed on pyrimidine derivatives and later applied to purines (see below). Uridine was chosen because if carbon branches could be introduced at C-3', then efficient methods were available for converting the uridine analogues to thymidines[215] and cytidines.[216]

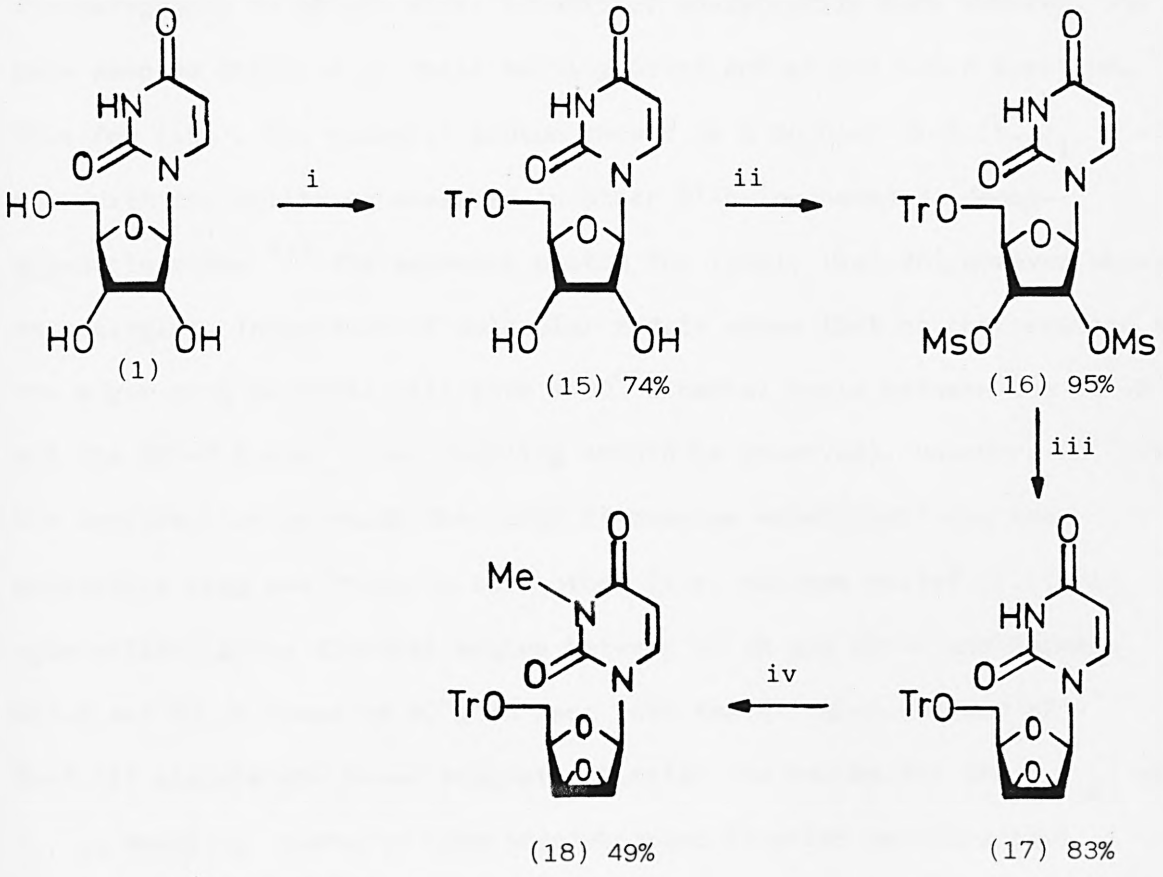
Thus 5'-O-trityl-2',3'-anhydro-lyxo-uridine was chosen as a suitable starting material. The reasons for the choice of the 5'-O-trityl protecting group were twofold. Firstly, it is very stable to organometallic reagents and secondly it would help to make the nucleosides soluble in diethyl ether or THF, the usual solvents for many organometallic reactions.[250] Scheme 2.4 outlines the preparation of the two uridine-2',3'-lyxo-epoxides used in this research work. The route used to arrive at (17) is a modification of the method employed to make the 2',3'-lyxo-epoxide of 6-azauridine.[129]

Uridine (1), was treated with triphenylchloromethane in pyridine at 100°C to afford 5'-O-trityl-uridine (15), in 74% yield. Reaction of (15) with excess methanesulphonylchloride in pyridine at 0°C for 18 hrs afforded the 2',3'-di-O-mesyl derivative (16) in essentially quantitative yield. Treatment of (16) with 3.5 equivalents of aqueous sodium hydroxide in ethanol gave 5'-O-trityl-2',3'-anhydro-lyxo-uridine (17) in 83% yield. We therefore have a convenient route, from readily available uridine (1) to a

derivative (17) ready to undergo nucleophilic attack at C-3', with an overall yield of ca. 61%. This sequence of reactions could conveniently be performed on up to 30g quantities of uridine.

The N^3-methyl derivative (18) was obtained in 49% yield from the epoxide (17) by treating the latter with sodium hydride and methyl iodide in THF for 24 hrs at ambient temperature.[217] This derivative was prepared to determine the effect of the exchangable N-3 proton on the course of the organometallic reactions to be investigated.

SCHEME 2.4 Preparation of Some Uridine 2',3'-lyxo-epoxides.



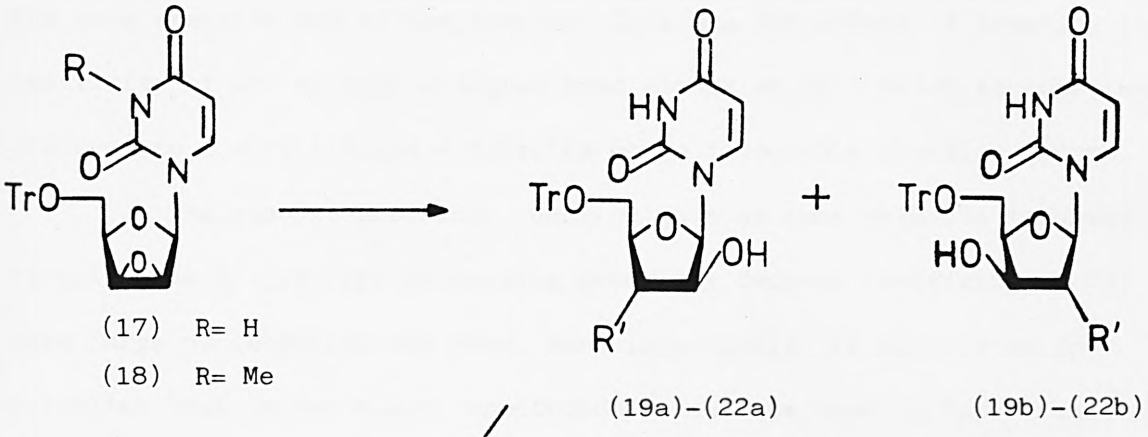
i) TrCl/Pyridine/110°C
ii) MsCl/Pyridine/0°C
iii) NaOH/EtOH/Reflux, RT
iv) NaH/MeI/THF/RT

2.2.3) Reaction of the Epoxide (17) with the Bromide Nucleophile.

The first reaction of epoxide (17) that was performed was attack by bromide (Scheme 2.5). This reaction has been performed on the unprotected[119] and the 5'-O-acetyl analogues[115] of (17) and exclusive attack at C-3' was reported in both cases. When (17) was heated with ammonium bromide in refluxing ethanol[119] reaction proceeded slowly and only after ca. 60 hrs had most of the starting material been consumed. Crystallisation from the reaction mixture gave a white solid which by proton n.m.r. was shown to be a 3:2 mixture of compounds (19a) and (19b). Separation of these two compounds proved inefficient requiring lengthy chromatography to obtain small amounts of analytically pure samples. The pure samples differed in their melting point and proton n.m.r spectrum. Thus for (19a), the anomeric proton showed as a doublet (δ=6.15, $J_{1',2'}$=7 Hz), with the splitting observed in other 3'-halogenated-3'-deoxy-ara-nucleosides.[218] The anomeric proton for (19b), (δ=5.96),however showed as a singlet. Inspection of molecular models shows that no conformation of the sugar ring in (19a) will give a 90° dihedral angle between the C1'-H and the C2'-H bonds (i.e. coupling should be observed). However, in (19b), the conformation in which the bulky 2'-bromine substituent and the pyrimidine ring are trans to each other (i.e. maximum relief of steric interaction) gives dihedral angles between C1'-H and C2'-H and between C2'-H and C3'-H close to 90°. In fact both the H1' (δ=5.96) and H2' (δ=4.18) signals are broad singlets. Similar low values for the $J_{1',2'}$ and $J_{2',3'}$ coupling constants have been observed in other xylofuranosyl nucleosides.[144,208,219]

The course of the above reaction is not in agreement with previously reported openings of nucleoside lyxo-epoxides using the bromide anion. However, the epoxides used previously were either unprotected at the 5'-position or bore a 5'-O-acetyl protecting group. In these cases reaction

SCHEME 2.5 Nucleophilic Opening of Uridine 2',3'-Lyxo-epoxides.



(17) R= H

(18) R= Me

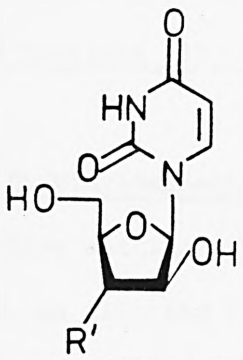
(19a)-(22a) (19b)-(22b)

(19) R'= Br

(20) R'= $CH{=}CH_2$

(21) R'= $C{\equiv}CH$

(22) R'= CH_3



(23) R'= $CH{=}CH_2$

(24) R'= $C{\equiv}CH$

(25) R'= CH_3

Starting Material	Reaction Conditions	Products (Yield)
17	NH_4Br/EtOH/Reflux	19a & 19b (low)
17	$CH_2{=}CHMgBr$/CuI/THF/-30°C	20a (11%) & 19a (30%)
18	"	No Reaction
17	$HC{\equiv}CLi$/DMSO/RT	21a(68%) & 21b(5%)
17	$LiCuMe_2$/THF/0°C	22a(63%) & 22b(7%) and 55b(5%)
20a	HCl/MeOH/RT	23 (57%)
21a	"	24 (90%)
22a	"	25 (90%)

was also much faster when using ammonium bromide (over in ca. 18 hrs).[119] These observations imply that the bulky 5'-O-trityl group in (17) is hindering approach of the large bromide nucleophile towards the C3' atom, the more reactive end of the epoxide. This has the effect of lowering the reactivity at C3' to such a degree that attack at C2', which is sometimes observed to a very limited extent, is now a favourable reaction course.

The results from this reaction gave us some valuable information. Firstly, the 5'-O-trityl protecting group may depress reactivity at C3' when large nucleophiles are used. More importantly, it allowed us to establish that proton n.m.r. spectroscopy could be used in later reactions to determine which end of the epoxide had been attacked.

2.2.4) Reactions of Epoxides (17) and (18) with Carbon Nucleophiles.[170]

Reactions with Vinylmagnesium Bromide.

Before setting out to try various organometallic reagents on epoxides such as (17) and (18) we wanted to see what effect the exchangeable proton on N^3 would have on the course of reaction. Treating (17) with an organometallic reagent would mean that the first equivalent of the reagent would be consumed in abstracting the N^3-proton, giving a negatively charged pyrimidine base, so that at least two equivalents of the reagent would be needed to open the epoxide. This was thought to be advantageous in that it would be expected to deactivate the pyrimidine ring to possible Michael addition at C-6, a reaction which has been shown to occur with lithium 1,3-dithian-2-yl in some pyrimidine nucleosides.[220,221]

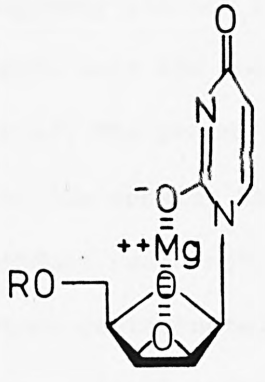
Compound (17) was treated with 2.5 equivalents of vinylmagnesium bromide and a catalytic amount of CuI in THF at -30° to $0^{\circ}C$.[252,253] Aqueous work-up led to the isolation of three compounds separable by chromatography and shown to be the bromohydrin (19a), (ca. 30%), starting material (17), (ca. 25%) and the required 3'-vinyl-3'-deoxy-ara-uridine derivative (20a),

(ca. 11%). Competing halohydrin formation has been observed in other CuI catalysed openings of carbohydrate epoxides by Grignard reagents.[254] This reaction, however, established the feasibility of a C-C bond-forming reaction at C3' of a nucleoside albeit in low yield. The position of attack at this stage was indicated by the proton n.m.r. spectrum of compound (20a) which showed the usual pattern of coupling constants associated with 3'-substituted-3'-deoxy-ara-nucleosides.[218] This was confirmed by double resonance studies (see Appendix I).

Interestingly, when compound (18) was treated with excess vinylmagnesium bromide and CuI in THF under several conditions, no opening of the epoxide ring was observed at all (not even by the halide anion). In neither of the reactions of (17) or (18) with vinylmagnesium bromide was attack at C6 observed.

A possible explanation for these observations is that the epoxide opening needs to be activated by coordination of a metal cation (Mg^{2+} in this case) to the oxirane oxygen. In (17), initial abstraction of the N-3 proton, could give rise to magnesium coordinated to the negatively charged pyrimidine ring and subsequently a cyclic coordinated intermediate such as (I), (Diag. 2.1) could arise.

DIAGRAM 2.1 Possible Intermediate in the Epoxide-opening Reaction.



(I)

Possibly, the presence of the bulky and hydrophobic 5'-O-trityl protecting group inhibits coordination by the magnesium cation to the oxirane ring unless the extra stabilisation of a cyclic intermediate such as (I) is possible. In (18), no exchangeable proton is present so that a negatively charged pyrimidine base cannot occur and any complex between the base and a magnesium cation might be weaker than in the case of (17). This factor, together with the extra steric interaction between the N^3-methyl and the 5'-O-trityl groups, might not allow a cyclic intermediate similar to (I) to occur for (18).

Reaction of Epoxide (17) with the Acetylide Anion.

The above studies established that C-C bond-forming reactions at C3' of nucleosides are feasible. The main problem encountered was competing attack at C3' by the halide counterion of the Grignard reagent. Also it was shown that the epoxide (17) reacted more readily than (18) and in the subsequent studies the former was used in consequence.

To overcome the competing reaction at C3' by any counterion which may be present in Grignard reagents, we decided to explore the use of organolithium reagents. Thus compound (17) was treated with excess of the lithium acetylide / ethylenediamine complex in DMSO at 20°C for 18 hrs.[222] Aqueous work-up gave a pale yellow foam which consisted of one major product (ca. 68% yield) together with a small amount of a minor product (ca. 5%). Column chromatography allowed isolation of the major product in a pure state in ca. 63% yield. This was shown to be the 3'-ethynyl-3'-deoxy-ara-uridine derivative (21a). The proton n.m.r. spectrum of (21a) was consistent with opening of the epoxide having occurred by attack at the C3'-atom.[218] The minor product could not be obtained pure, but the proton n.m.r. spectrum of a mixture contaminated with (21a) indicated that it could be compound (21b) as a signal present in the region expected for the anomeric proton (δ=6.02) was present and this had a very small coupling

constant ($J_{1',2'}$<1Hz at 250 MHz). Also, signals for the pyrimidine base protons as well as those of most of the sugar ring protons could be seen. Unfortunately, the region where the acetylenic proton would be expected to occur was obscured by the signals attributed to the H-5' of both (21a) and (21b). However, the presence of the usual two doublets for the pyrimidine H-5 and H-6 protons indicated that attack had not occurred on the base and the minor component had resulted from reaction on the sugar ring.

Thus an efficient and convenient route to 3'-alkynyl-3'-deoxy-ara-uridines has been established. During this research work several preparations using 5g quantities of (17) were carried out. Having demonstrated the introduction of an alkynyl and an alkenyl group at C3' of a nucleoside our next aim was to introduce a saturated alkyl chain at the same position.

Reaction of Epoxide (17) with Lithium Dialkylcuprates.

In the above studies a Grignard and an organolithium reagent were used to introduce an ethenyl and an ethynyl substituent respectively into uridine systems. In our aim to introduce a 3'-alkyl substituent we decided to look at a third class of organometallic reagents, namely the lithium dialkylcuprates. This group of reagents is known to react with epoxides under mild conditions and in high yields[223,224] and would allow a wide range of carbon nucleophiles to attack (17), (Scheme 2.5).

Thus, when compound (17) was treated with 3.5 equivalents of lithium dimethylcuprate in THF at -20° to 0°C for 18 hrs, followed by aqueous work-up, a glassy solid was obtained which consisted of one major product (22a), (ca. 63% yield) together with two minor products. These were the regio-isomer (22b), (ca. 7% yield) and the 2'-keto derivative (55b), (ca. 5% yield). It proved possible to separate the three compounds by column chromatography although only (22a) could be obtained crystalline. Again, proton n.m.r. spectroscopy was used to distinguish between the two

regio-isomers (22a) and (22b) and to establish the site of attack by the organometallic reagent.[218] The structure of compound (55b) was indicated by its proton n.m.r spectrum showing the anomeric proton as a singlet at δ=5.42, the upfield shift being presumably a consequence of shielding by the anisotropic effect of the 2'-keto group. A two proton, 8 line multiplet centred at δ=2.72 attributable to the C3' protons was also present. The same compound but bearing a 5'-O-benzoyl protecting group has been reported previously and its proton n.m.r. spectrum shows sugar proton resonances similar to those of (55b).[164] The ^{13}C n.m.r. spectrum of (55b) showed a signal at δ=206.7 for the 2'-keto group. Furthermore, the i.r. spectrum showed an absorption at 1770 cm^{-1} consistent with that expected for a ketone in a five membered ring.

The compounds (20a), (21a) and (22a) were all deprotected using methanolic HCl to give the free, crystalline nucleosides (23), (24) and (25) respectively.

Thus in this section of the work we have established routes to introduce directly, alkyl, alkenyl and alkynyl side-chains at C3' of nucleosides using three classes of organometallic reagents. The routes are short, convenient and two of them efficient, so that starting from uridine, 3'-methyl-3'-deoxy-ara-uridine (25) and 3'-ethynyl-3'-deoxy-ara-uridine (24) were obtained in 30% and 36% overall yield, in only six steps.

In the literature, the preparation of some related compounds, namely some 3'-alkyl-3'-deoxy-uridines[171] and 3'-methyl-3'-deoxy-adenosine[175] has previously been performed starting with a keto sugar in overall yields in the region of 10% or less and involving more steps (usually about 10).

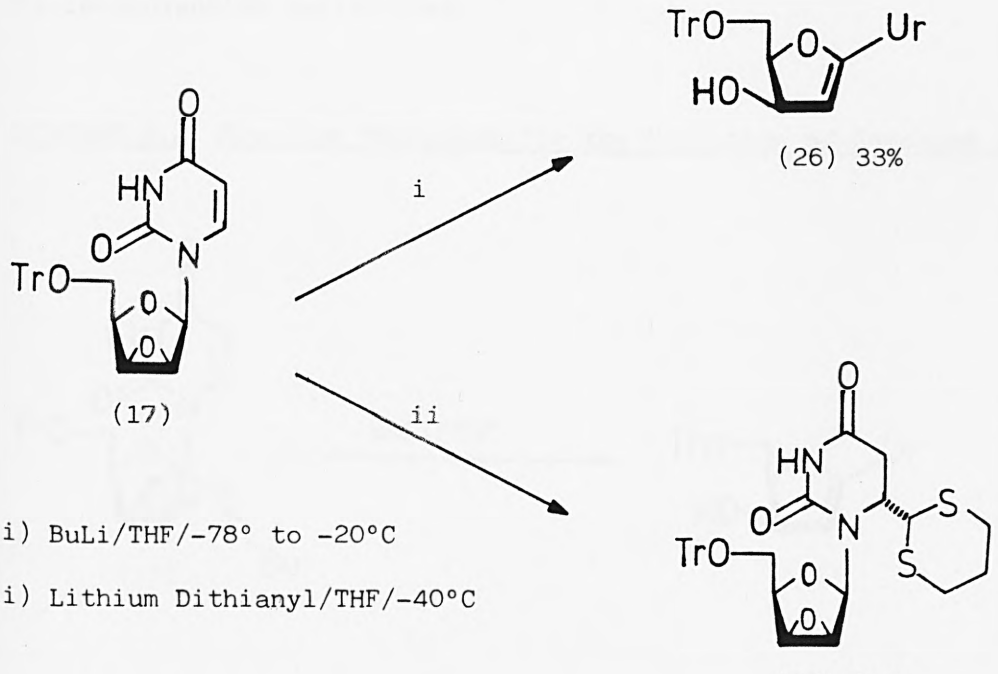
2.2.5) Reactions of Epoxide (17) not Leading to Attack at C3'.

Reaction with Butyllithium.

The reaction of alkyllithium reagents with (17) in the absence of CuI was explored for several reasons. Firstly, reagents such as butyllithium are extremely basic (pK_a = 40), much more so than the lithium acetylide (pK_a=22) and the lithium dialkylcuprates already used in this work. Thus, abstraction of either the 1'- or the 4'-proton might compete with epoxide opening. We also wished to establish whether the nucleoside molecule could withstand such basic conditions.

A solution of the epoxide (17) in THF at -78°C was treated with 2.5 equivalents of butyllithium and allowed to warm slowly to -20°C. Aqueous work-up gave a white foam consisting of two components separable by column chromatography. These were shown to be recovered starting material and the 1',2'-unsaturated nucleoside (26), (Scheme 2.6).

SCHEME 2.6 Reactions of Epoxide (17) not Leading to Attack at C3'.

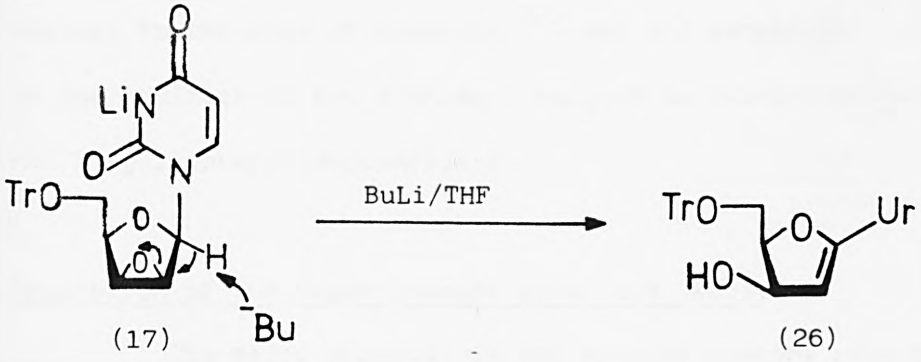


i) BuLi/THF/-78° to -20°C

ii) Lithium Dithianyl/THF/-40°C

The proton n.m.r. spectrum of (26) showed no signal in the region δ=5.8-6.2 (the usual range for anomeric protons), whereas a signal at δ=4.5 attributable to the 4'-proton was observed. A vinylic proton (δ=5.35, $J_{2',3'}$=3Hz) attributable to H-2' was present and was coupled to a signal at δ=4.74, (dd, $J_{3',4'}$=6Hz, $J_{2',3'}$=3Hz, H-3'). Also two exchangeable protons were present (i.e. for $\underline{N}^3$-H and 3'-OH), whereas the starting material (17) had only one. No attempt was made to establish the configuration at C3'. However, the proton n.m.r. spectrum of the unprotected nucleoside having the opposite configuration at C3', compared to (26), has been reported.[71] The sugar proton resonances for this compound have very similar chemical shifts to those of compound (26). However, $J_{2',3'}$=2.5Hz (cf. 3Hz for (26)) and $J_{3',4'}$=3Hz (cf. 6Hz for (26)), indicate a different angular relationship between H-2', H-3' and H-4' in this compound compared to (26). Thus,if the configuration at C3' of compound (26) is as shown (Scheme 2.6), it probably arises by initial abstraction of the anomeric proton by butyllithium. The epoxide ring then opens by an intramolecular elimination of the C2'-O bond (Diag. 2.2). These two steps probably occur in a concerted fashion and are akin to a β-elimination which has been used to generate various unsaturated sugar nucleosides from a number of cyclo-nucleoside derivatives.[71]

DIAGRAM 2.2 Possible Mechanism for the Formation of Compound (26).



(17) (26)

Reaction with Lithium 1,3-Dithian-2-yl.

The reaction of lithium 1,3-dithian-2-yl[225,226] with (17) was of interest for several reasons. From a synthetic point of view, introducing a dithianyl group at C3' followed by its oxidative hydrolysis and reduction would lead to a 3'-hydroxymethyl substituted nucleoside. Attack of this reagent on sugars bearing an epoxide function has been reported[227] so that the extension to nucleoside chemistry might be feasible. Secondly, Rosenthal[220,221] has shown that the reaction of this dithianyl reagent with 5'-O-trityl-3'-O-acetyl-O^2,2'-cyclo-uridine and 5-bromo-5'-O-trityl-2',3'-O-isopropylidene-uridine results in a Michael addition at C6 of the pyrimidine ring. Thus in compound (17) we have the possibility of attack at C3' or C6 as well as possible abstraction of a sugar proton.

The epoxide (17) was added to 2.5 equivalents of lithium 1,3-dithian-2-yl in THF at -30°C and after 2 hrs at 0°C aqueous work-up followed by column chromatography led to the isolation of some starting material and a product containing a dithianyl group. The spectroscopic data for this new compound were consistent with attack having occurred at C6 to give (27). Thus the u.v. spectrum showed no strong absorption at ca. 260 nm where the uracil base absorbs. The proton n.m.r. spectrum showed the sugar ring resonances for (27) to be almost identical to those of (17) and furthermore, the pair of doublets typical of the 5- and 6-protons of the uracil base were absent. Although no attempt was made to determine the absolute configuration at C6, it was assigned the 6-(R) configuration by analogy to the work of Rosenthal[220] who did establish the stereochemistry of the addition of the dithianyl reagent to 5-bromo-5'-O-trityl--2',3'-O-isopropylidene-uridine.

2.2.6) Separation of the Regio-isomers (28a) and (28b).

The major products in the epoxide opening reactions described above arise by attack at C3' to give 3'-substituted-3'-deoxy-ara-

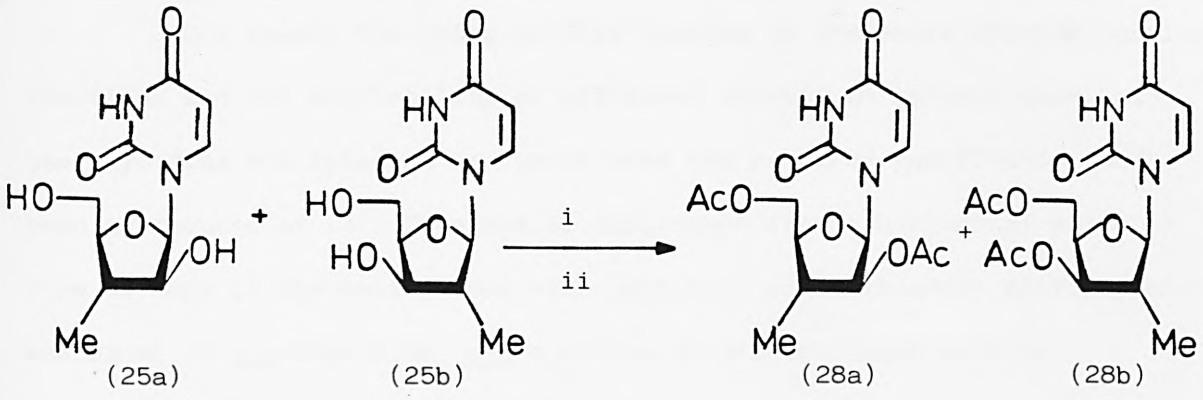
nucleosides. However, in most cases a minor product could be detected by t.l.c. and proton n.m.r. spectroscopy which might be the product arising by attack at C2', resulting in a 2'-substituted-2'-deoxy-xylo-nucleoside. In the case of the reaction of (17) with ammonium bromide it proved possible to separate the two products of reaction as the 5'-O-trityl derivatives (i.e. 19a and 19b). However, for the two pairs of compounds (21a) and (21b) and (22a) and (22b) this did not prove possible. In the reaction of vinylmagnesium bromide with (17) none of the product arising from attack at C2' by the vinyl anion was detected. However, since epoxide opening at C3' by the vinyl anion occurred in only 11% yield, any product resulting by attack at C2' would be produced in ca. 0.5% yield, making it very difficult to detect.

T.l.c. and proton n.m.r. spectroscopy indicated that the reaction of lithium dimethylcuprate and (17) gave the best yields of the product arising by attack at C2'; a 7:1 mixture of compounds (22a) and (22b) respectively being obtained. This seemed the best system to try and obtain the minor product in a pure state.

Deprotection of the 7:1 mixture of compounds (22a) and (22b) followed by acetylation to the diacetates (28a) and (28b) was performed by conventional methods (Scheme 2.7).

Fractional crystallisation allowed the isolation of pure (28a) and (28b). These compounds exhibited different melting points and spectral data. Of greatest value were the proton n.m.r. spectra of the two compounds which showed coupling constants for the sugar ring protons analogous to those of compounds (19a) and (19b). Thus the 3'-methyl derivative showed a doublet for the anomeric proton at δ=6.24 ($J_{1',2'}$=6Hz) and the expected coupling constants for the rest of the sugar protons.[218] The compound (28b) however, showed a singlet at δ=5.80 attributable to the anomeric proton and small coupling constants throughout the ring protons.[219] Furthermore, the observed chemical shifts for the anomeric protons in (28a) and (28b)

SCHEME 2.7 Preparation and Separation of the Isomers (28a) and (28b).



i) Ac_2O/Pyridine/RT

ii) Fractional Crystallisation

indicated the C1'-proton in (28a) to be in a more deshielded environment than the corresponding proton in (28b). This is a result of (28a) having an electron-withdrawing acetoxy group at C2' whereas (28b) has an electron-releasing methyl group at C2'. Possibly the carbonyl function of the acetoxy group at C2' may also be deshielding the C-1' proton in (28a).

2.2.7) 3'-Branched Thymidine and Cytidine Analogues.

Introduction.

One reason for using uridine species in the above epoxide opening reactions was the availability of efficient methods to convert them into the thymidine and cytidine analogues once the required modification had been introduced at C3'. This was of importance from a biological point of view as many of the nucleosides with antiviral and anticancer activity are analogues of ara-thymidine, ara-cytidine or 5-substituted uridine derivatives (See Tables 1.1 & 1.2).

3'-Branched-3'-deoxy-ara-thymidine Derivatives.

