Dedicated to My Parents

Studies of the Chemistry of Some 5-Diazopyrimidine Nucleosides

A thesis presented to The City University

in part fulfilment of the requirements for the

degree of Doctor of Philosophy

by

Zhong Yin

Department of Chemistry,

The City University,

London.

September 1994

Contents

Acknowledgements

I would like to give my special thanks to my supervisor, Professor S.A. Matlin, for his encouragement, invaluable advice and dedication throughout this work.

I would like to thank the World Laboratory for financially supporting the final year of my research.

I would like to thank Dr. S. Chen for running the high resolution mass spectra, Mr. I.K. Katyal for running mass spectra, Mr. J.J. Hastings for running the nmr spectra and Dr. W. Errington for running the X-ray crystallography of my compounds.

I would like to thank my wife for her invaluable support and assistance in typing this thesis.

Abstract

Uridine plays a very important role in the process of *de novo* synthesis of DNA and RNA. Many derivatives and analogues of uridine have shown anti-cancer and anti-viral activities. A search for new uridines with potential anti-cancer and anti-viral activity is described in this thesis, which includes the preparation of novel uridine derivatives from 5-diazo-5',6-cyclo-uridine and -deoxyuridine, and some chemical studies of the reactivity of 5-diazo-5',6-cyclo-uridines.

The results of the study are as follows:

1) The key intermediates, 5-diazo-5',6-cyclo-uridine and 5-diazo-5',6-cyclo-2'-deoxyuridine have been synthesised in a new procedure which is more efficient and safer than the reported one.

2) A number of novel 5-aryloxy-uridines, 5-aryloxy-2'-deoxyuridines, 5-(3"-aryl-1"-triazeno)-uridines and 5-(3"-aryl-1"-triazeno)-2'-deoxyuridines have been synthesised from 5-diazo-5',6-cyclo-uridine and 5-diazo-5',6-cyclo-2'-deoxyuridine, respectively.

3) The successful synthesis of 5-diazo-6-methoxy-5'-trityl-uridine and 5-diazo-6-isopropoxy-5'-trityl-uridine provides a convenient route for the preparation of some 5-diazo-6-aryloxy-uridines and -2'-deoxyuridines, from which some 5,6-disubstituted uridines and -2'-deoxyuridines may be prepared.

4) The reactivity of 5-diazo-5',6-cyclo-uridine has been studied in some organic solvents. A new intramolecular rearrangement of 5-diazo-5',6-cyclo-2'-deoxyuridine and of the corresponding 5-diazo-5',6-cyclo-2'-uridine via carbene intermediates has been discovered.

The structures of all of twenty eight new compounds prepared in this work have been confirmed spectroscopically and biological tests on many of these compounds are in progress.

Abbriviations

AMP	=	adensine monophosphate
ATP	=	adensine triphosphate
CDP	=	cytidine diphosphate
CI-MS	=	chemical ionisation mass spectrometry
CMP	=	cytidine monophosphate
DMF	=	N,N-dimethylformamide
DMSO	=	dimethyl sulphoxide
DNA	=	deoxyribonucleic acid
dTTP	=	deoxythymidine triphosphate
EI-MS	=	eletron impact mass spectrometry
FAB	=	fast atom bombardment mass spectrometry
GMP	=	guanosine monophosphate
HEPT	=	1-(2-hydroxyethoxy)-methyl-6-(phenylthio)-thymine
HIV	=	Human Immunodeficiency Virus
HPLC	=	high performance liquid chromatography
IR	=	infrared spectroscopy
LDA	=	lithium diisopropylamide
+M	=	positive inductive effect
-M	=	negative inductive effect
m.p.	=	melting point
mRNA	=	messenger RNA
MS	=	mass spectrometry
m/z	=	mass to charge ratio
NBS	=	N-bromosuccinimide
NHS	=	N-hydroxysuccinimide
NMR	=	nuclear magnetic resonance
nOe	=	nuclear Overhauser enhancement
PRPP	=	1-pyrophosphate-5-phospho-ribose
PY	=	pyridine
RNA	=	ribonucleic acid
rRNA	=	ribosomal RNA
THF	=	tetrahydrofuran
TLC	=	thin layer chromatography
tRNA	=	transfer RNA
UDP	=	uridine diphosphate
UTP	=	uridine triphosphate
UV	=	ultraviolet spectroscopy

Chapter 1. Basic Biochemistry and Medicinal Chemistry of Pyrimidine Nucleosides

1.1 Basis for nucleic acid

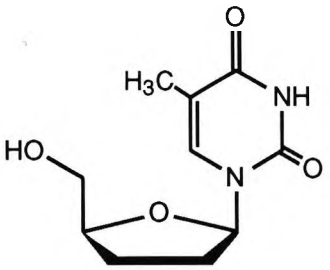
Nucleic acids play a crucial role in storage of genetic information and in protein biosynthesis [1,2]. There are two types of nucleic acid; ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). DNA is the molecule in which genetic information is stored in cells. It carries the information necessary for the exact duplication of the cell and, in fact, for the construction of the entire organism. There are at least three general types of RNA. All of them are involved in the biosynthesis of proteins.

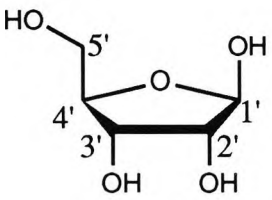
Both DNA and RNA are biopolymers in which the repeating monomer units are called nucleotides. A nucleotide is a complex molecule made up of one unit each of a phosphate, a sugar and a heterocyclic base. For each class of nucleic acid there are four main nucleotide monomers. In RNA, the sugar is the pentose ribose (1.1) and the heterocyclic base is a pyrimidine, uracil (1.2) or cytosine (1.3) or a purine, adenine (1.4) or guanine (1.5). In DNA, the sugar is 2-deoxyribose (1.6) and the heterocyclic bases are the same except that thymine (1.7) replaces uracil.

Base-catalysed hydrolysis of a nucleotide removes the phosphate group and yields a nucleoside. Nucleosides are glycosides formed from the pentose and the heterocyclic base. The nucleosides of RNA are cytidine (1.8), uridine (1.9), adenosine (1.10) and guanosine (1.11). For DNA, the nucleosides are the corresponding 2-deoxy analogues, with 2-deoxythymidine (1.12) replacing uridine.

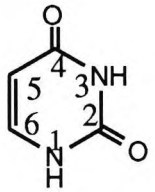
The nucleic acid backbone is a phosphodiester copolymer in which the phosphoric acid connects either ribose or 2-deoxyribose at C3 and C5, with a heterocycle linked to C1 of each of the pentose units (Fig 1.1).

DNA is a double-stranded helix of two individual molecules. The two chains are held together by reciprocal hydrogen bonding between pairs of bases in opposite positions in the two chains (Fig 1.2).

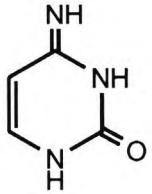




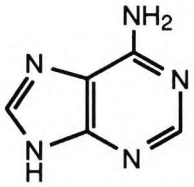
1.1



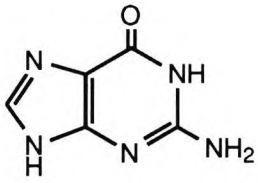
1.2



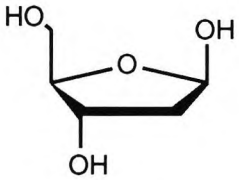
1.3



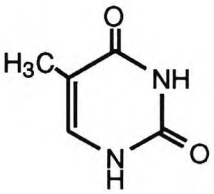
1.4



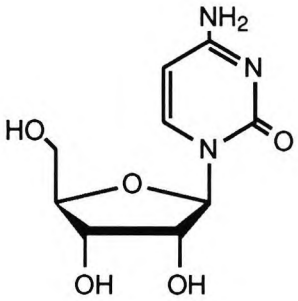
1.5



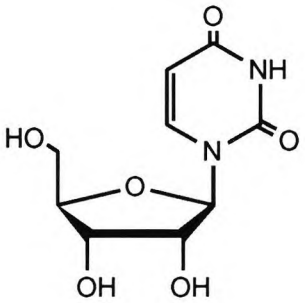
1.6



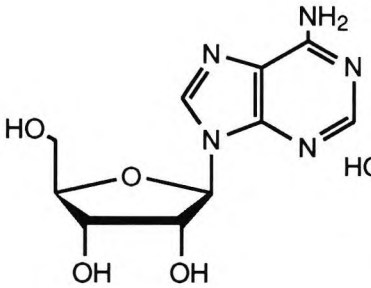
1.7



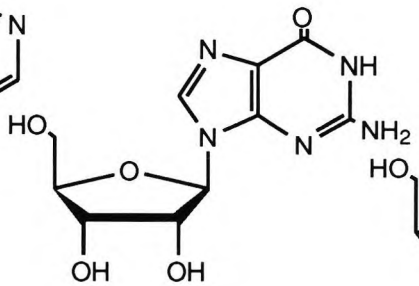
1.8



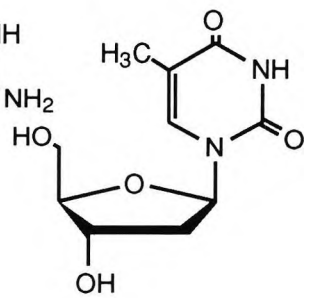
1.9



1.10



1.11



1.12

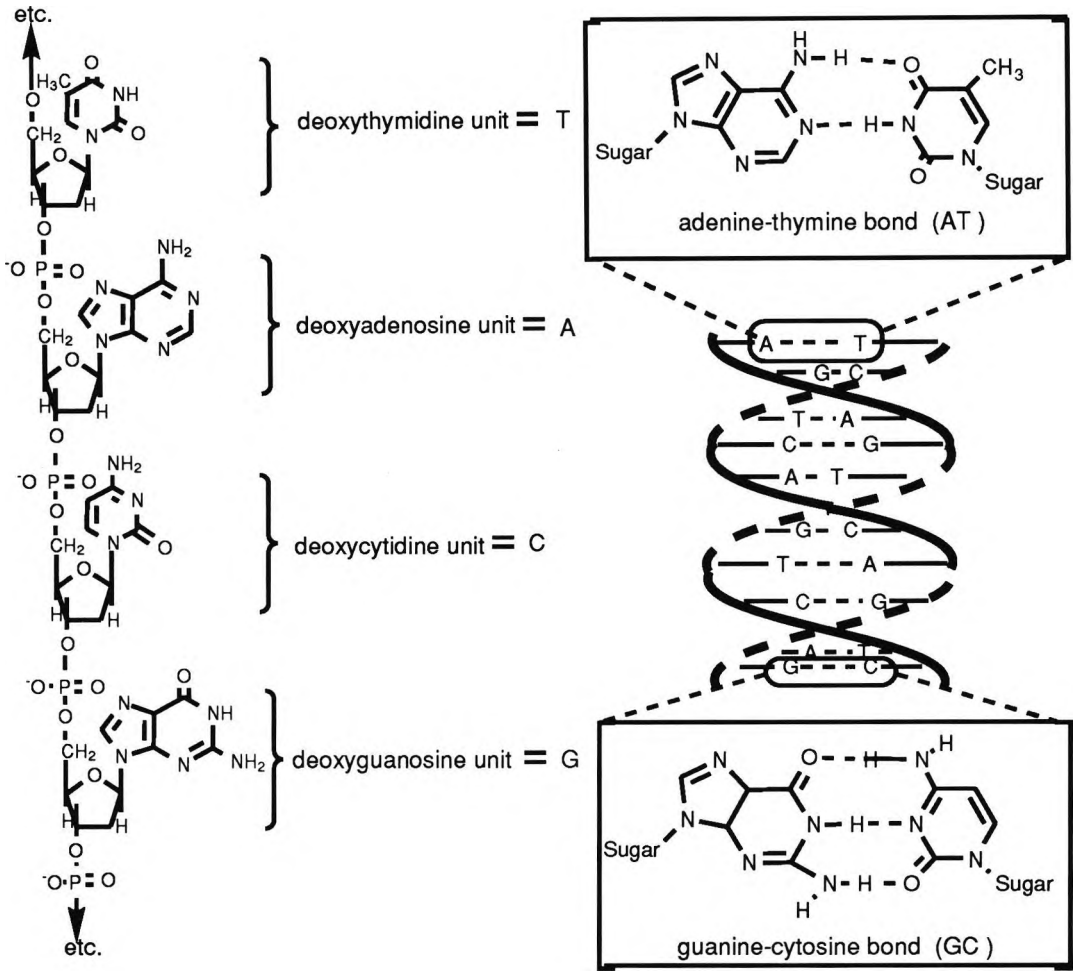


Fig. 1.1 Fig. 1.2

The principal function of DNA is as the master blueprint for the production of proteins, the essential catalysts for all cellular reactions. The information regarding primary structure of given proteins is encoded in the exact sequence of the four heterocyclic bases along the phosphodiester polymer in large assemblies of the molecules, known as genes [3,4]. In the process of DNA replication, the genetic code of the daughter cell could be induced to differ from that of the parent. The chemical substances which can cause such changes to the genetically-linked

properties of cells might be significant for medical purposes.

Unlike DNA, RNA molecules exist as single strands with rather irregular structures. Based on the individual function, three types of them can be classified, ribosomal RNA (rRNA), messenger RNA (mRNA) and transfer RNA (tRNA). All of them are involved in the protein biosynthesis.

1.2 Importance of nucleosides

The importance of nucleosides is illustrated by a review of their role in normal cellular physiology as follows [5-9]:

1. Precursors of DNA and RNA;
2. Intermediates in biosynthesis;
   a) UDP sugars: glycogen, glycoprotein
   b) CDP-diacylglycerol: phosphoglycerides
   c) CMP-N-acetylneuraminic acid: glycoprotein
3. Energy donor: ATP
4. Components of important coenzymes:
   a) Nicotinamide adenine dinucleotide
   b) Flavin adenine dinucleotide
   c) Coenzyme A
5. Metabolic regulators: cyclic AMP, cyclic GMP and cyclic CMP

Thus, it is known that metabolic derivatives of naturally occurring purine and pyrimidine nucleosides play a very important role in the life of a cell. It is not surprising that compounds which interfere with either their synthesis or function exert anticancer and/or antiviral activity.

1.3 Biosynthesis of nucleosides

1.3.1 Introduction

In view of the importance of nucleosides for the life of the cell, it is appropriate briefly to review the biosynthesis of nucleosides in mammalian cells and indicate the site of action of representative analogues.

1.3.1.1 *De novo* biosynthesis of pyrimidine nucleotides [10-15]

The basic difference in synthesis of pyrimidine and purine nucleosides is that the pyrimidine ring is made first and then ribose phosphate is attached, whereas the purine ring is sequentially built up starting with the ribose moiety. In both cases ribose phosphate is donated by 1-pyrophospho-5-phospho-ribose (PRPP) (Fig 1.3).

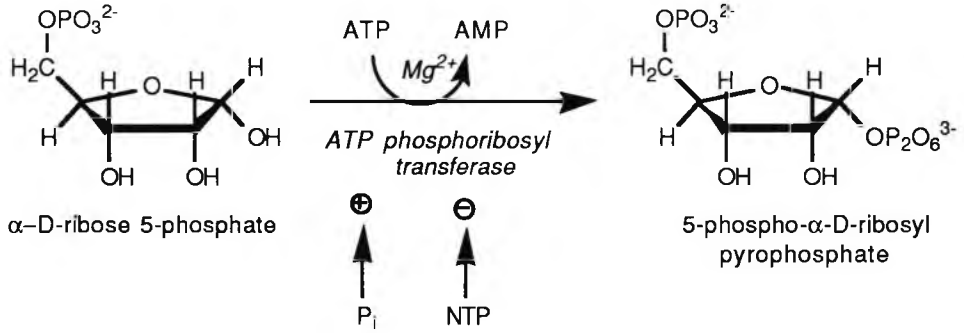


Fig.1.3

The elements of the base ring are contributed by glutamine, CO_2 and aspartate. The process begins by a reaction between carbamoyl phosphate and aspartate to form carbamoylaspartate. Carbamoylaspartate is formed for this purpose by obtaining the amino group from L-glutamine. Importantly, the enzyme is inhibited by UTP, which, therefore, regulates the initial step in its own production.

The simple removal of water from carbamoylaspartate forms a reduced pyrimidine, dihydroorotate, which is then oxidised to orotate.

Orotidine 5'-phosphate is converted to uridine 5'-phosphate by a simple decarboxylation. The enzyme catalysing the reaction has no known cofactors.

Uridine monophosphate, like the other nucleoside phosphates, is converted to its diphosphate by a specific kinase utilising ATP, and the diphosphate is converted to the triphosphate by a general nucleoside diphosphokinase, also at the expense of ATP. Part of the uridine triphosphate is consumed to make cytidine triphosphate by a transfer of the amide group of glutamine, driven by the cleavage of ATP (Fig. 1.4).

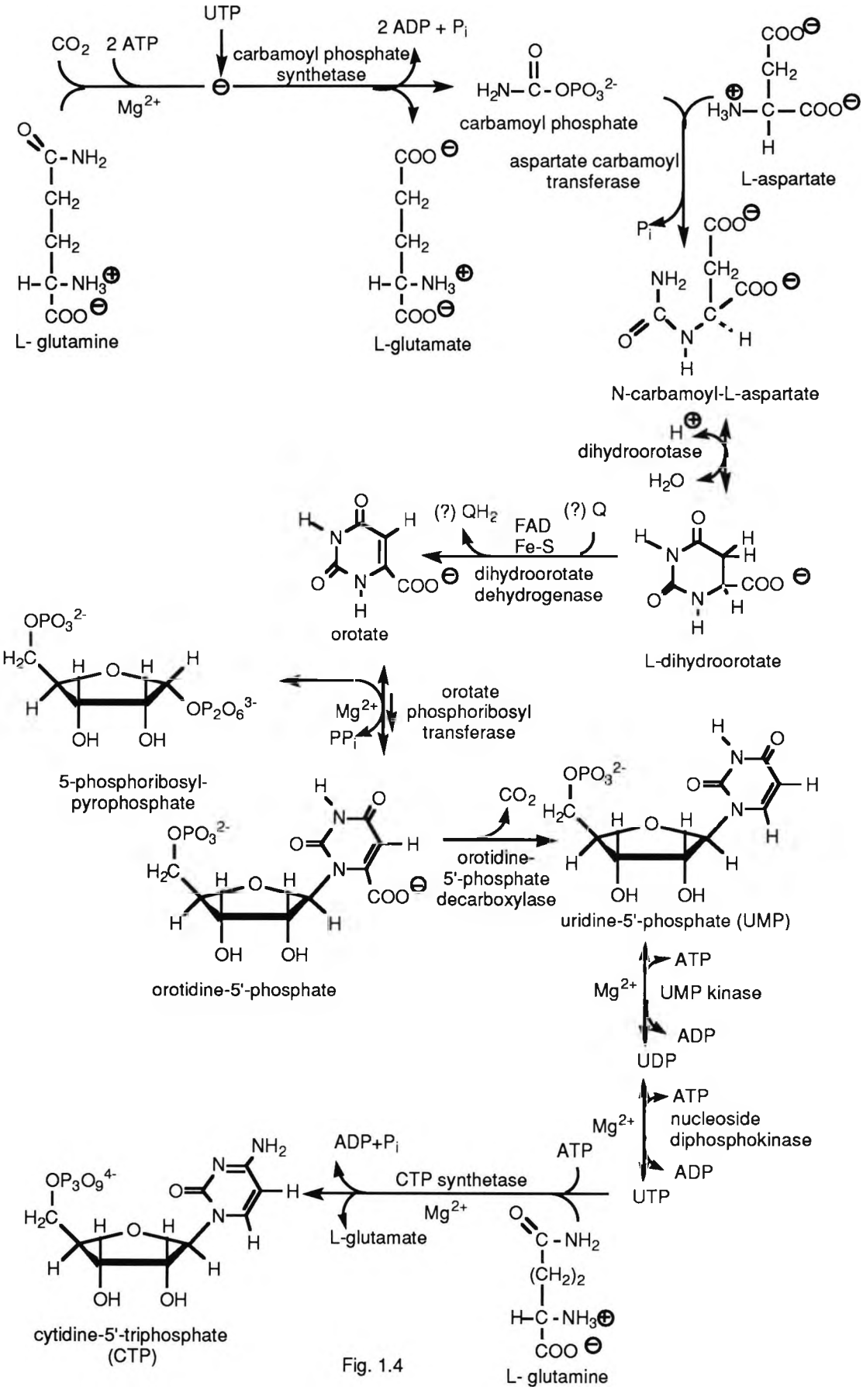

UTP
CO_2 2 ATP
Mg^{2+}
carbamoyl phosphate synthetase
2 ADP + P_i
$H_2N-C-OPO_3^{2-}$
carbamoyl phosphate
L- glutamine
L-glutamate
aspartate carbamoyl transferase
L-aspartate
P_i
N-carbamoyl-L-aspartate
dihydroorotase
H_2O
(?) QH_2
(?) Q
FAD
Fe-S
dihydroorotate dehydrogenase
orotate
L-dihydroorotate
5-phosphoribosyl-pyrophosphate
Mg^{2+}
PP_i
orotate phosphoribosyl transferase
CO_2
orotidine-5'-phosphate decarboxylase
orotidine-5'-phosphate
uridine-5'-phosphate (UMP)
ATP
Mg^{2+}
UMP kinase
ADP
UDP
ATP
nucleoside diphosphokinase
Mg^{2+}
ADP
UTP
ADP+P_i
CTP synthetase
ATP
Mg^{2+}
L-glutamate
L- glutamine
cytidine-5'-triphosphate (CTP)

Fig. 1.4

1.3.1.2 Formation of deoxyribonucleotides [16,17]

The deoxyribonucleoside phosphates required for DNA synthesis are generated by reduction of the 2'-carbon atom in the ribonucleoside diphosphate. The reaction is catalyzed by thioredoxin, a small protein containing a protruding disulphide group (Fig. 1.5).

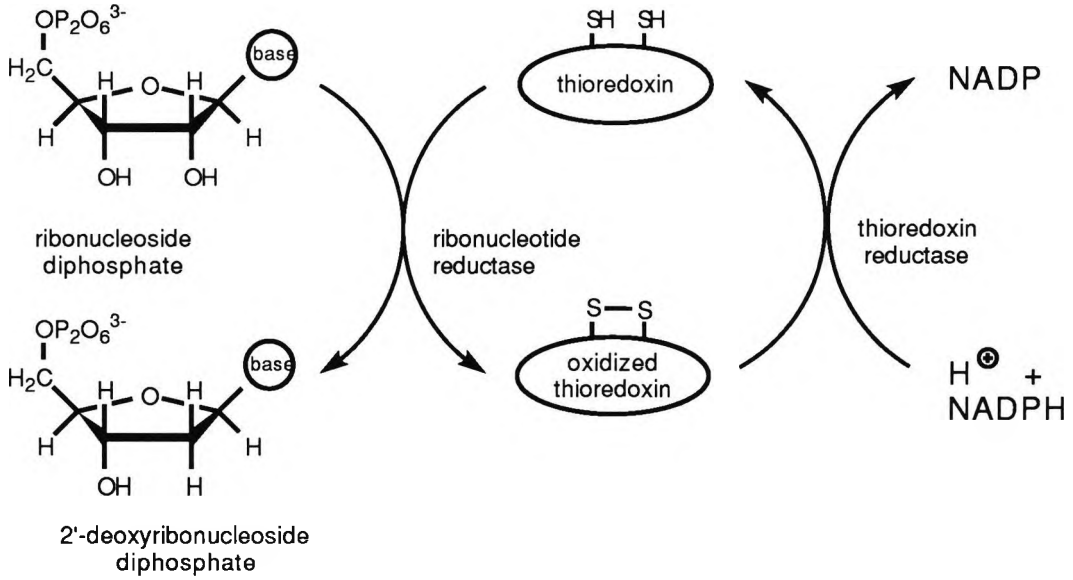


Fig. 1.5

The formation of dTTP as the fourth precursor of DNA involves a special route. The thymine moiety is created by an unusual reaction catalysed by an enzyme named thymidylate synthase (Fig. 1.6).

1.3.1.3 Salvage pathway for synthesis of nucleotides [18]

Preformed pyrimidines can be taken up by cells and converted into nucleotides by a salvage pathway. This may proceed by direct reaction with PRPP or via a phosphorylase and kinase (Fig. 1.7).

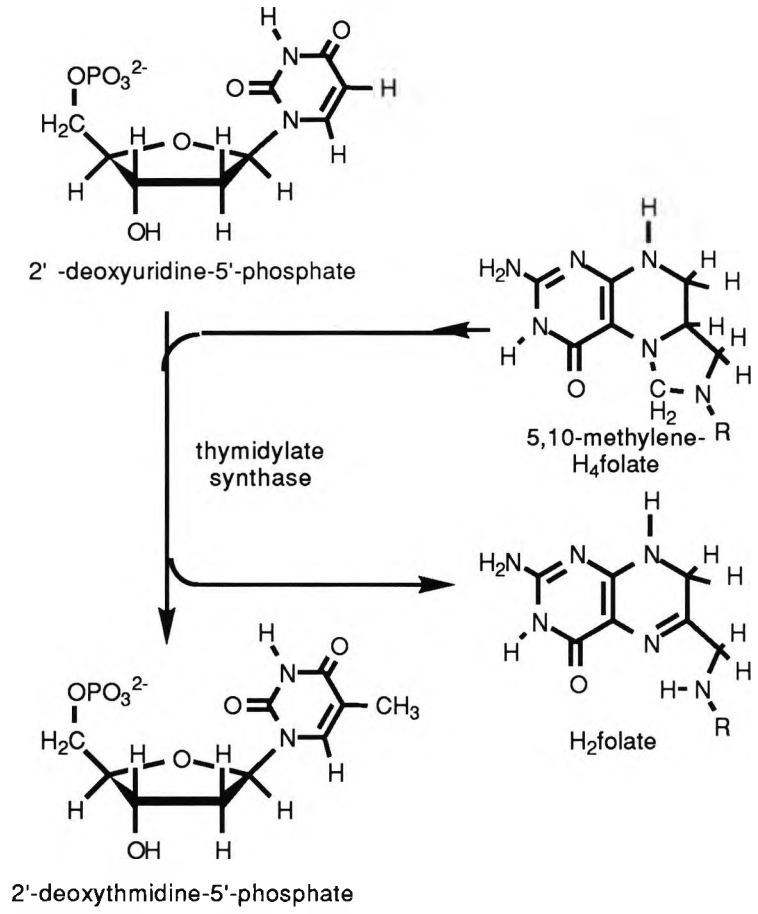

2' -deoxyuridine-5'-phosphate
thymidylate synthase
5,10-methylene-H4folate
H2folate
2'-deoxythmidine-5'-phosphate

Fig.1.6

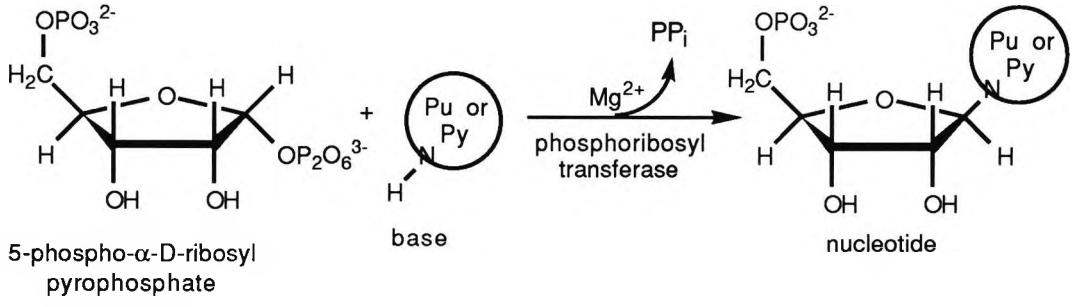

5-phospho-α-D-ribosyl pyrophosphate
base
Pu or Py
PPi
Mg2+
phosphoribosyl transferase
nucleotide

Fig.1.7

1.4 Anti-cancer and anti-viral effect of nucleosides

Because of the critical role of nucleic acids in the replicating process, medicinal chemists have synthesised a large number of derivatives and analogues of pyrimidine nucleosides which are intended to effect inhibition of the biosynthesis of nucleic acids in cancer cells and virus-infected cells.

In general, biological activity of analogues and derivatives of nucleosides can be classified into three kinds: inhibition of enzymes so as to stop certain bioreactions; inhibition of cell growth in some way, such as limitation of energy supply; insertion into the chain of DNA or RNA.

As a potential drug, a desirable compound should possess some of the following advantages:

1. As simple as possible a structure to make synthesis easy;

2. High medicinally useful activity but low side effects and toxicity, i.e. high chemotherapeutic index;

3. High selectivity for the indicated use and the lowest possible concentration in body fluids which is compatible with effectiveness.

1.4.1 Basis for selectivity [19-24]

There are several problems in the search for selective drugs. One is the identification of a target which is unique to the cancer cell or virus whether it be anatomical, biochemical or physical.

The concept of seeking exploitable differences or unique targets is not new. Despite considerable efforts, success, particularly in the cancer field, is rather limited.

1.4.1.1 Nucleosides as anti-cancer agents

Unfortunately, as far as it is known, there are no qualitative differences in the pathways used for the synthesis of pyrimidine nucleosides in neoplastic cells, normal cells or virus-infected cells. The partial specificity in the therapy of neoplasia apparently lies in differences in metabolism, uptake, pharmacokinetics, potential

modifying factors or repair mechanisms.

Therefore, effort has been concentrated on a number of areas which could form the basis for selectivity in cancer therapy [25-27];

1. preferential transport into the neoplastic cell [28-30],
2. preferential degradation by normal tissues [31,32],
3. preferential interaction with a biochemical target in the neoplastic cell [33-37]
4. preferential activation by the neoplastic cell,
5. preferential repair of drug induced damage,
6. pyrimidine activation of drug by lower pH value in some cancer cells may provide selectivity [38],
7. selectivity based on decreasing toxicity to normal tissues [27],
8. selectivity based on redox potential of solid tumour [39-41],

From the viewpoint of the chemist, seeking compounds which are capable of inhibiting enzymes in cancer cell remains one of the most useful goals.

Thymidylate synthase is responsible for the *de novo* synthesis of dTMP and therefore is a key target in cancer chemotherapy. Other targets involved are DNA polymerase, ß-deaminase, decarboxylase etc.

Obviously these approaches are not completely selective, since they are not directed at unique biochemical targets in cancer cells. Nevertheless, until we are able to find such unique targets, the basis for successful anti-cancer chemotherapy lies in the manipulation of drugs, the intelligent application of pharmacokinetic data, by decreasing host toxicity, etc [42].

1.4.1.2 Nucleosides as anti-viral agents

In contrast to cancer chemotherapeutics, the prospect for viral chemotherapeutics is more optimistic, because there are many identifiable targets that may be attacked. Some of them are unique to the interaction of virus with a host cell. Some of them are selective in the process of its reproduction.

In contrast to animal, plant or bacterial cells, the virus is an intracellular parasite

and it can only reproduce within an animal, plant or bacterial cell. After adsorption to the surface of the host cell [43-45], penetration into the cell [43,44,46], and uncoating [47-49], the processes of replication, transcription, translation and assembly take place [50-53]. Because of these specific biochemical processes, there are some identifiable enzymes of which we may take advantage. For example, in Herpes simplex virus, the virus-induced thymidine kinase differs from that of the host cell, as well as DNA polymerase, CDP-reductase, deoxycytidine deaminase, deoxyribonuclease, etc. [54-60]

1.5 Some research on uridine analogues

In the process of pyrimidine biosynthesis, uridine plays as a important role. From uridine-5'-phosphate, thymidine and cytidine can be derived. Therefore, a large number of analogues of uridine and uracil have been synthesised and investigated biologically in order to find useful drugs and to explore and understand the relationships between their structure and activity.

In general, biological activity of analogues of uridine and uracil can be classified into three kinds: inhibition of enzymes, inhibition of bacterial growth and insertion into the chain of DNA and RNA.

1.5.1 Research in biochemistry and pharmacology

For potential medicinal purposes, these compounds have been investigated in many areas, including anti-tumour, anti-viral, antibiotic and anti-malarial actions.

Biological investigations show that uridine is relatively nontoxic to mammalian cells, and can inhibit the enzyme cytosine nucleoside deaminase [60-62]. This inhibition appears to correlate with hydrophobic bonding between the enzyme and substituted uracils [63,64]. It is reasonable to consider that the hydrophobic bonding region is adjacent to the active site of cytosine nucleoside deaminase [63].

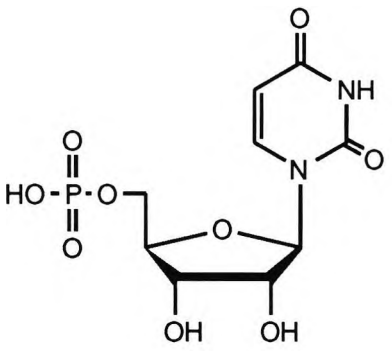
Many analogues and derivatives of uracil, deoxyuridine and uridine, as well as other nucleoside analogues have proved to be inhibitors, *in vitro* and/or *in vivo*, of enzymes involved in the *de novo* synthesis of nucleosides and nucleotides [65],

which include adenosine deaminase [66-68], phospho-diesterase [69], cytidine triphosphate synthetase [70,71], orotidine 5'-phosphate decarboxylase [72-74], and thymidylate synthetase [75].

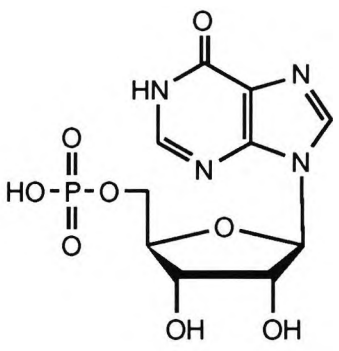
Since nucleoside analogues and derivatives play a wide range of biological roles in the metabolism of nucleic acids and biosynthesis of proteins, nucleoside analogues have been a major source of anti-viral and anti-tumour agents. Their effect probably comes from disruption of the *de novo* synthesis of nucleotides and nucleic acids in situations of rapid proliferation, which is symptomatic of viral infection or tumour growth.

The manner in which these compounds exert their influence is not completely understood. Inhibition of the enzymes involved in replication is a logical suggestion for a mode of action.

Other enzymes which would form suitable targets are the "post synthetic" modifiers of uridine monophosphate (1.13) and inosine monophosphate (1.14).



1.13



1.14

Mammalian cells synthesise uracil nucleotides by two separate pathways: a *de novo* pathway utilising glutamine, aspartate and bicarbonate and a salvage pathway utilising the enzyme uridine kinase [76]. Inhibitors of *de novo* pyrimidine biosynthesis have had only limited success in the clinic as single agents [77-80], since these agents inhibit only one of the pyrimidine biosynthetic pathways. Their chemotherapeutic efficacy could be diminished by the utilisation of exogenous uridine present in plasma.

A superior approach to the chemotherapeutic use of pyrimidine antimetabolites would, therefore, be a two-pronged approach in which both the *de novo* and salvage pathways of pyrimidine biosynthesis are blocked.

The design of nucleosides that have diagnostic and/or chemotherapeutic applications is assisted by a knowledge of the mechanisms through which they exert their effects *in vivo*. It is important to determine the ability of a drug to cross the cell membrane since entry into the cell is a requisite for efficacy. Nucleosides are known to enter cells by a facilitated diffusion mechanism which is sensitive to structural changes in the nucleoside [81,82].

The conformation of a compound is an important factor which can influence its interaction, as a substrate, with an enzyme. For example, it was initially considered that phosphorylases did not distinguish the anti or syn conformations of uridine and its analogues. However, it is now known that initial conversion of uridine and other 5-substituted uridines to the syn conformation occurs during the interaction of the compounds with phosphorylases [83]. This means that the conformation about the glycosidic bond is probably of importance and the conformational requirements of the uridine phosphorylase reaction may be of relevance in relation to the activity of some chemotherapeutic agents.

1.5.2 Research in medicinal chemistry

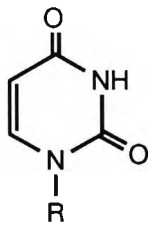
In order to find suitable compounds, a large amount of chemical work has been done on structural modifications of uridine. The main modifications have been focused on 1- and 5-substituted uracil, 5-substituted uridine and 3'- and 5'-substituted uridine. Recently, 6-substituted uridine analogues were reported.

A. 1-Substituted analogues of uridine

The analogues of uridine in which the cyclic carbohydrate moiety is replaced by acyclic chains possess the capability of interfering with the activity of enzymes for which the natural nucleosides or nucleotides serve as substrates [65]. Consequently, several series of alkyl, aryl and aralkyl and other aliphatic derivatives (1.15) were

prepared as potential inhibitors of cytosine nucleoside deaminases and phosphodiesterases and as antiviral compounds [69,84].

Investigations showed that some of the compounds can become complexed to the enzyme with hydrophobic bonding, so that they possess inhibitory and antiviral activity in vitro. Furthermore, it can be concluded that there is a bulk tolerance for these groups within the enzyme inhibitor complex.



1.15

R:		
	-CH_3	-$(CH_2)_4C_6H_5$
	n-C_4H_9	-$(CH_2)_5C_6H_5$
	n-C_5H_{11}	-$(CH_2)_2OC_6H_5$
	i-C_5H_{11}	-$(CH_2)_3OC_6H_5$
	-cyclopentyl	-$CH_2C_6H_4COOH$-*p*
	t-C_6H_{13}	-$CH_2C_6H_4NHCOCH_2Br$-*m*
	-C_6H_5	-$CH_2C_6H_4NHCOCH_2Br$-*o*
	-$CH_2C_6H_5$	-$(CH_2)_3C_6H_4COOC_2H_5$-*p*
	-$(CH_2)_2C_6H_5$	-$CH_2O(CH_2)COOC_6H_5$
	-$(CH_2)_3C_6H_5$	-$CH_2OCH_2CH_2OH$

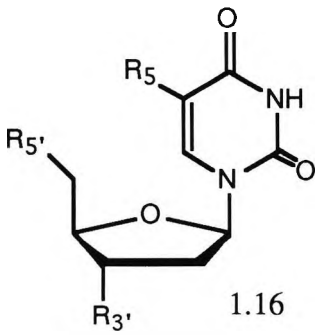
B. Chemical changes in the cyclic carbohydrate moiety of uridine

Interest in chemical changes in the cyclic carbohydrate moiety has concentrated on changes to the 5'-OH and 3'-OH of uridine, 2'-deoxyuridine and their 5-substituted analogues.

Chu et al [85] reported a number of nucleosides (1.16) which have been identified as potential anti-Human Immunodeficiency Virus Type 1 (anti-HIV-1) agents. The study indicated that a 3'-azido group on the sugar ring exhibited the most potent antiviral activity. The 5'-hydroxyl group was necessary for phosphorylation.

Moyer et al [76] have modified pyrimidine nucleosides (1.17) as inhibitors of uridine kinase in vitro and of uridine salvage by intact L 1210 cells. The research

showed that the tolerance of uridine kinase for large substituents at the 5'-position appeared limited and compounds with large substituents at this position were ineffective as inhibitors.

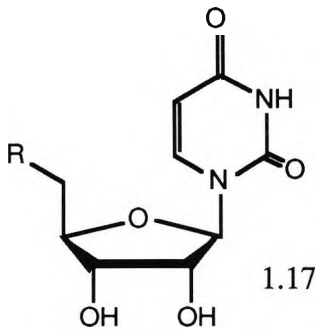


1.16

R_5: -H, -CH_3, -C_2H_5, -C_3H_7, -CH=CHBr, -Br, -I.

$R_{5'}$: -OH, -N_3, -NH_2.

$R_{3'}$: -N_3, -NH_2, -I, -H, -2',3'-unsaturated.

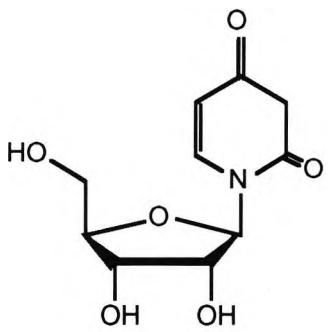


1.17

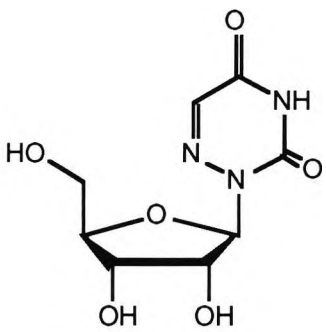
R: -H, -F, -Cl, -I, -N_3, -NH_2, -OSO_2CH_3, -$NHCH_2CO_2H$, -O-benzoyl, -O-trityl, -ONO_2, -OCH_2CO_2H, -$OPO(OH)CH_3$, -*p*-O-glycopyranosyl-monophosphate.

C. Interchange of nitrogen and carbon atoms in heterocyclic base

Design and synthesis of isosteric analogues of uridine has led to the synthesis of the biologically active nucleosides 3-deazauridine (1.18) and 6-azauridine (1.19).



1.18



1.19

3-Deazauridine [93], a molecule in which a carbon atom has been substituted for the nitrogen atom in the 3-position of uridine, is a substrate for uridine kinase and is converted to its triphosphate nucleotide, which exerts its action through inhibition of

the pyrimidine *de novo* synthesis enzyme, cytidine triphosphate synthetase [70,71]. 3-Deazauridine has been found to be active [86,87] both *in vitro* and *in vivo* as an antibacterial and as an antitumur and antiviral agent [88,90].

6-Azauridine [91,92], an isostere of uridine containing a nitrogen atom in the 6-position, is also phosphorylated by uridine kinase to the biologically active 6-azauridine 5'-phosphate, which then acts as a competitive inhibitor of orotidine 5'-phosphate decarboxylase [73,75].

D. 5-Substituted derivatives of uridine, 2-deoxyuridine and uracil

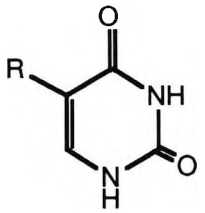
Substitution at the C5 position in many uracil, uridine and deoxyuridine derivatives has been a subject of great interest. Many uracil derivatives have shown biological activity as inhibitors of enzymes and of bacterial growth. Most deoxyuridine derivatives have shown anti-tumour or anti-viral activity.

The derivatives of uridine have shown mainly anti-viral activity or inhibition of protein biosynthesis via disruption of translation.

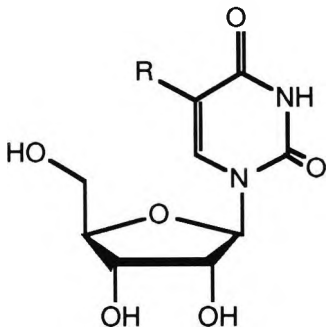
So far , the C5-position of the uracil base has proved to be the most useful position for structural modification to produce inhibition of viral and tumour replication. This involves the replacement of the olefinic proton with other functions, which, it will be noted, are relatively far away from the carbohydrate moiety. Consequently, little conformational change will occur when the C5-proton is replaced.

The C5-position of uracil is adjacent to the C4 carbonyl group. Chemical replacement of C5-H with other groups happens easily on this position and consequently, 5-substituted derivatives of uracil, uridine and deoxyuridine have been extensively investigated both in chemistry and in biology.

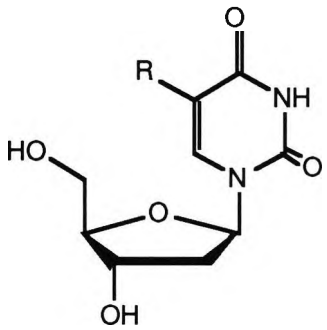
Some 5-substituted compounds which possess biological activity are shown below.



R: -F, -Cl, -Br, -I, $-NH_2$, $-NO_2$, $-N_3$, -OH, $-CH_2OH$, $-CF_3$, $-N(CH_2CH_2Cl)_2$.



R: $-CH_2N(Et)_2$ $-CH_2OH$ $-NH_2$ $-NO_2$ -F -Cl -Br -I

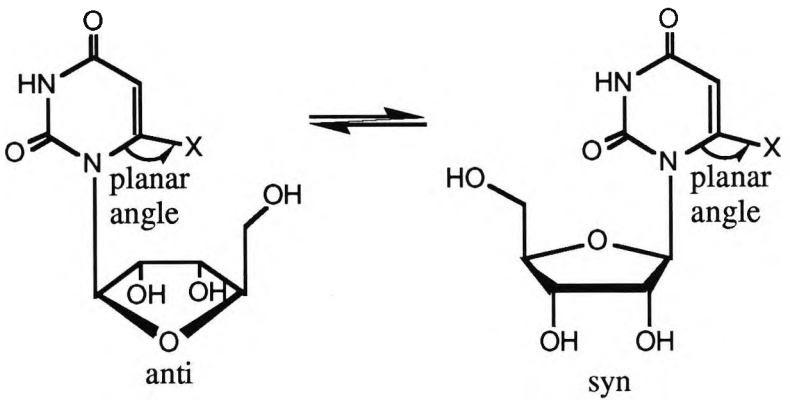


R: $-CH_2OH$, $-CH_2OCH_3$, $-CH_2SH$, $-CH_2SCH_3$, -CHO, -CN, $-CF_3$, $-C_2H_5$, $-C_3H_7$, $-CH_2CH=CH_2$, $-CH=CH_2$, -CH=CHBr, -CH=CHI, -C=CH, -NHR, $-NO_2$, $-OCH_2C=CH$, -SH, -SCN, -F, -Br, -I.

E. 6-Substituted analogues of uridine

Although, 6-substituted analogues and derivatives of uracil have been studied since the 1960's [53], little information is available concerning 6-substituted uridines, presumably due to the difficulty in their chemical preparation. Therefore, exploration of possible new routes to C6-substitution is significant for a better understanding of the biological properties of pyrimidine-2.4-diones.

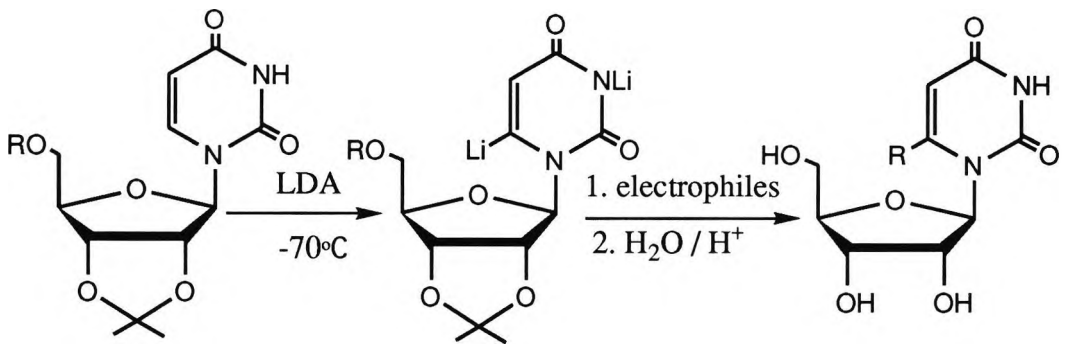
Uridine can assume two conformations; syn and anti, depending upon the relative position of the base with respect to the sugar residue, i.e. depending upon rotation about the glycosidic C1'-N1 bond [94]. (Scheme 1.1)



Scheme 1.1

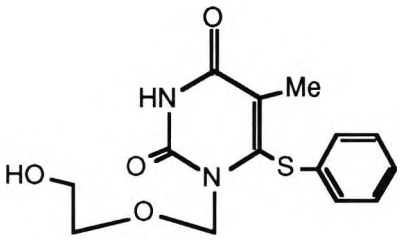
Analysis of the rotation barriers and calculation of the energy profile of this rotation indicate that the anti-conformation is favoured slightly over the syn-conformation for uridine itself [95-97]. However, substituents on uridine at C6 are known to favour predominantly or exclusively the syn-conformation about the glycosidic bond [98]. In addition, the planar angle N1-C_6-X is variable based on different steric interferences of X groups with the sugar ring.

