STRUCTURE-ANTI HERPES ACTIVITY STUDIES OF PYRIMIDINE NUCLEOSIDE ANALOGS

A Thesis

Submitted to the College of Graduate Studies and Research

in Partial Fulfillment of the Requirements for

the Degree of Doctor of Philosophy

in the Department of Chemistry

University of Saskatchewan

Saskatoon

by

Wajdi Michel Zoghaib

Fall 1996

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Canada

UNIVERSITY OF SASKATCHEWAN

College of Graduate Studies and Research

SUMMARY OF DISSERTATION

Submitted in partial fulfillment

of the requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

Wajdi Michel Zoghaib

Department of Chemistry
University of Saskatchewan

Spring 1996

Examining Committee:

Dr. R.B. McKercher	Dean/Associate Dean/Dean's Designate, Chair College of Graduate Studies and Research
Dr. D.B. Russell	Chair of Advisory Committee, Department of Chemistry
Dr. G. Tourigny	Supervisor, Department of Chemistry
Dr. S. Abrams	Department of Chemistry
Dr. J.W. Quail	Department of Chemistry
Dr. V.S. Gupta	Department of Veterinary Physiological Sciences

External Examiner:

Dr. E.E. Knaus
College of Pharmacy
University of Alberta
Edmonton, AB
T6G 2N8

STRUCTURE-ANTI HERPES ACTIVITY STUDIES OF PYRIMIDINE NUCLEOSIDE ANALOGS

Earlier studies have shown that 5-methoxymethyl-2'deoxycytidine (MMdCyd (**1**)) and 5-bromovinyl-2'-deoxycytidine (BrVdCyd (**2**)) are potent and selective inhibitors of Herpes simples virus type-1 (HSV-1) replication with a high therapeutic index.[1] The antiviral potency of BrVdCyd (**2**) and especially MMdCyd (**1**) against HSV-1 is greatly increased in the presence of tetrahydro deoxyuridine (H_4dUrd).[1] H_4dUrd is an inhibitor of both Cyd/dCyd deaminase and deoxycytidylate (dCMP) deaminase. A major drawback for the therapeutic use of cytidine (Cyd) and deoxycytidine (dCyd) derivatives is their tendency to undergo deamination in the presence of deaminating enzymes.[2] These enzymes are present in blood and mammalian cells catalyze the deamination of Cyd and dCyd derivatives to their corresponding uridine (Urd) and deoxyuridine (dUrd) analogs. Deoxycytidine analogs resistant to deamination would have the advantage of simplifying treatment regimens as well as maintaining other advantages of using dCyd compounds instead of deoxyuridine analogs, such as metabolic stability and selectivity because these drugs will be anabolized in virus infected cells by dCyd/dCMP kinase pathway.

The principal goals and objectives of this research programme were to synthesize deaminase resistant analogs of BrVdCyd (**2**) and MMdCyd (**1**) and to have some understanding of optimal conformational parameters essential for antiherpes activity.

Analogs of MMdCyd synthesized are: N^4-acetyl-MMdCyd (**7**), N^4-propanoyl-MMdCyd (**8**), N^4-butanoyl-MMdCyd (**9**), N^4-pivaloyl-MMdCyd (**10**) and N^4-benzoyl-MMdCyd (**11**); N^4,N^4-dimethyl-MMdCyd (**20**), N^4-phenyl-

MMdCyd (**21**), N^4-benzyl-MMdCyd (**22**), N^4-acetyl-N^4-methyl-MMdCyd (**23**) and 3,4-etheno-MMdCyd (**61**). Analogs of BrVdCyd synthesized are: N^4-methyl-BrVdCyd (**68**), N^4-acetyl-BrVdCyd (**62**), N^4-propanoyl-BrVdCyd (**63**) and N^4-butanoyl-BrVdCyd (**64**). Six of these analogs had anti HSV-1 activity, N^4-acetyl-MMdCyd (**7**), N^4-propanoyl-MMdCyd (**8**) and N^4-butanoyl-MMdCyd (**9**) were more potent than their parent compound MMdCyd (**1**); N^4-acetyl-BrVdCyd (**64**), N^4-propanoyl-BrVdCyd (**64**) and N^4-butanoyl-BrVdCyd (**64**) were also more potent than BrVdCyd (**2**). The rest of the analogs were inactive.

Stability studies of N^4-acyl derivatives of MMdCyd and BrVdCyd at different pH values indicate that these analogs are stable in the range of pH 3 to pH 9.

2-Amino-5-methoxymethyl-2'-deoxyuridine (2-amino-MMdUrd (**39**)) and 2-amino-5-methoxymethyl-2',3'-didehydrodideoxycytidine (2-amino-MMddCyd (**59**)) were prepared and were devoid of activity against HSV-1.

The systematic conformational analysis of these nucleosides was used to identify the hypothetical pharmacophoric pattern that binds with the receptor. The skeleton of a pyrimidine deoxynucleoside substrate has four major modes of conformational flexibility: rotation about the glycosidic bond, (torsion angle χ: $O_{4'}$-$C_{1'}$-N_1-C_2), puckering of the furanose ring described by the pseudorotation phase angle P, rotation about the exocyclic $C_{5'}$ side chain; site of phosphorylation (torsion angle ψ: $C_{3'}$-$C_{4'}$-$C_{5'}$-$O_{5'}$) and where applicable the orientation of the N^4-substituent of the pyrimidine ring.[3]

The conformation of both active and inactive analogs was investigated by NMR spectroscopy. The aim was to understand the structural constraints for recognition by the virus-induced deoxythymidine/deoxycytidine (dThd/dCyd) kinase responsible for the anabolism of 5-substituted pyrimidine deoxyribonucleosides. Analogs with the N^4-substituent *proximal* to C_5, an *anti*

glycosidic torsional preference and a predominant g^+ exocyclic rotamer population were active against HSV-1. In contrast, analogs with the N^4-substituent *proximal* to N_3 or with a *syn* glycosidic torsional preference were inactive against HSV-1.

The furanose population was in the range of 40/60 and 45/55 north/south distribution for all compounds. Thus there appears to be no direct correlation between antiviral activity and puckering of the furanose ring described by pseudorotational parameters.

The optimum geometry of a nucleoside required for recognition by the viral-induced kinase for biological activity has been determined.

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Head of the Department of Chemistry
University of Saskatchewan
Saskatoon, Saskatchewan, S7N 0W0

ABSTRACT

Earlier studies have shown that 5-methoxymethyl-2'deoxycytidine (MMdCyd (**18**)) and (E)-5-(2-bromovinyl)-2'-deoxycytidine (BrVdCyd (**17**)) are potent and selective inhibitors of Herpes simples virus type-1 (HSV-1) replication with a high therapeutic index.[1] The antiviral potency of BrVdCyd (**17**) and especially MMdCyd (**18**) against HSV-1 is greatly increased in the presence of tetrahydro deoxyuridine (H_4dUrd (**13**)), an inhibitor of both Cyd/dCyd deaminase and deoxycytidylate (dCMP) deaminase.[1] A major drawback for the therapeutic use of compounds in the cytidine (Cyd) / deoxycytidine (dCyd) family of derivatives is their tendency to undergo deamination in the presence of deaminating enzymes present in blood and mammalian cells.[2] Deoxycytidine analogs resistant to deamination would have the advantage of simplifying treatment regimens as well as maintaining other advantages such as metabolic stability and selectivity since these drugs will be anabolized in virus infected cells by dCyd/dCMP kinase pathway.

The principal goals and objectives of this research program were to synthesize deaminase resistant analogs of BrVdCyd (**17**) and MMdCyd (**18**) and to have some understanding of optimal conformational parameters essential for antiherpes activity.

Analogs of MMdCyd (**18**) synthesized are: N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**), N^4-butanoyl-MMdCyd (**32**), N^4-pivaloyl-MMdCyd (**33**) and N^4-benzoyl-MMdCyd (**34**); N^4,N^4-dimethyl-MMdCyd (**43**), N^4-phenyl-MMdCyd (**44**), N^4-benzyl-MMdCyd (**45**), N^4-methoxy-MMdCyd (**54**), N^4-hydroxy-MMdCyd (**55**), N^4-acetyl-N^4-methyl-MMdCyd (**57**) and 3,4-etheno-

MMdCyd (**94**). Analogs of BrVdCyd (**17**) synthesized are: N^4-methyl-BrVdCyd (**101**), N^4-acetyl-BrVdCyd (**95**), N^4-propanoyl-BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**). Six of these analogs had anti HSV-1 activity, and were more potent than their parent compounds MMdCyd (**18**) and BrVdCyd (**17**). These active analogs are **30**, **31**, **32**, **95**, **96** and **97**. The rest of the analogs were inactive.

Stability studies of N^4-acyl derivatives of MMdCyd (**18**) and BrVdCyd (**17**) at different pH values indicate that these analogs are stable in the range of pH 3 to pH 9.

The 2-imino-5-methoxymethyl-2'-deoxyuridine (2-imino-MMdUrd (**66**)) and 2-imino-5-methoxymethyl-2',3'-didehydrodideoxycytidine (2-imino-MMddCyd (**90**)) analogs were prepared and were devoid of activity against HSV-1.

Systematic conformational analysis by NMR spectroscopy of these nucleosides was used to identify the hypothetical pharmacophoric pattern that binds with the receptor (The skeleton of a pyrimidine deoxynucleoside substrate has four major modes of conformational flexibility).[3]

Analogs with the N^4-substituent *proximal* to C_5 and an *anti* glycosidic torsional preference were active against HSV-1. In contrast, analogs with the N^4-substituent *proximal* to N_3 or with a *syn* glycosidic torsional preference were inactive against HSV-1.

The furanose population was in the range of 40/60 and 45/55 north/south distribution for all compounds. The **g+** exocyclic rotamer population was predominant (45-57 %) for all compounds also. Thus this study could not conclude whether a correlation exists between antiviral activity and these two conformational parameters.

Optimum geometrical features of a nucleoside required for recognition by the viral-induced kinase for biological activity have been determined.

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ACKNOWLEDGEMENTS

This study would not have been possible without the support of a number of people and institutions. I wish to express my sincere gratitude to them:

To my supervisor, Dr. Guy Tourigny, for his patience, guidance and encouragement throughout the duration of my studies.

To Dr. V.S. Gupta, for his guidance and help in time of need. His valuable assistance and positive criticism enabled the completion of this thesis.

To Dr. V.P.S. Kumar, for his help and guidance in synthetic work.

To Dr. K.C. Brown and Dr. R.S. Reid for their valuable help and advice on NMR work.

To members of my supervisory committee: Drs. S. Abrams, D.B. Russell and W. Quail.

To my external examiner Dr. E.E. Knaus, who is an outstanding academic and gentleman.

To Dr. H.Z. Kamaly for performing the biological assays and Dr. A.L. Stuart for his cooperation and encouragement.

To Mr. Vijay Joshi for help in stability studies.

To the Chemistry Department, University of Saskatchewan for providing funds for consumables.

To the College of Graduate Studies and Research, University of Saskatchewan, for scholarship support.

To the Medical Research Counsil of Canada.

To my family, my beloved wife Joan, my mother Asceen, my brother Akram,

my sister Wafa and my father and mother in-law Rob and Helen Wilson, I wish to express my deep appreciation for their prayers and constant support.

Finally I wish to express my deepest gratitudes and thanksgivings to the Lord my God. A verse from a great hymn comes to my mind:

Who like Thyself my guide and stay can be

through cloud and sunshine, O abide with me.

May this work and every work I do in the future be to His Honor and Glory.

DEDICATION

I dedicate this thesis to my late father Michel Bishara Zoghaib who passed away shortly after I started my university studies.

His love, guidance and encouragement to excel in academics has left a lasting impression of him on me.

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LIST OF ABBREVIATIONS

ACV	Acyclovir (Acycloguanosine)
Ara-A	Arabinosyl-adenine
AIDS	Acquired immunodeficiency syndrome
AZT	3'-Azidothymidine
AcCl	Acetyl chloride
BrVdUrd	(E)-5-(2-Bromovinyl)-2'-deoxyuridine
BrVdCyd	(E)-5-(2-Bromovinyl)-2'-deoxycytidine
BzCl	Benzoyl chloride
BzCN	Benzoyl cyanide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCTPS	1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane
^{13}C NMR	Carbon-13 nuclear magnetic resonance
χ	Glycosidic torsion angle
dCyd	2'-Deoxycytidine
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulfoxide
DMTSCl	Dimethylthexylsilyl chloride
DNA	Deoxyribonucleic acid
dThd	2'-Deoxythymidine
dUrd	2'-Deoxyuridine
EtdUrd	5-Ethyl-2'-deoxyuridine
EtdCyd	5-Ethyl-2'-deoxycytidine
Et_3N	Triethylamine

ε	Extinction coefficient
EtOH	Ethanol
FAB	Fast atom bombardment
FCC	Flash column chromatography
F_3dThd	Trifluorothymidine
FBS	Fetal bovine serum
g+	Gauche-gauche conformation
g-	Gauche-trans conformation
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
HSV-1	Herpes simplex virus-type 1
HSV-2	Herpes simplex virus-type 2
H_4dUrd	Tetrahydrodeoxyuridine
^{1}H NMR	Proton nuclear magnetic resonance
h	Hour
IdCyd	5-Iodo-2'-deoxycytidine
IdUrd	5-Iodo-2'deoxyuridine
MeCN	Methylcyanide (acetonitrile)
MEM	Minimum essential medium
MeOH	Methanol
min	Minute
MMdCyd	5-Methoxymethyl-2'-deoxycytidine
MMdUrd	5-Methoxymethyl-2'-deoxyuridine
5-Me-dCyd	5-Methyl-2'-deoxycytidine
mp	Melting point

MsCl	Mesyl chloride
MS	Mass spectrum
NMR	Nuclear magnetic resonance
nOe	Nuclear Overhauser enhancement
N	North pseudorotational parameter
PANIC	Parameter adjustment in NMR through iteration calculation
PSEUROT	Software for pseudorotational analysis
PTLC	Preparative thin layer chromatography
P	Pseudorotational phase angle
rt	Room temperature
S	South pseudorotational parameter
TBAF	Tetrabutylammonium fluoride
TBDMSCl	Tertiarybutyl dimethylsilyl chloride
THF	Tetrahydrofuran
TK	Thymidine kinase
TK^-	Thymidine kinase negative
TLC	Thin layer chromatography
TMSCl	Trimethylsilyl chloride
τ_m	Maximum puckering amplitude
TsCl	*p*-Toluenesulfonyl chloride
t	Trans-gauche conformation
UV	Ultraviolet
v/v	Volume relative to volume measure
ψ	C_4'-C_5' Exocyclic torsion angle

1- GENERAL INTRODUCTION

1.1) Importance of Viral Diseases and Approaches for Treatment

Viral infections are among the greatest causes of human morbidity and resulting economic loss. Since viruses are obligatory parasites, viral infections induce profound changes in the host's physiological processes. Infections due to viruses are either localized to mucosal or cutaneous tissues or generalized by neurogenic spread to distant organs. The approaches to combat viral infections include vaccination, potentiation of immune response, stimulation of interferon and chemotherapy.

The subject matter of this thesis pertains primarily to Herpes simplex virus (HSV); therefore infections due to HSV only will be covered in greater detail in this section.

1.2) Herpes Viruses: Etiology and Infection.

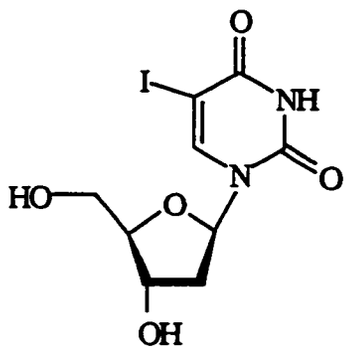
Herpes viruses are the most common cause of viral infections in humans. Ten to fifteen percent of the population on this continent over the age of 18 years have recurrent HSV infection three or more times every year.[4] The herpes simplex virus has been designated into two subgroups, namely, HSV type 1 (HSV-1) and HSV type 2 (HSV-2) based on their serology and molecular characteristics.

HSV-1 is a source of frequent infections in humans with varying degree of severity from mild cutaneous infection (cold sores) to serious ocular infection including impairment of vision (*herpes keratitis*) and life threatening disease (*herpes encephalitis*). More than twenty million people on this continent suffer

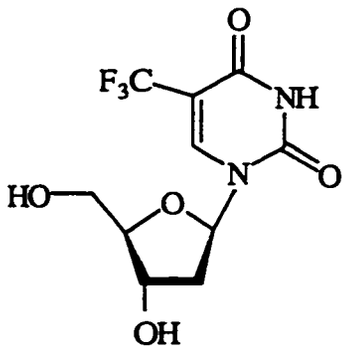
from cold sores.[5] Herpes keratitis is the leading cause of blindness and approximately 300,000 to 500,000 cases are reported worldwide each year. Herpes encephalitis is a serious infection of the central nervous system and causes significant mortality (40 - 50%) and neurological damage in surviving individuals. Herpes genitalis is predominantly caused by HSV-2. The incidence of herpes genitalis has been on the rise and it is estimated that approximately 25 to 30 million people are infected on the North American continent. The virus persists in latent form and causes recurrent disease, usually at the same site. Infections due to HSV-2 can lead to miscarriage, fetal death, congenital fetal infections and congenital abnormalities.[6] According to the American Academy of Pediatrics, 60 percent of babies born with HSV infection will die, and half of the survivors will suffer severe damage to the brain, nervous system and eyes.[7]

1.3) Chemotherapy of Herpes Simplex Virus Infections.

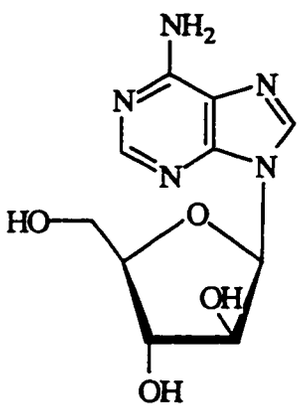
Infections due to herpes viruses in humans have not proven amenable to control by immunization. However, some measure of control has been achieved with the use of drugs. History of the development of drugs useful for the treatment of HSV Infections is covered briefly. Drugs currently approved for the treatment of HSV infections are nucleoside analogs (Fig. 1.1).
(Chemical structures of nucleoside analogs and pyrimidine bases mentioned in chapter 1 are shown in figure 1.3. Structures of deaminase inhibitors and nucleotides are shown in firgure 1.4.)



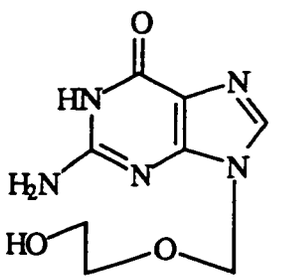
5-Iodo-2'-deoxyuridine
(IdUrd) **1**



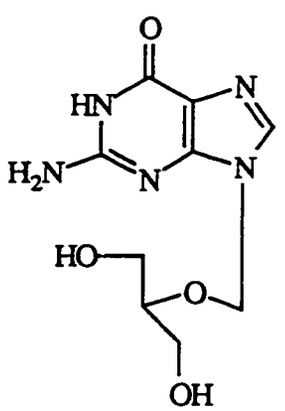
Trifluorothymidine
(F_3dThd) **2**



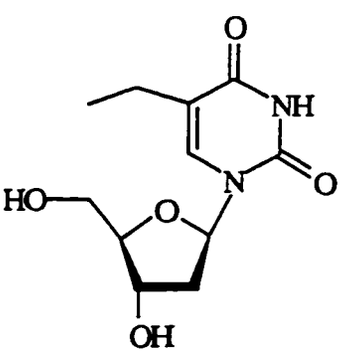
Arabinosyl adenine
(Ara-A) **3**



Acyclovir
(ACV) **4**



Ganciclovir
(GCV) **5**



5-Ethyl-2'-deoxyuridine
(EtdUrd) **9**

Fig. 1.1: Drugs currently approved for the treatment of HSV infections

A major advance in antiviral chemotherapy was the discovery that IdUrd (**1**) inhibits replication of herpes viruses.[8] Further investigations by Kaufman (1962)[9] established the efficacy of IdUrd (**1**) in the treatment of herpes keratitis in humans. Trifluorothymidine (F_3dThd (**2**)) and arabinosyl adenine (Ara-A (**3**)) were approved for the treatment of herpes keratitis in the early eighties.[10,11] Subsequently, Ara-A (**3**) was shown to be effective in the treatment of herpes encephalitis in humans. However, toxicity of IdUrd (**1**), F_3dThd (**2**) and Ara-A (**3**) remained a serious concern for the use of these drugs in the treatment of systemic herpes infections.[12]

A major breakthrough in establishing the usefulness of chemotherapeutic approach for the treatment of herpes infections was made with the discovery that acyclovir (ACV (**4**)) is a selective agent for the treatment of herpes genitalis and herpes encephalitis.[13] More recently, other acyclic nucleosides, such as ganciclovir (GCV (**5**)) have been approved for the treatment of HSV infection in humans.[14]

1.4) 5-Substituted Pyrimidine Nucleoside Analogs with Antiviral Activity Against HSV. (Brief Review).