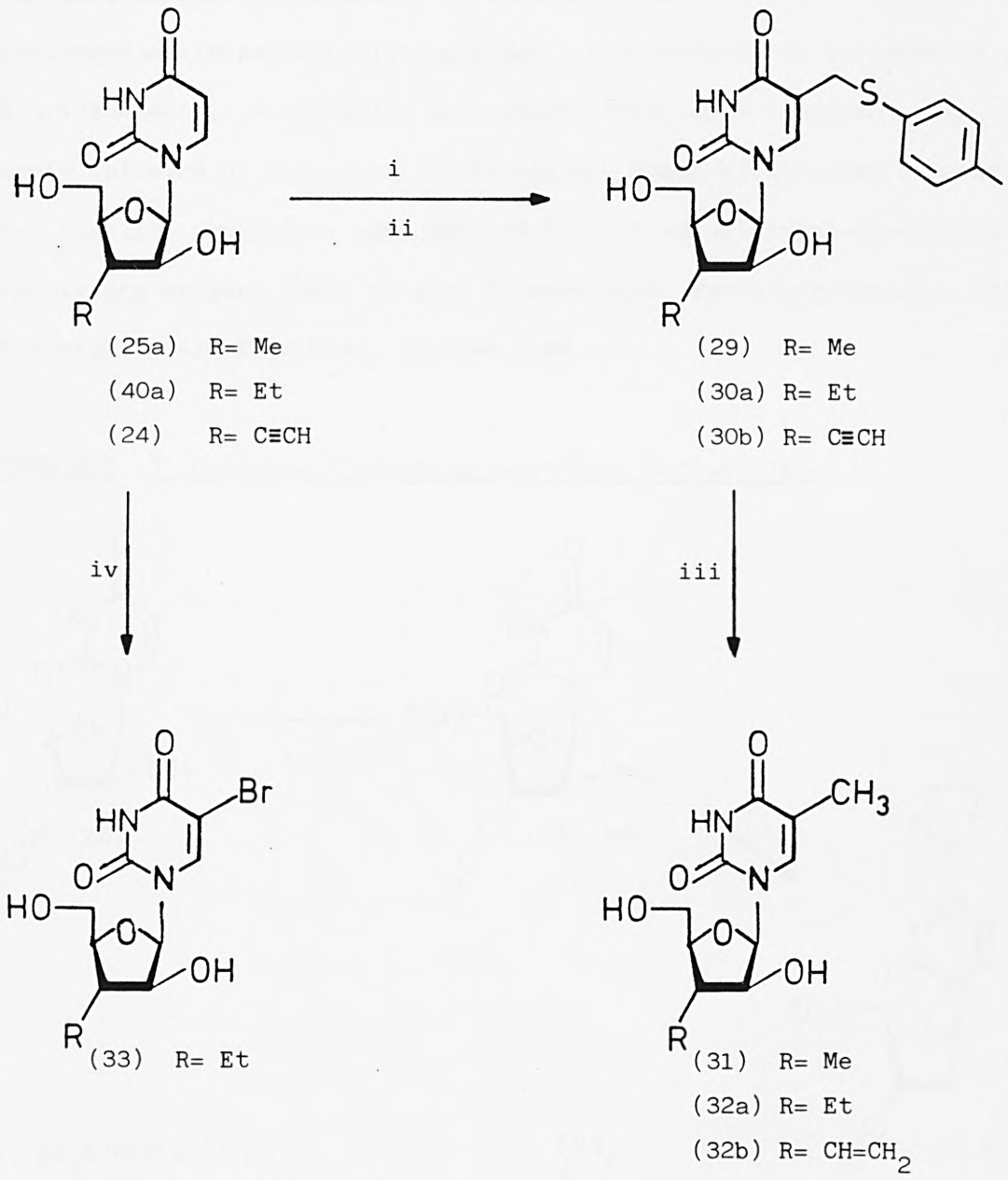
A mild and efficient method for the conversion of uridines to thymidines has been recently described by Reese et al.[215] The method involves a Mannich reaction between a uridine species, formaldehyde and pyrrolidine to introduce a methyl pyrrolidine substituent at C5 of the uridine molecule. This is then attacked by p-thiocresol to give a methylthiotolyl group at C5 which on treatment with Raney nickel is desulphurised to the 5-methyl (i.e thymidine) derivative (Scheme 2.8).

These conditions were applied to 3'-ethynyl-3'-deoxy-ara-uridine (24). However the final desulphurisation was carried out with a large excess of Raney nickel and this also hydrogenated the 3'-ethynyl group to give a mixture of two products. This consisted mostly of the 3'-ethyl-3'-deoxy-ara-thymidine (32a) with some of the 3'-vinyl analogue (32b). Hydrogenation of this mixture over 10% Pd/C gave pure compound (32a). The same conditions when applied to 3'-ethyl-3'-deoxy-ara-uridine (40a), also gave compound (32a) in 42% overall yield from (40a). Similarly, 3'-methyl-3'-deoxy-ara-uridine (25a) was converted to the ara-thymidine analogue (31) in 55% overall yield.

Finally, 3'-ethyl-3'-deoxy-ara-uridine (40a) was brominated with

NBS in acetic acid[228] to give 5-bromo-3'-ethyl-3'-deoxy-ara-uridine (33) in ca. 31% yield (Scheme 2.8).

SCHEME 2.8 3'-Branched-3'-deoxy-ara-thymidine Derivatives.

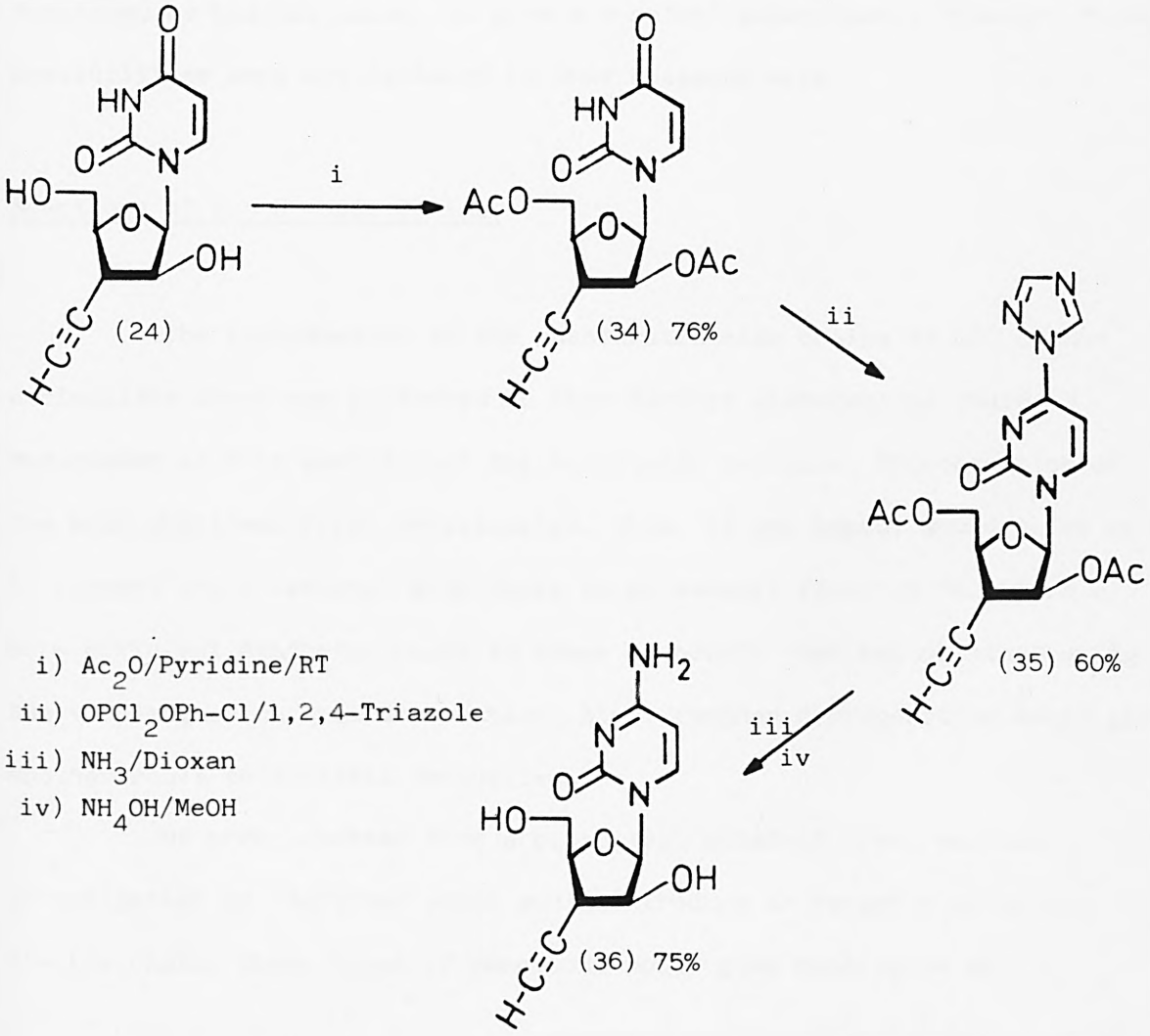


i) Pyrrolidine/CH_2O/H_2O
ii) p-Thiocresol/CH_3CN
iii) Raney Ni/EtOH
iv) NBS/Acetic Acid/135°C

3'-Branched-3'-deoxy-ara-cytidine Derivatives.

Various methods are available for converting uridine to cytidine derivatives. A mild and efficient method has been described recently[216] and has been applied to some 3'-azido-uridine systems.[229] The reaction conditions involve treatment of an acetylated uridine with (4-chlorophenyl)phosphodichloridate and 1,2,4-triazole in pyridine to give the uridine-4-(1,2,4-triazole) derivative. This, upon treatment with ammonia followed by deprotection affords the required cytidine derivative. These reaction conditions were applied to 2',5'-di-O-acetyl-3'-ethynyl-3'-deoxy-ara-uridine (34), to give 3'-ethynyl-3'-deoxy-ara-cytidine (36) in 45% overall yield from (24), (Scheme 2.9).

SCHEME 2.9 3'-Branched-3'-deoxy-ara-cytidine Derivatives.



Thus ara-thymidine and ara-cytidine derivatives with a branch at C3' are now accessible from the 3'-branched uridine species already described. In the ara-uridine and ara-cytidine series containing an ethynyl group at C3' further elaboration of the side chain is possible to give other 3'-branched nucleoside analogues. Some initial modifications were attempted in this research work and are described below. Unfortunately, in the conversion of (24) to the thymidine analogue, hydrogenation of the 3'-ethynyl group also occurred in the final desulphurisation reaction so that no thymidine analogues with an unsaturated side chain at C3' were obtained pure in this research work. However, suitable modifications to the conditions of the final desulphurisation reaction (i.e. use of less Raney nickel) may well afford thymidine analogues with unsaturated side chain at C3'. Another possibility involves introducing a hydroxymethyl substituent at C5,[252] and converting this to the -O-mesyl derivative which is then displaced by hydride anion, to give a 5-methyl substituent. However, these possibilities were not explored in this research work.

2.2.8) Reactions of the 3'-Side Chains.

The introduction of the unsaturated side chains at C3' of the nucleosides above was performed so that further elaborations could be undertaken at this position of the nucleoside molecule. Hydrogenation of the side chain was first investigated. This, it was hoped, would allow us to convert the 3'-ethynyl side chain to an ethenyl function to afford a more efficient synthetic route to these compounds than was obtained using the vinylmagnesium bromide reaction. Also, further hydrogenation would give another route to 3'-alkyl derivatives.

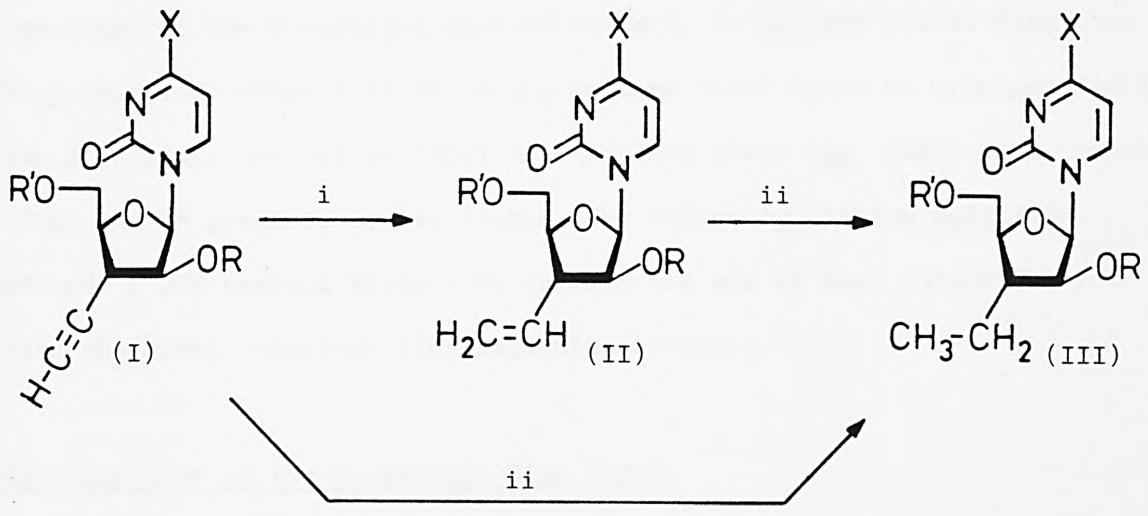
Of more interest from a biological point of view, was the investigation of reactions which would introduce an oxygen atom in the 3'-side chain. These types of reactions would give homologues of

ara-nucleosides, a class of compounds with potent anti-viral and anti-tumour activity.

Hydrogenation.

Hydrogenation of the unsaturated side chains in a number of the 3'-branched derivatives described above was performed. Scheme 2.10 shows the derivatives subjected to hydrogenation.

SCHEME 2.10 Hydrogenation of the 3'-Unsaturated Side Chains.



i) Lindlar Catalyst/H_2

ii) 10% Pd/C/H_2

			COMPOUND		
R'	R	X	(I)	(II)	(III)
H	H	NH_2	36	37	38
H	H	OH	24	23	39
Ac	Ac	OH	34	40	
Tr	H	OH	21a	20a	

Thus in the unprotected series, both 3'-ethynyl-3'-deoxy-ara-uridine (24) and 3'-ethynyl-3'-deoxy-ara-cytidine (36) could be

hydrogenated in excellent yields to their 3'-ethenyl derivatives (23, 95%) and (37, 93%) respectively using the Lindlar catalyst. The corresponding 3'-ethyl derivatives, (39) and (38), could also be obtained in excellent yields by the hydrogenation of either the 3'-ethynyl compounds (24) and (36), or the 3'-ethenyl derivatives (23) and (37) over a 10% Pd/C catalyst. The preparation of compound (23) was thus achieved by two routes; the one using lithium acetylide being the more efficient, giving (23) in ca. 34% overall yield from uridine (1).

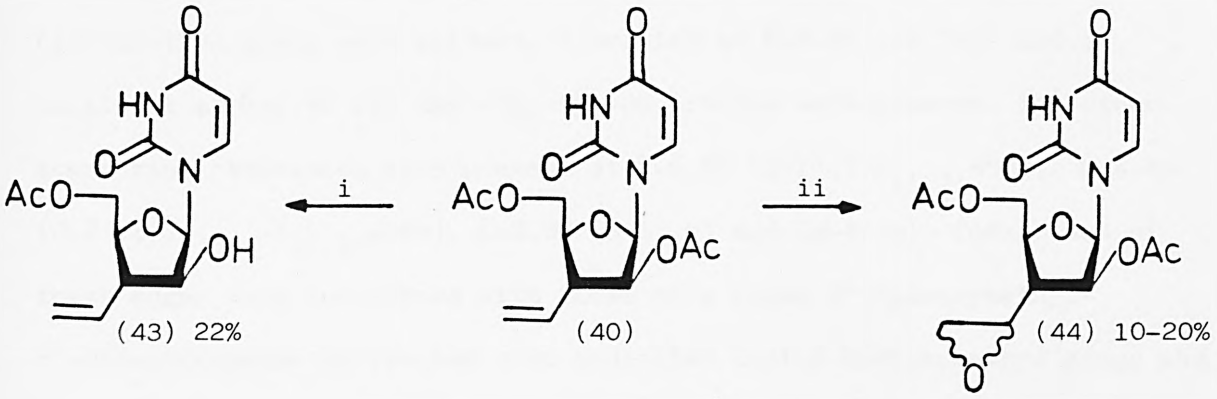
The applicability of the hydrogenation reaction using the Lindlar catalyst on some protected 3'-branched nucleosides was also investigated. Thus 2',5'-di-O-acetyl-3'-ethynyl-3'-deoxy-ara-uridine (34), could be converted to the 3'-ethenyl derivative (40), in ca. 95% yield. Also, the 5'-O-trityl-3'-ethynyl-3'-deoxy-ara-uridine (21a) could be hydrogenated to the 3'-ethenyl derivative (20a) in excellent yield (ca. 95%). Thus compound (20a) can be prepared by two routes. The one using lithium acetylide affords a 37% overall yield from uridine (1) and is much more efficient than the route involving vinylmagnesium bromide.

Hydroboration of the 3'-Ethenyl Side Chain.

If hydroboration of the 3'-ethenyl group in compounds (40) or (45) could be achieved then 3'-hydroxyethyl derivatives would be obtained (Schemes 2.11 & 2.12).

Thus treatment of 2',5'-di-O-acetyl-3'-ethenyl-3'-deoxy-ara-uridine (40) with 9-BBN in THF followed by aqueous alkaline oxidation[230,231] led to the isolation of compound (43) together with some recovered starting material. That no attack on the 3'-ethenyl group had occurred was shown by the proton n.m.r. spectrum of (43) which had vinyl resonances almost identical to those of compound (40). The use of diborane was then investigated. When compound (40) was treated with 1/3 molar equivalent of the BH_3/THF complex, followed by the usual work up, starting

SCHEME 2.11 Attempts at Epoxidation and Hydroboration of the 3'-Vinyl Side-chain of some Diacetylated Nucleosides.



iii

No Reaction

iv

No Reaction /
or Loss of Acetyl Groups

i) 9-BBN/THF
ii) MCPBA/$ClCH_2CH_2Cl$/Radical Inhibitor/Reflux
iii) MCPBA/CH_2Cl_2/Reflux
iv) BH_3/THF

material only could be isolated from the reaction mixture. On using excess diborane, very little material could be isolated by extracting the reaction mixture with ether. This would seem to indicate that the acetate protecting groups are not stable to the hydroboration or work-up conditions.

To overcome this problem, compound (45), made by acetylating (20a), was used in some hydroboration studies (Scheme 2.12). Treatment of (45) with 1/2 molar equivalent of the BH_3/THF complex led to the isolation of (20a). Thus, loss of the acetate group was occurring more readily than attack on the 3'-ethenyl group. On treating (45) with excess diborane, the

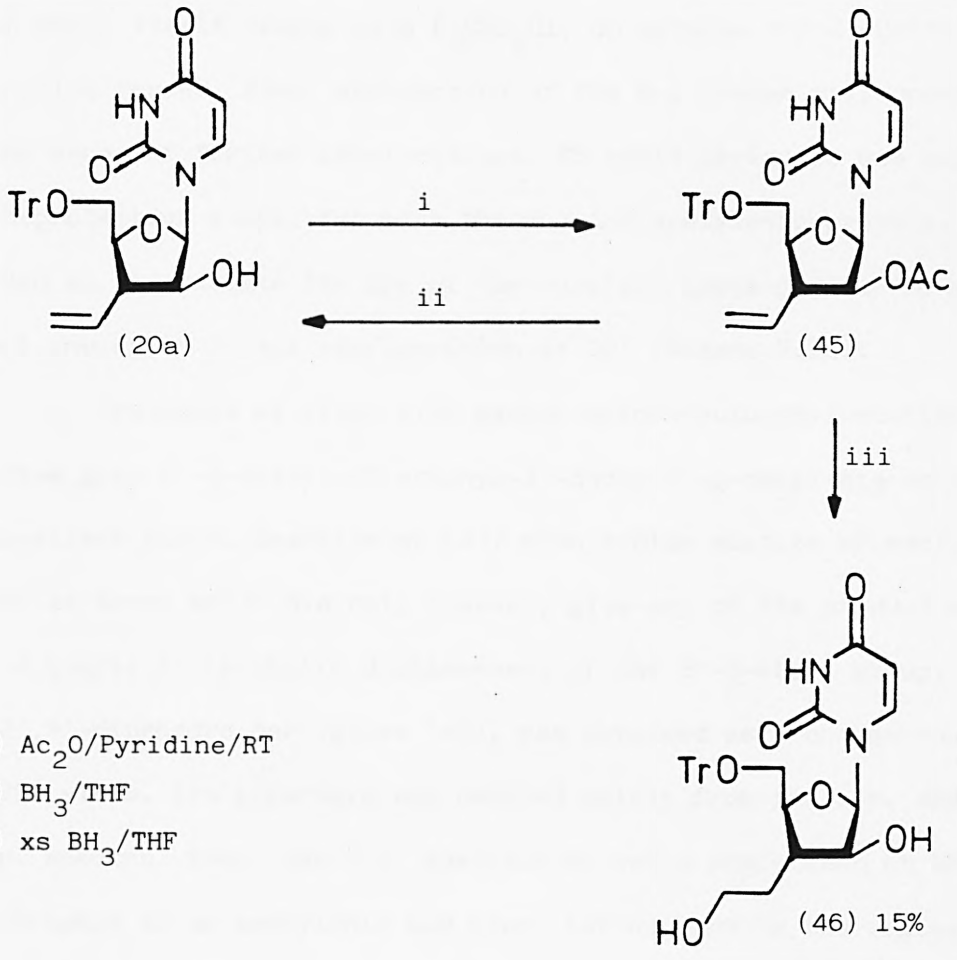
usual work-up led to the isolation of a compound with similar R_f on t.l.c. to 5'-O-trityl-uridine (15). This compound was obtained in low yield (ca. 15%) and could not be crystallised. However, its proton n.m.r. spectrum did not show any vinyl resonances and new signals consistent with a hydroxyethyl group were evident. A triplet at δ=3.52 (J= 7Hz) and a multiplet at δ=1.60 for the $-CH_2-CH_2-OH$ protons were present. The other sugar ring resonances were present at δ=5.88 (H-1',d,$J_{1',2'}$=5Hz), δ=4.44 (H-2',t,$J_{1',2'}=J_{2',3'}$=5Hz), δ=3.30 (H-5',m) and (H-4',m). Comparison of these sugar ring resonances with those of a known 3'-hydroxymethyl-3'-deoxyadenosine derivative also indicated that a hydroxymethyl group was present in the product.[176] However, further work to optimise the yield and to characterize the product fully needs to be done.

Epoxidation of the 3'-Ethenyl Side Chain.

The diacetate (40) was treated with MCPBA in dichloromethane under reflux for 4 hrs (Scheme 2.11). However, no epoxidation occurred and starting material was recovered. The reluctance of terminal olefins to undergo epoxidation has been reported in the literature and has been overcome by the use of MCPBA under forcing conditions in the presence of a radical inhibitor.[232] Therefore, compound (40) was treated with MCPBA and 3-t-butyl-4-hydroxy-5-methyl-phenylsulphide in 1,2-dichloroethane under reflux for 3 hrs. Addition of ether caused a white solid to crystallise out. The proton n.m.r. spectrum of this solid, although different to that of starting material, was very complex. This might be expected if epoxidation had occured without stereospecificity to give a mixture of diastereoisomers. Or, possibly incomplete epoxidation had occurred and the product had co-crystallised with starting material. The mass spectrum of the product did show a molecular ion 16 units higher than that expected for the starting material, indicating that possibly an oxygen atom had been added and epoxidation had occurred. However, the yield of this reaction was

low (10-20%) and insufficient of the product was obtained to allow full characterisation, so that further work is required to establish conclusively that epoxidation has occurred.

SCHEME 2.12 Hydroboration of the 3'-Vinyl Sidechain.



i) Ac_2O/Pyridine/RT
ii) BH_3/THF
iii) xs BH_3/THF

2.2.9) Some Attempts at Inverting the Configuration at C2'.

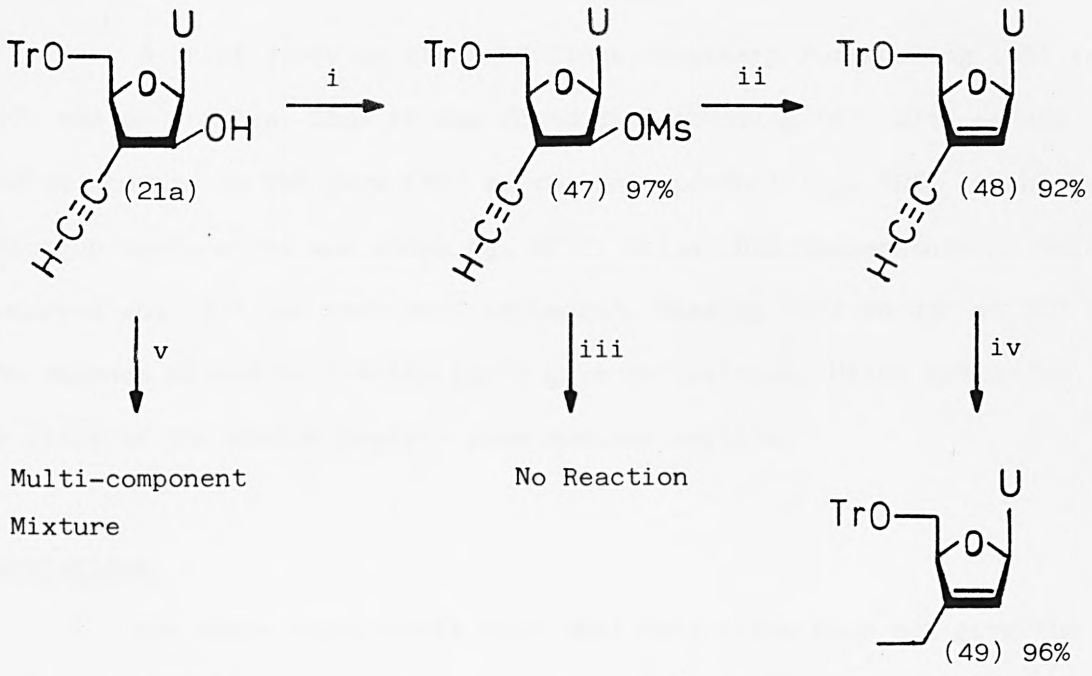
The inversion of the configuration at carbon in some sugars and nucleosides has been achieved by converting a hydroxyl group to a sulphonate ester and then displacing it with an oxygen nucleophile such as acetate[233] or benzoate.[234] Some of the mildest conditions have been described by Ranganathan[86,92] who converted an ara-adenosine derivative to the 2'-O-triflate ester. The 2'-O-triflate leaving group was then displaced

by the action of sodium acetate in HMPA at ambient temperature.

However, when compound (21a) was treated with sodium hydride followed by trifluoromethanesulphonyl chloride in THF,[86,92] a complex mixture of products was obtained, from which none of the required material could be isolated. Possibly the presence of the acetylenic proton complicates matters in being abstracted by the sodium hydride to give an anion which itself reacts with F_3CSO_2Cl, or attacks any 2'-O-triflate derivative formed. Also, abstraction of the N-3 proton will occur and may be the cause of further complications. To avoid having to use protection and deprotection steps, for both the N-3 and acetylenic protons, it was decided to investigate the use of the mesylate leaving group in order to effect inversion of the configuration at C2' (Scheme 2.13).

Treatment of (21a) with excess methanesulphonylchloride in pyridine gave 5'-O-trityl-3'-ethynyl-3'-deoxy-2'-O-mesyl-ara-uridine (47) in excellent yield. Reaction of (47) with sodium acetate or sodium benzoate in DMF at above 90° C did not, however, give any of the product arising from a simple nucleophilic displacement of the 2'-O-mesyl group. Instead, the 2',3'-didehydro derivative (48), was obtained as a homogeneous foam in ca. 90% yield. Its structure was deduced mainly from its i.r. and proton n.m.r. spectra. Thus, the i.r. spectrum showed a sharp band at 3300 cm^{-1} attributable to an acetylenic C-H bond, but no band in the region expected for an acetate carbonyl group. The proton n.m.r. spectum of (48) showed no three proton signal attributable to a mesylate or acetate group (i.e. the 2'-O-mesylate although lost had not been replaced by the acetate). Also the resonance attributable to the acetylenic C-H (δ=3.35) was not a doublet as in the spectra of compounds (21a) and (47), but a sharp singlet (i.e. the 3'-proton had been lost). Furthermore, the signals due to the sugar ring protons of (48), had similar splitting patterns to those of other 2',3'-unsaturated derivatives prepared in this work (i.e. 6a, 6b and 54 and 55a) although, as would be expected, there were differences in chemical shifts.

SCHEME 2.13 Attempted Inversion of Configuration at C2'.



i) MsCl/Pyridine/0°C

ii) xs NaOAc/DMF/ $\geqslant$ 90°C or Sodium Benzoate/DMF/Δ

iii) DMF/150°C or xs NaOAc/DMF/ $<$ 90°C

iv) 10% Pd/C /H_2

v) NaH/CF_3SO_2Cl/THF

Compound (48) was partially hydrogenated over a 10% Pd/C catalyst. It was found that rapid hydrogen absorption occurred till ca. 2 molar equivalents of the gas had been taken up. Stopping the reaction at this stage led to the isolation of a white homogeneous foam which was assigned the structure (49). The i.r. spectrum of this compound had no acetylenic C-H absorption at 3,300 cm^{-1}. Its proton n.m.r. spectrum similarly had no signal for an acetylenic C-H, but a triplet (δ=1.16, J=7.5Hz) and a multiplet (δ=2.05) for the ethyl group were now present. Also, a signal in the vinylic region for the 2'-proton was still present

(δ=5.52). The signals assigned to the sugar ring protons of (49) had similar splittings to those of the other 2',3'-unsaturated nucleosides already mentioned (i.e. 6a, 6b and 54 and 55a) and differed only in chemical shift.