Recently, a new approach [99-100] to prepare 6-substituted uridine and 6-substituted acyclouridine derivatives of uridine has been developed, involving initial protection of the hydroxyl groups in the sugar ring, then lithiation on positions 6 and 3 of the base using lithium diisopropylamide (LDA) below -70°C, followed by the reaction of the lithio derivative with electrophiles. (Scheme 1.2)



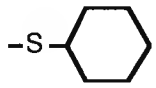
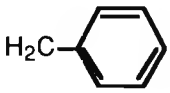
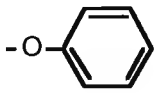
Scheme 1.2

Biological investigation revealed that some 6-substituted acyclouridine derivatives possessed anti-HIV-1 activity in vitro. Among them, 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)-thymine (HEPT) (1.20) [101] exhibited a potent and selective inhibition of HIV-1 replication. Further studies [100] on the structure-activity relationships showed that the phenylthio group at the C6-position of HEPT could be substituted by a cyclohexylthio group, a benzyl group, or a phenoxy group. However, substitution by the latter two groups weakened the activity. Furthermore, other substitution was totally ineffective. The results of C6 modified analogues suggested the necessity of a ring structure in the C6-substituent for this type of compound to be active against HIV-1[100].



HEPT

1.20

R:  ,  , 

Because of unique structural and biological features of HEPT and its congeners, these compounds are being further pursued as candidate drugs for anti-AIDS chemotherapy.

In summary, the activity of HEPT and its analogues in inhibition of HIV-1 replication suggests that the C6-position can contribute to activity and further investigation can be reasonably expected to complement the understanding of the biological properties of pyrimidine-2,4 diones.

1.5.3 Some medicinal chemistry aspects of pyrimidines

Many pyrimidine-2,4 diones have been proved to possess extensive biological activities. Unfortunately, the data available are still insufficient for establishment of

relationships between the structure and activity of the compounds which can properly describe all the biological activities in terms of factors such as electronic substituent parameters, hydrophobic constituent parameters and steric parameters.

However, based on previous investigations, some primary concepts could be deduced as follows:

1. Suitable substituents at the C1-position of uracil contribute to biological activity against tumour cells and viruses.

2. Substitution at 3'-OH and 5'-OH on the sugar ring of deoxyuridine can also contribute to biological activity. However, amongst such compounds, it is only when the 3'-OH is replaced by $-N_3$ that potent antiviral activity seems to be observed, so that the value of substitution at C3' seems to be limited. In addition, the steric effects of 5'-substituents might be a crucial factor relating to the activity.

3. The C5-position of the pyrimidine series - uracil, deoxyuridine and uridine - is a key site. It is probable that further investigations of C5-substituted compounds can lead to new, active drugs.

4. The C6-position might be another "active" site in pyrimidine-2,4-dione compounds based on recent evidence. Furthermore, C6-substitution can cause conformational change about the glycosidic bond. It would be expected that increasing interest might be focused on selective inhibition of enzymes by 6-substituted compounds.

5. According to the bioisosteric principle, compounds having interchange of nitrogen and carbon atoms in the heterocyclic base can retain some biological activity.

References:

1. R.W. McGilvery and G. Goldstein, "Biochemistry" , W.B. Saunders Co., Philadelphia, 2nd edition, 1979, 43.
2. R.L.P. Adams, J.T. Knowler and D.P. Leader, "The Biochemistry of the Nucleic Acids ", Chapman & Hall, London, 11th edition, 1992, 451.
3. B.J. Bachmann, *Microbiol. Rev.,* 1990, 54, 130.
4. K.E. Sanderson and J.R. Roth, *Microbiol. Rev.,* 1988, 52, 485.
5. J.D. Watson, "Molecular Biology of the Gene ", W.A. Benjamin Inc., California, 3rd edition, 1976, 52, 129 & 147.
6. L. Stryer, "Biochemistry " Freeman, San Francisco, 1975, 528.
7. J.D. Watson, "Molecular Biology of the Gene ", W.A. Benjamin Inc., California, 3rd edition, 1976, 113.
8. G.A. Robison, R.W. Butcher and E.W. Sutherland, "Cyclic AMP", Academic Press, New York, 1971
9. A. Bloch, *Biochem. Biophys. Res. Commun.,* 1975, 58, 652
10. R.W. McGilvery and G. Goldstein, "Biochemistry", W.B. Saunders Co., Philadelphia, 2nd edition, 1979, 657.
11. A. Kornberg, "DNA Replication", Freeman, San Francisco, 1980.
12. P. Reichard, *Adv. Enzymol.,*1959, 21, 263.
13. I. Lieberman, *J. Biol. Chem.,* 1956, 222, 765.
14. G.R. Stark, *Trends Biochem. Sci.,* 1977, 2, 64.
15. D.R. Grayson, L. Lee and D.R. Evans, *J. Biol. Chem.,* 1985, 260, 15840.
16. A. Larsson and P. Reichard, *Mol. Biol.,* 1967, 7, 303.
17. L. Thelander and P. Reichard, *Annu. Rev. Biochem.,* 1979, 48, 133.
18. J.Neuhard, "Metabolism of Nucleotides, Nucleosides and Nucleobases in Microorganisms", Academic Press, London, 1983, 95.
19. A.C. Sartorelli and D.G. Johns, "Antineoplastic and Immunosuppresive Agents", Springer, Berlin, 1975, vol. 1-2,

20. R.E. Harmon, R.K. Robis and L.B. Townsend, "Chemistry and Biology of Nucleosides and Nucleotides", Academic Press, New York, 1978.

21. A. Bloch, "Chemistry, Biology and Clinical Uses of Nucleoside Analogues" *Ann. N. Y. Acad. Sci.,* 1975.

22. E. DeClercq and P.F. Torrence, *J. Carb. Nucleosides, Nucleotides,* 1978, 5, 187.

23. W.E.G. Muller and R.K. Zahn, *Research in Molecular Biology,* 1973, 9, 1.

24. W.H. Prusoff and D. Ward, *Biochem. Pharmacol.,* 1976, 25, 1233.

25. J.F. Henderson, in "Antineoplastic and Immunosuppressive Agents Part I", Ed. A.C. Sartorelli and D.G. Johns, Springer, Berlin, 1974, 340,

26. W.C.J. Ross, in "Antineophastic and Immunosuppressive Agents Part I", Ed. A.C. Sartorelli and D.G. Johns, Springer, Berlin, 1974, 33,

27. J.Bertino, *Cancer Res.,* 1979, 39. 293.

28. P.G.W. Plagemann, R. Marz, and R.M. Wohlhuetter, *J. Cell. Physiol.,* 1978, 97, 49.

29. C.E. Cass and A.R.P. Paterson, *Exper. Cell, Res.,* 1977,105, 427.

30. J.M. Oliver and A.R.P. Paterson, *Canad. J. Biochem.,* 1971, 49, 262.

31. G.L. Neil, J.E. Moxley and R.C.Manak, *Cancer Res.,* 1970, 30, 2166.

32. C.D. Steuart and P.J. Burke, *Nature New Biology* , 1971, 233, 109.

33. P.V. Danenberg, *Biochim. Biophys. Acta.,* 1977, 473, 73.

34. A. Fridland, *Biochemistry,* 1977, 16, 5308.

35. E.C. Cadman, E.D. Douglas and R.E. Handschumacher, *Cancer Res.,* 1978, 38, 682.

36. L.J. Lu and K. Randerath, *Cancer Res.*, 1979, 39, 940.

37. M. Bobek, Y.C. Cheng and A. Bloch, *J. Med. Chem.*, 1978, 21, 597.

38. T.A. Connors, B.C.V. Mitchley, V.M. Rosenoer and W.C.J. Ross, *Biochem. Pharmacol.,* 1964, 13, 395.

39. H.W. Moore, *Science,* 1977, 197, 527.

40. A.J. Lin, L.A. Cosby and A.C. Sartorelli, "ACS Symposium Series, Number 30; Cancer Chemotherapy", American Chemical Society, Washington, 1976, 71.

41. K.A. Kennedy, S. Rockwell and A.C. Sartorelli, *Proc. Amer. Assoc. Cancer Res.*, 1979, 20, 278.

42. C.G. Zubrod, *Cancer Res.*, 1978, 38, 4377.

43. S.E. Luria, J.E. Darnell, Jr., D. Baltimore and A. Campbell, "General Virology", John Wiley and Sons, New York. 3rd edition, 1978, 225.

44. K. Lonberg and L. Philipson, "Early Interactions Between Viruses and Cells", Karger, New York, 1974.

45. F.M. Steele and F.L. Black, *J. Virol.*, 1967, 1, 653.

46. S. Dales, *Bact. Rev.*, 1973, 37, 103.

47. N. Kato and H.J. Eggers, *Virology,* 1969, 37, 632.

48. H.J. Eggers, *Virology,* 1977, 78, 241.

49. B. Rosenwirth and H.J. Eggers, *Nature,* 1977, 267, 370.

50. D.J. McCorquodal, A.E. Oleson and J.M. Buchanan, "The Molecular Biology of Viruses", Academic Press, New York, 1967, 29.

51. R.S. Edgar and W.B. Wood, *Sci. Am.*, 1967, 217(1), 60.

52. S. Casjens and J. King, *Annu. Rev. Biochem.*, 1975. 44, 555.

53. S.B. Primrose and N.J. Dimmock,"Introduction to Modern Virology", Blackwell Scientific, Oxford, 1980

54. H.G. Klemperer, G.R. Haynes, W.I.H. Shedden and D.H. Watson, *Virology,* 1967, 31, 120.

55. S. Kits and D.R. Dubbs, *Biochem. Biophys. Res. Commun.*, 1963, 11, 55

56. D.R. Dubbs and S. Kit, *Virology,* 1964, 22, 493.

57. H.M. Keir and E. Gold, *Biochim. Biophys. Acta.*, 1963, 72, 263.

58. G.J. Cohen, *Biochim. Biophys. Acta.*, 1972, 72, 263..

59. T. Chan, *Proc. Natl. Acad. Sci.*, USA, 1977, 74, 1734.

60. G.W. Camiener and C.G. Smith, *Biochem. Pharmacol.*, 1965, 14, 1405.

61. G.W. Camiener, *Biochem. Pharmacol.*, 1967, 16, 1691.

62. S.S. Cohen and H.D. Barner, *J. Biol. Chem.*, 1957, 226, 631.

63. B.R. Baker and J.L. Kelley, *J. Med. Chem.*, 1968, 11, 682.

64. B.R. Baker and J.L. Kelley, *J. Med. Chem.*, 1968, 11, 686.

65. A.C. Schroeder, R.G. Hughes and A. Bloch, *J. Med. Chem.*, 1981, 24, 1078.

66. H.J. Schaeffer, S. Gurwara, R. Vince, S. Bittner, *J. Med. Chem.*, 1971, 14, 367.

67. H.J. Schaeffer, in "Topics in Medicinal Chemistry", Ed. J.L. Rebinowitz and R.M. Myerson, Wiley Interscience, New York, 1970, 1.

68. H.J. Schaeffer and C.F. Schwender, *J. Med. Chem.*, 1974, 17, 6.

69. Y.C. Chen and A.Bloch, *J. Biol. Chem.*, 1978, 253, 2522.

70. R.P. Mepartland, M.C. Wang, A. Bloch, H. Weinfeld, *Cancer Res.*, 1974, 34, 3107.

71. R.W. Brokman, S.C. Shaddix, M. Williams, J.A. Nelson, L.M. Rose and F.M. Schabel, *Ann. N.Y. Acad. Sci.*, 1975, 255, 501.

72. J. Skoda, *Progr. Nucleic Acid Res. Mol. Biol.*, 1963, 2, 197.

73. J. Skoda, in "Antineoplastic and Immunosuppressive Agents Part II", Ed. A.C. Sartorelli and D.G. Johns, Sringer, Berlin, 1975, 348.

74. A. Cihak, *Collect. Czech. Chem. Commun.*, 1974, 39, 673.

75. D V. Santi, *J. Med. Chem.*, 1980, 23, 103.

76. J.D. Moyer, J.M. Karle, N. Malinowski, V.E. Marquez, M.A. Salam, L. Malspeis and R.L. Cysye, *Molecular Pharmacology*, 1973, 28, 454.

77. E.C. Cadman, D.E. Dix, R.E. Handschumacher, *Cancer Res.*, 1978, 38, 682.

78. E.S. Casper, R.J. Gralla, D.P. Kelsen, A. Houghton, R.B. Golbey and C.W. Young, *Cancer Treat. Rep.*, 1980, 64, 705.

79. T.J. Ervin, R.H. Blum, M.W. Meshed, D.W. Kufe, R.K. Johnson and G.P.

Cannellos, *Cancer Treat. Rep.*, 1980, 64, 1067.

80. R.D. Hart, T. Ohnuma and J.F. Holland, *Cancer Treat. Rep.*, 1980, 64, 617.
81. W.P. Gati, E.E. Knaus and L.I. Wiebe, *Mol. Pharmacol.*, 1983, 23, 146.
82. W.P. Gati, H.K. Misra, E.E. Knaus and L.I. Wiebe, *Biochem.Pharmacol.*, 1984, 33, 3325.
83. E. Krajewska and D. Shugar, *Biochem. Pharmacol.*, 1982, 31, 1097.
84. G.B. Elion, P.A. Furman, J.A. Fyfe, P. DeMiranda, L. Beauchamp and H.J. Schaeffer, *Proc. Natl. Acad. Sci.*, U.S.A., 1977, 74, 5716.
85. C.K. Chu, R.F. Schinazi, M.K. Ahn, G.V. Ullas and Z.P. Gu, *J. Med. Chem.* 1989, 32, 6112.
86. M.J. Robins, B.L. Currie, R.K. Robins and A. Bloch, *Proc. Am. Assoc. Cancer Res.*, 1969, 10, 73.
87. A.Bloch, G.Dutschman, B.L.Currie, R.K.Robins and J.M.Robins, *J. Med. Chem.*, 1973, 16, 294.
88 G .P. Khare, R.W. Sidwell, J.H. Huffman, R.L. Tolman and R.K. Robins, *Proc. Soc. Exp. Biol. Med.*, 1972, 140, 880.
89. W.M. Shannon, R.W. Brockman, L. Westbrook, S. Shaddix and F.M. Schabel, Jr., *J. Natl. Cancer Inst.*, 1974, 52, 199.
90. W.M. Shannon, G. Arnett and F.M. Schable, Jr., *Antimicrob. Agents Chemother.*, 1972, 2, 159.
91. R.E. Handschumacher, *J. Biol. Chem.*, 1976, 235, 764.
92. A. Capek, E. Svatek and M. Tadra, *Folia Microbiol.*, 1963, 8, 304.
93. M.J. Robins and B.L. Currie, *Chem. Commun.*, 1968, 1547.
94. D. Suck and W. Seanger, *J. Am. Chem. Soc.*, 1972, 94, 6502.
95. A.E.V. Haschemeyer and A. Rihc, *J. Mol. Biol.*, 1967, 27,369.
96. A.V. Lakshminarayanan and V. Sasisekharan, *Biochim. Biophys. Acta.*, 1970, 204, 49.
97. S. Kang and B. Pullman, *J. Mol. Biol.*, 1971, 58, 297.

98. D.B. Davies, *Prog. Nucl. Magn Reson. Spectrosc.*, 1978, 12, 135.

99. M. Shimizu, H. Tanaka, H. Hayakawa and T. Miyasaka, *Tetrahedron Letters* 1990, 31(9) 1295.

100. H. Tanaka, M. Baba, H. Hayakawa, T. Sakashi, T. Miyasaka, M. Ubasawa, H. Takashima, K. Sekiya, I. Nitte, S. Shigete, R.T. Walker, J. Balzarini and E. DeClercq, *J. Med. Chem.*, 1990, 34, 349.

101. T. Miyasaka, H. Tanaka, M. Baba, H. Hayakawa R.T. Walker and J. Balzarini, *J. Med. Chem.*, 1989, 32, 2507.

Chapter 2. Chemistry of Diazo Compounds

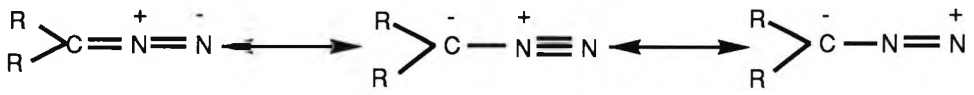
2.1 Introduction

2.1.1 General

In 1858, at a time when modern organic chemistry was just beginning, the aromatic diazo compounds were discovered [1]. There has subsequently been considerable interest in compounds containing diazonium and diazo groups, particularly because of their usefulness as intermediates and more recently as a consequence of work on the reactivity and structure of carbenes (R_2C:) which are intermediates formed in the photolysis and thermolysis of diazoalkanes.

2.1.2 Structure of the diazo group

In the diazoalkanes examined to date, the C-N bond length (1.28-1.32Å) is between a C-N single bond (1.47Å) and a CN double bond (>C=N-, sp^2, 1.23-1.26 Å) [2]. At the same time, the N-N distance (1.12-1.13Å) is greater than that found for dinitrogen and significantly greater than that found in the diazonium group in benzenediazonium chloride (1.09 Å) [3]. It is generally agreed [4] that this evidence suggests that the $>CN_2$ group is a resonance hybrid between the canonical forms (2.1) - (2.3). Form (2.3) is commonly not reported since its contribution is very small compared to that of (2.1) and (2.2), as it possesses fewer covalent bonds and there is a larger charge separation (Scheme 2.1).



2.2 2.1 2.3

Scheme 2.1

Furthermore, both structure (2.1) and (2.2) reflect rather strong polarity. Actually, the polarity of the diazo group in diazo compounds is not high. This is because the *p*-electrons of one carbon and two nitrogen atoms of diazo group overlap together to form a π -bond (Fig. 2.1).

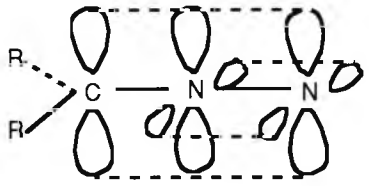


Fig. 2.1

2.2 Preparation of diazo compounds

Synthesis of diazo compounds can be systematically classified in the following way:

1. Condensation of two compounds which each possess a nitrogen-containing functional group , as in the diazotization of amines or the Forster reaction.

2. Conversion of compounds containing a functional group with two nitrogen atoms into diazo-function by dehydrogenation (hydrazones) or cleavage (tosyl-hydrazones, N-acyl-N-nitrosoamines)

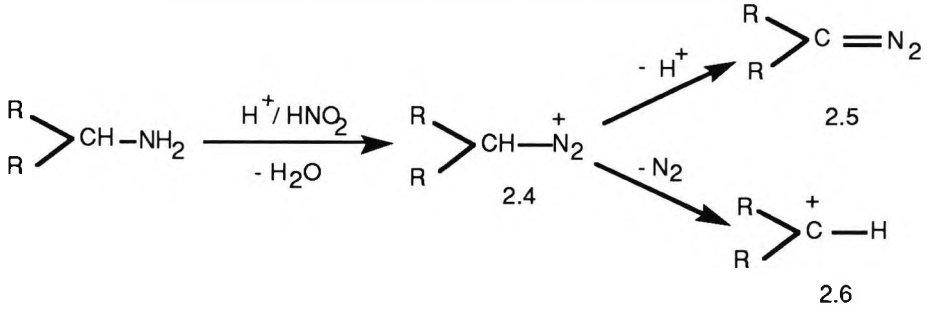
3. Transfer of a diazo group from a donor (usually tosyl azide) to an acceptor (diazo group transfer).

4. Substitution reactions of diazo compounds which leave the diazo group unchanged.

Among the synthetic routes to diazo compounds above, the approaches listed under 1 and 2 are most often used.

2.2.1 Diazotization of amines

The most common method of diazotization has been the treatment of the relevant amine with hydrochloric acid and sodium nitrite (Scheme 2.2).



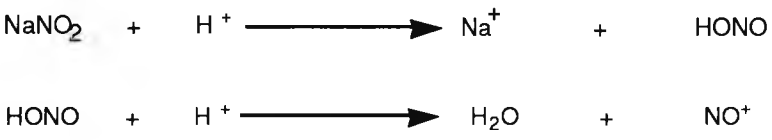
Scheme 2.2

The diazotization of primary amines is generally carried out in acid media, in which diazoalkanes are subject to elimination of nitrogen [5]. Therefore, the temperature for the reaction is usually maintained at about 0 °C. Moreover, it is necessary to maintain the pH value of the solvent below 2 for the reasons below [6]:

1. At higher pH values the equilibrium between ammonium ion and amine changes in favour of the free base which is much less soluble in water,

2. At a low concentration of hydrogen ions, the diazo compound formed reacts with the free base of as yet unattached amine to produce the diazoamino compound,

3. With increasing pH, the reactive forms of the diazotizing agent are converted into ineffective ones, namely, free nitrous acid, HNO_2 and the nitrite ion, NO_2^- since the formation of the nitrosonium ion which is involved in nitrosating needs 2 mol of hydrogen ion (Scheme 2.3).



Scheme 2.3

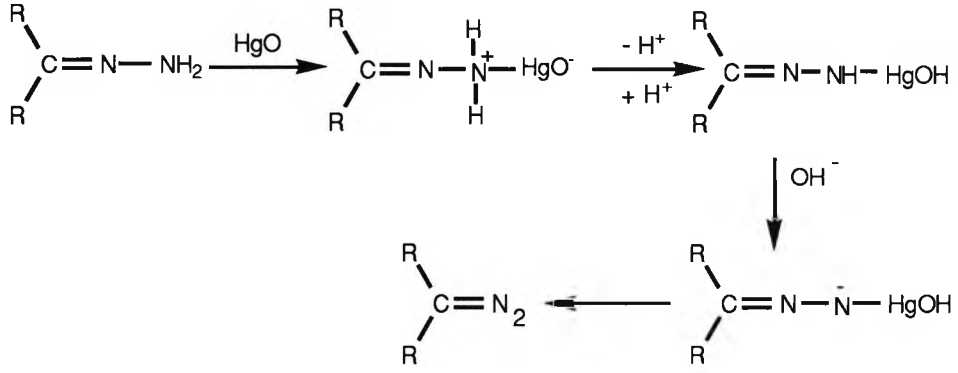
2.2.2 Diazotization in organic solvents

As the amines become more weakly basic, the normal method of diazotization becomes progressively more difficult. The equilibrium between free amine and ammonium salt increasingly favours the former which, usually because of its poor solubility in water, is prevented from taking part in the reaction. Therefore, in order to increase the concentration of amine in solution a higher concentration of aqueous mineral acid is used for weakly basic amines. However, this causes many problems related to the instability of the compounds in the highly acidic solvent. Consequently, investigations [7,8] have been made of diazotization in organic solvents. The obstacle to diazotization with nitrite in organic solvents is the poor solubility of the salts of nitrous acid. In place of nitrous gases or alkali nitrite as nitrosating agent, esters of nitrous acid are used, especially ethyl and amyl nitrite. With these,

diazotization can be carried out in alcohol, acetic acid, dioxane and other media.

2.2.3 Dehydrogenation of hydrazones with metal oxides

Dehydrogenation of hydrazones to diazo compounds is one of the oldest methods of diazo compound synthesis known [9]. While mercuric oxide is the dominant dehydrogenation reagent, silver oxide and manganese dioxide have also found wide application. A general mechanism has been postulated for conversion of hydrazones to diazo compounds (Scheme 2.4).



Scheme 2.4

The reaction is usually carried out in organic solvents such as benzene, toluene, chloroform, or light petroleum with admixture of compounds capable of binding water, such as sodium sulphate, and the reaction is catalysed by an ethanolic solution of potassium hydroxide. Since no intermediates have been isolated in the reaction, and dehydrogenations apparently proceed via hydrazone anions [10], the function of the hydroxyl ion can justifiably be assumed to lie in primary deprotonation. Other reagents include silver oxide [11], lead tetraacetate [12] and iodine [13a]. An extensive discussion is given in the book "The Chemistry of Diazonium and Diazo Groups" [13b].

2.3 Diazoketones

The conformations adopted by α–diazoketones depend on the conjugation between the diazo group, which in this case exerts a +M effect and the carbonyl

group, which exerts a -M effect. The atomic orbital scheme involved in this delocalisation is shown in Fig. 2.2. For the only X-ray molecular structure of a cyclic α-diazoketone solved to date, 1,4-bis-(diazo)-2,3-butanedione [14], the following values are obtained: the C2-N1-N2, 176.8° bond angle and C1-C2-N1 bond angle 116.5°. These indicate that in the molecule the carbon atom C2 is basically sp^2 hybridised and both nitrogen atoms have sp hybridisation. The π_y orbits are localised on the latter two atoms, whilst the π_x orbits are delocalized over several atoms. The central C1-C2 bond becomes partially double bonded, because of delocalisation of the electrons. It follows that two rotational isomers may exist.

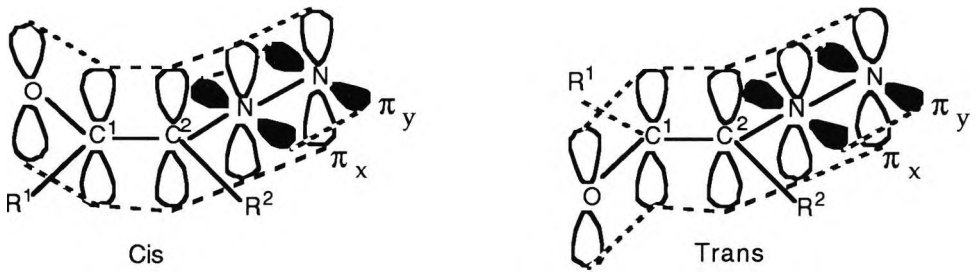
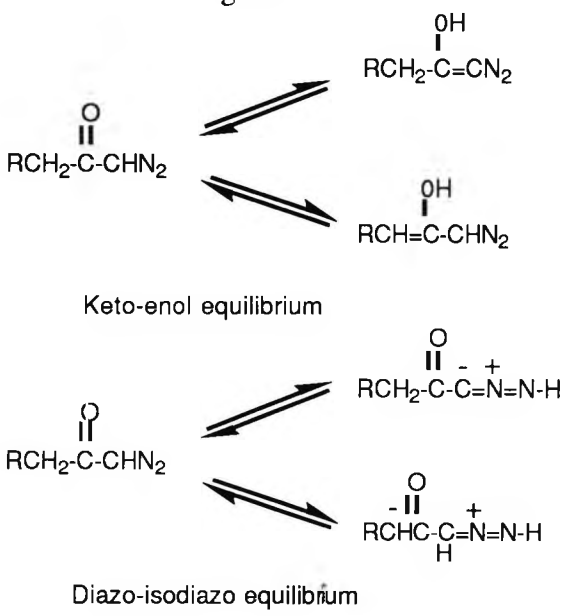


Fig. 2.2



Scheme 2.5

α–Diazoketones may also, in theory, show structural isomerism due to the migration of the α/α'-hydrogen atom to the carbonyl oxygen or to the terminal nitrogen, leading to keto-enol or diazo-isodiazo isomerism respectively (Scheme

2.5). Moreover, diazoketones may form an intermolecular hydrogen bond with another diazoketone molecule or with a hydroxylic compound [15].

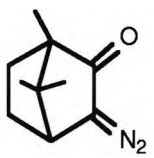
2.3.1 UV and IR spectra

The UV spectra of α-diazoketones in mixtures of non-polar and hydroxylic solvents show characteristic behaviour. The $-COCN_2$ group has an intense band at 245-250nm, which becomes weaker with increase in the hydroxylic/non-polar solvent ratio, and another band at 270-290 nm which at the same time increases in intensity [16]. There are no shifts in the bands on going from one solvent to the other.

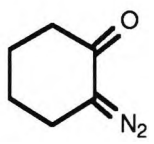
The presence of this phenomenon is linked to the availability of the carbonyl lone pair of electrons. In mixed solvents there is formation of a hydrogen bond between the diazo compound and hydroxylic solvent. The evidence is that:

1. The phenomenon is also observed for molecules which do not contain transposable hydrogen atoms, e.g. 3-diazocamphor (2.7) [17]. Therefore, the presence of the phenomenon is not because of the migration of an α–hydrogen atom.

2. The phenomenon is also found in the spectra of compounds for which conformational equilibrium between syn and anti forms is not possible, e.g. α–diazocyclohexanone (2.8), etc. [18].



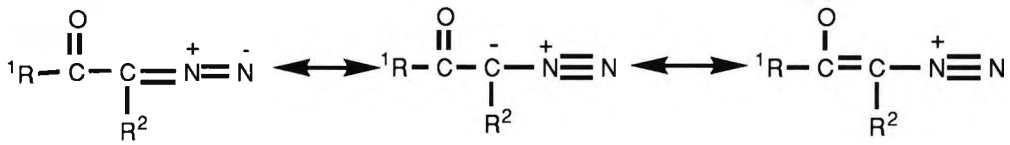
(2.7)



(2.8)

In the infrared spectra of α-diazoketones in mixed solvents, the integrated intensities and frequencies of the N-N stretching vibration band at about 2130 cm^{-1} are almost invariant, but the C=O stretching band at about 1660 cm^{-1} decreases in intensity with increasing quantity of phenol in C_2Cl_4 [19]. The possibility of the terminal nitrogen being involved in the hydrogen bonding can be excluded, because

the I.R. spectrum of phenol in the presence of diphenyldiazomethane (0.004M) in CCl_4 shows no evidence of its presence [20]. Thus, the only atom for H-bonding available is the carbonyl oxygen. Moreover, compared with the corresponding ketones [21] and diazohydrocarbons [22], V_{CO} of α–diazoketones moves to lower frequency and V_{NN} to higher frequency. This indicates that, in these molecules, the carbonyl group has less double-bond character than in diazohydrocarbons of the same order of substitution. In other words, a third limiting form (2.11), which occurs at the expense of form (2.10) is also present in diazoketones in addition to the canonical forms (2.9) and (2.10). The participation of the form (2.11) gives the central C-C bond a partial double bond character. The contribution of forms (2.10) and (2.11) is greater the higher the frequency of the diazo band and the lower its intensity. The opposite holds for contributions of forms of the type (2.9) (Scheme 2.6) [23].



(2.9) (2.10) (2.11)

Scheme 2.6

2.3.2 ^{1}H NMR spectra of α-diazoketones

α–Diazoketones with general formula $RCOCHN_2$ have been widely studied using ^{1}H NMR [24]. Temperature- dependent ^{1}H NMR spectra have been observed for some molecules. The spectra of some compounds show a single sharp peak at room temperature for the diazomethine proton, which broadens and splits into a doublet on lowering the temperature, while only a single peak is observed in the spectra of some other compounds with constant intensity at different temperatures. In the first type, observations indicate that two forms are present in conformation equilibrium, whilst the second case indicates only a single species.

Moreover, the ^{13}C-H coupling constant for high-temperature time-average

species is the same as that of the species which predominate at low temperature for the first type of compounds [24]. This phenomenon can exclude the possibilities of keto-enol and diazo-isodiazo structural isomerisms.

2.3.3 Conclusion

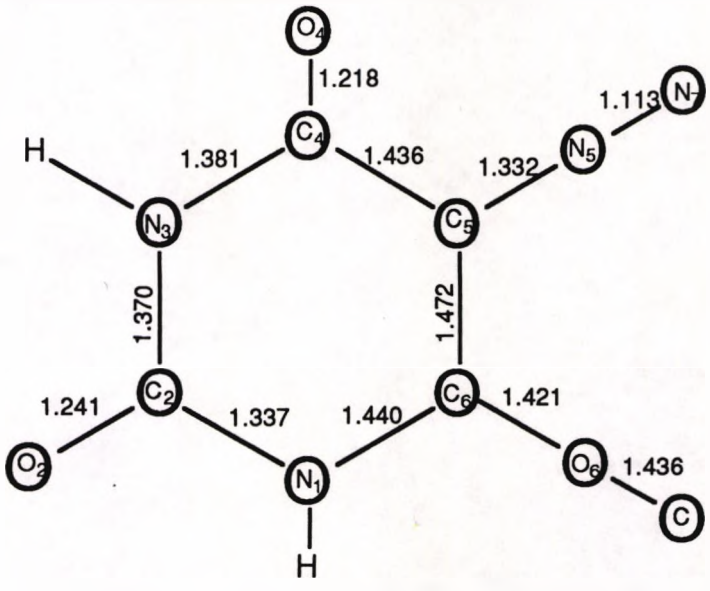
The results obtained to date show that the isomerism present in α–diazoketones is geometrical isomerism. This arises because of conjugation between diazo and carbonyl group, which makes the central C-C bond partially double-bonded. This suggestion is supported by the frequency of the N-N and C-O stretching vibrations compared with those in diazohydrocarbons and ketones. The presence of two conformers is confirmed by the low-temperature doubling of the methine proton in NMR spectra of several diazoketones of the type $RCOCHN_2$.

2.4 Bond length and structure characters of 5-diazouridine and uracil

The bond distances of 5-diazo-6-methoxy-6-hydrouracil and 5-diazo-6-methoxy-5',6-cyclo-2'-deoxyuridine have been determined by X-ray crystallography [25].

2.4.1 5-Diazo-6-methoxy-6-hydrouracil (2.12)

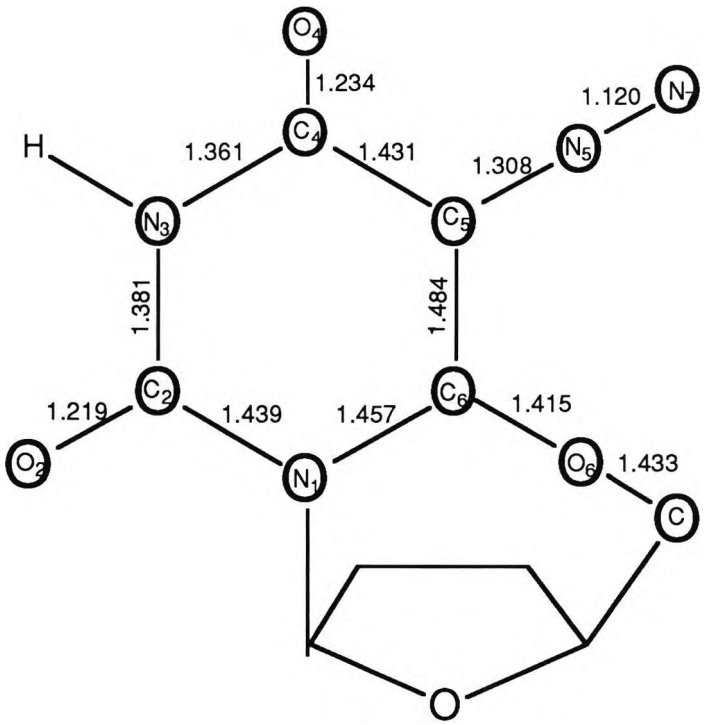
R=0.056; Standard deviation < 0.007 Å



(2.12)

2.4.2 5-Diazo-5',6(s)-cyclo-2'-deoxyuridine (2.13)

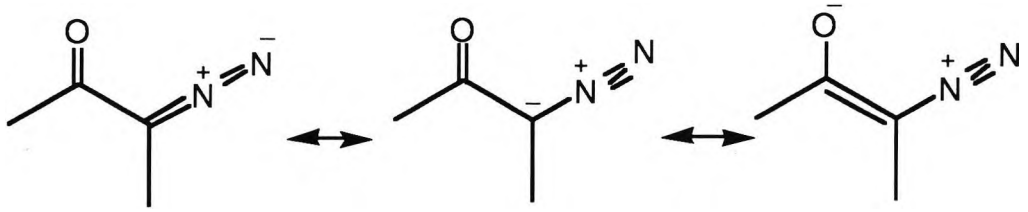
R=0.052; Standard deviation <0.007 Å



(2.13)

2.4.3 Discussion and conclusion

The bond distances observed for (2.12) and (2.13) suggest that the uracil ring is in its keto form with a resonance hybrid between the canonical forms (Scheme 2.7).

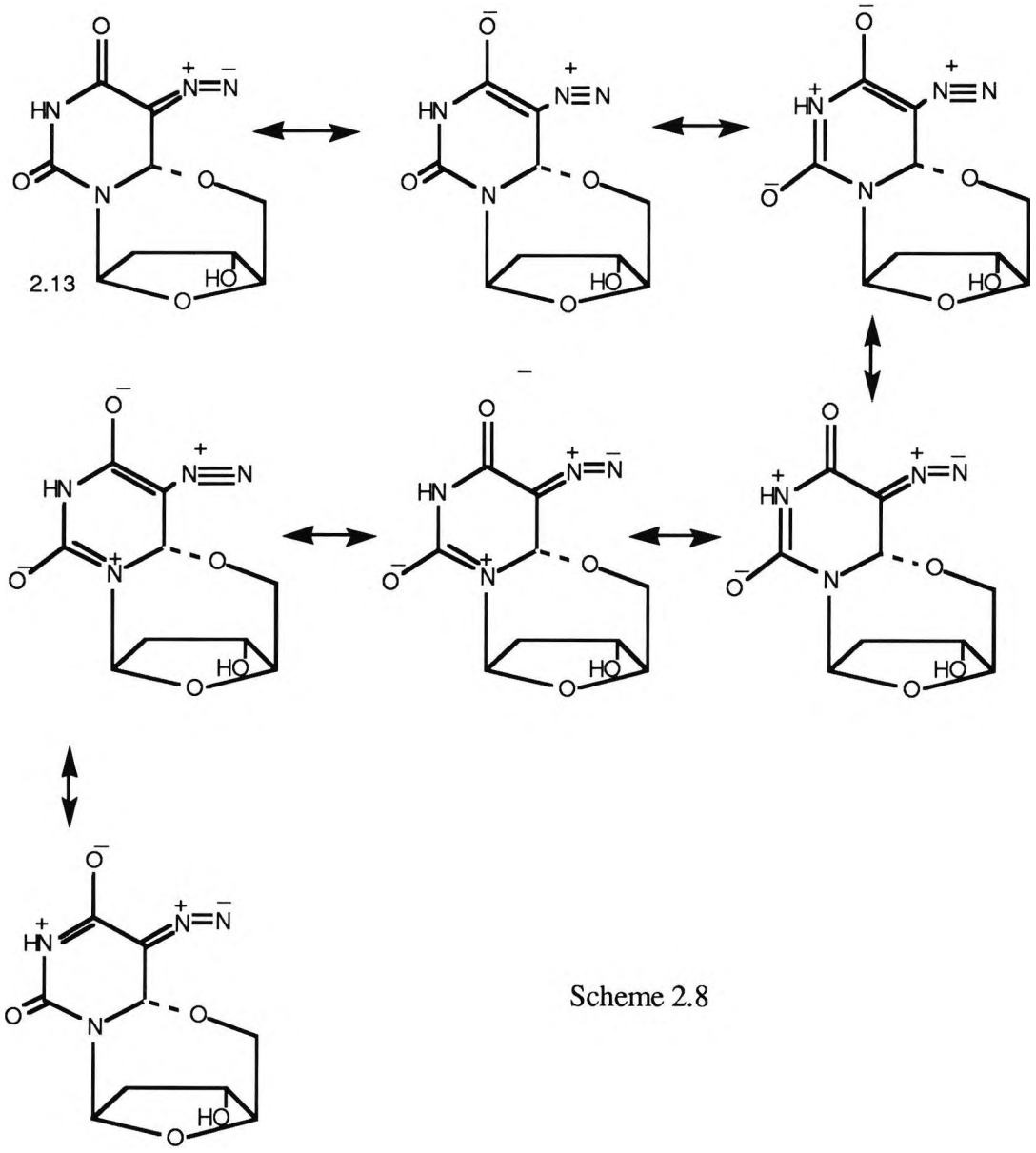


Scheme 2.7

The N-N bond length of the diazo group is greater than the bond length for a diazonium group but less than that of an azo group. The carbonyl bond length is between that of a typical carbonyl in a ketone and a C-O single bond. In addition, the length of C4-C5 (about 1.43 Å) is consistent with the hypothesis of a resonane

hybrid bond of C4-C5, and indicates the partial delocalisation of $\pi_{N\text{-}N}$.

Since there is no α–hydrogen in the 5-diazouridine (2.13), the possibility of the presence of tautomerism can be excluded. The comparison of bond distances in the uracil ring with typical bond lengths (Table 2.1), shows that every bond length in the pyrimidine ring in (2.13) is between the length of a single and double bond, except the typical single bond of N1-C6. This implies that there is a delocalized resonace hybrid system in N7-N5-C5-C4-N3-C2-N1 (Scheme 2.8).



Scheme 2.8

Table 2.1 Average Bond Lengths (Å) In Molecules

Bond label	CC	CN	NN	CO
Single	1.537	1.472	1.451	1.430
Double	1.335	1.320	1.250	1.200
Triple	1.202	1.157	1.098 (N_2)	

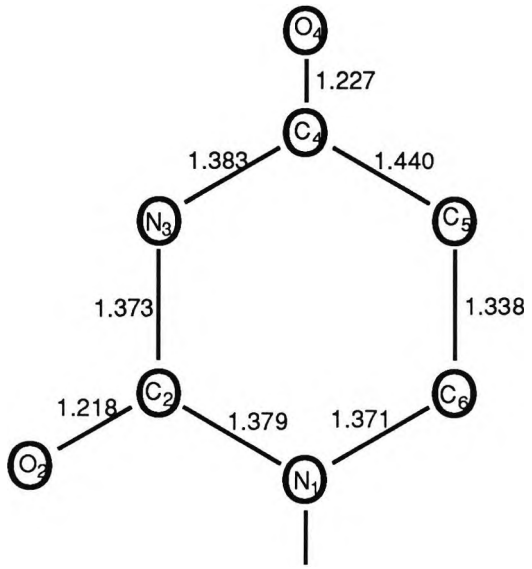
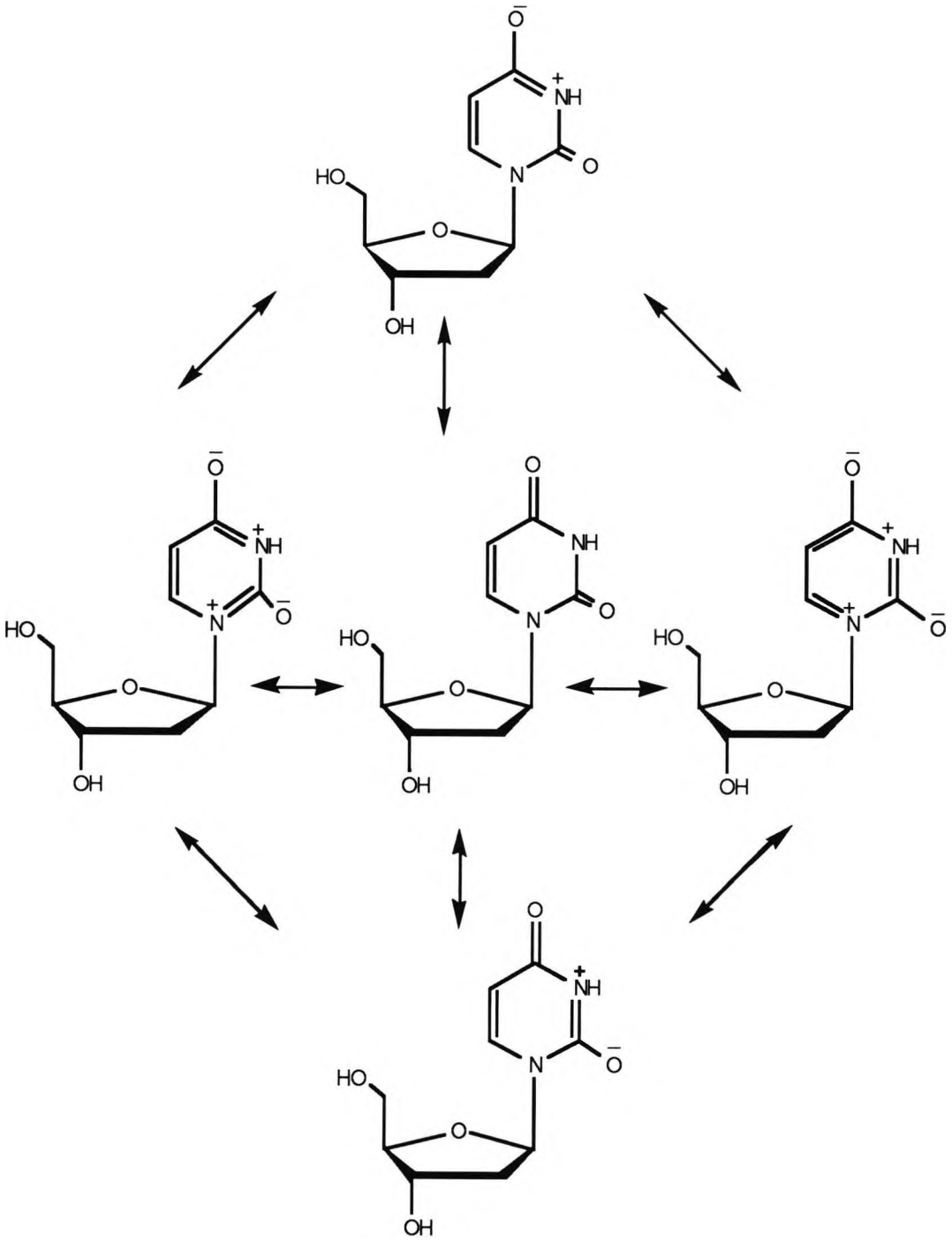


Fig. 2.3

On examination of the bond lengths in uridine (Fig. 2.3), the length of 1.371 Å between N1 and C6 indicates that bond N1-C6 possesses a high degree of double bond character involving conjugation of N1 with the double bond C6-C5. From consideration of the similarity in the length of the rest of the bonds in 5-diazouridine and uridine, it can be predicted that there is a cyclically delocalised resonance hybrid

system in the pyrimidine ring of uridine (Scheme 2.9). Therefore, after the diazo-resonance hybrid system is broken due to some reaction of the diazo function, the heterocyclic base resonance hybrid re-establishes easily.



Scheme 2.9

2.5 Chemical reactivity of diazo compounds

2.5.1 Formation of carbenes from diazo compounds

Carbenes can be obtained by pyrolysis or photolysis of diazo compounds (Scheme 2.10) [26].

$$R_2CN_2 \xrightarrow{\Delta / hv} R_2C: + N_2$$

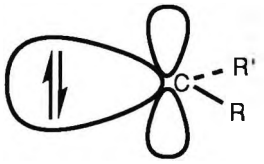
Scheme 2.10

In aprotic solvents, decomposition of the diazo compound yields only the carbene, but in proton-rich solvents protonation of the diazo compound may yield the diazonium ion, and hence the carbonium ion (Scheme 2.11). The borderline between these reactions is often ill-defined.

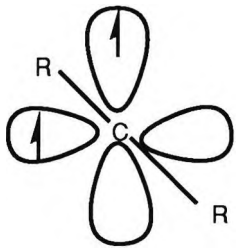
$$R_2CN_2 + H^+ \longrightarrow R_2C(H)-N^+\equiv N \; X^- \longrightarrow R_2HC^+$$

Scheme 2.11

A carbene is a neutral divalent species, which has two covalent bonds to other groups and possesses two non-bonding orbits containing two electrons. With respect to their quantum spin number, the two non-bonding orbits may be paired or unpaired. Two electronic configurations may occur: a bent singlet configuration (A) or a linear triplet configuration (B) (Scheme 2.12).



(A)



(B)

Scheme 2.12

Having less than the usual tetravalency, the carbene behaves like a very strong electrophile and its reactivity means that it exists only as a transient intermediate.