1.4.1) 5-Substituted-2'-Deoxyuridine Analogs: (atomic numbering system for pyrimidine nucleosides is discussed in section 4.3.1, page 90)

5-Methoxymethyl-2'-deoxyuridine, MMdUrd (**6**) was the first nucleoside analog with selectivity of action against HSV[15] synthesized at the University of Saskatchewan in the early seventies. MMdUrd (**6**) was found to have potent antiviral activity against HSV-1 and a higher therapeutic index than other antiviral drugs.[15] MMdUrd (**6**), found to be devoid of toxicity to lymphoid functions in *vitro*, non immunosuppressive in *vivo*, is effective in the treatment of herpes keratitis in rabbits and has very low mammalian toxicity. Subsequent

studies in Dr. Gupta's laboratory revealed that MMdUrd (**6**) was a moderate inhibitor of HSV-2 replication and showed synergistic activity when used in combination with other antiviral compounds in cell culture.[16] Combination of MMdUrd (**6**) and Ara-A (**3**) was efficacious in eradicating herpes genitalis in animal models.[16] MMdUrd (**6**) was found to have only moderate activity against herpes encephalitis in mice because of poor brain penetration by the drug.[16] MMdUrd (**6**) is selectively phosphorylated by the HSV-induced viral thymidine kinase to its monophosphate (MMdUrd-MP (**7**)). The lack of cytotoxicity and low mammalian toxicity of MMdUrd (**6**) is most likely due to the fact that it does not serve as a substrate for mammalian thymidine kinase.[16]

Synthesis and antiviral properties of (E)-5-(2-bromovinyl)-2'-deoxyuridine, BrVdUrd (**8**) were reported by De Clercq and his colleagues.[17] BrVdUrd (**8**) is more selective and more potent against HSV-1 in cell cultures than all known 5-substituted-2'-deoxyuridine compounds. BrVdUrd (**8**) is anabolized only in HSV-infected cells and does not appear to serve as a substrate for host cellular kinases and thus has low cytotoxicity.[17] In animal models of herpetic skin lesions, BrVdUrd (**8**) suppressed the development of skin lesions. However, the glycosyl bond of BrVdUrd (**8**) is cleaved very rapidly by phosphorylases in *vivo*, and thus its usefulness for the treatment of systemic infections due to herpes simplex virus is greatly diminished.[17]

5-Ethyl-2'-deoxyuridine, EtdUrd (**9**) was synthesized by Swierkowski *et al.* (1969).[18] The rationale for synthesizing EtdUrd (**9**) was that an ethyl group is electronically closer to the methyl of the natural analog 2'-deoxythymidine (dThd (**10**)) than an iodine atom (e.g. IdUrd (**1**)).[18] Thus the pKa of the proton on N_3 (which is responsible for base pairing in DNA) in EtdUrd (**9**) would be similar to that in dThd (**10**) and hence EtdUrd (**9**) in principle should be an ideal antiviral agent. EtdUrd (**9**) has potent activity against HSV-1, HSV-2 as

well as other DNA viruses.[18] Despite the fact that EtdUrd (**9**) is incorporated into DNA, it has been shown to be non-mutagenic and non-immunosuppressive. Clinical studies have shown EtdUrd (**9**) to be effective in the treatment of herpetic keratitis and herpes genitalis after topical application.[19] EtdUrd (**9**) is considerably more cytotoxic to rapidly growing cells than MMdUrd (**6**) and BrVdUrd (**8**).[12] It is also rapidly cleaved to inactive 5-ethyluracil (**11**) by phosphorylases in *vivo*. In addition to MMdUrd (**6**), BrVdUrd (**8**) and EtdUrd (**9**), a number of other 5-substituted-2'-deoxyuridines with selectivity of action against HSV have been synthesized.

Thus the most significant development has been the discovery of drugs which elicit antiviral activity by selectively utilizing or inhibiting virus specific functions to a greater degree than host cells. This new generation of antiviral agents includes compounds such as acyclovir (**4**), gancyclovir (**5**), BrVdUrd (**8**) and MMdUrd (**6**). The selectivity of these nucleoside analogs is due to the specific interaction with two virus induced enzymes, namely, pyrimidine viral-kinase and DNA polymerase.[2] The molecular basis for the selectivity of BrVdUrd (**8**) and MMdUrd (**6**) is that these 5-substituted pyrimidine deoxyribonucleosides are preferentially phosphorylated (anabolism) to their corresponding monophosphates by the virus-induced thymidine kinase. After conversion to their corresponding triphosphates, they selectively inhibit viral DNA polymerase.[2]

1.4.2) 5-Substituted-2'-Deoxycytidines.

One major limitation of BrVdUrd (**8**), EtdUrd (**9**) and MMdUrd (**6**) for therapeutic purposes is that 5-substituted-2'-deoxyuridines are rapidly catabolized to inactive pyrimidine bases by thymidine and/or uridine phosphorylases.[2] This limits their usefulness for the treatment of systemic infections. Gupta *et al.* [2] hypothesized that it should be possible to overcome

the limitation of metabolic instability by use of deoxycytidine (dCyd) analogs provided these compounds can be channeled through the dCyd kinase-deoxycytidylate (dCMP) kinase pathway in infected cells by the addition of deaminase inhibitors H_4Urd (**12**), H_4dUrd (**13**) and H_4dUrd-MP (**14**) (Fig.1.2).[1]

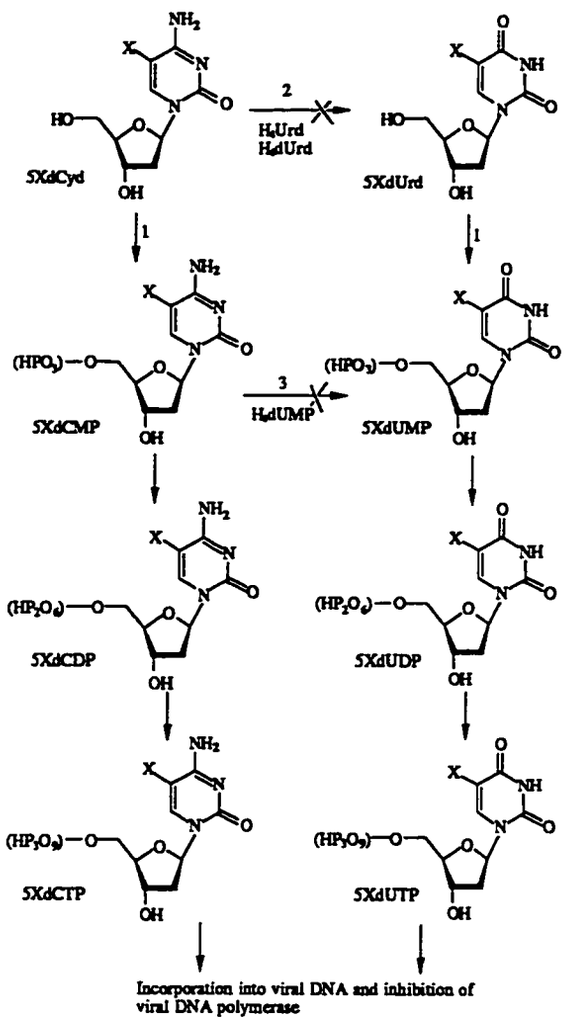


Fig.1.2: Proposed pathways for metabolism of 5-substituted -2'-deoxycytidines (5XdCyd) in HSV-1 infected cells.

Note: X = methoxymethyl or bromovinyl.

dCyd, dCydMP, dCydDP and dCydTP = 2'-deoxycytidine, 2'-deoxycytidine-5'-monophosphate, 2'-deoxycytidine-5'-diphosphate and 2'-deoxycytidine-5'-triphosphate respectively.

dUrd, dUrdMP, dUrdDP and dUrdTP = 2'-deoxyuridine, 2'-deoxyuridine-5'-monophosphate, 2'-deoxyuridine-5'-diphosphate and 2'-deoxyuridine-5'-triphosphate respectively.

H_4Urd, H_4dUrd and H_4dUrdMP = tetrahydrouridine (dCyd deaminase), tetrahydro-2'-deoxyuridine (dCyd deaminase) and tetrahydro-2'-deoxyuridine-5'-monophosphate (dCydMP deaminase) respectively.

1: dThd/dCyd kinase (virus-induced);

2: dCyd deaminase;

3: dCydMP deaminase.

Prior to systematic investigations initiated in Dr. Gupta's laboratory at the University of Saskatchewan, dCyd analogs had not received wide attention as antiherpes agents. Greer and colleagues reported that 5-halogenated-2'-deoxycytidines were more selective inhibitors of HSV than their corresponding 5-halogenated-2'-deoxyuridine analogs.[20] The basis for their selectivity is that 5-iodo-2'-deoxycytidine (IdCyd (**15**)) is more effectively phosphorylated by the pyrimidine nucleoside kinase of HSV.[21] After conversion to the corresponding triphosphate, IdCyd-TP (**16**) may inhibit DNA polymerase or be utilized as an alternate substrate by viral DNA polymerase (Fig 1.2). Of considerable interest was the finding that IdCyd (**15**) co-administered with tetrahydrodeoxyuridine (H_4dUrd (**13**), a deaminase inhibitor) was incorporated into viral DNA without undergoing deamination to its corresponding uridine (IdUrd (**1**)).[22]

Studies by Aduma *et al.*[1] have provided ample evidence in support of the hypothesis that 5-substituted dCyd analogs are potent and selective inhibitors of HSV. These investigations have shown that 5-bromovinyl-2'-deoxycytidine, BrVdCyd (**17**) and 5-methoxymethyl-2'-deoxycytidine, MMdCyd (**18**) are potent and selective inhibitors of HSV when co-administered with H_4dUrd (**13**) in infected cells. The potency of BrVdCyd (**17**) and MMdCyd (**18**) against HSV-1 was dependent on the dCyd deaminase/ dCMP deaminase content of the cell line used for antiviral assays.[1] Furthermore, the findings that BrVdCyd (**17**) and MMdCyd (**18**) were devoid of activity against thymidine kinase negative (TK^-) strain of HSV-1 corroborates the hypothesis that metabolic activation by viral-induced pyrimidine nucleoside kinase (deoxythymidine / deoxycytidine kinase) is an obligatory step for the antiviral activity of dCyd analogs.[1]

Besides metabolic stability, another useful attribute of dCyd analogs for the treatment of HSV infections is that these compounds may have some benefit in

reducing the incidence of recurrent herpes infections by preventing latency or emergence of viral activation from latent state.[23] The basis for this hypothesis is that deoxycytidine compounds would behave as analogs of 5-methyl-cytosine (**19**) (a modified pyrimidine base found in viral DNA) and thus may interfere with the synthesis of non-methylated DNA. The latter has been postulated to play an important part in virus maturation and productive infection.[23]

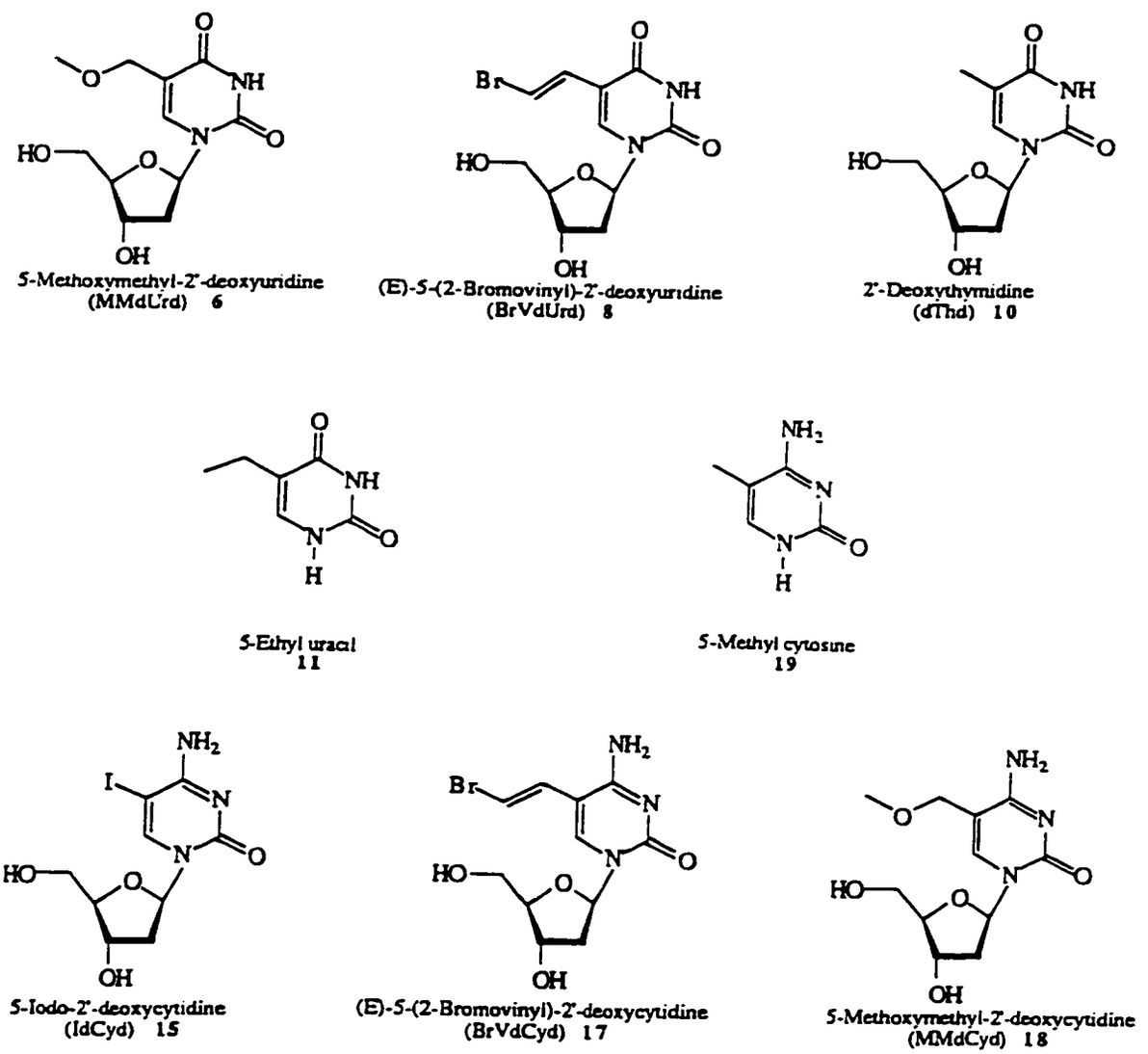


Fig. 1.3: Chemical structures of some nucleoside analogs and pyrimidine bases

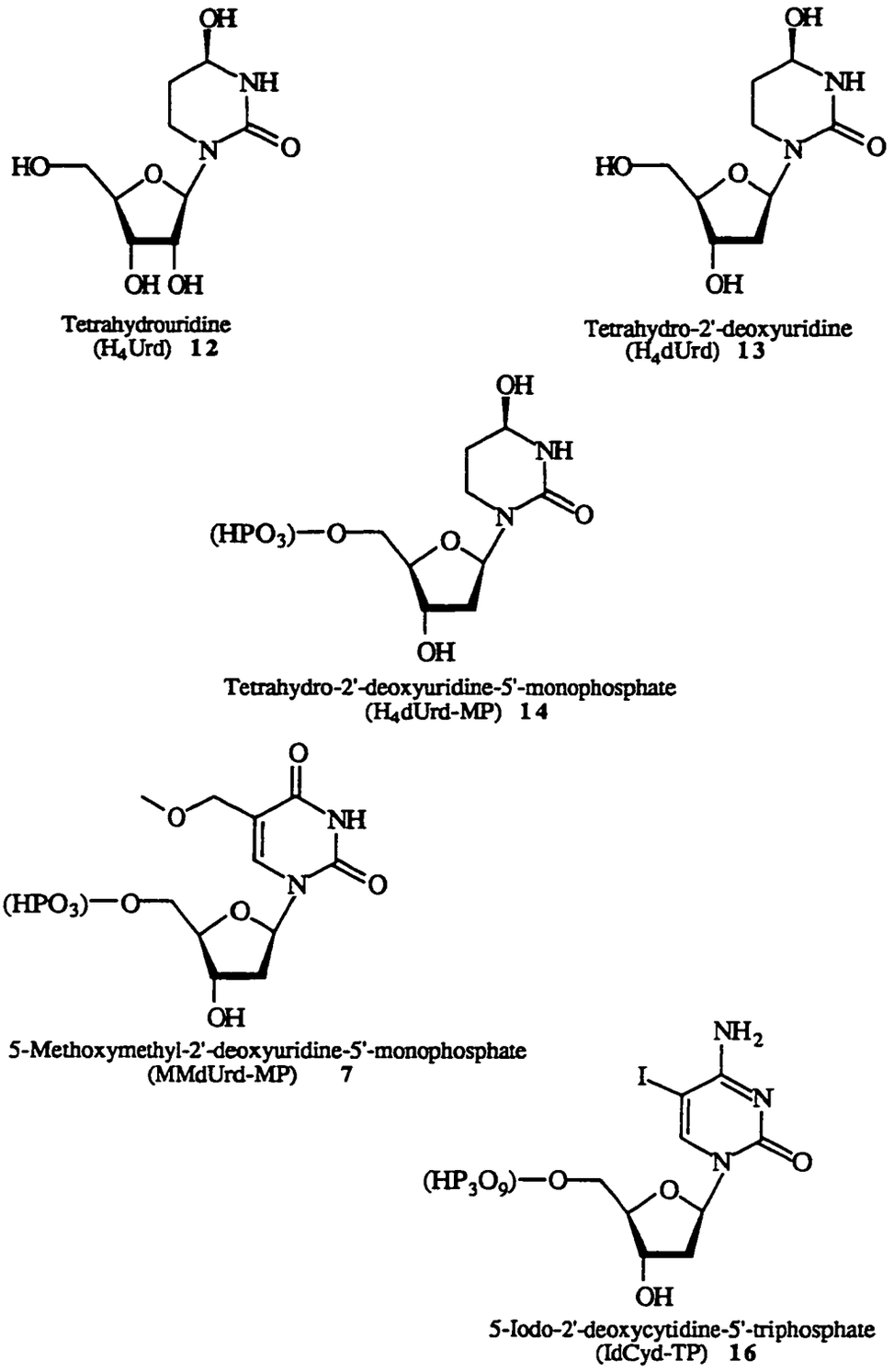
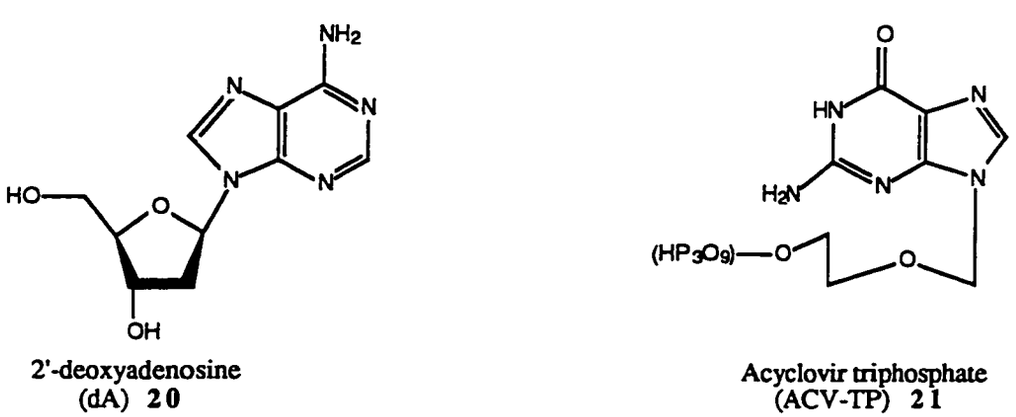


Fig. 1.4: Chemical structures of some deaminase inhibitors and nucleotides.