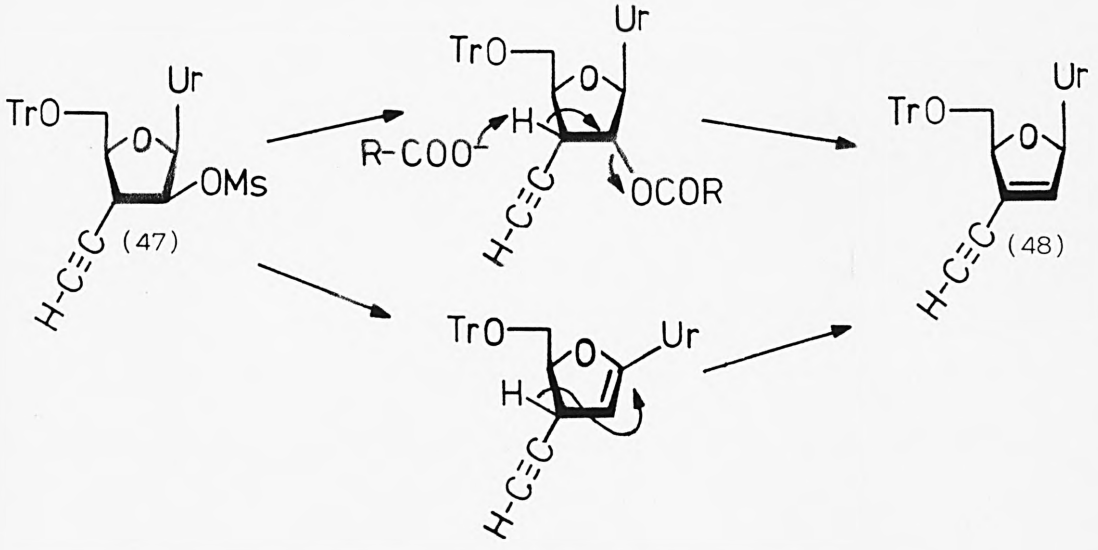
A brief study of the conditions necessary for forming (48) from (47) was undertaken. Thus it was found that treating (47) with excess sodium acetate in DMF gave (48) as the sole product (ca. 90%), when the reaction temperature was above ca. 90°C. Below this temperature no reaction occurred and (47) was recovered unchanged. Heating (47) in DMF at 150°C in the absence of sodium acetate again gave no reaction. Using sodium benzoate in place of the sodium acetate gave similar results.

Conclusions.

The above experiments show that heat alone does not give the 2',3'-unsaturated nucleoside (48) from (47). This means that a syn-elimination of the mesylate group is unlikely to give rise to compound (48), (Diag. 2.3).

The presence of acetate or benzoate therefore seems to be required in the formation of (48). These can either act as bases or as nucleophiles. If they act as bases, then abstraction of the anomeric proton

DIAGRAM 2.3 Possible Mechanisms for the Formation of Compound (48).



may be the first step to give a 1',2'-unsaturated nucleoside (as shown in Diag. 2.3) which can then undergo a 1,3-sigmatropic shift. The antarafacial nature of such a shift[235] would give the required product (48). However, although this mechanism is feasible, few such 1,3-sigmatropic shifts are known and this mechanism therefore seems unlikely.

A more likely route would involve initial nucleophilic displacement of the 2'-O-mesylate group by acetate (or benzoate) followed by rapid β-elimination of the newly introduced 2'-O-acetyl (or 2'-O-benzoyl) group (Diag. 2.3), the driving force for the final elimination, presumably being the formation of the conjugated eneyne system in (48).

2.2.10 Reactions of Some 2',3'-Unsaturated-2'-Chloro Nucleosides.

The 5'-O-trityl-2'-halo-2',3'-didehydro-2',3'-dideoxy-uridines (6a) and (6b), prepared in Section 2.1.2 have received little attention in the literature. In fact, the 5'-O-benzoyl derivative of (6a) has only been described once[169] and was obtained as a minor side product in the preparation of a 2',3'-dideoxy-2',3'-dichlorinated uridine. Similarly, the unprotected parent nucleoside of (6b) has only been reported once in the literature.[166] Consequently, the reactions of the 2',3'-unsaturated function have received limited attention. In fact the only reaction reported for such a system was hydrogenation of unprotected (6b). The authors reported that hydrogenation on a $Pd/BaSO_4$ catalyst led to only one product, identified as 2',3'-dideoxy-uridine (50), (Scheme 2.14) and concluded that hydrogenation of the 2',3'-double bond and hydrogenolysis of the 2'-bromo group occurred simultaneously.

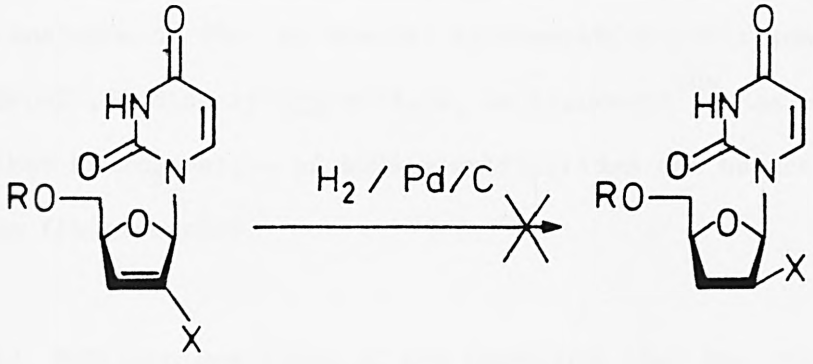
In Section 2.1.2, a convenient and efficient route to compounds (6a) and (6b) was established and we therefore decided to explore the reactions of the 2',3'-halovinyl function in these compounds. Two main groups of reactions were investigated. Firstly, addition reactions to the 2',3'-double bond were explored in the hope that novel substitution patterns at C2' and C3' could be obtained and these are described in this section. Secondly, vinyl halides are known to undergo C-C bond-forming reactions with various organometallic reagents[236] and it was hoped this would lead to 2'-branched nucleoside analogues. These reactions are described in Section 2.2.11.

Hydrogenation.

It has already been established that hydrogenation of deprotected (6b) using a $Pd/BaSO_4$ catalyst gives 2',3'-dideoxyuridine (50), with no intermediate being detected.[166] It is also known that hydrogenolysis of

nucleosides containing halogenated sugars, to yield deoxy derivatives, is more easily achieved using bromo- and iodo- rather than chloro-derivatives. It was therefore decided to hydrogenate the compounds (6a) and (8a) to see if the 2'-chloro function could be retained. These compounds might be expected to become hydrogenated from the less hindered ∝-side of the sugar ring, so that if the chlorine atom could be retained, 2'-chloro-2',3'-dideoxy-ara-uridine derivatives would be obtained (Diag. 2. 4). In Table 1.2 a number of pyrimidine 5-substituted-2'-halo-2'-deoxy-ara-nucleosides are shown. These compounds have high antiviral activity. The reaction anticipated in Diagram 2.4 would lead to 3'-deoxy analogues of such compounds and thus be of interest from a biological viewpoint.

DIAGRAM 2.4 Attempted Preparation of 2'-Chloro-2',3'-dideoxy-ara-uridine.

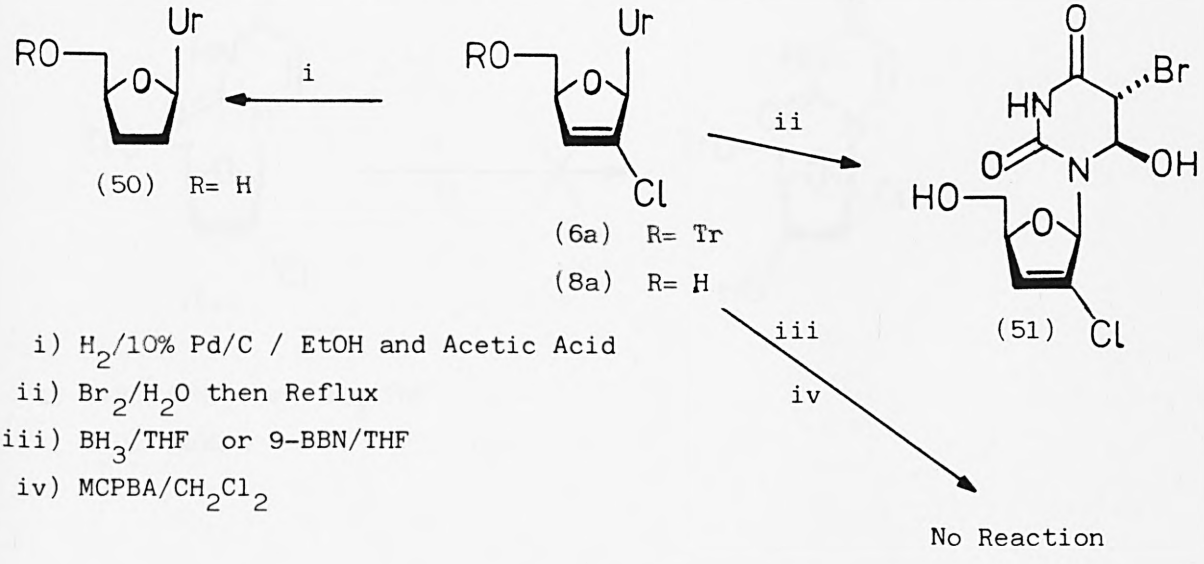


(6a) R= Tr, X= Cl
(8a) R= H, X= Cl

Compound (6a) in ethanol was stirred under an atmosphere of hydrogen and over a 10% Pd/C catalyst at ambient temperature. The reaction was, however, complicated by concomitant detritylation, but prolonged

reaction times afforded one nucleosidic product, namely 2',3'-dideoxyuridine (50) in ca. 60% yield (Scheme 2.14). The spectroscopic properties of this compound were identical to those given in the literature[166] and microanalysis showed no chlorine was present. In an attempt to stop the dehalogenation reaction an acidic solvent[237] and the deprotected nucleoside (8a) were used. However, when (8a) was hydrogenated in acetic acid over 10% Pd/C and the reaction followed by t.l.c. and stopped as soon as all the starting material had been consumed, only 2',3'-dideoxyuridine (50) was obtained. Reactions which were stopped well before completion and analysed by t.l.c. and proton n.m.r. spectroscopy showed only mixtures of compounds (50) and (8a) to be present. Thus in compounds (8a) and deprotected (6b)[166] it would appear that hydrogenation and dehalogenation occur either simultaneously or, if in discrete steps, the concentrations and lifetimes of any intermediates formed are too low to allow their detection by t.l.c. and proton n.m.r. spectroscopy. However, it is possible that subjecting the 2'-fluoro analogue of (8a) to similar hydrogenation conditions might lead to 2'-fluoro-2',3'-dideoxy-ara-uridine, as Blackburn[238] has recently reported that hydrogenation of some vinylfluorides can be achieved without loss of the fluorine atom.

SCHEME 2.14 Addition Reactions of the Compounds (6a) and (8a).



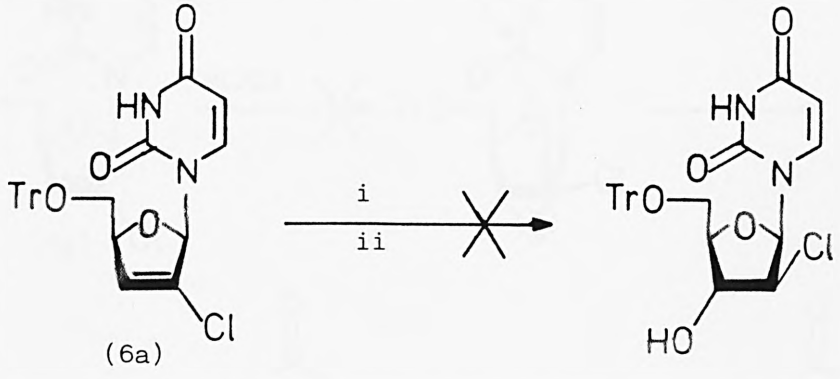
i) H_2/10% Pd/C / EtOH and Acetic Acid
ii) Br_2/H_2O then Reflux
iii) BH_3/THF or 9-BBN/THF
iv) MCPBA/CH_2Cl_2

Hydroboration.

Hydroboration of compound (6a) was an attractive goal as the major product might be expected to arise by attack at C3' from the less hindered side of the sugar ring and afford 2'-halo-2'-deoxy-ara-uridine species, analogues of the highly active antiviral compounds already described and shown in Table 1.2. If the hydroboration step could be made to occur (Diag. 2.5) then we would have a shorter route to these compounds than that already available (which involves preparation of the appropriate sugar which is then coupled with the base giving anomeric mixtures of the final product).[21]

Compound (6a) was treated with 9-BBN in THF as this reagent is known to react with a high degree of regio- and stereo-selectivity, giving the product arising by attack at the less substituted end and from the less hindered side of a double bond.[230,231] However no reaction occurred between (6a) and 9-BBN at ambient temperature or under reflux in THF and (6a) was always recovered in high yield. Even treatment of (6a) with the more powerful hydroboration reagent, the BH_3.THF complex, gave no evidence of reaction.

DIAGRAM 2.5 Attempted Preparation of 2'-Chloro-2'-deoxy-ara-uridine.



i) 9-BBN/THF or BH_3/THF

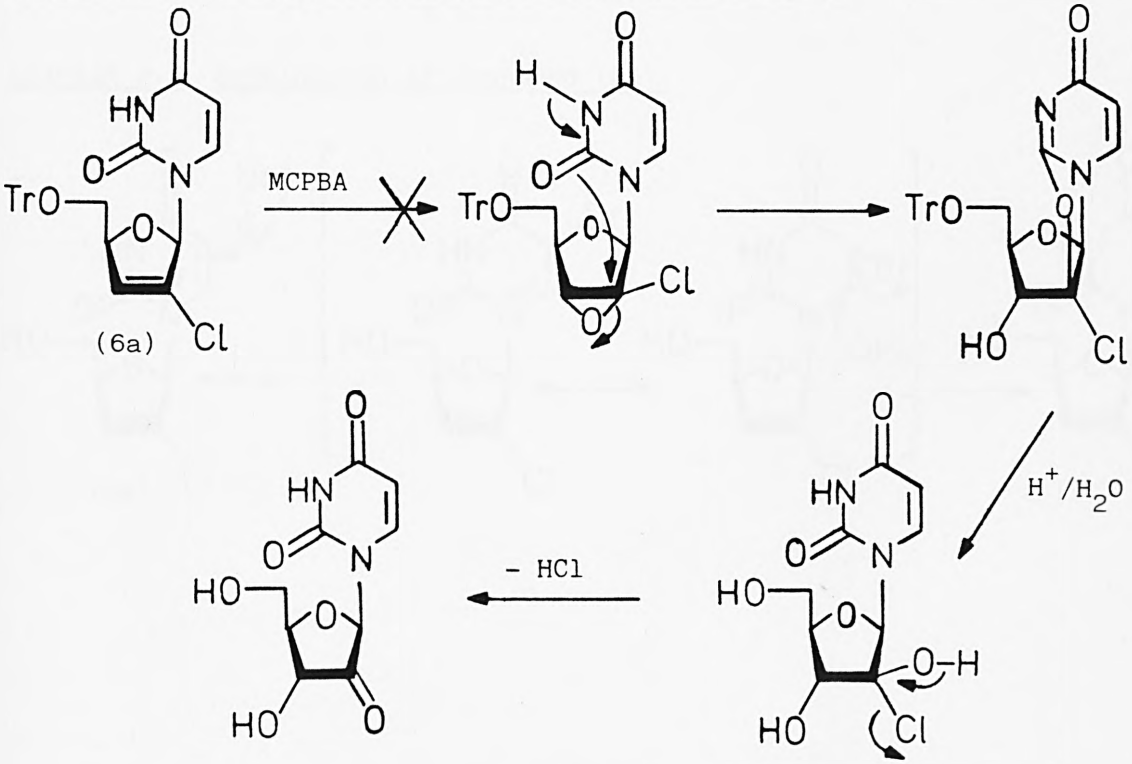
ii) H_2O_2/NaOH

Epoxidation.

Epoxidation of the 2',3'-unsaturated function in (6a) was of interest in that if the reaction took place and approach of the epoxidising reagent occurred from the less hindered side of the sugar ring, then a ribo-epoxide would be expected as the first product of reaction. Now ribo-epoxides in 2-keto-pyrimidine nucleosides are generally unstable to attack by the O2 atom to give the isomeric O^2,2'-cyclic derivative (as shown in Diag. 2.6). The cyclo-derivative, upon hydrolysis with aqueous acid, would yield the ara-analogue which might be expected to lose HCl resulting in the formation of a 2'-keto-nucleoside (in fact see later for reactions of 6b leading to some other 2'-keto compounds). This would constitute a new route to such compounds, which until recently[67] have not been readily available.

Treatment of compound (6a) with MCPBA in dichloromethane at ambient temperature and under reflux for 18 hrs gave no reaction, however. Using 1,2-dichloroethane as solvent and higher reflux temperatures made no difference; compound (6a) always being recovered in high yield.

DIAGRAM 2.6 Possible Results of Epoxidation of (6a).

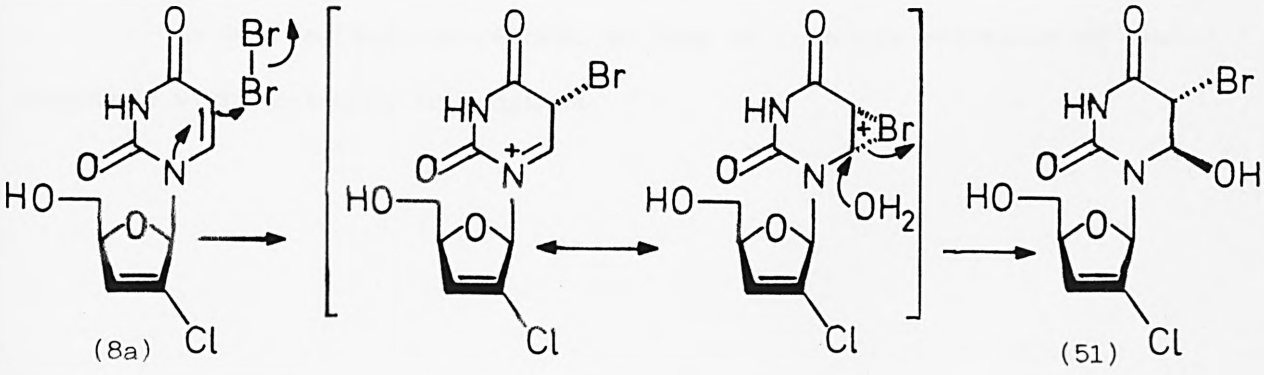


Bromination.

In compound (6a), while the 2',3'-chlorovinyl function was disappointingly inert to hydroboration and epoxidation, so also was the 5,6-double bond. Bromination of the 5,6-double bond occurs readily [240] so it would be interesting to see which of the two double bonds in (8a) would be more reactive under bromination conditions.

Compound (8a) in ethanol/water was treated with bromine until a faint bromine colour persisted. A crystalline solid was obtained which showed no u.v. absorption above 220 nm indicating that addition across the 5,6-double bond had occurred. Proton n.m.r. spectroscopy and elemental analysis indicated that a bromohydrin had been formed. The proton n.m.r. spectrum had sharp signals, indicating that only one diastereoisomer had been produced. Attack of halides on the uracil ring of nucleosides has been shown to occur stereospecifically to give a cationic intermediate of the type shown below (Diag. 2.7)[239,241] This is then attacked by water at C6, from the opposite side of the uracil ring relative to the halide group. Thus the trans bromohydrin (51) is obtained. The trans arrangement of the H-5 and H-6 protons was confirmed by the ^{1}H-^{1}H coupling constant between them which was 3 Hz.[241]

DIAGRAM 2.7 Bromination of Compound (8a).



Compound (51) was heated under reflux in ethanol for 12 hrs, without dehydration occurring to restore the 5,6-double bond. Presumably, since in (51) the 6-OH group does not have a hydrogen atom trans to itself, efficient elimination of water cannot occur. Similarly, efficient elimination of HBr cannot occur to restore the 5,6-double bond. However, dehydration in other uridine-5,6-bromohydrins has been shown to occur to restore the 5,6-double bond.[240] Possibly in these instances, trace amounts of basic impurities might have been present and caused equilibration of the configuration at C5 (this position being adjacent to a carbonyl group), thus allowing trans-elimination of water.

Conclusions.

The studies presented in this section show that the 2',3'-halovinyl function in (6a) and (8a) is inert towards electrophilic attack. In the three types of reaction described above, the initial step involves attack by the pi-electrons of a double bond on the electrophilic centre of the reagent molecule. In compounds (6a) and (8a), the presence of the electron-withdrawing chlorine atom on the 2',3'-double bond lowers the availability of the pi-electrons towards reaction with electrophiles and results in the lack of reactivity observed in these compounds. This low reactivity towards electrophiles is common in vinyl halides.[242]

It was decided, therefore, to look at possible reactions of these compounds with nucleophilic reagents.

2.2.11 Carbon-Carbon Bond-Forming Reactions at the 2'-Position of Uridine Nucleosides.

Introduction.

Our success at forming C-C bonds directly at the 3'-position of some uridine nucleosides (Section 2.2.4) encouraged us to attempt some C-C bond forming reactions at the 2'-position of nucleosides. This type of reaction was only recently reported by Ueda[199] who made suitably protected 2'-keto-uridine and 2'-keto-adenosine species and subjected them to Wittig reactions to afford 2'-branched-uridine and adenosine compounds. These have already been discussed in Section 1.6.2 and Table 1.16. Our observations (Section 2.2.10) that compounds (6a), (6b) and (8a) were inert to electrophilic reagents at the 2',3'-double bond suggested that nucleophilic reactions at C2' might be more feasible.

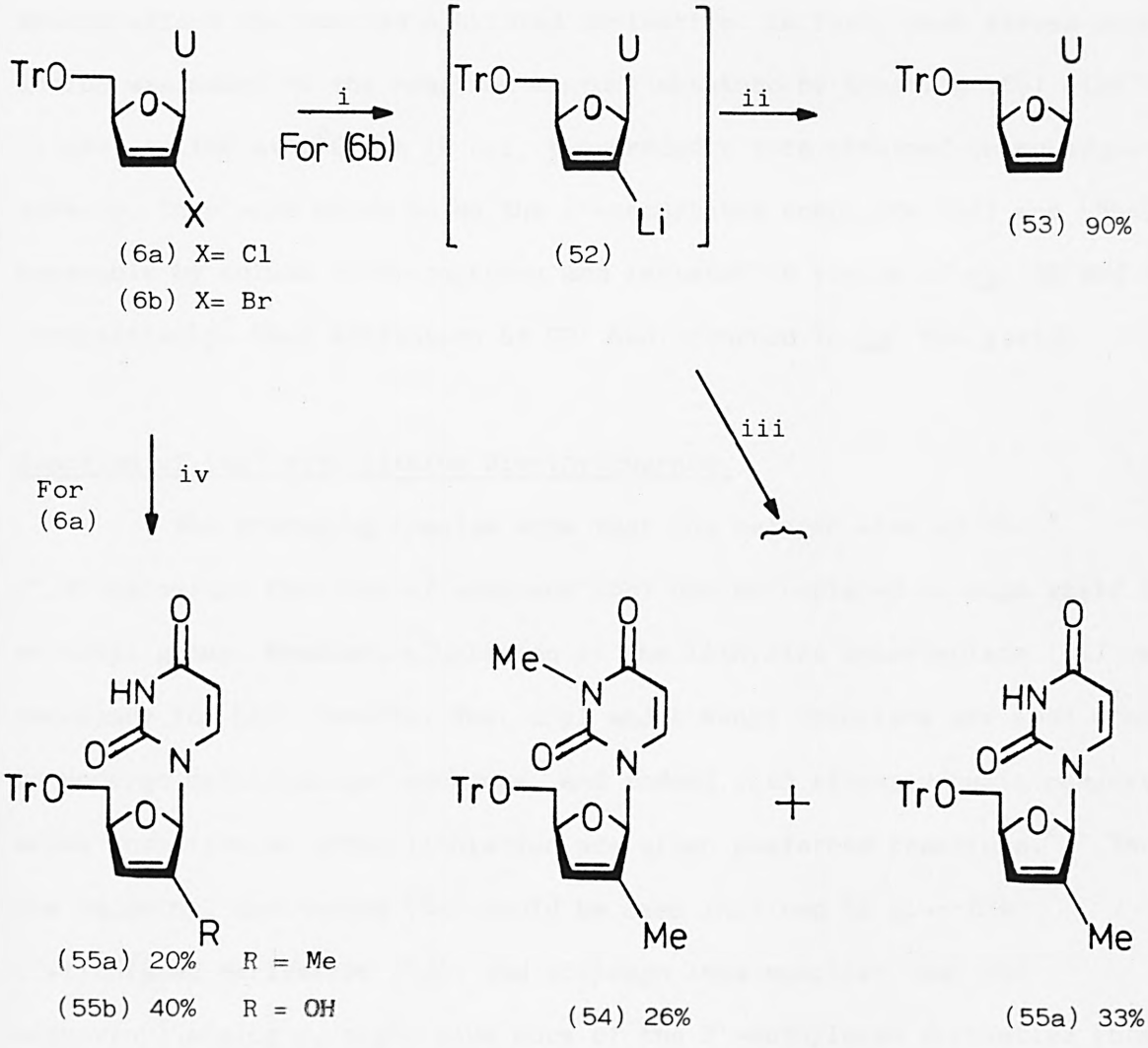
It is well established that various aryl and alkenyl halides can be alkylated by organometallic reagents by nucleophilic displacement of the halogen atom.[223,236] Of these, the lithium dialkylcuprates (already used in section 2.2.4) are highly nucleophilic and have found wide applications in reactions with halovinyl groups. Generally, bromovinyl groups have been found to react more readily with $LiCuR_2$ reagents than do chlorovinyl functions. Thus it was decided to look first at the reactions of the 2',3'-unsaturated derivative (6b) with these cuprate reagents as this seemed more likely to undergo the desired C-C bond-forming reaction at C2'.

Reaction of (6b) With Lithium Dimethylcuprate.

The bromovinyl derivative (6b) was treated with excess $LiCuMe_2$ (ca. 5 equivalents) in THF at ice-bath temperature for 18 hrs under a nitrogen atmosphere. Quenching with ammonium chloride led to the isolation of a white foam which by proton n.m.r. spectroscopy was shown to consist mostly of the 2',3'-unsaturated nucleoside (53), (Scheme 2.15). However, a

weak singlet at δ=1.70, possibly due to a methylated nucleoside (i.e. 55a, see below) could be seen although its intensity indicated that it was present in only ca. 5% yield. The yield of compound (53) was ca. 90% and the analytically pure nucleoside could be crystallised directly from the mixture. Repeating this reaction at 25°C for 48 hrs led essentially to the same product mixture.

SCHEME 2.15 Preparation of Some 2'-C-Substituted Nucleosides.



i) $LiCuMe_2$/THF/0°C
ii) Aqueous NH_4Cl
iii) MeI/NH_4Cl
iv) $LiCuMe_2$/THF/RT

It thus appears that two competing reactions may be occurring. The predominant reaction seems to be metal-halogen exchange to give the intermediate (52) lithiated at C2', which upon aqueous work-up gives the 2',3'-unsaturated nucleoside (53) in excellent yield. The second and minor reaction is the nucleophilic displacement of the 2'-bromo group which seems to be occurring in around 5% yield.

If lithiation at C2' is the main reaction competing with nucleophilic displacement of the 2'-bromo atom, then treatment of the lithiated intermediate with an alkylating agent prior to aqueous work-up should afford the desired alkylated derivative. In fact, when excess methyl iodide was added to the reaction mixture obtained by treating (6b) with $LiCuMe_2$ in THF at $0^{\circ}C$ for 18 hrs, two products were obtained on subsequent work-up. They were shown to be the 2'-methylated compounds (54) and (55a), separable by column chromatography and isolated in yields of ca. 26 and 33% respectively. Thus alkylation at C2' had occurred in ca. 60% yield.

Reaction of (6a) With Lithium Dimethylcuprate.

The preceding results show that the halogen atom in the 2',3'-halovinyl function of compound (6b) can be replaced in high yield by an alkyl group. However, alkylation of the lithiated intermediate (52) was necessary for best results. Now, aryl and alkenyl chlorides are less prone to undergo metal-halogen exchange, and indeed with strongly basic reagents, aryne formation or ortho-lithiation are often preferred reactions.[243] Thus the halovinyl derivative (6a) would be less inclined to give the 2'-lithiated derivative (53), and although less reactive than its bromovinyl analogue, might give more of the 2'-methylated derivative (55a) by direct nucleophilic attack by the cuprate reagent.[236]

When (6a) was treated with excess $LiCuMe_2$ in THF at $0^{\circ}C$ for 18 hrs, the usual work-up led to starting material being recovered, although some minor products could be detected by t.l.c. and proton n.m.r.

spectroscopy. Repeating the reaction at a higher temperature (20°C) and with a larger excess of the cuprate reagent (ca. 7 equivalents) for 24 hrs, led to a mixture of products containing starting material together with compounds (55a) and (55b). The latter pair of products were present in a ca. 1:2 ratio (ca. 20 and 40% yield respectively). Column chromatography led to the separation of these compounds from starting material, although only (55b) was obtained pure in this preparation.

Thus direct nucleophilic displacement of the 2'-chloro group in (6a) does occur to a greater extent than does the analogous direct displacement of the 2'-bromo group in (6b). However, another reaction not observed with (6b) was shown to occur to give the 2'-keto derivative (55b).

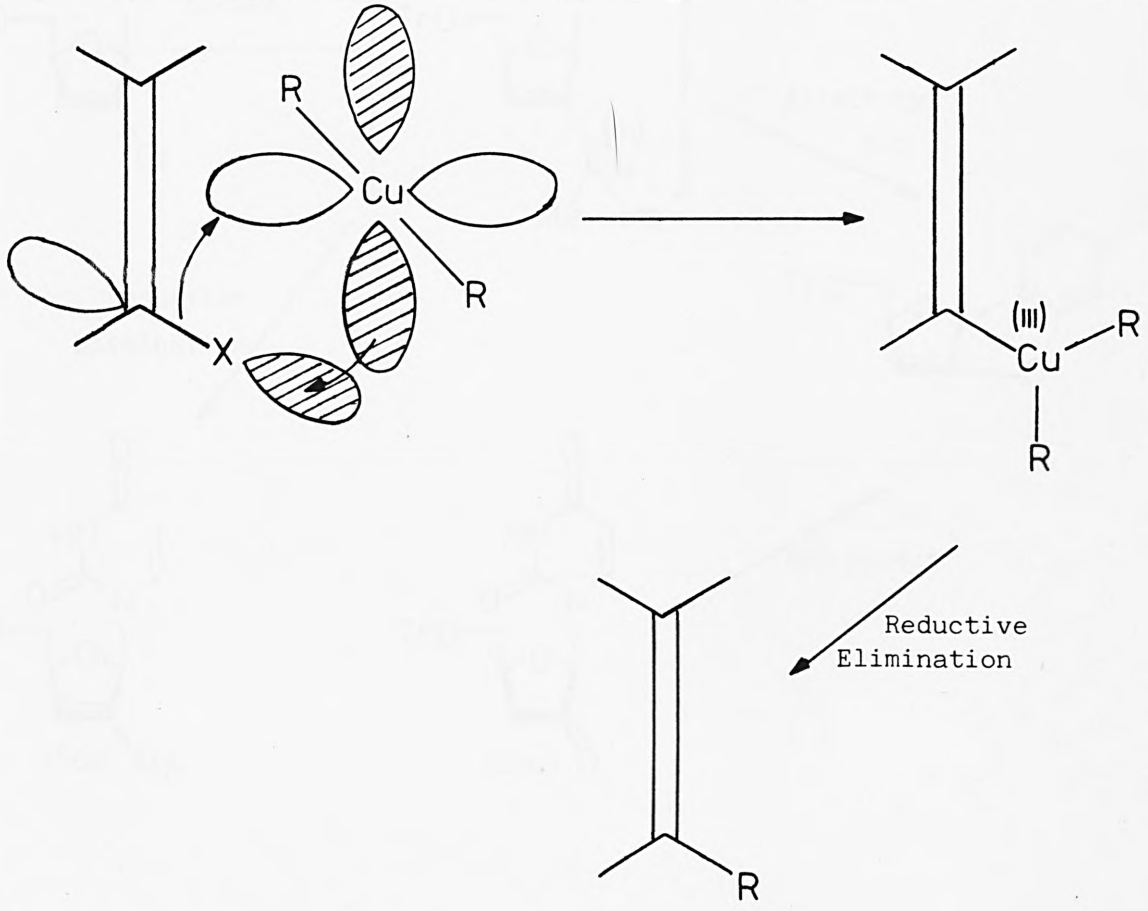
Conclusions.