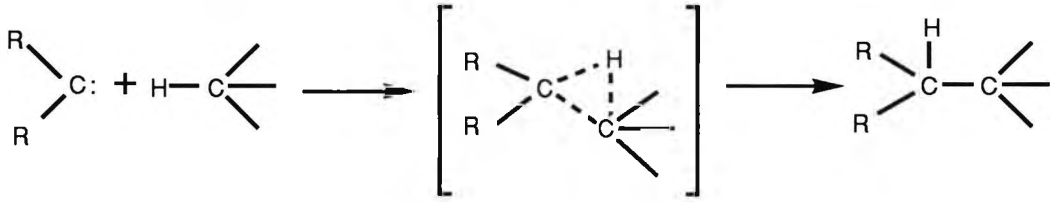
Thermal decomposition of diazo compounds often gives rise to products in which new bonds are formed simultaneously with the departure of N_2.

In those cases in which thermal decomposition of a diazo compound does yield a carbene, the carbene is probably formed as a singlet, that is with a vacant orbital and an electron pair [27]. It can then either react directly as the singlet, or form the triplet state in which it has two unpaired electrons and behaves as a diradical.

2.5.2 Reactions involving the carbene

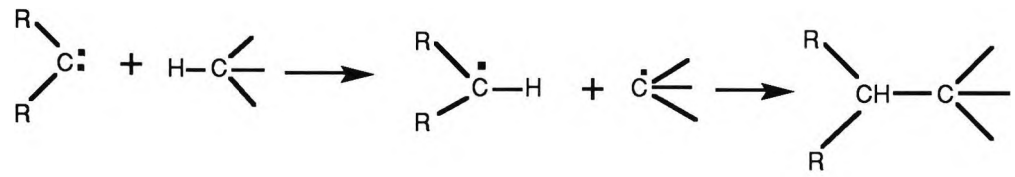
A. Insertion into a C-H bond

Consideration of spin conservation leads to the conclusion that the insertion of a singlet carbene into a C-H bond can be a concerted process involving a three- centre transition state (Scheme 2.13).

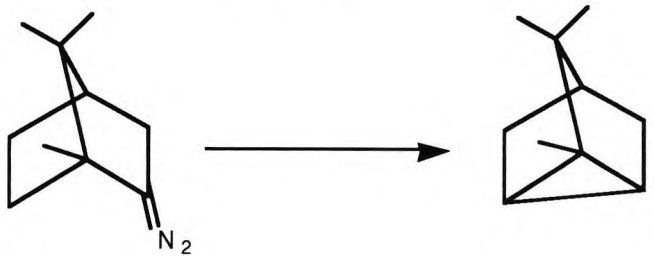


Scheme 2.13

By similar reasoning, the insertion of a triplet carbene (diradical species) into a C-H bond requires a two-step process involving radical formation and combination (Scheme 2.14).



Scheme 2.14

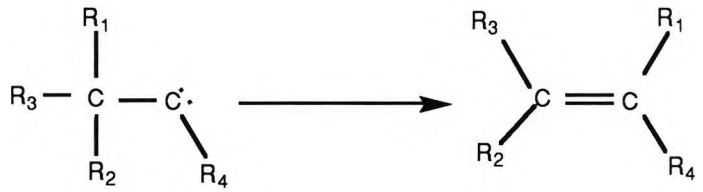


Scheme 2.15

Insertion of a highly energetic carbene, such as methylene, takes place almost equally readily into primary, secondary and tertiary C-H bonds, but phenyl carbene is more selective, and the stable chloromethylene even more selective. The reaction proceeds even better in cyclic systems, where diazocamphane gives tricyclene in 100% yield (Scheme 2.15) [28].

B. Olefin-forming insertion

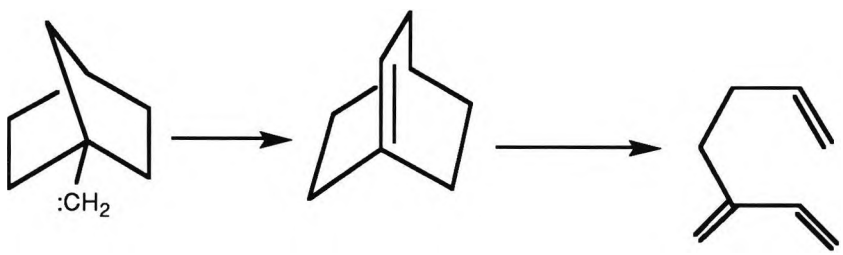
These reactions are of the general type (Scheme 2.16):



Scheme 2.16

The reaction consists of an intramolecular insertion of the carbene into one of the bonds on the α-carbon atom. These reactions are frequently described as 1,2-shift reactions [29].

Olefin-forming insertion reactions have recently been used to generate unstable olefins. An example of this type of reaction is that of the norbornyl derivative shown below (Scheme 2.17) [30].

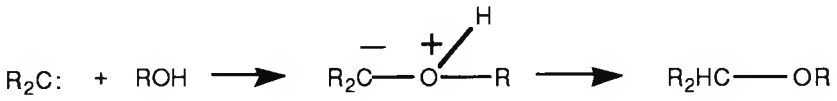


Scheme 2.17

C. Reaction with nucleophiles

The reaction of a carbene with a hydroxyl group shows an overall similarity to insertion reactions of the type discussed in the previous sections, but the mechanism

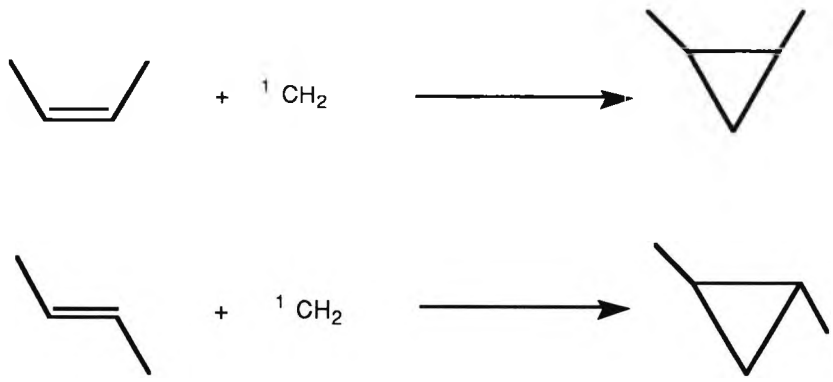
is different. From a detailed study [31], it is concluded that the reaction involves attack of the carbene on the hydroxyl oxygen to give an ylide; this then undergoes prototropic shift to yield the alcohol or ether (Scheme 2.18).



Scheme 2.18

D. Addition to multiple bonds

Addition to an olefin to yield a cyclopropane is one of the characteristic reactions of a carbene. It is generally accepted that addition of a singlet carbene proceeds stereospecifically, while triplet carbene gives non-stereospecific addition [32]. Singlet carbene adds stereospecifically in a concerted step. Both bonds of the cyclopropane are formed simultaneously, since such a step may occur with spin conservation. The additions of $^1CH_2$ (from photolysis of diazomethane in liquid phase) to cis-2-butene and trans-2-butene yield only cis-1,1-dimethylcyclopropane and trans-1,2-dimethylcyclopropane, respectively (Scheme 2.19).

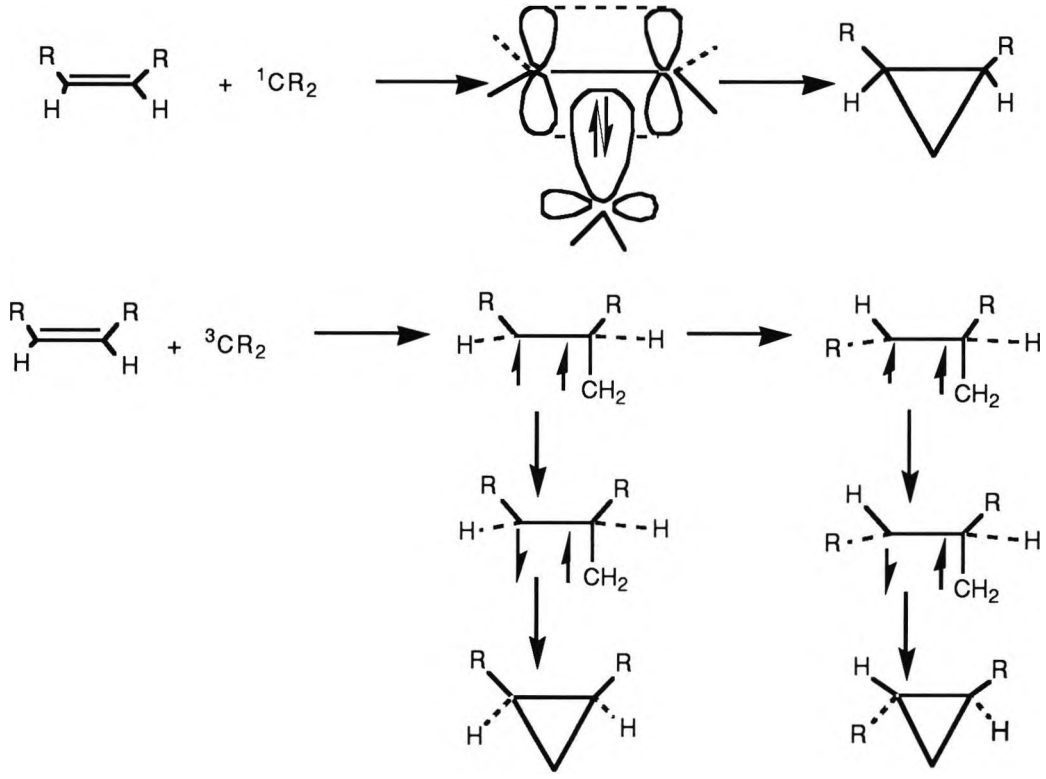


Scheme 2.19

This behaviour has been taken as evidence that the addition of singlet carbene to an olefin is a direct reaction and no diradical intermediate intervenes.

The addition of a triplet carbene to an olefin would generate a triplet trimethylene intermediate, in which rotation about the single C-C bonds would be at least as rapid

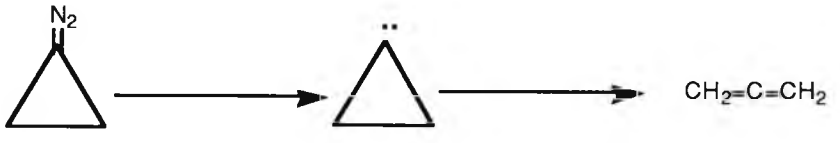
as the spin inversion needed before formation of the final cyclopropane C-C bond could occur. The addition of a triplet carbene to cis- or trans-2-butene should then yield both cis-and trans-1,2-dimethylcyclopropanes. On the other hand, addition of a singlet carbene could be stereospecific, since there would be no spin restriction on the simultaneous formation of both cyclopropane C-C bonds (Scheme 2.20).



Scheme 2.20

E. Fragmentation Reactions

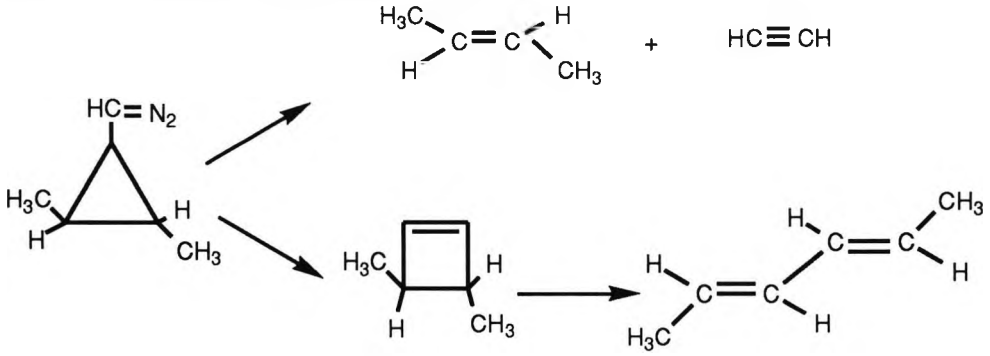
Cyclopropylidene (2.15) is readily generated by thermal decomposition of diazocyclopropane (2.14), and rearranges to yield allene (2.16) (Scheme 2.21) [33].



(2.14) (2.15) (2.16)

Scheme 2.21

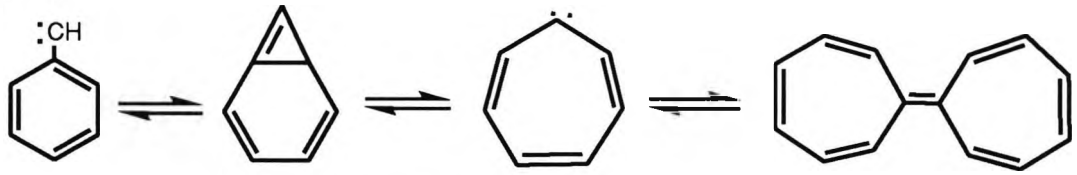
When a carbene is generated next to a cyclopropyl ring, a completely different reaction is observed (Scheme 2.22) [34].



Scheme 2.22

F. Carbene to carbene rearrangements

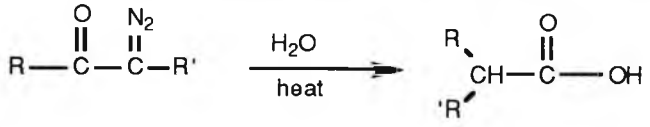
True carbene to carbene rearrangements are uncommon, since carbenes are highly reactive species which usually decompose more readily than they rearrange. Consequently, rearrangement offers a lower energy pathway than does any other reaction only when both the carbenes involved are unusually stable (Scheme 2.23) [35].



Scheme 2.23

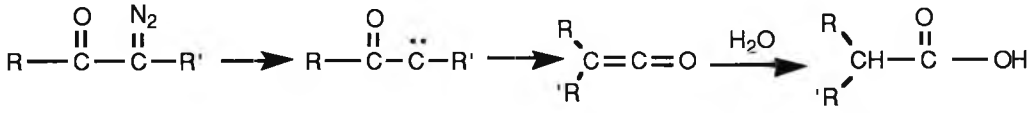
G. The Wolff rearrangement

Thermal or photolytic decomposition of a diazo compound which has a carbonyl group α to the diazo group does not proceed via a simple carbene reaction. The reaction does not involve a carbene which can be trapped by any conventional technique, and in the presence of water gives a rearranged carboxylic acid. The reaction is known as the Wolff rearrangement (Scheme 2.24) [36].



Scheme 2.24

The basic mechanism proposed by Wolff is via the route (Scheme 2.25):

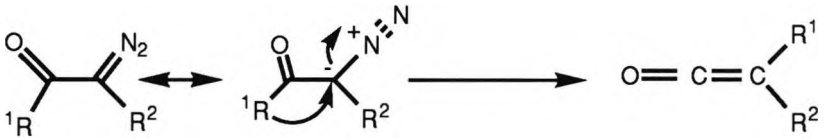


Scheme 2.25

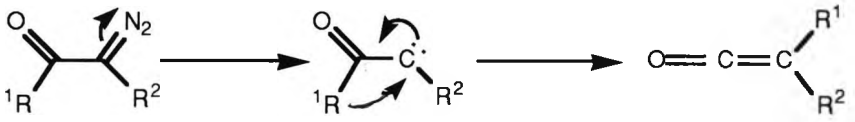
The intermediacy of ketenes seems well established, since ketenes or their decomposition products have been isolated from diazoketone thermolysis in aprotic solvents [37].

Three possible mechanisms for conversion of diazoketones into ketenes have been proposed:

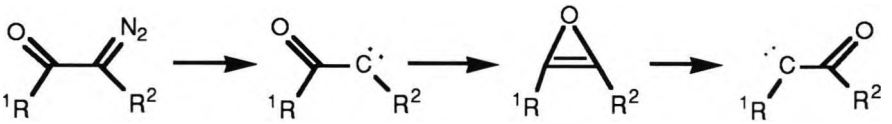
A) a concerted shift:



B) reaction via a ketocarbene:

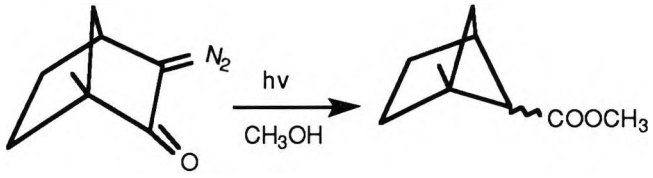


C) reaction involving an oxirene (2.17), probably formed from, or in equilibrium with, a ketocarbene:



2.17

The Wolff rearrangement is a very useful synthetic reaction, as it provides a valuable method of making strained cyclic systems by ring contraction (Scheme 2.26) [38].



Scheme 2.26

H. Carbenoid reactions

Carbenoids have been defined [39] as intermediates which exhibit reactions qualitatively similar to those of carbenes without necessarily being free divalent carbon species. Frequently the carbenoid species has a metal associated with the organic fragment, and carbenoid activity is often found in the reactive species formed when diazoalkanes decompose under the influence of metal elements.

$ZnCl_2$, $HgCl_2$, CuCl, $CuCl_2$ and $CuSO_4$ are often used as catalysts in formation of carbenoids. Recently, rhodium acetate has been found to be a superior catalyst to copper salts [40] in the reactions of 5-diazouracil.

Rhodium acetate is a catalyst which has received an increasing amount of attention in the last few years [41].

A partial crystal-structure determination of rhodium acetate (Fig. 2.4) showed it to be dimeric, consisting of two rhodium (II) ions bridged by four acetate groups.

A Rh-Rh bond unit is stable and can react with ligands to form axially-bound adducts L-Rh-Rh-L (Fig. 2.5) [41].

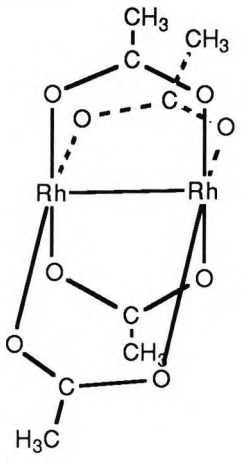


Fig. 2.4

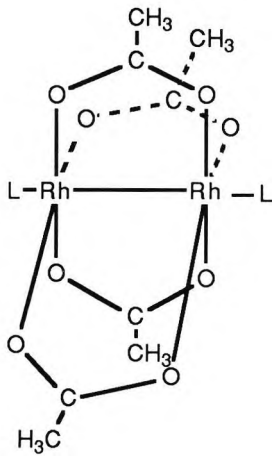
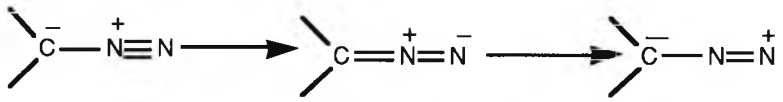


Fig. 2.5

2.5.3 Some other reactions involving the diazo group

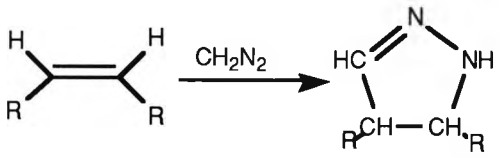
The structure of a diazo group may be described in the valence-bond formalism in terms of the canonical structures (2.1), (2.2) and (2.3), and reactions may be classified on this basis. Thus diazo compounds may react as 1,3-dipoles (2.3) or as nucleophiles through carbon (2.1) or through the terminal nitrogen (2.2). In addition, the unsaturated nature of these compounds renders possible the reduction of a multiple bond.



2.1 2.2 2.3

A. 1,3-Dipolar addition

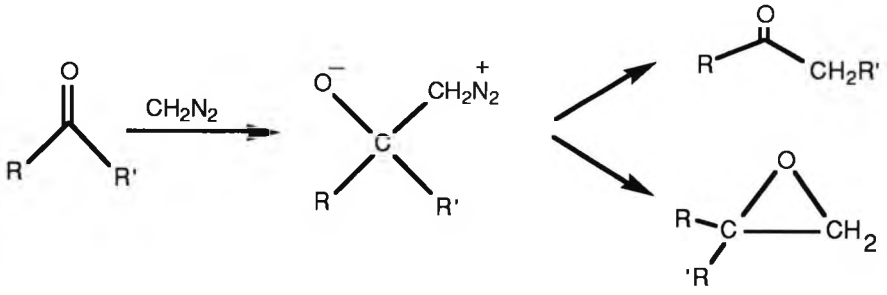
Diazoalkanes readily behave as 1,3-dipoles (e.g. 2.3), and as such will undergo thermal cycloaddition reactions to give pyrazolines (Scheme 2.27) [42].



Scheme 2.27

B. Nucleophilic addition

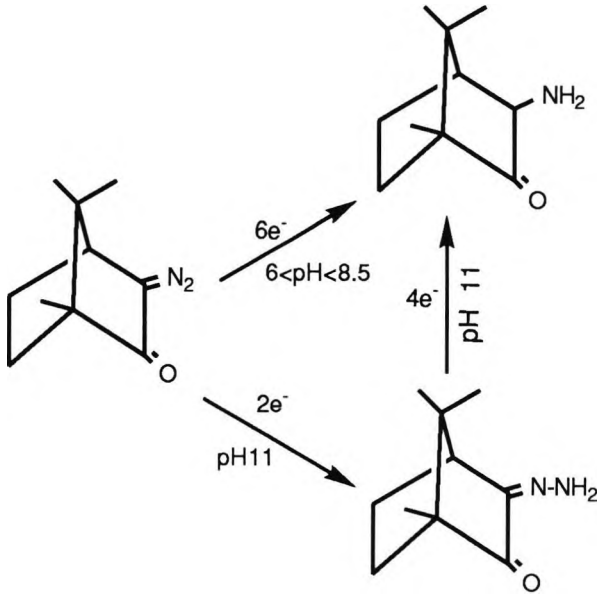
Diazomethane reacts readily with carbonyl compounds to yield epoxides and homologous carbonyl compounds (Scheme 2.28).



Scheme 2.28

C. Reduction of diazo-group

The electrochemical reduction of diazocarbonyl compounds has been reported [43]. In different conditions, the reduced product is different. (e.g. Scheme 2.29) [44]:



Scheme 2.29

References:

1. P. Griess, *Ann.,* 1858, 106, 123.
2. R. Allmann, in "The Chemistry of Hydrazo, Azo and Azoxy Groups", Ed. S. Patai, Wiley Interscience, London, 1975.
3. B.P. Stoicheff, *Canad.J. Phys.,* 1954, 82, 630.
4. O.P. Studzinskij and I.K. Korobitsyna, *Uspekikhim,* 1970, 39, 1754.
5. M. Regitz, in "The Chemistry of Diazonium and Diazo Groups", Ed. S. Patai, Wiley Interscience, London, 1978, 661.
6. H. Zollinger, "Azo and Diazo Chemistry", Wiley Interscience, London, 1961, 15.
8. E. Knoevenagel, *Ber.,* 1890, 23,1995.
9. T. Curtius, *Ber. dt. Chem. Ges,* 1889, 22, 2161.
10. W. Fischer and J.P. Anselme, *J. Amer. Chem. Soc.,* 1967, 89, 5312.
11. K. Heyns and A. Heins, *Liebigs Ann. Chem.,* 1957, 604, 133.
12. E.T. Mcbee and K.J. Sienkowski, *J. Org. Chem.,* 1973, 38, 1340.

13a. D.M. Barton, R.E. O'Brien and S. Sternhell, *J. Chem. Soc.,* 1962, 470. 1

13b. M. Regitz, in "The Chemistry of Diazonium and Diazo Groups", Ed. S. Patai, Wiley Interscience, London, 1978, 659.

14. H. Hope and K.T. Black, *Acta Cryst.,* 1972, B28, 3632.
15. J.B.F.N. Engberts and G. Zuidena, *Rec. Trav. Chim.,* 1970, 89, 741.
16. L.L. Leveson and C.W. Thomas, *Tetrahedron,* 1966, 22, 209.
17. E.Fahr, *Chem. Ber.,* 1959, 92, 398.
18. I.G. Csizmadia, S.A. Houlden, O. Meresz and P. Yates, *Tetrahedron,* 1969, 25, 2121.
19. C. Pecile, A. Foffani and S. Ghersetti, *Tetrahedron,* 1964, 20, 823.
20. R. Cataliotti, S. Sorriso and G. Paliani, *Z. Naturforsch.,* 1972, b27, 875.
21. L.J. Bellamy, "Advances in Infrared Group Frequencies", Methuen, London, 1968, 58.

22. A. Foffani, C. Pecile and S. Ghersetti, *Tetrahedron,* 1960, 11, 285.

23. E. Fahr and K.H. Keil, *Justus Liebigs Ann. Chem.,* 1963, 663, 5.

24. F. Kaplan and G.K. Meloy, *J. Amer. Chem. Soc.,* 1966, 88, 950.

25. D.J. Abraham, T.G. Cochran and R.D. Rosenstein, *J. Amer. Chem. Soc.,* 1971, 93, 6279.

26. D. Bethell, *Adv. Phys. Org. Chem.,* 1969, 7, 153.

27. W. Kirmse, "Carbene Chemistry", Academic Press, London, 1971.

28. J.W. Powell and M.C. Whiting, *Tetrahedron,* 1959, 7, 305.

29. W.E. Slack, C.G. Mosley, K.A. Gould and H. Shechter, *J. Amer. Chem. Soc.,* 1974, 96, 7596.

30. A.D. Wolf and M. Jones, Jr., *J. Amer. Chem. Soc.,* 1973, 95, 8209.

31. D. Bethell, A.R. Newall, G. Stevens and D. Whittaker, *J. Chem. Soc.,* 1969, B, 749.

32. P.S. Skell and R.C. Woodworth, *J. Amer. Chem. Soc.,* 1956, 78, 4496.

33. W.M. Jones and J.M. Walbrick, *J. Org. Chem.,* 1969, 34, 2217.

34. A. Guarino and A.P. Wolf, *Tetrahedron Lett.,* 1969, 8, 655.

35. W.M. Jones, R.C. Jones and J.A. Myres, *J. Amer.. Chem. Soc.,* 1973, 95, 826.

36. L. Wolff, *Ann. Chem.,* 1912, 394, 23.

37. G. Schroeter, *Ber. Deut. Chem. Ges.,* 1909, 42, 2336.

38. T. Gibson and W.F. Erman, *J. Org. Chem.,* 1966, 30, 3028.

39. G.L. Closs and R.A. Moss, *J. Amer. Chem. Soc.,* 1964, 86, 4042.

40. L.K.J. Aw, " Some Chemistry of 5-Diazouracil and Its Derivatives", Ph. D. Thesis, City University, 1989, 222.

41. G.W. Eastland and M.C.R. Symons, *J. Chem. Soc. Dalton Trans.,* 1984, 2193.

42. R. Huisgen, R. Grashey and J. Sauer. in "The Chemistry of Alkenes", Ed. S. Patai, Wiley Interscience, London, 1964, 739.

43. M. Bailes and L.L. Leveson, *J. Chem. Soc.*, 1970, B, 34.

44. A.J. Fry and R.G. Reed, "Double-bond Functional Groups", Wiley Interscience, London, 1977.

Chapter 3. Some Spectroscopic Studies of Nucleosides

3.1 Nuclear magnetic resonance studies of conformational properties of nucleosides

3.1.1 Introduction

Conformational and configurational properties of nucleosides are critical determinants of their functional behaviour in biological systems. For this reason, a great deal of research has been devoted to the conformational analysis of these molecules. Most of the quantitative structural data, e.g., bond lengths, bond angles, interatomic distances, have been derived from X-ray crystallographic measurements in the crystalline state [1-3].

From this information, it has been possible to identify preferred regions of conformational space for nucleoside and nucleotide conformational bonds. Thus, a majority of purine and pyrimidine nucleosides adopt an anti orientation of the base ring with respect to the sugar ring [1-3]. For the D-ribose and D-deoxyribose sugar rings, the favoured out-of-plane ring puckers are those in which either the C2' or C3' atom is furthest from the plane of the other atoms of the ribose ring. In the common ß-nucleosides, the third major functional group, i.e. the exocyclic carbonyl, exists preferentially in a gauche-gauche staggered rotamer form about the C4'-C5' bond [3].

Investigations of structural and conformational behaviour of nucleosides and nucleotides in solution are performed using NMR spectroscopy.

3.1.2 Conformational features of nucleosides

A typical nucleoside can be viewed as possessing three principal conformational feature. Firstly, rotational freedom is possible about the glycosidic C-N bond joining the base and sugar ring. A second source of conformational flexibility exists in the sugar ring where atoms may adopt a variety of ring pucker modes. Finally, the exocyclic groups at the C2',C3'and C4' positions are capable of rotational isomerism about the C2'-O2', C3'-O3' and C4'-C5' (and C5'-O5') bonds respectively.

3.1.3 NMR parameters for conformational analysis

Three NMR parameters, chemical shifts (δ), spin-spin coupling constants (J) and lattice relaxation times (T_1) are generally used to obtain conformational information for nucleosides. Chemical shifts are perhaps of greatest value for identification of H-bonding [4,5], base-stacking interactions [6-8] and, in certain instances, the existence of conformational preferences about glycosidic bonds [9]. However, quantitative evaluation of chemical shift/conformation dependencies is difficult [16].

Spin-spin coupling constants are important for determination of conformation of nucleosides. This utility is directly traceable to the unique dependence of vicinal coupling constant magnitudes on dihedral angle (q) [10]. Much effort has been made to try to establish a linkage of q with J. Two relatively successful equations are:

1. $J_{H,H} = 10.2\ \mathrm{Cos}^2\ q - 0.8\ \mathrm{Cos}\ q$
2. $J_{H,H} = A\ \mathrm{Cos}^2 q - B\ \mathrm{Cos}\ q + C$

(A, B and C are parameters about certain substituent groups bonded to atoms along the coupling path).

Some exceptions have been reported [11], in which it has been found that the theoretically calculated cis H-H coupling in the pentose ring of a nucleoside did not follow any simple dihedral angle relationship; trans couplings, i.e. $90° < q < 180°$, on the other hand, are adequately represented by the dihedral dependence.

Increasing use is being made of spin-lattice relaxation time [12,13] and nuclear Overhauser enhancement (nOe) measurements [14,15] to resolve conformational features of nucleosides.

3.1.4 Orientations about the glycosidic bond

Relative to the sugar moiety, the base can adopt two main orientations about the glycosyl bond of C1-N, called syn and anti [16, 17].

In anti, the bulk of the heterocycle, O2 in pyrimidines is pointing away from the sugar, and in syn, it is over or toward the sugar (Fig. 3.1). The torsion angle X

(O4'-C1'-C2) is used for description of the syn and anti conformations.

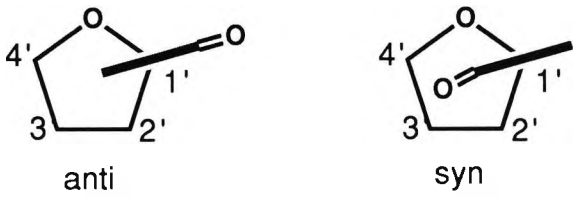


Fig. 3.1

3.1.5 Sugar ring pucker

The five membered furanose ring is generally nonplanar [18]. It can be puckered in an envelope (E) form with four atoms in a plane and the fifth atom out, or in a twist (T) form with two adjacent atoms displaced on opposite sides of a plane through the other three atoms (Fig. 3.2). Atoms displaced from these three or four-atom planes and on the same side as C5' are called endo, those on the opposite side are called exo (Fig. 3.2).

In an abbreviated notation, superscripts for endo atoms and superscripts for exo atoms precede or follow the letter E or T, depending on major or minor puckering. An unsymmetrical C3'-endo-C2'-exo twist with major C3' and minor C2' puckering is represented as $^{3}T_{2}$. The same twist in symmetrical form is designated as $^{3}_{2}T$. A C2'- endo envelope is given as ^{2}E, a C3'-endo envelope as ^{3}E (Fig. 3.3).

The preceding methods for describing sugar puckering are only approximate and they are inadequate. In cyclopentane, conformational changes do not proceed via a planar intermediate but the maximum pucker rotates virtually without potential energy barriers, giving rise to an "infinite" number of conformations Therefore, the pseudorotation phase angle P is used to describe the maximum torsion angle (degree of pucker) (Fig. 3.4). If the five-membered ring is unsymmetrically substituted as in a nucleoside, the "pseudorotation" can be limited by potential energy,which leads to preferred puckering modes of the ring [19].

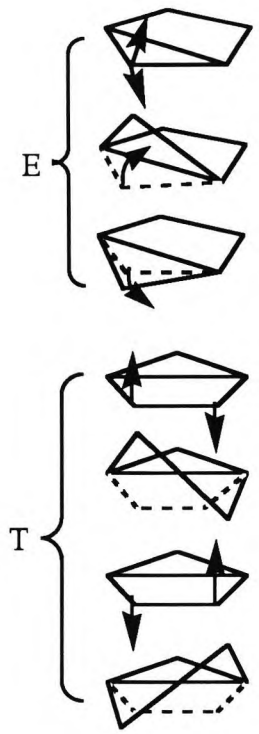


Fig. 3.2

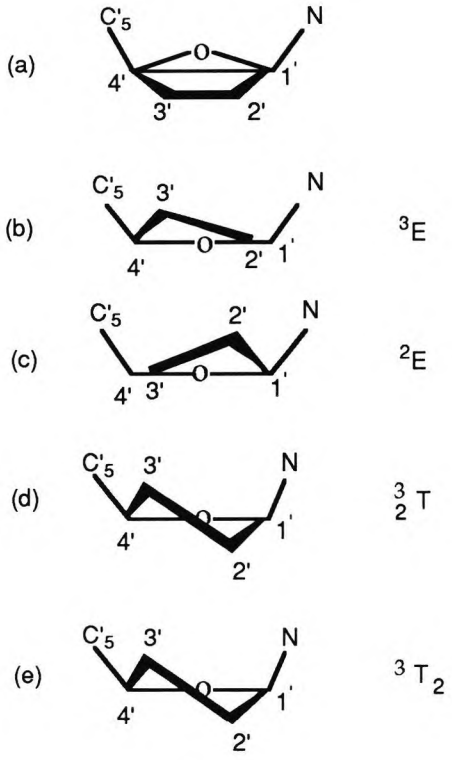


Fig. 3.3

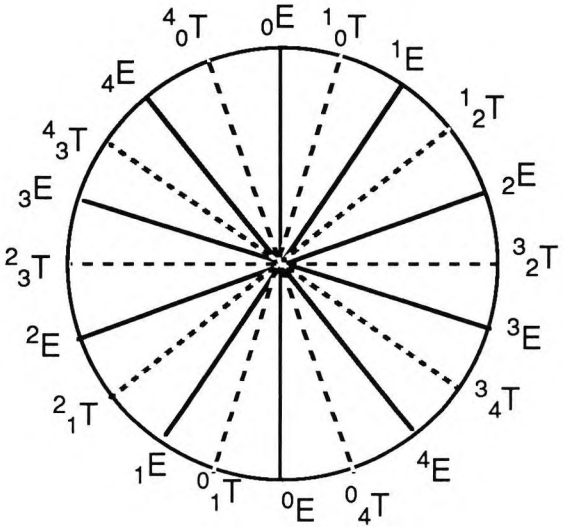


Fig. 3.4

The orientation of substituents with different sugar puckering modes of nucleosides can be summarised in Table 3.1, in which *e* and *a* mean equatorial and axial bonds. *b* means bisectional, i.e. halfway between *a* and *e*..

Table 3.1 Oritation of substituents with different sugar pucking modes

Ribose pucker	Atoms							
	N	H1'	H2'	O2'	H3'	O3'	H4'	C5'
C2'- endo	e	a	a	e	e	a	b	b
C3'- endo	b	b	e	a	a	e	a	e
O1'- endo	e	a	b	b	b	b	a	e
O1'- exo	a	e	b	b	b	b	e	a

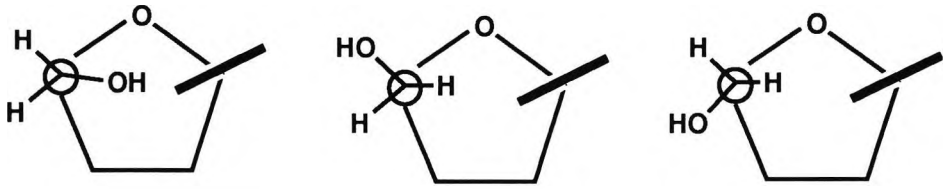
Several methods have been proposed for evaluation of pentose ring conformational properties from coupling data [20-22]. Among the methods, the pseudorotational approach of Altona and Sundaralingam (AS) [22] is conceptually the most sophisticated. In the AS approach, the pentose ring is described in terms of two pseudorotational parameters, P, the angle of pseudorotation and *r*, the degree of pucker [24]. However, the AS approach is also questionable as there are many exceptions [9,11,23].

While crystallographic data show a preponderance of C3'-endo (^{3}E) pucker for ribose and deoxyribose rings of nucleosides [1-3] and a smaller number of C2'-endo (^{2}E) forms in the solid state, the available evidence in solution overwhelmingly indicates that the pentose ring does not possess a unique rigid structure but is in a dynamic equilibrium between at least two or more puckered conformations [9,21,22,24]. In the crystalline state a specific nucleoside exists in one puckered mode [24], whereas in solution ribose and deoxyribose rings are rapidly equilibrating between two puckered conformations. A preference for ^{3}E pucker is exhibited by pyrimidine with the ratio about 9:1 for ^{3}E to ^{2}E.

3.1.6 C4'-C5' Rotamers

Rotation about the exocyclic C4'-C5' bond allows O5' to assume different positions relative to the furanose [25]. Three main conformations with all

substituents in staggered positions are possible (Fig. 3.5). It is common usage to describe these three conformations with the two torsion angles f_{oo}(O5'-C5'-C4'-O4') and f_{oc} (O5'-C5'-C4'-C3') or to use the angular ranges (+) or (-), gauche or trans.



gauche, gauche or (+), (+) | gauche, trans or (+), (-) | trans, trans or (-), (-)

Fig. 3.5

3.2 ^{1}H Chemical shift data for some nucleosides

The protons in nucleosides can be classified into protons in the sugar ring and in the base ring, some of which are exchangeable.

3.2.1 Non-exchangeable protons in base ring

The chemical shift of non-exchangeable protons in the heterocyclic base components of nucleic acids have been reported (Table 3.2) [21].

Table 3.2 Chemical shift (d) of non-exchangeable protons in base ring

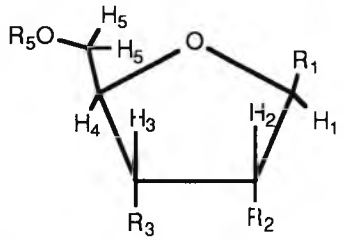
Compounds	H2	H5	H6	H8
Adenine	8.79			8.79
Guanine				8.31
Cytosine		6.63	8.54	
Uracil		6.46	8.4	
Adenosine		8.7		8.95
Guanosine				8.59
Cytidine		6.81	8.54	
Uridine		6.61	8.41	

3.2.2 Non-exchangeable protons in ribofuranose ring

The protons on the sugar moiety which do not readily exchange with D_2O have not always been assigned because of overlapping signals and less accurate coupling constants owing to the broadening effects of the hydroxyl groups.

Table 3.3 Chemical shift (δ) of non-exchangeable protons in ribofuranose ring

Cpd.	H1'	H2'	H3'	H4'	H5'	H5'
a.	5.90	4.35	4.25	4.15	3.82	
b.	6.30	5.51	5.86	4.93	4.76	4.61
c.	6.58	5.47	5.82	4.87	4.86	
d.	5.80	5.55	5.74	4.74	4.68	4.42
e.	6.30	5.56	5.82	4.63	4.63	
f.	6.02	5.01	4.87	4.35	4.00	4.10
g.	2.91	1.60	1.33	1.18	0.85	0.83
h.	6.09	4.71	4.75	4.37	4.60	4.39



Cpd.	R^1	R^2	R^3	R^5
a.	uracil	H	H	H
b.	F	PhCO-	PhCO-	PhCO-
c.	PhCO-	$MeSO_2$-	PhCO-	PhCO-
d.	F	Ac-	PhCO-	PhCO-
e.	Ac-	Ac-	PhCO-	PhCO-
f.	uracil	$(F_3C)_2C(OH)$-	$(F_3C)_2C(OH)$-	$(F_3C)_2C(OH)$-
g.	adenine	H	H	HPO_3-
h.	adenine	H	$O^{\overset{O^-}{P}}O^-$	

The anomeric proton (H1') is the most easily assignable in most cases. It often appears the most downfield compared with other protons in the sugar ring, because of the geminal oxygen and nitrogen, both with a negative inductive effect.

Some chemical shifts of ribofuranose ring protons in some derivatives have been collected in Table 3.3 [26-33].

Some general trends can be observed in the data:

1. The most downfield proton is H1' and the order of chemical shift of the remaining sugar protons is typically H2' > H3' > H4' > H5';

2. Apart from H1', differences of the chemical shifts between adjacent protons are relatively small because of similarity of electronic environments;

3. In some cases, chemical shifts of H2', H3' reverse owing to some

substituent on the α-hydroxyl group;

4. There is no difference between the two protons of the 5' position because of free rotation about C4'-C5' bond.

3.3 ^{13}C NMR chemical shift data for some uracils and uridine

^{13}C NMR spectra are in general much more informative than ^{1}H spectra for structural analysis of molecules, because the ^{13}C chemical shifts are more sensitive to structural changes. In the case of uracils, ^{13}C chemical shifts of the four carbons are showed in Table 3.4.

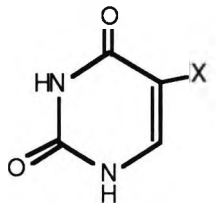


Table 3.4 ^{13}C NMR data (δ) of some uracils

X	C4	C2	C6	C5
H	165.2	152.74	142.98	100.95
CH_3	165.6	152.19	138.4	108.4
F	158.67	150.84	129.93	140.6
Cl	160.69	151.36	140.45	106.66
Br	160.83	151.56	142.91	95.1
I	162.28	152.04	147.77	67.26

The variations for ^{13}C chemical shifts of some uridine phosphates are collected in Table 3.5.

Table 3.5 ^{13}C NMR data (δ) of some uridine phosphates

	C4	C2	C6	C5	C1'	C4'	C2'	C3'	C5'
uridine	167.3	125.3	143	103.4	90.5	85.4	74.8	70.6	61.9
5'-UMP	167.6	153.2	143.2	103.7	89.6	85.1	75	71.1	64.4
3'-UMP	167.4	152.9	143	103.5	90.3	84.9	74.6	73.1	61.9
2'-UMP	167.7	153.2	144	103.7	90.3	85.3	76.5	71	62.3

In addition, ^{13}C chemical shift data are more sensitive in different solvents than ^{1}H. The ^{13}C chemical shift data of uridine in some solvents are given in Table 3.6.

Table 3.6 ^{13}C NMR data of uridine in some solvents

solvent	C4	C2	C6	C5	C1'	C4'	C2'	C3'	C5'
DMSO	164.6	152.3	142.3	103.3	89.3	86.3	75.1	71.4	62.4
Py	164.6	152.3	141.2	102.5	90.5	86.3	76.0	71.2	61.9
D_2O	167.3	152.8	143.0	103.4	90.6	85.5	74.8	70.6	62.0
H_2O	167.4	152.9	143.1	103.5	90.7	85.5	75.0	70.8	62.2

On examination of the ^{13}C chemical shift data of uridine, the order of the shifts is C4>C2 > C6 > C5 > C1' > C4' > C2' > C3' > C5'. The substitution of phosphate on the sugar hydroxyls causes limited variation in the chemical shift. The 5-substitution of the heterocyclic base causes great variation for the C5 and C6 shifts.

3.4 Mass spectrometry

The success of mass spectrometry as an analytical technique is due to its potential to supply definitive qualitative and quantitative information on molecules based on their structural composition. The technique has been extensively modified and developed. The selection of the best combination of sample introduction and ionisation methods is dependent on the nature of this ionisation and the molecular structure.

3.4.1 Comparison of ionisation methods

In the ion source of an EI mass spectrometer, ionisation is induced in volatilised sample molecules by collision with a beam of energetic electrons. Removal of an electron from the molecule in the collision process produces a radical molecular ion:

$$M + e \longrightarrow M^{+\cdot} + 2e$$

There is a drawback for applicability of EI, which is that only a limited number of molecules are sufficiently thermally stable to be volatilised and thus suitable for EI. The stability of molecular ions of many compounds is low and as a result they may be absent or of low relative abundance compared to fragment ions. The usefulness of the EI method is sometimes limited only to information about fragmentation. The high energy during ionisation of molecule by EI can be reduced

during chemical ionisation (CI) of molecules, in which a reagent gas is ionised in an EI-type ion source and the reactive ionised plasma is used to ionise sample molecules by ion molecule interaction. The stability of the quasi-molecular ion in CI often gives a gain in sensitivity over EI. CI is used to provided molecular weight information and for the production of ions prior to collision-induced dissociation experiments.

Fast atom bombardment (FAB) is a method which involves the desorption and ionising of a solid sample in a matrix, using a bombarding beam of fast atoms. The application of FAB provides a technique for analysis of thermally labile and non-volatile compounds. The disadvantage of FAB is the relatively high background level of chemical noise giving ions at every mass in the spectrum in addition to intense cluster ions from the matrix. This leads to much lower sensitivity of FAB ionisation in comparison with EI and CI.

The negative mass technique can be achieved, for example in FAB. Negative ionisation provides different and complementary structural information to positive ionisation.

3.4.2 Analysis of nucleosides using mass spectrometry

The major limitation of mass spectrometry in the analysis of nucleic acid and nucleosides as well as nucleotides is the thermally labile and non-volatile nature of these classes of compounds.

The mass spectral analysis of thermally labile or non-volatile compounds has undergone a profound revolution with the introduction of FAB as an ionisation technique. This is the method of choice in the analysis of nucleosides, and the advantages of FAB over other ionisation methods have been described [34].

In addition to the standard EI, CI and FAB , a number of other techniques have been developed for the mass spectral analysis of nucleosides, such as FD (desorption chemical ionisation), SIMS (secondary ion mass spectra) and DCI (desorption chemical ionisation). However, these methods do not appear to have significant advantage over EI, CI and FAB [35]. Therefore, they are used only for completeness

of analysis of nucleosides at the present time.

A comparison of EI, CI and FAB ionisation methods for nucleosides spectra of the eight major nucleosides found in RNA and DNA, and pseudouridine has been reported (Table. 3.7) [36]. In general, the acquisition of FAB spectra of nucleosides is a relatively straightforward procedure and provides important information concerning molecular weight and the mass of the aglycone. Moreover, the amount of structural information in a normal FAB spectrum is limited relative to the classical EI data. Therefore, use of the FAB technique in addition to EI or CI can provide more detailed data for structural analysis.