2- DEVELOPMENT OF ANTIVIRAL AGENTS

2.1) **Antiviral Agents** (historical review)

Serendipity has played a major role in the discovery of drugs for the treatment of malignancy and viral infections. Interestingly, drugs currently approved for the treatment of HSV infections were originally designed and synthesized as anti-cancer agents.[24] IdUrd (**1**) and F_3dThd (**2**) were synthesized as antimetabolites of dThd (**10**).[10] Ara-A (**3**) was synthesized as a structural analog of 2'-deoxyadenosine (**20**) to mimic its replacement in the DNA of cells.[11] Similarly, acyclovir (**4**), the drug now most widely used for the treatment of HSV infections was designed as an antitumor agent.[25] The rationale was that ACV (**4**) after conversion to its active form acyclovir triphosphate (ACV-TP (**21**)), would be incorporated into the DNA of cells and act as a chain terminator. Unfortunately, none of these compounds were found to have good antitumor activity. IdUrd (**1**) and F_3dThd (**2**) also lacked selectivity of action.[10]



2'-deoxyadenosine (dA) **20**

Acyclovir triphosphate (ACV-TP) **21**

Fig. 2.1: Chemical structure of 2'-deoxyadenosine and acyclovir triphosphate

Fortunately, the antiviral screen of these compounds against DNA

viruses and more specifically herpes viruses, led to the discovery of acyclovir (**4**) as a potent and very selective antiherpes agent with remarkably low toxicity in humans even after prolonged use.[26] Thus the discovery and introduction of ACV (**4**) in therapeutics, laid the foundation that viral infections can be controlled by chemotherapy.

More recently, the use of drugs for the treatment of human immunodeficiency virus (HIV), the etiological agent of Acquired Immunodeficiency Syndrome (AIDS) has led further credence to the usefulness of chemotherapeutic approach for prophylaxis and treatment of viral infections.[24, 27]

2.2) Scope of the Problem

The most common approach in the design of drugs has been a "hit or miss" process based on empirical observations. This approach involves repeated structural modifications of a leading compound until optimal biological activity is obtained, with an acceptable therapeutic index. This empirical approach[28] perhaps could be termed "rational serendipity", because structural modifications are carried out and a large number of derivatives are synthesized. This process is inefficient and it has been estimated that a newly synthesized compound, designed on the basis of empirical observations, has one chance in ten thousand of being therapeutically valuable.[29] The probability of producing a pharmacophore with desirable therapeutic properties can be improved by combining structure-activity relationships with computer-graphic model building.

Drugs interrupt or alter biological processes by interacting with receptors.[29,30] Therefore an optimal and more logical approach for the development of selective and more effective therapeutic agents would be to

develop molecules based on the knowledge of the molecular structure of the receptor (usually a protein) and the steric conformations most likely to fit the receptors.[31] Nuclear magnetic resonance (NMR) spectroscopy is useful in determining the conformation of compounds in solution,[32] and computer graphics can be used to examine the docking of drugs to the active site of the receptors.[30] The knowledge gained from these investigations can then be utilized to determine the most suitable molecular conformation for a pharmacophore.

2.3) Objectives and Goals

Correlation of biological activity to molecular structure is indispensable to the systematic design of drugs.[2,21,33] Receptors recognize and discriminate molecules by a process of highly specific binding and thereby initiate biochemical reactions that lead to the characteristic physiological or pharmacological response.

The thesis that conformation plays a very important role in influencing molecular interactions of substrates with receptors is now widely accepted.[21,33,34,35]

The principal goal of these studies was to synthesize dCyd analogs resistant to deaminases and have some understanding of the conformational parameters essential for antiviral activity. For these investigations, BrVdCyd (**17**) and MMdCyd (**18**) were chosen as model compounds. In order to accomplish these objectives, the following studies were undertaken:

(i) Analogs of BrVdCyd (**17**) and MMdCyd (**18**) were prepared by attaching various groups to the exocyclic NH_2 functional group at position 4 of the pyrimidine moiety and conformation was determined by NMR spectroscopy.

(ii) 2-Imino analogs of MMdUrd (**6**) and MMdCyd (**18**) were prepared and their

conformation was determined.

(iii) Antiviral activity and cytotoxicity of compounds prepared and stability of active analogs was determined.

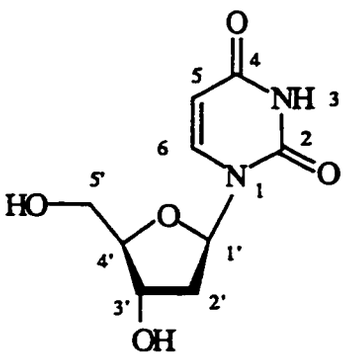
Results of these investigations are the subject matter of this thesis.

3- CHEMICAL SYNTHESIS

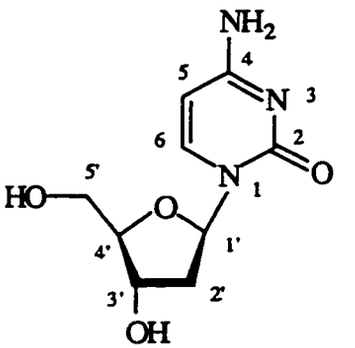
3.1) Introduction

Pyrimidine nucleosides are major components of nucleic acids. The basic repeating motif in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) is the nucleotide. A nucleotide is a nucleoside phosphorylated at one of the free sugar hydroxyls. The chemistry of nucleosides began with the isolation of nucleic acids and efforts to elucidate the chemical structures of the monomeric components. Nucleosides are composed of a cyclic furanoside-type sugar (β-D ribose in RNA or β-D-2'-deoxyribose in DNA) which is substituted at $C_{1'}$ by one of four different heterocycles attached by a β-glycosyl $C_{1'}$-N linkage. The heterocycles are the purine bases adenine and guanine and the pyrimidine bases cytosine and uracil or thymine. Structures of pyrimidine nucleosides are shown in fig. 3.1.

Pyrimidine nucleoside analogs can be roughly subdivided into two major groups, those containing modified heterocyclic bases, and those in which the sugar moiety has been altered and is no longer the ribose or 2'-deoxyribose. Base analogs have in general been prepared either via N-glycosylation of suitably modified heterocycles or by structural transformations of the base moiety in existing nucleosides. The analogs containing modified carbohydrate moieties have been frequently prepared via glycosylation of a pyrimidine base with a suitable derivative of the desired sugar. Alternatively, a great deal of effort has been devoted to the development of specific chemical transformations suitable for introducing the desired modifications into the sugar moiety of intact nucleosides.[36]



2'-deoxyuridine (**22**)



2'-deoxycytidine (**23**)

Fig. 3.1: Chemical structure of 2'-deoxyuridine and 2'-deoxycytidine

The discovery of 5-substituted-2'-deoxyuridines with selectivity against herpes simplex virus was a major development in the area of viral chemotherapy.[10] The basis for selective antiherpes activity of 5-alkyl-2'-deoxyuridines is primarily due to the specific interaction with two virus-induced enzymes, the deoxythymidine-deoxycytidine (dThd/ dCyd) kinase and the DNA polymerase. Cleavage of the N-glycosidic bond of many of these 5-substituted-2'-deoxyuridines by nucleoside phosphorylases rapidly leads to loss of biological activity.[21] One possible approach to overcome this limitation is to use deoxycytidine analogs. A brief survey of deoxycytidine analogs was introduced in section 1.4.

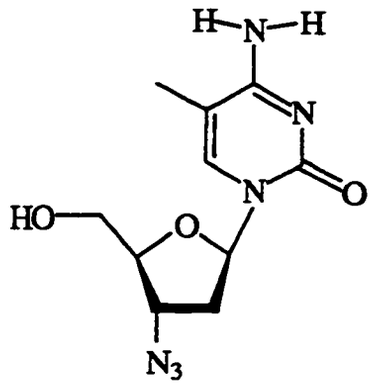
A major drawback for the therapeutic use of deoxycytidine compounds is their tendency to undergo deamination in the presence of deaminating enzymes. These enzymes are usually present in blood and mammalian cells and catalyze the deamination of the deoxycytidine compounds to the corresponding uridine analogs, which are either less active or do not display selectivity.[21] The problem of deamination can be overcome by modification of the deoxycytidine compounds to induce resistance to deaminases.[37]

Of considerable interest are the findings that alkyl derivatives of IdCyd (**15**) (N^4-methyl, -ethyl and -isopropyl) were incorporated into viral DNA without deamination.[25] 5-Methoxymethyl-2'-deoxycytidine (**18**) has potent activity against HSV-1 when co-administered with a deaminase inhibitor.[34] 5-Methoxymethyl-N^4-methyl-2'-deoxycytidine (N^4-methyl-MMdCyd, (**24**)) was prepared to make the molecule resistant to the action of deaminases. However, N^4-methyl-MMdCyd (**24**) was found to be devoid of antiherpes activity.[35]

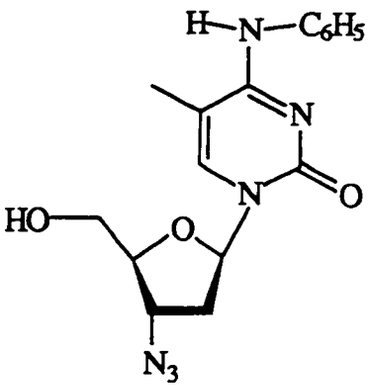
N^4-Substituted derivatives of 3'-azido-2',3'-dideoxy-5-methylcytidine (**25**), such as N^4-phenyl-3'-azido-2',3'-dideoxy-5-methylcytidine (**26**) and N^4,N^4-dimethyl-3'-azido-2',3'-dideoxy-5-methylcytidine (**27**) in particular, showed marked anti-HIV activity comparable to that of AZT (**28**).[38] These analogs are not deaminated in situ to AZT (**28**) and thus their activity is intrinsic.[38] Pathways through which anti-HIV and anti-HSV agents are metabolized are different. Any structural modifications that maintain or enhance the activity of anti-HIV agents may have the opposite effect on anti HSV agents.

Phosphorylation of 5-substituted pyrimidine deoxyribonucleosides by HSV-induced pyrimidine kinase to the monophosphate is an essential step for anti-HSV activity.[23] Studies were undertaken with the objective of defining the *structural requirements* for recognition by HSV-induced kinase. Compounds were synthesized by attaching various groups to the exocyclic amino group at N^4 position of MMdCyd (**18**) and BrVdCyd (**17**). The rationale was that by introducing these changes, deaminase resistant analogs of MMdCyd (**18**) and BrVdCyd (**17**) may be obtained. Conformational studies were undertaken to understand the effect of changes in structure (the various torsion angles) on antiviral activity. Studies on analogs of 5-Me-dCyd (**29**) were undertaken to complete studies on the dCyd series. Other objectives were to prepare and

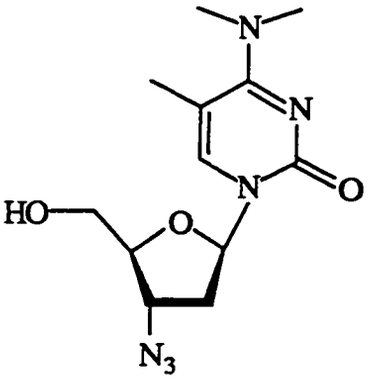
study the conformation of 2-imino derivatives of **6** and **18**.



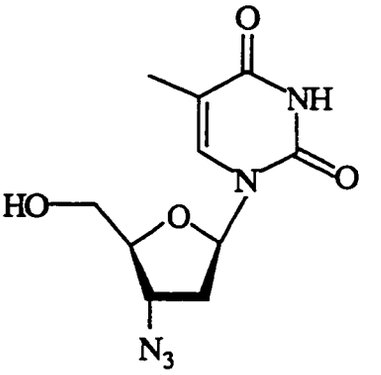
3'-Azido-2'-3'-dideoxy-5-methylcytidine **25**



N^4-phenyl-3'-Azido-2'-3'-dideoxy-5-methylcytidine **26**



N^4,N^4-dimethyl-3'-Azido-2'-3'-dideoxy-5-methylcytidine **27**



3'-Azido-2'-3'-dideoxythymidine (AZT) **28**

Fig 3.2: Chemical structures of anti-HIV nucleoside analogs

3.2) Results and Discussion

Since N^4-methyl-MMdCyd (**24**) has no antiherpes activity, it was suggested that the conformation of the molecule has been altered and this might have been the cause for loss of activity.[35] It was decided to undertake a systematic study of analogs of MMdCyd (**18**) by substitution on the exocyclic N^4 position of the pyrimidine and to study the effect on conformation and activity. In this

series, N^4-acyl and N^4-aroyl analogs of MMdCyd (**18**) were synthesized. The analogs are: N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**), N^4-butanoyl-MMdCyd (**32**) and N^4-trimethylacetyl (pivaloyl)-MMdCyd (**33**); were prepared from MMdCyd (**18**).[39] Fig. 3.3 shows the preparation of the four analogs **30**, **31**, **32** and **33**.

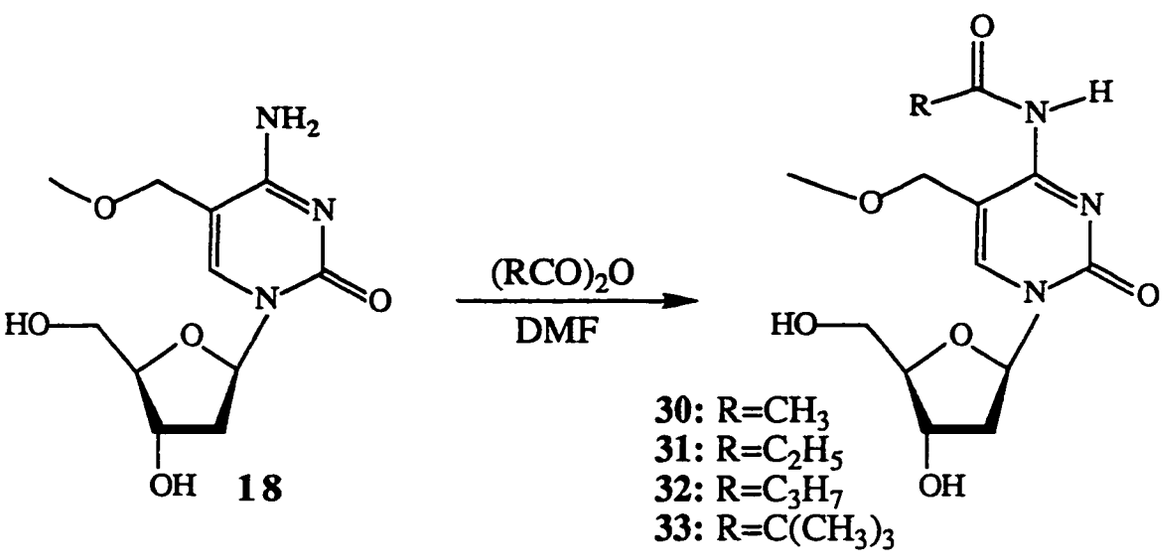


Fig 3.3: Preparation of N^4-acyl-MMdCyd analogs

To prepare N^4-benzoyl-MMdCyd (**34**), a different approach was employed. A procedure reported in the literature making use of benzoyl cyanide in acetonitrile as the benzoylating agent was attempted.[40] MMdCyd (**18**) was dissolved in DMF and benzoyl cyanide was added. After all of the starting material was consumed, workup of the reaction, purification and examination of the two products by NMR, indicated that the desired benzoyl amide was not present. Instead the two compounds 5'-benzoyl-MMdCyd (**35**) and 3',5'-dibenzoyl-MMdCyd (**36**) were formed as shown in fig. 3.4.

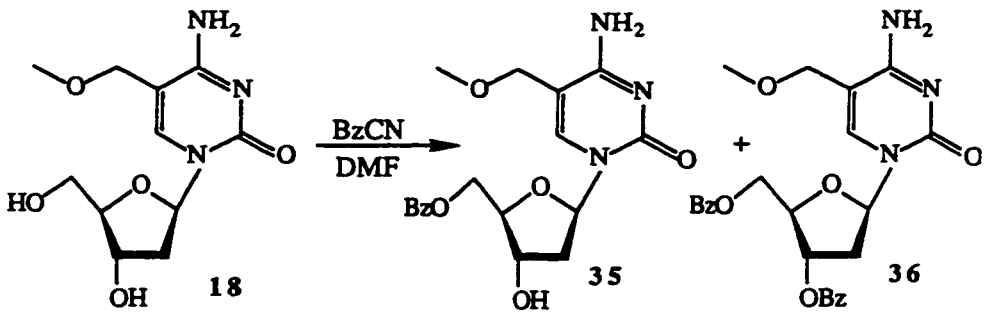


Fig. 3.4: Preparation of 5'-benzoyl and 3',5'-dibenzoyl-MMdCyd

Another procedure was employed to prepare N^4-benzoyl-MMdCyd (**34**) by protecting the 3' and 5' positions of MMdCyd (**18**) with trimethylsilyl groups, benzoylating the N^4-position using benzoyl chloride and an organic base and finally deprotecting the deoxyribose hydroxyls without affecting the N^4-benzoyl position.[41] Fig. 3.5 describes the preparation of N^4-benzoyl-MMdCyd (**34**).

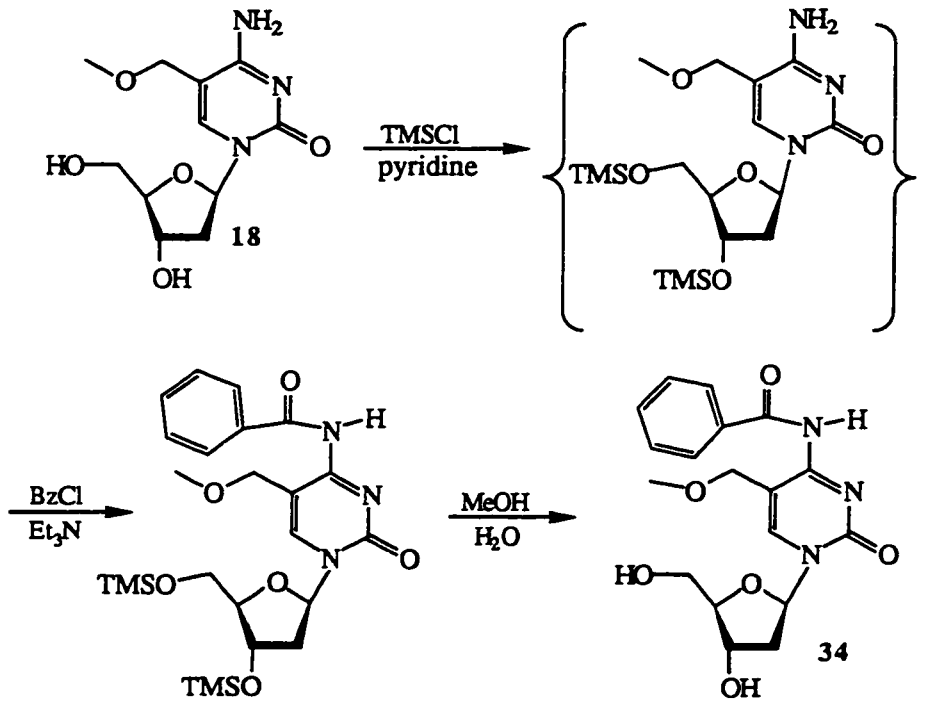


Fig. 3.5: Preparation of N^4-benzoyl-MMdCyd

Other N^4-alkyl and alkenyl analogs were prepared to study the effect of a wide range of N^4-substituents on conformation and activity. The compound N^4,N^4-dimethyl-MMdCyd (**43**) was prepared to determine if substitution of a dimethyl group at N^4 might restore the conformation required for activity. Three more analogs were prepared using the same methodology used for the preparation of **43**.[21] These are N^4-phenyl-MMdCyd (**44**), N^4-benzyl-MMdCyd (**45**) and N^4-amino-MMdCyd (**46**).