In the brief study described in this section the direct formation of a C-C bond at the C2'-position of some uridine nucleosides has been established. The most efficient route involved the formation of a 2'-lithiated derivative (52), the first time that such a nucleoside derivative has been reported. Upon treatment with an alkylating agent (MeI in these studies) this gave the 2',3'-didehydro-2',3'-dideoxy-2'-alkylated uridine derivatives (54) and (55a), alkylation at C2' being effected in above 60% yield. Direct nucleophilic attack at C2' of both (6a) and (6b) also seemed to take place, although the best yields were obtained when using (6a) and were only ca. 20%.

Two other major products from the above reactions were also obtained. If the lithiated intermediate (52) was treated with a proton source instead of an alkylating agent, then 2',3'-didehydro-2',3'-dideoxy-uridine (53) was obtained in excellent yields. When (6a) was treated with the lithium dimethylcuprate reagent, the major product was the 2'-keto derivative (55b). The formation of (55b) from (6a) but not from (6b) can be explained as follows. Firstly, since lithium-halogen exchange does not seem

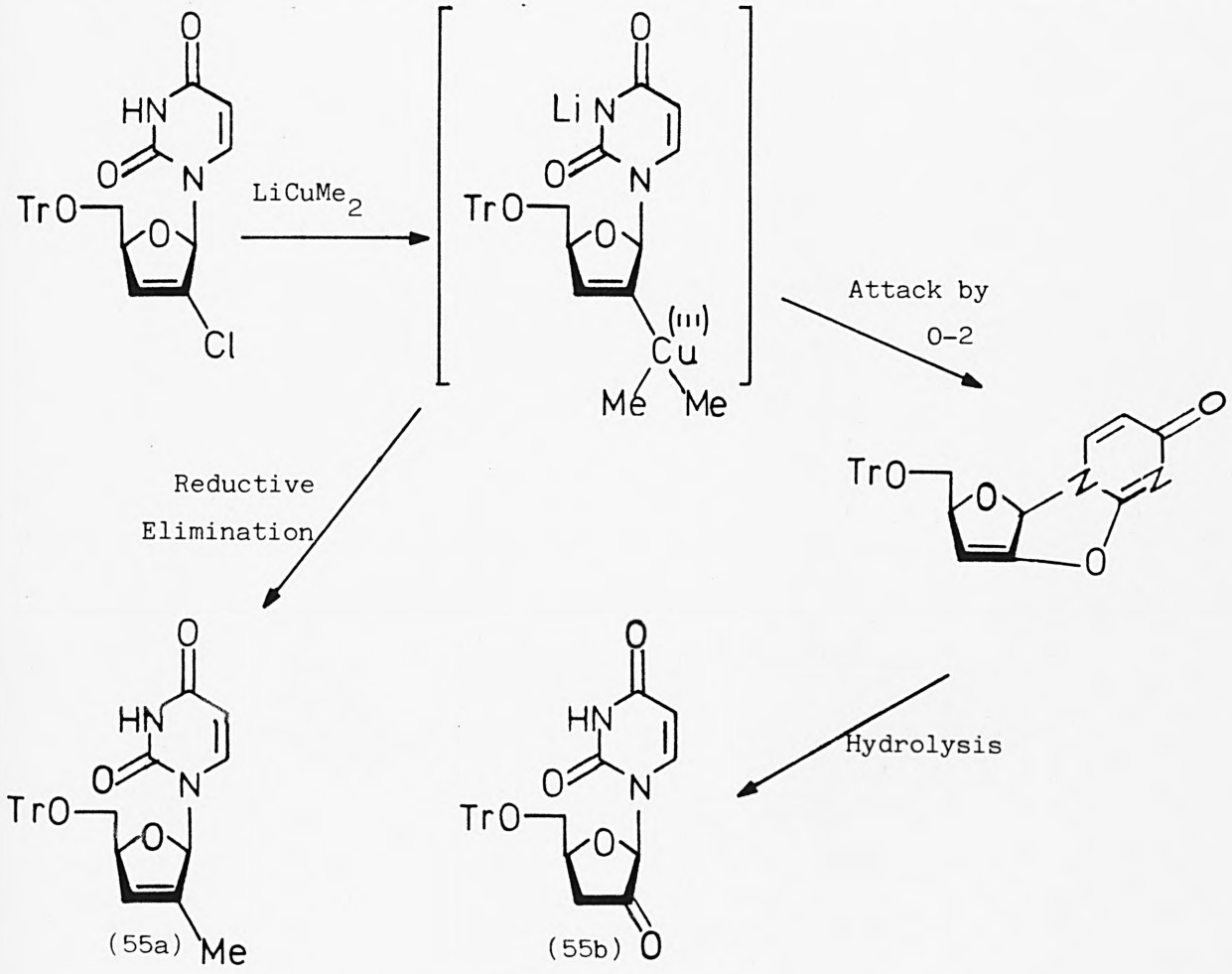
to occur in (6a), then nucleophilic attack at C2' is not inhibited by formation of an anion at that position as it is in (6b). Thus C2' in (6a) is methylated to a greater extent (ca. 20%) by the cuprate reagent than is C2' in (6b),(ca. 5%). To give a 2'-keto group, the C2' position must be attacked by an oxygen containing nucleophile. This might arise if there were any small leaks of moisture or oxygen into the reaction vessel which might accumulate to a significant level during the long reaction time (24 hrs) used. Thus water would give hydroxide ions and oxygen can react with organometallic reagents to give peroxides and alkoxides which might attack the C2'-position. This type of reaction might be facilitated by the presence of the Cu(I) ions, as it is known that halogen exchanges occur readily in aromatic systems under the influence of Cu(I) ions.[244] In (6b) the fact that an anion is present at C2' means that any moisture leaking in would protonate this position to give (53).

DIAGRAM 2.8 Possible Mechanism of the $LiCuR_2$ Reaction with Vinyl Halides.



However, another reaction course which may account for the formation of both compounds (55a) and (55b) from the same intermediate is possible. A detailed mechanism for the reaction between a vinyl halide and the $LiCuR_2$ reagents has recently been reported.[245] In this mechanism (Diag. 2.8), an electron pair from a copper d-orbital of a $[CuR_2]^-$ species, enters the C-X antibonding lobe at X, with a simultaneous bonding interaction occurring between the developing electron pair at carbon and the same copper d-orbital. In such a transition state, negative charge is transferred from copper to the halide atom and a Cu-X bond forms as the carbon-halogen bond is broken. The resulting Cu(III) intermediate then undergoes a reductive elimination to give the cross-coupled product.

DIAGRAM 2.9 Possible Mechanism for the Formation of (55a) and (55b) from (6a)



If such a reaction course were to take place between $LiCuMe_2$ and the nucleoside (6a), then a Cu(III) intermediate of the type shown in Diagram 2.9 would result. This could then undergo the usual reductive elimination to give the expected 2'-methylated derivative (55a). Alternatively, if it is attacked by the O-2 atom of the pyrimidine ring, an $\underline{O}^2$,2'-cyclic derivative, as shown in Diagram 2.9, would result. Molecular models show that such a structure is possible and a similar $\underline{O}^2$,2'-anhydro-2',3'-didehydro-nucleoside was considered as a possible intermediate in the hydrolysis of a 2',3'-didehydro-2'-$\underline{O}$-tosyl-uridine derivative to give the 5'-$\underline{O}$-benzoyl derivative of (55b).[164] In fact, if the $\underline{O}^2$,2'-anhydro-2',3'-didehydro-nucleoside in Diagram 2.9 is labile to hydrolysis, then attack of water at either C-2 or C-2', upon aqueous work-up, would give rise to the 2'-keto-nucleoside (55b).

2.2.12 Synthetic Routes to 3'-Branched Adenosine Nucleoside Derivatives.

Introduction.

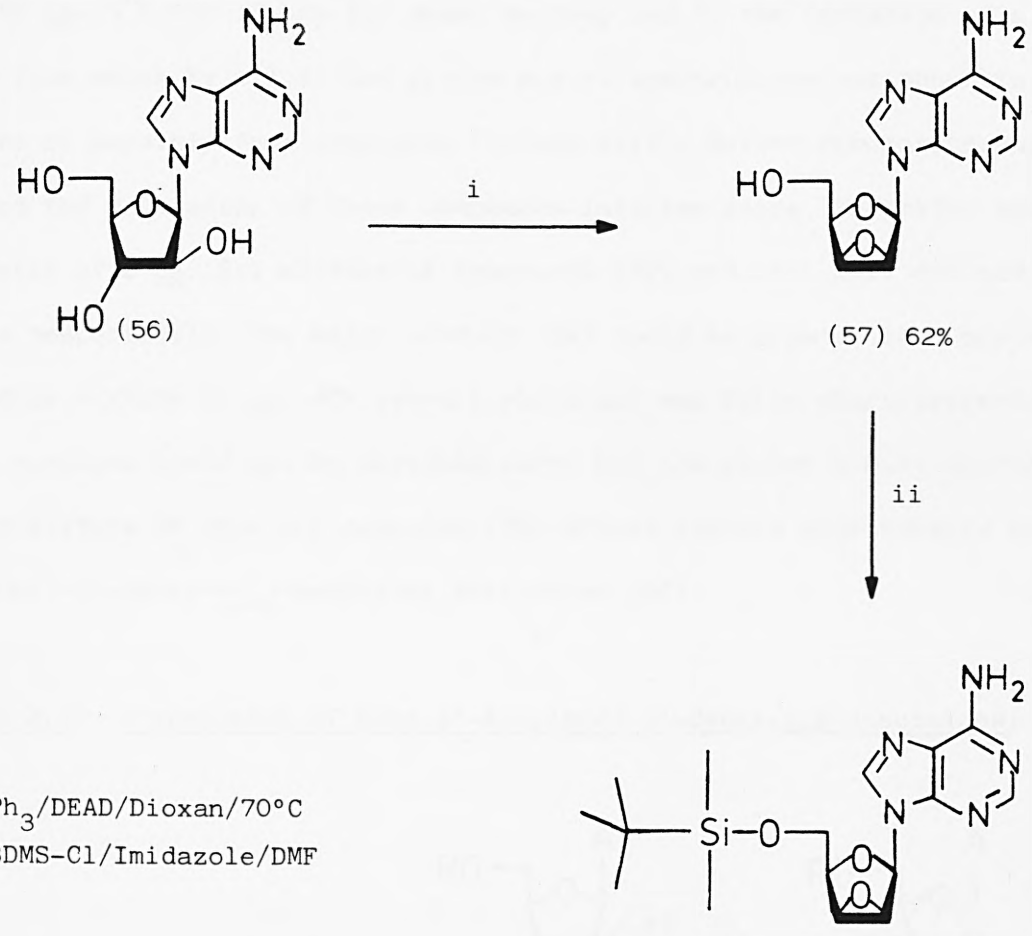
In the preceding sections, new methods for the formation of C-C bonds at C3' and C2' of various uridine derivatives were established. Also, some of the 3'-branched derivatives were converted by known methods to the cytidine, thymidine and 5-halo-uridine analogues. We now wanted to see if some of these reactions could be applied in the purine series. We decided to look at the epoxide-opening reactions that we had applied in uridine systems as both ribo- and lyxo-epoxides are readily available in both the adenosine and guanosine[139-144] series. Our first attempts at C-C bond-forming reactions directly on purine nucleosides were made on an adenosine-2',3'-lyxo-epoxide derivative as this would be expected to give 3'-alkylated-3'-deoxy-ara-adenosine derivatives, analogues of the antiviral nucleoside ara-adenosine (see Table 1.2).

Preparation of a Suitably Protected Adenosine-2',3'-lyxo-epoxide (58).

Various methods for preparing adenosine-2',3'-lyxo-epoxides are available and have been already described (Section 1.5.5). We were attracted to the one step conversion of ara-adenosine (56) to the lyxo-epoxide (57), reported to occur quantitatively[157] (Scheme 2.16). Ara-adenosine (56) is available commercially or can be conveniently prepared from adenosine by a seven-step procedure in ca. 20-30% overall yield.[76,77] In these studies ara-adenosine from both these sources was used.

When ara-adenosine (56) was treated with triphenylphosphine and diethyl azodicarboxylate in dioxan, under a nitrogen atmosphere at 70°C, the 2',3'-lyxo-epoxide was obtained as a crystalline solid in 62% yield (Sceme 2.16). This compound was protected at the 5'-position by reaction with t-butyldimethylsilylchloride and imidazole in DMF at 20°C [247] to give the 5'-O-t-butyldimethylsilyl ether (58) in ca. 67% yield.

SCHEME 2.16 Preparation of a 5'-O-Protected Adenosine-2',3'-lyxo-epoxide (58).



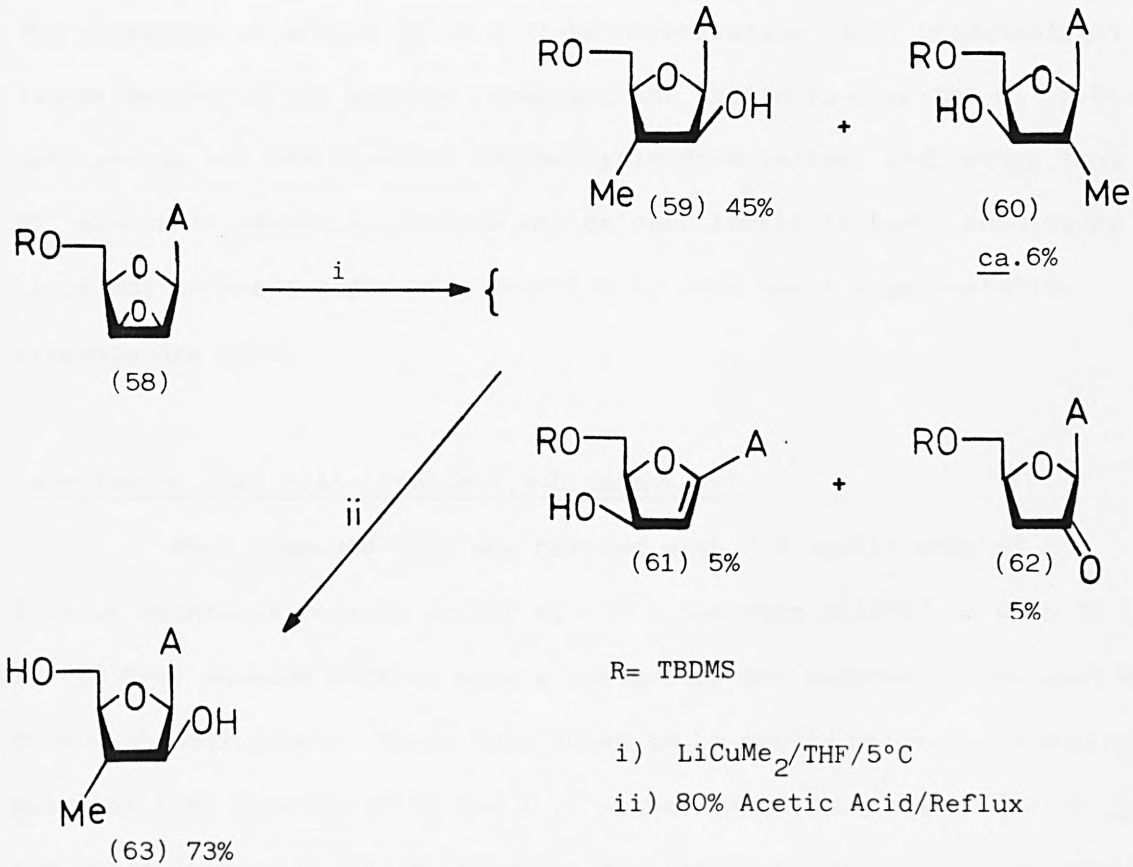
i) PPh_3/DEAD/Dioxan/70°C
ii) TBDMS-Cl/Imidazole/DMF

This protecting group was chosen for several reasons. Firstly, it can be introduced in higher yields and under milder conditions than the trityl group. There was thus less danger that the epoxide would be opened by the halide counter-ion. Also, we wanted to see what other protecting groups would render the nucleoside soluble in THF and withstand the organometallic reagents to be used.[250]

Reaction of (58) with Lithium Dimethylcuprate.

Compound (58) was added to 3.5 equivalents of the cuprate reagent in THF at -30°C and under a nitrogen atmosphere. After being allowed to warm to ca. 5°C for 18 hrs the usual work-up led to the isolation of a white foam which by t.l.c. and proton n.m.r. spectroscopy was shown to be a mixture of possibly four compounds (Scheme 2.17). Column chromatography allowed the separation of these compounds into two pairs. The major pair consisted of a ca. 8:1 mixture of compounds (59) and (60) i.e. 45% and 6% yields respectively. The major product (59) could be crystallized directly from this mixture in ca. 40% overall yield and was fully characterised. The minor compound could not be obtained pure, but the proton n.m.r. spectrum of the mixture of this and compound (59) showed signals attributable to a 2'-methyl-2'-deoxy-xylo-adenosine derivative (60).

SCHEME 2.17 Preparation of Some 3'-Alkylated-3'-deoxy-ara-adenosines.



The mixture of the other pair of compounds appeared by proton n.m.r. spectroscopy to be an approximately 1:1 mixture of the 1',2'-unsaturated derivative (61) and the 2'-keto derivative (62), (each in 5-10% yield). Although these compounds could not be separated the proton n.m.r. spectrum of the mixture showed signals for (61), which was obtained pure in a different preparation (see below) and the remaining signals consisted of resonances for the adenine base (δ=7.88 and 8.36), and for the TBDMS group (δ=0.10 and 0.90) while the remaining sugar ring resonances were almost identical to those of the analogous 2'-keto-uridine derivative (55b) which was also obtained pure in another preparation (Section 2.2.11)

Compound (59) was deprotected by 80% aqueous acetic acid to give the free 3'-methyl-3'-deoxy-ara-adenosine (63) in 73% yield.[251]

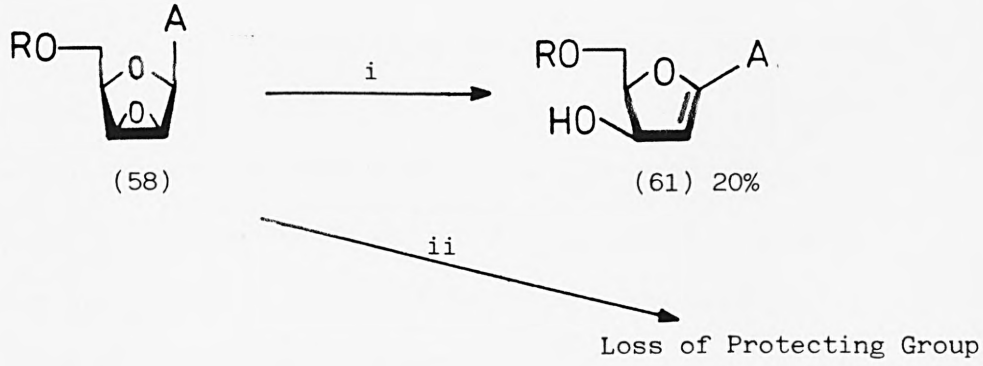
Thus, as in the uridine series, attack of lithium dimethylcuprate on the adenosine-2',3'-lyxo-epoxide occured predominantly at C3' to give a 3'-alkyl-3'-deoxy-ara-adenosine derivative (59) in good yield. As in the uridine series, a few percent of attack at C2' also occurred together with the formation of around 5% of a 2'-keto derivative (62), presumably by isomerisation of the epoxide. However, the abstraction of the C1' proton by the cuprate was not observed in the pyrimidine series, indicating that the C1' proton in adenosine systems may be more labile to basic conditions and hence may become a major side reaction if more basic organometallic reagents are used.

Reaction of (58) with Lithium 1,3-Dithian-2-yl.

When compound (58) was reacted with 2.5 equivalents of the lithium dithianyl reagent in THF at -30°C and then allowed to warm to 5°C for 18 hrs, aqueous work-up gave a mixture of two compounds separable by column chromatography. These were shown to be mostly recovered starting material (58) together with the 1',2'-unsaturated derivative (61) in ca. 11% yield (Scheme 2.18). No evidence for attack at C3' or C2' was obtained.

The reasons for this may be twofold. Firstly, as indicated in the reaction of this reagent with the uridine-2',3'-lyxo-epoxide (17), the dithianyl anion has a low reactivity towards the epoxide. In fact in some reactions of the lithium dithianyl reagent with certain carbohydrate epoxides higher temperatures and longer reaction times were required.[227] In the compounds (17) and (58) the presence of the bulky 5'-protecting group may further inhibit reaction by sterically hindering attack at the more reactive end of the epoxide (i.e. the C3' end). In the pyrimidine series, the presence of the α-β-unsaturated amide system of the base allowed the dithianyl reagent an alternative reaction course leading to Michael addition at C6 (Section 2.2.5). This reaction cannot occur in adenosine systems and the higher acidity of H1' in (58) as compared to (17), already mentioned above, means that abstraction of this proton is the only course of reaction available to the dithianyl anion thus leading to (61). The structure of compound (61) was confirmed by its proton n.m.r. spectrum which showed sugar resonances almost identical to those of the analogous uridine-1',2'-unsaturated derivative (26). Furthermore, the free nucleoside having the opposite configuration at C3' relative to (61) is known and its proton n.m.r. spectrum differs only in its values for the $J_{2',3'}$ and $J_{3',4'}$ coupling constants, indicating a difference in configuration at C3'.[98]

SCHEME 2.18 Other Reactions of (58) with Organometallic Reagents.



i) Lithium Dithianyl/THF/5°C

ii) LiC≡CH/DMSO/RT

Attempted Opening of the Epoxide in (58) with Lithium Acetylide.

Compound (58) was treated with the lithium acetylide-ethylene diamine complex in DMSO at ca. 20°C for 18 hrs. However, aqueous work-up followed by extraction into ether, led to little material being obtained in the organic extracts. Furthermore, the little that was extracted was shown by proton n.m.r. to be mostly t-butyldimethylsilyl alcohol, indicating that the 5'-O-TBDMS protecting group is unstable under these conditions. This was confirmed by the fact that the aqueous extracts had a u.v. absorption at ca. 258 nm.

Biological Studies

The biological properties of some of the novel nucleoside analogues prepared during this research work are currently being investigated by other workers at Glaxo Group Research Ltd. (Ware), and are therefore not discussed in this thesis.

3) EXPERIMENTAL

Melting points are uncorrected, in degrees centigrade and were determined in glass capillaries. Infra-red analysis was performed on nujol mulls or chloroform solutions of the compounds (as indicated in the experimental details below) on a Perkin-Elmer 159 G spectrometer. Frequencies of maximum absorption (μ_{max}) are quoted in wavenumbers (cm^{-1}) using a polystyrene film as a reference. Ultra-violet analysis was performed on a Pye-Unicam SP 1800 and a Perkin-Elmer Lambda 5 spectrometer using quartz cells. Wavelengths of maximum absorbance (λ_{max}) are given in nanometers along with molar extinction coefficients (ε). Mass spectra were determined on AEI MS30 and MS9 instruments. All ^{1}H n.m.r. spectra were determined at 100 MHz using a JEOL MH100 spectrometer, unless otherwise stated. For those determined at 250 MHz, a Brucker WM250 instrument was used. ^{13}C n.m.r. spectra were determined on a JEOL FX60 instrument at 15 MHz. All n.m.r. measurements were made in deuterated solvents (as indicated in each experiment below) with teramethylsilane (T.M.S) as an internal standard. Chemical shifts are quoted in δ and coupling constants (J) in Hz. T.l.c. investigations were performed on 0.2 mm thick Merck Kieselgel GF_{254} silica-gel plates and monitored by irradiation of the plates with ultra-violet light or by exposure to iodine vapour. Column chromatography was perfomed using Merck Kieselgel 60 (0.063-0.200 mesh). The sample / column weight ratio is given for each individual experiment. "Flash- chromatography" was performed using Merck Kieselgel 60 (Art. 9385) following the literature procedure.[153] Solvents were purified and dried by the methods described in "Purification of Laboratory Chemicals".[132]

3.1) O^2,2'-cyclouridine (2).

Prepared by the method of Verheyden et al.[41]

A solution of uridine (1), (5.05g, 20.6mmol) and diphenyl carbonate (5.88g, 27.5mmol) in dry (4A mol. sieve) HMPA (20 ml) was stirred at 145°C. After 5 mins. sodium bicarbonate (0.125g, 1.5 mmol) was added. An effervescence soon occurred and lasted for 15 mins. Heating was continued for a further 10 mins. The mixture was allowed to cool, diluted with water (150 ml) and extracted with dichloromethane (3x70 ml). The aqueous layer was evaporated to a yellow solid which crystallized from methanol, (3.82g, 82%).

M.p. 240-242°C Lit. 239-240°C.[40]

U.v. (0.01 M HCl): λ_{max} 250 nm, 223 nm Lit. 249 nm, 223 nm.[40]

I.r. (nujol, cm^{-1}): 3000-3400 (m), 1650 (s), 1620 (s), 1520 (s), 830 (s)

^{1}H n.m.r. ($DMSO_{d6}$, δ): 3.40 (2H,bs,H-5'), 4.15 (1H,m,H-4'), 4.48 (1H,bs,H-3'), 5.04 (1H,m,OH), 5.28 (1H,d,H-2', $J_{1',2'}$=6Hz), 5.94 (1H,d,H-5, $J_{5,6}$=8Hz), 5.96 (1H,bs,-OH), 6.40 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.94 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 47.79; H, 4.46; N, 12.39.

found : C, 47.77; H, 4.49; N, 12.25.

3.2) 2'-Chloro-2'-deoxyuridine (3a).

Prepared by the method described by White.[94]

A solution of (2), (10.0g, 44.0 mmol) in 2M HCl (25 ml, 50 mmol) was evaporated to dryness at 30°C to give a white solid. DMF (60 ml) was added and the solution was evaporated to dryness again. Further DMF (60 ml) was added and the solution stirred at 100°C for 70 mins. It was evaporated down to a yellow gum which crystallized from methanol, (9.75g, 84%).

M.p. 207-209°C Lit. 207-212°C.[115]

U.v. (MeOH): λ_{max} 261 nm Lit. (H_2O) 260 nm [115]

I.r. (nujol, cm^{-1}): 3440 (m), 3340 (m), 3200 (m), 3060 (w), 1680 (s,br), 1100 (m), 1040 (m), 830 (s).

^1H n.m.r. ($DMSO_{d6}$,δ): 3.70 (1H,bs,5'OH), 3.84 (2H,bs,H-5'), 4.16 (1H,m,H-4'), 4.24 (1H,m,H-3'), 4.76 (1H,bt,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=5 Hz), 5.56 (1H,bs,$\underline{N}^3$-H), 5.92 (1H,d,H-5, $J_{5,6}$=8Hz), 6.08 (1H,d,H-1', $J_{1',2'}$=6Hz), 8.00 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 41.15; H, 4.22; N, 10.67.

found : C, 41.20; H, 4.24; N, 10.51.

3.3) 5'-O-Trityl-2'-chloro-2'-deoxyuridine (4a) from (3a).

A solution of (3a), (3.63g, 1.4 mmol) and triphenylchloromethane (4.14g, 1.5 mmol) in dry (4A mol. sieve) pyridine (30 ml) was stirred at 20°C for 18 hrs. The dark orange solution was then heated at 80° C for 3 hrs, allowed to cool and evaporated to an oil. This was dissolved in methanol (15 ml) and poured onto crushed ice. The precipitate was filtered, washed with water and recrystallized from methanol, (5,47g, 78%).

M.p. 176-178°C Lit. 164-166°C.[152]

U.v. (MeOH): λ_{max} 260 nm Lit. 259 nm [152]

I.r. (nujol, cm^{-1}): 3340 (w), 3160 (w), 3060 (w), 1700 (s), 1680 (s), 1100 (m), 1060 (m), 820 (m), 700 (m).

^1H n.m.r. ($CDCl_3/DMSO_{d6}$,δ): 3.36 (1H,bs,3'-OH), 3.54 (2H,bs,H-5'), 4.14 (1H,m,H-4'), 4.54 (2H,m,H-2' & H-3'), 5.30 (1H,d,H-5, $J_{5,6}$=8Hz), 5.98 (1H,bs,$\underline{N}^3$-H), 6.14 (1H,d,H-1', $J_{1',2'}$=3Hz), 7.48 (15H,bm,aromatics), 7.80 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 66.60; H, 4.99; N, 5.55.

Found : C, 66.60; H, 4.94; N, 5.43.

3.4) 5'-O-Trityl-2'-chloro-2'-deoxyuridine (4a) from (2).

Prepared by the method of Ogilvie et al.[152]

A solution of (2), (1.0g, 4.4 mmol) and triphenylchloromethane (1.5g, 5.4 mmol) in dry pyridine (20 ml) was stirred at 20°C for 18 hrs. The temperature was raised to 105°C and stirring continued for 8 hrs. The pyridine was evaporated off to leave a gum which was dissolved in methanol (15 ml) and poured onto crushed ice. The precipitate was filtered, washed with water and crystallized from methanol, (0.83g, 38%).

M.p. 178-180°C Lit. 164-166°C.[152]

U.v., i.r., ^{1}H n.m.r and t.l.c. data were identical for the sample prepared in section (3.3).

Microanalysis requires: C, 66.60; H, 4.99; N, 5.55.

found : C, 66.70; H, 5.01; N, 5.55.