Table 3.7. A comparison of the major ions and their relative intensities of nucleoside ionized using FAB, EI and CI

compounds	m/z(%)	MH (a,b) m/z(%)	B+44 (c) m/z(%)	B+30 (d) m/z(%)	B+2H m/z(%)	Sugar m/z(%)	Other
adenosine	FAB	268(92)	178(3)	164(7)	136(100)	133(3	[MH+G]: 360 (28)
	CI	268(59)	178(3)	164(3)	136(100)	133(2)	
	EI	267(62)	178(35)	164(70)	136(78)	133(2)	BH: 135(100)
guanosine	FAB	284(62)	194(5)	180(3)	152(100)	133(2)	[MH+G]: 386 (12)
uridine	FAB	245(58)	155(6)	141(1)	113(100)	133(11)	[M+G]:337(13)
	CI	245(19)	155(3)	141(17)	113(100)	133(33)	
	EI	244(9)	155(6)	141(20)	113(100)	133(57)	BH: 112(22)
cytidine	FAB	244(63)	154(4)	140(4)	112(100)		[MH+G]: 336(12)
	CI	244 (2)	154(2)	140(2)	112(100)		
	EI	243(1)	154(6)	140(43)	112(100)	133(2)	
deoxy-	FAB	252(54)	162(7)	164(4)	136(100)	117(4)	[MH+G]: 344(8)
adenosine	CI	252(10)	162(6)	164(23)	136(100)	117(5)	
	EI	251(10)	162(37)	164(12)	136(30)	117(2)	BH: 135(100)
deoxy- guanosine	FAB	268(43)	178(4)	180(10)	152(100)	117(2)	[MH+G]: 360(3)
deoxy-	FAB	228(46)	138(6)	140(10	112(100)	117(5)	[MH+G]: 320(5)
cytidine	CI	138(2)	140(4)	112(100)	117(20)		
	EI	227(2)	138(15)	140(<1)	112(63)	117(15)	BH: 111(100)
thymidine	FAB	243(42)	153(2)	155(24)	127(100)	117(38)	[MH+G]: 335
	CI	243(42)	53(2)	155(24)	127(100)	117(49)	
	EI	242(8)	153(4)	155(<1)	127(27)	117(100)	BH: 126(32)
pseudo-	FAB	245(100)	155(29)	141(8)	113(15)		
uridine	CI	245(64)	155(100)	141(19)	113(28)	133(2)	
	EI	244(1)	155(8)	141(8)	141(100)	113(10)	

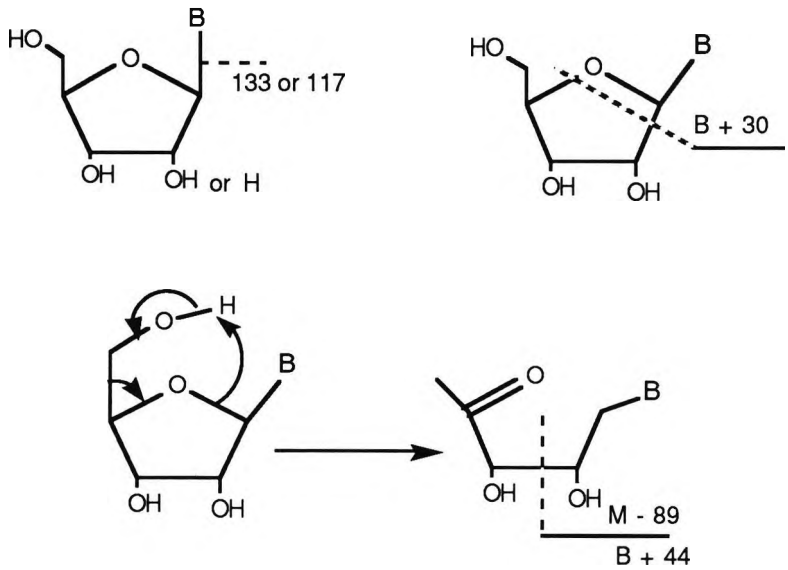
a: Molecular ion species in EI is M;
b: Methane used as reagent gas in CI mode;
c: In the deoxyseries, the ion corresponding to B+44 ion is the B+28 ion;
d: The relative contributions of $BH{\cdot}C_2H_5$ and $B{\cdot}CH_2O$ to the B+30 ion in the CI mode (methane) are not currently known.

3.4.3 Fragmentation of nucleosides

The heterocyclic base fragment is often identified by the observation of B + 1 and B + 2 peaks which correspond to the mass for the base fragment plus either one or two hydrogens [37].

The D-ribose unit is characterised by a peak at m/z 133, whilst deoxyribose is characterised by a peak at m/z 117. One fragment which can be used to characterise the glycosidic link in free nucleosides is the base + 30 peak. Labelling experiments show this to be composed of base component, the anomeric carbon and its hydrogen and the furanoside oxygen with any abstracted proton [38]. A similar fragment containing the base and the C1' and C2' carbons might be observed at the base + 44.

The nucleosides can be characterised by masses M + 30 and M - 89 which correspond to the fragmentation of the sugar moiety (Scheme 3.1).

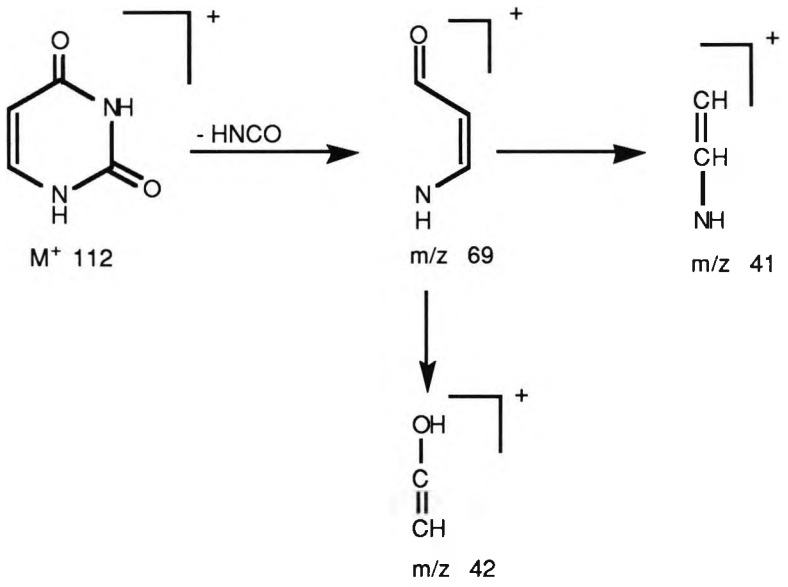


Scheme 3.1

3.4.4 Fragmentation of pyrimidines

In general, pyrimidines are sufficiently volatile for EI and CI at temperatures between 130-200 °C [38]. The fragmentation of uracil and its derivatives has been studied [39]. In the EI mass spectrum of uracil, the molecular ion occurs at m/z 112 and the main fragments at m/z 69, m/z 42 and m/z 41 are assigned to the loss of

HNCO and then either HCN or CO (Scheme 3.2).



Scheme 3.2

The loss of HNCO from pyrimidine is a common feature also seen in the EI spectra of cytosine, thymine,etc [40]. Other common features are the loss of HCN from pyrimidine-type compounds.

References

1. M. Sundaralingam, in: "Conformations of Biological Molecules and Polymers", Proceedings of the Jerusalem Symposium V, Ed. Bergmann and B. Pullman, Israel Academy of Sciences and Humanities, Jerusalem, 1973, 417.
2. W. Saenger, *Angew. Chem. Int. Ed. Engl.,* 1973, 12, 591.
3. M. Sundaralingam, *Biopolymers* , 1969, 7, 821.
4. L. Katz and S. Penman, *J. Mol. Biol.,* 1966, 15, 220.
5. C.D. Poulter and C.L. Liringston, *Tetrahedron Letters*, 1979, 9, 755.
6. M.P. Schweizer, S.I. Chan and P.O.P. Ts'o , *J. Amer. Chem. Soc.,* 1965, 87, 5241.
7. F.E. Hruska and S.S. Danyluk, *Biochem. Biophys. Acta,* 1968, 157, 238.
8. P.O.P. Ts'o, N.S. Kondo, M.P. Schweizer and D.P. Hollis, *Biochemistry*, 1969, 8, 997.
9. D.B. Davies and S.S. Danyluk, *Biochemistry*, 1974, 13, 4417.
10. M. Karplus, *J. Chem. Phys.,* 1959, 30, 11.
11. A. Jaworski, I. Ekiel and D. Shuar, *J. Amer. Chem,.Soc.,* 1978, 100, 14.
12. C. Chachaty, T. Zemb, C. Langlet, Son. Tran-Dinh, H. Buc and M. Morange. *Eur. J. Biochem.*, 1976, 62, 45.
13. K. Akasaka, T. Imoto and H. Hatano, *Chem. Phys. Letters*, 1973, 21, 398.
14. P.A. Hart and J.P. Davis, *J. Amer. Chem. Soc.,* 1971, 93, 753.
15. P.A. Hart, *J. Biophysical*, 1978, 24, 833.
16 J. Donohue and K.N. Trueblood, *J. Mol. Biol.,* 1960, 2, 363.
17. A.E.V. Haschemeyer and A. Rich, *J. Mol. Biol.,* 1967, 369.
18. H.P.M. DeLeeuw, C.A.G. Haasnoot and C. Altona, *Isr. J. Chem.,* 20, 108.
19. A.A. Lugovskoi and V.G. Dashevskii, *Mol. Biol.,* 1972, 6, 354.
20. M. Smith and C.D. Jardetzky, *J. Mol. Spectrosocopy*, 1968, 28, 70.
21. F.E. Hruska, in: "Conformation of Biological Molecules and Polymers", Proceedings of the Jerusalem Symposium V, Ed. Bergmann and B. Pullman,

Israel Academy of Sciences and Humanities, Jerusalem, 1973, 345.

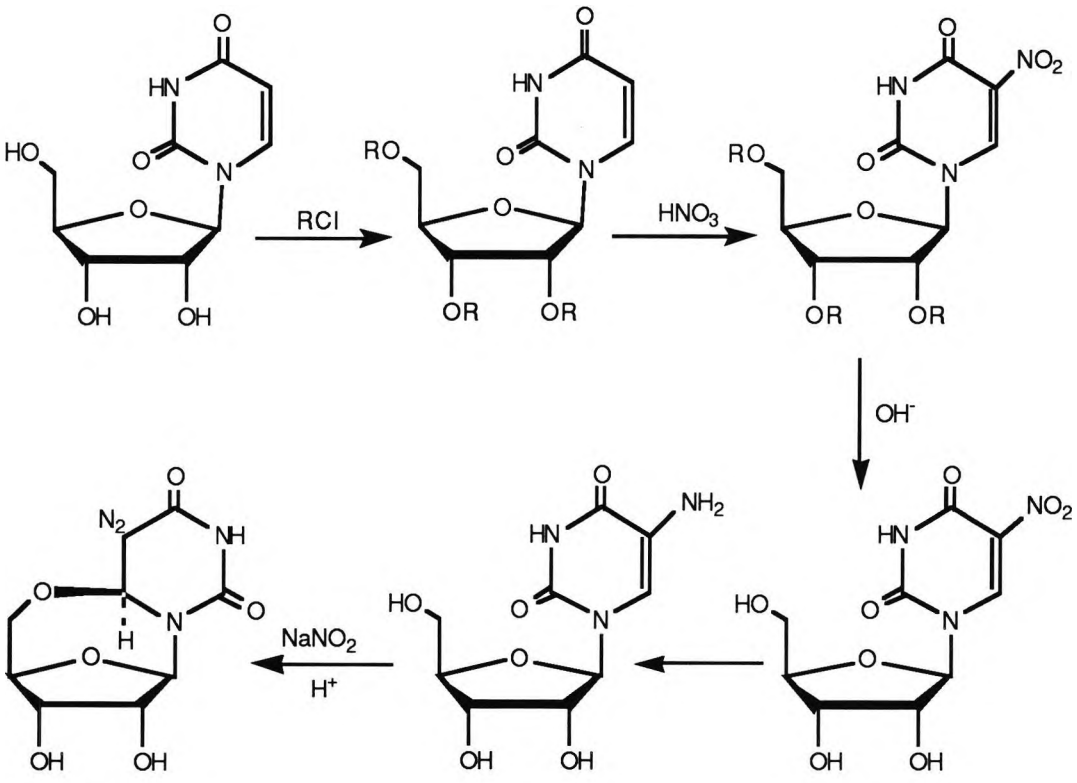
22. C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.,* 1973, 95, 2333.
23. F.E. Evans and R.H. Sarma, *J. Biol. Chem.,* 1974, 249, 4754.
24. C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.,* 1972, 94, 2333.
25. J.L. Sussman, N.C. Seeman, S.H. Kim and H. Berman, *J. Mol. Biol.,* 1972, 66, 403.
26. C.D. Jardetzky and O. Jardetzky, *J. Amer. Chem. Soc.,* 1960, 82, 222.
27. W. Regel and E. Stengele, *Org. Magn. Res.,* 1974, 6, 558.
28. A.A. Gray, I.C.P. Smith and F. E. Hruska, *J. Amer. Chem. Soc.,* 1971, 93, 1765.
29. F.E. Hruska, A.A. Crey and I.C.P. Smith, *J. Amer,.Chem. Soc.,* 1971, 93, 1765.
30. I.D. Hall, P.R. Steiner and C. Pedersen, *Can. J. Chem.,* 1970, 48, 1155.
31. T. Nishiwki, *Tetrahedron,* 1967, 23, 2657.
32. J.D. Stevens and H. Fletcher, *J. Org. Chem.,* 1968, 33, 1799.
33. R.H. Sarma and R.J. Mynott, *J. Amer. Chem. Soc.,* 1973, 95, 1641.
34. M. Barber, R.S. Bordoli, G.J. Elliott, R.D. Sedgwick and A.N. Tyler, *Anal. Chem.,* 1982, 54, 645A-647A.
35. A.M. Lawson, "Mass Spectrometry", de Gruyter, Berlin, 1989, 534.
36. D. Slowikowski and K.H. Schram, *Nucleosides Nucleotides,* 1985, 4, 347.
37. K. Biemann and J.A. McCloskey, *J. Amer. Chem. Soc.,* 1962, 84, 2005.
38. J.M. Rice and G.O. Dudek, *J. Amer. Chem. Soc.,* 1967, 89, 2719.
39. J.M. Rice, G.O. Dudek and M. Barber, *J. Amer. Chem. Soc.,* 1965, 87, 4569.
40. T. Nishiwaki, *Tetrahedron,* 1967, 23, 1153.

Chapter 4. Preparation of 5-Diazo-5',6(s)-cyclo-uridine and 5-Diazo-5',6(s)-cyclo-2'-deoxyuridine

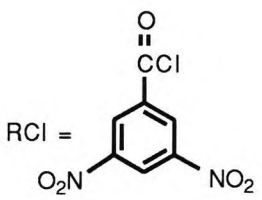
4.1 Introduction

5-Diazo-5',6(s)-cyclo-uridine (4.1) and 5-diazo-5',6(s)-cyclo-2'-deoxyuridine (2.13) are potentially very useful intermediates for the preparation of 5-substituted uridines, because the 5-diazo function is very reactive.

The reported preparation [1] of compound (4.1) from uridine included 5 stages, which were protection of the hydroxyl groups in the sugar ring, nitration of the protected uridine to produce the 5-nitro derivative, deprotection of the compound by hydrolysis, reduction of the 5-nitrouridine to 5-aminouridine and diazotization of 5-aminouridine to afford the final product, compound (4.1) (Scheme 4.1)

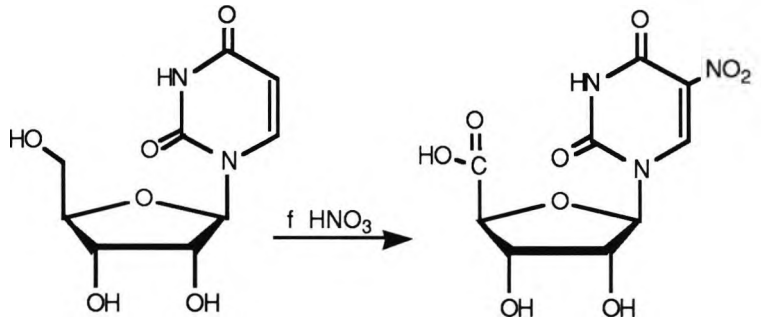


(4.1)



Scheme 4.1

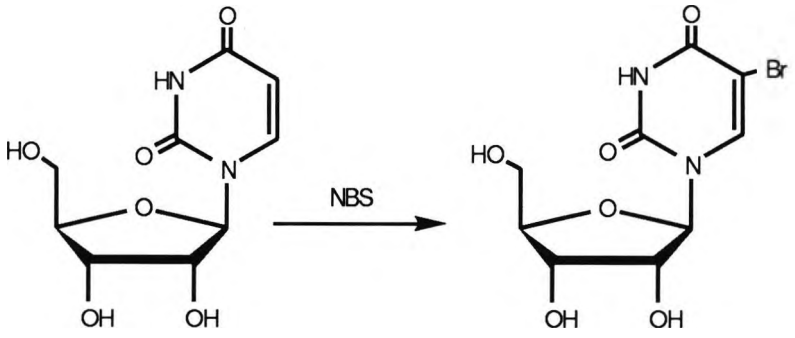
Protection in the first stage was necessary, otherwise uridine gave an oxidised nitronucleoside when nitrated directly with nitric acid [2]. Subsequent work [1] revealed the product to be the 5-nitro-1-(b-D- riboyl-uronic acid)-uracil in which the 5'-hydroxyl group had been oxidised (Scheme 4.2).



Scheme 4.2

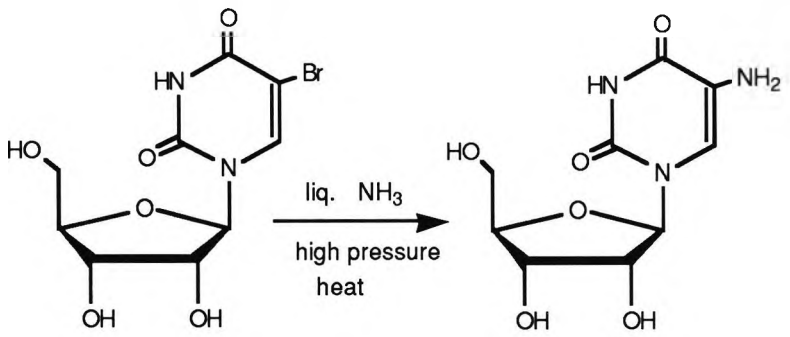
Furthermore, the selective reduction of the nitro group to the amine without affecting the double bond has proved to be difficult. In view of these difficulties, alternative routes to the 5-diazo derivatives of uridine have been explored.

Srivastava and Nagpal [3] reported that 5-bromouridine (4.2) was synthesised by bromination of uridine with N-bromosuccinimide (NBS) (Scheme 4.3).



Scheme 4.3 (4.2)

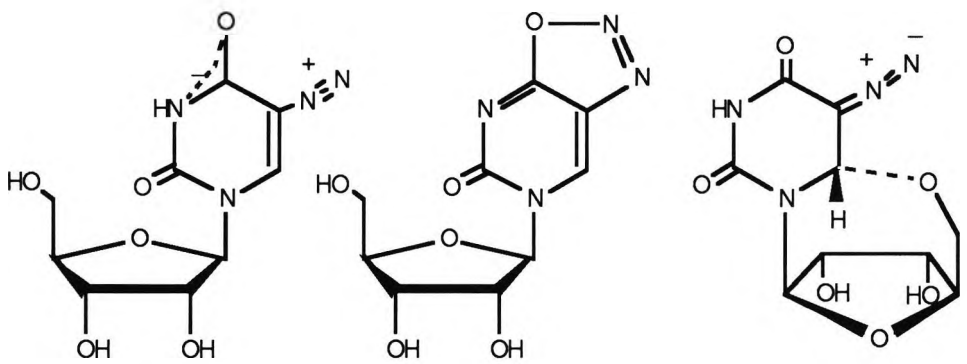
According to the paper of Roberts and Visser [4], 5-aminouridine (4.3) could be prepared by using liquid ammonia for ammoniation of 5-bromouridine under high pressure (Scheme 4.4).



(4.2) (4.3)

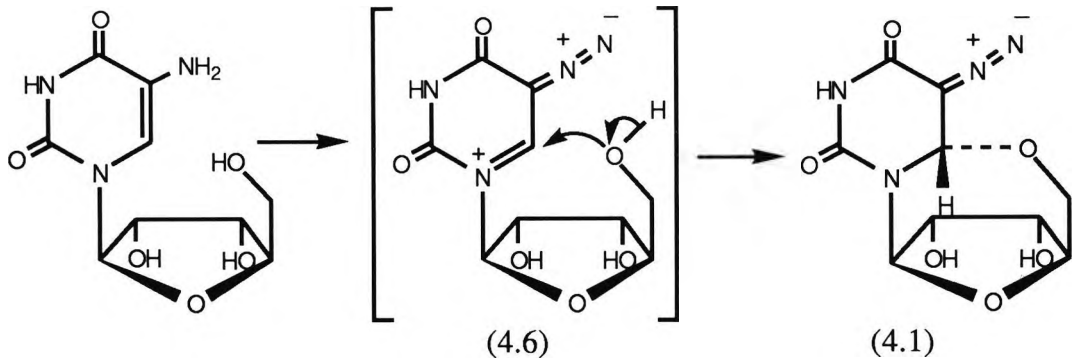
Scheme 4.4

Originally in the literature, tentative structures (4.4 and 4.5) were assigned to the diazotisation product from 5-aminouridine [4,5]. However, the structures proposed seemed at odds with the ^{1}H NMR of the compound and the third structure (4.1) was assigned to it [6]. The structure (4.1) was also confirmed by X-ray crystallography [7].



(4.4) (4.5) (4.1)

Therefore, it was postulated that the formation of the diazo compound (4.1) from 5-aminouridine took place via an unstable intermediate of structure (4.6) (Scheme 4.5) in which there is a strongly electrophilic character at the 6 position. The close proximity of the 5'-hydroxyl group could then allow cyclonucleoside formation to occur rather than cleavage of the glycosidic bond.



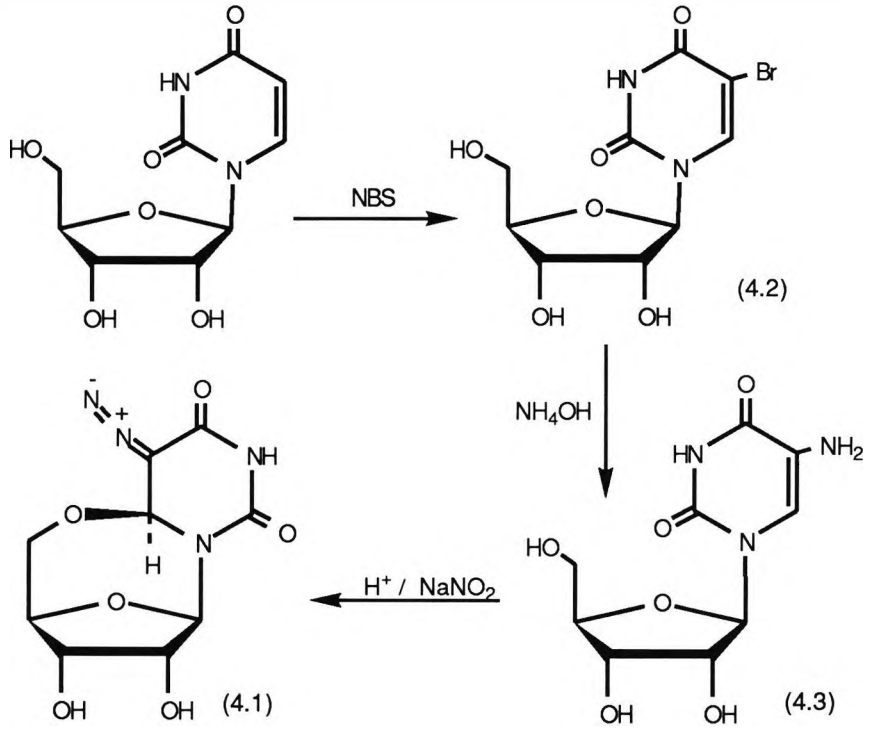
(4.6) (4.1)

Scheme 4.5

4.2 Results and discussion

In the present work, we linked the two reactions shown in Schemes 4.3 and 4.4 to form a highly efficient pathway to prepare compound (4.1).

Whilst 5-bromouridine (4.2) can be easily prepared from uridine by bromination with NBS, the ammoniation of 5-bromouridine is a difficult step in this route. Ammoniation by heating with liquid ammonia can cause very high pressure in the reaction container. Consequently, the scale of the preparation is limited and it was found that the sealed tube was often broken owing to the high pressure.



Scheme 4.6

Therefore, we tried to modify the ammoniation to prepare 5-aminouridine by using saturated aqueous ammonia solution instead of liquid ammonia. Success in using this modification of the ammoniation was achieved and this greatly reduced the reaction pressure. Moreover, the yield in the modified ammoniation is the same as that of the original ammoniation. As a result of the modification, this is a highly efficient and simple method for the preparation of 5-diazo-5',6(s)-cyclo-uridine (4.1) (Scheme 4.6).

4.2.1 Preparation of 5-bromouridine (4.2)

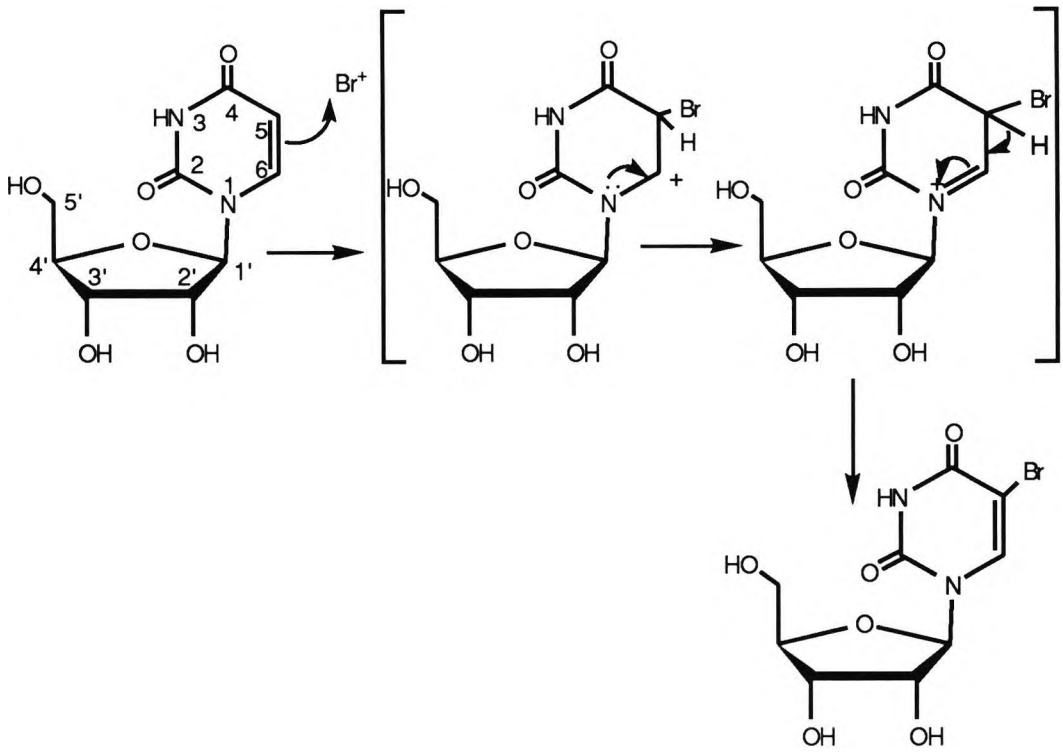
5-Bromouridine (4.2) was prepared from commercially available uridine in DMF using N-bromosuccinimide (NBS) at room temperature for 16 h. NBS releases a positive bromine ion as the electrophilic agent. Electrophilic substitution of uridine is favoured at the 5-position where the enamine- like contribution from the electron pair of the nitrogen (N1) enhances the nucleophilic character of C5 (Scheme 4.7).

The product was characterised in its I.R. spectrum by strong peaks at 1678 cm^{-1} and 1625 cm^{-1} which were attributed to the carbonyls of the uracil ring. The peaks around 3350 cm^{-1} were taken as evidence for the presence of the hydroxyl group and imido group.

Since 5-bromouridine (4.2) was prepared from uridine, it is helpful to first analyse the ^1H NMR spectrum of uridine. ^1H NMR data of uridine are given in Table 4.1.

Table 4.1. ^1H NMR data of uridine

chemical shift δ		int.	assignment
11.86	bs	1H	H3 exchangeable
7.87	d(J=4Hz)	1H	H6
5.70	d(J=4Hz)	1H	H5
5.84	d(J=3Hz)	1H	H1'
4.20-3.83	bm	3H	H2' H3' H4'
3.80-3.5	bm	2H	2xH5'
5.35-4.90	bm	3H	3x-OH exchangable



Scheme 4.7 (4.2)

In comparison with the ^1H NMR spectrum of uridine, the ^1H NMR spectrum of 5-bromouridine showed that bromination resulted in the absence of an H5 peak and a loss of coupling and a downfield shift of the H6 peak to δ 8.48. whereas, the δ value for H1' was almost identical to that in the starting material. The signals of other protons of the compound were similar to those of uridine (Table 4.2).

Table 4.2. ^1H NMR data of 5-bromouridine (4.2)

chemical shift δ		int.	assignment
11.87	bs	1H	H3 exchangeable
8.46	s	1H	H6
5.87	d(J=3Hz)	1H	H1'
5.6-4.8	bs	3H	3x-OH exchangeable
4.2-3.8	bm	3H	H2' H3' H4'
3.75-3.55	bm	2H	2xH5'

EI-MS data of 5-bromouridine are shown in Table 4.3.

Table 4.3. EI-MS data of 5-bromouridine (4.2)

m/z	intensity	assignment
325	0.27	[M+1]
324	2.75	[M] (^{81}Br isotope)
323	0.23	[M+1]
322	2.73	[M] (^{79}Br isotope)
193	22.43	[M-sugar+2H]
192	25.75	[M-sugar+H]
191	23.09	[M-sugar+2H]
190	26.84	[M-sugar+H]
149	34.20	[M-sugar-HNCO+H]
147	37.04	[M-sugar+HNCO+H]
133	94.24	[sugar]
115	16.42	[sugar-H_2O]
82	39.5	[Br+H]
80	35	[Br+H]

4.2.2 Preparation of 5-aminouridine hydrochloride (4.3·HCl)

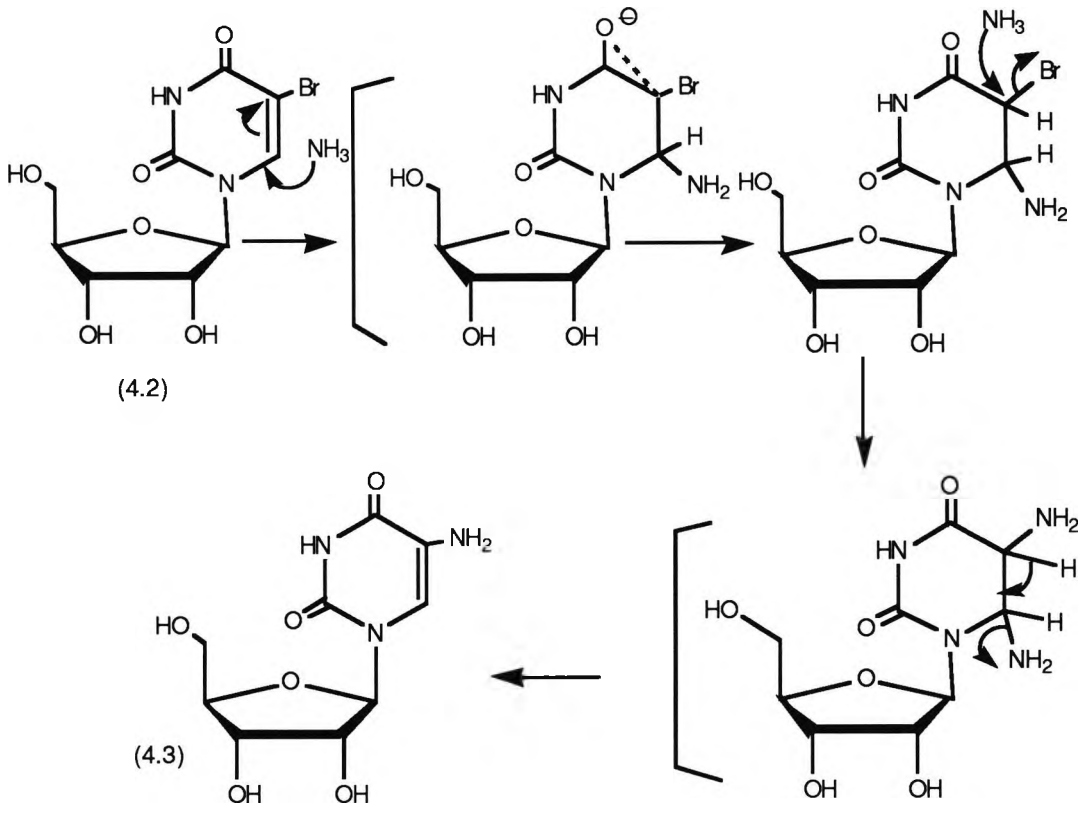
A) Ammoniation of 5-bromouridine in liquid ammonia

5-Bromouridine (4.2) was aminated with liquid ammonia in a bomb tube at 50-55 °C for 120 h. Then the ammonia was allowed to boil off. A minimum amount of hot water and 2 volumes of isopropyl alcohol were added and 5-aminouridine hydrochloride (4.3·HCl) was crystallised at low temperature (yield: 63%).

B)Ammoniation of 5-bromouridine in aqueous ammonia solution

Saturated aqueous ammonia water solution was prepared by passing ammonia gas into water at -18°C in a thick-walled tube, then 5-bromouridine was added and the tube was heated to 50 - 55 °C for 120 h. After evaporation of the solution to dryness, a minimum amount of hot water and 2 volumes of isopropyl alcohol were added. The pH of the solution was adjusted with HCl to 1. 5-Aminouridine hydrochloride (4.3·HCl) crystallised from the solution on cooling (yield: 63%).

Direct nucleophilic replacement of a leaving group at C5 would not be expected to occur. It is likely that displacement at C5 occurs via an addition reaction at C6 followed by displacement at C5 and subsequent elimination from C5 and C6 to re-establish extended conjugation (Scheme 4.8).



Scheme 4.8

The ^1H NMR spectrum of 5-aminouridine hydrochloride in $DMSO_{d6}$ was similar to that of 5-bromouridine. The slight difference was that the chemical shift of H6 in the ammonium compound was at δ 8.32 rather than at δ 8.46. Moreover, the chemical shift of H6 reflected the electronic influence of the 5-substituent. Thus, the chemical shift of H6 in the free base form of 5-aminouridine was at δ 7.32 instead of δ 8.32. In addition, the chemical shift of protons in the amine group was variable based on the concentration of the sample.

The FAB-MS spectrum of 5-aminouridine gave a pseudomolecular ion [P] at m/z 260 of high intensity (100%) and molecular ion [M] at m/z 257 of low intensity (2.05%). The spectrum was also characterised by [M-NH_2] species at m/z 243, [P-sugar+H] species at m/z 180 and sugar species at m/z 133.

C) Investigation of suitable reaction conditions for the ammoniation of 5-bromouridine

As solvents, both liquid ammonia and aqueous ammonia solution are adequate to dissolve 5-bromouridine. As reagents, the difference between them is that liquid ammonia is a single reagent, whilst aqueous ammonia solution contains two nucleophiles, NH_3 and OH^-. Occasional failure to obtain 5-aminouridine from aqueous ammonia raised the question of what are the best conditions for ammoniation of 5-bromouridine in aqueous ammonia.

There appears to be four factors important for the ammoniation: 1. concentration of ammonia in water; 2. time and temperature for the reaction; 3. volume of container; 4. the ratio of 5-bromouridine to ammonia. It appears that in the preparation of 5-aminouridine, the saturated aqueous ammonia solution should occupy about 95% of the space in the container. Otherwise, at high temperature, the residual space provides a reservoir to hold a large proportion of the ammonia molecules, which escape from the aqueous solution. As a result, the concentration of the aqueous ammonia decreases considerably. Therefore, the factor of space is linked to the concentration of ammonia. Preferably, a large volume of saturated aqueous ammonia should always be used, so that the ammonia is always in great excess to the 5-bromouridine.

Therefore, failure in the preparation of 5-aminouridine is attributed to the concentration of ammonia, reaction time and temperature. As a result of the investigation, three successful preparations confirmed that it was necessary to keep the concentration of ammonia in water over 74.3% (w/v). Meanwhile, the temperature for the reaction must be 50 - 55 °C. Under these conditions, the reaction time can be shortened from 120 h to 96 h.

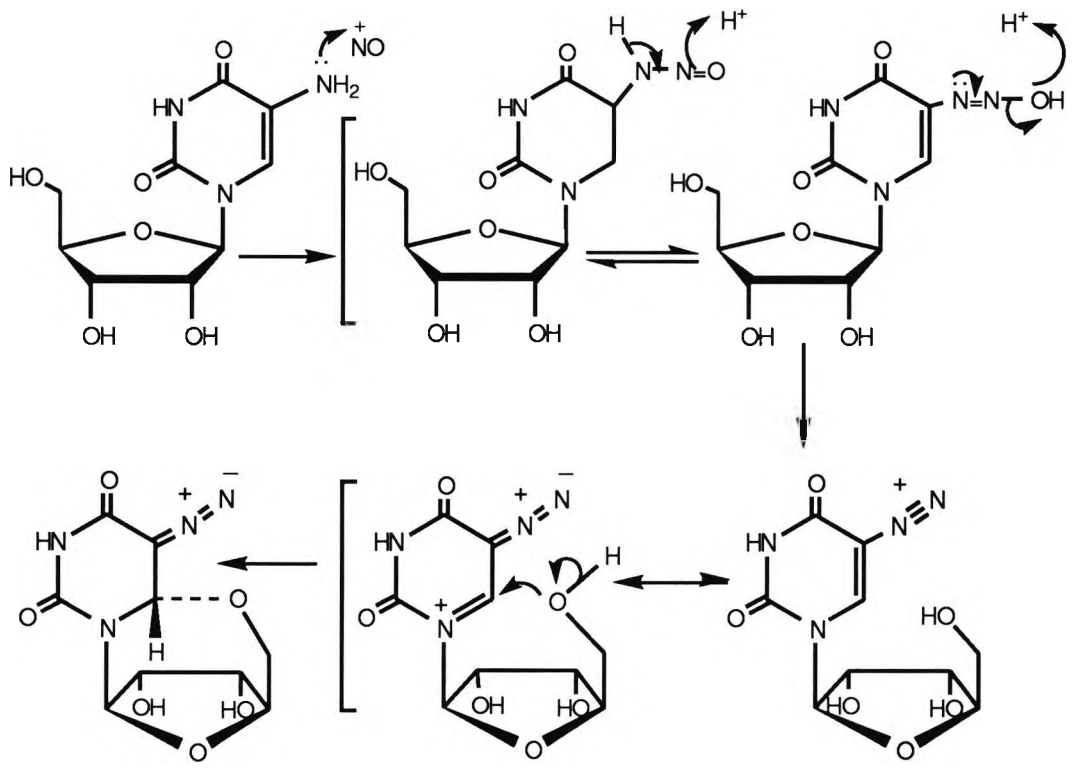
4.2.3 Preparation of 5-diazo-5',6(s)-cyclo-uridine (4.1)

Diazotization of 5-aminouridine hydrochloride with hydrochloric acid and sodium nitrite was carried out at 0°C and pale yellow crystals which formed in less

than five minutes were obtained by rapid filtration and recrystallization from methanol, affording a light yellow crystalline product.

Sodium nitrite affords NO^+ ion in dilute acid solution, which is the reagent responsible for diazotization of 5-aminouridine. The structure of compound (4.1) was assigned as 1-(b-D-ribofuranosyl)-O-5',6(s)cyclo-5-diazo-1,6-dihydropyrimidine-2,4(3H.6H)-dione (Scheme 4.9).

The I.R. spectrum of compound (1) displayed a strong absorption at 2128 cm^{-1}, characteristic of the diazo functional group. In the region around 3424 cm^{-1}, there was a broad absorption which was assigned to the imido N-H bond and hydroxy OH bond. A broad and strong absorbance was seen for the carbonyls between 1740 cm^{-1} and 1580 cm^{-1}.



Scheme 4.9

In the ^1H NMR spectrum (Table 4.4), there was a large upfield chemical shift for H6 (δ 6.15). Moreover, there were two exchangeable protons in the sugar moiety instead of three and the anomeric proton (H1') appeared as a singlet instead of the

usual doublet. These data were consistent with cyclonucleoside formation through the C6 and O5' positions.

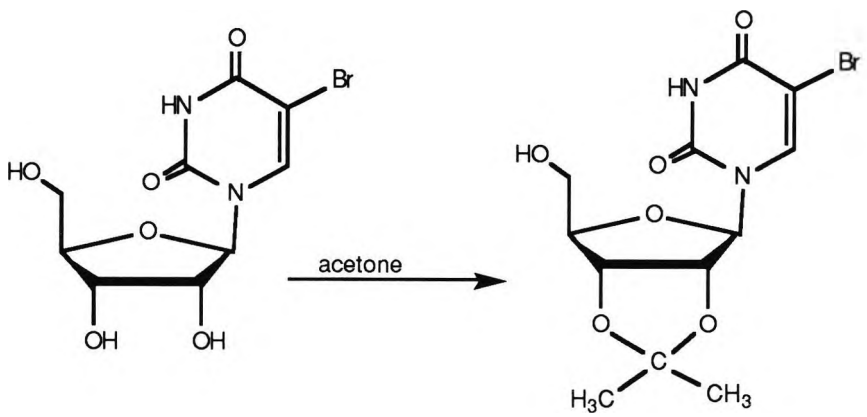
The FAB-MS spectrum gave the strongest peak (100%) at m/z 271 which was assigned to [M + H], a pseudomolecular ion. The molecular ion was weak (1.7%) at m/z 270, but a peak at m/z 293 was reasonably strong (15.4%) and was assigned to the species [M+Na]. The spectrum also contained peaks at m/z 243 assigned [P-N_2], at m/z 228 [P-HNCO] and at m/z 136 [M-sugar-H].

Table 4.4. ^1H NMR data of 5-diazo-5',6(s)-cyclo-uridine (4.1)

chemical shift δ		int.	fragment
10.42	bs	1H	H3 exchangeable
6.15	s	1H	H6
5.26	s	1H	H1'
5.14	d(J=4Hz)	1H	-OH exchangeable
4.93	d(J=6Hz)	1H	-OH exchangeable
4.0-4.3	m	3H	H2'
			H3'
			H4'
3.78	s	2H	2 X H5'

4.2.4 Preparation of 2',3'-isopropylidene-5-bromouridine (4.7)

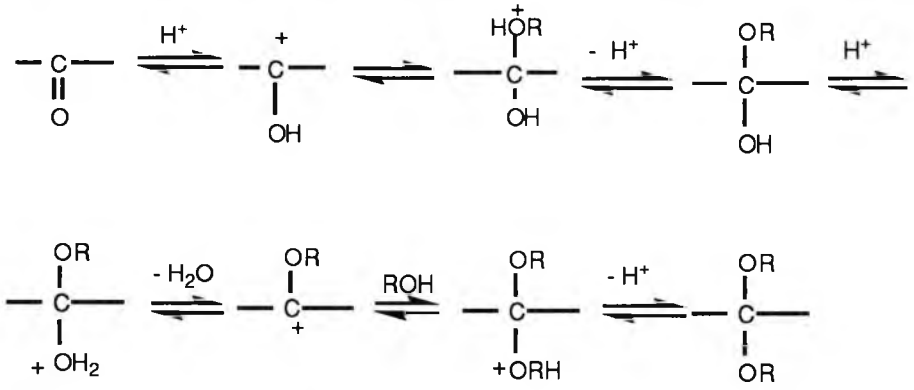
When crude 5-bromouridine (4.2) was recrystallized from acetone, a derivative of uridine was produced, which was identified as 2',3'-isopropylidene-5-bromouridine (4.7) (Scheme 4.10).



(4.2) (4.7)

Scheme 4.11

Compound (4.7) was probably formed by reaction of the 5-bromouridine with acetone in presence of an acid catalyst, e.g. succinimide, which was produced as a by-product during the previous bromination. The mechanism, which involves initial formation of a hemiacetal, is the reverse of acetal hydrolysis (Scheme 4.11).



Scheme 4.11

The high resolution ^1H NMR spectrum of the compound was characterised by a broad singlet at δ 11.9 which integrated for one proton (H3), a sharp singlet at δ 8.36 which integrated for one proton (H6), two singlets at δ 1.4 and δ 1.6 which integrated for six protons altogether and were assigned to hydrogens of the isopropylidene group (Table 4.5).

Table 4.5. ^1H NMR data of 2',3'-isopropylidene-5-bromouridine (4.7)

chemical shift δ		int.	assignment
11.98	bs	1H	H3 exchangeable
8.37	s	1H	H6
5.93	d	1H	H1'
5.22	t	1H	-OH exchangeable
4.915	d	1H	H2'
4.75	d	1H	H3'
4.10	d	1H	H4'
3.64	m	2H	2xH5'
1.47	s	3H	$-CH_3$
1.27	s	3H	$-CH_3$

* Deuterated sample was prepared by dissolving the compound (4.7) in deuterated acetone and adding D_2O followed by stirring for 30 min and then evaporation of the mixture to dryness.

The ^{13}C NMR spectrum of the compound (4.7) showed twelve carbon peaks, in addition to those of the solvent. The two most downfield peaks were assigned to the carbonyls of the uracil ring and two of the most upfield signals were assigned to the two methyl groups of the isopropylidene function. The signal at δ 97 was assigned to the quaternary carbon of the isopropylidene group (Table 4.6).

Table 4.6. ^{13}C NMR data of 2',3'-isopropylidene-5-bromouridine (4.7)

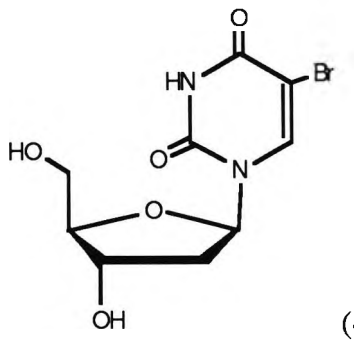
chemical shift δ	assignment
162	C4
151.7	C2
143.1	C6
115	C5
97	iso-C of $CH(CH_3)_2$
94	C1'
88.7	C4'
86	C2'
82	C3'
63	C5'
27.5	$-CH_3$
25.5	$-CH_3$

The FAB-MS spectrum of compound (4.7) provided peaks at m/z 363 and m/z 365 in almost equal intensity (M^+, ^{79}Br and ^{81}Br isotopes). Other peaks which were assigned to some important fragments were at m/z 285, m/z 191, m/z 193 (in equal intensity with the peak at m/z 191) and m/z 173. The fragment at m/z 285 was assigned to the [M-Br] species. Both ions at m/z 191 and m/z 193 corresponded to [M-sugar] species. whilst the peak at m/z 173 was assigned to the [2',3'-isopropylidene-sugar] fragment.

Elemental analysis of the product was consistent with the theoretical data for compound (4.7).

4.2.5 Preparation of 5-bromo-2'-deoxyuridine (4.8)

5-Bromo-2'-deoxyuridine was prepared from commercially available 2'-deoxyuridine and N-bromosuccinimide in dry DMF. After 16 hours stirring at room



(4.8)

temperature, the solution was evaporated to dryness and the crude product was recrystallized from acetone. A white crystalline solid was obtained.

The I.R. spectrum of the white product showed a strong and broad peak at about 3400 cm^{-1} which could be taken as evidence of the stretch of the hydroxyl groups and imide group. The peaks at 1670 cm^{-1} and 1730 cm^{-1} were assigned to carbonyls of the uracil ring. Moreover, the I.R. spectrum of the product was identical to the standard I.R. spectrum in the Sadtler I.R. Spectrum Collection.

The ^{1}H NMR data of the product were consistent with the proposed structure (Table 4.7).

Table 4.7. ^{1}H NMR data of 5-Bromo-2'-deoxyuridine (4.8)

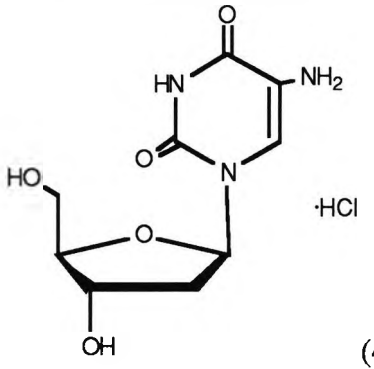
chemical shift δ		int.	assignment
10.5	bs	1H	H3 exchangeable
8.4	s	1H	H6
6.15	t	1H	H1'
4.6-5.9	bs	2H	2x-OH exchangeable
4.5	d	1H	H3'
3.9	d	1H	H4'
3.65	m	2H	2xH5'
2.1	m	2H	2xH2'

The FAB-MS spectrum provided two peaks at m/z 307 and m/z 309 which were assigned to molecular ions (Br^{79} and Br^{81} isotopes). In addition, the spectrum showed two peaks at m/z 329 and m/z 331 in about equal intensity which were assigned to species [M + Na]. The fragments at m/z 191 and m/z 193 with about the same intensity were assigned to the species [M - sugar + 2H] (Table 4.8).