The analogs **43**, **44**, **45**, and **46** were prepared from MMdUrd (**6**).[21] The protected compound 3',5'-diacetyl-MMdUrd (**37**)[21], was transformed to the intermediate 4-triazolyl-3',5'-diacetyl-MMdUrd (**38**) which was treated with the proper base (dimethylamine, aniline, benzylamine and hydrazine) in four separate experiments to get the four corresponding protected deoxycytidine derivatives **39**, **40**, **41** and **42** as described in fig. 3.6. Deprotection yielded the desired crude analogs **43**, **44**, **45** and **46**. One analog that proved impossible to purify was the compound N^4-amino-MMdCyd (**46**). Crude **46** was identified by NMR in DMSO-d_6 but decomposed partially upon purification by FCC and was never isolated in pure form. The same problems were reported for the isolation of an N-amino derivative of a purine nucleoside.[42]

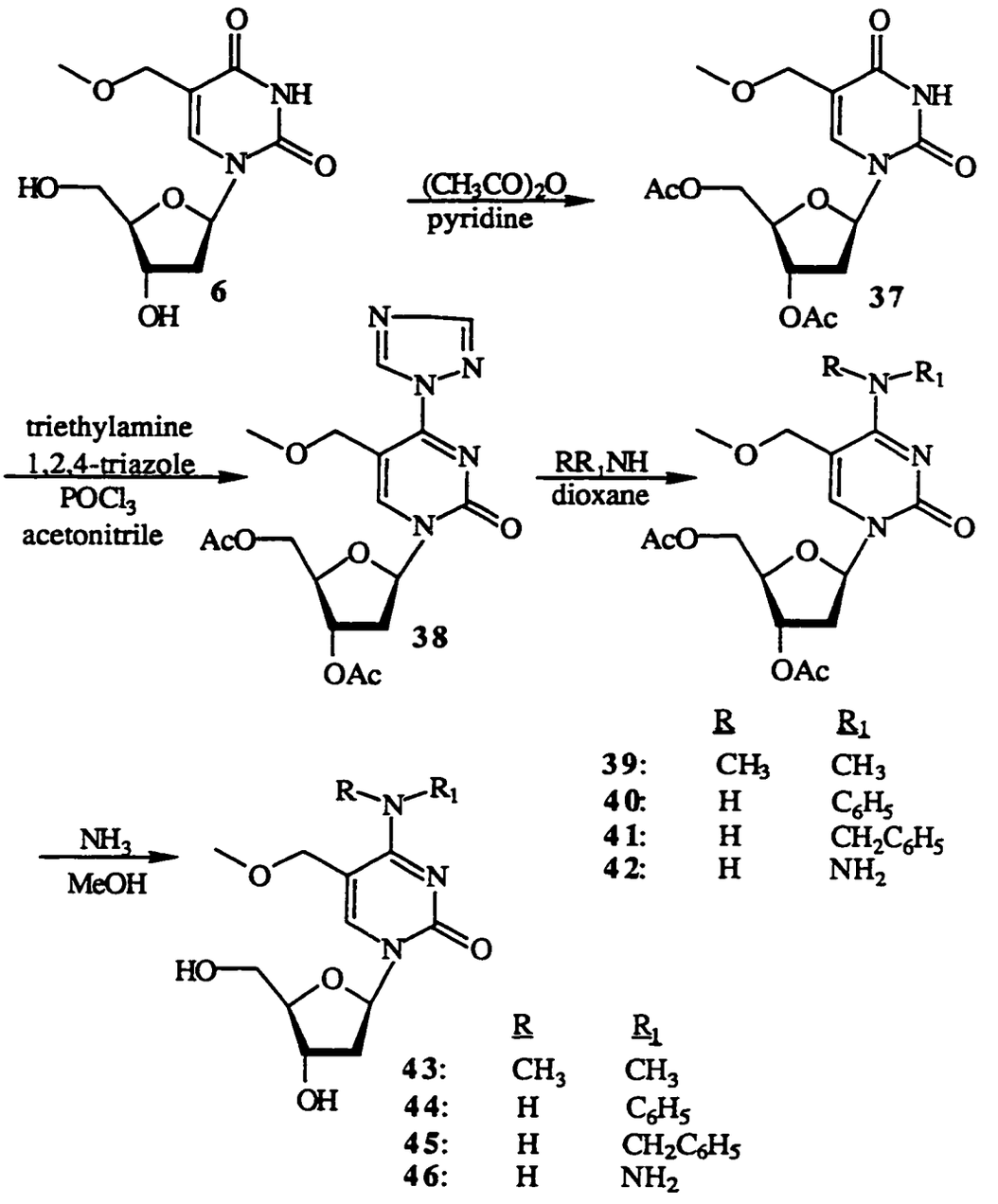


Fig. 3.6: Preparation of N^4-alkyl, N^4-aryl and N^4-amino-MMdCyd

The synthesis of two more analogs of MMdCyd (**18**), namely N^4-methoxy-MMdCyd (**54**) and N^4-hydroxy-MMdCyd (**55**) was undertaken. The 3',5'-hydroxyls of MMdUrd (**6**) were protected with benzoyl groups to give compound **47**.[43] Tosylation at the N^4 position of **47** to give **48**, followed by

treatment with the proper amine hydrochloride yielded compounds **50** and **51**. An attempt to remove the 3' and 5'-dibenzoyl protecting groups of **50** and **51** with sodium methoxide in methanol[43] resulted in deamination yielding the starting material MMdUrd (**6**). The unsuccessful synthetic route for the preparation of **54** and **55** is described in fig. 3.7.

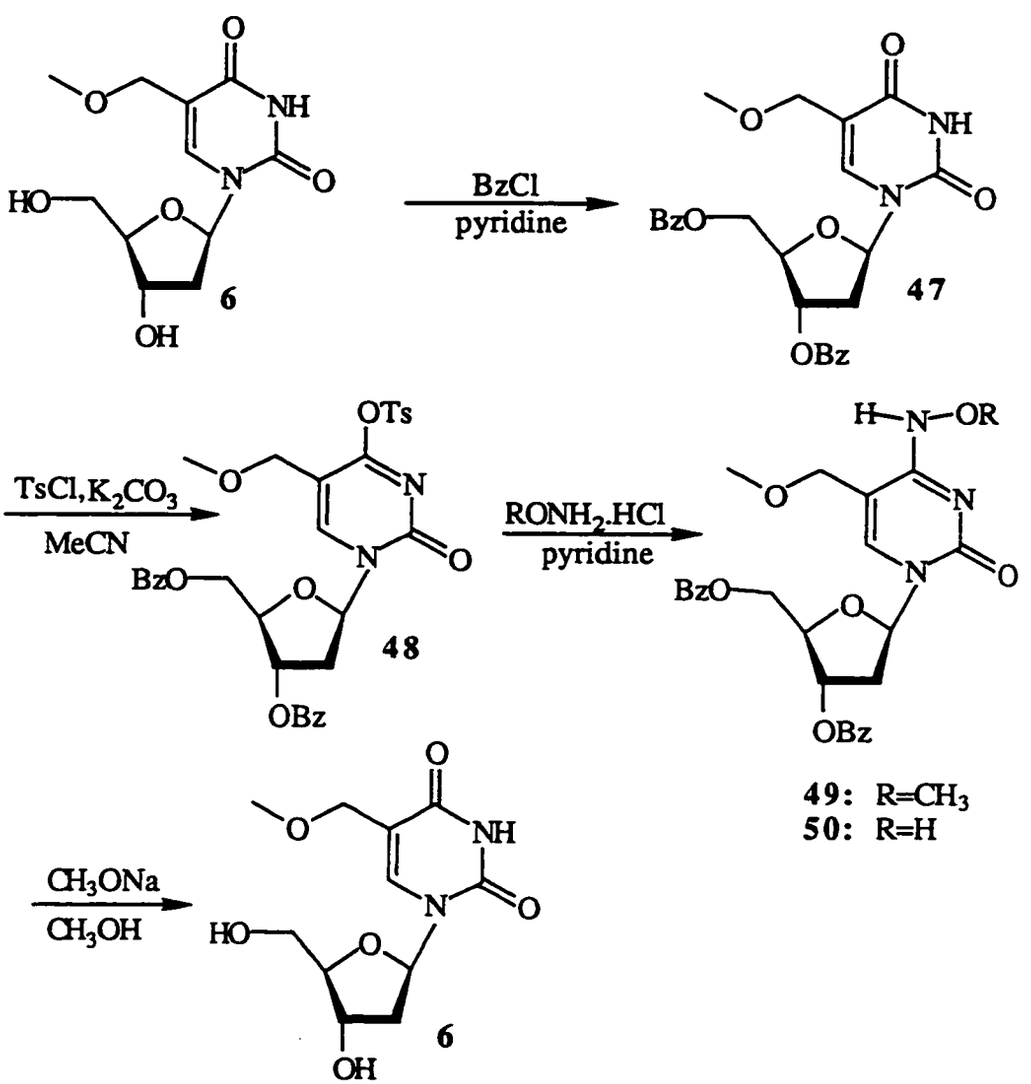


Fig. 3.7: Attempt to prepare N^4-methoxy and N^4-hydroxy-MMdCyd

After this a successful attempt at preparing **54** and **55** proceeded via protecting the deoxyribose hydroxyls with labile groups that can be removed using milder reaction conditions. 3',5'-Diacetyl-MMdUrd (**37**) was prepared as before and the unstable N^4-tosylate intermediate **51** was added to the proper amine hydrochloride in pyridine to give the protected products **52** and **53**. Deprotection of the furanose hydroxyls with methanolic ammonia afforded **54** and **55**. Fig. 3.8 describes this synthesis.

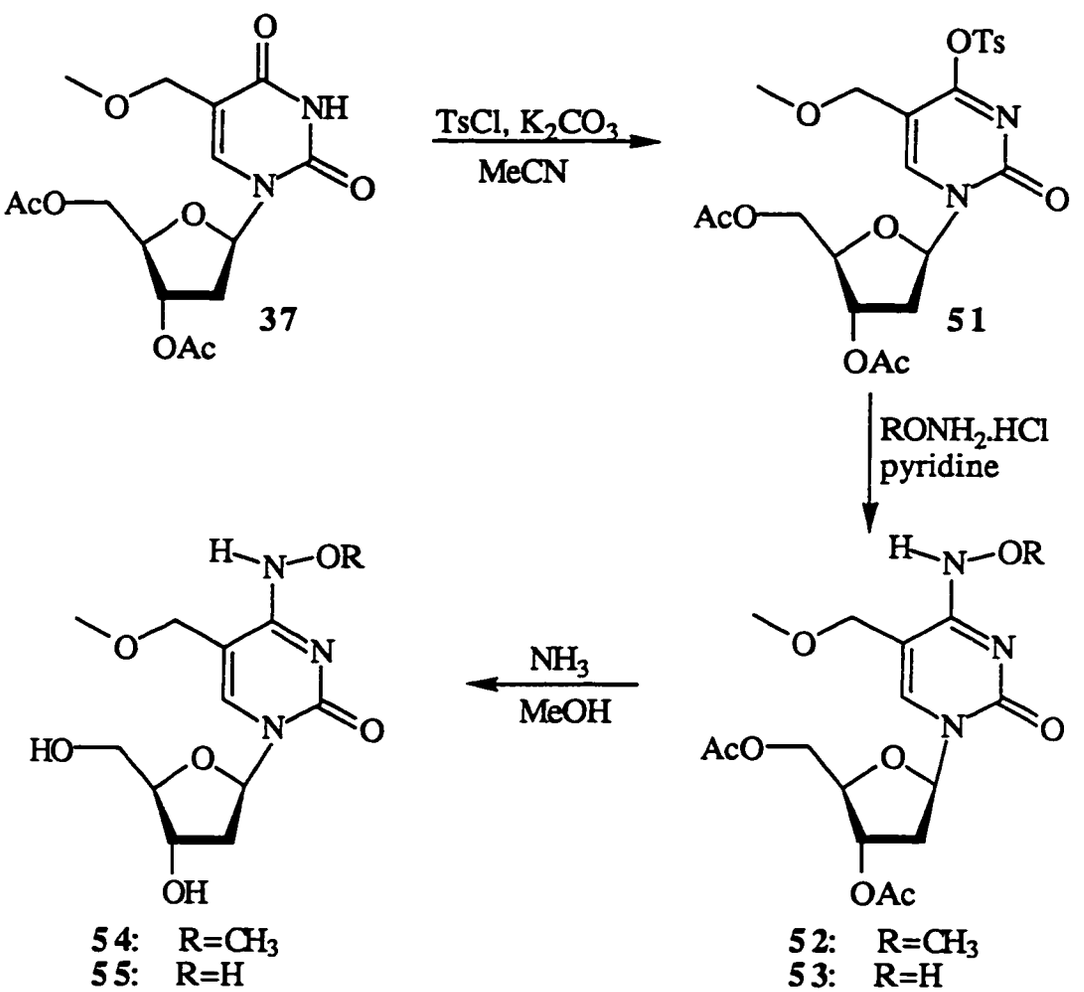


Fig. 3.8: preparation of N^4-methoxy and N^4-hydroxy-MMdCyd

Preliminary studies were carried on compounds to determine their activity against HSV. Since N^4-methyl-MMdCyd (**24**) is inactive and N^4-acetyl-MMdCyd (**30**) was active (biological activity will be addressed in chapter 5), it was interesting to find out whether an analog that carries both functional groups would be active or inactive. An initial attempt to prepare N^4-acetyl-N^4-methyl-MMdCyd (**57**) was unsuccessful.

Acetylation of N^4-methyl-MMdCyd (**24**) in DMF[39] and examination of the single product after its isolation, revealed that the product isolated was 5'-acetyl-N^4-methyl-MMdCyd (**56**) and not the desired compound (fig. 3.9).

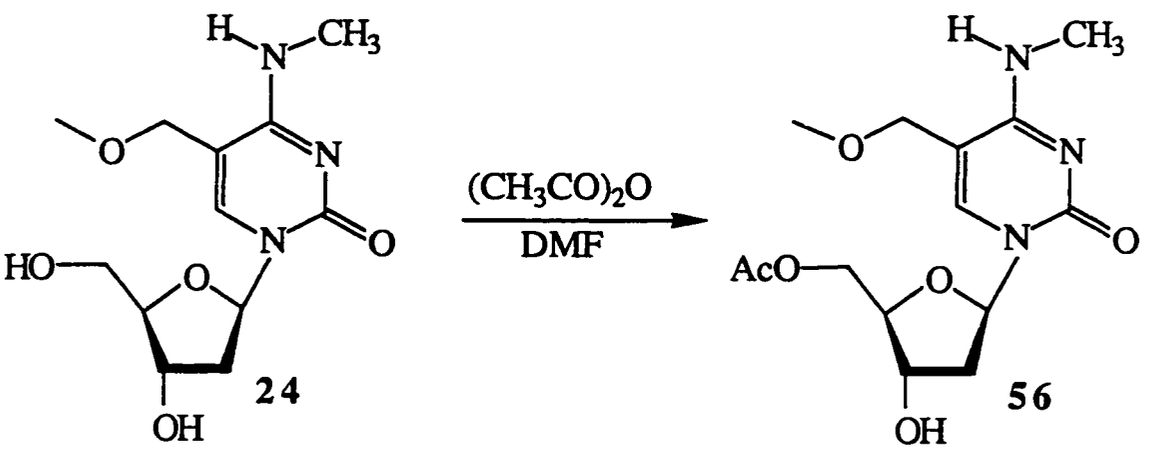


Fig. 3.9: Attempt to prepare N^4-acetyl-N^4-methyl-MMdCyd

While it was quite facile to prepare N^4-acyl analogs of MMdCyd (**18**) using the above one step methodology, it is assumed that the steric bulk of an N^4-methyl substituent likely prevented an easy access of the acetic anhydride to N^4. Another process was used to prepare **57**, one similar to that used for the preparation of N^4-benzoyl-MMdCyd (**34**).[41] Through protection of the furanose hydroxyls and acetylation with acetyl chloride, the preparation of **57** was achieved as shown in fig. 3.10.

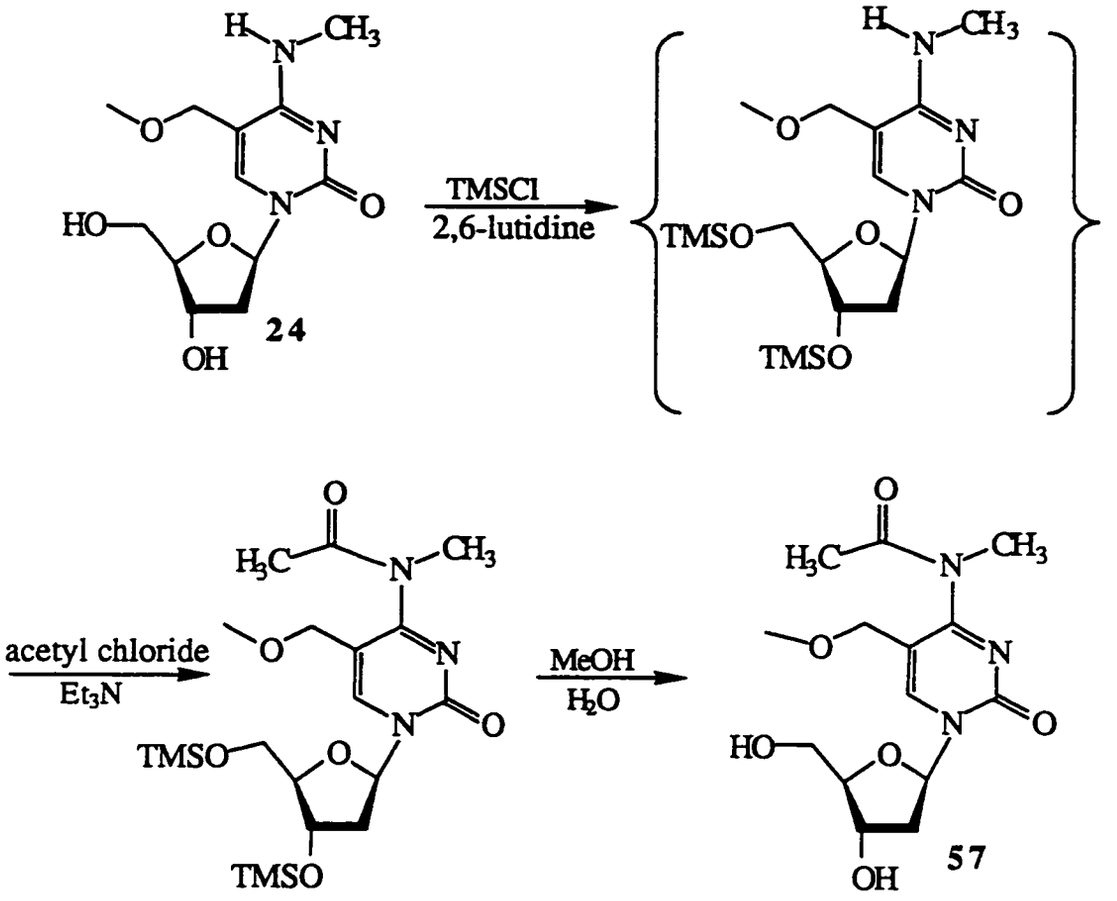


Fig 3.10: Preparation of N^4-acetyl-N^4-methyl-MMdCyd

Another compound of interest is the imide N^4,N^4-diacetyl-MMdCyd (**60**). This compound should have one acetyl group proximal to N_3, and the other acetyl group proximal to C_5 of the pyrimidine ring. (discussion on restricted rotation around C_4-N^4 bond is found in chapter 4 section 4.4.4.). Preparation of this analog began by protecting the furanose hydroxyls of **18** with a dichlorotetraisopropyl disiloxane in pyridine.[41] The protected product 3',5'-tetraisopropyl disioloxane-MMdCyd (**58**) was acetylated in 2,6-lutidine.[44] The product 3',5'-tetraisopropyl disiloxane-N^4,N^4-diacetyl-MMdCyd (**59**) was deprotected using tetrabutyl ammonium fluoride in THF.[45] This step led to

removal of the silicone protecting group as well as disintegration of the imide functional group. The products of this reaction were MMdUrd (**6**) and MMdCyd (**18**). Noncyclic imides have been reported to be very unstable.[46] Fig. 3.11 describes the attempt.