3.5) 5'-O-Trityl-3'-O-mesyl-2'-chloro-2'-deoxyuridine (5a).

Methanesulphonylchloride (5.4ml,7.9g, 70 mmol.) was added drop by drop to a cooled (-20°C) solution of (4a),(9.1g,18mmoml) in dry pyridine (150 mls). After standing at ca. 0°C. for 18 hrs the solution was treated with methanol (25 mls) and left at ambient temperature for 1hr. The pyridine was removed under reduced pressure and the resulting gum dissolved in methanol (25 mls) and poured onto crushed ice. The white precipitate was filtered, washed with water and dried in a vacuum oven at 50°C for 18hrs. Yield 10.3g. It was used in later reactions with no further purification. Yield: 98%

M.p.118-120°C.

U.v.(MeOH) : λ_{max} 260 nm

I.r. : (nujol,cm^{-1}): 3160(w), 1660(s,br), 1660(m), 760(m), 700(m).

^{1}H n.m.r. ($CDCl_3$,δ): 3.00 (3H,s,$C\underline{H}_3SO_2$-), 3.60 (2H,bs,H-5'), 4.36 (1H,m,H-4'), 4.60 (1H,t,H-3',$J_{2',3'}$=5Hz, $J_{3',4'}$=5Hz), 5.20 (1H,t,H-2', $J_{1',2'}$=5Hz, $J_{2',3'}$=5Hz), 5.32 (1H,d,H-5, $J_{5,6}$=8Hz), 6.08 (1H,d,H-1', $J_{1',2'}$=5Hz), 7.30 (15H,bm,aromatics), 7.64 (1H,d,H-6, $J_{5,6}$=8Hz), 9.40 (1H,bs,$\underline{N}^3$-H).

Microanalysis requires: C, 59.79; H, 4.67; N, 4.81; Cl, 6.08; S, 5.50.

found : C, 58.98; H, 4.67; N, 4.74; Cl, 6.13; S, 4.88.

3.6) 2'-Bromo-2'-deoxyuridine (3b).

Prepared by the same method used for (3a). Yield: 69%

M.p. 188-190°C Lit. 186-190°C.[56]

U.v. (MeOH): λ_{max} 262 nm Lit. (H_2O) 262 nm[56]

I.r. (nujol, cm^{-1}): 3340 (m), 3240 (m), 3120 (m), 3040 (w), 1695 (s), 1675 (s), 1100 (m), 1040 (m), 835 (m).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 3.40-3.70 (1H,bs,5'-OH), 3.70 (2H,bs,H-5'), 4.04 (1H,m,H-4'), 4.64 (1H,bt,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=5Hz), 5.00-5.40 (1H,bs,$\underline{N}^3$-H), 5.74 (1H,d,H-5, $J_{5,6}$=8Hz), 5.94 (1H,m,3'-OH), 6.18 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.96 (1H,d,H6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 35.19; H, 3.61; N, 9.12.

found : C, 35.08; H, 3.58; N, 8.92.

3.7) 5'-O-trityl-2'-bromo-2'-deoxyuridine (4b).

Prepared by the same method used for (4a) in Section 3.3. Yield 54%

M.p. 170-172°C Lit.162-163°C[52]

U.v. (MeOH): λ_{max}262 nm Lit. 261 nm[52]

I.r. (nujol, cm^{-1}): 3360 (m), 3260 (w), 3080 (w), 1710 (s), 1650 (s), 1100 (s), 710 (s).

^1H n.m.r. ($CDCl_3/DMSO_{d6}$,δ): 3.44 (2H,bs,H-5'), 4.16 (1H,m,H-4'), 4.30-4.60 (2H,m,H-2' & H-3'), 5.16 (1H,d,H-5, $J_{5,6}$=8Hz), 5.60 (1H,bs,3'-OH), 6.12 (1H,d,H-1', $J_{1',2'}$=4Hz), 7.30 (15H,bs,aromatics), 7.70 (1H,d,H-6, $J_{5,6}$=8Hz), 11.10 (1H,bs,$\underline{N}^3$-H).

Microanalysis requires: C, 61.21; H, 4.58; N, 5.09.

found : C, 61.03; H, 4.59; N, 5.14.

3.8) 5'-O-Trityl-3'-O-mesyl-2'-bromo-2'-deoxyuridine (5b).

Prepared by the same method used for (5a). Yield 95%.

U.v. (MeOH): λ_{max} 262 nm.

^1H n.m.r. ($CDCl_3$,δ): 3.00 (3H,s,-$SO_2\underline{Me}$), 3.56 (2H,m,H-5'), 4.35 (1H,m,H-4'), 4.52 (1H,t,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=6Hz), 5.08 (1H,br t,H-3',$J_{2',3'}=J_{3',4'}$=6Hz), 5.32 (1H,d,H-5, $J_{5,6}$=8Hz), 6.20 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.30 (15H,m,aromatics), 7.56 (1H,d,H-6, $J_{5,6}$=8Hz), 8.50 (1H,br s,$\underline{N}^3$-H).

3.9) Heating 5'-O-Trityl-3'-O-mesyl-2'-chloro-2'-deoxyuridine (5a) in pyridine.

A stirred solution of (5a), (0.28g, 5.0 mmol) was heated in dry pyridine (10 mls) at 60°C for 2.5 hrs. Analytical t.l.c. showed only starting material to be present. The solution was heated at 100°C for 18 hrs, then allowed to cool and evaporated to an oil which was dissolved in methanol (10 mls) and poured onto crushed ice. The precipitate was filtered, washed with water and dried in vacuo at 60°C for 18 hrs. T.l.c and ^1H n.m.r. analysis showed it to be starting material.

3.10) Reaction of 5'-O-Trityl-3'-O-mesyl-2'-chloro-2'-deoxyuridine (5a) with NaCl/DMSO.

Compound (5a), (1.0g, 1.72 mmol) was dissolved in dry DMSO (10 ml) and treated with NaCl (1.0g, 17 mmol). The stirred mixture was heated in an oil bath at 140°C for 5 hrs , allowed to cool and poured onto crushed ice. The precipitate was filtered, washed with water and dried in vacuo at 50°C for 2 hrs to give a white solid (0.5g).

T.l.c. and ^{1}H n.m.r. spectroscopy showed it to be starting material.

3.11) 5'-O-Trityl-3'-O-mesyl-O^2, 2'-cyclouridine (7).

DBU (0.35 ml, 2.2 mmol) was added drop by drop to a stirred solution of (5a), (1.17g, 2.0 mmol) in dry DMF (25 mls). The solution was stirred at 20°C for 18 hrs and then evaporated to a yellow oil which was dissolved in methanol (20 mls) and poured onto crushed ice. The white precipitate was filtered, washed with water and dried in a vacuum oven at 60°C for 8 hrs to give an off-white solid (0.7g, 64%). This was used in the next step with no further purification.

T.l.c : R_f=0.5 ($CHCl_3$:MeOH, 4:1, v/v)

U.v. (MeOH): λ_{max} 250 nm (shoulder)

I.r. (nujol, cm^{-1}): 1650 (s,br), 1550 (s), 1180 (m), 1080 (m), 830 (m), 710 (m).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 3.20 (2H,m,H-5'), 3.52 (3H,s,-SO_2Me), 4.88 (1H,m,H-4'), 5.74 (1H,bs,H-3'), 5.94 (1H,d,H-2', $J_{1',2'}$=5Hz), 6.16 (1H,d,H-5, $J_{5,6}$=8Hz), 6.80 (1H,d,H-1', $J_{1',2'}$=5Hz), 7.40-7.70 (15H,bm,3x-Ph), 8.32 (1H,d,H-6, $J_{5,6}$=8Hz).

3.12) Reaction of (5a) with sodium benzoate in DMF.

i) To give (6a).

Compound (5a), (1.29g, 2.2mmol) in DMF (10 ml) was added drop by

drop (over ca. 10 mins) to a stirred suspension of sodium benzoate (1.72g, 12mmol) in dry DMF (60 ml) at 110°C. After 40 mins at this temperature the mixture was allowed to cool and filtered. The filtrate was evaporated to a sticky mass which was dissolved in methanol (20 ml) and poured onto crushed ice. The white precipitate was filtered, washed with water, dissolved in dichloromethane (100 ml) and dried over $MgSO_4$. The solution was evaporated down to give a white solid which recrystallized from methanol (0.60g, 55%).

An alternative work up using column chromatography to purify the product gave better isolated yields (ca. 70%).

M.p. 183-185°C.

U.v.(MeOH): λ_{max} 260 nm, ε=8990

I.r.(nujol,cm^{-1}): 3260 (w), 1730 (s), 1695 (s), 1640 (m), 1270 (s), 1090 (m), 720 (m).

^1H n.m.r. ($CDCl_3$,δ): 3.40 (2H,d,H-5', $J_{4',5'}$=3Hz), 4.84 (1H,m,H-4'), 5.04 (1H,d,H-5, $J_{5,6}$=9Hz), 5.96 (1H,t,H-1', $J_{1',3'}$=1.5Hz, $J_{1',4'}$=1.5Hz), 6.80 (1H,dd,H-3', $J_{1',3'}$=1.5Hz, $J_{3',4'}$=3Hz), 7.10-7.40 (15H,bm,3x-Ph), 7.70 (1H,d,H-6, $J_{5,6}$=9Hz), 9.50 (1H,bs,$\underline{N}^3$-H).

^{13}C n.m.r ($CDCl_3$,δ): 64.45 (t,C-5', ^1J=142Hz), 84.44 (dd,C-4', ^1J=150Hz, ^3J=10Hz), 88.02 (s,-O$\underline{C}$-Tr), 88.86 (dd,C-1', ^1J=174Hz, ^3J=7Hz), 103.39 (d,C-5, ^1J=177Hz), 127.96 (d,C-3', ^1J=160Hz), 127.79 & 128.32 & 129.04 (dm,$\underline{C}$-2- & $\underline{C}$-3- & $\underline{C}$-4-Tr, ^1J=165Hz), 129.31 (s,C-2'), 146.62 (dt,C-6, ^1J=183Hz, ^3J=4Hz), 143.49 (s,$\underline{C}$-1-Tr), 151.23 (d,C-2, ^3J=7Hz), 163.47 (d,C-4, ^3J=11Hz).

Microanalysis requires: C, 69.06; H, 4.76; N, 5.75; Cl, 7.28.

found : C, 69.07; H, 4.85; N, 5.57; Cl, 7.38.

Mass spec.(E.I.): (No M+ formed), 260 (7), 244 (13), 243 (18), 154 (32), 133 (59), 112 (71), 105 (89), 102 (100), 101 (83), 69 (51).

ii) To give (6a) and (7).

To a stirred suspension of sodium benzoate (1.5g, 11mmol) in DMF (50 mls) heated at 105°C, was added compound (5a), (1.2g, 2 mmol) in one portion. After 1 hr the mixture was allowed to cool and filtered. The filtrate was evaporated to a sticky mass, dissolved in methanol (20 ml) and poured onto crushed ice. The precipitate was filtered, washed with water and dried in vacuo for 17 hrs. T.l.c. analysis indicated that the solid was a mixture of two compounds (R_f=0.8 and 0.45, $CHCl_3$:MeOH 4:1). The solid was loaded onto a silica gel column (60g, 0.063-0.200 mesh) and eluted with chloroform:methanol (15:1, v/v). Seventeen fractions of 10 mls were collected. Fractions 6 and 7 were combined and evaporated to give a white solid (0.32g, 26%).

T.l.c., u.v., i.r. and ^{1}H n.m.r. data for this compound proved identical to those of compound (6a).

Fractions 12 to 17 were combined and evaporated down to a white solid (0,3g, 24%).

T.l.c., u.v., i.r. and 1H n.m.r. data for this compound proved identical to those of compound (7).

3.13) Reaction of (5a) with sodium methoxide in methanol to give (6a).

Sodium (1.60g, 70mmol) was dissolved in methanol (100 ml) and the solution heated to reflux temperature. A solution of (5a), (14.0g, 24mmol) in methanol (150 ml) was added drop by drop (over ca. 10 mins). After 1 hr, benzoic acid (5.6g, 46mmol) was added and the mixture evaporated down to give a solid. This was partitioned between water (300 ml) and dichloromethane (300 ml). The aqueous layer was extracted with further dichloromethane (100 ml and 50 ml). The combined organic extracts were washed with water (100 ml), dried over $MgSO_4$ and evaporated to give an oil. Methanol was added and the solution evaporated down to a crispy foam which

was recrystallized from methanol, (8.07g, 69%).

T.l.c., m.p., u.v., i.r., ^{1}H n.m.r. data for this compound proved identical with those of compound (6a) prepared in Section 3.12.i.

Microanalysis requires: C, 69.06; H, 4.76; N, 5.75.

found ; C, 69.17; H, 4.48; N, 5.50.

3.14) Heating 5'-O-Trityl-3'-O-mesyl-2'-chloro-2'-deoxyuridine (5a) in HMPA.

A stirred solution of (5a), (0.3g, 0.5 mmol) in dry HMPA (20 ml) was stirred at 145°C for 9 hrs. The dark brown solution was poured onto crushed ice and the precipitate was filtered, washed with water and dried in vacuo at 60°C for 18 hrs. An off-white solid was obtained (0.19g). T.l.c. and ^{1}H n.m.r. showed it to be approximately a 1:1 mixture of (6a) and (7).

3.15) 2'-Chloro-2,3'-didehydro-2',3'-dideoxyuridine (8a).

i) Using TFA/BuOH[249]

Compound (6a) ,(0.55g, 2.2mmol) was dissolved in a 3:1 (v/v) mixture of butanol:TFA (40 ml) and stirred for 0.5 hr at 20°C. Excess butanol was then added (60 ml) and the solution evaporated to give an oil. Analytical t.l.c. showed that the reaction was far from completion.

ii) Using HCl/MeOH

The oil obtained from the TFA/BuOH reaction was dissolved in methanol and treated with 2M HCl (1 ml, 2mmol) and stirred at 20°C for 24 hrs. The reaction mixture was evaporated to an oil, loaded onto a silica gel column (45g, 0.063-0.200 mesh) and eluted with $CHCl_3$:MeOH (10:1, v/v). Fractions of 10 ml were collected. The combined fractions 11 to 16 were evaporated to a solid (0.22g, 80%) which was crystallized from benzene:ethanol (10:1, v/v).

M.p. 154-156°C T.l.c. Rf=0.50 ($CHCl_3$:MeOH,4:1, v/v)

U.v. (MeOH): λ_{max} 260 nm ε=9,255

I.r. (nujol,cm^{-1}): 3400 (m,br), 3160 (w), 3100 (w), 1705 (s), 1690 (s), 1650 (m,shoulder), 1420 (m), 1260 (m), 1100 (m).

^1H n.m.r. ($DMSO_{d6}$,δ): 3.67 (2H,m,H-5'), 4.94 (1H,m,H-4'), 5.20 (1H,bs,5'-OH), 5.75 (1H,d,H-5, $J_{5,6}$=7Hz), 6.68 (1H,t,H-1', $J_{1',3'}$=1.5Hz, $J_{1',4'}$=1.5Hz), 6.82 (1H,dd,H-3', $J_{1',3'}$=1.5Hz, $J_{3',4'}$=3Hz), 7.96 (1H,d,H-6, $J_{5,6}$=7Hz).

Double resonance studies in Appendix I.

Microanalysis requires: C, 44.18; H, 3.71; N, 11.45; Cl, 14.49.

found : C, 44.30; H, 3.88; N, 11.25; Cl, 13.84.

Mass spec. (EI): M+ 245 (0.2), 135 (23), 133 (70), 112 (80), 105 (34), 103 (100), 102 (68), 69 (61).

3.16) 3'-O-Mesyl-O^2,2'-cyclouridine (9).

Compound (7), (0.7g,1.3 mmol) was dissolved in a mixture of butanol:TFA (3:1,v/v,40 mls) and stirred at 20°C for 3 hrs. T.l.c. of the solution showed the reaction to be incomplete. Butanol was added and the mixture evaporated to an oil. On addition of ether (30 mls) a solid formed which was washed with more ether (2x30 mls) and then methanol (20 mls). The white solid obtained (0.15g, 41%) was homogeneous on t.l.c. R_f=0.15 ($CHCl_3$:MeOH,4:1).

M.p.=210-213°C.

U.v. (MeOH): λ_{max} 226 nm (ε=8,100), 250 nm (ε=9,290)

I.r. (nujol,cm^{-1}): 3200(m), 3080 (w), 3040 (w), 1670 (s), 1620 (s), 1530 (s), 1180 (s), 1060 (s), 960 (s), 830 (s).

^1H n.m.r. ($DMSO_{d6}$,δ): 3.50 (2H,bs,H-5'), 3.56 (3H,s,$-SO_2Me$), 4.70 (1H,m,H-4'), 5.40 (1H,bs,5'-OH), 5.68 (1H,bs,H-3'), 5.86 (1H,d,H-2',

$J_{1',2'}$=5Hz), 6.12 (1H,d,H-5, $J_{5,6}$=8Hz), 6.70 (1H,d,H1', $J_{1',2'}$=5Hz), 8.16 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 39.47; H, 3.98; N, 9.21; Cl, 0.0; S, 10.54.

found : C, 39.63; H, 3.94; N, 9.40 ; Cl, 0.0; S, 10.34.

Mass spec.(EI): M+ 304(20), 179(65), 177(100), 149(32), 137(88), 112(17), 96(63), 79(73), 70(53), 69(75), 68(65), 64(80).

3.17) Reaction of (5b) with sodium benzoate to give (7).

Compound (5b) was subjected to the same reaction conditions as was (5a) in Section 3.12.i. The only product isolated (ca. 50% yield) was shown to be identical to (7) by: T.l.c., u.v., i.r. and ^{1}H n.m.r.

3.18) 5'-O-Trityl-2'-bromo-2',3'-didehydro-2',3'-dideoxyuridine (6b).

Compound (6b) was prepared from (5b) by the method described in Section 3.13. Yield: 66%

M.p. 142-143°C.

U.v. (MeOH): λ_{max} 258 nm ε=9,060

I.r. (nujol,cm^{-1}): 3180 (w), 3060 (w), 1720 (s), 1680 (s), 1630 (m), 1260 (m), 1080 (m), 1030 (w).

^{1}H n.m.r. ($CDCl_3$,δ): 3.45 (2H,br d,H-5', $J_{4',5'}$=3Hz), 4.85 (1H,m,H-4'), 5.10 (1H,d,H-5, $J_{5,6}$=8.5Hz), 6.24 (1H,t,H-1', $J_{1',3'}$=1.5Hz, $J_{1',4'}$=1.5Hz) 6.92 (1H,dd,H-3', $J_{1',3'}$=1.5Hz, $J_{3',4'}$=3.5Hz), 7.20-7.50 (15H,m,3x-Ph), 7.75 (1H,d,H-6, $J_{5,6}$=8.5Hz), 9.50 (1H,br s,N^3-H).

Microanalysis requires: C, 63.28; H, 4.36; N, 5.27.

found : C, 63.01; H, 4.34; N, 5.35.

3.19) 5'-O-Trityl-O^2,2'-cyclouridine (10) from (4a).

Compound (4a) ,(1.0g, 2.0mmol) was dissolved in dry HMPA (15 ml) together with diphenyl carbonate (0.46g, 2.15mmol) and stirred at 140°C for 15 mins. Sodium bicarbonate (ca. 0.2g, 2.4mmol) was added and a mild effervescence resulted. After 45 mins it was removed from the oil bath, allowed to cool and poured onto crushed ice. The precipitate was filtered, washed with water, dissolved in ethyl acetate, dried over $MgSO_4$ and evaporated to give a white solid (0.6g, 65%) which crystallized from methanol.

M.p. 194-196°C. Lit. 205-208°C[52]

U.v. (MeOH): λ_{max} 250 nm (shoulder)

I.r. (nujol,cm^{-1}): 3280 (w), 3080 (w), 3040 (w), 1650 (s),1630 (s), 1530 (s), 1100 (m), 1040 (m), 710 (m).

^1H n.m.r. ($CDCl_3$/$DMSO_{d6}$,δ): 3.00 (2H,m,H-5'), 4.40 (2H,m,H-3' & H-4'), 5.32 (1H,d,H-2', $J_{1',2'}$=5Hz), 5.95 (1H,d,H-5, $J_{5,6}$=8Hz), 6.00 (1H,d, 3'-OH, $J_{3'-H,-OH}$=5Hz), 6.44 (1H,d,H-1', $J_{1',2'}$=5Hz), 7.40 (15H,m,3x-Ph), 7.88 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 71.02; H, 5.11; N, 5.92.

found : C, 71.28; H, 5.01; N, 6.10.

3.20) 5'-O-Trityl-2'-chloro-2',3'-didehydro-2',3'-dideoxyuridine (6a) from (4a).

Compound (4a), (1.0g, 2.0mmol) and triphenylphosphine (0.8g, 3.0mmol) were dissolved in dry THF (40 ml) and stirred for 30 mins at 20°C under a dry nitrogen atmosphere. A solution of diethyl azodicarboxylate (0.5ml, 3.0mmol) in THF (15 ml) was added drop by drop over 15 mins. One hour after the addition was complete the solution was evaporated to give a thick oil. Chloroform (20 ml) was added and the mixture evaporated again.

This was repeated once more.

T.l.c. and ^{1}H n.m.r. analysis of the crude oil showed it to contain (6a) as the sole nucleoside product of the reaction.

3.21) 3'-O-Mesyl-2'-chloro-2'-deoxyuridine (11).

Compound (5a), (3.64g, 6.2mmol) was dissolved in methanol (50 ml) and treated with 2M HCl (5 ml, 10mmol) and stirred at 20°C for 18 hrs. The reaction mixture was evaporated to dryness, triturated with ether (20 ml) and the solid residue recrystallized from methanol (1.51g, 71%).

M.p. 170-172°C

U.v. (MeOH): λ_{max} 260 nm, ε=9,750

I.r. (nujol, cm^{-1}): 3440 (m,br), 3200 (w), 3040 (w), 1700 (s,br), 1270 (m,br), 1180 (s), 1100 (m), 900 (m), 820 (m).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 3.50 (4H,bs,5'-OH &-SO_2Me), 3.90 (2H,bs,H-5'), 4.56 (1H,bd,H-4', $J_{3',4'}$=3Hz), 5.16 (1H,bt,H-2', $J_{1',2'}$=7Hz, $J_{2',3'}$=6Hz), 5.50 (1H,dd,H-3', $J_{2',3'}$=6Hz, $J_{3',4'}$=3Hz), 5.70 (1H,bs,N^3-H), 6.00 (1H,d,H-5, $J_{5,6}$=8Hz), 6.30 (1H,d,H-1', $J_{1',2'}$=7Hz), 8.24 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 35.25; H, 3.84; N, 8.22.

found : C, 34.87; H, 3.77; N, 7.86.

3.22) 2',5'-Di-O-Trityl-uridine (12).

Prepared by the method of Yung et al.[106]

A solution of uridine (5.0g, 20.5mmol) and triphenylchloromethane (17.0g, 61mmol) in dry pyridine was stirred at 20°C for 18 hrs. It was then heated at 110°C for 4.5 hrs, allowed to cool and evaporated to a thick oil. The oil was dissolved in methanol (20 ml) and poured onto crushed ice to give a sticky solid which was triturated with hot water (2x300 ml). The residual solid was dissolved in dichloromethane (250 ml), dried over $MgSO_4$ and

evaporated to an oil. This was recrystallized twice from benzene/ether to give a white solid (3.95g, 26%).

M.p. 220-222°C Lit. 224-225°C[106]

U.v. (MeOH): λ_{max} 264 nm

I.r. (nujol,cm^{-1}): 3510 (w), 3380 (w), 1725 (s), 1700 (s), 1500 (m), 1275 (m), 1095 (w), 710 (s).

^{1}H n.m.r. ($CDCl_3$,δ): 2.44 (1H,s,3'-OH), 2.88 (1H,bd,H-3', $J_{2',3'}$=5Hz), 3.20 (2H,bs,H-5'), 4.08 (1H,bs,H-4'), 4.60 (1H,dd,H-2', $J_{1',2'}$=7.5Hz, $J_{2',3'}$=5Hz), 5.24 (1H,d,H-5, $J_{5,6}$=8Hz), 6.70 (1H,d,H-1', $J_{1',2'}$=7.5Hz), 7.20-7.70 (30H,m,6x-Ph), 7.80 (1H,d,H-6, $J_{5,6}$=8Hz) 9.80 (1H,bs,$\underline{N}^3$-H).

Microanalysis requires: C, 77.45; H, 5.53; N, 3.84.

found : C, 77.45; H, 5.61; N, 3.61.

3.23) 3'-O-Mesyl-2',5'-di-O-trityl-uridine (13).

Prepared by the method of Yung et al.[106]

The mesylation procedure used in Section 3.5 was applied and crystallization from ethanol was performed. Yield: 80%

M.p. 230-232°C Lit. 225-226°C[106]

U.v. (MeOH): λ_{max} 262 nm

I.r. (nujol,cm^{-1}): 3320 (w), 1730 (s), 1500 (m), 1180 (s), 1150 (m), 710 (s).

^{1}H n.m.r. ($CDCl_3$,δ): 2.92 (3H,s,-SO_2Me), 3.28 (2H,bs,H-5'), 4.32 (1H,bd,H-3', $J_{2',3'}$=5Hz), 4.48 (1H,bs,H-4'), 4.70 (1H,dd,H-2',$J_{1',2'}$=8Hz, $J_{2',3'}$=5Hz), 5.22 (1H,d,H-5, $J_{5,6}$=9Hz), 6.36 (1H,d,H-1', $J_{1',2'}$=8Hz), 7.00-7.80 (31H,m,6x-Ph & H-6), 9.75 (1H,bs,$\underline{N}^3$-H).

Microanalysis requires: C, 71.45; H, 5.25; N, 3.47.

found : C, 71.65; H, 5.33; N, 3.29.

3.24) 2',5'-Di-O-Trityl-O^2,3'-cyclouridine (14).

i) Attempt using DBU/DMF.

The same procedure and conditions used to prepare (7) in Section 3.11 were tried.

However t.l.c. and proton n.m.r. showed that the product was identical to starting material.

ii) Using sodium benzoate in DMF.

Prepared by the method of Yung et al.[106]

Compound (13), (2.0g, 2.5mmol) and sodium benzoate (4.0g, 28mmol) in dry DMF (100 ml) were stirred at 130°C for 20 hrs. The mixture was allowed to cool, filtered and the filtrate evaporated to give a sticky solid. This was dissolved in methanol (25 ml) and poured onto crushed ice. The precipitate was filtered, washed with water and recrystallized from ethanol, (0.53, 30%).

M.p. 239-240°C Lit. 237°C[106]

U.v. (MeOH): λ_{max} 252 nm (shoulder)

I.r. (nujol, cm^{-1}): 1650 (s), 1635 (s), 1515 (s), 1495 (m), 1180 (s), 710 (s).

^{1}H n.m.r. ($CDCl_3$, δ): 3.32 and 3.40 (2H, 2xs, H-5'), 3.60 (1H,s,H-3'), 4.48 (1H,s,H-1' or -2' or -4'), 4.54 (2H,bs,H-1' or -2' or -4'), 5.78 (1H,d,H-5, $J_{5,6}$=7Hz), 6.36 (1H,d,H6, $J_{5,6}$=7Hz), 6.80-7.80 (30H,m,6x-Ph).

Microanalysis requires: C, 79.42; H, 5.38; N, 3.94.

found : C, 79.33; H, 5.41; N, 4.14.

3.25) Reaction of (13) with KCl/18-crown-6/DMF.

Compound (13), (0.41g, 0.5mmol) in dry DMF (5 ml) was added to a stirred solution of potassium chloride (0.19g, 2.5mmol) and 18-crown-6 (0.135g, 0.5mmol) in DMF at 130°C and kept at this temperature for 4 hrs The mixture was allowed to cool and evaporated to an oil. Addition of methanol (20 ml) caused a solid to form which was filtered, washed with water (20 ml), then methanol (20 ml) and dried *in vacuo* (0.315g). M.p., t.l.c. and ^{1}H n.m.r. showed this solid to be starting material.

3.26) Reaction of (14) with LiCl/DMF.

A stirred solution of compound (14), (0.28g, 0.4mmol) and lithium chloride (0.1g, 2.3mmol) in dry DMF was heated at 105°C. Benzoic acid (0.05g, 0.4mmol) was added slowly over *ca*. 3 hrs. After 3.5 hr the solution was allowed to cool and evaporated to afford an oil. This was dissolved in methanol (10 ml) and poured onto crushed ice. The precipitate was filtered, washed with water, dissolved in dichloromethane (50 ml) and dried over $MgSO_4$. The solution was concentated to give a white solid (0.25 g). T.l.c. and proton n.m.r. analysis showed this to be starting material.

3.27) 5'-O-Trityl-uridine (15).

Prepared by the method of Levene *et al*.[58]

A solution of uridine (1), (10.0g, 41mmol) and triphenylchloromethane (13.0g, 47mmol) in dry pyridine (130 ml) was stirred at 20°C for 18 hrs. The dark orange solution was then heated at 100°C for 3 hrs, allowed to cool and evaporated to a gum. This was dissolved in methanol (30 ml) and poured into cold water (800 ml) to give a sticky solid. The mixture was heated on a steam bath, allowed to cool and the water decanted off. This process was repeated once more. The residual solid was recrystallized from ethanol (14.67g, 74%).

M.p. 197-198°C Lit. 198-201°C[53b]

U.v. (MeOH): λ_{max} 262 nm ε=9,100

I.r. (nujol, cm^{-1}): 3100-3550 (m), 3050 (w), 1710 (s), 1675 (s), 1650 (m), 1265 (m), 1110 (s), 1055 (m), 770 (m).

^1H n.m.r.(250 MHz, $DMSO_{d6}$, δ): 3.28 (1H,br d,H-5', $J_{5',5'}$=10Hz), 3.38 (1H,dd,H-5', $J_{5',5'}$=10Hz, $J_{4',5'}$=5Hz), 4.04 (1H,m,H-4'), 4.1-4.2 (2H,m,H-2' & H-3'), 5.39 (1H,d,H-5, $J_{5,6}$=8Hz), 5.83 (1H,d,H-1', $J_{1',2'}$=3Hz), 7.2-7.5 (15H,m,3x-Ph), 7.79 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 69.12; H, 5.39; N, 5.76.

found : C, 69.21; H, 5.56; N, 5.61.

3.28) 5'-O-Trityl-2',3'-di-O-mesyl-uridine (16).

Compound (15) was mesylated by the standard procedure described in Section 3.5 to give compound (16) as a homogeneous foam in excellent yields (95-100%).