Table 4.8. FAB-MS data of 5-bromo-2'-deoxyuridine (4.8)

m/z	intensity (%)	assignment
331	4	[M+Na] (^{81}Br isotope)
329	4.1	[M+Na] (^{79}Br iostope)
309	7.6	[M+H]
307	8	[M+H]
193	12.8	[M - sugar + 2H]
191	12.1	[M - sugar + 2H]
117	32	[sugar]

4.2.6 Preparation of 5-amino-2'-deoxyuridine hydrochloride (4.9)



(4.9)

A) Preparation of 5-amino-2'-deoxyuridine in ammonia-water solution

Owing to the success in preparation of 5-aminouridine, 5-amino-2'-deoxyuridine was initially prepared in the same way. After sealing the tube containing 5-bromo-2'-deoxyuridine in concentrated ammonia solution, it was stood in a water bath at 50 °C for 120 hours. Then the tube was opened and the ammonia and water were evaporated in vacuo. A red-brown solid was obtained, which changed to light yellow after added HCl (36%) to pH 1. TLC (THF, SiO_2) showed the solid was a new product, more polar than 5-bromo-2'-deoxyuridine.

The FAB-MS spectrum of the yellow solid was consistent with the required 5-amino-2'-deoxyuridine, with the strongest peak (100 %) at m/z 244 which was attributed to the pseudomolecular ion [M + H].

However, when this yellow product was used for the next reaction of diazotization, it failed to provide the required diazo compound and further examination indicated that this yellow product was not pure enough for use. The

impurity might be a product of reaction of 5-bromo-2'-deoxyuridine with OH^- in water, or products of decomposition of 5-amino-2'-deoxyuridine because the latter is not stable in water [8].

B) Preparation of 5-amino-2'-deoxyuridine in ammonia-acetonitrile solution

In order to avoid possible side reactions and increase the yield of the ammoniation, the aqueous solvent had to be replaced. The new solvent should possess enough polarity to allow both ammonia and 5-bromo-2'-deoxyuridine to dissolve properly. Moreover, it should be aprotic, and should exclude the possibility for the reaction of the solvent as a nucleophile with 5-bromo-2'-deoxyuridine. A possible solvent seemed to be acetonitrile and its use for the ammoniation was investigated.

Ammonia gas was passed into acetonitrile in a stainless steel tube cooled to -20 °C and then 5-bromo-2'-deoxyuridine was added. After the tube had been sealed, it was stood in a water bath at 50 °C for 120 hours. Then the pressure inside the tube was carefully released. After evaporation of ammonia and acetonitrile in vacuo, a solid product was obtained. The solid was acidified with hydrochloric acid (36%) and on addition of 2-propanol to the acidic solution a white solid was obtained.

The I.R. spectrum of the white product was characterised by a broad absorption at 3350 cm^{-1} which was attributed to hydroxyl groups and imide group, and two strong and sharp peaks at 1730 cm^{-1} and 1660 cm^{-1} which were assigned to carbonyls.

The ^1H NMR spectrum of the white product was consistent with the required structure (4.9) (Table 4.9).

The FAB-MS of the product provided a single peak (100 %) at m/z 244 which was the pseudomolecular ion of 5-aminouridine. The EI-MS provided two strong peaks at m/z 127 and m/z 117 which were assigned to the fragments of [M-sugar+H] and [sugar]. In addition, the CI-MS displayed the similar peaks at m/z 117 and m/z 128 which were attributed to the same fragments.

Table 4.9. ^1H NMR data of 5-amino-2'-deoxyuridine (4.9)

chemical shift δ		int.	assignment
11.1	bs	1H	H3
8.2	s	1H	H6
6.1	t	1H	H1'
4.3	dd	1H	H3'
3.8	dd	1H	H4'
3.6	m	2H	2xH5'
2.2	m	2H	2xH2'
2-5	bs	4H	2x-OH and $-NH_2$

C) Preparation of 5-amino-2'-deoxyuridine using crude 5-bromo-2'-deoxyuridine

After acetonitrile was adopted as the solvent for the ammoniation, the yield of 5-amino-2'-deoxyuridine from 5-bromo-2'-deoxyuridine was apparently quantitative, according to TLC, and the scale of the preparation could be increased by use of a larger stainless steel tube with a volume of 250 ml. In addition, based on TLC detection, the bromination of 2'-deoxyuridine was also quantitative. However, only about 64% of the bromo compound was obtained after recrystallization from acetone. The main impurity in the reaction was succinimide (NHS) which was formed from N-bromosuccinimide. The difference in polarity between 5-bromo-2'-deoxyuridine and NHS was limited. Therefore, loss of 5-bromo-2'-deoxyuridine in the recrystallization from organic solvents could not be avoided. However, after it was converted into 5-amino-2'-deoxyuridine hydrochloride, there would be considerable difference in polarity between the hydrochloride and NHS. Therefore, they could be separated in this stage.

Consequently, in order to increase the yield, the crude 5-bromo-2'-deoxyuridine was directly used as the starting material for ammoniation. After ammoniation for 120 h, the residue was acidified and the product precipitated with propanol. In this way, the yield of 5-amino-2'-deoxyuridine hydrochloride was over 94% based on 2'- deoxyuridine.

4.2.7 Preparation of 5-diazo-5',6(s)-cyclo-2'-deoxyuridine (2.13)

5-Amino-2'-deoxyuridine hydrochloride (4.9) was diazotized with sodium nitrite in cold 50% aqueous acetic acid. The resulting yellow precipitate was filtered off and washed with cold water and dried whilst being kept at low temperature (-15°C).

The presence of a diazo group in the product was confirmed by a very strong absorption at 2130 cm^{-1} in the I.R. spectrum. Other useful signals were a broad peak in range of 3450-3330 cm^{-1} assigned to a hydroxyl group and two peaks at about 1680 cm^{-1} and 1640 cm^{-1} which were assigned to carbonyl groups.

The ^{1}H NMR spectrum of the product revealed the presence of an H3 absorption at δ 10.55, and an upfield chemical shift for the H6 proton at δ 6.15. The anomeric proton appeared as a quartet at δ 5.9, and only one exchangeable proton for the carbohydrate portionappeared. Other signals were at δ 5.1, 4.2, 3.8, 2.38 and 2.05 which were assigned to H3', H4' which were overlapped with -OH, H5', endo-H2' and exo-H2'.

The ^{13}C NMR spectrum of the product revealed nine peaks at δ 161, 148, 90.5, 89.3, 80, 72, 71, 60 and 46.5 which were attributed to C4, C2, C6, C5, C1', C4', C3', C5' and C2'.

References

1. I. Wempen and I.L. Doerr, *J. Am. Chem. Soc.,* 1960,82, 1624
2. P.A. Levene and F.B. Laforge, *Ber.,* 1912,45, 608
3. P.C. Srivastava and K.L. Nagpal, *Experientia*, 1920, 26, 220
4. M. Roberts and D.W. Visser, *J. Am. Chem. Soc.,* 1952, 74, 668
5. J.P. Paolini and R.K. Robins, *Biochim. Biophys. Acta* ., 1963, 72, 114
6. T.C. Thurber and L.B.Townsend, *J. Heterocycl . Chemistry* , 1972, 9, 626
7. D.J. Abraham, T.G. Cochran and R.D. Rosenstein, *J. Amer, Chem. Soc.,* 1971, 93.6279.
8. M. Friedland and D.W. Visser, *Biochim. Biophys. Acta.,* 1961, 51,148.

Chapter 5. Synthesis and Spectroscopic Studies of 5-Alkoxyuridines and -2'-Deoxyuridines

Part A: 5-Alkoxyuridines

5.1 Introduction

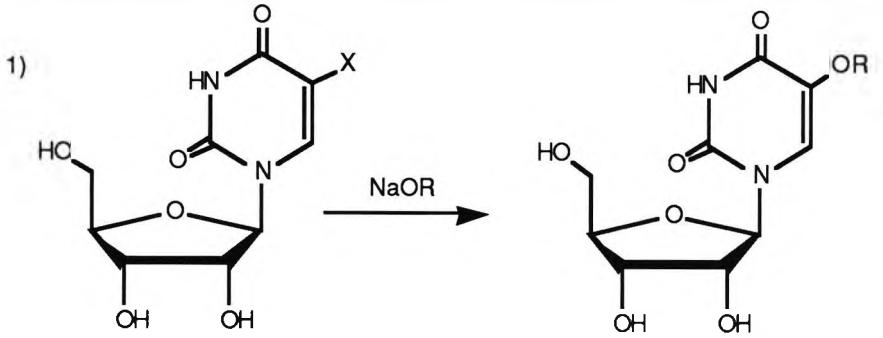
Interest in the preparation of 5-alkoxyuridines is based on the following:

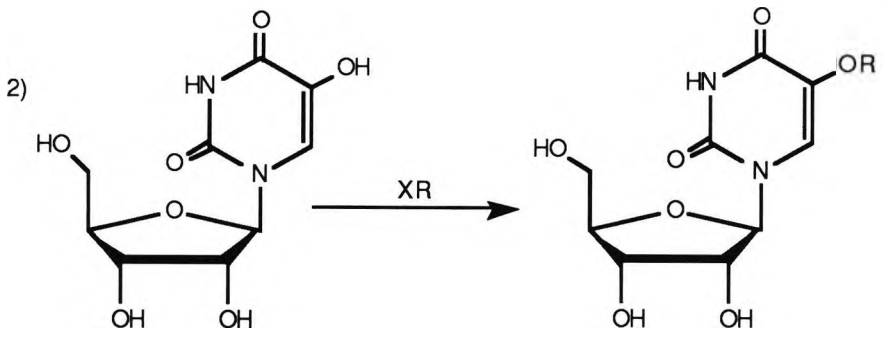
1. Up to now, almost no information about the chemistry or biology of 5-alkoxy uridines is available.

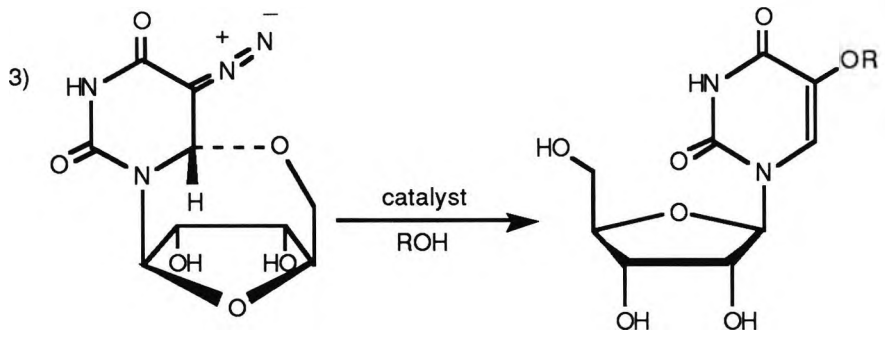
2. The major substituents previously attached at the 5-position of uridine have been halogens and some alkyl and vinyl groups, such as $-CH_3$, $-CH=CH_2$ and -CH=CHX. The influence of electron-donor groups on the double bond of the uracil ring should be explored.

3. Uridine in the first position of the anticodon of transfer RNA (tRNA) is always "post transcriptionally" modified into 5-methoxyuridine as well as other derivatives of uridine [1]. Therefore, the alkoxyuridines might have influence on protein biosynthesis.

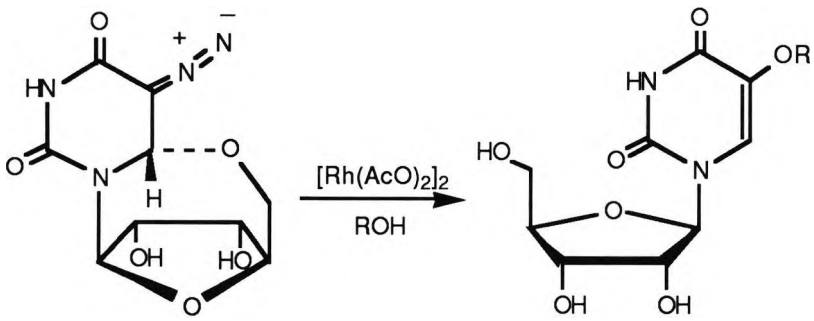
Theoretically, 5-alkoxyuridines could be prepared in several ways:







In the first route, the reaction conditions required cause breakdown of the uridine molecule. Selectivity in the second route would certainly be poor, since there are several -OH and >NH functions in the molecule. Consequently, we decided to examine the potential use of the carbenoid pathway shown in the third. In the third preparation of 5-alkoxyuridine, rhodium acetate has been chosen as the catalyst because it is known to be a superior catalyst to copper for many carbenoid reactions [2] (Scheme 5.1).



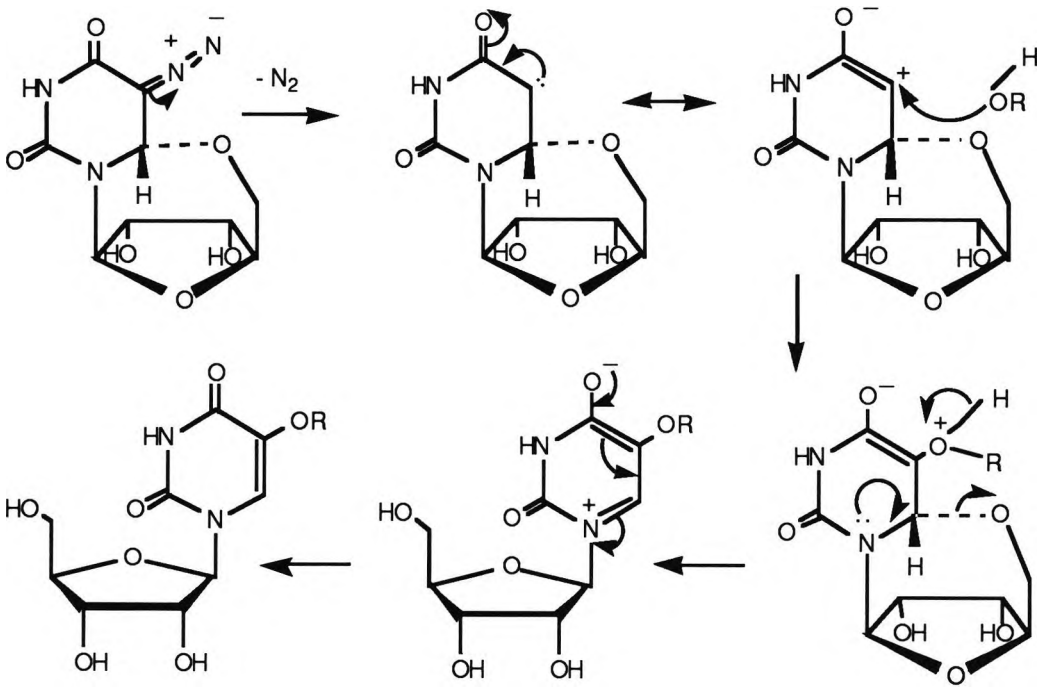
Scheme 5.1

Like most diazoalkanes, 5-diazo-5',6-cyclo-uridine is unstable to light and heat, decomposing into a carbene and nitrogen. Since the carbene is a highly reactive electrophilic intermediate, it combines with alcohols. The use of metal catalysts can reduce the activation energy of the reaction, the reactive species may then be a metal-carbenoid complex rather than the free carbene [3].

The concentration of rhodium acetate required for the reaction of a carbene with a hydroxylic bond has been tested by Paulissen et al [4]. The result showed that the reaction was nearly quantitative at room temperature, even with very low catalyst

concentrations.

The mechanism for the preparation of 5-alkoxyuridine includes production of carbene (or carbenoid), combination of the carbene and alcohol, cleavage of the C6-O5' bond with the re-establishment of extended conjugation (Scheme 5.2).



Scheme 5.2

5.2 Results and discussion

In the present work, the reaction of 5-diazo-5',6-cyclo-uridine was explored with methanol, ethanol, n-butanol and benzyl alcohol and the products were examined by spectroscopic methods.

5.2.1 Spectroscopic characteristics of 5-alkoxyuridines

Since the diazo moiety is no longer present, the characteristic peak of the diazo group is absent at 2129 cm^{-1} in the I.R. spectrum of 5-alkoxyuridine.

Becauce the C6-O5' bond has been broken, C6-H is incorporated into an olefinic system again (Scheme 5.2). The ^1H NMR spectrum reveals a downshift of C6-H from δ 6.5 to a range of δ 7.5 to δ 8.0 and change in the anomeric proton (C1'-H) from a singlet to a doublet ($J_{1'2'}$ =7Hz) due to the conformational change of C1' and

C2'. Furthermore, C5 has became attached to an alkoxyl group, whose protons show signals at characteristic positions in the ^1H NMR spectra.

The alkoxyuridines show similar characteristics in their mass spectra. A typical fragmentation process is the loss of the alkoxyl radical. Therefore, the spectra all contain peaks at around m/z 243, which are assigned to [M-OR] or its pseudo-molecular ion fragment as well as [M+H-OR]. Moreover, in many cases a peak at m/z 133 can be observed which is assigned to the fragment of the sugar ring. Sometimes, several common fragmentation pathways, such as [M-HNCO], [M-H_2O], can be observed.

In general, a high intensity peak is observed at m/z [M-sugar] which means that decomposition of the glycosidic bond (C1'-N1) is a dominant fragmentation process (Fig. 5.1).

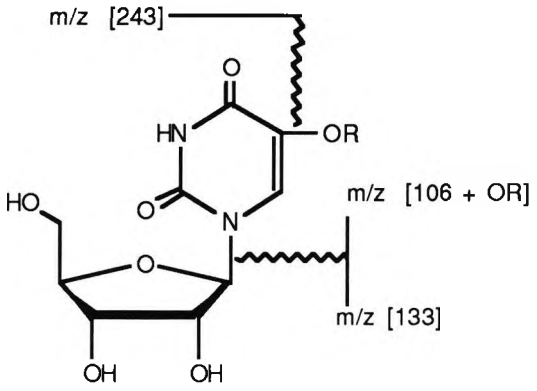
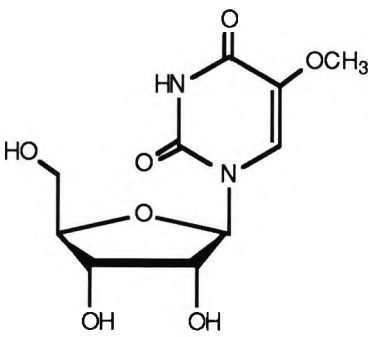


Fig. 5.1

5.2.2 Preparation of 5-methoxyuridine (5.1)



(5.1)

Freshly prepared 5-diazo-5',6-cyclo-uridine (4.1) was dissolved in boiling CH_3OH, then a little rhodium acetate was added and the solution was refluxed for 4 hours. The solution was evaporated to dryness to afford a green-yellow material. Cold acetone was used to wash the material several times in small portions to get rid of the green-yellow colour of rhodium acetate. The white solid was recrystallized from CH_3OH.

The I.R. spectrum of the white solid showed the absence of a diazo stretch around 2129 cm^{-1} but the presence of two absorption band at 1711 cm^{-1} and 1663 cm^{-1} of high intensity which were assigned to two carbonyls of the uracil base.

^1H NMR data of the product are shown in Table 5.1.

Table 5.1. ^1H NMR data of 5-methoxyuridine (5.1)

chemical shift δ		int.	assignment
11.22	bs	1H	H3 exchangeable
7.55	s	1H	H6
5.66	d(J=7)	1H	H1'
5.03-5.40	m	3H	3x-OH exchangeable
4.39	d(J=7)	1H	H2'
4.17	m	2H	H3'
			H4'
3.83	s	3H	$-CH_3$
3.65	d	2H	2xH5'

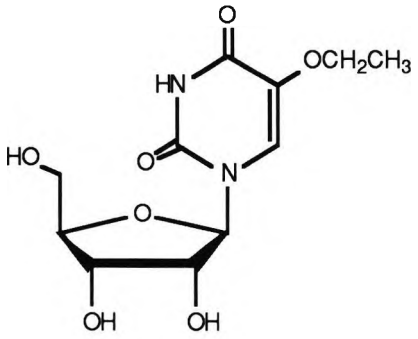
Table 5.2. EI-MS data of 5-methoxyuridine (5.1)

m/z	intensity(%)	assignment
274	0.25	[M]
242	1.01	[M-OCH_3-H]
214	2.77	[M-OCH_3-CO]
142	100	[M-sugar+H]
141	5.13	[M-sugar]
133	3.9	[sugar]
115	6.49	[sugar-H_2O]
99	12.23	[M-sugar-HNCO+H]
98	12.13	[M-sugar-HNCO]

The EI-MS spectrum of 5-methoxyuridine (5.1) was characterised by peak a at m/z 274 which corresponded to the molecular ion. The loss of methoxy group could

be deduced from the peak at m/z 242 which corresponded to [M-OCH_3-H] species. The intensity of the [M-sugar+H] species was highest (100%) (Table 5.2).

5.2.3 Preparation of 5-ethoxyuridine (5.2)



(5.2)

Freshly prepared 5-diazo-5,6-cyclo-uridine (4.1) and a catalytic amount of rhodium acetate were dissolved in boiling ethanol and the solution was refluxed for 4 hours. The residue was separated with a silica gel column, affording a white solid.

The I.R. spectrum of the white solid showed no diazo stretch around 2129 cm^{-1} but showed two absorption bands at 1703 cm^{-1} and 1665 cm^{-1} of high intensity which were assigned to two carbonyls.

The ^1H NMR data of the product are shown in Table 5.3.

Table 5.3. ^1H NMR data of 5-ethoxyuridine (5.2)

chemical shift δ		int.	assignment
11.43	bs	1H	H3 exchangeable
7.63	s	1H	H6
5.80	d(J=9Hz)	1H	H1'
5.5-4.8	m	3H	3x-OHexchangeable
4.25-4.0	m	3H	H2' H3' H4'
3.8	q	2H	$-CH_2-$
3.6	bs	2H	2xH5'
1.2	t	3H	$-CH_3$

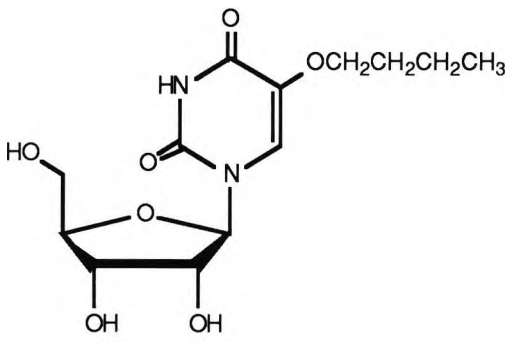
The FAB-MS of the compound provided a base peak (100%) at m/z 289 and a weak signal at m/z 288 which corresponded to the pseudomolecular ion [P] and molecular ion [M]. From the high intensity of peak at m/z 157 which corresponded

to [P-sugar+H] species, it was evident that the ready cleavage of the glycosidic bond was occurring. Moreover, the peak at m/z 243 suggested loss of the ethoxyl group to be a ready process as well (Table 5.4).

Table 5.4. FAB-MS data of 5-ethoxyuridine (5.2)

m/z	intensity(%)	assignment
311	5	[M+Na]
291	12.5	[P+2H]
290	21.2	[P+H]
289	100	[P]
288	5	[M]
275	10	[P-CH_3+H]
262	6	[P-C_2H_5+2H]
261	4	[P-C_2H_5+H]
243	7.5	[M-OC_2H_5]
199	10.2	[M-OC_2H_5-HNCO]
171	9	[M-OC_2H_5-HNCO-CO]
157	98	[P-sugar+H]
133	25	[sugar]
115	33	[sugar-H_2O]

5.2.4 Preparation of 5-butoxyuridine (5.3)



(5.3)

Freshly prepared diazo compound (4.1) and a trace of rhodium acetate were added to n-butanol and the solution kept at 65 °C for 4 hours. The excess butanol was removed under reduced pressure to afford a green-yellow solid. This was separated on a silica gel column. A white solid was obtained after removal of solvent.

The I.R. spectrum of the white product showed no diazo group stretch around 2129 cm^{-1}, but showed a broad absorption band at 1743 cm^{-1} to 1680 cm^{-1} of high intensity which corresponded to two carbonyls in the molecule.

The ^1H NMR spectrum of the product was consistent with required structure (5.3) (Table 5.5).

Table 5.5. ^1H NMR data of 5-butoxyuridine (5.3)

chemical shift δ		int	assignment
11.4	bs	1H	H3 exchangeable
7.8	s	1H	H6
5.75	d	1H	H1'
5.5-4.8	m	3H	3x-OH exchangeable
4.3-3.5	m	7H	H2' H3' H4' 2xH5' $-OCH_2-$
1.88-1.0	m	4H	$-CH_2CH_2-$
0.91	t	3H	$-CH_3$

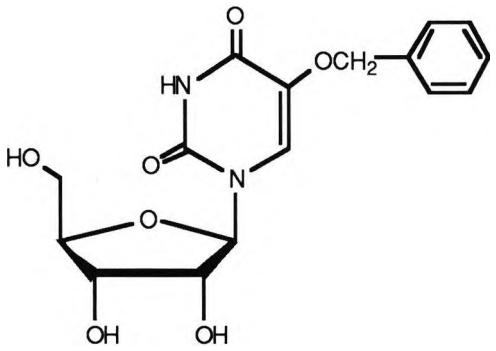
The FAB-MS data of the compound are given in Table 5.6.

Table 5.6: FAB-MS data of 5-butoxyuridine (5.3)

m/z	intensity(%)	assignment
355	1.25	[M+K]
339	17	[M+Na]
318	16	[M+2H]
317	74	[M+H]/[P]
316	13	[M]
315	12	[M-H]
289	13	$[P-C_2H_5+H]$
273	3	$[M-C_3H_7]$
259	4	$[M-C_4H_{10}]$
243	5	$[M-OC_4H_{10}]$
185	100	[P-sugar+H]
184	30	[P-sugar]
183	8	[M-sugar]
133	20	[sugar]

5.2.5 Preparation of 5-benzyloxyuridine (5.4)

Reaction of the diazo compound (4.1) with excess benzyl alcohol in refluxing $CHCl_3$ containing a trace of rhodium acetate afforded 5-benzyloxyuridine (5.4) as a light yellow solid.



(5.4)

Data of ^1H NMR and FAB-MS of the product are shown in Tables 5.7 and 5.8.

Table 5.7. ^1H NMR data of 5-benzyloxyuridine (5.4)

chemical shift δ		int.	assignment
11.3	bs	1H	H3 exchangeable
7.93	s	1H	H6
7.70-7.40	m	5H	aromatic-H
5.9	d	1H	H1'
5.5-5.0	m	3H	3x-OH exchangeable
5.2	m	1H	H2'
5.0	m	1H	H3'
4.6	m	1H	H4'
4.2-4.0	bs	2H	benzyl-CH_2-
3.8-3.6	m	2H	2xH5'

Table 5.8. FAB-MS data of 5-benzyloxyuridine (5.4)

m/z	intensity(%)	fragment
373	8	[M+Na]
353	8	[P+2H]
352	11	[P+H]
351	100	[P]
350	43	[M]
349	7	[M-H]
219	50	[P-sugar+H]
218	25	[P-sugar]
217	23	[M-sugar]

The I.R. spectrum of the product showed no diazo group stretch around 2129 cm^{-1}, but showed broad absorption bands in the range of 1750cm^{-1} to 1600cm^{-1} in high intensity which corresponded to two carbonyls in the molecule.

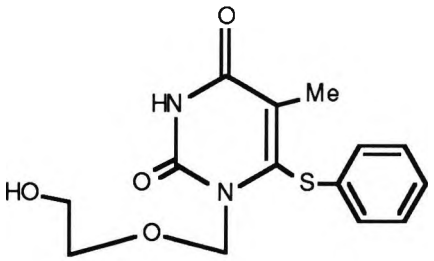
Part B. 5-Arylalkoxyuridines and -2'-Deoxyuridines

5.3 Introduction

Some 5-substituted uridines which had been initially synthesised were tested biologically in vitro. The results revealed that all of the compounds were inactive as antiviral agents. However, two of them, 5-benzyloxyuridine (5.4) and 5-(3"-benzyl-1"-triazeno)-uridine (6.6) showed some activities against cancer cells [5]. The two anti-cancer active compounds possess side chains at the 5-position which have different lengths. On the terminal of the chain, there is a phenyl group attached.

Since the nucleosides which are clinically used as anti-viral agents contain the 2'-deoxy-ribose moiety, a series of 5-substituted deoxyuridines were synthesised in the hope that they would possess anti-viral activity.

A recent paper has reported [6] that some 6-substituted uridine derivatives possess good bioactivity, such as 1-[(2-hydroxy)-methyl]-6-(phenylthio)-thymine (HEPT) (5.5).



HEPT

(5.5)

It may be suspected that, in the receptor sites of the enzymes with which these compounds interacted as substrates, there might be a steric space which could accept the hydrophobic aryl group at the 5- and 6-position of the pyrimidines. After study of the interaction of mammalian uridine phosphorylase (Walker 256) with a series of uridine analogues which are 1-substituted, 6-substituted and 5-substituted uracils

Baker [7] proposed a diagram to depict the mode of binding of these analogues in the active site of the receptor (Fig. 5.2).

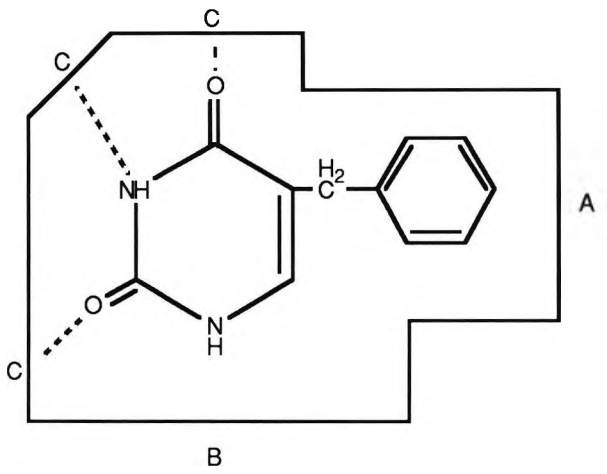


Fig. 5.2

A is the hydrophobic region of the enzyme, B is one of block tolerance and C can accommodate groups that are compatible with and can interact with both the oxo and amino moieties of uracil.

Based on this prediction, we designed a series of 5-substituted uridine and 2'-deoxyuridine derivatives which had side chains of different lengths and a phenyl group at the terminal. It is expected that with the increasing length of the side chain, the flexibility of side chain increases as well. Thus, it might be possible for this steric space to hold the hydrophobic group more easily, owing to a relatively low rotational energy barrier.

5.4 Results and discussion

5.4.1 General approach for the preparation, separation and purification of the compounds

The alcohols were used both as solvents and reagents, since all of them are liquid at about 60 °C and with appropriate polarity to allow the starting materials to dissolve. In the first half hour, the formation of a foam was usually observed, due to the liberation of N_2 from the diazo compound. The reactions were followed by TLC

(ethyl acetate) and/or I.R. After about 3.5 h, the diazo compound could no longer be detected and reactions were normally worked up after 4 h.

The excess of solvent was separated from the products in different ways which were:

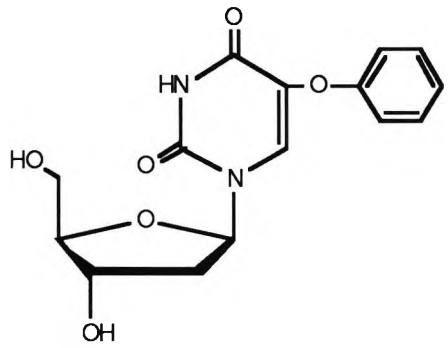
A) Evaporation in vacuo for sufficiently low boiling point alcohols, such as phenol and benzyl alcohol. Then, the residues were washed with ether and ethyl acetate to remove impurities;

B) Using a chromatographic column (silica gel and dichloromethane). After the alcohols emerged, the crude products were quickly eluted with methanol. Then, the crude products were reloaded on a silica column very carefully and purified by elution with diethyl ether. When the green band of the rhodium acetate had moved to about the middle position of the column, the ethyl ether was replaced by ethyl acetate as the mobile phase. Two products were obtained. One, a side product, came out before the green band, the second, which was the required compound, came out after the green band. The polarities of the target compounds, side products, and rhodium acetate were only very slightly different. The elution for each product took about 7 days and the confirmation of the identities of fractions relied considerably on ^{1}H NMR as well as TLC;

C) A few products were obtained by adding an excess of hexane to the reaction solution, followed by addition of dichloromethane dropwise to make the solution clear. Then, a precipitate of the target compound could be obtained after the solution was cooled. However, using this method the yield was very low.

In general for these compounds, it was extremely difficult to obtain crystals by recrystallization, despite many efforts. In most cases, only a gum was obtained. Consequently, there was more than 0.3% of error in the micro-analysis of the compounds.

5.4.2 Preparation of 5-phenoxy-2'-deoxyuridine (5.6)



(5.6)

A mixture of phenol, 5-diazo-5',6-cyclo-2'-deoxyuridine (2.13) and a trace of rhodium acetate was stirred at 60 °C for 4 h. The crude product was obtained by method A and it was purified by method B.

The I.R.spectrum of the product showed the absence of a peak at about 2130 cm^{-1}, indicating loss of the diazo group. The broad absorption in the range of 3420 cm^{-1}-3200 cm^{-1} indicated that the molecule contained hydroxyl and/or imido groups. Strong absorptions at 1700 cm^{-1} and 1650 cm^{-1} were assigned to carbonyls. The weak peak at 1598 cm^{-1} might be assigned to C=C vibration of phenyl ring.

The ^1H NMR spectrum of the product was consistent with the required structure (5.6) (Table 5.9).

Table 5.9. ^1H NMR data of 5-phenoxy-2'-deoxyuridine (5.6)

chemical shfit δ	int.	multiplicity	assignment
10.4	1H	bs	H3
8.2	1H	s	H6
7.3-7.0	5H	m	$5xH_{arom}$
6.35	1H	t	H1'
4.5	1H	m	H3'
3.95	1H	m	H4'
3.75	2H	dd	2xH5'
3.4-2.6	2H	bs	2x-OH
2.3	2H	m	2xH2

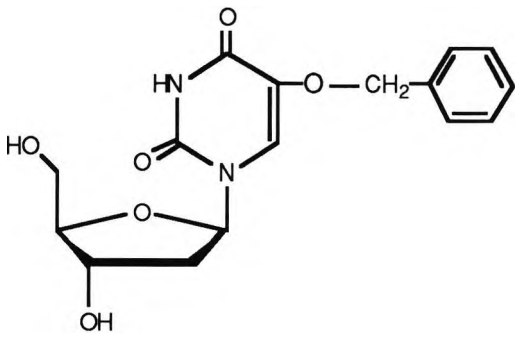
The ^{13}C NMR spectrum of the product provided 13 peaks, which was consistent with the required structure (5.6) (Table 5.10).

Table 5.10. ^{13}C NMR data of 5-phenoxy-2'-deoxyuridine (5.6)

chemicalshift δ	assignment	chemical shift d	assignment
159.7	C4	116	$2xC_{o\text{-arom}}$
159	$C_{t\text{-arom}}$	88.7	C1'
150.5	C2	86	C4'
132.7	C6	72	C3'
131	C5	62.5	C5'
130	$2xC_{m\text{-arom}}$	41	C2'
123	$C_{p\text{-arom}}$		

FAB-MS of the product revealed a peak at m/z 321 which was attributed to pseudomolecular ion [M + H], a peak at m/z 227 was assigned to the species [M - phenoxy] and a peak at m/z 117 was assigned to the fragment deoxy-ribose.

5.4.3 Preparation of 5-benzyloxy-2'-deoxyuridine (5.7)



(5.7)

A mixture of benzyl alcohol, 5-diazo-5',6(s)-cyclo-2'-deoxyuridine (2.13) and a trace of rhodium acetate was stirred at 60 °C for 4 h. The excess benzyl alcohol was removed by evaporation in vacuo and the residue was separated by using preparative TLC (ethyl acetate). Two bands of white solid were collected. The solid with slightly more polarity was confirmed to be the required compound (5.7). The other one was confirmed to be compound (8.1) (see Section 8.1, Chapter 8).

The I.R. spectrum of the product showed that there was no absorption around

2130 cm^{-1} for an N_2. However, the absorptions around 3381 cm^{-1}, 1685 cm^{-1} and 1632 cm^{-1} indicated the presence of hydroxyl, imido and carbonyl groups in the molecule.

The ^1H NMR spectrum of the product revealed data which were consistent with the required structure (Table 5.11).

Table 5.11. ^1H NMR data of 5-benzyloxy-2'-deoxyuridine (5.7)

chemical shift δ	int.	mult.	assignment
10.2	1H	bs	H3
7.8	1H	s	H6
7.4	5H	m	$5xH_{arom}$
6.3	1H	t	H1'
4.5	1H	m	H3'
4.6-4.3	2H	bs	2x-OH
3.97	1H	m	H4'
3.8	2H	m	2xH5'
2.23	2H	m	2xH2

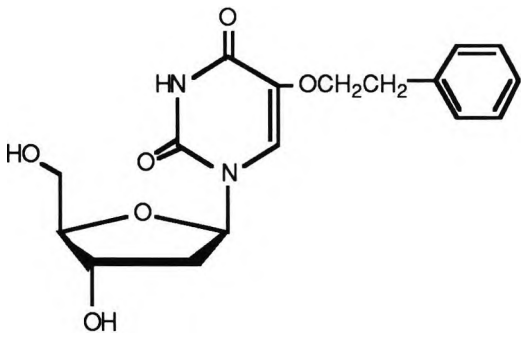
The ^{13}C NMR spectrum of the product provided 14 peaks which were attributed to 16 carbon atoms in the molecule (Table 5.12).

Table 5.12. ^{13}C NMR data of 5-benzyloxy-2'-deoxyuridine (5.7)

chemicalshift δ	assignment	chemical shift d	assignment
160	C4	125	C5
150	C2	89	C1'
136.5	$C_{t\text{-}arom}$	87	C4'
135	C6	73	$-CH_2-$
129.2	$2xC_{m\text{-}arom}$	72	C3'
129	$2xC_{o\text{-}arom}$	63	C5'
128.8	$C_{p\text{-}arom}$	41	C2'

The FAB-MS of the product provided a peak at m/z 335 which was assigned to pseudomolecular ion [M + H]. A peak at m/z 242 was assigned to species [M - benzyl - H]. The presence of a benzyloxyl group was confirmed by a group of peaks at m/z 107, m/z 91 and m/z 77 which were assigned to the species benzyloxy, benzyl and phenyl, respectively.

5.4.4 Preparation of 5-(2''-phenethoxy)-2'-deoxyuridine (5.8)



(5.8)

A mixture of 2-phenylethanol, 5-diazo-5',6(s)-cyclo-2'-deoxyuridine (2.13) and a trace of rhodium acetate was stirred at 60 °C for 4 h. The product was separated and purified by method B. The product from the second fraction was the target compound.

The I.R. spectrum of the product revealed the absence of an absorption at 2130 cm^{-1}. A broad strong absorption in the range of 3500 cm^{-1}-3100 cm^{-1} was assigned to the hydroxyl and imido groups. The peaks at 1684 cm^{-1} and 1641 cm^{-1} were attributed to carbonyls.

The ^1H NMR data of the product are shown in Table 5.13.

Table 5.13. ^1H NMR data of 5-(2''-phenethoxy)-2'-deoxyuridine (5.8)

chemical shift δ	int.	mult.	assignment
10.5	1H	bs	H3
7.9	1H	s	H6
7.2	5H	m	$5xH_{arom}$
6.4	1H	t	H1'
5.1-4.9	2H	bs	2x-OH
4.5	1H	m	H3'
4.1	2H	bt	$-CH_2O-$
3.9	1H	dt	H4'
3.75	2H	d	2xH5'
3.0	2H	t	$-CH_2-$
2.2	2H	m	2xH2'

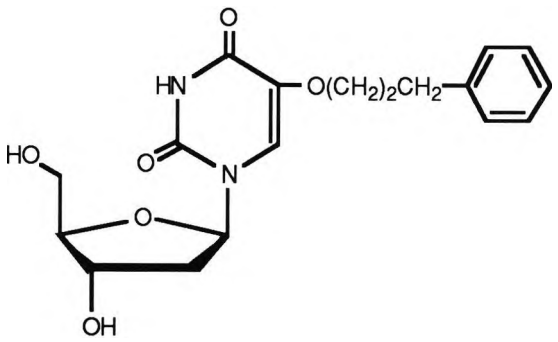
The ^{13}C NMR spectrum of the product provided 15 peaks which were readily assigned (Table 5.14).

Table 5.14. ^{13}C NMR data of 5-(2''-phenethoxy)-2'-deoxyuridine (5.8)

chemical shift δ	assignment	chemical shift d	assignment
160	C4	88	C1'
150	C2	86	C4'
139	$C_{t\text{-arom}}$	72	$-CH_2O-$
135	C6	71.5	C3'
129	$2xC_{o\text{-arom}}$	62	C5'
128	$2xC_{m\text{-arom}}$	42	C2'
126	$C_{p\text{-arom}}$	36	$-CH_2-$
123	C5		

The FAB-MS spectrum of the product displayed a peak at m/z 349 which was assigned to a pseudomolecular ion [M + H] and a peak at m/z 371 which was assigned to the species [M + Na]. The peak at m/z 233 was assigned to [M - sugar + 2H]. The peak at m/z 226 was assigned to the fragment [M - O-CH_2-CH_2-Ph +H].

5.4.5 Preparation of 5-(3''-phenyl-1''-propoxy)-2'-deoxyuridine (5.9)



(5.9)

A solution of 3-phenyl-1-propanol, 5-diazo-5',6-cyclo-2'-deoxyuridine (2.13) and a trace of rhodium acetate was stirred at 60 °C for 4 h. The product was separated and purified by method B.

The I.R. spectrum of the product showed no absorption at 2130 cm^{-1}. Around 3280 cm^{-1}, there was a broad absorption corresponding to the presence of hydroxyl and imido hydrogens. At about 1720 cm^{-1} and 1658 cm^{-1} two strong peaks were assigned to the carbonyls.

^1H NMR and ^{13}C NMR data of the product are shown in Tables 5.15 and 5.16.

Table 5.15. ^1H NMR of 5-(3"-phenyl-1"-propoxy)-2'-deoxyuridine (5.9)

chemical shift δ	int.	mult.	assignment
10.2	1H	bs	H3
7.8	1H	s	H6
7.2	5H	m	5xH_{arom}
6.35	1H	t	H1'
4.5	1H	t	H3'
4.3-3.8	7H	m	2x-OH 2xH5' H4, -CH_2O-
2.8	2H	t	-CH_2-Ph
2.0	2H	m	2xH2'
1.9	2H	m	-CH_2-

Table 5.16. ^{13}C NMR data of 5-(3"-phenyl-1"-propoxy)-2'-deoxyuridine (5.9)

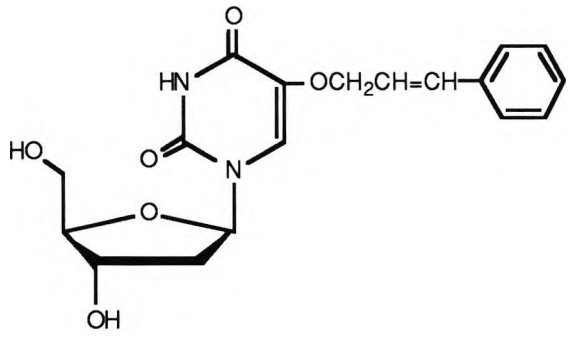
chemical shift δ	assignment	chemical shift d	assignment
160	C4	88	C1'
150	C2	83	C4'
142	$C_{t\text{-arom}}$	76.5	-CH_2O-
136	C6	70.5	C3'
129	2x$C_{m\text{-arom}}$	62.5	C5'
128.8	2x$C_{o\text{-arom}}$	41.5	C2'
127	$C_{p\text{-arom}}$	32	-CH_2-Ph
124	C5	31	-CH_2-

The FAB-MS spectrum of the product revealed a peak at m/z 363 which was assigned to the pseudomolecular ion [M + H]. The peak at m/z 385 was attributed to [M + Na]. The base peak was assigned as [M - sugar + 2H] at m/z 247. The peak at m/z 227 was assigned to the fragment [M - $O(CH_2)_3$-Ph].

5.4.6 Preparation of 5-cinnamyloxy-2'-deoxyuridine (5.10)

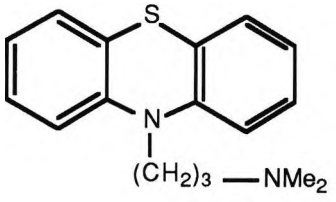
Introduction

The carbon-carbon double bond adds rigidity to the carbon chain. Insertion of a C-C double bond into a carbon chain permits study of the effect on drug activity caused by changing the flexibility of the chain.

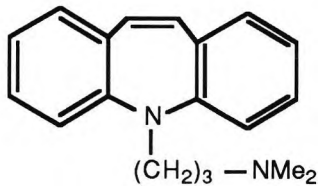


(5.10)

Moreover, in many cases, further delocalisation of a p-electronic system by addition of a vinyl bond adjacent to a conjugate aromatic ring can maintain and even extend the biological activities. The substitution of the sulphur atom in phenothiazine by the -CH=CH- group is an example, with compound (5.11) possessing activity as a neuroleptic, whereas compound (5.12) possesses activity as an antiemetic.



(5.11)



(5.12)

In addition, from the chemical viewpoint, the carbene species can react both with nucleophiles such as various alcohols to afford ethers, and with double bonds to yield cyclopropanes. Therefore, when both hydroxyl function and vinyl function exist in the same molecule, the favoured selectivity between the two reactions is of interest.

Results and discussion

Cinnamyl alcohol was heated to 60 °C. 5-Diazo-5'6-cyclo-2'-deoxyuridine (2.13) and a trace of rhodium acetate were added and the mixture was stirred at 60 °C for 4 h. The product was separated and purified by method B. The product eluted after the green band was compound (5.10).

In the I.R. spectrum of the compound, there was no diazo absorption at about

2130 cm^{-1}. A broad absorption in the range of 3500 cm^{-1} to 3200 cm^{-1} was associated with the hydroxyl and imido groups. At 1680 cm^{-1} and 1642 cm^{-1} there were two strong absorptions corresponding to the carbonyls.

^{1}H NMR and ^{13}C NMR data of the product are shown in Tables 5.17 and 5.18.