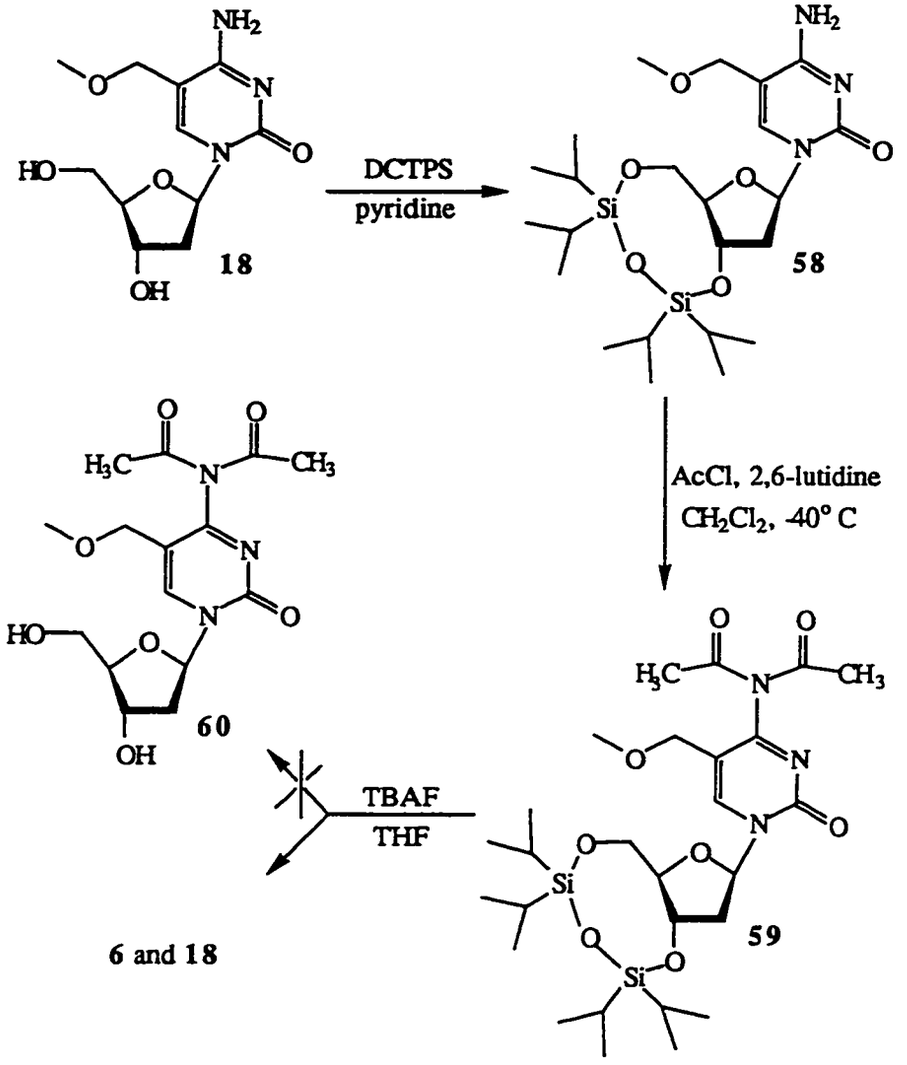


Fig. 3.11: Attempt to prepare N^4,N^4-diacetyl-MMdCyd

One final compound in this N^4-substituted series that was desired is N^4-ethylcarbamoyl-MMdCyd (**63**). Attempts at preparing this analog were

unsuccessful. One reference uses ethyl chloroformate in acetone in the presence of dry K_2CO_3 to attach the ethyl formate group to N^4.[47] However, MMdCyd (**18**) is too polar to dissolve even in refluxing acetone (DMF and DMSO reacted with ethylchloroformate). To solve the problem of poor solubility in acetone, two silyl groups were placed on the deoxyribose hydroxyls using dimethylthexylsilyl chloride and imidazole in DMF. This protected compound 3',5'-bis(dimethylthexylsilyl)-MMdCyd (**61**) was soluble in acetone and its reaction with ethyl chloroformate did yield a product (**62**) as shown in fig. 3.12. The crude NMR of the reaction mixture did show signals pertaining to an ethyl formate group (a triplet and a quartet at 1.4 and 4.5 ppm, respectively). One portion of the above crude mixture was subjected to desilylation using TBAF in THF, the use of which proved to be disadvantageous due to TBAF streaking on the FCC column and hence contaminating all fractions. Further purification of the above fractions led apparently to hydrolysis of the ethylformate group and only starting material **18** was recovered. No further attempts for the preparation of N^4-ethylcarbamoyl-MMdCyd (**63**) were pursued.

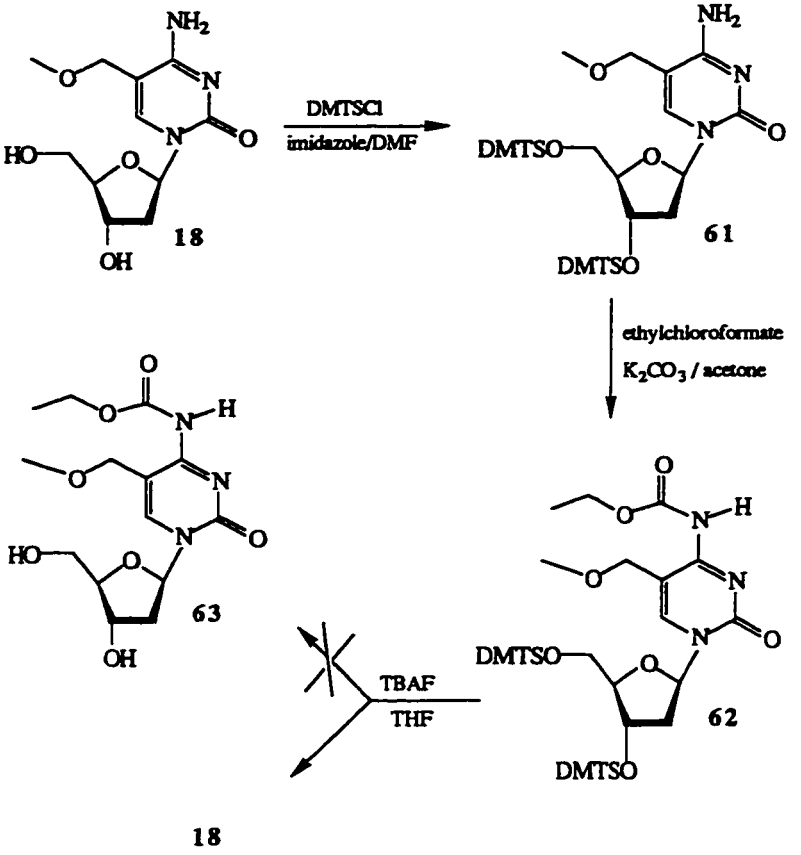


Fig. 3.12: Attempt to prepare N^4-ethylcarbamoyl-MMdCyd

Of interest also to this study was the preparation of the compound 2-imino-MMdUrd (**66**). The leaving group *para*-toluenesulfonyl was placed at the 5' position of MMdUrd (**6**) using TsCl in pyridine.[48] The product 5'-tosyl-MMdUrd (**64**) was transformed to the unstable intermediate 2-O-5'-anhydro-MMdUrd (**65**) which gave the product 2-imino-MMdUrd (**66**) upon reaction with ammonia in methanol as can be seen in fig. 3.13.

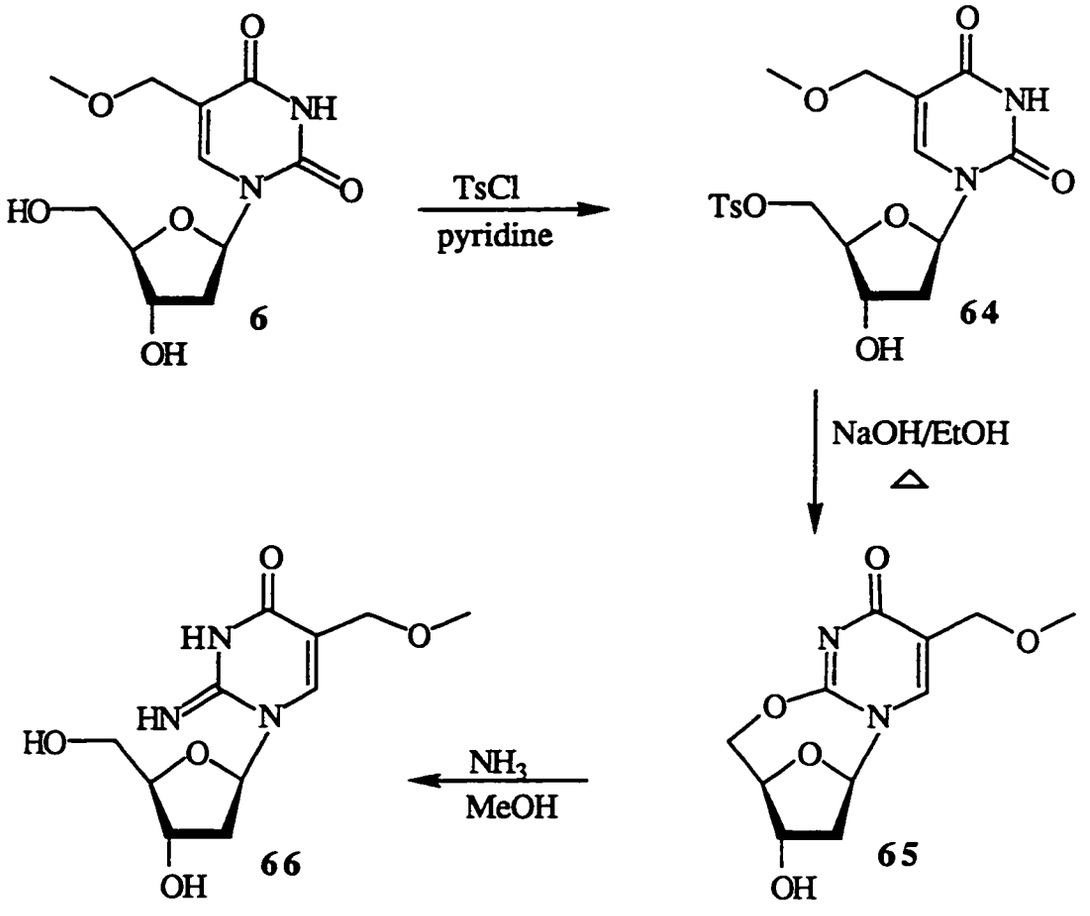
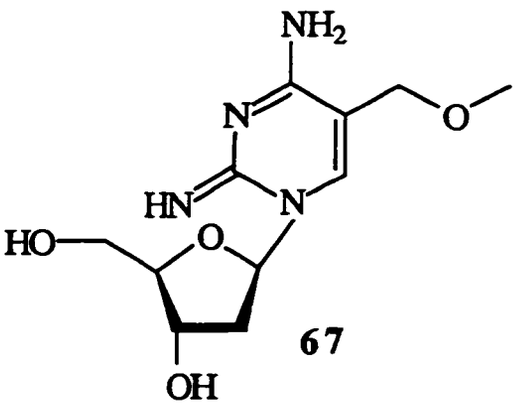


Fig. 3.13: Preparation of 2-imino-MMdUrd

Following 2-imino-MMdUrd (**66**), the focus of this research shifted to preparing 2-imino-MMdCyd (**67**). Such analogs which have an imino group at position 2 of the pyrimidine ring have proven resistant to deaminase enzymes and thus more effective as antiviral agents.[49]



2-imino-MMdCyd

At first, the route adopted aimed at preparing 2-imino-MMdCyd (**67**) starting from 2-imino-MMdUrd (**66**) via a triazolyl intermediate at position 4 of the pyrimidine ring.[21] However, the reaction did not go forward and no triazolyl intermediate was produced. Another approach, was to prepare the compound 2-O-5'-anhydro-MMdCyd (**70**) and treat it with ammonia in methanol to obtain **67**. Tosylation (or mesylation)[48] to produce **68** (or **69**) followed by treatment with DBU in acetonitrile (or 1,4-dioxane) as shown in fig. 3.14, failed to give the desired anhydro derivative **70**.

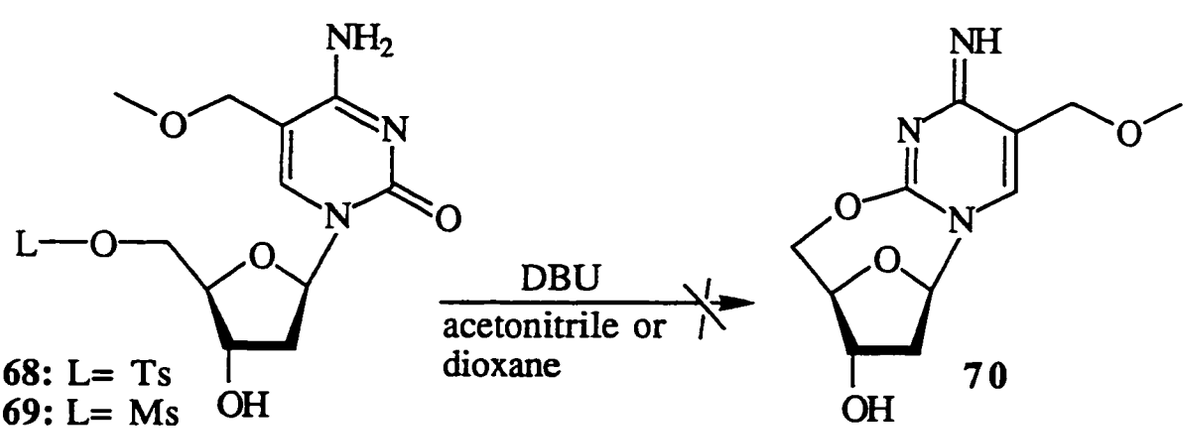


Fig. 3.14: Attempt to form 2-O-5'-anhydro-MMdCyd

After several trials it was found that the starting material, 5'-tosyl-MMdCyd

(**68**), decomposes totally when refluxed for two hours in the presence of either a strong base such as DBU or a weak base such as pyridine. Another attempt aimed at refluxing 5'-tosyl-MMdCyd (**68**) in ethanol or in 1:1 ethanol/water mixture to achieve an intramolecular 2-O-5'-anhydro formation.[51] The amino group on position 4 of **68** is an internal base and there is precedent for such an anhydro formation to proceed rather easily.[51] This attempt also failed.

A careful literature search had no reference to any 2-O-5'-anhydro in the deoxycytidine nucleoside family. The only 2-O-5'-anhydro-cytidine compounds reported in the literature are those in which the sugar ring is a ribose and the 2' and 3' oxygens are tied together in an isopropylidine protecting group. In such a molecule, the X-ray crystal structure indicated that the ribose ring is in a conformation whereby $C_{5'}$ is sufficiently close to O_2 to facilitate the formation of a 2-O-5' anhydro bond.[52] Since the molecule being dealt with (MMdCyd (**18**)) has a 2'-deoxyribose, and hence no 2'-OH is available, it is impossible to tie together $O_{2'}$ and $O_{3'}$ in an isopropylidene link.

Choosing the 5' position to prepare the 2-O-5'-anhydro compound **70** was the synthetically easier approach, since the 5'-carbon is not a stereogenic center and thus no inversion of configuration can possibly take place. The alternative strategy was to prepare the 2-O-3'-anhydro bond in an MMdCyd (**18**) molecule that has a protecting group at 5' OH. This process is feasible yet it involves inversion of configuration at the 3' stereogenic carbon. The 5' hydroxyl group of MMdCyd (**18**) was protected with a DMTS group to give 5'-dimethylthexylsilyl-MMdCyd (**71**),[53] the 3' hydroxyl group of **71** was subsequently mesylated[51] to provide the compound 3'-(R)-mesyl-5'-dimethylthexylsilyl-MMdCyd (**72**). This compound **72** was dissolved in 1:1 ethanol/water mixture and refluxed. The product 2-O-3'-anhydro-5'-dimethylthexylsilyl-MMdCyd (**73**) (mesylate salt form) was treated with

ammonia in methanol.[54] The resulting product 3'-(S)-hydroxy-5'-dimethylthexylsilyl-2-amino-MMdCyd (**74**) was obtained. The crucial step now was to invert the configuration of the 3' carbon from (S) to (R). The 3'-hydroxyl group of **74** was mesylated[51] to yield 3'-(S)-mesyl-5'-dimethylthexylsilyl-2-imino-MMdCyd (**75**). What should have been an easy nucleophilic substitution reaction resulting in inversion of configuration at 3' was not feasible. 3'-(S)-Mesyl-5'-dimethylthexylsilyl-2-imino-MMdCyd (**75**) was refluxed in aqueous pyridine (pyridine-water 2:1 v/v) to convert 3'-(S) into 3'-(R).[51] The reaction progress was monitored by TLC which showed no distinct new spot rather a streak of material along the plate which proved to be decomposition products upon purification of a portion of the solution by FCC. Another attempt using a different nucleophile to invert the 3' configuration was to add 1M NaOH to a solution of **75** in pyridine. The product obtained from this experiment was the 5' desilylated compound, namely 3'-(S)-mesyl-2-imino-MMdCyd (**76**) without inversion at 3'.

Another attempt to invert the configuration at 3' of **75** was carried. Compound **75** was dissolved in methanol and 2 equivalents of 1M NaOH were added at room temperature. After stirring for a few hours, no change was observed. The reaction mixture was then refluxed and monitored until all of the starting material was consumed. After purification by PTLC, two products were identified which are compounds **76** that was previously identified and 5'-dimethylthexylsilyl-2',3'-dideoxy-2',3'-didehydro-2-imino-MMdCyd (**77**) in minor amount. The synthetic scheme is shown in fig. 3.15.