U.v. (MeOH): λ_{max} 258 nm ε=9,400

I.r. (nujol,cm^{-1}): 3360 (m), 1720 (s), 1690 (s), 1360 (m), 1180 (m).

^1H n.m.r. (250 MHz, $CDCl_3$, δ): 3.09 (3H,s,$-SO_2Me$), 3.21 (3H,s,$-SO_2Me$), 3.61 (2H,m,H-5'), 4.37 (1H,m,H-4'), 5.3-5.5 (3H,m,H-2' & H-3' & H-5), 6.00 (1H,d,H-1', $J_{1',2'}$=4Hz), 7.30 (15H,m,3x-Ph), 7.72 (1H,d,H-6, $J_{5,6}$=8.5Hz), 9.72 (1H,br s,N^3-H).

3.29) 1-(2,3-Epoxy-5-O-trityl-β-D-lyxofuranosyl) uracil (17).

Prepared by a modification of the method described by Brokes et al.[129]

A stirred suspension of (16), (33.0g, 51 mmol) in 80% ethanol (500 ml) was treated with 6M NaOH (9.5ml, 57mmol) and heated under reflux for 15 mins. The resulting solution was allowed to cool to 40°C, treated with 6M NaOH (20ml, 120mmol) and stirred at 25°C for 3 hrs. 2M acetic acid was added to

neutralise the solution which was then evaporated to a sticky mass. This was partitioned between water (450 ml) and dichloromethane (450 ml). The aqueous layer was extracted with further dichloromethane (450 ml) and the combined organic layers were dried over $MgSO_4$ and evaporated to a solid which recrystallized from IPA (20.06g, 83%).

M.p. 120-121°C.

U.v. (MeOH): λ_{max} 259 nm ε=10,000

I.r. (nujol, cm^{-1}): 3370 (w), 1705 (s), 1685 (s), 760 (m), 700 (m).

^1H n.m.r. ($CDCl_3$, δ): 3.35 (2H,m,H-5'), 3.80 (2H,m,H-2' & H-3'), 4.10 (1H,t,H-4', $J_{4',5'}$=6Hz), 5.55 (1H,d,H-5, $J_{5,6}$=8Hz), 6.10 (1H,s,H-1'), 7.0-7.4 (16H,m,H-6 & 3x-Ph), 9.50 (1H,br s,N^3-H).

Microanalysis requires: C, 71.78; H, 5.16; N, 5.98.

found : C, 71.52; H, 5.22; N, 5.77.

3.30) 1-(2,3-epoxy-5-O-trityl-β-D-lyxofuranosyl)-N^3-methyluracil (18).

A stirred solution of (17), (1.0g, 2.1mmol) and methyliodide (2.42g, 17mmol) in dry THF (10 ml) and under a nitrogen atmosphere, was treated with sodium hydride (50% in oil, 0.25g, 5mmol) and kept at 25°C for 22 hr. T.l.c. indicated that the reaction was still incomplete. Saturated ammomium chloride (75 ml) was added and the mixture extracted with ether (3x100ml). The ether extracts were washed with water (75 ml), dried over $MgSO_4$ and evaporated to a gum. 'Flash chromatography' [153] (Ethyl acetate:hexane, 1:1) was used to separate the two components of the mixture, fractions of 10 ml being collected. Fractions 25 to 43 were evaporated down to a white foam (0.3g). This was the required product. Fractions 85 to 120 yielded recovered starting material (t.l.c. and ^1H n.m.r.), (0.4g).

Yield: 49%

U.v. (MeOH): λ_{max} 260 nm

^{1}H n.m.r. (60 MHz, $CDCl_3$,δ): 3.30 (3H,s,$\underline{N}^3$-Me), 3.40 (2H,m,H-5'), 3.80 (2H,m,H-2' & H-3'), 4.20 (1H,t,H-4', $J_{3',4'}$=6Hz, $J_{4',5'}$=6Hz), 5.60 (1H,d,H-5, $J_{5,6}$=8Hz), 6.15(1H,s,H-1'), 7.10-7.50 (16H,m,H-6 & 3x-Ph).

3.31) Reaction of (17) with ammonium bromide to give (19a) and (19b).

A solution of (17), (1.53g, 3.3mmol) and ammomium bromide (0.8g, 7.2mmol) in ethanol (25 ml) was heated under reflux for ca. 60 hrs. The solution was evaporated to a white solid, this was dissolved in methanol and evaporated again to a solid. Crystallization from methanol gave a white solid (0.49 g) which by t.l.c. and proton n.m.r. consisted of two components. Separation of these two compounds proved inefficient but was achieved as follows: the crude reaction product was loaded onto a silica gel column (0.063-0.200 mesh).

i) Eluting with CH_2Cl_2:MeOH, 13:1 (v/v) allowed separation of a small amount of pure compound (19a) which crystallized from methanol.

M.p. 195-196°C

U.v. (MeOH): λ_{max} 260 nm

^{1}H n.m.r. ($DMSO_{d6}$,δ): 3.40 (2H,m,H-5'), 4.25 (2H,m,H-3' & H-4'), 4.65 (1H,t,H-2', $J_{1',2'}$=7Hz, $J_{2',3'}$=7Hz), 5.30 (1H,d,H-5, $J_{5,6}$=8Hz), 6.15 (1H,d,H-1', $J_{1',2'}$=7Hz), 7.25-7.50 (15H,m,aromatics), 7.75 (1H,d,H-6, $J_{5,6}$=8Hz), 11.50 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: C, 61.21; H, 4.59; N, 5.10.

found : C, 61.45; H, 4.64; N, 5.37.

ii) Alternatively eluting the column with ethyl acetate / ether, 3:2 (v/v) allowed the isolation of pure compound (19b) which crystallized from methanol.

M.p. 216-218°C

U.v. (MeOH): λ_{max} 260 nm

I.r. (nujol,cm^{-1}): 3500-3300 (m), 3150 (m), 3050 (w), 1670 (br s), 1260 (m), 1120 (m), 1090 (m), 710 (m).

^{1}H n.m.r. ($CDCl_3$,δ): 3.30 (2H,m,H-5'), 4.18 (2H,br s,H-2' & H-3'), 4.44 (1H,m,H-4'), 5.28 (1H,d,H-5, $J_{5,6}$=8Hz), 5.76 (1H,br s,3'-OH), 5.96 (1H,s,H-1'), 7.00-7.40 (16H,m,H-6 & 3-Ph).

Microanalysis requires: C, 61.21; H, 4.59; N, 5.10; Br, 14.54.

found : C, 61.20; H, 4.50; N, 5.04; Br, 14.33.

3.32) Reaction of (17) with vinylmagnesium bromide/CuI in THF.

A stirred suspension of CuI (0.10g, 0.53mmol) in dry THF (5 ml), kept at -30°C and under a nitrogen atmosphere, was treated with a solution of vinylmagnesium bromide (1M in THF, 5.3 ml, 5.3mmol) added drop by drop. A grey-green suspension soon formed and the mixture was stirred at -30°C for 15 min. A solution of (17), (1.0g, 2.1mmol) in dry THF (8 ml) was added slowly and the mixture kept at -30°C for 15 min then allowed to warm slowly (0.5 hr) to 0°C and stirred for 2 hr. Saturated ammonium chloride (100 ml) was added and the mixture extracted with ether (100 ml and 2x50 ml). The combined ether layers were washed with sat. NH_4Cl (50 ml), dried over $MgSO_4$ and evaporated to a pale yellow foam. T.l.c. analysis showed three components to be present. The foam was passed down a 'flash chromatography' column eluted with EtOAc / Cyclohexane, 1:1 (v/v). The fractions containing the slowest running component were combined and evaporated to a white foam (0.12g, 11%), which was shown to be compound (20a).

U.v. (MeOH): λ_{max} 263 nm.

I.r. (nujol,cm^{-1}): 3400 (br m), 3060 (w), 1710 (s), 1690 (s), 1620 (m), 1270 (m), 1050 (m), 700 (m).

^{1}H n.m.r. (250 MHz,$DMSO_{d6}$,δ): 2.83 (1H,m,H-3'), 3.25 (2H,m,H-5'), 3.90 (1H,dt,H-4', $J_{3',4'}$=10Hz, $J_{4',5'}$=3.5Hz), 4.33 (1H,dd,H-2',

$J_{1',2'}$= 6 Hz, $J_{2',3'}$=8.5Hz), 5.2 (2H,2 x dd, C=CH_2, J_{cis}=10Hz, J_{trans}=17Hz), 5.27 (1H,d,H-5, $J_{5,6}$=8Hz), 5.72 (1H,m, vinyl=C-H), 6.10 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.4 (15H,m,3x-Ph), 7.87 (1H,d,H-6, $J_{5,6}$=8Hz). Double resonance studies in Appendix I.

The other two components isolated from the column were shown by t.l.c. and proton n.m.r. to be starting material (0.30g) and compound (19a) (0.25g, 21%).

3.33) Reaction of (18) with vinylmagnesium bromide/CuI in THF.

Compound (18) was subjected to the same reaction conditions as described in Section 3.32 using:

i) 1.7 equivalents of vinylmagnesium bromide.

ii) 4.0 equivalents of vinylmagnesium bromide.

In both cases no reaction occured and starting material was recovered as shown by t.l.c. and proton n.m.r.

3.34) Reaction of (17) with lithium acetylide / diaminoethane / DMSO to give (21a).

A suspension of the lithium acetylide / diaminoethane complex (5.0g, 54 mmol) in dry DMSO (50 ml) and under a dry nitrogen atmosphere was treated with a solution of (17), (6.0g, 13mmol) in dry DMSO (50 ml) and stirred at 20°C for 17 hrs. Saturated ammonium chloride (250 ml) was added and the mixture extracted with ether (3x250ml). The combined ether extracts were washed with water (150 ml), brine (200 ml), dried over $MgSO_4$ and evaporated to a yellow foam. Purification was effected by 'flash chromatography' using dichloromethane : ethanol, 20:1 (v/v) as eluent and collecting fractions of 100 ml. The combined fractions 12 to 20 were evaporated down to a foam (3.9g, 62%) which was shown to be compound (21a).

U.v. (MeOH): λ_{max} 263 nm.

I.r.(nujol,cm^{-1}): 3600 (m), 3450 (m), 3300 (sharp, s), 1685 (br s), 1640 (m), 1060 (m), 760 (m).

^{1}H n.m.r. (250 MHz,$CDCl_3$,δ): 3.08 (1H,dt,H-3', $J_{2',3'}$=8.5Hz, $J_{3',4'}$=8.5Hz, $J_{3',\equiv CH}$=3Hz), 3.26 (1H,d,-CH, $J_{3',\equiv CH}$=3Hz), 3.3-3.5 (2H,m,H-5'), 4.02 (1H,br dt,H-4', $J_{3',4'}$=8.5Hz), 4.50 (1H,dd,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=8.5Hz), 5.36 (1H,d,H-5, $J_{5,6}$=8Hz), 6.05-6.20 (1H,br s,2'-OH), 6.15 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.30-7.50 (15H,m, 3x-Ph) 7.70 (1H,d,H-6, $J_{5,6}$=8Hz), 11.4 (1H,br s,$\underline{N}^3$-H).

Fraction 11 was evaporated to a foam (0.9g) which by t.l.c and proton n.m.r. was shown to be a 4:1 mixture of two compounds. The major component was (21a), (i.e. total yield ca.70%), while the minor component although not identified may prove to be (21b). The proton n.m.r. spectrum of the above mixture had signals attributable to compound (21a) together with the following signals possibly due to compound (21b).

^{1}H n.m.r. ($CDCl_3$,δ): 4.25 (br s, H-2'), 4.35 (m, H-3'), 5.56 (d,H-5, $J_{5,6}$=8.5Hz), 6.03 (d,H-1', $J_{1',2'}$=1 Hz), 7.30-7.50 (br m, Trityl), 7.56 (d,H-5, $J_{5,6}$=8.5 Hz).

3.35) Reaction of (17) with $LiCuMe_2$/THF to give (22a), (22b) and (55b).

Copper(I) iodide (1.14g, 6.0mmol) in dry THF (20 ml) was stirred at -30°C, under a dry nitrogen atmosphere and treated, slowly, with a solution of methyllithium in ether (1.5M, 8.0 ml, 12.0mmol). The mixture was kept at this temperature for ca. 0.5 hr during which time the CuI dissolved. A solution of compound (17), (0.94g, 2.0mmol) in dry THF (10 ml) was added drop by drop. The mixture was allowed to slowly warm up to 0°C and stirred at this temperature for 18 hr. Saturated ammonium chloride (50 ml) was added and the mixture extracted with ether (3x75 ml). The combined

ether layers were washed with sat. NH_4Cl (2x50 ml), then brine (50 ml), dried over $MgSO_4$ and evaporated down to a white foam (0.95g). T.l.c. and proton n.m.r. analysis of this foam showed it to contain three components in the approximate ratio of 7:1:1.

i) Work-up 1.

The major component (22a), was obtained pure by fractional crystallization from the above foam using methanol (Yield: ca. 32%).

ii) Work-up 2.

The foam was passed down a silica gel column (100g, 0.063-0.200 mesh) and eluted with CH_2Cl_2:MeOH, 10:1 (v/v). Two of the components were obtained pure.

Compound (22a).

Yield: 50%

M.p. 196-197°C

U.v. (MeOH): λ_{max} 263 nm. ε=10,340.

I.r. (nujol, cm^{-1}): 3390 (br m), 3060 (w), 1710 (s), 1680 (s), 1620 (w), 1280 (m), 1115 (m), 1040 (m), 700 (m).

^1H n.m.r. ($CDCl_3$, δ): 1.00 (3H,d,3'-Me, $J_{3',-Me}$=6.75Hz), 2.35 (1H,m,H-3'), 3.30 (1H,m,1xH-5'), 3.60 (2H,m,H-4' & 1xH-5'), 4.20 (1H,dd,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=9Hz), 5.40 (1H,d,H-5, $J_{5,6}$=8Hz), 6.10 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.30-7.60 (15H,m, 3x-Ph), 8.20 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 71.88; H, 5.83; N, 5.78.

found : C, 71.92; H, 5.84; N, 5.79.

Compound (55b).

Yield 6%.

T.l.c. and proton n.m.r. of this compound were identical with those for the alternative preparation in Section 3.76 below.

3.36) 3'-C-Ethenyl-3'-deoxy-ara-uridine (23) from (20a).

The detritylation procedure described in Section 3.21 was used. Compound (23) recrystallized from EtOH / EtOAc.

Yield: 57% m.p. 187.5-188.5°C

U.v. (MeOH): λ_{max} 263 nm. ε=10,300

I.r. (nujol,cm^{-1}): 3400 (s), 3300 (s), 3040 (w), 1680 (s), 1640 (s), 1400 (m), 1270 (s), 1140 (m), 1080 (m), 930 (w).

^{1}H n.m.r. (250 MHz,$DMSO_{d6}$,δ): 2.65 (1H,m,H-3'), 3.54 (1H,m,1xH-5'), 3.7-3.8 (2H,m,H-4' & 1xH-5'), 4.32 (1H,m,H-2'), 5.1-5.3 (3H,m,=CH_2 & 5'-OH), 5.61 (1H,d,H-5, $J_{5,6}$=9Hz), 5.65 (1H,d,2'-OH), 5.78 (1H,m,=CH-), 6.06 (1H,d,H-1', $J_{1',2'}$=7Hz), 7.94 (1H,d,H-6, $J_{5,6}$=9Hz), 11.3 (1H,br s,$\underline{N}^3$-H).

Mass spec.(C.I./$NH4^+$): $(MNH4)^+$272 (15), $(MH)^+$ 255 (100), 237 (10), 130 (12), 113 (25)

An alternative preparation is given in Section 3.54 below.

3.37) 3'-C-Ethynyl-3'-deoxy-ara-uridine (24) from (21a).

The detritylation procedure described in Section 3.21 was used. Compound (24) was crystallized from methanol / ether.

Yield: 94% m.p. 199-200°C

U.v. (MeOH); λ_{max} 262 nm ε=10,400

I.r. (nujol,cm^{-1}): 3370-3240 (m), 3295 (sharp, s), 1685 (s), 1655 (s), 1280 (m), 1120 (m), 1050 (m), 830 (m).

^{1}H n.m.r. (250 MHz,$DMSO_{d6}$,δ): 2.93 (1H,dt,H-3', $J_{2',3'}$=7.5Hz, $J_{3',4'}$=7.5Hz, $J_{3',\equiv CH}$=3Hz), 3.25 (1H,d,-CH, $J_{3',\equiv CH}$=3Hz), 3.63 and 3.80 (2H,ABX,2xH-5'), 3.90 (1H,br dt,H-4', $J_{3',4'}$=7.5Hz), 4.49

(1H,dd,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=7.5Hz), 5.64 (1H,d,H-5, $J_{5,6}$=9Hz), 6.11 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.80 (1H,d,H-6, $J_{5,6}$=9Hz).

Microanalysis requires: C, 52.38; H, 4.80; N, 11.11.

found : C, 52.19; H, 4.66; N, 10.89.

Mass spec. (C.I./NH4+): (MNH_4)+ 270 (5), (MH)+ 253 (100), 141 (3), 130 (4), 113 (46), 94 (4), 81 (8), 70 (5), 53 (5).

3.38) 3'-C-Methyl-3'-deoxy-ara-uridine (25) from (22a).

The detritylation procedure described in Section 3.21 was used. Compound (25) was recrystallized from ethanol.

Yield: 97% m.p. 202-203°C

U.v. (MeOH): λ_{max} 263 nm ε=9,960

I.r. (nujol,cm^{-1}): 3420-3200 (m), 3040 (w), 1680 (s), 1660 (s), 1630 (m), 1270 (m), 1130 (m), 1060 (m).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 1.05 (3H,d,3'-Me, $J_{3',-Me}$=6.75Hz), 2.00 (1H,m,H-3'), 3.50-3.90 (3H,m,H-4' & H-5'), 4.05 (1H,m,H-2'), 5.15 (1H,t,5'-OH, $J_{5',-OH}$=5Hz), 5.60 (1H,d,2'-OH, $J_{2',-OH}$=6Hz), 5.70 (1H,d,H-5, $J_{5,6}$=8Hz), 6.10 (1H,d,H-1', $J_{1',2'}$=6.75Hz), 8.05 (1H,d,H-6, $J_{5,6}$=8Hz), 11.5 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: C, 49.58; H, 5.83; N, 11,57.

found : C, 49.45; H, 5.85; N, 11.45.

Mass spec. (C.I./NH4+): (MNH_4)+ 260 (5), (MH)+ 243 (100), 148 (10), 130 (20), 113 (85), 95 (5), 83 (5), 70 (7).

3.39) Reaction of (17) with butyllithium / THF to give (26).

A solution of butyllithium in ether (2.7M, 2.0ml,5.4mmol) was added to dry THF (10 ml), under a dry nitrogen atmosphere. The resulting solution was cooled to -78°C with stirring. Compound (17), (1.0g,2.1mmol) in dry THF (15 ml) was added slowly and stirring continued at this temperature for 1 hr. An orange colouration developed. The solution was allowed to slowly warm up to -20°C (ca. 1 hr.), treated with saturated ammonium chloride (50 ml) and allowed to warm to 20°C. Water (50 ml) was added and the mixture extracted with ether (3x50 ml). The combined ether layers were washed with water (50 ml), then brine (50 ml), dried over $MgSO_4$ and evaporated to a yellow foam (0.9g). T.l.c. and proton n.m.r. showed starting material to be present together with a new product in approximately equal amounts. This foam was loaded onto a silica gel column (60g,0.063-0.200 mesh) and eluted with CH_2Cl_2: MeOH, 15:1 (v/v), fractions of 10 ml being collected. The combined fractions 15 to 20 were evaporated to a solid which gave an amorphous white solid from ethyl acetate / ether (0.33g, 33%).

M.p. 75-76°C

U.v. (MeOH): λ_{max} 258 nm. ε =9,160

I.r. (nujol,cm^{-1}): 3500-3200 (br m), 3040 (w), 1740-1650 (br s), 1270 (m), 1160 (m), 760 (m), 700 (m).

^1H n.m.r. ($DMSO_{d6}/CDCl_3$,δ): 3.50 (2H,m,H-5'), 4.40-4.60 (2H,m,H-4' & 3'-OH), 4.74 (1H,dd,H-3', $J_{2',3'}$=3Hz, $J_{3',4'}$=6Hz), 5.40 (1H,d,H-2', $J_{2',3'}$=3Hz), 5.64 (1H,d,H-5, $J_{5,6}$=8Hz), 7.05-7.40 (15H,m, 3x-Ph), 7.60 (1H,d,H-6, $J_{5,6}$=8Hz), 10.50 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: C, 71.78; H, 5.16; N, 5.98.

found : C, 71.36; H, 5.27; N, 5.93.

3.40) Reaction of (17) with lithium 1,3-dithian-2-yl / THF to give (27).

A stirred solution of 1,3-dithiane (0.64g, 5.3mmol) in dry THF (20 ml) at -40°C and under a dry nitrogen atmosphere, was treated with a solution of butyllithium in hexane (2.7M,2.0 ml,5.5mmol) added drop by drop. The clear solution was kept at -40 to -25°C for 2hrs. Compound (17), (1.0g,2.1mmol) in dry THF (12 ml) was added slowly at -30°C. The solution which became orange in colour, was allowed to warm up slowly to 0°C (ca. 1 hr) and kept at this temperature for 2 hrs. Saturated ammonium chloride (50 ml) was added and the mixture was extracted with ether (3x50 ml). The combined ether extracts were washed with brine (50 ml), dried over Na_2SO_4 and evaporated to a solid. This was loaded onto a silica gel column (90g, 0.063-0.200 mesh) and eluted with CH_2Cl_2:EtOH, 14:1 (v/v); fractions of 20 ml being collected. The combined fractions 11 to 16 were evaporated to a solid (0.20g) which by t.l.c. and proton n.m.r. was shown to be recovered (17).

Fractions 8 to 10 were evaporated to a solid which crystallized from ethyl acetate / ether (0.43g, 34%).

M.p. 208-210°C

U.v. (MeOH): λ_{max} 257 nm. ε=3,470

I.r. (nujol,cm^{-1}): 3200 (w), 3060 (w), 1720 (m), 1700 (s), 1680 (s), 1230 (m), 1070 (m), 1050 (m).

^1H n.m.r. ($CDCl_3$,δ): 1.95 (2H,m,-S-C-CH_2), 2.60-3.00 (6H,m,-SCH_2- & H-5), 3.28 (2H,d,H-5', $J_{4',5'}$=6Hz), 3.70-3.95 (3H,m,H-2' & H-3' & H-6), 4.15 (1H,t,H-4', $J_{4',5'}$=6Hz), 4.40 (1H,d,S-CH-S, $J_{SCH,6}$=6Hz), 5.90 (1H,s,H-1'), 7.10-7.40 (15H,m, 3x-Ph), 7.95 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: C, 65.28; H, 5.48; N, 4.76; S, 10.89.

found : C, 65.29; H, 5.50; N, 4.75; S, 10.65.

3.41) Preparation and separation of the diacetates (28a) and (28b).

The mixture of isomers (22a) and (22b) obtained in the reaction described in Section 3.35, (1.20g, 2.5mmol) was subjected to the detritylation procedure described in Section 3.21 to give (0.50g, 2.0mmol) of a mixture of (25a) and (25b). This mixture was dissolved in dry pyridine (50 ml), treated with excess acetic anhydride (5 ml) and left at 20°C for 18 hrs. Methanol (5 ml) was added, the solution left at 20°C for 1 hr and then evaporated to a gum. This gum was dissolved in ethyl acetate (150 ml) and washed with 1M HCl (2x50ml), then brine (50 ml), dried over $MgSO_4$ and evaporated to a solid. Crystallisation from ethyl acetate gave two crops of compound (28a), (0.385g, 57%).

M.p. 134-135°C.

U.v. (MeOH): λ_{max} 260 nm. ε=10,270

I.r. (nujol, cm^{-1}): 3100 (w), 1745 (s), 1730 (s), 1700 (s), 1680 (s), 1610 (w), 1260 (m), 1220 (m), 1130 (m), 1070 (m).

^1H n.m.r. ($CDCl_3$, δ): 1.10 (3H,d,3'-Me, $J_{3',-Me}$=7.5Hz), 2.00 (3H,s,-OAc), 2.12 (3H,s,-OAc), 2.20 (1H,m,H-3'), 3.90 (1H,m,H-4'), 4.36 (2H,d,H-5', $J_{4',5'}$=4.5Hz), 5.20 (1H,t,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=6Hz), 5.76 (1H,d,H-5, $J_{5,6}$=8Hz), 6.24 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.62 (1H,d,H-6, $J_{5,6}$=8Hz), 9.70 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: C, 51.53; H, 5.56; N, 8.59.

found : C, 51.50; H, 5.58; N, 8.49.

Mass spec. (C.I./NH4+): (MNH_4)+ 344 (4), (MH)+ 327 (100), 215 (70), 113 (6), 95 (8), 43 (8).

Crystallisation of the mother liquors from ethyl acetate/ether gave compound (28b), (0.035g, 5%).

M.p. 113-114°C.

U.v. (MeOH): λ_{max} 260 nm. ε=8,800

^{1}H n.m.r. ($CDCl_3$,δ): 1.30 (3H,d,2'-Me, $J_{Me,2'}$=7.5Hz), 2.00 (3H,s,-OAc), 2.08 (3H,s,-OAc), 2.38 (1H,m,H-2'), 4.20-4.40 (3H,m,H-4' & H-5'), 5.05 (1H,m,H-3'), 5.76 (2H,s & d,H-1' & H-5, $J_{5,6}$=8Hz), 7.64 (1H,d,H-6, $J_{5,6}$=8Hz), 9.28 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: C, 51.53; H, 5.56; N, 8.59.

found : C, 51.48; H, 5.61; N, 8.37.

Mass spec. (C.I./NH4+): (MNH_4)+ 344 (7), (MH)+ 327 (90), 215 (43), 113 (7), 112 (9), 95 (100), 43 (4).

3.42) 5-(p-Tolylthiomethyl)-3'-C-methyl-3'-deoxy-ara-uridine (29).

Prepared by the method of Reese et al.[215]

A solution of (25a), (0.41g,1.7mmol), pyrrolidine (0.7ml,8.5mmol) and 40% aqueous formaldehyde (0.7 ml) in water (15 ml) was heated under reflux for 1.5 hrs. The solution was evaporated to dryness and then co-evaporated with toluene (50 ml), ethanol (3x25 ml) and chloroform (25 ml). The resulting oil was dissolved in dry acetonitrile (25 ml), treated with p-thiocresol (0.42g, 3.4mmol) and heated under reflux for 1.25 hrs. The mixture was then evaporated to a pale green oil. This was loaded onto a silica gel column (100g, 0.063-0.200 mesh) and eluted with dichloromethane : methanol, 15:1 (v/v); fractions of 25 ml being collected. The combined fractions 26 to 36 were evaporated to a foam (0.46g, 72%).

^{1}H n.m.r. ($CDCl_3$,δ): 1.05 (3H,d,3'-Me, $J_{3',-Me}$=7Hz), 1.90 (1H,m,H-3'), 2.28 (3H,s,-Ar-Me), 3.45-3.90 (3H,m,H-4' & H-5'), 3.70 (2H,s,Ar-CH_2-S), 4.08 (1H,dd,H-2', $J_{1',2'}$=5Hz, $J_{2',3'}$=7Hz), 5.95 (1H,d,H-1', $J_{1',2'}$=5Hz), 7.20 (4H,dd,Ar), 7.65 (1H,s,H-6).

This was used in the next section with no further purification.

3.43) 3'-C-Methyl-3'-deoxy-ara-thymidine (31).

Compound (29), (0.44g,1.2mmol) and excess Raney nickel (3.0g) in ethanol (25 ml) were heated under reflux for 1 hr. The mixture was filtered through celite, the catalyst being washed with ethanol (2x20 ml). The filtrate was evaporated to to a solid which crystallized from ethanol (0.23g, 77%).

M.p. 203-204°C

U.v. (MeOH): λ_{max} 272 nm ε=10,000

I.r. (nujol,cm^{-1}): 3440-3200 (m), 3040 (w), 1680 (s), 1640 (s), 1260 (m), 1120 (m), 1060 (m), 780 (w).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 1.04 (3H,d,3'-Me, $J_{3',-Me}$=7Hz), 1.80 (3H,s,5-Me), 2.00 (1H,m,H-3'), 3.40-3.80 (3H,m,H-4' & H-5'), 3.96 (1H,m,H-2'), 5.08 (1H,br t,5'-OH), 5.40 (1H,d,2'-OH), 5.95 (1H,d,H-1', $J_{1',2'}$=6.5Hz), 7.80 (1H,s,H-6), 11.20 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: C, 51.56; H, 6.29; N, 10.93.

found : C, 51.44; H, 6.30; N, 10.95.

Mass spec. (C.I./NH4+): (MH)+ 257 (10), 127 (10).

(E.I.) : (M)+ 256 (7), 238 (2), 207 (9), 131 (45), 130 (7), 127 (77), 126 (85), 113 (62), 112 (13), 103 (18), 83 (80).

3.44) 5-(p-Tolylthiomethyl)-3'-C-ethyl-3'-deoxy-ara-uridine (30).

Compound (40) was converted to (30) by the same procedure described in Section 3.42. The compound was isolated as a foam and used in the next step with no further purification.

Yield: 67%

^{1}H n.m.r. (250 MHz,$DMSO_{d6}$,δ): 0.95 (3H,t,3'-CH_2-$\underline{Me}$, $J_{-CH_2,-Me}$=7.5Hz) 1.45 (2H,m,3'-$C\underline{H}_2$-Me), 1.85 (1H,m,H-3'), 2.28 (3H,s,Ar-$\underline{Me}$), 3.20-3.70 (3H,m,H-4' & H-5'), 3.78 (2H,s,Ar-$C\underline{H}_2$-), 4.05 (1H,m,H-2'), 5.10 (1H,br

s,-OH), 5.40 (1H,br s,-OH), 5.90 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.40 (4H,dd,Ar), 7.84 (1H,s,H-6), 11.4 (1H,br s,$\underline{N}^3$-H).

3.45) 3'-C-Ethyl-3'-deoxy-ara-thymidine (32).

Compound (32) was prepared from (30) by the same procedure used in Section 3.43.

Yield: 62%

M.p. 183-184°C.

U.v. (MeOH): λ_{max} 268 nm ε=9,000

I.r. (nujol,cm^{-1}): 3430-3200 (m), 1690 (br,s), 1270 (m), 1130 (m), 1070 (m), 720 (m).