Table 5.17. ^{1}H NMR data of 5-cinnamyloxy-2'-deoxyuridine (5.10)

chemical shift δ	int.	mult.	assignment
10.3	1H	bs	H3
7.83	1H	s	H6
7.35	5H	m	$5xH_{arom}$
6.67	1H	d	=CH-Ph
6.4	1H	m	=CH-
6.36	1H	t	H1'
4.6-4.4	5H	m	H3' 2x-OH $-CH_2O-$
4.0	1H	d	H4'
3.9	2H	m	2xH5'
2.2	2H	m	2xH2'

Table 5.18. ^{13}C NMR data of 5-cinnamyloxy-2'-deoxyuridine (5.10)

chemical shift δ	assignment	chemical shift d	assignment
161	C4	125	$C_{p\text{-arom}}$
151	C2	124	C5
138	$C_{t\text{-arom}}$	89	C1'
136	C6	87	C4'
134	=CH-	72.5	$-CH_2-$
129	$2xC_{o\text{-arom}}$	72	C3'
128	=C-	63	C5'
127	$2xC_{m\text{-arom}}$	41	C2

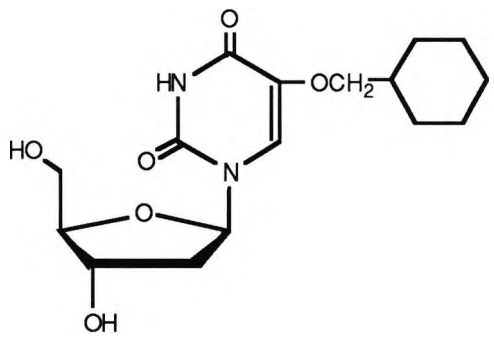
The FAB-MS of the product provided a peak at m/z 361 which was assigned to the pseudomolecular ion [M + H], a peak at m/z 383 which was assigned to [M + Na], a strong peak at m/z 117 which was assigned as the species $[sugar]^{+}$ and a peak at m/z 245 which was assigned as [M - sugar + 2H].

The negative FAB-MS of the product provided a group of complementary peaks. A peak at m/z 359 was assigned to the pseudomolecular ion [M - H]. A peak at m/z 243 was assigned as [M - sugar].

Consequently, the data, especially the ^{1}H and ^{13}C NMR spectra, proved that the structure of the compound was 5-cinnamyloxy-2'-deoxyuridine.

Therefore, as expected [8,9], the favoured course for the reaction of the carbene from 5-diazo-5',6-cyclo-2'-deoxyuridine with cinnamyl alcohol is to form the ether type product instead of undergoing cyclopropanation.

5.4.7 Preparation of 5-cyclohexylmethoxy-2'-deoxyuridine (5.13)



(5.13)

The method for preparation of 5-cyclohexylmethoxy-2'-deoxyuridine is similar to the preparation of the 5-alkoxyl products described above. The separation and purification of the product on a silica column resulted in two products. One has been identified as a novel nucleoside, structure (8.1) (See Section 8.1, Chapter 8). The other was the expected product (5.13).

The I.R. spectrum of the product (5.13) did not contain a diazo absorption at 2130 cm^{-1}.

^{1}H NMR and ^{13}C NMR data of the product are shown in Tables 5.19 and 5.20.

The FAB-MS of the product gave peaks at m/z 341 (20%) and m/z 363 (40%) assigned as pseudomolecular ions [M + H] and [M + Na]. The base peak at m/z 225 was associated with the fragment [M - sugar + 2H].

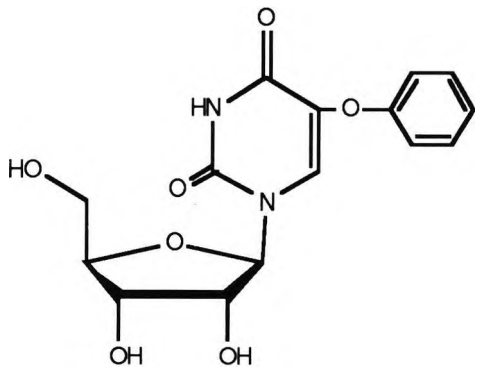
Table 5.19. ^{1}H NMR data of 5-cyclohexylmethoxy -2'-deoxyuridine (5.13)

chemical shift δ	int.	multiplicity	assignments
10.2	1H	bs	H3 exchangeable
7.7	1H	s	H6
6.25	1H	t	H1'
4.45	1H	m	H3'
4.35	2H	bs	2 x OH exchangeable
3.95	1H	m	H4'
3.8	2H	dd	2 x H5'
3.6	2H	m	2 x H1"
2.25	2H	m	2 x H2'
1.9-0.9	11H	m	cyclohexane

Table 5.20. ^{13}C NMR of 5-cyclohexylmethoxy-2'-deoxyuridine (5.13)

Chemical shift δ	assignments	Chemical shift d	assignments
160	C4	72	C5'
150	C2	62	C1'
136.5	C6	41	C2'
122	C5	38	C2"
88	C1'	30	C3"orC5"
86	C4'	27	C4"
76	C3'	26.5	C5"orC3"

5.4.8 Preparation of 5-phenoxyuridine (5.14)



(5.14)

5-Diazo-5',6(s)-cyclo-uridine (4.1) and a trace of rhodium acetate were added to phenol which was heated to 60 °C. The mixture was stirred at 60 °C for 4 h. The crude product was obtained by method A. and, then, recrystallized from ethyl acetate.

The I.R. spectrum of the product (5.14) showed absence of a peak at 2130 cm^{-}. A strong and broad absorption around 3500 cm^{-1} - 3100 cm^{-1} was assigned to hydroxyl and imido groups in the structure. A broad and strong absorption around 1730 cm^{-1} - 1630 cm^{-1} was associated with carbonyls.

The ^{1}H NMR spectrum of the product provided signals which were consistent with the expected structure (Table 5.21).

Table 5.21. ^{1}H NMR data of 5-phenoxyuridine (5.14)

chemical shift δ	int.	mult.	assignment
10.4	1H	bs	H3
8.2	1H	s	H6
7.3-7.0	5H	m	$5xH_{arom}$
5.95	1H	d	H1'
4.7	1H	sb	-OH
4.45	1H	sb	-OH
4.35	1H	sb	-OH
4.3	2H	s	H2', H3'
4.05	1H	s	H4'
3.9-3.7	2H	m	2xH5'

The ^{13}C NMR spectrum of the product provided 13 peaks which corresponded to the 15 carbon atoms in the structure (Table 5.2).

Table 5.22. ^{13}C NMR data of 5-phenoxyuridine (5.14)

chemical shift δ	assignment	chemical shift d	assignment
160	C4	116	$C_{p\text{-arom}}$
159	$C_{t\text{-arom}}$	89	C1'
151	C2	85	C4'
133	C6	75	C3'
131	C5	71	C2'
130	$2xC_{m\text{-arom}}$	61	C5'
123	$2xC_{o\text{-arom}}$		

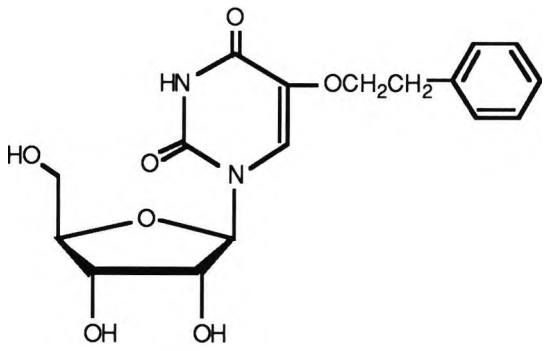
Unusually, the EI-MS of the product provided a peak for a pseudomolecular ion, [M + H] at m/z 337 owing to self- protonation. Peaks at m/z 205 and m/z 204 corresponded to species [M - sugar + H] and [M - sugar] (Table 5.23).

Table 5.23. EI-MS data of 5-phenoxyuridine (5.14)

m/z	intensity (%)	assignment
337	50	[M + H]
319	8.8	M - H_2O + H]
243	6	[M - O-Ph]
205	100	[M - sugar + H]
204	86	[M - sugar]
133	13	[sugar]
94	5	[-O-Ph + H]
77	8	[-Ph]

The CI-MS of the product revealed a group of similar peaks. Peaks at m/z 337 (93%) and m/z 354 (12%) corresponded to pseudo molecular ion, [M + H] and [M + NH_4]. Peaks at m/z 205 (100%) and 222 (64%) were attributed to [M - sugar + H] and [M - sugar + NH_4].

5.4.9 Preparation of 5-(2''-phenethoxy)-uridine (5.15)



(5.15)

5-Diazo-5',6(s)-cyclo-uridine (4.1) and a trace of rhodium acetate were added to a solution of 2-phenylethanol, and heated to 60 °C and stirred for 4 h. The product was separated and purified by method B. A white product was obtained.

The I.R. spectrum of the product did not contain an absorption at 2130 cm^{-1} but provided a broad absorption around 3410 cm^{-1} which corresponded to hydroxyl and imido groups and strong absorptions at 1698 cm^{-1} and 1650 cm^{-1} which were assigned to carbonyls.

The ^{1}H NMR specrum of the product supported the proposed structure (Table 5.24).

Table 5.24. ^{1}H NMR data of 5-(2"-phenethoxy)-uridine (5.15)

chemical shift δ	int.	multiplicity	assignment
10.4	1H	bs	H3 exchangeable
7.8	1H	s	H6
7.3-7.1	5H	m	$5xH_{arom}$
5.95	1H	d	H1'
4.8	1H	bs	OH exchangeable
4.5	1H	bs	OH exchangeable
4.4	1H	bs	OH exchangeable
4.2	2H	dd	H2' and H3'
4.05	2H	t	$-CH_2O-$
4.0	1H	m	H4'
3.83	2H	m	2xH5'
3.0	2H	t	$-CH_2-Ph$

The ^{13}C NMR spectrum of the product displayed 15 signals which corresponded to the 17 carbons in the structure (Table 5.25).

Table 5.25. ^{13}C NMR data of 5-(2"-phenethoxy)-uridine (5.15)

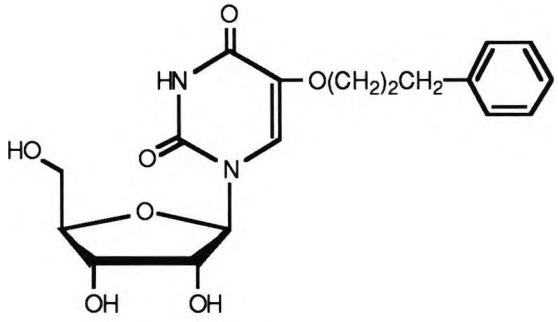
chemical shift δ	assignment	chemical shift d	assignment
160	C4	90	C1'
150	C2	86	C4'
139	$C_{t\text{-arom}}$	75	C2'
136	C6	72	C3'
129.8	$2xC_{m\text{-arom}}$	71.1	$-CH_2O-$
129	$2xC_{o\text{-arom}}$	62	C5'
127	$C_{p\text{-arom}}$	36	$-CH_2-Ph$
123	C5		

The FAB-MS of the product is shown in Table 5.26.

Table 5.26. FAB-MS data of 5-(2"-phenethoxy)-uridine (5.15)

m/z	intensity (%)	assignment
366	10	[M + 2H]
365	45	[M + H]
364	13	[M]
289	68	[M - Ph + 2H]
287	15	[M - Ph]
242	14	[M- OCH_2CH_2-Ph - H]
233	40	[M - sugar + 2H]
232	12	{M - sugar + H]

5.4.10 Preparation of 5-(3''-phenyl-1''-propoxy)-uridine (5.16)



(5.16)

A mixture of 3-phenyl-1-propanol, 5-diazo-5',6-cyclo-uridine (4.1) and catalyst was stirred at 60 °C for 4h. Then, after addition of excess hexane, dichloromethane was added to the mixture dropwise with stirring until it became homogeneous. The solution was placed in a freezer (-20°C) and an off-white solid was obtained.

The I.R. spectrum of the solid showed the absence of any absorption at 2130 cm^{-1}. A broad absorption around 3500 cm^{-1} - 3200 cm^{-1} corresponded to carboxyl and imido groups. Two strong absorptions at 1660 cm^{-1} and 1630 cm^{-1} were attributed to carbonyls.

The ^{1}H NMR spectrum of the compound provided signals which were consistent with the structure of the target product (Table 5.27).

Table 5.27. ^{1}H NMR data of 5-(3''-phenyl-1''-propoxy)-uridine (5.16)

chemical shift δ	int.	mult.	assignment
10.6	1H	bs	H3
7.8	1H	s	H6
7.2	5H	m	$5xH_{arom}$
5.9	1H	d	H1'
4.2	2H	s	H2', H3'
3.95	1H	m	H4'
3.8 (around)	4H	m	2xH5' $-CH_2O-$
3.7-2.6	3H	bs	3x-OH exchangeable
2.7	2H	t	$-CH_2$-Ph
1.8	2H	m	$-CH_2-$

The ^{13}C NMR spectrum of the compound was characterised by 16 peaks which

represented the 18 carbon atoms in the molecule (Table 5.28).

Table 5.28. ^{13}C NMR data of 5-(3"-phenyl-1"-propoxy)-uridine (5.16)

chemical shift δ	assignment	chemical shift d	assignment
160	C4	89.3	C1'
150	C2	83	C4'
142	$C_{t\text{-arom}}$	75	C2'
135	C6	70.5	C3'
128.4	$2xC_{o\text{-arom}}$	69.6	$-CH_2O-$
128	$2xC_{m\text{-arom}}$	61	C5'
126	$C_{p\text{-arom}}$	32	$-CH_2-Ph$
123	C5	31	$-CH_2-$

The FAB-MS of the compound showed a peak at m/z 379 (20%) corresponding to the pseudomolecular ion [M + H]. A peak at m/z 247 (40%) corresponded to [M - sugar + 2H]. The sugar fragment was observed at m/z 133 (8%). Two peaks at m/z 119 and m/z 77 were assigned to fragments [Ph-$(CH_2)_3$] and [Ph].

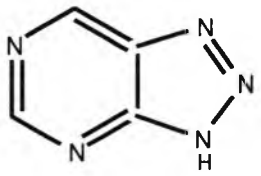
References

1. S. Yokoyama and T. Meyazawa, *J. Mol. Structure* , 1985, 126, 563.
2. L.K.J. Aw, "Some Chemistry of 5-Diazouracil and Its Derivatives" Ph.D. Thesis, City University, 1989.
3. G. Kobrich, *Angew. Chem. Int.*, 1967 6, 41.
4. R. Paulissen, H. Reimlinger, E. Hayez, A.J. Hubert and Ph. Teyssie, *Tetrahedron Letters*, 1973, 24, 2233.
5. S.A. Matlin, Z. Yin, unpublished material.
6. T. Miyasaka, H. Tanaka, M. Baba, H. Hayakawa, R.T. Walker and J. Balzarini, *J. Med. Chem.*, 1989, 32, 2507.
7. B.R. Baker and J.L. Kelley, *J. Med. Chem.*, 1970, 13(3), 456
8. A.F. Noels, A. Demonceau, N. Petiniot, A.J. Hubert and Ph. Teyssie, *Tetrahedron*, 1982, 38, 2733.
9. G.G. Cox, C.J. Moody, D.J. Austin and A. Padwa, *Tetrahedron,* 1993, 49, 5109.

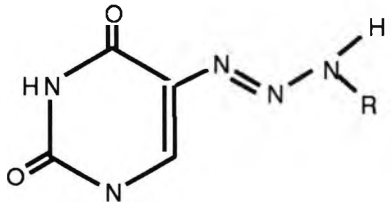
Chapter 6 Synthesis and Spectroscopic Studies of 5-(3''-Alkyl-1''-triazeno)-uridines and -2'-Deoxyuridines

6.1 Introduction

A logical extension of reported work on acyclic equivalent bases of purine would be the synthesis of acyclic analogues (6.2) of 8-azapurines (6.1).

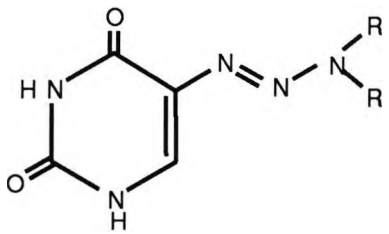


(6.1)



(6.2)

When reacted with an amine, 5-diazo-5',6-cyclo-uridine and 5-diazo-5', 6-cyclo-2'-deoxyuridine would lead to the formation of 5-(3"-alkyl-1"-triazeno)-uridine and -2'-deoxyuridine, which could be considered as acyclic analogues of azapurine. This structure is of interest because 5-(3'3'-dimethyl-1'-triazeno)-uracil (6.3; R=Me) and 5-(3',3'-diethyl-1'-triazeno)-uracil (6.3; R=Et) have been reported to possess significant activity against a number of common bacteria and fungi [1].



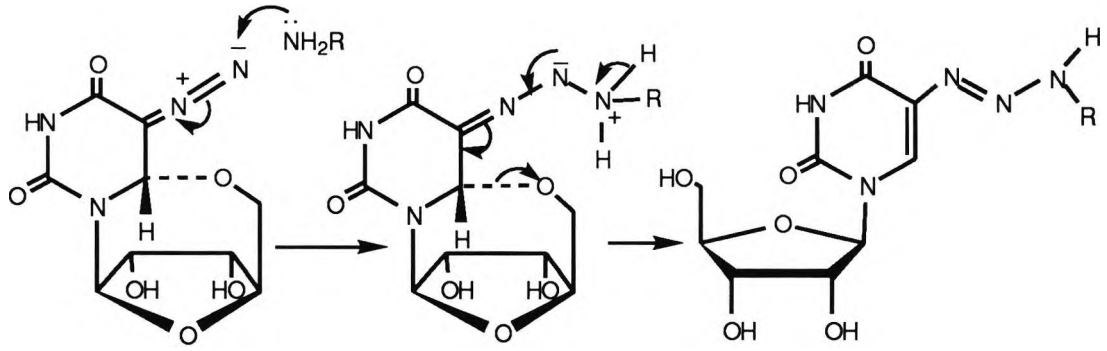
R: methyl or ethyl

(6.3)

In addition, based on the induced-fit theory in which the enzyme arranges itself to fit the ligand, 5-(3"-alkyl-1"-triazeno)-uridine and -2'-deoxyuridine could perhaps inhibit some enzymes with which purine nucleosides can react as substrates, owing to similarities in structure as well as being conformationally more flexible open chain structures which can decrease the energy required to fit the enzymes.

We proposed to use the following route to prepare 5-(3"-alkyl-1"-triazeno)-

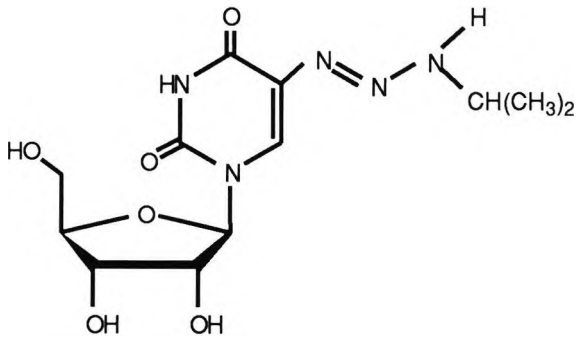
uridines and -2'-deoxyuridines (Scheme 6.1).



Scheme 6.1

6.2 Results and discussion

6.2.1 Preparation of 5-(3''-isopropyl-1''-triazeno)-uridine (6.4)



(6.4)

The orange solution of 5-diazo-5',6-cyclo-uridine (4.1) in freshly distilled isopropylamine was stirred for 16 h, evaporated to dryness and the yellow residue was purified using a silica column to give an off-white solid.

The I.R. spectrum of the product did not contain the absorption band for the diazo group at 2130 cm^{-1}. The presence of the uridine moiety was inferred from the carbonyl absorption around 1693 cm^{-1} and hydroxyl as well as imido absorptions around 3300 cm^{-1}.

The ^{1}H NMR spectrum of the product was consistent with the proposed structure (Table 6.1).

Table 6.1. ^{1}H NMR data of 5-(3"-isopropyl-1"-triazeno)-uridine (6.4)

chemical δ	int.	mult.	assignment
10.3	1H	bs	H3
8.95	1H	s	H6
8.7	1H	d	=NH exchangeable
5.3	1H	dd	H1'
5.2	1H	d	-OH exchangeable
5.0-4.8	3H	m	2x-OH exchangeable >CH-
3.9	1H	dd	H2'
3.8	1H	dd	H3'
3.7	1H	m	H4'
3.45	2H	m	2xH5'
1.55	6H	d	2x-CH_3

There are several features of the ^{1}H NMR spectrum which support the structure of 5-(3"-alkyl-1"triazeno) uridine. Examination of the chemical shifts of the protons in the alkyl group clearly indicated that the amine is attached to a strongly electron withdrawing group. This feature is made manifest by the downfield chemical shifts of protons in alkyl group, when compared with their shifts in the unreacted alkyl amine. The cleavage of the C6-O5' bond is indicated by the down field shift of the singlet for H6 from δ 6.15 to >δ 8.5 which showed that C6 is once again incorporated into an aromatic system. A split signal for one proton around δ 8.1-8.4, exchageable with D_2O, was assigned to the proton attached to N3 of the triazeno group. The splitting of the signal was attributed to the fact that the N2-N3 bond of the triazene could not spin freely because of the p-Π conjugation between the N1-N2 double bond and the lone pair of electrons of the N3. Therefore, the chemical and magnetic environments for the protons on the two sides are different.

^{13}C NMR of the product revealed 11 peaks which represented the 12 carbon atoms in the structure (Table 6.2).

The FAB-MS of the product provided two peaks at m/z 330 (27%) and m/z 352 (18%) which were assigned to the pseudomolecular ion [M + H] and [M + Na]. The peak at m/z 272 was assigned to the fragment [M - $NHCH(CH_3)2$]. The peaks at

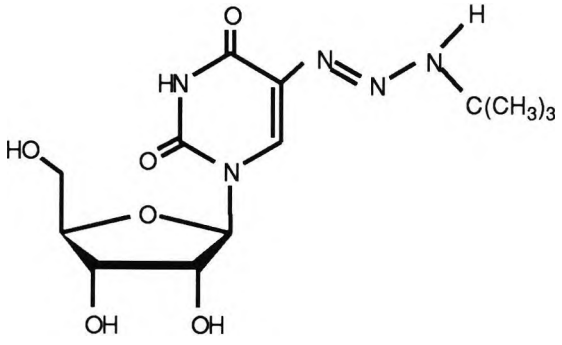
m/z 226, m/z 198 and m/z 197 were assigned to fragments [M - $C_4H_8N_3$ + H] [M - sugar + 2H] and [M - sugar + H].

The negative FAB-MS of the product gave a pseudomolecular ion [M - H] at m/z 228 with intensity of 35 %.

Table 6.2. ^{13}C NMR data of 5-(3"-isopropyl-1"-triazeno)-uridine (6.4)

chemical shift δ	assignment	chemical shift d	assignment
160	C4	74	C2'
152	C2	70	C3'
140	C6	61.5	C4'
126	C5	52	C5'
85	>CH	22	2 X CH_3
84.5	C1'		

6.2.2 Preparation of 5-(3"-t-butyl-1"-triazeno)-uridine (6.5)



(6.5)

5-Diazo-5',6-cyclo-uridine (4.1) was stirred in neat t-butylamine at room temperature for 16 h and the mixture was evaporated to dryness in vacuo at room temperature. The residue was purified by silica column chromatography, giving an off-white solid.

The I.R. spectrum of the product contained no diazo absorption around 2130 cm^{-1}. The presence of a broad and strong absorption around 3200 cm^{-1} was assigned to the hydroxyl and imido groups. The strong absorption around 1690 cm^{-1} was assigned to the carbonyl groups.

The ^1H NMR spectrum of the product was consistent with the required structure

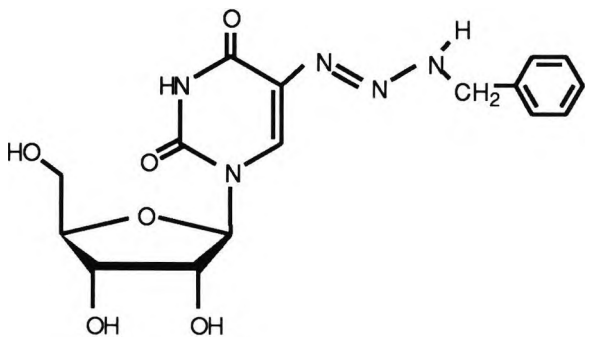
(6.5) (Table 6.3).

Table 6.3. ^{1}H NMR data of 5-(3"-t-butyl-1"-triazeno)-uridine (6.5)

chemical shift δ	int.	mult.	assignment
10.4	1H	bs	H3
9.22	1H	s	H6
8.96	1H	d	=NH exchangeable
5.5	1H	d	H1'
5.4	1H	bs	-OH exchangeable
5.2	1H	bs	-OH exchangeable
5.0	1H	bs	-OH exchangeable
4.2-3.6	5H	m	H2' H3' H4' 2xH5'
1.7	9H	s	3x-CH_3

The FAB-MS of the product provided two peaks at m/z 344 (35%) and m/z 366 (100%) which were attributed to pseudo-molecular ions [M + H] and [M + Na]. The peak at m/z 212 corresponded to the fragment [M -sugar + 2H].

6.2.3 Preparation of 5-(3"-benzyl-1"-triazeno)-uridine (6.6)



(6.6)

5-Diazo-5',6-cyclo-uridine (4.1) was added to a solution of chloroform and freshly distilled benzylamine and the mixture was stirred for 16 h at room temperature. The mixture was evaporated to dryness and the red residue passed through a silica column and a white solid product obtained.

The I.R. spectrum of the product did not contain an absorption for a diazo group. The presence of the uridine structure was inferred from hydroxyl and imido

absorption around 3460 cm^{-1}, 3300 cm^{-1} and carbonyl absorption around 1688 cm^{-1}.

The ^{1}H NMR spectrum of the product was consistent with the required structure (6.6) (Table 6.4).

Table 6.4. ^{1}H NMR data of 5-(3"-benzyl-1"-triazeno)-uridine (6.6)

chemical shift δ	int.	mult.	assignment
10.4	1H	bs	H3
9	1H	s	H6
8.68	1H	d	=NH exchangeable
7.4-7.3	5H	m	$5xH_{arom}$
5.7	2H	s	$-CH_2-$
5.3	1H	dd	H1'
5.2	1H	d	-OH exchangeable
4.95	1H	t	-OH exchangeable
4.85	1H	t	-OH exchangeable
3.95	1H	dd	H2'
3.8	1H	dd	H3'
3.7	1H	dd	H4'
3.4	2H	m	2xH5'

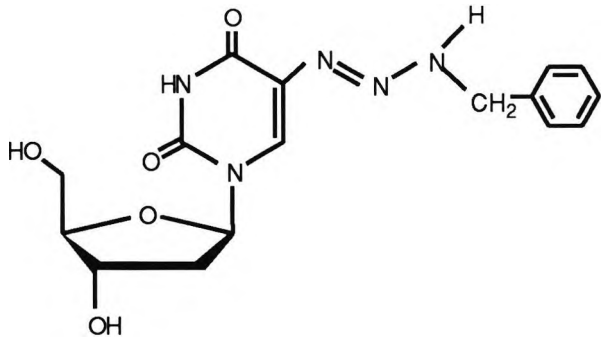
The ^{13}C NMR spectrum of the product provided evidence to support the proposed structure (Table 6.5).

Table 6.5. ^{13}C NMR data of 5-(3"benzyl-1"-triazeno)-uridine (6.6)

chemical shift δ	assignment	chemical shift δ	assignment
160	C4	127	C5
152	C2	84.5	$-CH_2-$
141	C6	84	C1'
135	$C_{t\text{-}arom}$	75	C4'
129	$2xC_{o\text{-}arom}$	70	C2'
128.5	$C_{p\text{-}arom}$	61.5	C3'
128	$2xC_{m\text{-}arom}$	53	C5'

The FAB-MS of the compound provided a clear and simple fragmentation. The peak at m/z 378 (55%) corresponded to the pseudo-molecular ion of [M + H]. The peak at m/z 272 (30%) corresponded to species [M - $NHCH_2Ph$ + H]. The peak at 242 (30%) corresponded to species of [M - sugar - H].

6.2.4 Preparation of 5-(3''-benzyl-1''-triazeno)-2'-deoxyuridine (6.7)



(6.7)

A) In chloroform and benzylamine

A mixture of chloroform, freshly distilled benzylamine and 5-diazo-5',6-cyclo-2'-deoxyuridine (2.13) was stirred for 16 h at room temperature. After about 8 h, a precipitate formed. Based on TLC and ^1H NMR spectrum of the precipitate, it was a polymer instead of the expected product.

B) In neat benzylamine

A solution of freshly distilled benzylamine and 5-diazo-5',6-cyclo-2'-deoxyuridine was stirred for 16 h at room temperature. The solution solidified to give a light-yellow polymer.

The I.R. spectrum of the polymer proved that the diazo group had reacted because of absence of the absorption around 2130 cm^{-1}.

From the ^1H NMR spectrum of the polymer, two weak signals, a singlet and a triplet, could be observed at δ 8.95 and δ 5.9 with intensity of 1:1, corresponding to H6 and H1' of the target compound. Therefore, it was concluded that, although most of the product was polymer, the target compound had also formed.

C) In acetonitrile and benzylamine

It was thought that the problem of polymerisation might be avoided if the benzylamine in the reaction was present in high dilution in about equivalent ratio to the diazo compound. Thus, the diazo compound and benzylamine (1:1.6 mol) were added to a large quantity of acetonitrile. The solution was stirred overnight at about

50 °C and, then for 5 h at 60°C. I.R.analysis revealed that there was no reaction. Therefore the temperature of the reaction was increased to 80 °C and an extra 1.6 equivalent of amine was added. About 4.5 h later, the 2120 cm^{-1} diazo peak had disappeared.

TLC and the ^1H NMR spectrum showed that the product was still a mixture. An attempt to purify the compound with a silica column was not successful.

In order to eliminate the polymerisation, a modification was adopted. While the solution of the diazo compound and acetonitrile was refluxing, a solution of acetonitrile containing about 2.1 equivalent benzyamine was added dropwise during about 12 hours. Although the product obtained was contaminated by polymer, the I.R., ^1H NMR and mass spectra provide clear evidence to prove the proposed structure.

The I.R. spectrum of the product did not contain an absorption for a diazo group at about 2120 cm^{-1}. The hydroxyl and carbonyl groups could be inferred from the presence of strong absorption around 3300 cm^{-1} and 1680 cm^{-1}.

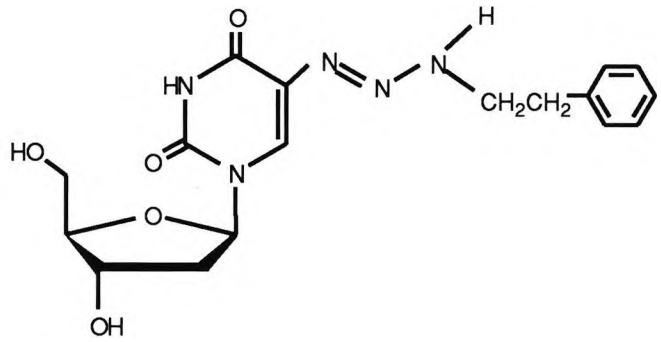
The ^1H NMR spectrum of the product did not provide satisfactory resolution and precise integration because of the presence of polymeric impurities. However, analysis of the spectrum, in comparison with the ^1H NMR of similar structures, provided some useful evidence to support the required structure (6.7) (Table 6.6).

Table 6.6. ^1H NMR data of 5-(3-"benzyl-1"-triazeno)-2'-deoxyuridine (6.7)

chemical shift δ	assignmet
10	H3
8.9	H6
8.6	=NH exchangeable
7.23	$5xH_{arom}$
5.8-5.5	H1', $-CH_2-$
5	-OH exchangeable
4.8	-OH exchangeable
4.31	H3'
4.1	H4'
3.6	2xH5'
2.0	2xH2

The negative FAB-MS of the product displayed signals consistent with the expected structure. The peak corresponding to pseudomolecular ion, [M - H] was at m/z 360 (100%). Peaks corresponding to benzyl triazenouracil were at m/z 271 [M - $C_3H_6O_3$] and at m/z 245 [M - sugar + H]. A peak at m/z 117 was assigned to [sugar]. A peak at m/z 254 was assigned to the fragment [M - $NHCH_2Ph$].

6.2.5 Preparation of 5-[3''-(2'''-Phenyl-1'''-ethyl)-1''-triazeno]-2'-deoxyuridine (6.8)



(6.8)

A) In neat phenylethylamine

A solution of 5-diazo-5',6(s)-cyclo-2'-deoxyuridine (2.13) and 2-phenylethylamine was stirred for 16 hours at room temperature. The solution solidified into a white solid.

The I.R. spectrum of the solid did not contain an absorption for a diazo group.

B) In a solution of phenylethylamine in acetonitrile

A solution of diazo compound, phenylethylamine (1:3 mol) and excess acetonitrile was stirred at 45 °C for 20 h and the I.R. peak at 2130 cm^{-1} disappeared. The solution was cooled and an off-white solid was obtained by filtration.

The I.R. spectrum of the product displayed no absorption at 2130 cm^{-1}. An absorption around 3315 cm^{-1} was assigned to hydroxyl and imido groups. Absorptions at 1723 cm^{-1} and 1690 cm^{-1} were assigned to carbonyl groups.

The ^1H NMR spectrum of the product was consistent with the required structure (6.8) (Table 6.7).

Table 6.7. ^{1}H NMR data of 5-[3"-(2"'-phenyl-1"'-ethyl)-1"-triazeno]-2'-deoxyuridine (6.8)

chemical shift δ	int.	mult.	assignment
10.2	1H	bs	H3
8.8	1H	s	H6
8.6	1H	d	=NH exchangeable
7.4-7.0	5H	m	$5xH_{arom}$
5.8	1H	dd	H1'
5.75	2H	t	$-CH_2$-NH-
5.1	1H	bs	-OH exchangeable
4.8	1H	bs	-OH exchangeable
4.2	1H	d	H3'
3.7	1H	d	H4'
3.4	2H	m	2xH5'
3.2	2H	t	$-CH_2$-Ph
2.1	2H	m	2xH2'

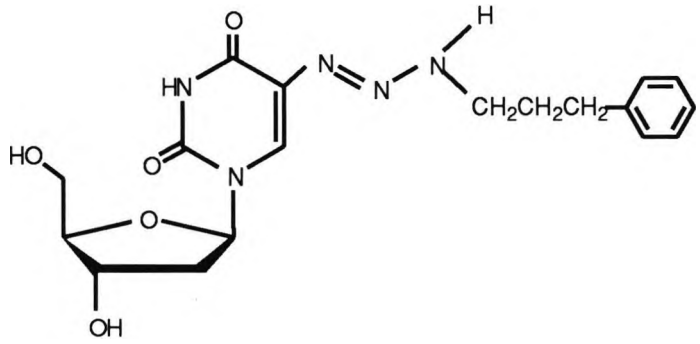
The ^{13}C NMR spectrum of the compound provided evidence to support the required structure in which 17 carbon atoms were represented by 15 signals in the spectrum (Table 6.8).

Table 6.8. ^{13}C NMR data of 5-[3"-(2"'-phenyl-1"'-ethyl)-1"-triazeno]-2'-deoxyuridine (6.8)

chemical shift δ	assignment	chemical shift δ	assignment
160.5	C4	87	C1'
152	C2	81	$-CH_2O$-
141	C6	71	C4'
138	$C_{t\text{-arom}}$	62	C3'
129	$2xC_{o\text{-arom}}$	51	C5'
128.8	$2xC_{m\text{-arom}}$	41	C2'
128.2	$C_{p\text{-arom}}$	36	$-CH_2$-Ph
127	C5		

The negative FAB-MS of the product showed the peak for the pseudomolecular ion, [M-H], at m/z 374. The peak at m/z 254 was assigned to [M-$NHCH_2CH_2Ph$]. The peak for [sugar] was at m/z 117.

6.2.6 Preparation of 5-[3''-(3'''-phenyl-1'''-propyl)-1''-triazeno]-2'-deoxyuridine (6.9)



(6.9)

5-Diazo-5',6-cyclo-2'-deoxyuridine (2.13) and 3-phenyl-1-propylamine (1:1.6 mol) were added to an excess of acetonitrile. The solution was stirred at 42 °C for 3 h and then at 60 °C for about 5 h. A light-yellow solid was obtained by precipitation.

The I.R. spectrum of the product did not contain an absorption at 2130 cm^{-1}. The presence of hydroxyl and carbonyl groups were inferred from absorption around 3300 cm^{-1} and 1685 cm^{-1}.

The ^1H NMR spectrum of the solid was consistent with the proposed structure (6.9) (Table 6.9).

Table 6.9. ^1H NMR data of 5-[3''-(3'''-phenyl-1'''-propyl) -1''-triazeno]-2'-deoxyuridine (6.9)

chemical shift δ	int.	mult.	assignment
10.3	1H	bs	H3
8.9	1H	s	H6
8.7	1H	d	=NH exchangeable
7.4-7.0	5H	m	$5xH_{arom}$
5.8	1H	dd	H1'
4.9	2H	bs	2x-OH exchangeable
4.45	2H	t	$-CH_2O-$
4.2	1H	m	H3'
3.7	1H	m	H4'
3.4	2H	d	2xH5'
2.6	2H	m	$-CH_2-Ph$
2.2	2H	t	$-CH_2-$
2.0	2H	m	2xH2'

The ^{13}C NMR spectrum of the solid revealed 16 signals which corresponded to the 18 carbon atoms in the molecule (Table 6.10).

Table 6.10. ^{13}C NMR data of 5-[3"-(3'"-phenyl-1'"-propyl)-1"-triazeno]-2'-deoxyuridine (6.9)

chemical shift δ	assignment	chemical shift δ	assignment
160	C4	87	C1'
152	C2	81	$-CH_2O-$
141.5	C6	72	C4'
141	$C_{t\text{-arom}}$	62	C3'
129	$2xC_{o\text{-arom}}$	50	C5'
128.8	$2xC_{m\text{-arom}}$	41	C2'
128.6	$C_{p\text{-arom}}$	32	$-CH_2$-Ph
126.5	C5	31	$-CH_2-$

The negative FAB-MS of the product provided a peak at m/z 388 which was assigned to the pseudomolecular ion, [M - H]. The peak at m/z 254 was assigned as [M - $NHCH_2CH_2CH_2Ph$]. The peak for the sugar fragment was at m/z 117.

Discussion:

1. Comparing the preparation of 5-(3"-benzyl-1"-triazeno)-uridine (6.6) and 5-(3"-benzyl-1"-triazeno)-2'-deoxyuridine (6.7), there was greater difficulty encountered in the case of the deoxyuridine for reasons which are unknown.

2. The greater the length of the alkyl side chain, the easier is the preparation of 5-(3"-aryalalkyl-1"-triazeno)-2'-deoxyuridines, apparently for steric reasons.

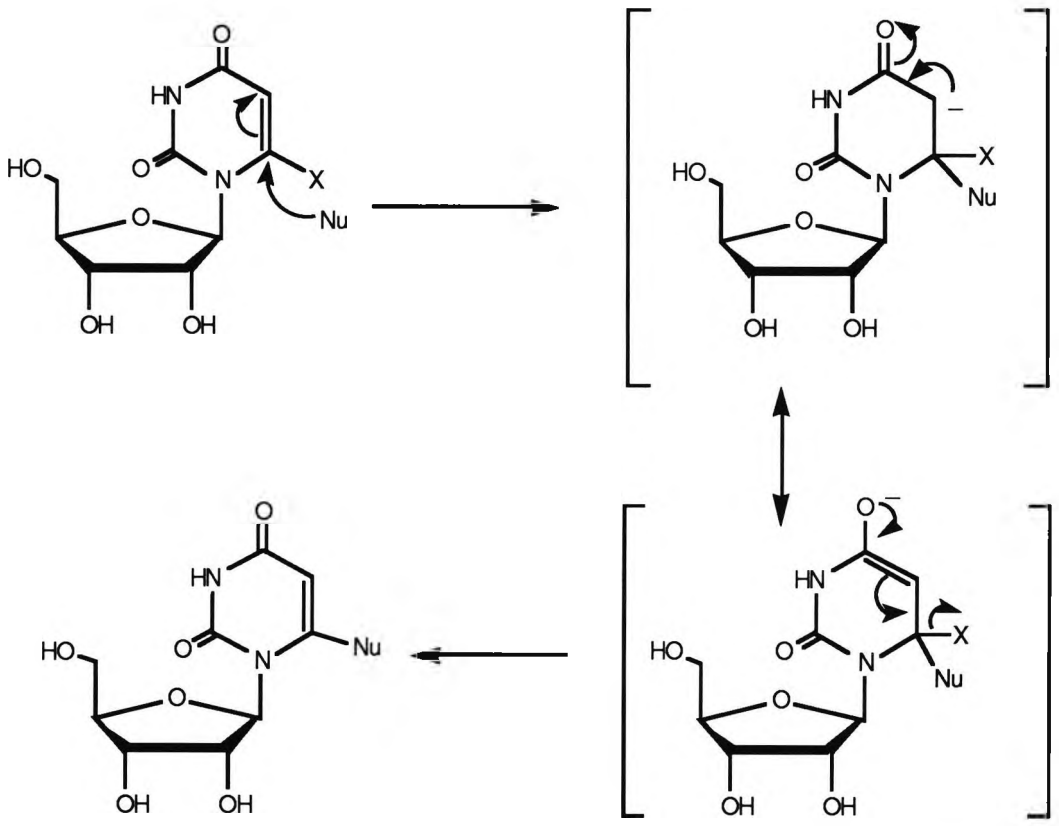
Reference:

1. L.B. Townsend, USA Patent 4,092,305 , 30 May 1978, *Chem. Abstr.*, 1978, 89,163904z.

Chapter 7. Preparation of 5'-O-Trityl-5-diazo-6-alkoxyuridines

7.1 Introduction

Attack of a nucleophile on uridine, as indicated in Scheme 7.1, results in favourable localisation of negative charge on the oxygen of O4. The leaving ability of group X is crucial. If X is H, the tendency for the hydrogen to leave with a negative charge is very low, limiting the utility of approach for the synthesis of 6-substituted uridines.

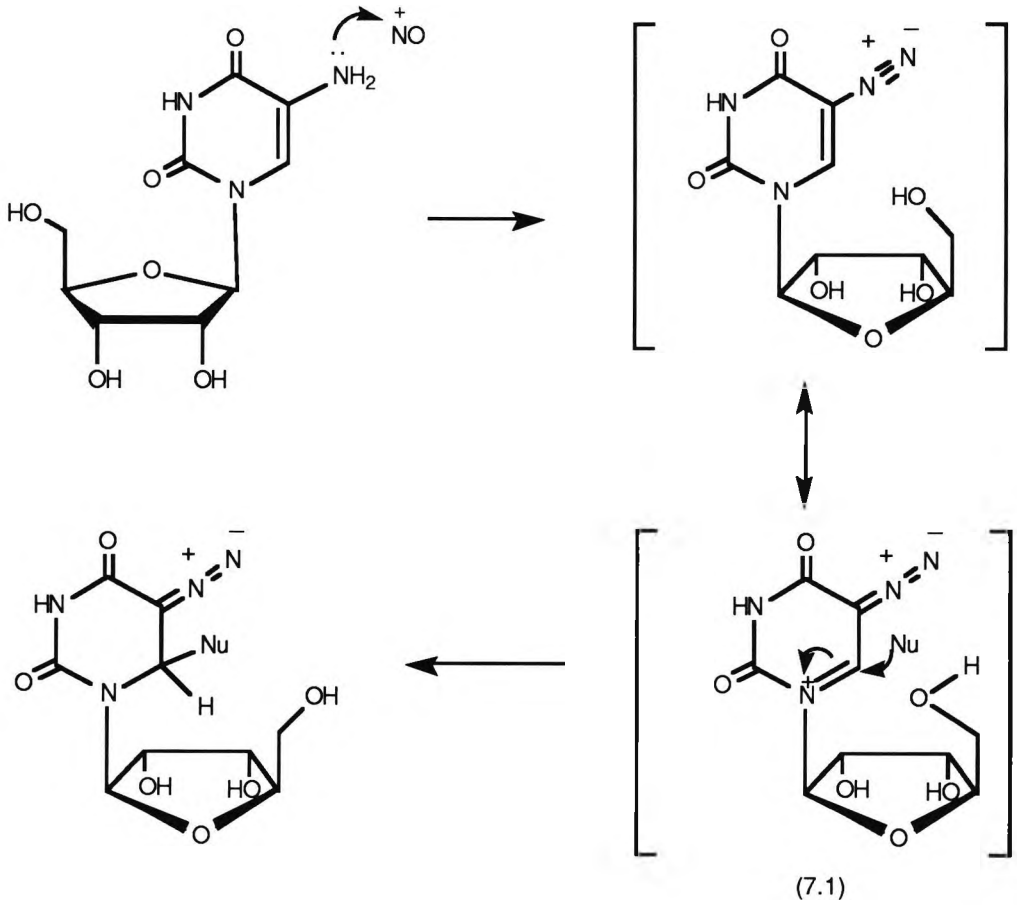


Scheme 7.1

During diazotization of 5-aminouridine, the formation of intermediate (7.1) provides a possibility for nucleophiles to attack C6, because of the presence of positive charge at the nitrogen N3 (Scheme 7.2).

Thus, it was reasoned that if the diazotization was carried out in nucleophilic circumstances, such as in an alcohol solvent, it might be possible to form 6-

substituted uridine derivatives. Therefore, an attempt was made to prepare 6-methoxy-5-diazo-uridine. The reason for methanol to be selected was that it possesses a low steric barrier to attack the C6. Unfortunately, the product of the preparation was confirmed to be 5-diazo-5',6(s)-cyclo-uridine (4.1).

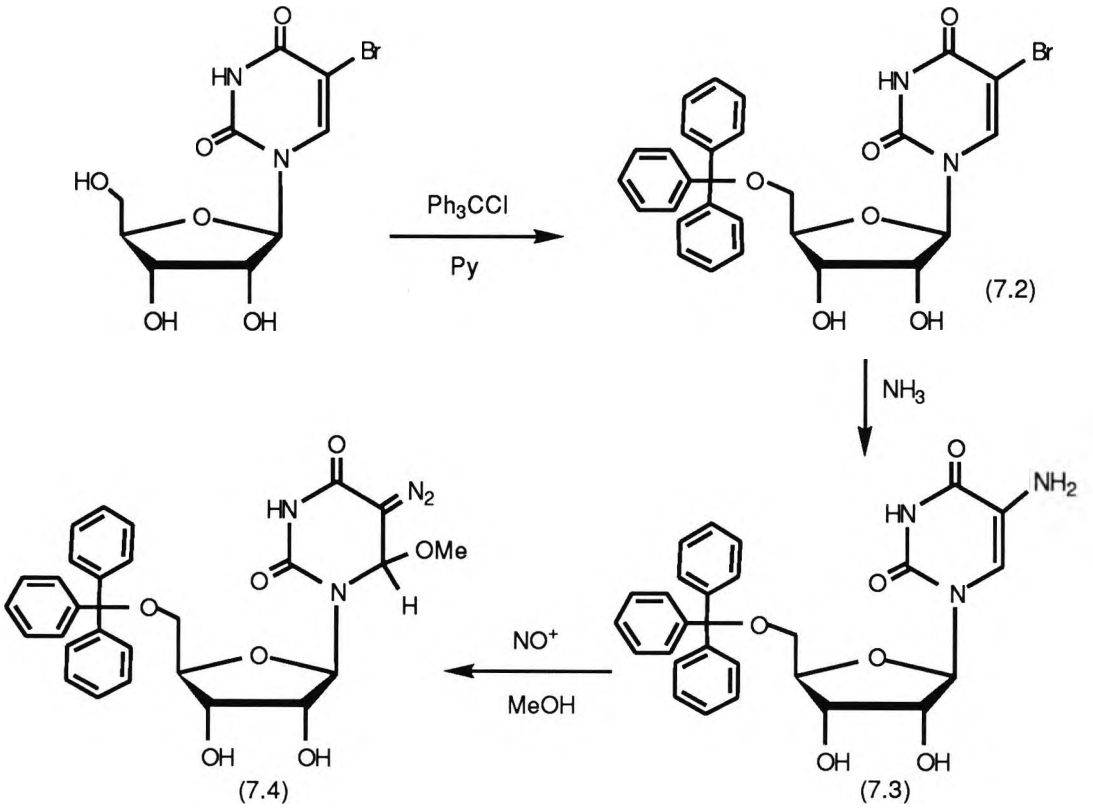


Scheme 7.2

The formation of 5-diazo-5',6(s)-cyclo-uridine (4.1) indicates that the 5'-hydroxyl group of uridine possesses a favourable geometric position from which to attack C6. Consequently, the synthesis might be successful under conditions of blocking of the 5'-hydroxyl group. Trityl chloride was chosen as a blocking agent, because it possesses the desired selectivity to react with primary hydroxyls.