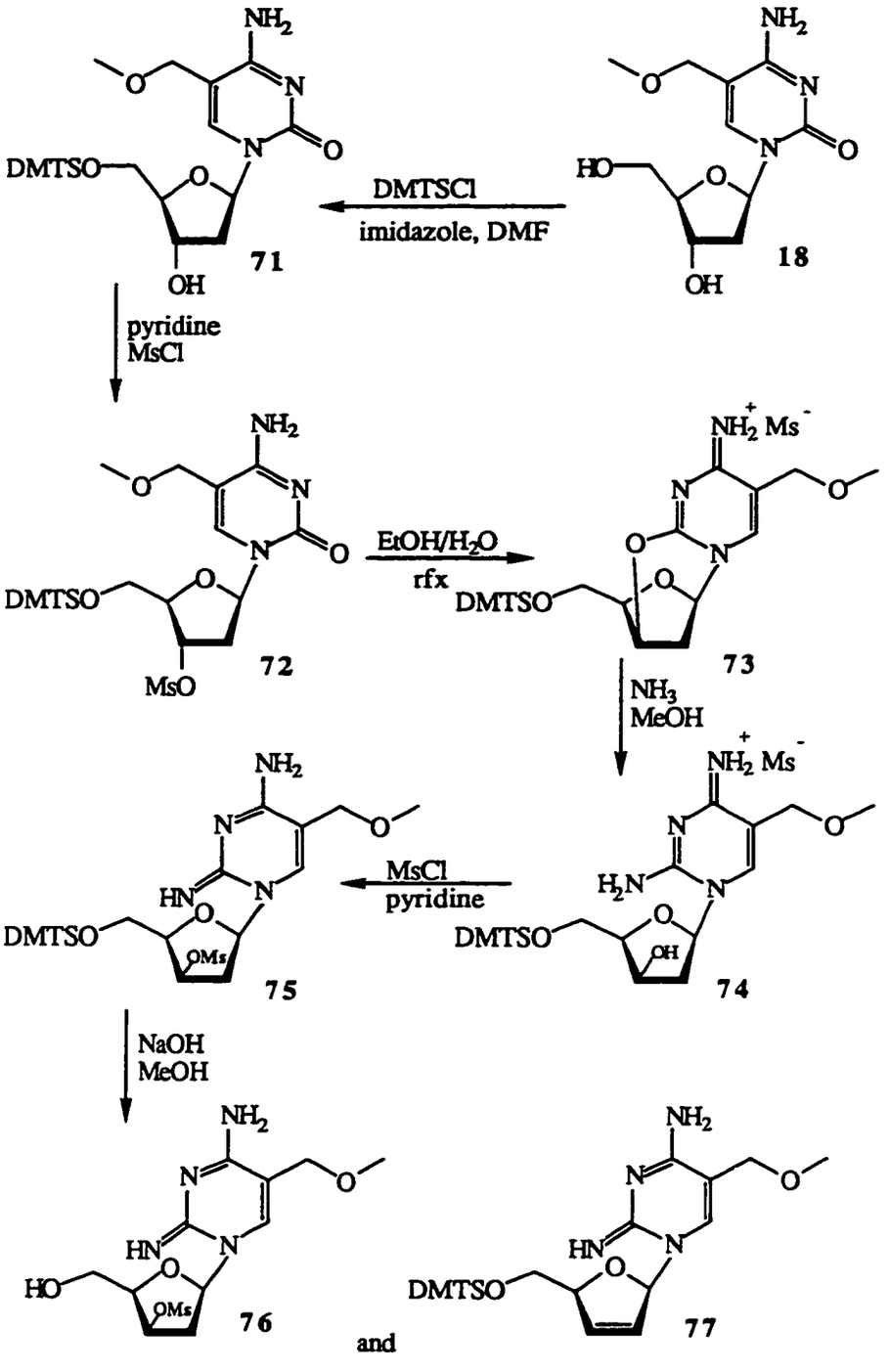


Fig. 3.15: Attempt to prepare 2-imino-MMdCyd (I)

At this stage, the preparation of 2-imino-MMdCyd (**67**) has proven much

more difficult than previously thought. However, due to the report[49] that a compound like **67** would be resistant to deamination and thus potentially be a very good antiviral agent, the attempts to prepare **67** were carried forward. Looking back at how easy it was in the literature to prepare 2-O-5'-anhydro cytidine compounds with an isopropylidine link between 2' and 3' hydroxyls [52], it was thought that attaching a protecting group to the 3'-OH group might imitate the "isopropylidene effect" leading to a change in conformation that will bring $C_{5'}$ and O_2 into close proximity for an anhydro 2-O-5' bond formation. Or by merely blocking the 3'-OH with a protecting group, the reaction might be forced to proceed via a 2-O-5' anhydro bond formation because the easier and energetically more favorable route, that is, the formation of a 2-O-3'-anhydro bond has been blocked. The compound 3'-tertbutyldimethylsilyl-5'-tosyl-MMdCyd (**78**) was prepared by first placing a tosyl leaving group on 5'-OH,[48] then by placing a tertbutyldimethylsilyl group on 3'-OH.[53] Refluxing of **78** in benzene-d6 resulted in decomposition rather than the anticipated 2-O-5'-anhydro bond formation. If the preparation of 3'-tertiarybutyldimethylsilyl-2-O-5'-anhydro-MMdCyd (**79**) was achievable, the target molecule 2-imino-MMdCyd (**67**) would have been easily prepared by treating **79** with ammonia in methanol followed by deprotection of 3' OH. Fig. 3.16 shows the failed attempt.

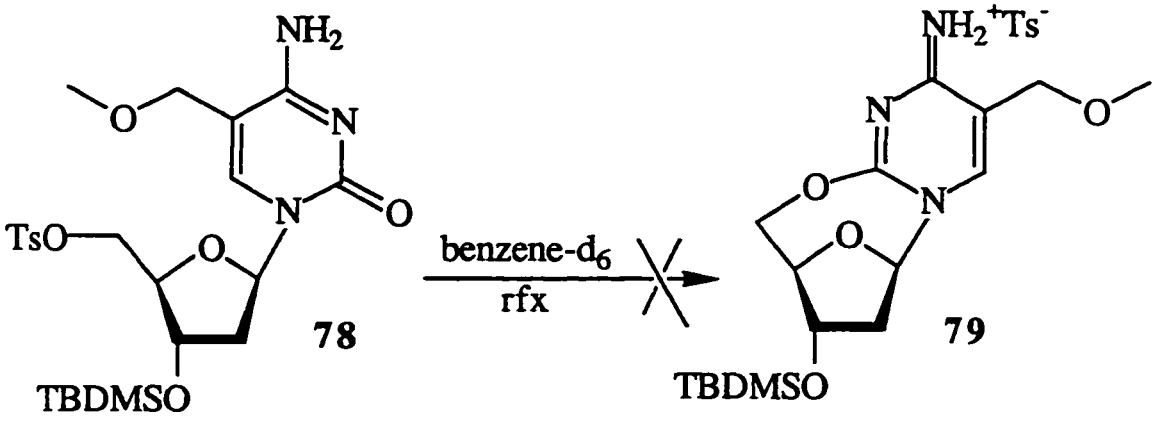


Fig. 3.16: Attempt to prepare 3-TBDMS-2-O-5'-anhydro-MMdCyd

Since thermal decomposition was observed in all the attempts to prepare the desired intermediate 2-O-5'-anhydro-MMdCyd (**70**), the thought of pursuing the intramolecular rearrangement (formation of 2-O-5'-anhydro bond) under non thermal reaction conditions came to mind. Under such conditions, the half-life of the starting material would be prolonged substantially giving ample reaction time for a cyclization to proceed. Two parallel experiments were conducted, one in which the compound 5'-tosyl-MMdCyd (**68**) was dissolved in D_2O and the other in acetone-d_6 as a solvent. The two solutions were placed in two NMR tubes and were sonicated using an ultrasound bath.[55] In D_2O, the ultra-sound waves produce air bubbles that collapse to give rise to two species of free radicals $D^{\cdot}$ and $DO^{\cdot}$ which might initiate the desired cyclization through a free radical mechanism.[55] While in an organic solvent such as acetone, the ultrasound waves would exert pressure on the system which might force the reaction forward. The temperature of the bath was maintained at operating temperature of 48°C and both reaction mixtures monitored by NMR. Again, no progress towards formation of 2-O-5'-anhydro linkage was observed even after 72 h. Decomposition of starting material in both NMR tubes was much less than in an equivalent period of thermal

reaction time.

Further attempts were made on the preparation of 2-imino-MMdCyd (**67**). These attempts were aimed at converting the 4-oxo group of MMdUrd (**6**) or a suitably substituted analog of **6** into a 4-thio group which then can be easily transformed into a 4-amino group by treating with methanolic ammonia.[54] The first of these attempts involved protection of the compound 2-imino-MMdUrd (**66**) with acetyl groups.[56] The 3',5'-N^2-triacetyl-2-imino-MMdUrd (**80**) was mixed with two equivalents of phosphorus pentasulfide (P_2S_5) in pyridine.[56] The reaction mixture was heated to 80°C and monitored by UV for the disappearance of the λ_{max} of the starting material (260 nm, in MeOH).[56] After 24 h, the ratio of the intensity of the new λ_{max} (322 nm in MeOH, expected for a thioketo chromophore) to that of the starting material was 9/1. The purified material was an unidentified side product and not the desired 3',5'-N^2-triacetyl-4-thio-2-imino-MMdUrd (**81**). Fig. 3.17 shows the failed attempt.

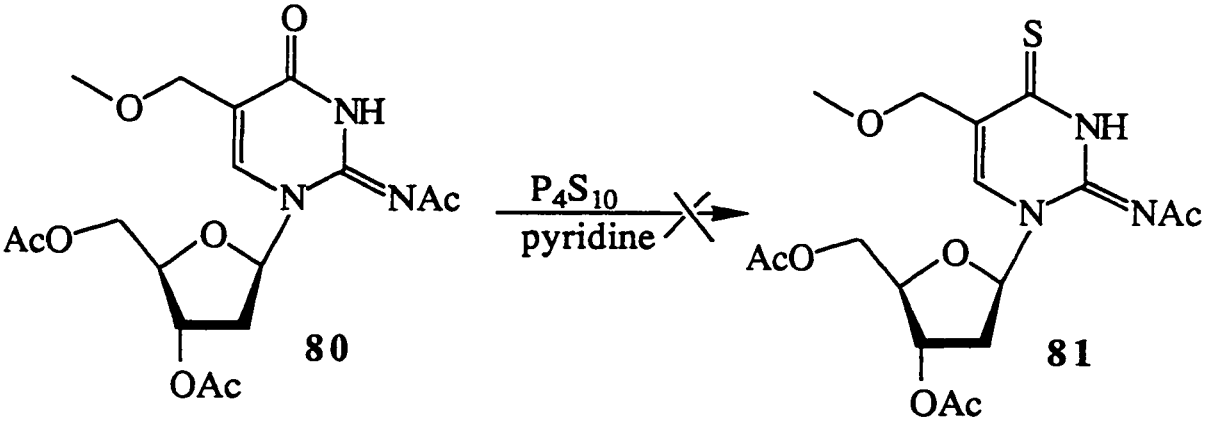


Fig. 3.17: Attempt to prepare 3',5'-N^2-triacetyl-4-thio-2-imino-MMdUrd

Another approach adopted for the preparation of 2-imino-MMdCyd (**67**) was to prepare the 3',5'-oxetane of **67**, (compound **84** starting from **82**) and then to achieve oxetane ring opening to the desired (R) configuration at

position 3' via either acid or base conditions. However, it was decided to test the possibility of such an oxetane ring opening on a test molecule first before using the expensive and hard to prepare MMdCyd (**18**). The 3',5'-thymidine oxetane (**85**) was chosen as a model (the desired ring cleavage would yield the naturally occurring 3'-R dThd (**10**)). Fig. 3.18 shows the proposed pathway to prepare **67**.

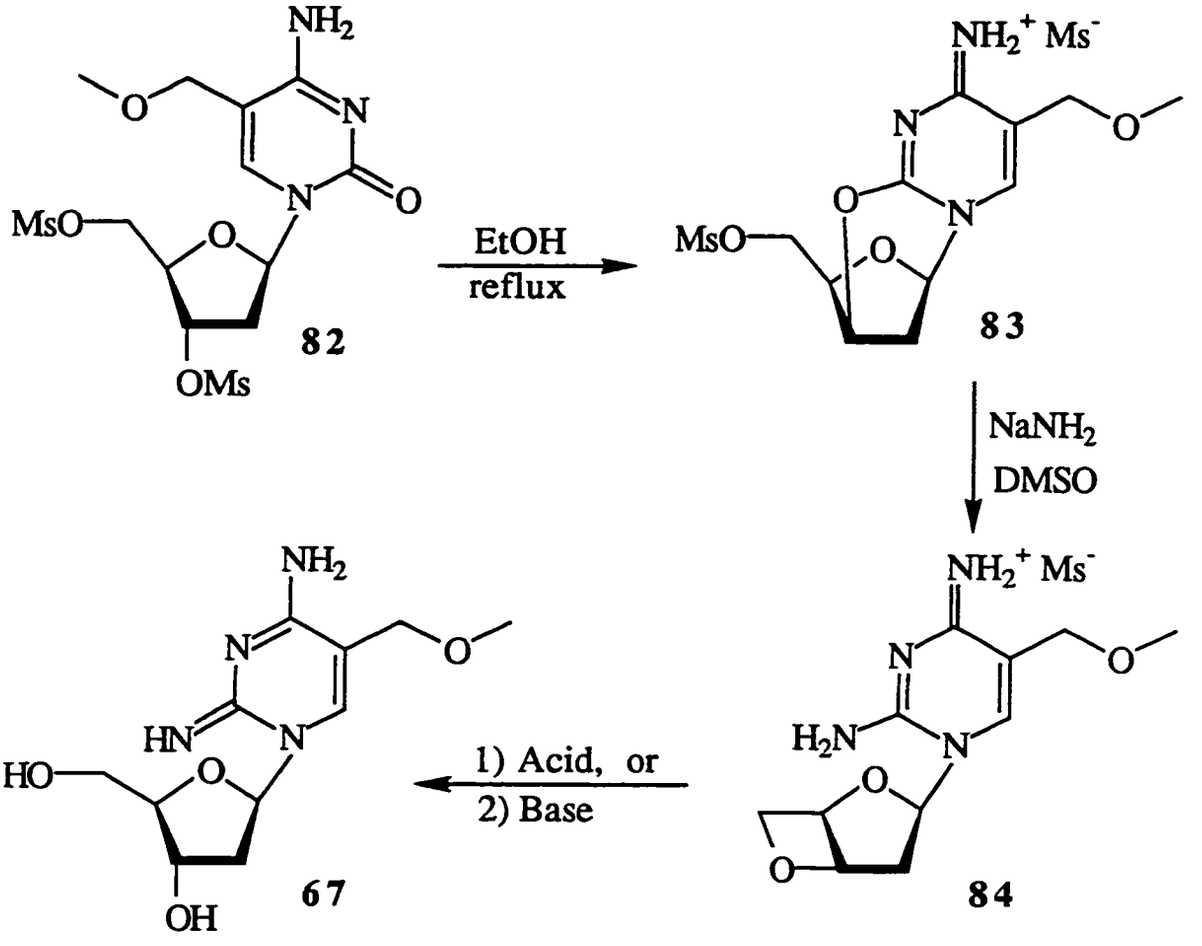


Fig. 3.18: Scheme to prepare 2-imino-MMdCyd (I)

A literature search on the opening of oxetane rings in nucleosides cited acid treatment of 3',5'-thymidine oxetane (**85**) to produce the undesired (S)

configuration at 3' position in low yield.[57] No cleavage with base was reported. A small amount of 3',5'-thymidine oxetane (**85**) was dissolved in 80% acetic acid and heated to 100° C for 1 h. TLC showed that no conversion to either dThd (**10**) or 3'-(S)-dThd (**86**) has occurred. The reaction was left for 24 h after which the oxetane ring in the starting material underwent partial hydrolysis to 3'-(S)- thymidine (**86**) as evidenced by TLC and NMR (fig. 3.19).

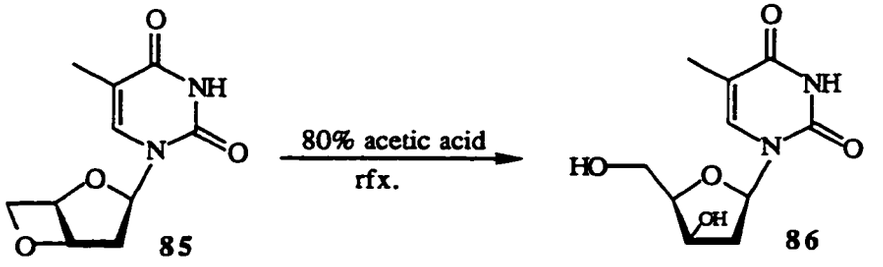


Fig. 3.19: Attempt to open thymidine oxetane with acid

Another small amount of **85** was dissolved in 1M NaOH and refluxed with occasional monitoring by TLC for 24 h. The thymidine oxetane maintained its integrity without being transformed into either dThd (**10**) or 3'-(S)-dThd (**86**) as can be seen in fig. 3.20. After workup, however, it was evident that there was some decomposition or cleavage at the glycosyl bond.

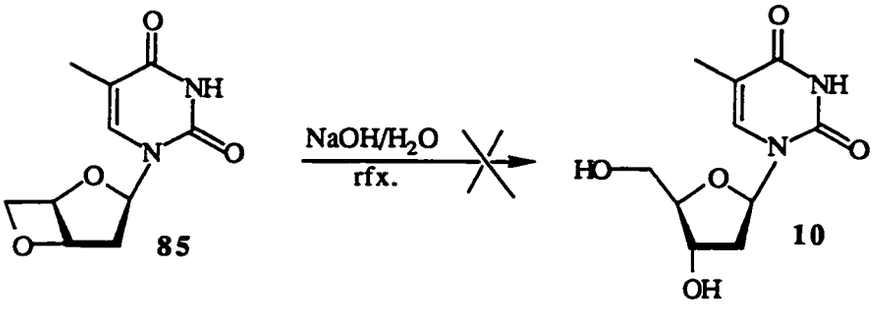


Fig. 3.20: Attempt to open thymidine oxetane with base

The final attempt on the preparation of **67** used non basic nucleophiles. In

one attempt, 3'-(S)-mesyl-5'-dimethylthexylsilyl-2-imino-MMdCyd (**75**) was dissolved in D_2O in an NMR tube and NaI was used as the nucleophile.[58] Another duplicate experiment was done in DMF as a solvent. The reaction was checked by NMR after one h of reaction time at room temperature. There was no progress in both NMR tubes so the temperature was raised to 100°C. Reaction progress was monitored by TLC, until all the starting material was consumed. NMR spectra of the crude reaction mixtures showed that decomposition had taken place. The two reaction mixtures were purified on PTLC which confirmed the original conclusion of decomposition.

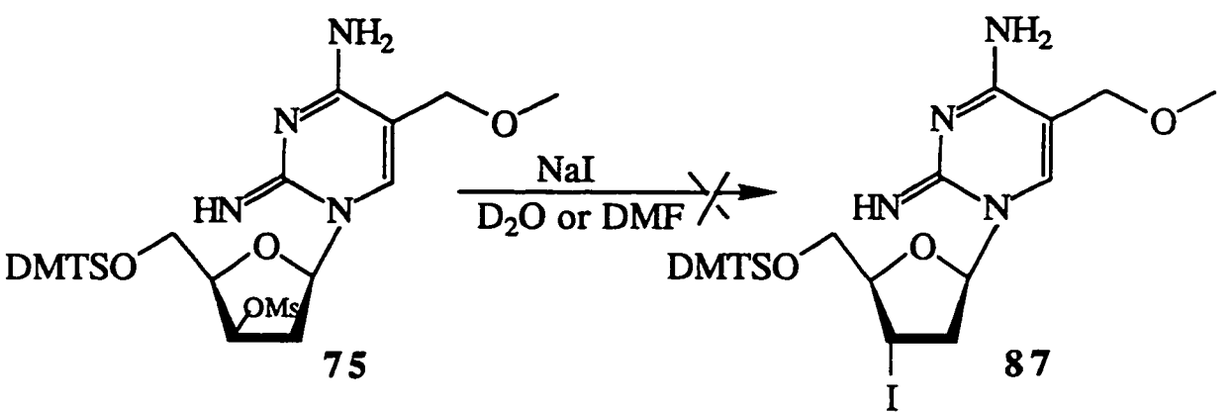


Fig. 3.21: Attempt to prepare 5'-DMTS--3'-iodo-2-imino-MMdCyd

The objective of the above experiment was to have the 3'-(R)-iodo compound **87** to facilitate an intramolecular substitution by the 2-imino group to form a 2-N-3'-anhydro bond which has been reported in the literature,[59] (compound **88**) and then attempt to open that link with an aqueous base to have the $C_{3'}$ (R) compound **89**. Fig. 3.22 explains the objective.