^{1}H n.m.r. (250 MHz,$DMSO_{d6}$,δ): 0.98 (3H,t,3'-CH_2-Me, $J_{CH_2,-Me}$=7.5Hz), 1.46 (2H,quintet,3'-$C\underline{H}_2$-), 1.88 (3H,s,5-Me), 1.92 (1H,m,H-3'), 3.5-3.8 (3H,m,H-4' & H-5'), 4.08 (1H,t,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=6Hz), 5.40 (2H,br s,2x-OH), 5.94 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.76 (1H,s,H-6), 11.2 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: C, 53.32; H, 6.71; N, 10.36.

found : C, 53.13; H, 6.77; N, 10.16.

Mass spec. (C.I./NH_4+): (MH)+ 271 (39), 253 (3), 162 (6), 144 (11), 127 (100), 112 (19), 109 (6).

3.46) 5-Bromo-3'-C-ethyl-3'-deoxy-ara-uridine (33).

A solution of (40), (0.4g,1.6mmol) in acetic acid (10 ml) was treated with NBS (0.36g, 2.0mmol) and heated at 130°C for 1hr. The solution was evaporated to dryness to give an oil. This was loaded onto a silica gel column (50g, 0.063-0.200 mesh) and eluted with dichloromethane : methanol, 9:1 (v/v). The required fractions were evaporated to a solid which crystallized from ethanol / ethylacetate (0.16g, 31%).

M.p. 165-166°C.

U.v. (water): λ_{max} 284 nm ε=9,620

I.r. (nujol,cm^{-1}): 3470 (sharp,s), 3350 (m), 3180 (m), 3060 (w), 1690 (s), 1650 (s), 1620 (m), 1270 (m), 1150 (m), 1080 (m), 770 (m).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 0.92 (3H,t,3'-CH_2-Me, $J_{CH_2,-Me}$=7.5Hz), 1.40 (2H,m,3'-CH_2-), 1.84 (1H,m,H-3'), 3.35-3.65 (3H,m,H-4' & H-5'), 3.92 (1H,m,H-2'), 5.00 (1H,br t,5'-OH, $J_{5',-OH}$=5Hz), 5.20 (1H,d,2'-OH, $J_{2',-OH}$=6Hz), 5.66 (1H,d,H-1', $J_{1',2'}$=6Hz), 8.00 (1H,s,H-6).

Microanalysis requires: C, 39.41; H, 4.51; N, 8.36.

found : C, 39.02; H, 4.45; N, 8.60.

Mass spec. (C.I./NH4+): M+ 336 (10) & 334 (10), 193 (3), 191 (3), 162 (17), 145 (15), 144 (20), 127 (14), 109 (7).

3.47) 5-(p-Tolylthiomethyl)-3'-C-ethynyl-3'-deoxy-ara-uridine (30b).

Compound (30b) was prepared from (24) by the method outlined in Section 3.42 above.

Yield: 70%

^{1}H n.m.r. ($CDCl_3$,δ): 2.34 (3H,s,-Ar-Me), 2.92 (1H,m,H-3'), 3.30-3.50 (3H,m,H-5' & ≡CH), 3.76 (2H,s,-CH_2Ar), 4.04 (1H,m,H-4'), 4.56 (1H,t,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=6Hz), 6.14 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.30 (4H,m,-Ar), 7.70 (1H,s,H-6).

The crude oil was used in the next step with no further purification.

3.48) Hydrogenation of (30b).

i) Using Raney nickel.

The compound (30b), (ca. 1.0g, 2.5mmol) was subjected to the same conditions as described in Section 3.43. A white solid was isolated (0.35g).

T.l.c. and proton n.m.r. however showed this to be a mixture of two compounds. The major component was 3'-ethyl-3'-deoxy-ara-thymidine (32); the minor one being the 3'-vinyl analogue. This mixture was used below in Section 3.48.ii.

ii) Using 10% Pd/C.

The mixture of compounds from above, (ca. 0.32g), was subjected to the hydrogenation procedure described in section 3.53. A white solid was obtained (0.30g, 93%). This was shown to be identical to compound (32) by m.p., u.v., i.r. and proton n.m.r.

Microanalysis requires: C, 53.32; H, 6.71; N, 10.37.

found : C, 52.90; H, 6.57; N, 10.16.

3.49) 2',5'-Di-O-Acetyl-3'-C-ethynyl-3'-deoxy-ara-uridine (34).

Compound (34) was prepared from (24) by the same procedure described in Section 3.41. Compound (34) was crystallized from ethyl acetate (yield: 76%).

M.p. 112-114°C.

U.v. (MeOH): λ_{max} 259 nm.

I.r. (nujol, cm^{-1}): 3290 (w), 3260 (sharp,m), 1750 (s), 1730 (s), 1690 (br,s) 1610 (s), 1260 (m), 1240 (m), 1220 (m), 1160 (m).

^{1}H n.m.r. ($CDCl_3$,δ): 2.04 (3H,s,-OAc), 2.14 (3H,s,-OAc), 2.40 (1H,d,≡CH, $J_{3',\equiv CH}$=3Hz), 3.10 (1H,m,H-3'), 4.30 (1H,m,H-4'), 4.46 (2H,m,H-5'), 5.60 (1H,t,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=6Hz), 5.80 (1H,d,H-5, $J_{5,6}$=8Hz), 6.35 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.55 (1H,d,H-6, $J_{5,6}$=8Hz), 9.45 (1H,br s,$\underline{N}^3$-H).

^{13}C n.m.r. ($CDCl_3$,δ):

20.51 ($-O_2C\underline{Me}$), 20.83 ($-O_2C\underline{Me}$), 37.43 (C-3'), 63.15 (C-5'), 74.22

(acetylenic), 75.10 (shoulder on $CDCl_3$, acetelynic), 76.43 (C-2'), 78.58 (C-4'), 84.70 (C-1'), 102.02 (C-5), 140.95 (C-6), 150.19 (C-2), 163.54 (C-4), 169.27 (-OCOMe), 170.76 (-OCOMe).

Microanalysis requires: C, 53.57; H, 4.80; N, 8.33.

found : C, 53.45; H, 4.89; N, 8.29.

3.50) Preparation of compound (35).

Prepared by the method of Sung.[216]

A solution of (34), (0.65g, 2.0mmol) in dry pyridine (50 ml) was cooled in an ice-bath and treated with (4-chlorophenyl)phosphodichloridate (0.74g, 3.0mmol) followed by 1,2,4-triazole (0.41g, 6.0mmol). The solution was then stirred at 20°C for 72 hrs. The dark orange solution was then evaporated (at ca. 30°C) to a gum which was dissolved in dichloromethane (50 ml) and extracted with water (2x60 ml), then saturated $NaHCO_3$ (60 ml) and dried over $MgSO_4$. The solution was evaporated to afford a pale brown solid (0.45g, 60%). It was used in the next step with no further purification.

^1H n.m.r. ($CDCl_3$,δ): 1.96 (3H,s,-OAc), 2.16 (3H,s,-OAc), 2.44 (1H,d,$\equiv$CH, $J_{3',\equiv CH}$=3Hz), 3.10 (1H,m,H-3'), 4.40-4.56 (3H,m,H-4' & H-5'), 5.84 (1H,t,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=6Hz), 6.44 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.20 (1H,d,H-5, $J_{5,6}$=8Hz), 8.24 (1H,s,Triazole), 8.35 (1H,d,H-6, $J_{5,6}$=8Hz), 9.40 (1H,s,Triazole).

3.51) 3'-C-Ethynyl-3'-deoxy-ara-cytidine (36).

Compound (35), (0.43g, 1.1mmol) was dissolved in a 3:1 (v/v) mixture of dioxane and 0.880 ammonia (20 ml) and stirred at 20°C for 4 hrs. The solution was evaporated to an oil, treated with a 1:1 (v/v) mixture of 0.880 ammonia and methanol (20 ml) and stirred at 20°C for 1.5 hrs. The solution was then evaporated to an oil which was loaded onto a silica gel column (50g, 0.063-0.200 mesh) and eluted with CH_2Cl_2 : MeOH, 4:1 (v/v).

The required fractions were evaporated to a white solid which crystallized from water / ethanol (0.21g, 75%).

M.p. 232-234°C

U.v. (water): λ_{max} 275 nm. ε=9,440

I.r. (nujol,cm^{-1}): 3480 (sharp,s), 3320-3100 (br,s), 3300 (sharp,s), 1660-1620 (br,s), 1530 (s), 1500 (s), 1290 (m), 1120 (m), 1050 (s), 795 (m).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 2.76 (1H,m,H-3'), 3.04 (1H,d,≡CH, $J_{3',\equiv CH}$=3Hz), 3.40-3.80 (3H,m,H-4' & H-5'), 4.20 (1H,m,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=6Hz), 4.96 (1H,br t,5'-OH), 5.50 (1H,d,H-5, $J_{5,6}$=8Hz), 5.60 (1H,d,2'-OH, $J_{2',-OH}$=6Hz), 5.90 (1H,d,H-1', $J_{1',2'}$=6Hz), 6.85 (2H,br s,$-NH_2$), 7.40 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 52.59; H, 5.21; N, 16.72.

found : C, 52.29; H, 5.30; N, 16.52.

Mass spec.(C.I.; NH_4+): (MH)+ 252 (20), 158 (6), 141 (8), 127 (5), 112 (100), 111 (10), 74 (3).

3.52) 3'-C-Ethenyl-3'-deoxy-ara-cytidine (37).

A solution of (36), (0.16g, 0.64mmol) in methanol (15 ml) and water (5 ml) was treated with 5% Pd/$BaSO_4$ poisoned with lead (0.03g) and stirred under an atmosphere of hydrogen, at 20°C, till one equivalent of the gas had been absorbed (ca. 50 min). The mixture was filtered through celite and the filtrate evaporated to a solid which crystallized from absolute alcohol (0.15g, 93%).

M.p. 235-237°C.

U.v. (water): λ_{max} 274 nm ε=9,260

I.r. (nujol,cm^{-1}): 3490 (s), 3320-3200 (br s), 1650 (s), 1610 (s), 1490 (s), 1290 (m), 1100 (m), 1050 (m), 800 (m).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 2.50 (1H,m,H-3'), 3.20-3.50 (3H,m,H-4' & H-5'), 4.05 (1H,m,H-2'), 4.90 (2H,br m,2x-OH), 5.05-5.20 (2H,m,-C=CH_2), 5.40-5.80 (2H,m,H-5 & -CH=), 5.85 (1H,d,H-1',$J_{1',2'}$=6Hz), 6.80 (2H,br s,NH_2), 7.52 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 52.16; H, 5.97; N, 16.60.

found : C, 52.31; H, 6.19; N, 16.54.

Mass spec.(C.I./NH_4+): (MH)+ 254 (5), 186 (7), 160 (3), 143 (2), 112 (100), 111 (7), 107 (18), 95 (3).

3.53) 3'-C-Ethyl-3'-deoxy-ara-cytidine (38).

A solution of (36), (0.16g, 0.64mmol) in a 1:1 (v/v) mixture of ethanol / water (15 ml) was treated with 10% Pd/C (0.025g) and stirred under an atmosphere of hydrogen, at 20°C, until absorption of the gas stopped (ca. 30 min). The mixture was filtered through celite and evaporated to a solid which crystallized from ethanol (0.16g, 98%).

M.p. 228-230°C

U.v. (water): λ_{max} 275 nm ε=9,260

I.r. (nujol,cm^{-1}): 3480 (s), 3260 (s), 3200 (m), 3100 (m), 1650 (s), 1630 (s), 1610 (s), 1520 (m), 1490 (s) 1280 (m), 1160 (m), 1030 (m), 780 (m).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 0.94 (3H,t,-CH_3,J_{CH_2,CH_3}=7.5Hz), 1.50 (2H,m,-CH_2-Me), 1.80 (1H,m,H-3'), 3.45 (3H,m,H-4' & H-5'), 3.85 (1H,t,H-2', $J_{1',2'}$= 6 Hz, $J_{2',3'}$= 6 Hz), 4.75 (1H,br s,-OH), 4.96 (1H,br s,-OH), 5.44 (1H,d,H-5, $J_{5,6}$=8Hz), 5.70 (1H,d,H-1', $J_{1',2'}$=6Hz), 6.70 (2H,br s,-NH_2), 7.40 (1H,d,H-6, $J_{5,6}$=8Hz).

Microanalysis requires: C, 51.76; H, 6.71; N, 16.46.

found : C, 51.54; H, 6.85; N, 16.20.

Mass spec. (C.I./NH_4+): (MH)+ 256 (9), 239 (7), 238 (6), 205 (21), 188 (19), 187 (14), 162 (16), 144 (10), 127 (8), 112 (100), 111 (6).

3.54) 3'-C-Ethenyl-3'-deoxy-ara-uridine (23) from (24).

Compound (24) was hydrogenated to give (23) by the procedure described in Section 3.52. The product crystallized from ethanol / ethyl acetate.

Yield: 95%

M.p., u.v., i.r. and proton n.m.r. were identical to those of the sample prepared by the alternative route in section 3.36.

Microanalysis requires: C, 51.97; H, 5.55; N, 11.02.

found : C, 51.99; H, 5.50; N, 11.26.

3.55) 3'-C-Ethyl-3'-deoxy-ara-uridine (39).

Compound (23) was hydrogenated by the procedure described in Section 3.53 to give compound (39) which crystallized from ethanol / ethyl acetate. Yield: 95%

M.p. 183-184°C.

U.v. (MeOH): λ_{max} 259 nm. ε=9,900

I.r. (nujol, cm^{-1}): 3400 (s), 3300(s), 3040 (w), 1700-1650 (br s), 1620 (m), 1400 (m), 1270 (s), 1130 (m), 1070 (s), 1020 (m), 770 (m).

^{1}H n.m.r. ($DMSO_{d6}$, δ): 0.98 (3H,t,-Me, $J_{CH_2,Me}$=7.5Hz), 1.48 (2H,m,-CH_2-Me), 1.98 (1H,m,H-3'), 3.60 (3H,m,H-4' & H-5'), 4.10 (1H,t,H-2', $J_{1',2'}$=5.5Hz, $J_{2',3'}$=5.5Hz), 5.10 (1H,br s,-OH), 5.50 (1H,br s,-OH), 5.60 (1H,d,H-5, $J_{5,6}$=8Hz), 5.96 (1H,d,H-1', $J_{1',2'}$=5.5Hz), 7.87 (1H,d,H-6, $J_{5,6}$=8Hz), 11.30 (1H,br s,N^3-H).

Microanalysis requires: C, 51.56; H, 6.29; N, 10.93.

found : C, 51.40; H, 6.21; N, 10.56.

Mass spec. (C.I./NH_4+): (MNH_4)+ 274 (29), (MH)+ 257 (100), 239 (10), 205 (10), 162 (31), 144 (23), 127 (11), 113 (73).

3.56) 2',5'-Di-O-Acetyl-3'-C-ethenyl-3'-deoxy-ara-uridine (40).

i) From (24).

Compound (24) was hydrogenated by the procedure described in Section 3.52 to give (40) which crystallised from ethyl acetate.

Yield: 95%

M.p. 158-159°C.

U.v. (MeOH): λ_{max} 259 nm. ε=9,900

I.r. (nujol, cm^{-1}): 3120 (w), 3100 (w), 1740 (s), 1710 (s), 1690 (s), 1610 (shrp m), 1240 (s), 1220 (s), 1120 (m), 1070 (m), 930 (m).

^{1}H n.m.r. (250 MHz, $CDCl_3$, δ): 2.00 (3H,s,-OAc), 2.13 (3H,s,-OAc), 2.82 (1H,m,3'-H), 4.05 (1H,m,H-4'), 4.2-4.4 (2H,m,H-5'), 5.2-5.35 (2H,m,C=CH_2), 5.44 (1H,dd,H-2', $J_{1',2'}$=5Hz, $J_{2',3'}$=6Hz), 5.7-5.9 (2H,m,H-5 & -CH=), 6.24 (1H,d,H-1', $J_{1',2'}$=5Hz), 7.57 (1H,d,H-6, $J_{5,6}$=8Hz), 9.20 (1H,br s,N^3-H).

^{13}C n.m.r. ($CDCl_3$,d): 20.51 (-OCOMe), 20.83 (-OCOMe), 49.28 (C-3'), 63.41 (C-5'), 76.04 (C-2'), 78.97 (C-4'), 84.05 (C-1'), 101.89 (C-5), 120.50 (Vinyl), 132.94 (vinyl), 140.95 (C-6), 150.39 (C-2), 163.54 (C-4), 169.52 (-OCOMe), 170.76 (-OCOMe).

Microanalysis requires: C, 53.25; H, 5.36; N, 8.28.

found : C, 53.08; H, 5.46; N, 8.17.

ii) From (23).

Compound (23) was acetylated by the procedure described in Section 3.41 to give (40) which crystallized from ethyl acetate.

Yield: 66%

M.p., u.v., i.r. and proton n.m.r. for this proved identical to those of the compound prepared in Section 3.56.i above.

Microanalysis requires: C, 53.25; H, 5.36; N, 8.28.

found : C, 53.30; H, 5.48; N, 8.17.

3.57) 5'-O-Trityl-3'-C-ethenyl-3'-deoxy-ara-uridine (20a) from (21a).

Compound (21a) was hydrogenated by the procedure described in Section 3.52 to give (20a) isolated as a homogeneous foam (ca. 95%). T.l.c., u.v., i.r. and proton n.m.r. for this compound proved identical to those from the alternative preparation in Section 3.32.

3.58) Reaction of (40) with 9-BBN/THF to give (43).

A stirred solution of 9-BBN (3.25 mmol) in dry THF (10 ml) and under a dry nitrogen atmosphere, was treated with a solution of (40), (0.51g, 1.5mmol) in dry THF (5 ml). The reaction mixture was kept at 24°C for 1hr. Ethanol (2 ml), 6M NaOH (0.65ml, 4.mmol) and 50% aqueous hydrogen peroxide (1 ml) were added in that order and the mixture heated at 55°C for 1 hr. Saturated ammonium chloride solution (50 ml) was added and the mixture was extracted with ether (4x100 ml). The combined ether extract was washed with brine (50 ml), dried over sodium sulphate and evaporated to an oil. T.l.c. analysis showed two components. The oil was loaded onto a 'flash chromatography' column eluted with CH_2Cl_2 : EtOH, 20:1 (v/v). Fractions 9 to 18 were combined and evaporated to a solid (0.30g) which was shown to be recovered (40) by t.l.c. and proton n.m.r.
The combined fractions 28 to 50 were evaporated down to a solid which crystallized from ethyl acetate / ether (0.10g, 22%).

M.p. 173-174°C.

U.v. (MeOH): λ_{max} 262 nm. ε=12,200

I.r. (nujol, cm^{-1}): 3340 (br m), 3180 (m), 3060 (w), 1740 (s), 1725 (s), 1705 (s), 1670 (s), 1240 (s), 1140 (s), 800 (m).

^1H n.m.r. (250 MHz, $DMSO_{d6}$, δ): 2.07 (3H,s,-OAc), 2.56 (1H,m,H-3'),

3.97 (1H,m,H-4'), 4.1-4.25 (2H,m,H-5'), 4.31 (1H,t,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=6Hz), 5.1-5.3 (2H,m,-C=CH_2), 5.63 (1H,d,H-5, $J_{5,6}$=8.5Hz), 5.7-5.9 (2H,m,=CH- & -OH), 6.08 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.58 (1H,d,H-6, $J_{5,6}$=8.5Hz), 11.30 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: C, 52.69; H, 5.44; N, 9.46.

found : C, 52.72; H, 5.45; N, 9.27.

3.59) Reaction of (40) with borane/THF.

i) With 1/3 molar equivalent of borane / THF.

A stirred solution of (40), (0.40g, 1.2mmol) in dry THF (15 ml) and under a dry nitrogen atmosphere was cooled to 0°C and treated with a 1M solution of borane in THF (0.5ml, 0.5mmol). The solution was stirred at 0°C for 1 hr and then treated with ethanol (1ml), 6M NaOH (0.3ml, 1.8mmol) and 30% aqueous hydrogen peroxide, in that order. The mixture was heated at 50°C for 1 hr. Saturated ammonium chloride solution was added (50 ml) and the mixture was extracted with ether (3x75 ml). The combined ether extracts were washed with brine (50 ml), dried over $MgSO_4$ and evaporated to a solid (0.20g, 50%). T.l.c. and proton n.m.r. showed this to be recovered (40).

ii) With 1 molar equivalent of borane / THF.

The same procedure as described above was followed. In this case extractive work up allowed recovery of even less starting material (ca. 10%).

3.60) Reaction of (40) with MCPBA in dichloromethane.

A solution of (40), (0.17g, 0.5mmol) in dichloromethane (15 ml) was treated with MCPBA (85% grade, 0.14g, 0.7mmol) and stirred at 20°C for 0.5 hr. T.l.c. analysis showed only starting material to be present. The solution was heated under reflux for 4 hrs. It was allowed to cool to 20°C and a 10% aqueous solution of sodium sulphite (10 ml) was added. The

mixture was stirred at 20°C for 0.5 hr, then washed with saturated $NaHCO_3$ (50 ml), water (2x50 ml), dried over potassium carbonate and evaporated to give a solid (0.16g).

T.l.c. and proton n.m.r. showed this to be recovered (40).

3.61) Reaction of (40) with MCPBA in 1,2-dichloroethane and a radical inhibitor.

A solution of (40), (0.1g,0.3mmol) in 1,2-dichloroethane (8 ml) was treated with MCPBA (85%, 0.2g, 1mmol) and a few milligrammes of 3-tert-butyl-4-hydroxy-5-methyl-phenylsulphide. The solution was stirred at 85°C for 3 hrs, allowed to cool and treated with an 10% solution of sodium sulphite (15 ml) and stirred at 20°C for 1 hr. Dichloromethane was added (100 ml) and the mixture was washed with water (2x50 ml), dried over $MgSO_4$ and evaporate to ca. 5 ml. Addition of ether (10 ml) caused a solid to crystallize out (0.02g).

m.p. 124-125°C.

U.v. (MeOH): λ_{max} 262 nm

I.r. (Nujol,cm^{-1}): 3100 (w), 1750 (s), 1730 (s), 1690 (s), 1610 (w), 1240 (s), 1220 (s), 1100 (m), 1070 (m).

^1H n.m.r. ($CDCl_3$,δ): 2.00 (s,-OAc), 2.15 (s,-OAc), 2.65 (m,?), 2.90 (m,?), 3.15 (m,?), 4.10 (m,H-4'), 4.36 (br d,H-5'), 5.20-5.50 (br m,H-2' & ?), 5.80 (d, H-5), 6.24 (2xd,H-1'), 7.64 (d,H-6), 9.4 (br s,$\underline{N}^3$-H).

Mass Spec. (C.I./NH_4^+): MNH_4^+ 372 (0.6), 356 (2.3), MH^+ 355 (10), 341 (5.8), 339 (9.2), 243 (3.4), 229 (2.7), 227 (3.5), 198 (0.7), 106 (0.6).

3.62) 5'-O-Trityl-2'-O-acetyl-3'-C-ethenyl-3'-deoxy-ara-uridine (45).

Compound (20a) was acetylated by the procedure described in Section 3.41 to give (45) isolated as a foam.

Yield: 83%

U.v. (MeOH): λ_{max} 263 nm.

I.r. ($CHCl_3$, cm^{-1}): 3060 (m), 1750 (s), 1710 (s), 1690 (s), 1460 (m), 1270 (m), 700 (s).

^1H n.m.r. ($CDCl_3$, δ): 1.94 (3H,s,-OAc), 3.11 (1H,m,H-3'), 3.40 (2H,m,H-5'), 3.85 (1H,m,H-4'), 5.02-5.54 (5H,m,H-2' & H-5 & $-C=CH_2$), 6.15 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.10-7.40 (15H,m,3x-Ph), 7.94 (1H,d,H-6, $J_{5,6}$=8Hz).

3.63) Reaction of (45) with 1 molar equivalent of borane/THF.

The procedure described in Section 3.59 was used, leading to the isolation of one product (0.125g, 90%). T.l.c. and proton n.m.r. showed this to be identical to compound (20a).

3.64) Reaction of (45) with 4 molar equivalents of borane/THF to give (46).

A stirred solution of (45), (0.27g, 0.5mmol) in dry THF (10 ml) and under a dry nitrogen atmosphere, was cooled to 0°C and treated with a 1M solution of borane in THF (2ml, 2mmol). After 2 hrs at this temperature, ethanol (2 ml), 6M NaOH (1.3ml, 0.8mmol) and 30% aqueous hydrogen peroxide (2.6 ml) were added, in that order and the mixture stirred at 15°C for 1 hr. The mixture was poured into ethyl acetate (100 ml), washed with water (2x30 ml), brine (50 ml), dried over $MgSO_4$ and evaporated down to a solid. This was loaded onto a silica gel column (30g, 0.063-0.200 mesh) and eluted with ethyl acetate; fractions of 5 ml being collected. The combined fractions 19 to 25 were evaporated to a crude solid (0.030g), possibly (46).

^{1}H n.m.r. ($CDCl_3/DMSO_{d6}$,δ): 1.60 (2H,m,$-CH_2CH_2OH$), 2.05 (1H,m,H-3'), 3.10 (1H,m,H-4'), 3.30 (2H,m,H-5'), 3.52 (1H,t,CH_2OH, $J_{CH2,CH2}$=7.5Hz), 4.44 (1H,t,H-2', $J_{1',2'}$=5Hz, $J_{2',3'}$=5Hz), 5.32 (1H,d,H-5, $J_{5,6}$=8Hz), 5.88 (1H,d,H-1', $J_{1',2'}$=5Hz), 7.0-7.40 (15H,m,3x-Ph), 7.64 (1H,d,H-6, $J_{5,6}$=8Hz).

3.65) 5'-O-Trityl-2'-O-mesyl-3'-C-ethynyl-3'-deoxy-ara-uridine (47).

Compound (21a) was mesylated by the procedure described in Section 3.5 to give (47) as a white foam homogeneous by t.l.c. This was used in the next step without further purification.

Yield: 97%

U.v. (MeOH): λ_{max} 259 nm.

I.r. (nujol,cm^{-1}): 3290 (shrp m), 3040 (w), 1700 (s), 1680 (s), 1270 (m), 1180 (m), 1120 (m), 1040 (m), 760 (s), 700 (s).

^{1}H n.m.r. ($CDCl_3$,δ): 2.36 (1H,d,≡CH, $J_{3',≡CH}$=3Hz), 3.00 (3H,s,$-SO_2Me$), 3.40-3.60 (3H,m,H-3' & H-5'), 4.00 (1H,m,H-4'), 5.36 (1H,t,H-2',$J_{1',2'}$=6Hz, $J_{2',3'}$=6Hz), 5.44 (1H,d,H-5, $J_{5,6}$=8Hz), 6.20 (1H,d,H-1', $J_{1',2'}$=6Hz), 7.2-7.5 (15H,m,3x-Ph), 7.76 (1H,d,H-6, $J_{5,6}$=8Hz), 9.30 (1H,br s,N^3-H).

An attempt to convert compound (47) to the 2'-O-triflate ester was made. Thus (47), (2.3g, 4.7mmol) in dry THF (50 mls), at 0°C and under a nitrogen atmosphere was treated with sodium hydride (0.20g, 8.3 mmol) for ca. 1.5 hrs. The reaction mixture was cooled to ca. -60°C and treated with trifluoromethylsulphonyl chloride (1.1 ml, 1.72g, 10.2mmol) and stirred at this temperature for ca. 1.25 hrs. The solution was allowed to warm up to -20°C and treated with saturated sodium bicarbonate solution (50 ml). The mixture was extracted with ether (3x70 ml). The combined ether extracts were washed with water (100 ml), sat. sodium chloride (100 ml), dried over $MgSO_4$ and

evaporated to a yellow solid (1.56g). T.l.c. and proton n.m.r. showed this to be a mixture of many components. Column chromatography did not lead to the isolation of any of the required product to be isolated.

3.66) Reaction of (47) with excess NaOAc / DMF / 150°C to give (48).

A stirred solution of compound (47), (0.60g, 1.05mmol) in dry DMF (25 ml) was treated with anhydrous sodium acetate (0.45g, 5.5mmol) and heated at ca. 150°C for 2 hrs. The solution was allowed to cool, evaporated to an oil and partitioned between water (100 ml) and ether (70 ml). The aqueous layer was extracted with further ether (2x70 ml). The combined ether layers were washed with water (50 ml), brine (50 ml), dried over $MgSO_4$ and evaporated to a pale yellow solid (0.46g, 92%) homogeneous on t.l.c. However it failed to crystallise from various solvents.

U.v. (MeOH): λ_{max} 258 nm.

I.r. ($CHCl_3$, cm^{-1}): 3300 (shrp m), 1700 (s), 1690 (s), 1620 (w), 1460 (m), 1260 (m), 700 (s).

^1H n.m.r. ($CDCl_3$, δ): 3.40 (1H,br d,1xH-5', $J_{5',5'}$=11Hz), 3.35 (1H,s,C=CH), 3.72 (1H,dd,1xH-5', $J_{4',5'}$=3Hz, $J_{5',5'}$=11Hz), 4.70-5.00 (2H,m,H-5 & H-4'), 6.12 (1H,br s,H-2'), 7.04 (1H,br d,H-1', $J_{1',4'}$=4Hz), 7.2-7.60 (15H,m,3x-Ph), 7.88 (1H,d,H-6, $J_{5,6}$=8Hz), 9.80 (1H,br s,$\underline{N}^3$-H).

3.67) Reaction of (47) with 1 equivalent of NaOAc / DMF

i) At 120°C.

Compound (47) was treated with one equivalent of anhydrous sodium acetate in dry DMF at 120°C for 2 hrs. Work-up of the reaction as described above (Section 3.66) lead to the isolation of one product identical to (48) by t.l.c. and proton n.m.r. Yield: 72%

ii) At 90°C.

The procedure described in Section 3.67.i was repeated at 90°C for 1 hr. The usual work-up lead to the isolation of a solid. T.l.c. and proton n.m.r showed this to consist of two compounds: starting material (47) and compoumd (48) in approximately equal amounts.

iii) At 70°C.

The procedure described in Section 3.67.i was repeated at 70°C for 0.5 hr. The usual work up lead to the isolation of a solid. T.l.c. and proton n.m.r showed this to be recovered starting material (47).

3.68) Reaction of (47) with sodium benzoate / DMF.

Compound (47), (0.25g, 0.44mmol) and sodium benzoate (0.063g, 0.44mol) in dry DMF (10 ml) were heated at 90°C for 2.25 hr. Work-up as in Section 3.66 lead to the isolation of a foam (0.18g).
T.l.c. and proton n.m.r. showed this to consist of a mixture of starting material (47) and compound (48).

3.69) Heating compound (47) in DMF.

A stirred solution of (47) (0.30g, 0.5mmol) in dry DMF (10 ml) was heated at 150°C for 2.5 hr. Work-up as described in Section 3.66 led to the isolation of a solid (0.28g) identical to starting material by t.l.c. and proton n.m.r.