The tritylation of uridine at 5'-OH has been reported [1], but there have been no similar reports for 5-aminouridine and 5-bromouridine. The first tritylation attempted

was with 5-aminouridine, because it was the latest intermediate on the pathway to the target compound. However, the tritylation was not successful. 5-Bromouridine was then chosen as the starting material. The pathway for the preparation of 5'-O-trityl-5-diazo-6-alkoxyuridine is shown in Scheme 7.3.



Scheme 7.3

7.2 Results and discussion

7.2.1 Preparation of 5'-O-trityl-5-bromouridine (7.2)

5-Bromouridine (4.2) and trityl chloride were reacted in pyridine at 110 °C for 2 h. After cooling and pouring into ice-water, the product was obtained as white crystals.

The I.R. spectrum of the product showed absorptions around 3416 cm^{-1} and 3200 cm^{-1} which were assigned to the hydroxyl and imido groups. Strong signals at

1709 cm^{-1} and 1670 cm^{-1} were attributed to carbonyls. At 1613 cm^{-1}, a new peak was associated with the C=C vibration of the benzene rings. At 1114 cm^{-1}, a sharp and strong absorption was assigned to C-O-C vibration.

In the ^1H NMR specrum of the product, a broad singlet at δ 11 was observed for H3; a singlet at δ 8.03 for H6; a group of signals around δ 7.4 for 15 protons of the trityl group, a doublet at δ 5.75 for H1'; two exchangeable doublets at δ 5.5 and 5.15 for two hydroxyl protons, a multiplet around δ 4.4- 4.2 for two protons H2' and H3'. The multiplet for one proton H4' was at δ 3.5. The signals for two protons 2xH5' were around δ 3.3.

Since electric charge can easily distribute around the whole conjugated system of the trityl species to form a stable fragment, the FAB-MS of the product provided a base peak at m/z 243 for the [Ph_3C], whereas the intensity of other fragments was very low. However, from the low intensity signals useful information could also be obtained. Peaks at m/z 587 and m/z 589 were assigned to the species [M + Na] Peaks at m/z 565 and m/z 567 were assigned to the pseudo-molecular ion, [M + H]. Peaks at 485 and m/z 484 were attributed to species [M - Br] and [M - Br - H]. A peak at m/z 307 corresponded to [M - Ph_3CO + 2H].

7.2.2 Preparation of 5'-O-trityl-5-aminouridine (7.3)

5'-O-Trityl-5-bromouridine (7.2) was added to ammonia in isopropanol and the mixture was heated in a sealed tube for 120 h at 55 °C. After purification, an off-white solid was obtained.

The I.R. spectrum of the product revealed mainly signals similar to those in the precursor bromo-compound. The absorption at 3353 cm^{-1} corresponded to hydroxyl groups. The broad absorption at 3202 cm^{-1} corresponded to amino and imido groups. The carbonyl absorption moved to 1692 cm^{-1} and 1642 cm^{-1} because of the electron donating effect of the amine group. The C=C vibration of the benzene rings was seen at 1596 cm^{-1}. A strong absorption at 1109 cm^{-1} corresponded to the presence of C-O-C in the molecule.

The ^1H NMR signals were consistent with the proposed structure. At δ 10.8, there was a broad singlet for H3; around δ 7.2, a group of signals with high intensity for 15 protons in the trityl group; at δ 6.7, a sharp singlet for H6; at δ 5.72, a doublet for H1'; at δ 5.25 and δ 5.0, two exchangeable signals for two hydroxyl protons; around δ 4.0-3.6, overlapped signals for H2', H3, and H4'; at δ 3.25, an exchangeable and broad signal for two amino protons; at δ 3.19, a signal for two protons H5'.

The EI-MS of the product did not provide a peak at m/z 559 required for the molecular ion. A strong peak at m/z 243 was assigned to the species [Ph_3C]. A significant fragment at m/z 260 was seen for [M - Ph_3C]. From the fragment at m/z 260, the conclusion could be made that the 5-bromine in the uridine was replaced by amine. The final evidence to prove the structure was that the product was successfully diazotised to the target compound (7.3).

7.2.3 Preparation of 5'-O-trityl-5-diazo-6-methoxyuridine (7.4)

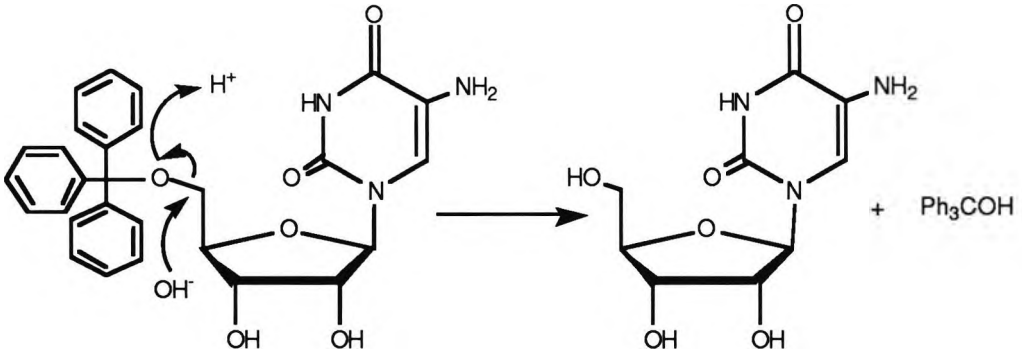
A) With sodium nitrite and HCl (aq) in methanol

5'-O-Trityl-5-aminouridine (7.3) and HCl (aq) were added to methanol at about -3 °C and a mixture of $NaNO_2$, MeOH and H_2O was added dropwise to the solution. After addition of cold water, a precipitate was obtained.

The I.R. spectrum of the product provided an absorption around 2120 cm^{-1}. The ^1H NMR spectrum showed signals which were consistent with those expected for 5-diazo-5',6-cyclo-uridine (4.1).

B) With sodium nitrite and methanolic HCl

It was thought that the loss of the trityl group from the uridine, which occurred under that condition, was caused by the presence of the water and acid (Scheme 7.4).



(7.3)

Scheme 7.4

In order to avoid the decomposition, a modified approach was tested. Gaseous HCl was passed into MeOH to prepare a solution with pH 1. Then, 5'-O-trityl-5-aminouridine was added to the solution at -2°C and the solution was stirred at -5°C and powdered $NaNO_2$ was added gradually. After 1 h, the solution was evaporated to dryness at 2 °C in vacuo. A yellow solid was obtained which was confirmed by ^1H NMR and FAB-MS to be 5-diazo-5',6(s)-cyclo-uridine (4.1).

C) With isoamyl nitrite and acetic acid

A mixture of 5'-O-trityl-5-aminouridine (7.3), methanol, isoamyl nitrite and acetic acid was stirred at room temperature for about 90 min, during which time the starting material disappeared and a product with lower polarity was detected by TLC. However, after the solvent was removed by evaporation, TLC detection revealed one more new spot of even lower polarity. The I.R. spectrum of the product revealed an absorption at 2170 cm^{-1} with very weak intensity compared with the carbonyl absorption.

From the TLC, it seemed that the desired reaction was occurring but some further change happened in the process of evaporation.

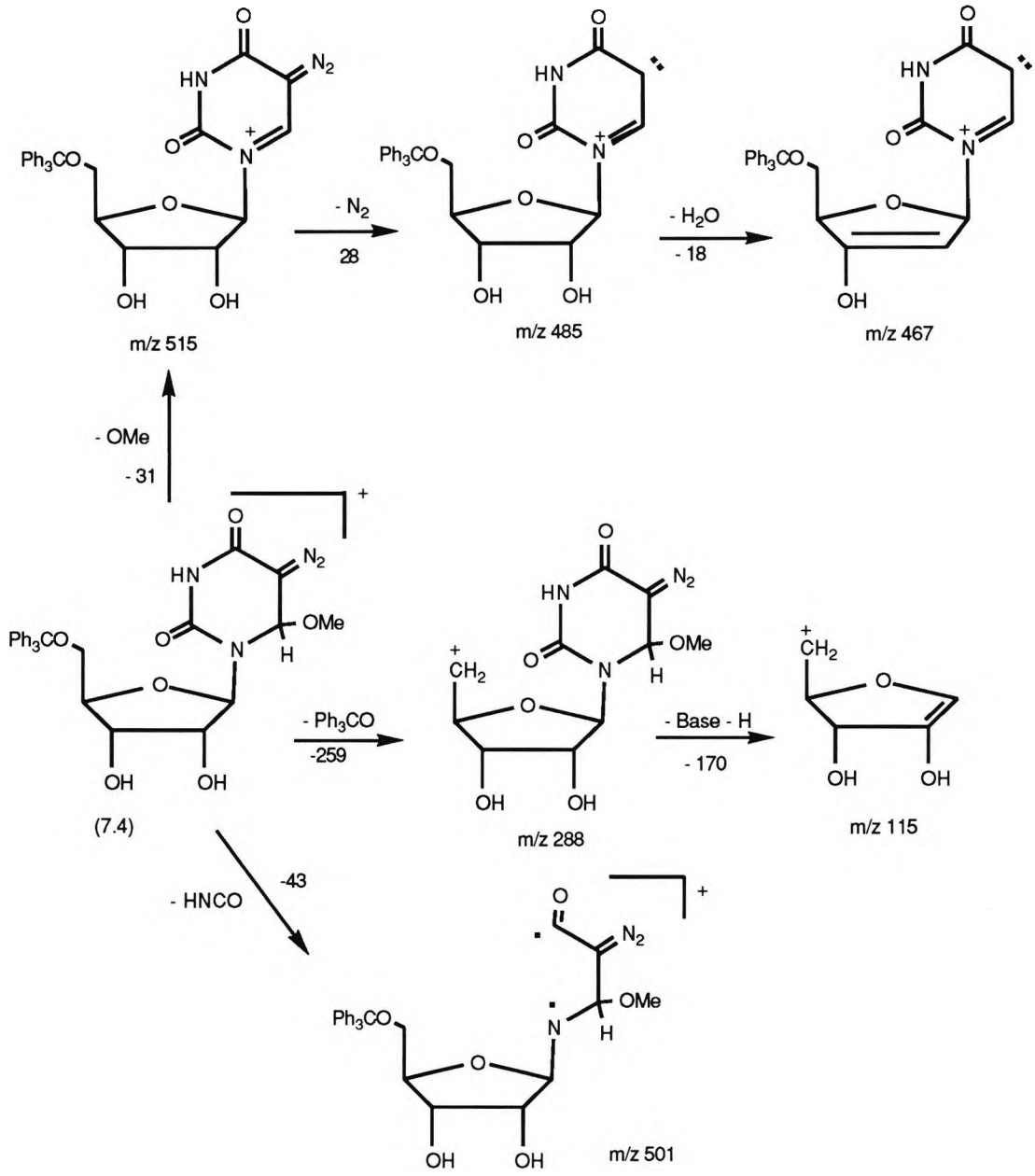
A modified procedure was adopted for the diazotization. The reaction was carried out under similar conditions and with TLC detection every 15 min. When the spot of the starting material disappeared, the temperature was decreased to about -10 °C and H_2O was added to the solution dropwise. An off-white precipitate was obtained by

quick filtration.

The I.R. spectrum of the product provided the required diazo absorption at 2118 cm^{-1} with about the same intensity as that of the carbonyl absorption. The other readily assigned absorptions were a broad bond around 3400 cm^{-1} for hydroxyl and imido groups, a strong and broad absorption around 1680 cm^{-1} for the carbonyls, an absorption at 3057 cm^{-1} for aromatic and olefinic C-H, absorptions at 2956 cm^{-1} and 2870 cm^{-1} for aliphatic C- H, and an absorption at 1597 cm^{-1} for C=C vibration of benzene rings.

The ^{1}H NMR spectrum of the compound provided signals which were consistent with the required structure (7.4). A singlet at δ 10.8 was assigned to H3. A multiplet around δ 7.5-7.1 was assigned to the 15 protons in the trityl group. The singlet for H6 moved to δ 6.25. There was a doublet at δ 5.65 for the anomeric proton of H1', a multiplet at around δ 4.1 for H2' and H3', signals at δ 3.9 and 3.2 for H4' and the two protons of H5'. The two exchangeable doublets at δ 5.3 and 5.05 were assigned to two hydroxyl protons. The singlet for the three protons of the 6-methoxy group appeared at δ 3.15.

The FAB-MS of the product also supported the proposed structure (7.4). Peaks at m/z 567 and m/z 545 were assigned to species [M + Na] and [M + H]. The base peak at m/z 243 and strong peak at m/z 259 corresponded to species [Ph_3C] and [Ph_3CO]. Other important fragments were at m/z 513, m/z 485, m/z 467, m/z 285, m/z 115 and m/z 501 which represented cleavages via the routes shown (Scheme 7.5).



Scheme 7.5

7.2.4 Purity and stability study of the product (7.4) using HPLC

7.2.4.1 Introduction

5'-O-Trityl-5-diazo-6-methoxyuridine (7.4) was found to be unstable to conditions examined for its isolation and purification. One way in which the crude product can be obtained is quick precipitation by adding water to the reaction solution at low temperature. The crude product can not be purified by recrystallization

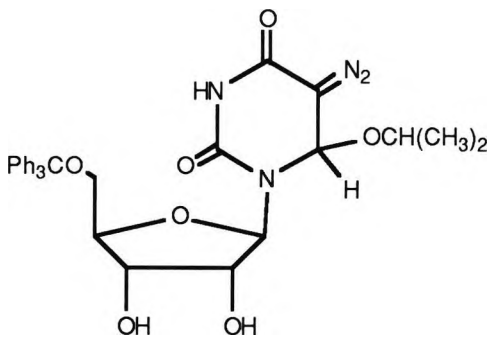
because the compound is thermally unstable. In addition,the trityl group in some compounds can be lost in chromatographic purification [2]. HPLC was therefore adopted for the assessment of purity of the product.

7.2.4.2 Results and discussion

HPLC analysis was carried out under reverse phase conditions (ODS-silica eluted with MeOH:H_2O) which showed that the crude product contained 4 components at retention times of 15 min. (A), 21 min. (B), 25 min. (C) and 31 min. (D) with an intensity ratio of 1 : 1.3 : 2.5 : 8.7. After the sample solution was stood at 0 °C for 192 h, one more peak occurred at 36 min. and the intensity of component D decreased slightly. Therefore, the component D was possibly the target compound (7.4). The comparison of four individual preparations by HPLC showed that the product from the fourth preparation, in which every reagent involved was purified before the reaction, contained the highest percentage of component D. The intensity ratio was 1:1.4:10:46. Moreover, the fifth component at 36 min. could not be detected eight days later.

Unfortunately, the isolation of component D and component C from the mobile phase was not successful. Therefore, further effort to obtain the pure compound was suspended.

7.2.5 Preparation of 5'-O-trityl-5-diazo-6-isopropoxyuridine (7.5)



(7.5)

A similar procedure was adopted for the preparation of 5'-trityl-5-diazo-6-isopropoxyuridine (7.5). After addition of water, a crude product was obtained.

The I.R. spectrum of the crude product revealed an absorption for the diazo group at 2115 cm^{-1} which was with about the same intensity as that of the carbonyl absorption at 1681 cm^{-1}. The presence of hydroxyl and phenyl groups was inferred from absorptions at 3442 cm^{-1} and 1598 cm^{-1}.

Because of impurities, the signals in the ^1H NMR of the product were not distinct enough to be assigned.

The FAB-MS of the crude product revealed a very similar spectrum to that of compound (7.4). Although the peak for the pseudo-molecular ion [M + H] was too weak to be detected, the signal at m/z 595 was readily assigned to the species [M + Na]. The signal at m/z 513 was due to fragment [M - propoxy]. The peak at m/z 553 corresponded to the fragment [M - H_2O + H] and the peak at m/z 485 was associated with the fragment [M - propoxy - N_2].

7.3 Conclusion and suggestions

The apparently successful generation of two 6-substituted diazo uridines indicates that this is a feasible route to prepare this type of compound. However, the method for isolation of the product from the solvent requires that the precursor of the 6-alkoxyl substituent must be a water-soluble alcohol.

An alternative method to isolate product could be feeze drying.

In addition, it would be possible to use the diazo compounds before they were thoroughly purified as intermediates in the synthesis of more stable products.

Further study might include the exploration of reduction of the 5-diazo group to different stages, hydrazone, amine, etc., without influencing the 6-substituent. In this way, a group of novel 6-substituted and 5,6-disubstituted uridines might be obtained.

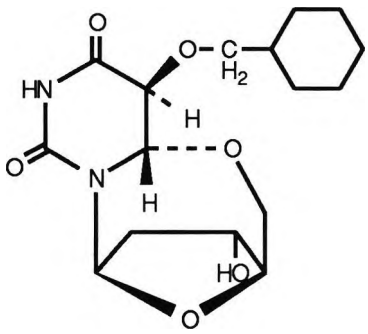
References:

1. Z. Zhang, Z. Xia, G. Zeng and F. Zhou, *Youji Huaxua (Organic Chemistry)*, 1986, 2, 125, *Chem. Abstr.*, 1986, 105, 172949u.
2. J. Lehrfeld, *J. Org. Chem.*, 1967, 32, 2544.

Chapter 8. Structural and Chemical Studies of Three Novel and Unexpected Products

8.1 5-Cyclohexylmethoxy-5'O,6-cyclo-5,6-dihydro-2'-deoxyuridine (5s,6s) (8.1)

In the preparation of 5-cyclohexylmethoxy-2'-deoxyuridine (5.13), another novel compound was formed in reasonably high yield (30%), which was identified as 5-cyclohexylmethoxy-5'O,6-dihydro-2'-deoxyuridine (5s,6s) (8.1).



(8.1)

Compound (8.1) contains one less hydroxyl group than the 5-cyclohexylmethoxy-2'-deoxyuridine (5.13). Therefore, it was eluted first from the silica column. The structure of compound (8.1) has been confirmed by micro-analysis, I.R., NMR, MS, and UV.

The I.R. of the compound did not contain the diazo absorption at 2130 cm^{-1}. The absorption at 3369 cm^{-1} was assigned to the hydroxyl and imido groups. The absorptions at 1726 cm^{-1} and 1691 cm^{-1} were assigned to the carbonyls.

The FAB-MS of the compound provided two peaks at m/z 363 (100%) and m/z 341 (63%) which were assigned to the species [M + Na] and the pseudomolecular ion [M + H]. The peak at m/z 225 was assigned to the species [M - sugar + H]. The negative FAB-MS of the compound provided a peak at m/z 340 (100%) .The peak at m/z 223 (20%) was assigned to fragment [M - sugar - H].

Based on the pseudomolecular ions and the data of the micro-analysis, the

formula of the compound was deduced to be $C_{16}H_{24}N_2O_6$.

The ^{13}C NMR spectrum of the compound revealed 14 peaks which were assigned to 16 carbon atoms in the molecule. Among them, the ^{13}C DEPT experiment revealed six methylenes at δ 79.3, 75.6, 45.8, 30.5, 27.3 and 26.5. The assignments for the carbons and ^{1}H-^{13}C COSY determination of some H-C attachments are given in Table 8.1.

Table 8.1. ^{13}C NMR and H-C attachments of compound (8.1)

chemical shift δ	assignment	attachments of H
168.8	C4	
150.1	C2	
89.5	C6	H (d 4.9)
89.2	C4'	H (d 4.3)
87.3	C1'	H (d 6.4)
79.2	C1"	H (d 3.71) H (d 3.45)
78.8	C5	H (d 4.05)
75.6	C5'	H (d 4.15) H (d 3.72)
74.2	C3'	H (d 4.4)
45.8	C2'	H (d 25) H (d 2.3)
39.0	C2"	
30.4	2x*Ca*	
27.3	*Ca*	
26.5	2x*Ca*	

a the remaining cyclohexane carbons have not been specifically assigned.

The details of ^{1}H NMR of product (8.1) are given in Table 8.2. The large coupling (8.3 Hz) observed between H5 and H6 confirmed that these two protons were attached to adjacent carbon atoms andhave a trans geometry.

The trans configuration in geometry of H5 and H6 was supported by nuclear Overhauser experiments (nOe) (Table 8.3). Irradiating H5, 5.5%, 4.8%, and 4.8% enhancements were observed at H1"a, H1"b and H6. Irradiating H6, 6.9% and 2.7% enhancement were observed at H5'a and H5.

Table 8.2. ^{1}H NMR data of compound (8.1)

chemical shift (δ) and int.	mult.	proton	couplings observed in COSY
9.2 (1H)	bs	H3	
6.4 (1H)	dd	H1'	H2', H2'
4.9 (1H)	d	H6	H5
4.4 (1H)	m	H3'	H2', H2', -OH,
4.3 (1H)	d	H4'	H3', H5'
4.2 (1H)	d	-OH	H3'
4.15(1H)	d	H5'	H5'
4.05(1H)	d	H5	H6
3.72(1H)	dd	H5'	H5', H4'
3.71(1H)	dd	H1"	H1", H2"
3.45(1H)	dd	H1"	H1", H2"
2.5 (1H)	m	H2'	H2', H3', H1'
2.3 (1H)	m	H2'	H2', H3', H1'
1.8-0.97(11H)	m	11xH in cyclohexane	

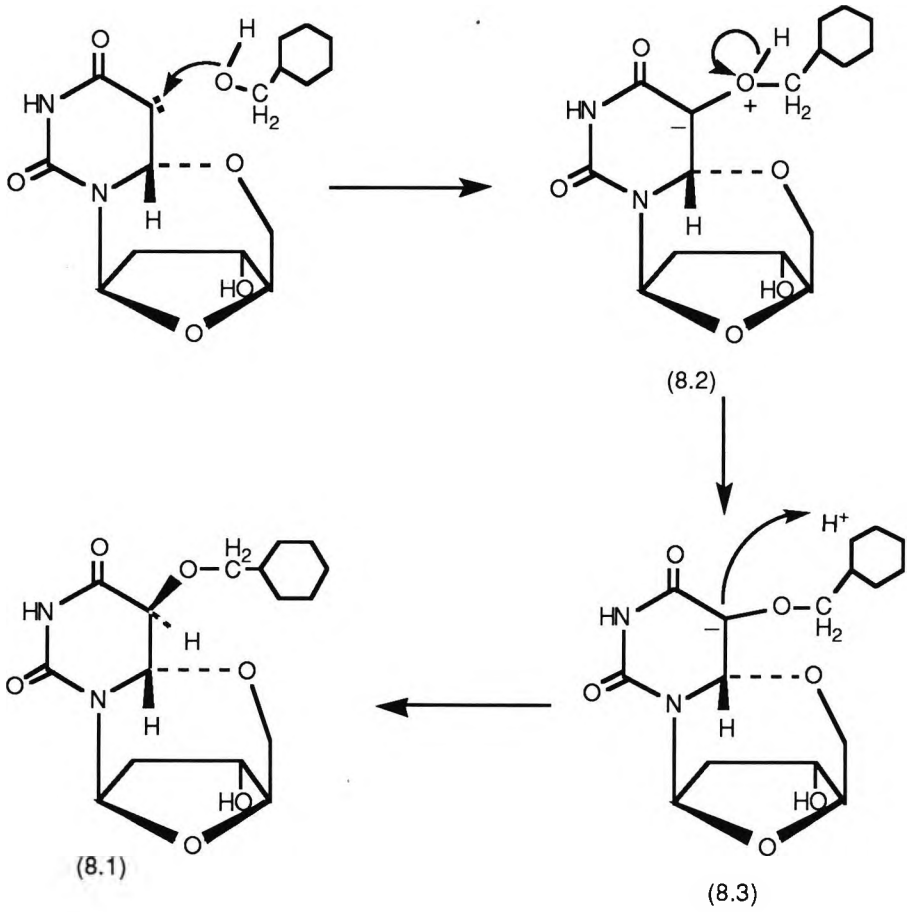
Table 8.3. nOe (%) caused by irradiating signals in the ^{1}H NMR spectrum of compound (8.1)

proton irradiated	enhancement (%)
H1'	H2'a(3.4)
H2'a(down)	H'"b(16.7), H1'(6.3)
H2'b(up)	H2'a(10), H3'(4.2)
H3'	H2'b(3.2), -OH(2.4), H5'b(2.4)
H4'	H5'a(3.5), H5'b(2.1)
H5'b	H5'a(18.6), H3'(4.4), H4'(3.3), H1"a(1.1)
-OH	H3'(6.4), H2'a(1.4)
H5	H1"A(5.5), H1"b(4.8), H6(4.8)
H6	H5'a(6.9), H5(2.7)
H1"a*	H1"b(12.7), H5(4.7), H5'b(0.9), H2'a(0.5), * and some cyclohexane H

Since the "s" geometrical conformation about C6 of 5-diazo-5',6-cyclo-2'-deoxyuridine (2.13) had been confirmed by single crystal X-ray determination [1], the geometrical configuration about C5 of compound (8.1) was confirmed to be "s".

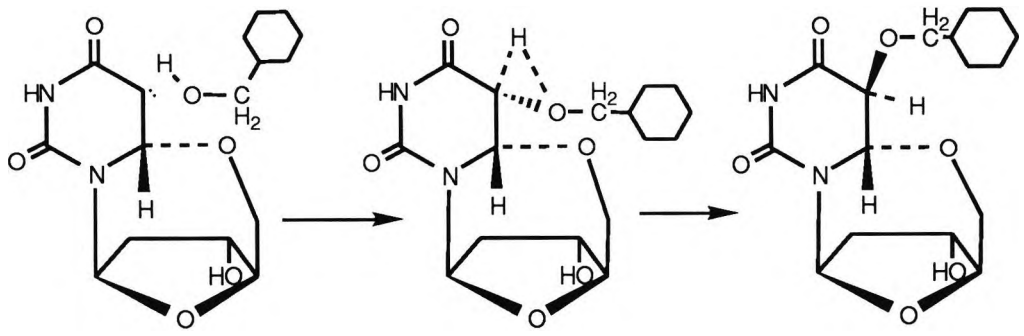
From a detailed study of the reaction of diphenyl carbene with alcohols and with water, it was concluded that the reaction involves attack of the carbene on the hydroxyl oxygen to give an ylide which yields the alcohol or ether [2]. Therefore,

the process for formation of compound (8.1) should be that after formation of ylide (8.2), anionic intermediate (8.3) forms by departure of the proton. Intermediate (8.3) can capture a proton from the front or back side to yield a pair of isomers. However, for steric reasons, only the process to form the product with 5S configuration is favourable (Scheme 8.1).



Scheme 8.1

Alternatively, since no trace of 5R isomer has been found, the mechanism for insertion of a singlet carbene into a C-H bond [3] could be reasonably adopted for the formation of compound (8.1) in a concerted process involving a three-centre transition state (Scheme 8.2).

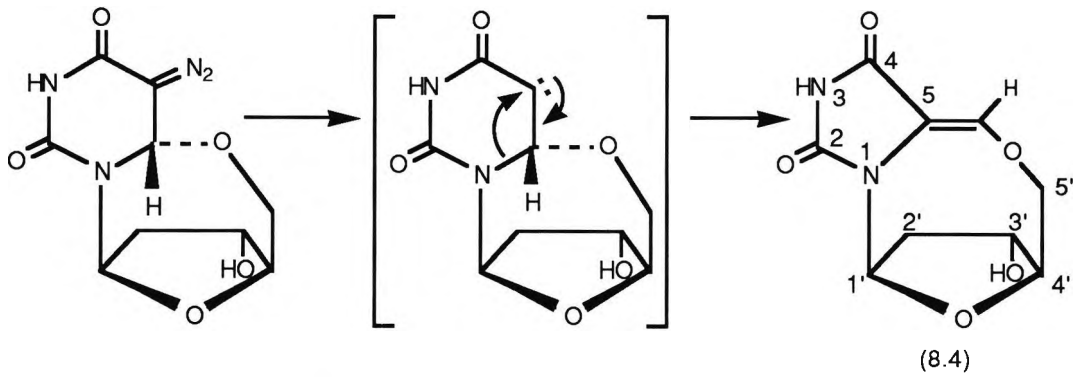


Scheme 8.2 (8.1)

8.2 1-(2'-Deoxy-β-D-ribofuranosyl)-5'-oxymethylene,5-cyclo-imidazolidine-2,4-dione (8.4)

Compound (8.4) is a side product obtained in the preparation of 5-arylalkoxy-2'-deoxyuridines (see Section 5.4, Chapter 5). It can also be prepared from 5-diazo-5',6(s)-cyclo-2'-deoxyuridine (2.13) by treatment with rhodium acetate in some solvents (see Section 9.3, Chapter 9).

The mechanism of formation of compound (8.4) is shown in Scheme 8.3. This reaction consists of an intramolecular rearrangement in which there is a carbene 1,2-shift to the electron-deficient centre.



(8.4)

Scheme 8.3

The I.R. spectrum of compound (8.4) did not contain the absorption of the diazo group around 2130 cm^{-1}. The band around 3429 cm^{-1} was assigned to the -OH and >NH groups. The absorption at 1718 cm^{-1} and 1666 cm^{-1} were related to the >C=O groups.

The ^{1}H NMR spectrum of compound (8.4) revealed signals which were consistent with the proposed structure (Table 8.4). The considerable difference in chemical shifts between two of the 5' protons provided a characteristic pattern [4] from which the presence of the cyclo form of the sugar moiety could be inferred.

Table 8.4. ^{1}H NMR data of compound (8.4)

chemical shift δ	int.	mult.	assignment
10.2	1H	bs	H3 exchangeable
6.8	1H	s	H6
6.4	1H	dd	H1'
4.6	1H	m	H3'
4.32	1H	dd	H5'
4.2	1H	m	H4'
3.67	1H	dd	H5'
3.0	1H	bs	-OH exchangeable
2.4	2H	m	2xH2'

^{13}C NMR analysis of compound (8.4) gave separate peaks for all 9 carbon atoms in the molecule (Table 8.5).

Table 8.5. ^{13}C NMR data of compound (8.4)

chemical shift δ	assignment	chemical shift d	assignment
164	C4	86	C4'
153	C2	81.5	C1'
133	C6	75	C5'
118.5	C5	72	C3'
42	C2'		

The COSY ^{13}C-^{1}H long-range correlation spectrum revealed that C4 and C5 were related to H6 and C6 was related to 2xH5'.

Nuclear Overhauser experiments showed that there were no enhancements observed in H2', H3', H4' and H5', when H6 was irradiated, and vice versa.

The structure of compound (8.4) was confirmed by X-ray crystallography (Fig. 8.1, Table 8.6 and Table 8.7).

In addition, the high resolution mass spectrum of compound (8.4) provided the

molecular ion at m/z 226.0825 (required 226.0604).

Table 8.6. Bond lengths (Å) of compound (8.4)

bond	length (Å)	bond	length
O3'-C3'	1.427	N1-C1'	1.458
O1'-C1'	1.408	N3-C4	1.366
O1'-C4'	1.429	N3-C2	1.368
O5'-C6	1.362	C1'-C2'	1.519
O5'-C5'	1.439	C2'-C3'	1.508
O4-C4	1.196	C3'-C4'	1.503
O2-C2	1.211	C4'-C5'	1.496
N1-C2	1.368	C4-C5	1.488
N1-C5	1.405	C5-C6	1.304

Table 8.7. Bond angles (deg) of compound (8.4)

bond	angle (deg)	bond	angle (deg)
C1'-O1'-C4'	109.9	C5'-C4'-C3'	115.3
C6-O5'-C5	116.7	O5'-C5'-C4'	112.9
C2-N1-C5	109.9	O2-C2-N1	125.5
C2-N1-C1'	120.9	O2-C2-N3	127.2
C5-N1-C1'	124.8	N1-C2-N3	107.2
C4-N3-C2	112.9	O4-C4-N3	126.8
O1'-C1'-N1	109.5	O4-C4-C5	129.0
O1'-C1'-C2'	106.9	N3-C4-C5	104.2
N1'-C1'-C2'	114.4	6-C5-N1	132.2
C3'-C2'-C1'	103.5	C6-C5-C4	122.1
O3'-C3'-C4'	106.3	N1-C5-C4	105.6
03'-C3'-C2'	112.2	C5-C6-O5'	126.6
C4'-C3'-C2'	102.8	O1'-C4'-C5'	111.6
O1'-C4'-C3'	103.5		

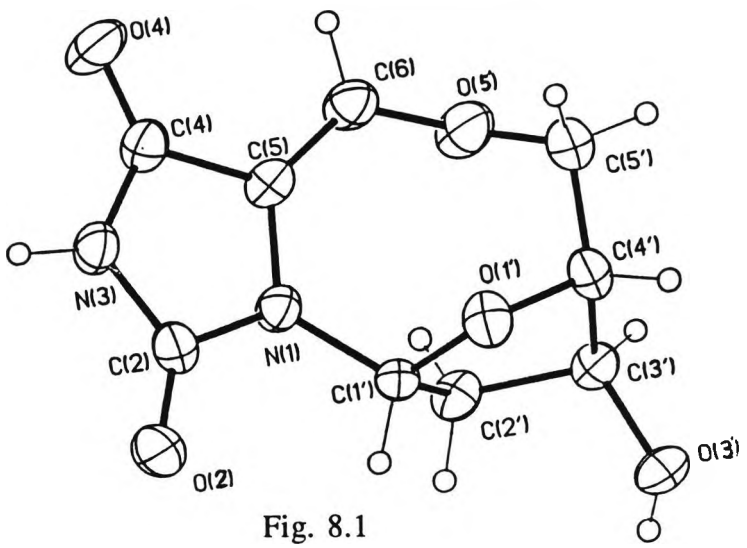
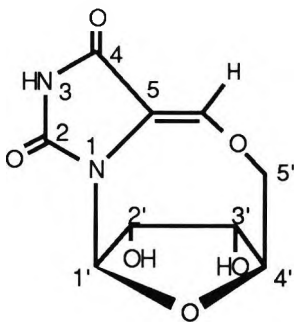


Fig. 8.1

8.3 1-(β-D-ribofuranosyl)-5'-oxymethylene-5-cyclo-imidazolidine-2,4-dione (8.5)

Compound (8.5) can be prepared from 5-diazo-5',6(s)-cyclo-uridine (4.1) in an analogous manner to that used for the deoxy analogue (8.4).

The ^{1}H NMR data of compound (8.5) (Table 8.8) showed similar signals to that of compound (8.4). However, the signal for H1' was shifted upfield to d 5.76 and there was a 2H multiplet at d 4.18 for H2', owing to the absence of the 2'-OH.



(8.5)

Table 8.8. ^{1}H NMR data of compound (8.5)

chemical shift (δ)	int.	mult.	assignment
11.4	1H	bs	H3 exchangeable
6.78	1H	s	H6
5.76	1H	d	H1'
5.4	1H	d	-OH exchangeable
5.1	1H	bs	-OH exchangeable
4.37	1H	m	H3'
4.28	1H	dd	H5'
4.18	2H	m	H2', H4'
3.8	1H	dd	H5'

The ^{13}C NMR spectrum of compound (8.5) was nearly identical to that of compound (8.4) (Table 8.9).

Table 8.9. ^{13}C NMR data of compound (8.5)

chemical shift (δ)	assignment	chemical shift (d)	assinment
164	C4	88	C4'
153	C2	86	C1'
133	C6	77	C5'
121	C5	75	C2'
		72	C3'

References:

1. D.J. Abraham, T.G. Cochran and R.D. Rosenstein, *J. Amer. Chem. Soc.*, 1971, 93, 6279.
2. D. Bethell, A.R. Newall, G. Stevens and D. Whittaker, *J. Chem. Soc.*, 1969, B, 749.
3. "The Chemistry of Diazonium and Diazo Groups", Ed. S. Patai, Wiley Interscience, London, 1978, 602.
4. D. Lipkin, C. Cori and M. Sano, *Tetrahedron Letters* , 5993, 57, 1968.

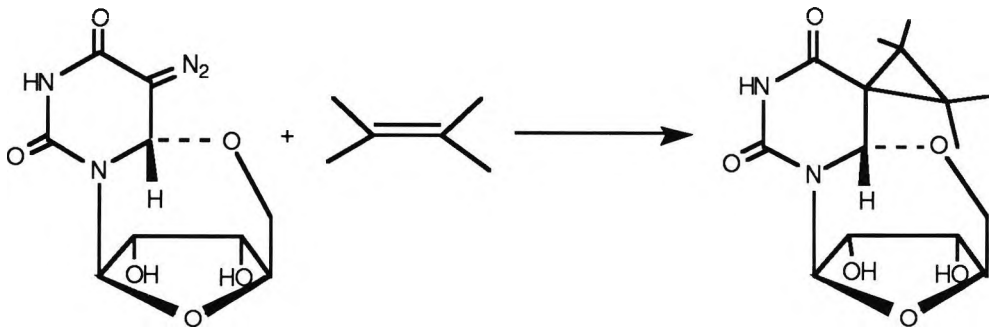
Chapter 9. Studies of the Reactions of 5-Diazo-5',6'-cyclo-uridine with Unsaturated Compounds and Its Stability in Some Solvents

9.1 Introduction

Much of the chemistry of diazo compounds may be explained by supposing the reacting species to be a carbene. Carbenes will readily react with electron rich molecules such as olefins to yield cyclopropanes. The mechanism of the cycloaddition has been suggested to be dependent on the nature of the intermediate carbene. A guide to the numerous examples in literature concerning the preparation of cyclopropanes from carbenes or carbenoids has been given elsewhere [1,2]. A singlet carbene usually results in a totally stereospecific addition. A triplet carbene results in a product without stereospecificity.

It has been demonstrated that with copper salts or finely divided copper, the carbenoid species formed behaves in the same way as a simple carbene [3,4]. Rhodium acetate also appears to behave in a similar manner.

The cyclopropanation reaction which was undertaken for the preparation of 5-spiro-uridines (9.1) is shown in Scheme 9.1.



Scheme 9.1 (9.1)

In principle, the reaction with olefins could be extended to other electron rich systems. For example, compounds containing carbonyls, imino groups, azo groups, or nitriles [5].

9.2 Results and discussion

9.2.1 Reaction with styrene

A) Neat styrene

5-Diazo-5',6-cyclo-uridine and rhodium acetate were added to styrene and the mixture kept at ambient temperature overnight in a flask shielded from light to avoid polymerisation of styrene. After about 14 h, the solution had completely solidified to afford a plastic solid.

B) Styrene in dichloromethane

In order to reduce the opportunity for polymerisation, a very dilute solution of styrene in dichloromethane was employed. Rhodium acetate dissolved in the solution but the diazo compound did not. The reaction mixture was stirred for 20 h at ambient temperature. However. the diazo compound still remained suspended and unreacted.

9.2.2 Reaction with ethyl vinyl ether and 2,3-dihydrofuran

It was thought that low solubility of the diazo compound in the non-polar solvents might be the main factor responsible for lack of successful reaction. Consequently, two more polar double bond-containing compounds, ethyl vinyl ether and 2,3-dihydrofuran were chosen for the reaction.

9.2.2.1 Ethyl vinyl ether

5-Diazo-5',6-cyclo-uridine and rhodium acetate were added to ethyl vinyl ether, but little of the diazo compound appeared to dissolve. The suspension was refluxed for 20 h, after which only the unreacted diazo compound was isolated.

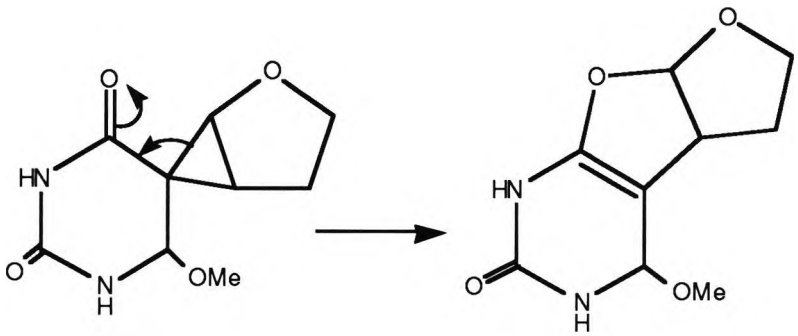
After consideration of the low boiling point of ethyl vinyl ether and low solubility of the diazo compound, a stainless steel sealing tube was used to increase the reaction temperature and solubility. However, apart from starting material, no identificable compounds could be isolated.

9.2.2.2 2,3-Dihydrofuran

The diazo compound and a trace of rhodium acetate were added to 2,3-dihydrofuran. The mixture was refluxed for 24 h. The diazo compound

progressively dissolved and, 16 h later, the solution was clear. The solvent was removed by evaporation in vacuo and the residue obtained was dissolved in ethyl acetate. The solution was washed with water to remove the rhodium acetate, then the organic phase was dried with Na_2SO_4. After removal of the ethyl acetate a yellow solid was obtained.

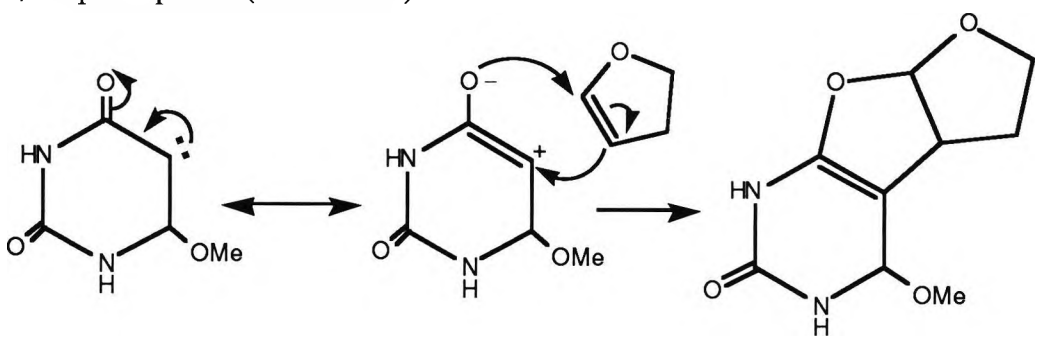
Earlier work in this laboratory has shown that 5-diazo-6-methoxyuracil reacts with dihydrofuran to produce a compound with a furanopyrimidine structure (9.3) [6]. One possible route to the tricyclo compound is via the formation of the cyclopropane derivative (9.2) which subsequently rearranges to provided the observed structure (Scheme 9.2).



(9.2) (9.3)

Scheme 9.2

An alternative mechanism would be to consider that the product arose from a 1,3-dipole species (Scheme 9.3).



Scheme 9.3 (9.3)

In the case of 5-diazo-5',6-cyclo-uridine, the I.R. of the product did not contain the diazo absorption around 2130 cm^{-1}. An absorption around 3400 cm^{-1} was

related to hydroxyl and imido groups. A strong and broad absorption around 1710 cm^{-1} to 1650 cm^{-1} was associated with carbonyls.

The ^{1}H NMR spectrum of the crude product provided very poorly resolved signals, which was attributed to the presence of the paramagnetic species Rh^{++} or some other impurities.

TLC gave very poor results with considerable streaking and the product was unexpectedly found to be very soluble in water.

The EI-MS of the product did not provide fragments above m/z 181, but CI and FAB-MS provided a peak at m/z 313 (8%) which corresponded to the pseudo-molecular ion, [M + H]. A peak at m/z 181 (38%) might be further evidence for the expected compound, probably corresponding to the species [M - sugar + H].

Consequently, it seems that the reaction of 5-diazo-5',6-cyclo-uridine (4.1) with 2.3-dihydrofuran had occurred. However, because of difficulty in purifying the product, work on it was suspended.

9.3 Stability and solubility studies of 5-diazouridine in some solvents

9.3.1 Introduction

The unsuccessful reactions of the diazo compound with styrene and ethyl vinyl ether drew attention to the low solubility of the compound, which could be the main reason for the failure of the reactions.

The presence of hydroxyl and diazo groups in 5-diazo-5',6-cyclo-uridine makes the molecule quite polar. Moreover, the diazo-compound might be quite reactive when it dissolves in some solvents.

An ideal solvent for synthetic work needs to satisfy the following requirements:

A. Possesses reasonable polarity so that the diazo-compound, olefins and catalyst will dissolve;

B. Possesses chemical inertness towards the diazo compound and olefins in the presence of rhodium acetate;

C. Does not affect the catalytic function of rhodium acetate.

Based on the points above, some solvents which are predicted to possess enough polarity to dissolve the substances have been tested. They are DMF, DMSO, Py, THF, acetone and acetonitrile. The test for each solvent was carried out without and with rhodium acetate at room temperature or 60 °C.

9.3.2 Results and discussion

9.3.2.1 Tetrahydrofuran (THF)

A) Without rhodium acetate

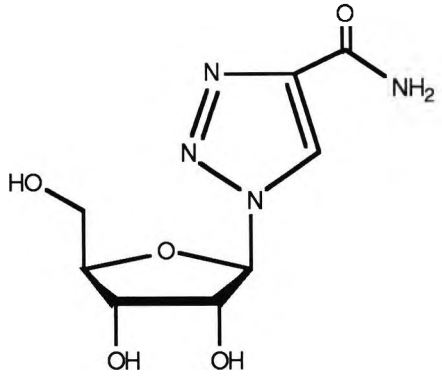
The diazo-compound was added to THF. The solution was stirred for 24 h at room temperature. The substance dissolved fully in THF. A yellow solid was obtained by evaporation of the solution to dryness.

The I.R. spectrum of the solid showed a sharp absorption at 2130 cm^{-1} with weaker intensity compared with the starting material. The solid was redissolved in THF and the solution was refluxed (65 °C) for 28 h. After the solution was cooled, a yellow precipitate was obtained. The I.R. of the product did not contain the diazo absorption at 2130 cm^{-1}.

The ^{1}H NMR spectrum of the product revealed 12 signals for 12 protons based on the integration. They were at δ 8.75 (s), 7.9 (bs), 7.5 (bs), 5.95 (d), 5.65 (d), 5.25 (d), 5.07 (t), 4.36 (dd), 4.12 (dd), 3.95 (dd), 3.6 (m) and 3.52 (m). Among them, 5 signals at δ 7.9, 7.5, 5.65, 5.25, and 5.07 were exchangeable by addition of D_2O. The ^{13}C NMR spectrum of the product provided 8 signals at δ 161.5, 143.3, 125.1, 92.5, 86.1, 75.5, 70.3 and 61.

Negative FAB-MS revealed a peak at m/z 243 which could be assigned to the pseudo molecular ion [M - H].

Based on the numbers of protons,carbons and the pseudomolecular ion, the product was confirmed to be 1-(β-D-ribofuranosyl)-1,2,3-triazole-4-carboxamide (9.4) , which has been reported by Thurber [7].