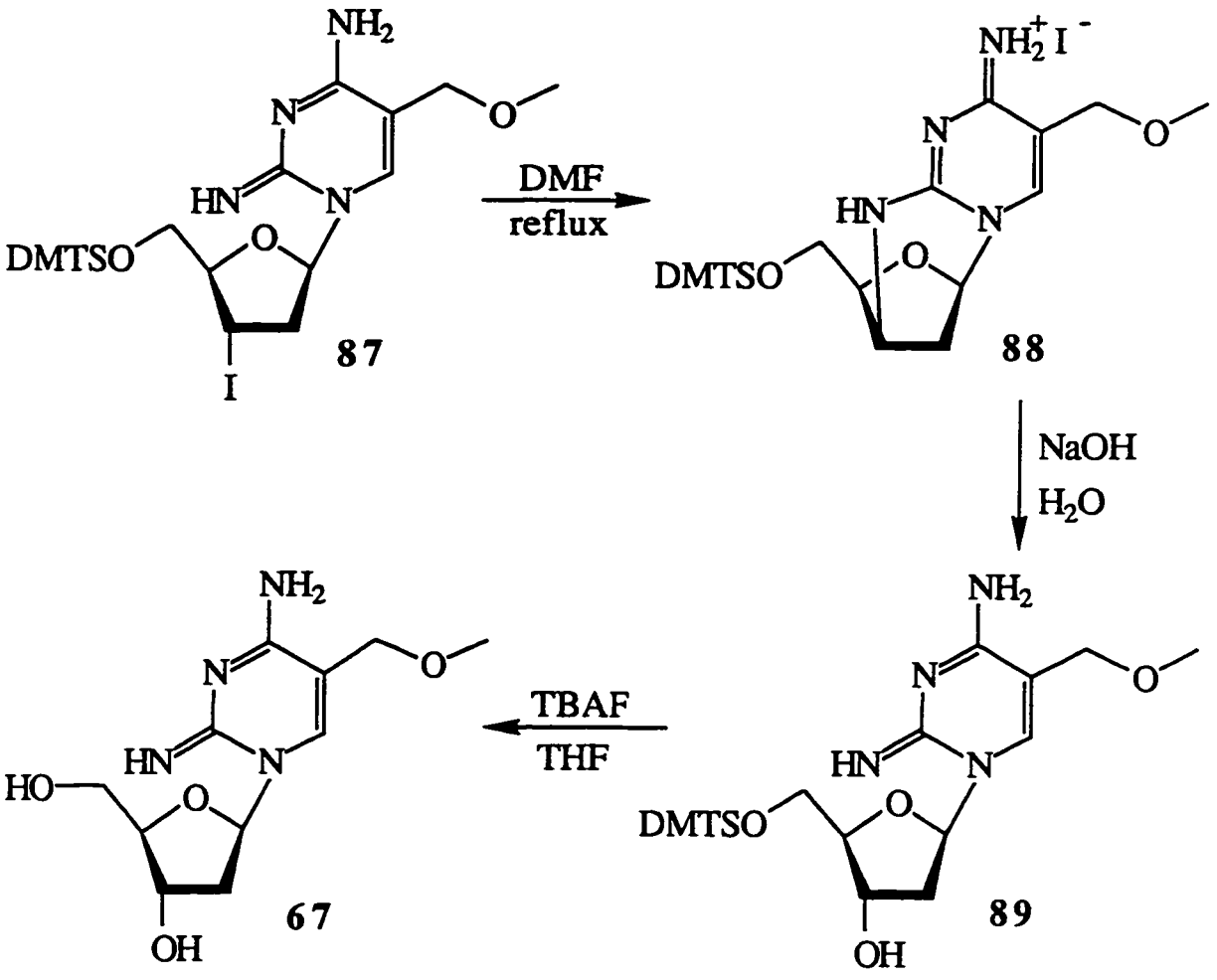


Fig. 3.22: Scheme to prepare 2-imino-MMdCyd (II)

The final attempt at the synthesis of **67** based on a literature precedent[60] was to use sodium acetate, or sodium benzoate as the nucleophile in DMF. This would have ended up with an acetate or a benzoate group with the proper (R) configuration at $C_{3'}$. Deprotection with ammonia of the acetate or benzoate groups would have exposed the desired 3'-(R)-hydroxy functional group. In one experiment, 3'-(S)-mesyl-5'-dimethylthexylsilyl-2-imino-MMdCyd (**75**) was dissolved in DMF and sodium acetate was added to it. After the reaction was over, the NMR of the crude product showed the presence of olefinic signals.

Purification of the crude mixture on PTLC and examination of the various products, proved that the desired substitution reaction and thus inversion of configuration did not take place. Instead, elimination took place and the compound 5'-dimethylthexylsilyl-2',3'-dideoxy-2',3'-didehydro-2-imino-MMdCyd (**77**) which was isolated in a previous experiment was obtained. Deprotection of **77** afforded 2',3'-dideoxy-2',3'-didehydro-2-imino-MMdCyd (**90**) (2-imino-MMddCyd) as can be seen in fig. 3.23.

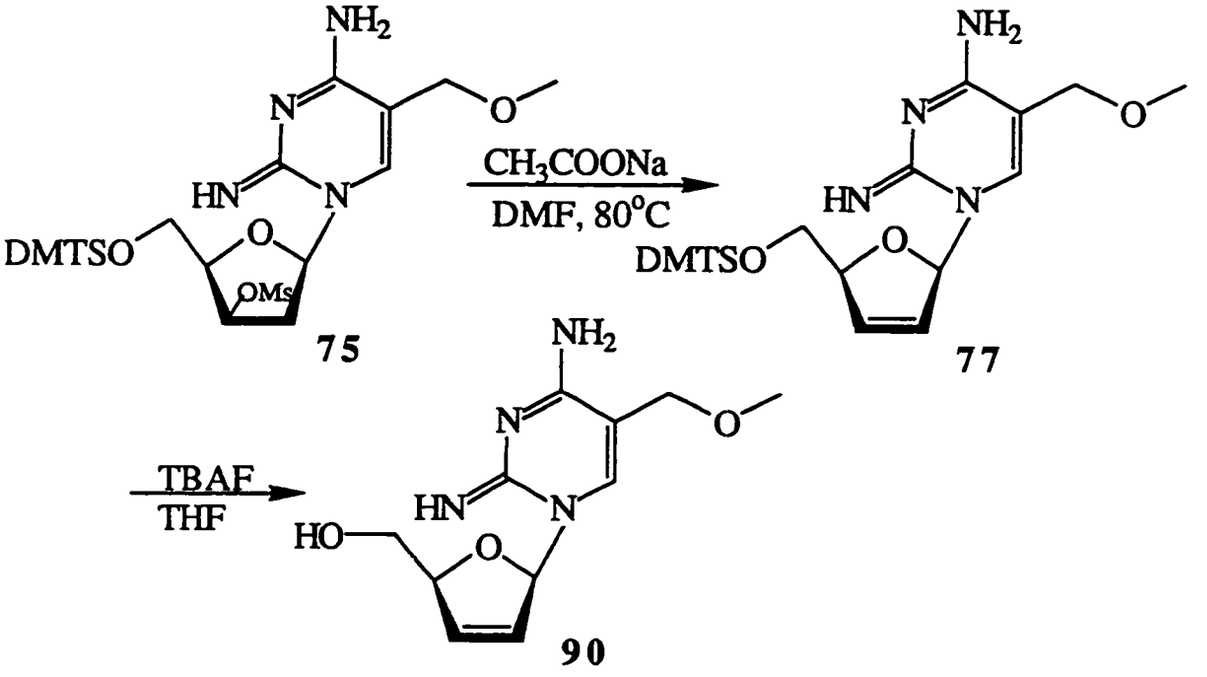


Fig. 3.23: Preparation of 2-imino-MMddCyd

The other attempt was made using the same starting material **75** in DMF, with 2 molar equivalents of sodium benzoate. The reaction mixture was heated to 80°C and monitored by TLC, and after 8 h it was evident that all starting material has been consumed. The crude reaction mixture was concentrated and examined by NMR. There were peaks indicating that a new non olefinic compound has been formed. The crude product was purified by PTLC and the

result was not the anticipated one to yield compound **91**. Instead of the nucleophilic attack occurring at $C_{3'}$, the attack took place at $C_{1'}$ giving rise to the deoxyribose **92** described in fig. 3.24.

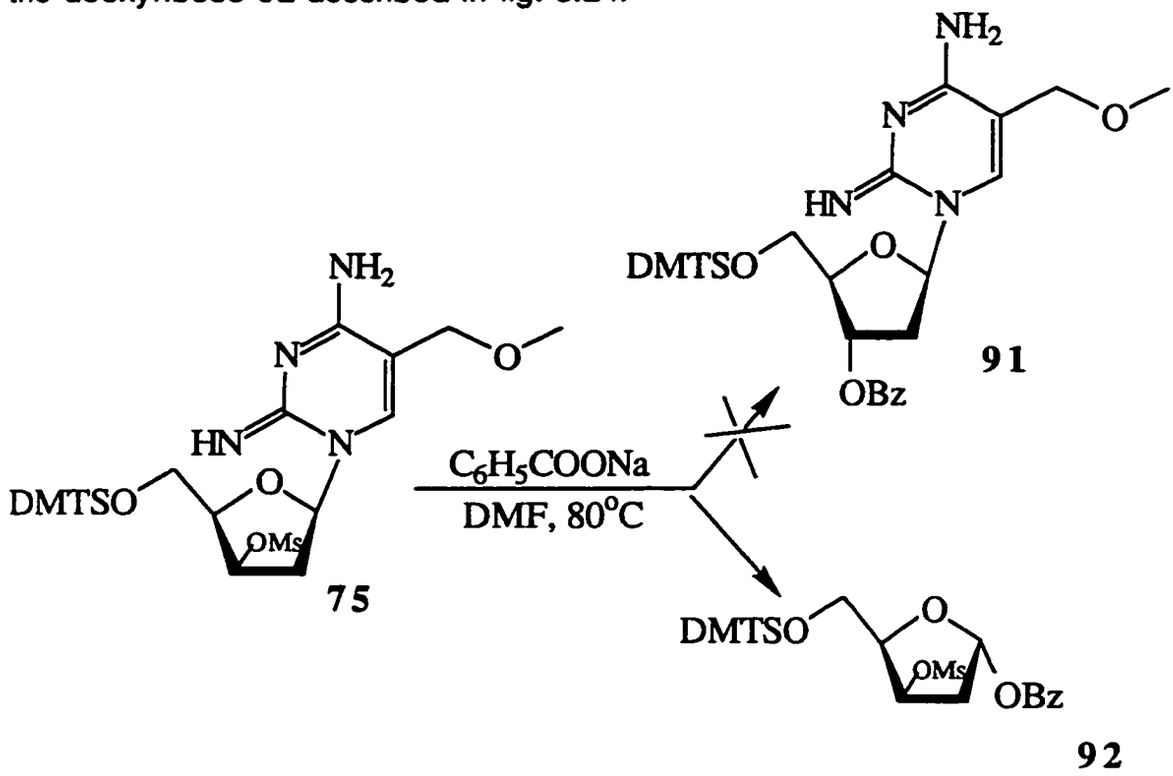


Fig. 3.24: Attempt to prepare 2-imino-MMdCyd (II)

Attempts to prepare 2-imino-MMdCyd (**67**) were terminated at compound **90** for the following reasons. The time and effort already spent on attempting to prepare one compound was out of proportion with the scope of this study aimed at preparing many compounds. Another reason is the extreme polarity of 2-imino analogs which may prove to be counterproductive for therapeutic applications even if the analog 2-imino-MMdCyd (**67**) was prepared and proved to be active. The third reason is that it became obvious at this point from conformational studies (chapter 4) that analogs with a *syn* glycosidic torsional preference such as 2-imino-MMdUrd (**66**) and 2-imino-MMddCyd

(**67**) are inactive.

The final analog of MMdCyd (**18**) in this section is a compound with a rigid link between N_3 and N^4. The compound 3,4-etheno-MMdCyd (**94**) was prepared using MMdCyd (**18**) and a large excess of aqueous chloroacetaldehyde.[61] Fig. 3.25 describes the preparation.

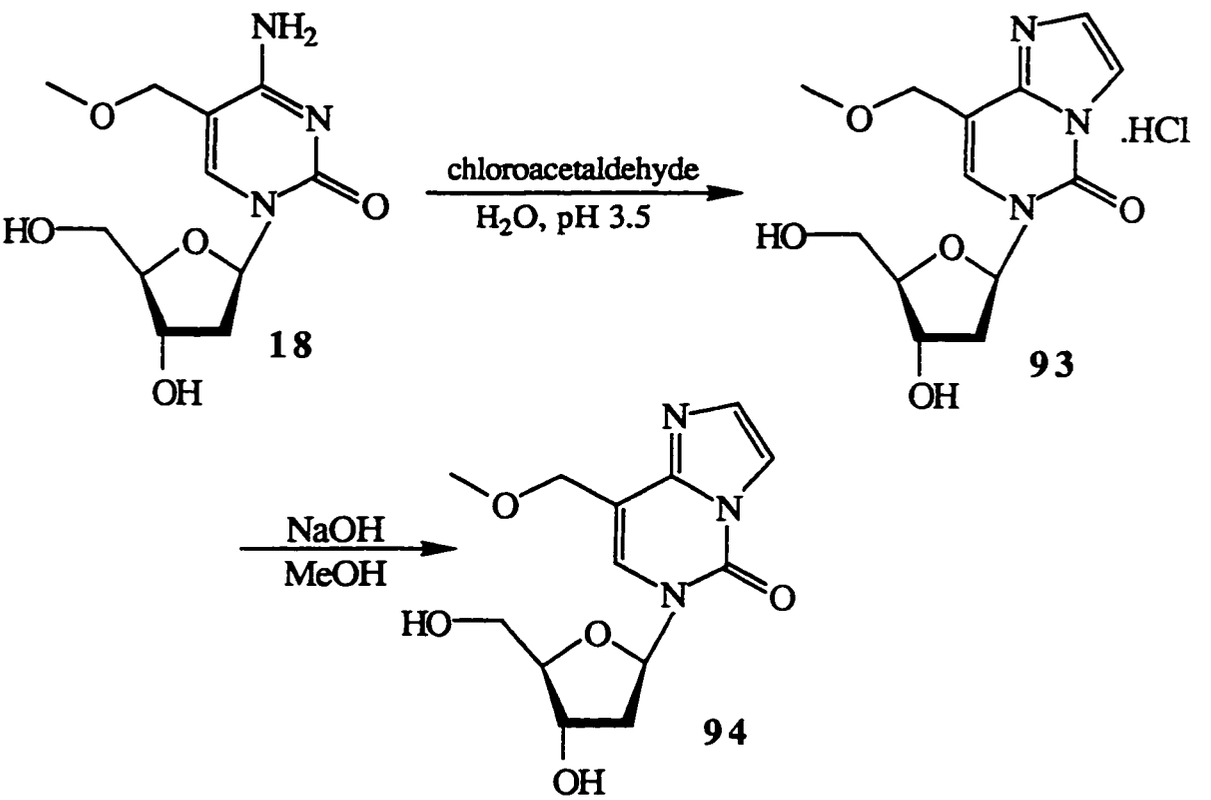


Fig. 3.25: Preparation of 3,4-etheno-MMdCyd

Moving to another front in synthetic preparations, analogs of BrVdCyd (**17**) were targeted. The analog BrVdCyd (**17**) is a potent antiherpes agent when deamination is prevented.[52] Three acyl analogs of **17** were prepared using the same methodology that was reported on earlier to prepare acyl analogs of MMdCyd (**18**).[39] These analogs are N^4-acetyl-BrVdCyd (**95**), N^4-propanoyl-

BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**). Fig. 3.26 shows the preparation of the three analogs **95**, **96** and **97**.

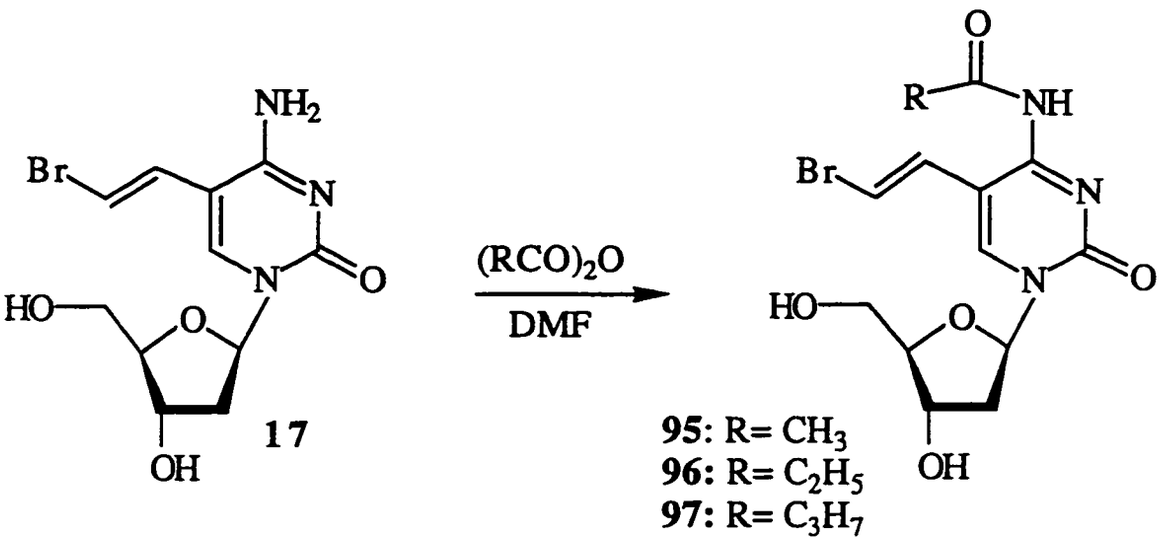


Fig. 3.26: Preparation of N^4-acyl BrVdCyd analogs

N^4-Methyl-BrVdCyd (**101**) was also prepared for the purpose of comparing it with its counterpart N^4-methyl-MMdCyd (**24**). Again, the same synthetic pathway to prepare **24** was adopted.[21] The 3' and 5' hydroxyl groups of the deoxyribose moiety of BrVdUrd (**8**) were protected with acetyl groups. The protected product 3',5'-diacetyl-BrVdUrd (**98**) was transformed to the N^4-triazolyl intermediate **99**, which was transformed into 3',5'-diacetyl-N^4-methyl-BrVdCyd (**100**), which in turn afforded the analog N^4-methyl-BrVdCyd (**101**) upon deprotection. Fig. 3.27 describes the steps used.

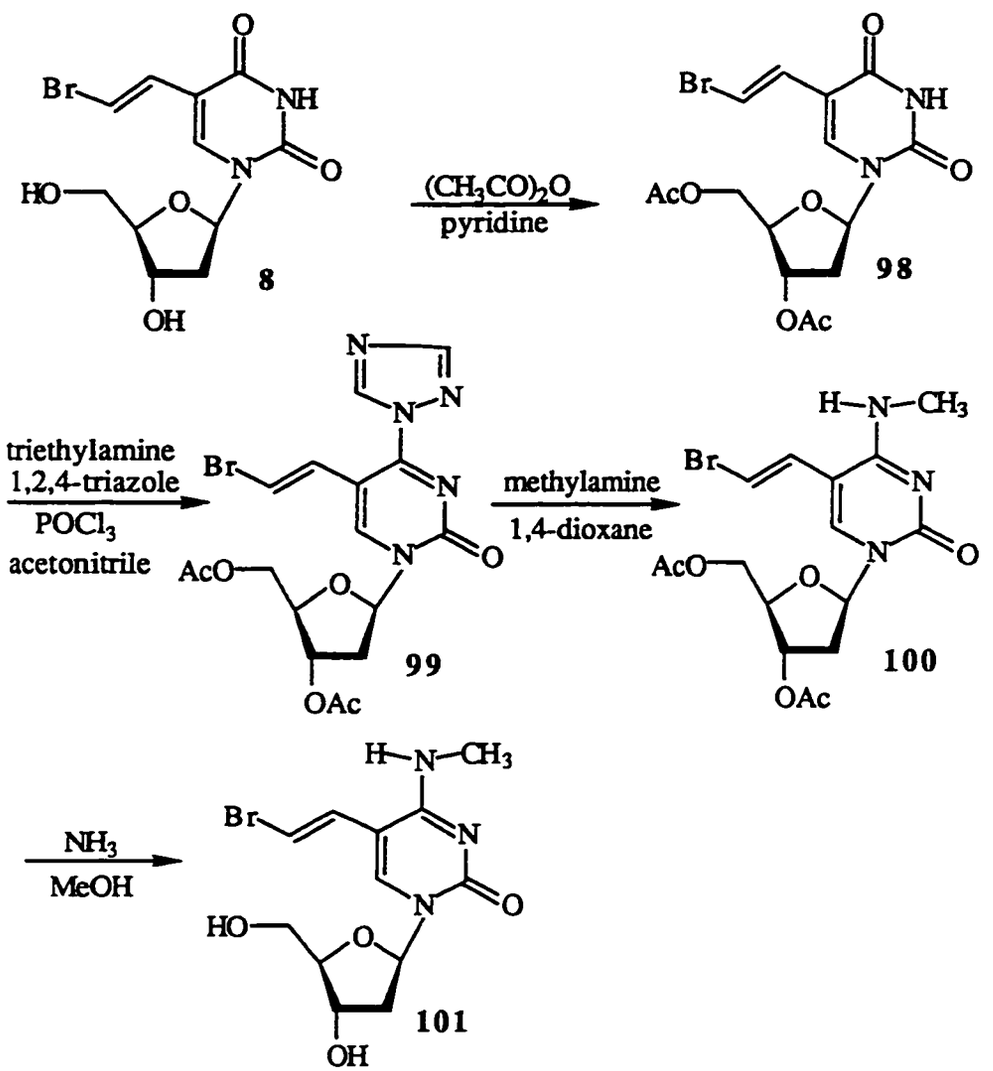


Fig. 3.27: Preparation of N^4-Me-BrVdCyd

The final two analogs in this series of preparations are derivatives of the nucleoside 5-methyl-2'-deoxycytidine (**29**) which has no activity against herpes simplex virus. These two analogs are N^4-methoxy-5-methyl-2'-deoxycytidine (**105**) and N^4-hydroxy-5-methyl-2'-deoxycytidine (**106**). The simple protocol of modifying the structure of 5-methyl-2'-deoxycytidine (**29**) and observing its effect on both activity and conformation was adopted.