3.70) Partial hydrogenation of (48) to give (49).

Compound (48), (0.30g, 0.6mmol) in methanol (20 ml) was treated with 10% Pd/C (0.05g) and stirred under an atmosphere of hydrogen gas, at 20°C, till rapid absorption of the gas ceased (ca. 30ml, 1.25 mmol were absorbed). The reaction mixture was filtered through celite and the

filtrate evaporated down to a white foam (0.29g, 96%). It did not prove possible to obtain a crystalline sample.

U.v. (MeOH): λ_{max} 262 nm.

I.r. ($CHCl_3$, cm^{-1}): 1710 (s), 1680 (s), 1460 (m), 1260 (m), 700 (s).

^1H n.m.r. ($CDCl_3$, δ): 1.16 (3H,t,-CH_3, J_{CH_2,CH_3}=7.5Hz), 2.05 (2H,m,-$C\underline{H}_2$-Me), 3.36 (1H,dd, 1xH-5', $J_{4',5'}$=3Hz, $J_{5',5'}$=12Hz), 3.64 (1H,dd,1xH-5', $J_{4',5'}$=3Hz, $J_{5',5'}$=12Hz), 4.68 (1H,m,H-4'), 4.96 (1H,d,H-5, $J_{5,6}$=8Hz), 5.52 (1H,br s,H-2'), 7.00 (1H,m,H-1'), 7.20-7.50 (15H,m,3x-Ph), 7.90 (1H,d,H-6, $J_{5,6}$=8Hz).

3.71) Reaction of (6a) with borane / THF.

A 1M solution of borane in THF (2.2ml, 2.2mmol) was added to dry THF (15 ml) under a dry nitrogen atmosphere. Compound (6a), (1.0g, 2.05mmol) in dry THF (10 ml) was added, drop by drop and the solution stirred at 20°C for 3 hrs. Ethanol (2 ml), 6M NaOH (0.8ml, 0.48mmol) and 30% aqueous hydrogen peroxide were added, in that order, and the mixture heated at 50°C for 1 hr. The mixture was then poured into water (100 ml) and extracted with ether (3x70 ml). The combined ether layer was washed with water (50 ml), brine (50 ml), dried over $MgSO_4$ and evaporated to give an off-white solid.

T.l.c. and proton n.m.r. showed this to be recovered starting material.

An analogous reaction using 2.5 equivalents of 9-BBN in THF also failed. Starting material only being recovered.

3.72) Reaction of (6a) with MCPBA in dichloromethane.

A solution of (6a), (0.49g, 1.0mmol) and MCPBA (85% grade, 0.23g, 1.1mmol) in dichloromethane (25 ml) was stirred at 20°C for 3 hrs. T.l.c. analysis showed only starting material to be present in the reaction

mixture. The solution was heated under reflux for 18 hrs. Work up as described in Section 3.60 lead to the isolation of a single product (0.45g).

T.l.c. and proton n.m.r. showed this to be recovered starting material.

3.73) 2',3'-dideoxyuridine (50).

Compound (8a), (0.14g, 0.6mmol) was dissolved in ethanol (20 ml) and stirred with 10% Pd/C (0.07g) under an atmosphere of hydrogen gas at 20°C for 18 hrs. The reaction mixture was filtered, evaporated to dryness, loaded onto a silica gel column (20g, 0.063-0.200 mesh) and eluted with CH_2Cl_2 : MeOH, 7:1 (v/v); fractions of 5 ml being collected. The combined fractions 8 and 9 were evaporated to give a white solid (0.08g, 66%).

This reaction, repeated under identical conditions and followed by t.l.c. was shown to be complete in 1.75 hrs. No intermediates in the reduction were detected by t.l.c.

U.v. (MeOH): λ_{max} 263 nm Lit. 262 nm.[166]

^{1}H n.m.r. ($DMSO_{d6}$,δ): 1.80-2.40 (4H,m,H-2' & H-3'), 3.84 (2H,m,H-5'), 4.04 (1H,m,H-4'), 4.90 (1H,br s,-OH), 5.68 (1H,d,H-5, $J_{5,6}$=8Hz), 5.90 (1H,dd,H-1'), 7.90 (1H,d,H-6, $J_{5,6}$=8Hz), 11.10 (1H,br s,$\underline{N}^3$-H).

Microanalysis requires: Cl, 0

found : Cl, 0

^{1}H n.m.r. double resonance studies:

Irradiating the multiplet centred at ca.δ=2.0 caused the doublet of doublets assigned to the C-1' proton to collapse to a broad singlet.

3.74) Bromination of (8a) to give (51).

To a stirred solution of (8a), (0.25g, 1.0mmol) in water (12 ml), at 24°C, bromine was added slowly until a faint yellow-orange colour persisted. The solution was evaporated to give a solid which was crystallized from methanol / water, (0.15g, 43%).

M.p. 117-119°C(dec.)

U.v. No absorbance above 220 nm.

I.r. (nujol,cm^{-1}): 3440 (m), 3200 (s), 3080 (s), 1730 (s), 1650 (shrp m), 1130 (m), 1050 (m), 840 (m).

^{1}H n.m.r. (250 MHz, $DMSO_{d6}$,δ): 3.55-3.75 (2H,m,H-5'), 4.56 (1H,d,H-5, $J_{5,6}$=3Hz), 4.84 (1H,br s,H-4'), 5.04 (1H,d,H-6, $J_{5,6}$=3Hz), 6.06 (1H,br s,-OH), 6.56 (1H,t,H-1', $J_{1',3'}$=2Hz, $J_{1',4'}$=2Hz), 6.65 (1H,dd,H-3', $J_{1',3'}$=2Hz, $J_{1',4'}$=4Hz), 7.14 (1H,br s,-OH), 11.13 (1H,br s,N^3-H).

Microanalysis requires: C, 31.65; H, 2.95; N, 8.20.

found : C, 31.57; H, 2.80; N, 8.04.

3.75) Attempted dehydration of (51).

A solution of (51), (0.08g, 0.23mmol) in ethanol (20 ml) was heated under reflux for 12 hrs, then evaporated down to a solid which crystallized from ethanol / hexane (0.04g).

T.l.c. and proton n.m.r. showed this to be recovered starting material.

3.76) Reaction of (6a) with $LiCuMe_2$/THF to give (55a) and (55b).

A suspension of CuI (1.33g, 7mmol) in dry THF (25 ml) was cooled to -10°C and stirred under a dry nitrogen atmosphere. A solution of methyllithium (1.5M, 9.5 ml, 14mmol) was added drop by drop. When the CuI had dissolved (ca. 10 mins), compound (6a), (0.5g, 1mmol) in THF (5 ml) was added slowly and the mixture stirred at 20°C for 24 hrs. The resulting dark brown mixture was treated with sat. NH_4Cl (50 ml) and extracted with ether

(3x70 ml). The ether extracts were washed with sat. NH_4Cl (2x50 ml), dried over $MgSO_4$ and evaporated to give a glassy solid (0.52g). This solid was loaded onto a silica gel column (50g, 0.063-0.200 mesh), eluted with CH_2Cl_2 : MeOH, 25:1, and fractions of 10 ml were collected.

Compounds 11 and 12 were combined and evaporated down to a foam (0.11g). This was compound (55b), 23%.

I.r. (nujol, cm^{-1}): 3040 (w), 1770 (s), 1700 (br s), 1260 (m), 1150 (m), 1070 (m), 700 (m).

^1H n.m.r. ($CDCl_3$, δ): 2.72 (2H,8 lines,H-3'), 3.48 (2H,8 lines,H-5'), 4.68 (1H,m,H-4'), 5.42 (1H,s,H-1'), 5.68 (1H,d,H-5, $J_{5,6}$=8Hz), 7.20-7.60 (16H,m,H-6 & 3x-Ph).

^{13}C n.m.r. ($CDCl_3$, δ): 36.72 (t,C-3', ^1J=134Hz), 65.62 (t,C-5', ^1J=144Hz), 75.46 (d,C-1', ^1J=152Hz), 86.13 (d,C-4', ^1J=162Hz), 87.24 (s,-O<u>C</u>-Tr), 103.32 (d,C-5, ^1J=176Hz), 128.96 & 128.12 & 127.47 (dm, <u>C</u>-2- & <u>C</u>-3- & <u>C</u>-4-trityl, ^1J=160Hz), 143.36 (dm,C-6, ^1J=181Hz), 143.75 (s,<u>C</u>-1-Tr), 150.32 (d,C-2, ^3J=8Hz), 163.67 (d,C-4, ^3J=11Hz), 206.64 (m,C-2').

Fraction 10 was evaporated to afford a glassy solid, 0.20g. T.l.c. and proton n.m.r. showed this to be a <u>ca</u>. 1:1 mixture of compounds (55a) and (55b). Physical data for pure (55a) are given below, Section 3.79.

3.77) <u>Reaction of (6b) with $LiCuMe_2$/THF/5°C to give (53).</u>

A stirred suspension of CuI (1.33g, 7mmol) in dry THF (20 ml) and under a dry nitrogen atmosphere, was cooled to -10°C. A 1.5M solution of methyl lithium in THF (10ml, 15mmol) was added drop by drop and stirring continued until the CuI had dissolved (<u>ca</u>. 15 min). Compound (6b), (0.53g, 1.0mmol) in dry THF (7 ml) was added slowly and the mixture was allowed to warm to <u>ca</u>. 5°C and left stirring for 18 hrs. Saturated ammonium chloride solution was added (100 ml) and the mixture extracted with ether (3x75 ml).

The combined ether layer was washed with ammonium chloride solution (2x50 ml), brine (50 ml), dried over $MgSO_4$ and evaporated to give a white solid (0.44g) which proton n.m.r. indicated was a ca. 10:1 mixture of (53) (i.e. ca.80% yield) and (55a) (i.e. ca. 10% yield).

Compound (53) was crystallized from the mixture using methanol.

M.p. 183-185°C. Lit. 195-197°C [116]

U.v. (MeOH): λ_{max} 259 nm. ε=10,430 Lit. (EtOH): 261 (9,770).[116]

I.r. (nujol, cm^{-1}): 3040 (w), 1720 (s), 1680 (s), 1630 (w), 1240 (m), 1170 (m), 700 (m).

^1H n.m.r. ($CDCl_3$, δ): 3.54 (2H,br d,H-5', $J_{4',5'}$=3Hz), 4.96 (1H,br s,H-4'), 5.08 (1H,d,H-5, $J_{5,6}$=8Hz), 5.84 (1H,br d,H-2' or H-3', $J_{2',3'}$=6Hz,), 6.30 (1H,br d,H-2' or H-3', $J_{2',3'}$=6Hz), 7.04 (1H,m,H-1'), 7.10-7.50 (15H,m,3x-Ph), 7.76 (1H,d,H-6, $J_{5,6}$=8Hz), 9.50 (1H,br s,N^3-H).

Microanalysis requires: C, 74.32; H, 5.35; N, 6.19.

found : C, 74.34; H, 5.41; N, 6.08.

3.78) Reaction of (6b) with $LiCuMe_2$/THF/20°C to give (53).

The same procedure as described in Section 3.77 was followed. The reaction mixture however was left at 20°C for 46 hrs. Work up as in Section 3.77 lead to the isolation of a white solid which was shown by t.l.c. and proton n.m.r. to be identical to compound (53).

3.79) Reaction of (6b) with $LiCuMe_2$/THF/5°C/MeI to give (54) and (55a).

A stirred suspension of CuI (0.48g, 2.5mmol) in dry THF (15 ml) and under a dry nitrogen atmosphere, was cooled to -30°C. A 1.5M solution of methyllithium in THF (3.3ml, 5mmol) was added drop by drop and stirring continued until the CuI had dissolved (ca. 15 min). Compound (6b), (0.265g, 0.5mmol) in dry THF (5 ml) was added slowly and the mixture was allowed to

warm to ca. 5°C and left stirring for 18 hrs. Excess methyl iodide (0.5 ml) was added and the mixture stirred at 5°C for 0.5 hr and then allowed to warm to 20°C and left for a further 5 hrs. Work up as described in Section 3.77 led to the isolation of a foam (0.196 g) which was a ca. 1:1 mixture of compounds (54) and (55a) by t.l.c. and proton n.m.r. The foam was loaded onto a silica gel column (30g, 0.063-0.200 mesh) and eluted with CH_2Cl_2 : MeOH, 24:1 (v/v); fractions of 5 ml being collected. The combined fractions 7 and 8 were evaporated to afford a glassy solid, compound (54) (0.065g, 26%).

U.v. (MeOH): λ_{max} 262 nm.

I.r. ($CHCl_3$,cm^{-1}): 3050 (m), 1710 (s), 1660 (s), 1630 (m), 1460 (s), 1290 (m), 1180 (m), 700 (s).

^{1}H n.m.r. ($CDCl_3$,δ): 1.70 (3H,s,2'-Me), 3.30 (3H,s,$\underline{N}^3$-Me), 3.38 (2H,br d,H-5', $J_{4',5'}$=4Hz), 4.88 (1H,m,H-4'), 5.24 (1H,d,H-5, $J_{5,6}$=8Hz), 5.84 (1H,br s,H-3'), 6.88 (1H,br s,H-1'), 7.20-7.60 (15H,m,3x-Ph), 7.68 (1H,d,H-6, $J_{5,6}$=8Hz).

Mass spec. (C.I./NH_4^+): 244 (51.7) , 243 (81.2), 223 (20.1), 183 (20.2) 165 (5.7), 127 (41.5), 114 (1.7), 113 (1.3), 106 (1.5), 105 (17) 95 (8.2).

The combined fractions 10 to 13 were evaporated to a glassy solid, compound (55a) (0.08g, 33%).

I.r. ($CHCl_3$,cm^{-1}): 3050 (m), 1710 (s), 1690 (s), 1630 (s), 1460 (s), 1250 (m), 790 (s).

^{1}H n.m.r. ($CDCl_3$,δ): 1.72 (3H,s,2'-Me), 3.40 (2H,br d,H-5', $J_{4',5'}$=4Hz), 4.88 (1H,m,H-4'), 5.16 (1H,d,H-5, $J_{5,6}$=8Hz), 5.85 (1H,m,H-3'), 6.90 (1H,m,H-1'), 7.30-7.60 (15H,m,3x-Ph), 7.76

(1H,d,H-6, $J_{5,6}$=8Hz), 9.90 (1H,br s,$\underline{N}^3$-H).

Double Resonance Studies: Irradiating the signal for the 3'-proton at δ=5.85 caused the signal at δ=1.72 (i.e. for the 2'-methyl) to sharpen.

^{13}C n.m.r. ($CDCl_3$,δ): 11.59 (q,3'-Me, ^{1}J=128Hz), 64.84 (t,C-5', ^{1}J=142Hz), 85.29 (d,C-4', ^{1}J=138Hz), 87.63 (s,-O-$\underline{C}$-Tr), 91.34 (d,C-1', ^{1}J=150Hz), 102.86 (d,C-5, ^{1}J=177Hz), 127.60 & 128.18 & 129.04 (m,$\underline{C}$-2- & $\underline{C}$-3- & $\underline{C}$-4-Tr, & C-3', ^{1}J=160Hz), 135.35 (s,C-2'), 141.21 (d,C-6, ^{1}J=158), 143.49 (s,$\underline{C}$-1-Tr), 151.23 (d,C-2, ^{3}J=8Hz), 163.47 (d,C-4, ^{3}J=12Hz).

Mass spec. (C.I./NH_4+): 243 (43.4), 226 (6.6), 209 (3.0), 183 (3.1), 165 (1.6), 130 (5.2), 114 (1.9), 113 (15.3), 112 (1.4), 105 (2), 95 (7).

3.80) Preparation of ara-adenosine (56).

Ara-adenosine was prepared from adenosine in seven steps by the method outlined by Chattopadhyaya and Reese[77] and Divakar and Reese[76] in overall yield of ca. 30%. This was shown to be identical to commercially available ara-adenosine (ex. Aldrich) which was also used in some of the experiments below.

3.81) 1-(2',3'-epoxy-β-D-lyxofuranosyl)-adenine (57).

Prepared by the method of Mengel et al.[157]

A suspension of ara-adenosine (57), (1.0g, 4.0mmol) and triphenylphosphine (1.57g, 6.0mmol) in dry dioxane (150 ml) and under a nitrogen atmosphere, was stirred at 70°C for 15 mins. A solution of diethyl azodicarboxylate (1.05g, 6.0mmol) in dioxane (15 ml) was added drop by drop. The temperature was slowly raised to 90°C (over ca. 0.5 hr) and stirring continued for 1 hr. The reaction mixture was allowed to cool, filtered and the filtrate was evaporated to an oil. Benzene (50 ml) was added and the precipitate was

filtered, washed with benzene, ether and crystallized from ethanol (0.58g, 62%).

M.p. 195-197°C Lit.208-210°C.[98]

U.v. (MeOH): λ_{max}259 nm. Lit. 258 nm.[98]

I.r. (nujol,cm^{-1}): 3400-3050 (br s), 1680 (s), 1610 (s), 1580 (m), 1330 (s), 1300 (s), 1200 (m), 1050 (s), 830 (m).

^{1}H n.m.r. ($DMSO_{d6}$,δ): 3.68 (2H,br d,H-5', $J_{4',5'}$=6Hz), 4.00-4.40 (3H,m,H-2' & H-3' & H-4'), 5.00 (1H,br s,-OH), 6.28 (1H,s,H-1'), 7.28 (2H,br s,$-NH_2$), 8.12 & 8.16 (2x 1H,2x s,H-2 & H-8).

3.82) 1-(5'-O-t-butyldimethylsilyl-2',3'-epoxy-β-D-lyxofuranosyl)-adenine (58).

A solution of (57), (0.50g, 2.0mmol) and imidazole (0.33g, 4.8mmol) in dry DMF was treated with t-butyldimethylsilylchloride (0.36g, 2.4mmol) and stirred at 20°C for ca. 20 hrs. The mixture was evaporated to an oil which was dissolved in ethyl acetate (100 ml), washed with water (2x50 ml), brine (50 ml), dried over $MgSO_4$ and evaporated to give a white solid which crystallized from ethyl acetate (0.492, 67%).

M.p. 166-167°C

U.v. (MeOH): λ_{max} 257 nm ε=15,180

I.r. (nujol,cm^{-1}): 3350 (s), 3160 (s), 1660 (s), 1600 (s), 1580 (s), 1330 (m), 1250 (m), 1090 (s), 900 (s), 830 (s).

^{1}H n.m.r. ($CDCl_3$,δ): 0.10 (6H,s,$Si-Me_2$), 0.92 (9H,s,$Si-^tBu$), 3.86 (2H,d,H-5', $J_{4',5'}$=6.5Hz), 4.00-4.24 (3H,m,H-2' & H-3' & H-4'), 6.32 (1H,s,H-1'), 6.50 (2H,br s,$-NH_2$), 8.16 & 8.32 (2 x 1H,2 x s,H-2 & H-8).

^{13}C n.m.r. ($CDCl_3$,δ): -5.66 (q,$-SiMe_2$, ^{1}J=119Hz), 18.10 (s,-Si-C≡), 25.65 (qm,-Si-CMe, ^{1}J=125Hz), 56.64 (d,C-3' or C-2', ^{1}J=194Hz), 56.97 (d,C-2' or C-3', ^{1}J=190Hz), 60.94 (t,C-5', ^{1}J=145Hz), 78.06 (dm,C-4', ^{1}J=148Hz), 80.86 (dd,C-1', ^{1}J=163Hz, ^{3}J=13Hz), 119.07 (d,C-5, ^{3}J=12Hz),

139.39 (dd,C-8, ^{1}J=215Hz, ^{3}J=4Hz), 149.87 (dd,C-4, ^{3}J=4Hz, ^{3}J=11Hz), 153.39 (d,C-2, ^{1}J=200Hz), 155.92 (d,C-6, ^{3}J=11Hz).

Microanalysis requires: C, 52.87; H, 6.93; N, 19.27.

found : C, 52.76; H, 6.97; N, 19.23.

3.83) Reaction of (58) with $LiCuMe_2$/THF/5°C.

A stirred suspension of CuI (1.35g, 7.1mmol) in dry THF (30 ml) and under a dry nitrogen atmosphere, was cooled to -10°C. A 1.5M solution of methyl lithium in THF (9.5ml, 14.3mmol) was added drop by drop and the mixture stirred at -10°C till the CuI had dissolved. Compound (58), (0.475g, 1.3mmol) in dry THF (12 ml) was added slowly and the solution was kept at -10°C for 1 hr, then at ca. 5°C for 18 hrs. Saturated ammonium chloride solution (100 ml) was slowly added and the mixture was extracted with ethyl acetate (3x70 ml). The combined organic layer was washed with ammonium chloride solution (2x50 ml), brine (50 ml), dried over $MgSO_4$ and evaporated to give a white solid (0.45g). This was loaded onto a silica gel column (50g, 0.063-0.200 mesh) and eluted with ethyl acetate; fractions of 10 mls being collected.

The combined fractions 30 to 54 were evaporated to afford a white solid (0.13g). T.l.c. showed that two components were present. Proton n.m.r. showed signals for compound (61), (see section 3.58) and for compound (62).

Compound (62).

^{1}H n.m.r. ($CDCl_3$,δ): 0.10 (6H,s,$-SiMe_2$), 0.90 (9H,s,$-Si^{t}Bu$), 2.96 (2H,8 lines,H-3'), 3.92 (2H,8 lines,H-5'), 4.64 (1H,br m,H-4'), 6.48 (2H,br s,$-NH_2$), 7.88 & 8.06 (2 x 1H,2 x s,H-2 & H-8).

The combined fractions 59 to 64 were evaporated to give a white solid (0.25g), which by proton n.m.r. was shown to consist of a ca. 8:1 mixture of the isomers (59) (i.e. ca. 45% yield) and (60) (i.e. ca. 6% yield). Recrystallisation of this mixture from chloroform / carbon tetrachloride

gave pure (59), (0.19g, 38%).

M.p. 139-140°C.

U.v. (MeOH): λ_{max} 259 nm. ε=15,300

I.r. (nujol,cm^{-1}): 3200 (m), 3150 (s), 1670 (s), 1600 (s), 1520 (m), 1300 (m), 1210 (m), 1100 (m), 1030 (m), 880 (m).

^{1}H n.m.r. ($CDCl_3$,δ): 0.15 (6H,s,Si-Me_2), 0.95 (9H,s,Si-^{t}Bu), 1.20 (3H,d,3'-Me, $J_{3',Me}$=7Hz), 2.45 (1H,m,H-3'), 3.90 (2H,br d,H-5', $J_{4',5'}$=9Hz), 4.10 (1H,dd,H-4', $J_{3',4'}$=4Hz, $J_{4',5'}$=9Hz), 4.25 (1H,dd,H-2', $J_{1',2'}$=6Hz, $J_{2',3'}$=7.5Hz), 5.70 (1H,br s,2'-OH), 6.23 (1H,d,H-1', $J_{1',2'}$=6Hz), 6.40 (2H,br s,-NH_2), 8.13 & 8.26 (2 x 1H,2 x s,H-2 & H-8).

^{13}C n.m.r. ($CDCl_3$,δ): -5.40 (q,-$SiMe_2$, ^{1}J=118Hz), 14.97 (q,3'-Me, ^{1}J=160Hz), 18.55 (s,-Si-C≡), 26.04 (qm,-Si-C$\underline{Me}_3$, ^{1}J=125Hz), 39.13 (d,C-3', ^{1}J=127Hz), 63.15 (t,C-5', ^{1}J=144Hz), 78.32 (dm,C-2', ^{1}J=130Hz), 84.77 (dm,C-4', ^{1}J=160Hz), 85.09 (d,C-1', ^{1}J=170Hz), 119.46 (d,C-5, ^{3}J=11Hz), 140.43 (dd,C-8, ^{1}J=214Hz, ^{3}J=4Hz), 149.80 (dd,C-4, ^{3}J=5Hz, ^{3}J=12Hz), 152.86 (d,C-2, ^{1}J=201Hz), 155.73 (d,C-6, ^{3}J=12Hz).

Microanalysis requires: C, 53.79; H, 7.70; N, 18.45.

found : C, 53.61; H, 7.74; N, 18.32.

The proton n.m.r. spetrum of the mixture of compounds (59) and (60) had the following resonances which may be attributable to the minor isomer (60).

^{1}H n.m.r. ($CDCl_3$,δ): 1.20 (d, J=6Hz).

3.84) 3'-C-methyl-3'-deoxy-ara-adenosine (63).

A stirred solution of compound (59), (0.11g, 3.0mmol) in 80% aqueous acetic acid (10 ml) was heated at 100°C for ½hr.[251] The mixture was evaporated to dryness and triturated with ether (2x15 ml) to leave a white solid (0.056g, 73%).

M.p. 227-228°C.

U.v. (MeOH): λ_{max} 259 nm. ε=14,480

I.r. (nujol, cm^{-1}): 3400-3200 (s), 1680 (s), 1610 (s), 1570 (m), 1330 (s), 1300 (s), 1230 (m), 1080 (s), 1040 (s).

^{1}H n.m.r. (360 MHz, $DMSO_{d6}$, δ): 1.07 (3H,d,-Me, $J_{3',Me}$=6.5Hz), 2.27 (1H,m,H-3'), 3.55-3.73 (3H,m,H-4' & H-5'), 4.14 (1H,m,H-2'), 5.11 (1H,t,5'-OH, $J_{5',OH}$=5Hz), 5.44 (1H,d,2'-OH, $J_{2',OH}$=5.5Hz), 6.17 (1H,d,H-1', $J_{1',2'}$=6.5Hz), 7.17 (2H,br s,$-NH_2$), 8.11 & 8.31 (2 x 1H,2 x s,H-2 & H-8).

For double resonance n.m.r. studies see Appendix I.

Mass spec.(C.I./NH_4+): MH^+ 266 (45.5), 248 (10.8), 178 (0.9), 164 (5.1), 148 (1.4), 136 (18.1), 135 (4.4), 130 (0.9), 113 (0.5).

3.85) Reaction of (58) with lithium 1,3-dithian-2-yl/THF.

A stirred solution of 1,3-dithiane (0.15g, 1.25mmol) in dry THF (10 ml) and under a dry nitrogen atmosphere, was cooled to -40°C. A 2.7M solution of butyl lithium (0.5ml, 1.3mmol) was added drop by drop and stirring continued at -30 to -20°C for 1.5 hrs. The solution was then cooled to -78°C and compound (58), (0.18g,0.5mmol) in dry THF (5 ml) was added slowly. The temperature was allowed to rise slowly to -30°C (ca. 0.5 hr). After 1 hr at -30°C the solution was allowed to warm up to ca. 5°C and

kept at this temperature for 18 hrs. Saturated ammonium chloride (50 ml) was added and the mixture was extracted with ether (3x50 ml). The combined organic layer was washed with brine (50 ml), dried over $MgSO_4$ and evaporated to give a white solid. T.l.c. and proton n.m.r. showed it to consist mostly of recovered (58) together with a minor new product. The solid was loaded onto a silica gel column (30g, 0.063-0.200 mesh) and was eluted with ethyl acetate; fractions of 15 ml being collected.
The combined fractions 23 to 29 were evaporated to afford a white solid (0.050g). T.l.c. and proton n.m.r. showed this to be recovered starting material.

The combined fractions 15 to 19 were evaporated down to a white solid, compound (61), (0.020g, 11%).

M.p. 188-190°C.

U.v. (MeOH): λ_{max} 257 nm.

I.r. ($CHCl_3$, cm^{-1}): 1670 (m), 1630 (s), 1580 (m), 1460 (m), 1250 (m), 850 (s).

^1H n.m.r. ($CDCl_3$, δ): 0.10 and 0.12 (6H, 2 x s, Si-Me_2), 0.90 (9H, s, Si-^tBu), 4.16 (2H, d, H-5', $J_{4',5'}$=4.5Hz), 4.60 (1H, m, H-4'), 5.16 (1H, dd, H-3', $J_{2',3'}$=3Hz, $J_{3',4'}$=7Hz), 5.88 (1H, d, H-2', $J_{2',3'}$=3Hz), 6.00 (2H, br s, $-NH_2$), 8.00 & 8.32 (2 x 1H, 2 x s, H-2 & H-8).

3.86) Reaction of (58) with lithium acetylide/diaminoethane/DMSO.

Compound (58) was subjected to the same reaction conditions described in Section 3.34. However very little material extracted into the organic phase and proton n.m.r. showed signals in the δ=0.1-1.0 region indicating that the protecting group was being lost in the reaction conditions. This was backed by the fact that the aqueous layer showed a u.v. absorbance centred at 258 nm. However it proved difficult to isolate any identifiable compound from the aqueous layer.

APPENDIX I

Double Resonance Proton n.m.r. Studies

Signal positions given in δ

Coupling constants J, given in Hz

Compound (8a)

Signal Irradiated	Signals Affected By Double Resonance						
	H-5'	H-4'	5'-OH	H-5	H-1'	H-3'	H-6
None	3.67 (bs)	4.94 (m)	5.20 (bs)	5.75 (d)	6.68 (t)	6.82 (dd)	7.96 (d)
3.67		dd $J_{1',4'}$=1.5 $J_{3',4'}$=3	Sharpens				
4.94	Sharpens				d $J_{1',3'}$=1.5	d $J_{1',3'}$=1.5	
5.20	d $J_{4',5'}$= 3						
5.75							s
6.68		d $J_{4',5'}$=3				d $J_{3',4'}$=3	
6.82					d $J_{1',4'}$=1.5		
7.96				s			

Compound (21a)

Signal Irradiated	Signals Affected By Double Resonance					
	H-3'	H-4'	H-2'	=CH2	-CH=	H-1'
None	2.83 (m)	3.90 (dt)	4.33 (dd)	5.20 (m)	5.72 (m)	6.10 (d)
2.83		br t $J_{3',4'}$=10	d $J_{1',2'}$=6		dd J_{cis}=10 J_{trans}=17.5	
6.10			d $J_{2',3'}$=8.5			

Compound (63)

Signal Irradiated	Signals Affected By Double Resonance						
	-Me	H-3'	H-4'&5'	H-2'	2'-OH	5'-OH	H-1'
None	1.07 (d)	2.27 (m)	3.60 (m)	4.14 (m)	5.10 (bs)	5.44 (bt)	6.17 (d)
1.07		br t $J_{2',3'}$=9.5 $J_{3',4'}$=9.5					
2.27	s			br t $J_{1',2'}$=6.5 $J_{2',OH}$=6			
4.14		6 lines				s	s
5.10			Sharpens				
5.44				dd $J_{1',2'}$=6.5 $J_{2',3'}$=9.5			
6.17				dd $J_{2',3'}$=9.5 $J_{2',OH}$=6			

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