(9.4)

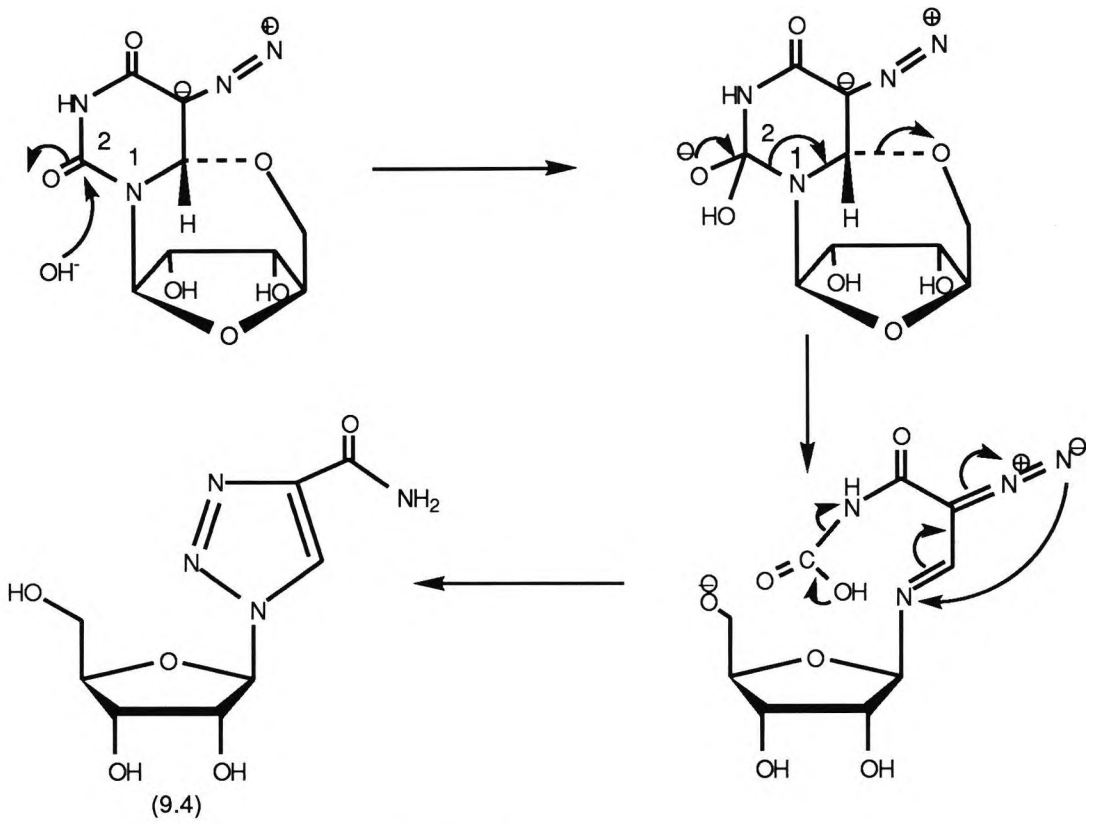
Table 9.1. Assignment of ^{13}C NMR for compound (9.4)

chemical shift δ	assignment
161.5	C6
143.3	C4
125.1	C5
92.5	C1'
86.1	C4'
75.5	C2'
70.3	C3'
61	C5'

Table 9.2. Assignment of ^{1}H NMR for compound (9.4)

chemical shift δ	mut.	int.	assignment
8.75	s	1H	H5
7.9	bs	1H	H7 exchangeable
7.5	bs	1H	H7 exchangeable
5.95	d	1H	H1'
5.65	d	1H	-OH exchangeable
5.25	d	1H	-OH exchangeable
5.07	t	1H	-OH exchangeable
4.36	dd	1H	H2'
4.12	dd	1H	H3'
3.95	dd	1H	H4'
3.6	m	1H	H5'
3.52	m	1H	H5'

A possible mechanism for the reaction is shown in Scheme 9.4.



Scheme 9.4

B) With rhodium acetate

A solution of the diazo compound in THF was refluxed in the presence of rhodium acetate for 4 h. No identifiable product could be isolated.

9.3.2.2 Acetonitrile

The diazo compound and rhodium acetate were added to acetonitrile and the suspension was stirred at 17 °C for 9 h. The unreacted diazo compound was isolated by filtration and the filtrate was evaporated to dryness. The I.R. spectrum of the residue was identical with that of the starting material.

The experiment was repeated at high temperature. After the solution was refluxed for 9 h, a solid was obtained by evaporation of the solution to dryness. The I.R. spectrum of the solid showed absence of diazo absorption. The ^{1}H and ^{13}C NMR spectra as well as MS of the solid revealed that its structure (8.5) was the result of a ring contraction (see Section 8.3, Chapter 8).

9.3.2.3 N,N-Dimethylformamide (DMF)

The solution of the diazo compound in DMF was heated to 70 °C for 21 h. Then, excess of dichloromethane was added. The yellow precipitate obtained was identified to be compound (9.4).

When a solution of the diazo compound and DMF in presence of rhodium acetate was stirred at 20 °C for only 40 min, the diazo absorption could not be detected.

9.3.2.4 Acetone

A) Without rhodium acetate

The solution of the diazo compound in acetone was refluxed for 8 h. Then, the solution was evaporated to dryness. A yellow product was obtained. The product was confirmed to be compound (9.4) by ^{1}H NMR.

B) With rhodium acetate

The solution of the diazo compound, rhodium acetate and acetone was refluxed for 20 h. The product was revealed to be compound (8.5) (see Section 8.3, Chapter 8).

C) Attempted protection of hydroxyl groups in the diazo compound

Because of the polarity of hydroxyl groups in the molecule, the diazo compound can be not dissolved in non-polar solvents. Therefore, decreasing the polarity by blocking the hydroxyl groups might be another route for increasing the solubility in some non-polar solvents.

Since 2',3'-isopropylidene-5-bromouridine (4.7) can be easily prepared by reaction of 5-bromouridine (4.2) with acetone in the presence of proton (see Section 4.2.4, Chapter 4), a similar reaction might be observed for the reaction of 5-diazo-5',6- cyclo-uridine (4.1) with acetone.

The diazo compound was added to the solution of acetone and acetic acid (2:1). The solution was refluxed for 2h. The product obtained showed a peak at 2130 cm^{-1} in I.R.spectrum. The FAB-MS of the product provided peaks at m/z 311 (41%), m/z 333 (10%), m/z 271 (100%) and m/z 293 (28%) among which the former two could

be assigned to the pseudomolecular ions [M + H] and [M + Na] of the expected compound and the latter two could be assigned to the pseudomolecular ion [M + H] and [M + Na] of the starting material.

However, after the reaction was carried out for 4 h more, the I.R. spectrum of the product revealed the absence of the diazo absorption at 2130 cm^{-1}.

9.3.2.5 Dimethyl sulphoxide (DMSO)

Rhodium acetate dissolved in DMSO to afford a pink solution instead of a green one. A solution of the diazo compound and rhodium acetate in DMSO was stirred at 65 °C. The reaction was followed by I.R analysis. The compound seemed to be reasonably stable in DMSO. After 52 h, the diazo-absorption disappeared.

9.3.2.6 Pyridine

Rhodium acetate dissolved in pyridine to yield a pink solution. A solution of the diazo compound and rhodium acetate in pyridine was heated to 60 °C for 8 h, then excess of dichloromethane was added to the solution. A solid was obtained. The I.R. and ^{1}H NMR spectra of the solid revealed that it was the starting material.

9.3.2.7 Attempted preparation of 5-methoxyuridine in DMSO and pyridine

5-Diazo-5',6-cyclo-uridine is reasonably stable in DMSO and pyridine in the presence of rhodium acetate, but the colour of the rhodium acetate changes from green to pink in these solvents. Whether the catalytic function of rhodium acetate is still operable or not is a further consideration. Therefore, the preparation of 5-methoxyuridine was attempted in these two solvents.

The negative results indicated that the catalytic function of rhodium acetate was lost or decreased greatly while it was in DMSO and pyridine.

9.3.2.8 Benzonitrile

The diazo compound in acetonitrile undergoes intramolecular conversion into compound (8.5) rather than reacting with the triple bond of the acetonitrile. Benzonitrile was also investigated as a solvent. In order to obtain better solubility, 5-

diazo-5',6-cyclo-2'-deoxyuridine instead of 5-diazo-5',6-cyclo-uridine was chosen as the starting material.

The mixture of the diazo deoxyuridine, rhodium acetate and benzonitrile was stirred at 80 °C for 18 h. An excess of dichloromethane was added to the mixture, giving a precipitate. The ^1H NMR spectrum of the precipitate proved that it was compound (8.4) (see Section 8.2, Chapter 8).

9.3.2.9 Furan

The diazo deoxyuridine, rhodium acetate and furan were refluxed for 6 h (32 °C). The I.R.spectrum of the product showed that it was identical with the starting material. Therefore, the reaction was attempted in a sealed tube at 75 °C for 12 h. The precipitate obtained by addition of excess of dichloromethane and hexane was identified as compound (8.4).

9.3.2.10 Summary

The results obtained from the above tests can be summarised as follows:

A. When heated in some solvents, 5-diazo-5',6-cyclo-uridine converts to compound (9.4) by intramolecular rearrangement.

B. When heated with rhodium acetate in some solvents, 5-diazo-5',6-cyclo-uridine (or deoxyuridine) can form a carbene with loss of N_2. However, the tendency for the carbenoid to convert to compound (8.5) (or 8.4) is predominant, rather than intermolecular reaction with the multiple bond in an olefin, ketone or nitrile.

References:

1. W. Kirmse, "Carbene Chemistry", Academic Press, London, 2nd Ed.,1971, 302
2. W. Ando, in "The Chemistry of Diazonium and Diazo Groups", Ed. S. Patai, Wiley Interscience, London, 1978, 367.
3. L. Wolff, *Justus Liebigs Ann. Chem.,* 1938, 23, 394.
4. M. Takebayashi, T. Ibata, H. Kohara and B.H. Kim, *Bull. Soc. Jap.,* 1967, 40, 2392.
5. R. Behrend and P. Enert, *Annalen der Chemie und Pharmacie,* 1890, 66.
6. L.K.J. Aw, "Some Chemistry of 5-Diazouracil and Its Derivatives", Ph. D. Thesis, City University, London, 1989, 229 .
7. T.C. Thurber and L.B. Townsend, *J. Org. Chem.,* 1967, 41(6), 1041.

Chapter 10. Experimental

Melting points were determined on a Gallenhamp Melting Point Apparatus and are uncorrected. I.R. measurements were performed on a Perkin-Elmer IR instrument (FT-IR 1720X spectrometer) with nujol mulls or thin films or KBr discs of the compounds and recorded as wavenumbers (cm^{-1}). ^{1}H NMR spectra were obtained using a Jeol NMR spectrometer (100 MHz) and a Bruker NMR spectrometer (250 MHz). ^{13}C NMR spectra were obtained on a Bruker NMR spectrometer (250 MHz) as well as a Bruker NMR spectrometer (400 MHz). FAB-MS spectra were partly obtained from the SERC Mass Spectrometery Service Centre, University College of Swansea. Negative FAB, EI, some positive FAB and high resolution mass spectra were obtained on a Kratos CONCEPT II HH four-sector mass spectrometer. Negative Liquid Secondary Ion Mass Spectrometry (LSIMS) was selected for high resolution mass determinations (resolution was more than 5000). All of UV data were determined on a UNICAM 8700 Series UV/VIS spectrometer. Silica gel H was used for column chromatography and TLC was performed on Kieselgel 60 F_{254}.

10.1 5-Bromouridine (4.2)

Uridine (2.44 g, 0.01 mole) was dissolved in DMF (20ml). N-bromosuccinimide (2 g, 0.011 mole) was added to the clear light yellow solution which was allowed to stand at room temperature for 16 h. The solution was evaporated in vacuo to remove DMF. The residue was recrystallised from methanol. 5-Bromouridine (1.97g, 61%) was obtained, m.p. 190-191 °C, (Lit:[1] 181 °C); ^{1}H NMR data are shown in Table 4.2 (page 72) and EI-MS data are shown in Table 4.3 (page 73).

10.2 5-Aminouridine hydrochloride (4.3·HCl)

To a thick walled glass tube (or a stainless steel tube) with vollume about 55 ml , 5-bromouridine (2 g, 0.0062 mole) and distilled water (25 ml) were added. Ammonia gas was passed into the solution at about -20 °C, until the volume of the

solution extended to over 50 ml (the concentration of the NH_4OH should be over 74.25% and the specific gravity should be below 0.88). The tube was sealed and stood in a water bath for 96 h at about 53 °C. Then, the tube was cooled to about -15 °C and opened carefully. The excess of ammonia and water were removed in vacuo (50°C/5mmHg). The solid residue was dissolved in minimum of isopropanol. Concentrated hydrochloric acid (36%) was added to the solution to pH 1. 5-Aminouridine hydrochloride (1.8 g, 98%) was obtained, m.p. 218 °C, (Lit:[2] 220 °C); ^1H NMR δ (acetone-d_6) 11.8 (bs, H3), 8.7-8.4 (bs,-NH_2), 8.32 (s, H6), 5.83 (d, H1'), 5.6-4.9 (bs, 3x-OH), 4.2-3.75 (bm, H2', H3', H4'), 3.7-3.5 (bm, 2xH5'); FAB-MS m/z: 257 (M^+; 2.05%), 243 (20%), 180 (23%), 133 (41%).

10.3 5-Diazo-5',6(s)-cyclo-uridine (4.1)

5-Aminouridine hydrochloride (1.5g, 0.0051 mole) was dissolved in 1M hydrochloric acid (9ml) and the solution was cooled to 0°C. A solution of sodium nitrite (4 ml, 6.9%, 0.0052 mole) was added dropwise at 0°C and pale yellow crystals formed in less than five minutes. Two volumes of 95% ethanol were added at -5°C and the solution was cooled to -10°C. Rapid filtration and recrystallization from methanol gave a light yellow crystalline product (0.45g, 40%), m.p. 177-178 °C, (Lit:[3] 178-182 °C); I.R. υ (KBr, cm^{-1}): 3424 (-OH, >NH), 2128 (-N_2), 1740-1580 (>C=O); ^1H NMR data are shown in Table 4.4 (page 77); FAB-MS m/e: 271 (100%), 270 (M^+; 1.7%), 293 (15.4%), 243 (21%), 228 (13%), 136 (16%).

10.4 2',3'-Isopropylidene-5-bromouridine (4.7)

Uridine (2.44g, 0.01 mole) and N-bromosuccinimide (2g, 0.011 mole) were dissolved in DMF (20 ml). The solution was stirred for 16 h at room temperature. The DMF was removed by evaporation in vacuo (50°C/5mmHg). The residue was dissolved in acetone (10 ml) and the mixture was refluxed for 30 min and then cooled to 0 °C. A white crystalline solid was obtained which was recrystallized from acetone, giving white needles (2.92g, 80 %), m.p. 227-228 °C, (Lit:[4] 207-8 °C); ^1H NMR and ^{13}C NMR data are shown in Tables 4.5 and 4.6 (page 78-9); FAB-MS

m/z: 365 (M^+; 25%), 363 (M^+; 24.7%), 285 (40%), 193 (37%), 191 (37.2%), 173 (54%).

10.5 5-Bromo-2'-deoxyuridine (4.8)

2'-Deoxyuridine (2.28 g, 0.01 mole) and N-bromosuccinimide (NBS) (2g, 0.011 mole) were added to dried DMF (20ml). The solution was stirred at room temperature for 16 hours. After about 2 hours, the yellow colour, which was caused by NBS, started to diminish and when the reaction over, the solution was nearly colourless. The solution was evaporated to dryness at about 70 °C, in vacuo. TLC detection (ethyl acetate) of the product showed that a new spot had formed. The crude product was recrystallized from acetone. White crystals were obtained (1.98g, 64%), m.p. 179-182 °C, (Lit:[5] 181-3 °C); I.R. υ (KBr. cm^{-1}): 3400 (-OH< >NH), 1730, 1670 (>C=O); data of ^1H NMR and FAB-MS are shown in Tables 4.7 (page 80) and 4.8 (page 81).

10.6 5-Amino-2'-deoxyuridine hydrochloride (4.9)

Ammonia gas was passed into acetonitrile (20 ml) in a stainless steel tube with volume of about 30 ml at -20 °C. When the volume of the solution extended to 25 ml, 5-bromo-2'-deoxyuridine (1g, 0.0033 mole) was added. After the tube was sealed, it was stood in a water bath at 50 °C for 120 hours, then the pressure was released carefully. After evaporation of ammonia and acetonitrile in vacuo, a solid product was obtained which was acidified to pH 1 with hydrochloric acid (36%). 2-Propanol (3 volumes) was added to the acidic solution. A white solid was obtained (0.89g, 98%), m.p.184-186 °C, (Lit:[6] 186-7 °C); I.R. υ (KBr, cm^{-1}): 3350 (-OH, >NH, $-NH_2$), 1730, 1660 (>C=O); ^1H NMR data are shown in Table 4.9 (page 83); FAB-MS m/z: 244 ($[M+1]^+$; 100%), 127 (17%), 117 (30%).

10.7 5-Diazo-5',6-cyclo-2'-deoxyuridine (2.13)

5-Amino-2'-deoxyuridine hydrochloride (2.3 g, 0.0089 mole) was dissolved in an aqueous solution of acetic acid (18 ml, 50 %). The solution was cooled to about 0-2 °C. and at this temperature, aqueous sodium nitrite solution (8.2 ml, 6.9%,

0.009 mole) was added dropwise. A yellow precipitate was produced in about 10 minutes. After the addition, the mixture was stirred for half an hour and the product was filtered and washed with cold water to pH 7, and dried over silica under high vacuum at -15 °C, to give a yellow solid (1.43 g, 68%), m.p. 167-169 °C: (Lit:[7] 163-5 °C); I.R.υ (KBr, cm^{-1}): 3450-3330 (-OH, >NH), 2130 ($-N_2$), 1680, 1640 (.C=O); ^1H NMR δ (acetone-d_6): 10.55 (bs, H3), 6.15 (s, H6), 5.9 (dd, H1'), 5.1 (dd, H3'), 4.2(m, H4', -OH), 3.8 (m, 2xH5'), 2.38 (m, endo-H2'), 2.05 (m, exo-H2'), ^{13}C NMR δ: 161 (C4), 148 (C2), 90.5 (C5), 89.3 (C1'), 80 (C2'), 72 (C3'), 71 (C4'), 60 (C6), 46.5 (C2').

10.8 5-Methoxyuridine (5.1)

Freshly prepared 5-diazo-5',6-cyclo-uridine (0.5g, 0.00185 mole) was dissolved in boiling CH_3OH (40 ml), then a little rhodium acetate was added and the solution was refluxed for 4 hours. The solution was evaporated to dryness to afford a green- yellow material. Cold acetone was used to wash the material several times in small portions to remove the green-yellow colour of rhodium acetate. Recrystallization from CH_3OH gave a white solid (0.3g, 60.9%), m.p.203-206°C; UV λ_{max}(MeOH): 201.3 (ε 7090), 294.2 nm (ε 10500); I.R.υ (KBr, cm^{-1}): 3500-3250 (-OH, >NH), 1711, 1663 (>C=O); data of ^1H NMR and EI-MS are shown in Tables 5.1 and 5.2 (page 89); high resolution FAB-MS m/e (M^+): 274.1145 ($C_{10}H_{14}N_2O_7$ requires 274.0821).

10.9 5-Ethoxyuridine (5.2)

Freshly prepared 5-diazo-5,6-cyclo-uridine (0.5g, 0.00185 mole) was dissolved in boiling ethanol(40 ml). A trace amount of rhodium acetate was added and the solution was refluxed for 4 hours and then evaporated to dryness in vacuo to afford a yellow residue. The residue was separated on a silica gel column (mobile phase THF/AcOEt 1:1). A white solid was obtained (0.28 g, 55.2 %), m.p. 176-178 °C), UV λ_{max}(MeOH): 203.2 nm (ε 6760), 293.9 nm (ε 10000); I.R. (KBr, cm^{-1}): 3450-3200 (-OH, >NH), 1703, 1655 (>C=O); data of ^1H NMR and FAB-MS are

shown in Tables 5.3 and 5.4 (page 90-1); high resolution FAB-MS m/e (M^+): 288.1783 ($C_{11}H_{16}N_2O_7$ requires 288.0980).

10.10 5-Butoxyuridine (5.3)

Freshly prepared 5-diazo-5',6-cyclo-uridine (1 g, 0.0037 mole) was dissolved in n-butanol (80 ml) at 65 °C. A trace amount of rhodium acetate catalyst was added and the solution was kept at 65 °C for 4 hours. The excess butanol was removed under reduced pressure to afford a green-yellow solid, which was separated on a silica gel column (mobile phase THF/AcOEt 1:1). A white solid was obtained after removal of the solvent (0.63g, 54%), m.p. 33 °C; UV λ_{max}(MeOH): 203.2 nm (ε 6760), 281.6 nm (ε 5200); I.R. υ (nujol, cm^{-1}): 3430-3280 (-OH,>NH), 1743-1680 (>C=O); data of ^1H NMR and FAB-MS are shown in Tables 5.5 and 5.6 (page 92); high resolution FAB-MS m/e (M^+): 316.1277 ($C_{13}H_{20}N_2O_7$ requires 316.1299).

10.11 5-Benzyloxyuridine (5.4)

To a mixture fo benzyl alcohol (10 ml) and chloroform (20 ml), freshly prepared 5-diazo-5',6-cyclo-uridine (1g, 0.0037 mole) was added. The solution was heated to reflux and a trace of $[Rh(AcO)_2]_2$ was added. The solution was refluxed for four hours and the diazo compound dissolved gradually. The chloroform was evaporated under reduced pressure and the mixture of product and excess benzyl alcohol was separated on a silica gel column (mobile phase $CHCl_3/CH_3OH$ 4:1). A light-yellow solid was obtained after removal of the solvent (0.6 g, 46.3 %), m.p. 146-149 °C; UV λ_{max}(MeOH): 205.9 nm (ε 13800), 285.3 nm (ε 6010); I.R. υ (KBr, cm^{-1}): 3540-3200 (-OH, >NH), 1750-1600 (>C=O); data of ^1H NMR and FAB-MS are shown in Tables 5.7 and 5.8 (page 93); high resolution mass spectrun m/e (M^+): 350.1141 ($C_{16}H_{18}N_2O_7$ requires 350.1140).

10.12 5-Phenoxy-2'-deoxyuridine (5.6)

5-Diazo-5',6-cyclo-2'-deoxyuridine (1.5 g, 0.0059 mole) and a trace of rhodium acetate were added to liquid phenol (20 ml) at 60 °C. The mixture was stirred for 4 h. at this temperature. Then, the solution was loaded on a silica column and the excess phenonl was eluted with dichloromethane. The crude product was quickly eluted with methanol and reloaded on a silica column and re-eluted, first with diethyl ether, until the green band of rhodium acetate moved to about the middle position of the column, then with ethyl acetate. A white product was obtained (0.5g, 26.5%) m.p. 110-112°C; UV λ_{max}(MeOH): 210.8 nm (ε 15900), 269.8 nm (ε 9520); I.R. υ (nujol, cm^{-1}), 3420-3200 (-OH, >NH), 1700, 1650 (>C=O), 1598 (C=C); data of ^{1}H NMR and ^{13}C NMR are shown in Tables 5.9 and 5.10 (page 97-8), EI-MS (negative) m/z: 320 (M^+; 35%), 244 (12%), 226 (43%); high resolution FAB-MS m/e (M^+): 320.0529 ($C_{15}H_{16}N_2O_6$ requires 320.1031).

10.13 5-Benzyloxy-2'-deoxyuridine (5.7)

To benzyl alcohol (20 ml) were added 5-diazo-5',6(s)-cyclo-2'-deoxyuridine (1.5g, 0.59 mole) and a trace of rhodium acetate. The solution was stirred at 60 °C for 4 h, the reaction being monitored by TLC (silica, ethyl acetate) until , after about 3.5 h, the spot of diazo compound disappeared.The excess benzyl alcohol was removed by evaporation in vacuo. The residue was purified using preparative TLC (silica, ethyl acetate). A white product was obtained (0.36 g, 27.7 %), m.p. 112-114 °C; UV λ_{max}(MeOH): 207.2 nm (ε 15400), 274.2 nm (ε 7780); I.R. υ (nujol, cm^{-1}): 3381 (-OH,>NH), 1685, 1643 (>C=O); data of ^{1}H NMR and ^{13}C NMR are shown in Tables 5.11 and 5.12 (page 99); FAB-MS m/z: 335 ($[M+1]^+$; 10%), 242 (4%), 107 (60%), 91 (55%), 77 (100%); high resolution FAB-MS m/e (M^+): 334.0658 ($C_{16}H_{18}N_2O_6$ required 334.1191).

10.14 5-(2''-Phenethoxy)-2'-deoxyuridine (5.8)

To 2-phenylethanol (20 ml) were added 5-diazo-5',6(s)-deoxyuridine (1.5 g, 0.005 mole) and a trace of rhodium acetate. The solution was stirred at 60 °C for 4 h. The peak at 2130 cm^{-1} disappeared within 3.5 h. After cooling, the solution was loaded on a silica column, excess 2-phenylethoxyl eluted first with dichoromethane and the residue eluted with methanol. The crude product was carefully reloaded on a silica column and re-eluted, first with diethyl ether until the green band of rhodium acetate moved to about the middle part of the column, then with ethyl acetate. A white solid was the target compound (5.8) (0.5g, 24.3%), m.p. 129-133 °C; UV λ_{max}(MeOH): 208.0 nm (ε 18000), 279.5 nm (ε 7970); I.R.υ (nujol, cm^{-1}): 3500-3100 (-OH, >NH), 1684, 1641 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 5.13 and 5.14 (page 100-1), FAB-MS m/z: 371 (25%), 349 ($[M+1]^+$; 52%), 259 (10%), 233 (72%), 226 (20%), high resolution CI-MS m/e (M^+): 348.2105 ($C_{17}H_{20}N_2O_6$ requires 348.1350).

10.15 5-(3''-Phenyl-1''-propoxy)-2'-deoxyuridine (5.9)

5-Diazo-5',6-cyclo(s)-2'-deoxyuridine (1.5g, 0.0059 mole) and a trace of rhodium acetate were added to 3-phenyl-1-propanol (20 ml). The solution was stirred at 60 °C for 4 h, the reaction being checked by I.R. every half hour. After about 3 hours, the diazo absorption disappeared. The solvent was separated from the mixture using a silica column with dichloromethane. Then, the crude product was eluted with methanol and re-loaded on a silica column. The product was purified by stepwise elution with diethyl ether and ethyl acetate. Eluent fractions were examined by ^{1}H NMR and TLC. A white product was the required product (5.9) (0.46 g, 21.5 %), m.p. 148-150 °C; UV λ_{max}(MeOH): 211.4 nm (ε 14700), 297.5nm (ε 9350); I.R.υ (nujol, cm^{-1}): 3280 (-OH,>NH), 1720, 1658 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 5.15 and 5.16 (page 102); FAB-MS m/z: 385 (33%), 363 ($[M+1]^+$; 10%), 247 (42%), 227 (10%), high resolution CI-MS m/z (M^+): 362.1934 ($C_{18}H_{22}N_2O_6$ requires 362.1509)..

10.16 5-Cinnamyloxy-2'-deoxyuridine (5.10)

5-Diazo-5'6-cyclo-2'-deoxyuridine (1.5g, 0.0059 mole) and a trace of rhodium acetate were added to liquid cinnamyl alcohol (20 g, 60°C). The mixture was stirred at 60 °C for 4 h and followed by I.R. Within 3.5 h, the diazo absorption disappeared. The reaction mixture was loaded on a silica column and the excess of cinnamyl alcohol eluted with dichloromethane. The crude product was eluted with methanol and carefully reloaded on a silica column. When the green band had moved to the middle of the column by elution with diethyl ether, the eluant was changed to ethyl acetate. The product after the green band was the required product (5.10) (0.6 g, 28.2%); m.p. 97-99 °C; UV λ_{max}(MeOH): 204.9 nm (ε 24900), 254.2 nm (ε 14300); I.R.υ (nujol, cm^{-1}): 3500-3200 (-OH, >NH), 1680, 1642 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 5.17 and 5.18 (page 104); FAB-MS m/z: 383 (5%), 361 ($[M+1]^+$; 2%), 245 (12%), 117 (10%); high resolution FAB-MS m/z (M^+); 360.1651 ($C_{18}H_{20}N_2O_6$ requires 360.1350).

10.17 5-Cyclohexylmethoxy-2'-deoxyuridine (5.13)

5-Diazo-5',6-cyclo-2'-deoxyuridine (1.5g, 0.0059 mole) and a trace of rhodium acetate were dissolved in cyclohexylmethanol (20 ml). The solution was stirred for 4h. at 60 °C. The excess of cyclohexylmethanol was eluted with dichloromethane on a silica column and the product was eluted with ethyl acetate. A white solid after the green band was identified as the required product (5.13) (0.6g, 30%), m.p. 119-121°C; UV λ_{max}(MeOH): 208.8 nm (ε 9230), 288.0 nm (ε 7550); I.R.υ (nujol, cm^{-1}): 3400 (-OH, >NH), 1680, 1640 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 5.19 and 5.20 (page 106); FAB-MS m/z: 363 (40%), 341 ($[M+1]^+$; 20%), 225 (100%); high resolution FAB-MS m/z (M^+): 340.2019 ($C_{16}H_{22}N_2O_5$ requires 340.1669).

10.18 5-Phenoxyuridine (5.14)

5-Diazo-5',6-cyclo-uridine (1.5g, 0.0056 mole) and a trace of rhodium acetate were added to phenol (20 ml) which had been pre-heated to 60 °C. The mixture was stirred at 60 °C for 4 h. The reaction was over within about 3.5 h based on TLC detection (methanol:ether 1:7). The mixture was evavporated to remove phenol in vacuo. The residue was recrystallised from a minimum amount of ethyl acetate. An off-white solid (0.5 g, 26.8 %)) was obtained, m.p. 187-189 °C; UV λ_{max}(MeOH): 203.2 nm (ε 14500), 268.6 nm (ε 8900); I.R. υ (nujol, cm^{-1}), 3500-3100 (-OH, >NH), 1730-1630 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 5.21 and 5.22 (page 107); EI-MS data are shown in Table 5.23 (page 108); high resolution FAB-MS m/z (M^+): 336.0960 ($C_{15}H_{16}N_2O_7$ requires 336.0980),

10.19 5-(2''-Phenethoxy)-uridine (5.15)

5-Diazo-5',6(s)-cyclo-uridine (1.5g, 0.0056 mole) and a trace of rhodium acetate were added to 2-phenylethanol (20 ml). The mixture was heated to 60 °C and stirred for 4 h. After about 3.5 h, the diazo absorption at 2130 cm^{-1} had disappeared. The unreacted 2-phenylethanol was eluted with dichloromethane on a silica column. and the crude product was eluted with methanol and reloaded on a silica column. The product was eluted with diethyl ether, followed by ethyl acetate. A white solid (0.53g, 26.2%) was the target compound (5.15), m.p. 118-120 °C, UV λ_{max}(MeOH): 207.6 nm (ε 16700), 280.0 nm (ε 7810); I.R.υ (nujol, cm^{-1}), 3410 (-OH,>NH), 1698, 1650 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 5.24 and 5.25 (page 109); FAB-MS data are shown in Table 5.26 (page 109); high resolution FAB-MS m/z (M^+): 364.1549 ($C_{17}H_{20}N_2O_7$ requires 364.1299).

10.20 5-(3''-Phenyl-1''-propoxy)-uridine (5.16)

5-Diazo-5',6-cyclo-uridine (1.5g, 0.0056 mole) and a trace of rhodium acetate were added to 3-phenyl-1-propanol (20 ml). The mixture was stirred at 60 °C for 4h. Then, after 150 ml of hexane was added to the mixture, dichloromethane was added to the solution dropwise with stirring until the mixture became homogeneous. The

mixture was placed in a freezer (-20 °C). An off-white solid was obtained by filtration and was washed with ether and then dried in vacuo. The product (5.16) (0.2g, 9.5 %) was obtained, m.p. 156-157°C, UV λ_{max}(MeOH): 208.1 nm (ε 17300), 280.5 nm (ε 7570); I.R.υ (nujol, cm^{-1}), 3500-3200 (-OH, >NH), 1660, 1630 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 5.27 and 5.28 (page 110-1); FAB-MS m/z: 379 ($[M+1]^+$; 20%), 247 (40%), 133(8%), 119 (18%), 77 (12%); high resolution FAB-MS m/z (M^+): 378.1342 ($C_{18}H_{22}N_2O_7$ requires 378.1459).

10.21 5-(3''-Isopropyl-1''-triazeno)-uridine (6.4)

5-Diazo-5',6-cyclo-uridine (0.98g, 0.0036mole) was mixed with freshly distilled isopropylamine (20ml) and the orange solution was stirred for 16 h at room temperature, whereupon a colour change to bright yellow occurred. The isopropylamine was removed in vacuo, giving a yellow residue which was purified using a silica column with $CHCl_3$-CH_3OH (4:1). An off-white product (0.4g, 36.5%) was obtained, m.p. 172-174°C; UV λ_{max}(MeOH): 230.8 nm (ε 11100); I.R. υ (nujol, cm^{-1}), 3300 (-OH, >NH), 1693 (>C=O), ^{1}H NMR and ^{13}C NMR data are shown in Tables 6.1 and 6.2 (page 114-5); FAB-MS m/z: 352 (18%), 330 ($[M+1]^+$; 27%), 272 (6%), 226 (20%), 198 (27%), 197 (23%); high resolution FAB-MS m/z (M^+): 329.1002 ($C_{12}H_{19}N_5O_6$ requires 329.1362).

10.22 5-(3''-t-Butyl-1''-triazeno)-uridine (6.5)

5-Diazo-5',6(s)-cyclo-uridine (0.44g, 0.00163 mole) was stirred in neat t-butylamine (20 ml) at room temperature for 16 h, during which time the colour of the mixture changed from red to yellow. The mixture was evaporated to dryness in vacuo at room temperature. The residue was passed through a silica column for purification ($CHCl_3$:CH_3OH = 4:1). An off-white solid (0.3g, 53.6%) was obtained, m.p.163-164 °C; UV λ_{max}(MeOH): 231.5 nm (ε 14500); I.R.υ (nujol, cm^{-1}), 3200 (-OH, >NH), 1690 (>C=O), ^{1}H NMR data are shown in Table 6.3 (page 116), FAB-MS m/z: 366 (100%), 344 ($[M+1]^+$; 35%), 212 (50%); high

resolution FAB-MS m/z (M^+): 343.1496 ($C_{13}H_{21}N_5O_6$ requires 343.1522).

10.23 5-(3''-Benzyl-1''-triazeno)-uridine (6.6)

5-Diazo-5',6(s)-cyclo-uridine (1g, 0.0037 mole) was added to 20 ml of a solution of chloroform and freshly distilled benzylamine (40 : 1). The mixture was stirred for 16 h at room temperature and evaporated to dryness in vacuo. The red residue was passed through a silica column for purification ($CHCl_3$: MeOH = 5:1). A white solid (0.6 g, 43 %) was obtained, m.p. 204-205°C; UV λ_{max}(MeOH): 203.2 nm (ε 8910), 230.2 nm (ε 8610); I.R. υ (nujol,cm^{-1}), 3460, 3300 (-OH, >NH), 1688 (>C=O); ^1H NMR and ^{13}C NMR data are shown in Tables 6.4 and 6.5 (page 117); FAB-MS m/z: 378 ($[M+1]^+$; 55%), 272 (30%), 242 (30%); high resolution FAB-MS m/z (M^+): 377.1355 ($C_{16}H_{19}N_5O_6$ requires 377.1362).

10.24 5-(3''-Benzyl-1''-triazeno)-2'-deoxyuridine (6.7)

5-Diazo5',6-cyclo-2'-deoxyuridine (1.5g, 0.0059.mole) and benzylamine (1g, 0.0094 mole) were added to acetonitrile (80 ml). The solution was stirred overnight at about 50 °C. I.R. revealed that no reaction had occurred, so the reaction temperature was increased to 60 °C. After 5 hours, the absorption at 2120 cm^{-1} was still strong, so the temperature was increased to 80 °C and extra amine (1g, 0.0094 mole) was added. The intensity of the absorption at 2120 cm^{-1} then decreased and after 4.5 h the peak had disappeared. The solution was kept at -20°C overnight. The crude solid product was obtained by filtration, I.R. υ (nujol, cm^{-1}), 3300 (-OH, >NH), 1680 (>C=O), ^1H NMR data are shown in Table 6.6 (page 119); FAB-MS (negative) m/z: 360 ($[M-1]^+$; 100%), 271 (57%), 254 (61%), 245 (87%), 117 (36%).

10.25 5-[3''-(2'''-Phenyl-1'''-ethyl)-1''-triazeno]-2'deoxyuridine (6.8)

5-Diazo-5',6-cyclo-2'-deoxyuridine (1.5g,0.0059 mole) and 2-phenylethylamine (0.67g, 0.0118 mole) were added to acetonitrile. The solution was stirred at 45 °C for 7 h. The intensity of the diazo bond at about 2130 cm^{-1} was decreased However,

there was no further change after stirring overnight (about 13 h) at room temperature. Therefore, one extra equivalent of amine (0.74g) was added and the temperature was increased to 45 °C for a further overnight period. The peak at 2130 cm^{-1} disappeared. The solution was stored cold and an off-white solid (0.5g, 25%) was obtained, m.p. 186-190 °C; UV λ_{max}(MeOH): 211.7 nm (ε 16400), 230.6 nm (ε 16000); I.R.υ (nujol, cm^{-1}), 3315 (-OH, >NH), 1723, 1690 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 6.7 and 6.8 (page 121); FAB-MS (negative) m/z: 374 ($[M-1]^+$; 38%), 254 (46%), 117 (81%); high resolution FAB-MS m/z (M^+): 375.1516 ($C_{17}H_{21}N_5O_5$ requires 375.0932).

10.26 5-[3''-(3'''-Phenyl-1'''-propyl)-1''-triazeno]-2'-deoxyuridine (6.9)

5-Diazo-5',6-cyclo-2'-deoxyuridine (1.5g, 0.0059 mole) and 3-phenyl-1-propylamine (0.62g, 0.0094 mole) (1:1.6) were added to acetonitrile. The solution was stirred at 42 °C for 3h. The diazo absorption at 2130 cm^{-1} decreased according to the I.R. detection. After standing overnight at room temperature, the solution was stirred at 60 °C for about 5 h, until the reaction finished. The solution was cooled to about -15 °C and left overnight. A light-yellow solid (0.6 g, 28.6%) was obtained, m.p. 168-170 °C; UV λ_{max}(MeOH): 209.8 nm (ε 18500), 230.5 nm (ε 16900); I.R. υ (nujol, cm^{-1}), 3300 (-OH, >NH0, 1680 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 6.9 and 6.10 (page 122-3); FAB-MS (negative) m/z: 388 ($[M-1]^+$; 21%), 254 (33%), 117 (46%); high resolution FAB-MS m/z (M^+): 389.1598 ($C_{18}H_{23}N_5O_5$ requires 389.1732).

10.27 5'-O-Trityl-5-bromo-uridine (7.2)

5-Bromouridine (3.07g, 0.01 mole) and trityl chloride 2.73g, 0.011mole) were added to freshly distilled pyridine. The mixture was heated to 110 °C and stirred for 2 h. then poured into ice-water giving a light yellow precipitate. After decanting the solution, the precipitate was dissolved in chloroform and washed with water, then dried with Na_2SO_4 overnight. The chloroform was removed in vacuo to give a white

solid. Yield 4.35g (81%); m.p. 154-156 °C; I.R. υ (KBr, cm^{-1}), 3200 (-OH, >NH), 1709, 1670 (>C=O), 1613 (C=C), ^1H NMR δ (acetone-d_6): 11 (bs, H3), 8.03 (s, H6), 7.4 (m, 15xH_{arom}), 5.75 (d, H1'), 5.5 (d, -OH), 5.15 (d, -OH), 4.4-4.2 (m, H2', H3'), 3.5 (m, H4'), 3.3 (m, 2xH5'); FAB-MS m/z: 589 (3%), 587 (3.5%), 567 ($[M+1]^+$; 2%), 565 (2%), 485 (1%), 484 (1%), 307 (3%), 243 (100%). The molecular ion was not stable enough to produce a sufficiently strong signal for accurate mass mesurement. The elemental analytical data did not match the requirement because the trityl group is partly lost during purification.

10.28 5'-O-Trityl-5-aminouridine (7.3)

Isopropanol (50ml) in a sealing tube was cooled to about -20°C, then ammonia gas passed in until the solution was saturated. 5'-O-Trityl-5-bromouridine (1.5g, 0.0026 mole) was added to the solution and the tube was sealed and heated in a water bath for 120 h at 55 °C. The tube was opened and the solution was evaporated at about 0°C to drive out the excess ammonia and then warmed to remove the solvent. A crude solid was obtained, which was recrystallized from isopropanol. An off-white solid was obtained (0.96g, 67%); m.p. 125-127°C; I.R. υ (KBr, cm^{-1}), 3353 (-OH), 3202 (>NH), 1692, 1642 (>C=O), 1596 (C=C) ^1H NMR δ (acetone-d_6): 10.8 (bs, H3), 7.2 (m, 15xH_{arom}), 6.7 (s, H6), 5.72 (d, H1'), 5.25 (bs, -OH), 5.0 (bs,-OH), 4.0-3.6 (m, H2', H3', H4'), 3.25 (bs,-NH_2), 3.19 (m, 2xH5'); EI-MS m/z: 260 ($[M-Ph_3C]^+$; 100%), 243 (74%). The molecular ion was not stable enough to produce a sufficiently strong signal for accurate mass mesurement. The elemental analytical data did not match the requirement because the trityl group is partly lost during purification.

10.29 5'-O-Trityl-5-diazo-6-methoxy-uridine (7.4)

A mixture of 5'-O-trityl-5-amino-uridine(1g, 0.0019 mole) methanol(20 ml), isoamyl nitrite (0.22g, 0.0021 mole) and acetic acid (0.18g, 0.003 mole) was stirred at room temperature with TLC detection every 15 min (ethyl acetate). The spot of the amino starting material diminished progressively and, in addition, a new spot of

lower polarity appeared. When the spot of the starting material disappeared, the temperature was decreased to about -10 °C and H_2O was added to the solution dropwise. An off-white precipitate was obtained by quick filtration (0.7g, 69%); m.p. 96-98°C; I.R. υ (KBr, cm^{-1}), 3400 (-OH, >NH), 3075 (=C-H), 2956 ($-CH_3$), 2870 ($-CH_2-$), 2118 =N=N), 1680 (>C=O), 1597 (C=C); ^1H NMR δ (DMSO d_6): 10.8 (bs, H3), 7.5-7.1 (m, 15x H_{Aro}), 6.25 (s, H6), 5.65 (d, H1'), 5.3 (bs, -OH), 5.05 (bs, -OH), 4.1 (m, H2', H3'), 3.9 (m, H4'), 3.2 (m, 2xH5'), 3.15 (s, $-OCH_3$); FAB-MS m/z: 567 (10%), 545 ($[M+1]^+$; 3%), 513 (13%), 501 (10%), 485 (5%), 467 (3%), 285 (2%), 259 (3%), 243 (100%), 115 (1.5%). The molecular ion was not stable enough to produce a sufficiently strong signal for accurate mass mesurement. The elemental analytical data did not match the requirement because the trityl group is partly lost during purification.

10.30 5'-O-Trityl-5-diazo-6-isopropoxy-uridine (crude) (7.5)

5'-O-Trityl-5-amino-uridine (0.1g, 0.0019 mole), isoamyl nitrite (0.22g, 0.0021 mole) and acetic acid (0.18g, 0.003 mole) were added to isopropanol (20 ml). The solution was stirred at room temperature until the starting material disappeared, then cooled to -10 °C and water (60 ml) was added to the solution dropwise to precipitate the product. A crude product was obtained by quick filtration. I.R. υ (KBr, cm^{-1}), 3442 (-OH, >NH), 2115 (=N=N), 1681 (>C=O), 1598 (C=C); FAB-MS m/z: 595 ($[M+Na]^+$; 3%), 578 (0.8%), 513 (11%), 553 (4.5%), 485 (7%), 243 (100%), 259 (74%);

10.31 5-Cyclohexylmethoxy-5'-O,6-cyclo-5,6-dihydro-2'-deoxyuridine (5s, 6s) (8.1)

5-Diazo-5',6-cyclo-2'-deoxyuridine (1.5g, 0.0059 mole) and a trace of rhodium acetate were dissolved in cyclohexylmethanol (20 ml) and the solution was stirred for 4h. at 60 °C. The excess of cyclohexylmethanol was eluted with dichloromethane on a silica column, then the product was separated with ethyl acetate. A white solid (0.54g, 27%) was identified as the required compound (8.1), m.p. 175-176°C; UV

λ_{max}(MeOH): 201.4 nm (ε 6770); I.R. υ (nujol, cm^{-1}), 3396 (-OH, >NH), 1726, 1691 (>C=O), ^{1}H NMR and ^{13}C NMR data are shown in Tables 8.2 and 8.1 (page 135-6); (Found: 56.08; 7.08; 8.17; $C_{16}H_{24}N_2O_6$ requires 56.47; 7.06; 8.24%); FAB-MS m/z: 363 (100%), 341 ($[M+1]^+$; 63%).

10.32 1-(β-D-ribofuranosyl)-1,2,3-triazole-4-carboxamide (9.4)

A solution of 5-diazo-5'-cyclo-uridine (1.0g, 0.0034 mol) in acetone (20ml) was refluxed for 8 h, then evaporated to dryness. A yellow product was obtained (0.50g, 61%), m.p. 195-198 °C (Lit:[8] 198-201 °C); ^{1}H NMR and ^{13}C NMR data are shown in Tables 9.1 and 9.2 (page 148).

10.33 1-(2'-Deoxy-β-D-ribofuranosyl)-5'-oxymethylene, 5-cyclo-imidazolidine-2,4-dione (8.4)

A mixture of 5-diazo-5',6'-cyclo-deoxyuridine (0.5g, 0.002 mole), a trace of rhodium acetate and excess acetone was refluxed for 20 h and then evaporated to dryness. The product (0.3g, 0.79%) was revealed to be compound (8.4), m.p. 235-240°C; UV λ_{max}(MeOH): 201.0 nm (ε 6410), 218.6 nm (ε 6520), 277.1 nm (ε 8480) I.R.υ (nujol, cm^{-1}), 3429 (-OH, >NH), 1718, 1666 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 8.4 and 8.5 (page 139); high resolution FAB-MS m/z (M^+): 226.0563 ($C_9H_{10}N_2O_5$ requires 226.0604). X-ray crystallography data are shown in Fig. 8.1, Tables 8.6 and 8.7 (140).

10.34 1-(β-D-ribofuranosyl)-5'-oxymethylene,5-cyclo-imidazolidine-2,4-dione (8.5)

A mixture of 5-diazo-5',6-cyclo-uridine (0.5g, 0.0017 mole), a trace of rhodium acetate and excess acetone was refluxed for 20 h and then evaporated to dryness. The residue (0.29g, 69%) was compound (28), m.p. 230-235°C; UV λ_{max}(MeOH): 211.5 nm (ε 8820), 275.3 nm (ε 3840); I.R.υ (nujol, cm^{-1}), 3392 (-OH, >NH), 1776, 1645 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in Tables 8.8 and 8.9 (page 141-142); high resolution FAB-MS m/z (M^+): 242.0927 ($C_9H_{10}N_2O_6$ requires 242.0533).

References

1. P.A. Leven and F.B. La Forge, *Chem. Ber.*, 1912, 45, 608
2. I. Wempen, I. L. Doerr, L. Kaplan and J.J. Fox, *J. Am. Chem. Soc.*, 1960, 82, 1624
3. J.P. Paolini, R.K. Robins and C.C. Cheng, *Biochem. Biophys. Acta.*, 1963, 72, 114
4. N.K. Kochetkov, E.I. Budovskii, V.N. Shibaev, G.I. Yeliseeva, M.A. Grachev and V.P. Demushkin, *Tetrahedron*, 1963, 19(8), 1207
5. T.J. Bardos, G.M. Levin, R.R. Here and H.L. Gordon, *J. Am. Chem. Soc.*, 1955, 77, 4279
6. T.Y. Shen, J.F. McPherson and B.O. Linn, *J. Med. Chem.*, 1966, 9, 366
7. F.G. Fisher and E. Fahr, *Ann. Chem.*, 1962, 651, 64
8. T.C. Thurber and L.B. Townsend, *J. Org. Chem.*, 1976, 41, 1041