The compound 3',5'-diacetyl-N^4-triazolyl-5-methyl-2'-deoxycytidine[63] (**102**)

was transformed to 3',5'-diacetyl-N^4-methoxy-5-methyl-2'-deoxycytidine (**103**) and 3',5'-diacetyl-N^4-hydroxy-5-methyl-2'-deoxycytidine (**104**) as described in a previous experiment.[43] It was noticed upon purification of **103** and **104** that partial hydrolysis of the acetyl groups had occurred. The compounds **103** and **104** were deprotected in methanolic ammonia to give N^4-methoxy-5-methyl-2'-deoxycytidine (**105**) and N^4-hydroxy-5-methyl-2'-deoxycytidine (**106**). Fig. 3.28 shows the route used for preparation.

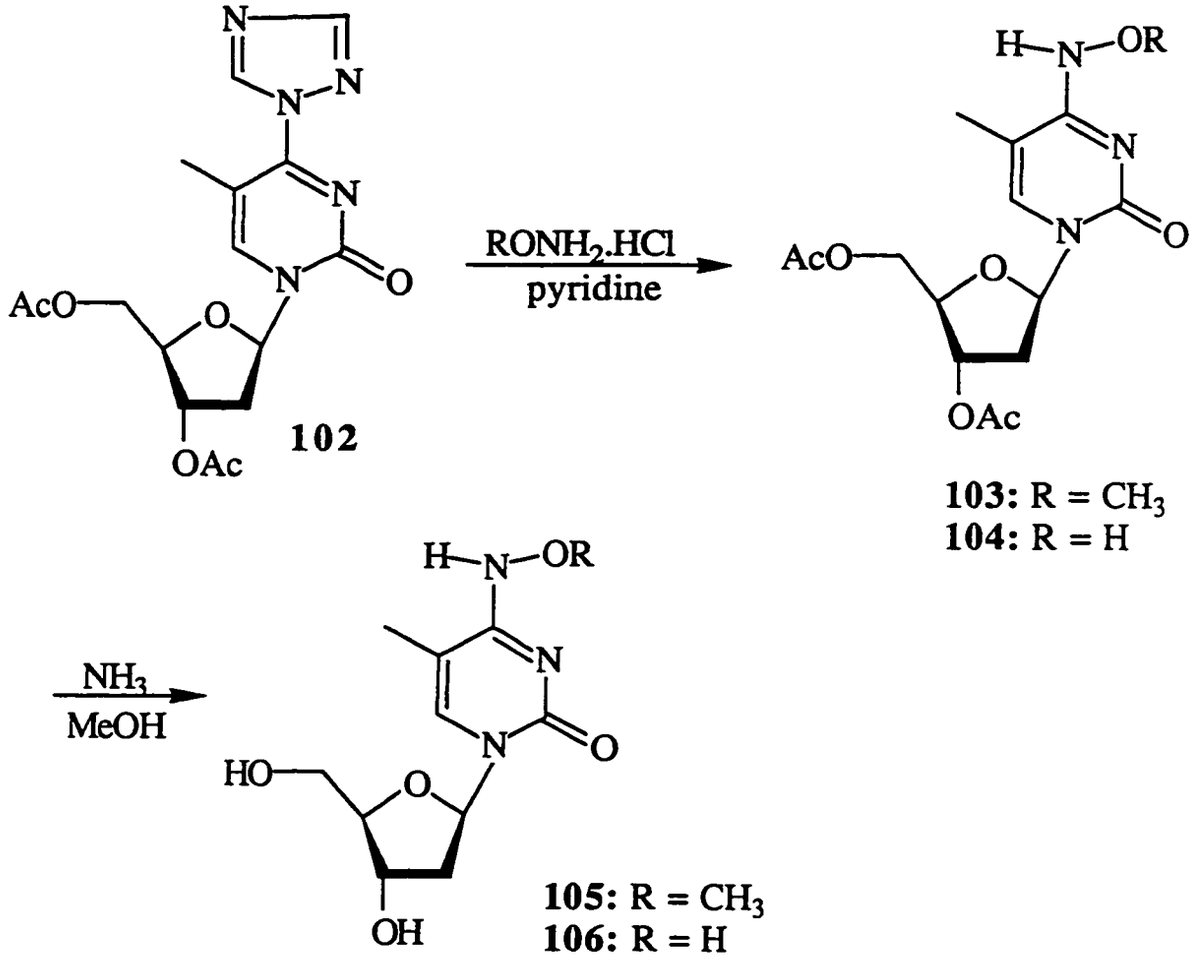


Fig. 3.28: Preparation of N^4-methoxy -5-Me-dCyd and N^4-hydroxy-5-Me-dCyd

3.3) Experimental

All solvents were distilled prior to use and stored in Aldrich sure seal bottles. Acetonitrile was stirred with CaH_2 and fractionally distilled. THF was purified by refluxing with and distilling from $LiAlH_4$.[64] Acetone was dried with anhydrous $CaSO_4$ and then distilled. DMSO was dried with Linde type 4A molecular sieves and then fractionally distilled at low pressure.[64] CH_2Cl_2 was distilled from P_2O_5.[64] 1,4-Dioxane was refluxed with and distilled from $NaBH_4$. $CHCl_3$ was fractionally distilled. Commercial anhydrous pyridine was used without purification. Acetic anhydride was dried by standing over P_2O_5 for 3 h followed by standing over ignited K_2CO_3 for a further 3 h, then purified by azeotropic distillation with toluene.[64] Unless otherwise noted, reactions were carried out under atmospheric air and reaction temperature refers to the bath. Preparative thin-layer chromatography (PTLC) was carried out on glass plates (20x20cm) precoated (0.25mm) with silica gel 60 F_{254}. Materials on a PTLC were detected by visualization under an ultraviolet lamp (254 nm) and/or by treating a small section removed from the plate with a solution of phosphomolybdic acid (5%) containing a trace of ceric sulfate in aqueous sulfuric acid (5% v/v), followed by charring on a hot plate. Flash column chromatography (FCC) was performed according to Still *et al.*[65] with silica gel, 60 Angstroms (40-63 μm).

^1H nuclear magnetic resonance (^1H NMR) spectra and ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra were measured on a Bruker AM-300 spectrometer. For ^1H NMR, residual $CHCl_3$ in $CDCl_3$ was employed as the internal standard and assigned as 7.26 ppm downfield from TMS. Residual HDO In D_2O was employed as the internal standard and assigned as 4.67 ppm downfield from TMS. The ^1H NMR assignments were made on the basis of chemical shift and where necessary, confirmed by homonuclear decoupling.

^1H NMR spectra were obtained with a digital resolution of 0.244 Hz/pt (sweep width = 4000 Hz, FID = 16 K data points). Actual spectra for second order analysis were obtained using NMR samples of 0.1 M concentration in D_2O without adjusting the pD. Experimental spectra for PSEUROT were obtained using the Brucker routine PANIC on an Aspect mini-computer of the 300-MHz NMR spectrometer with coupling constants from observed spectra used as a starting point.

Ultraviolet spectra were obtained using a Kontron Uvikon 860 spectrophotometer with a Kontron printer. Samples were dissolved in a phosphate buffer solution (pH 7.2). Extinction coefficients were rounded to the nearest zero.

Mass spectra were obtained on a FISONS VG70SE high resolution mass spectrometer with FAB capacity. The matrix used to dissolve the samples was 3-nitrobenzyl alcohol.

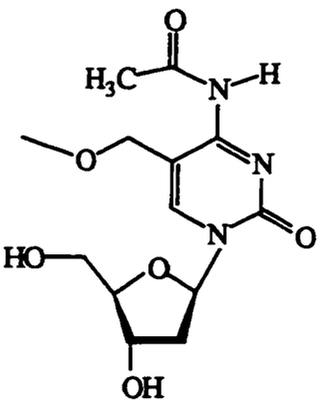
Melting points were measured on an Electothermal melting point apparatus and are uncorrected.

Notes

-The phrase "room temperature" refers to an ambient temperature of 21-24° C.

-The phrase "freeze-dried" refers to dissolving a compound in a sufficient amount of distilled water, freezing the solution and placing it in a freeze-drier until all of the solvent has evaporated.

N^4-acetyl-5-methoxymethyl-2'-deoxycytidine. (**30**)



The nucleoside 5-methoxymethyl-2'-deoxycytidine (**18**) (150 mg, 0.55 mmol) was dissolved in DMF (1.5 mL) at room temperature. The solution was cooled in an ice water bath under argon and acetic anhydride (63 µL, 0.66 mmol) was added. The reaction mixture was left stirring at room temperature for 24 h. A TLC (15% MeOH in CH_2Cl_2) indicated complete reaction. The entire solution was concentrated to dryness under high vacuum and the crude mixture was applied on a silica gel plug 3 cm in depth and eluted with several column volumes of 5% methanol in dichloromethane to yield a white solid product (122 mg, 0.39 mmol, yield 70%). This product was freeze-dried to obtain the final white powder, which was submitted for biological testing.

^{1}H NMR (D_2O): 8.25 (1H, s, H_6), 6.08 (1H, dd, $H_{1'}$), 4.30 (3H, m, CH_2 and $H_{3'}$), 4.00 (1H, ddd, $H_{4'}$), 3.75 (1H, dd, $H_{5'}$), 3.65 (1H, dd, $H_{5''}$), 3.28 (3H, s, OCH_3) 2.48 (1H, m, $H_{2''}$), 2.19 (4H, m, acetyl CH_3 and $H_{2'}$).

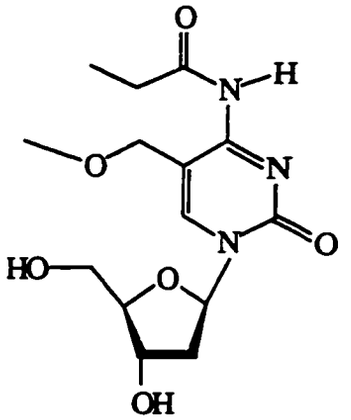
^{13}C NMR (D_2O): 89.6 (d, $C_{1'}$), 42.2 (t, $C_{2'}$), 72.1 (d, $C_{3'}$), 89.8 (d, $C_{4'}$), 63.0 (t, $C_{5'}$), 158.6 (s, C_2), 163.7 (s, C_4), 109.0 (s, C_5), 147.1 (d, C_6), 69.9 (t, CH_2), 59.6 (q, OCH_3), 175.7 (s, acetyl CO) 26.9 (q, CH_3)

UV: λ_{min}: 266 nm, λ_{max}: 298 nm (ε, 7500)

MS: FAB mass 314.1 (M^++1), acc. mass found 314.1347, calc. (M^++1) 314.1335

mp: 138-140°C

N^4-propanoyl-5-methoxymethyl-2'-deoxycytidine. (**31**)



The compound 5-methoxymethyl-2'-deoxycytidine (**18**) (100 mg, 0.37 mmol) was dissolved in DMF (1.0 mL) at room temperature. The solution was cooled in an ice water bath under argon and propanoic anhydride (53 μL, 0.41 mmol) was added using a syringe. The reaction mixture was stirred at room temperature for 24 h. A TLC (20% MeOH in CH_2Cl_2) indicated complete reaction. One mL of absolute ethanol was added to the crude reaction mixture and stirring for 5 min, the reaction mixture was concentrated to dryness under high vacuum. The oily crude mixture was dissolved in chloroform and applied on a silica gel plug 3 cm in depth and eluted with several column volumes of 5% methanol in dichloromethane to yield a white solid product (88.3 mg, 0.27 mmol, yield 74%). The product was freeze-dried and white amorphous solid was submitted for biological testing.

^{1}H NMR (D_2O): 8.21 (1H, s, H_6), 6.02 (1H, dd, $H_{1'}$), 4.33 (3H, m, CH_2O and $H_{3'}$), 3.95 (1H, ddd, $H_{4'}$), 3.75 (1H, dd, $H_{5'}$), 3.60 (1H, dd, $H_{5''}$), 3.20 (3H, s, OCH_3), 2.55-2.40 (3H, m, CH_2 and $H_{2''}$), 2.15 (1H, ddd $H_{2'}$), 0.95 (3H, t, CH_3).

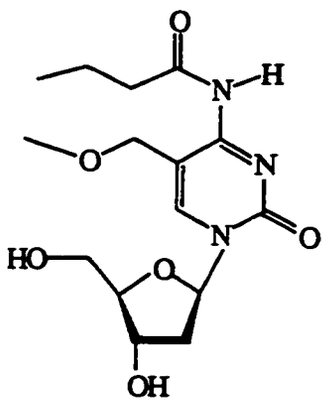
^{13}C NMR (D_2O): 89.5 (d, $C_{1'}$), 42.2 (t, $C_{2'}$), 72.1 (d, $C_{3'}$), 89.8 (d, $C_{4'}$), 63.0 (t, $C_{5'}$), 158.6 (s, C_2), 163.8 (s, C_4), 108.9 (s, C_5), 147.0 (d, C_6), 70.0 (t, CH_2O), 59.5 (q, OCH_3), 178.9 (s, propanoyl CO), 33.2 (t, CH_2), 10.7 (q, CH_3).

UV: λ_{min}: 266 nm, λ_{max}: 298 nm (ε, 8040)

MS: FAB mass 328.1 (M^++1), acc. mass found 328.1506, calc. (M^++1) 328.1463

mp: 123-125°C

N^4-butanoyl-5-methoxymethyl-2'-deoxycytidine. (**32**)



The nucleoside 5-methoxymethyl-2'-deoxycytidine (**18**) (140 mg, 0.52 mmol) was dissolved in DMF (1.5 mL) at room temperature. The solution was cooled in an ice water bath under argon and butyric anhydride (93 µL, 0.57 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. A TLC (15% MeOH in CH_2Cl_2) indicated complete reaction. One mL of absolute ethanol was added to the reaction mixture and stirred for 5 min. The solution was concentrated to dryness under high vacuum, the oily crude mixture was dissolved in chloroform and applied on a silica gel plug 3 cm in depth. Elution with several column volumes of 5% methanol in dichloromethane yielded a white solid product (120 mg, 0.35 mmol, yield 68%). The product was freeze-dried and white amorphous solid was submitted for biological testing.

^{1}H NMR (D_2O): 8.21 (1H, s, H_6), 6.05 (1H, dd, $H_{1'}$), 4.24 (3H, m, CH_2O and $H_{3'}$), 3.95 (1H, ddd, $H_{4'}$), 3.75 (1H, dd, $H_{5'}$), 3.57 (1H, dd, $H_{5''}$), 3.18 (3H, s, OCH_3), 2.50-2.35 (3H, m, α CH_2 and $H_{2''}$), 2.15 (1H, ddd $H_{2'}$), 1.50 (2H, m, β CH_2), 0.78 (3H, t, CH_3).

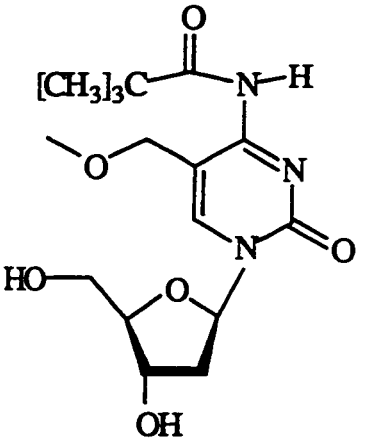
^{13}C NMR ($CDCl_3$): 87.7 (d, $C_{1'}$), 40.4 (t, $C_{2'}$), 69.5 (d, $C_{3'}$), 87.9 (d, $C_{4'}$), 61.2 (t, $C_{5'}$), 153.5 (s, C_2), 160.2 (s, C_4), 106.4 (s, C_5), 144.0 (d, C_6), 68.8 (t, CH_2O), 58.2 (q, OCH_3), 170.9 (s, CO), 34.2 (t, α CH_2), 25.3 (t, β CH_2), 13.6 (q, CH_3).

UV: λ_{min}: 268 nm, λ_{max}: 300 nm (ε, 7800)

MS: FAB mass 342.1 (M^++1), acc. mass found 342.1671, calc. (M^++1) 342.1632

mp: 100-102° C

N^4-trimethylacetyl-5-methoxymethyl-2'-deoxycytidine. (**33**)



The compound 5-methoxymethyl-2'-deoxycytidine (**18**) (50 mg, 0.19 mmol) was dissolved in DMF (0.5 mL) at room temperature. The solution was cooled in an ice water bath under argon and trimethylacetic anhydride (41 μL, 0.20 mmol) was added using a syringe. The reaction mixture was left at room temperature for 24 h. A TLC (10% MeOH in CH_2Cl_2) indicated complete reaction. The reaction mixture was concentrated to dryness under high vacuum. The crude mixture was dissolved in chloroform, applied on a FCC column 3 cm in length and eluted with several column volumes of 5% methanol in dichloromethane. The product was isolated as a white solid (57 mg, 0.16 mmol, yield 84%). The product was freeze-dried and submitted for biological testing.

^{1}H NMR (D_2O): 8.22 (1H, s, H_6), 6.05 (1H, dd, $H_{1'}$), 4.40-4.20 (3H, m, CH_2 and $H_{3'}$), 4.00 (1H, m, $H_{4'}$), 3.75 (1H, dd, $H_{5'}$), 3.65 (1H, dd, $H_{5''}$), 3.28 (3H, s, OCH_3), 2.45 (1H, m, $H_{2''}$), 2.15 (1H, m, $H_{2'}$), 1.12 (9H, s, $(CH_3)_3$).

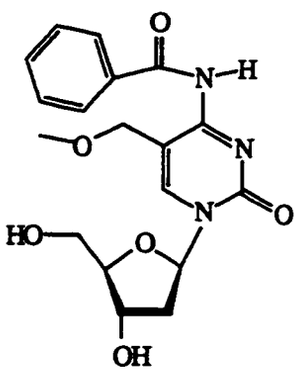
^{13}C NMR (D_2O): 89.6 (d, $C_{1'}$), 42.2 (t, $C_{2'}$), 72.2 (d, $C_{3'}$), 89.8 (d, $C_{4'}$), 63.1 (t, $C_{5'}$), 158.7 (s, C_2), 164.3 (s, C_4), 108.7 (s, C_5), 146.4 (d, C_6), 70.7 (t, CH_2), 59.7 (q, OCH_3), 182.2 (s, pivaloyl CO), 28.5 (q, pivaloyl $(CH_3)_3$).

UV: λ_{min}: 269 nm, λ_{max}: 301nm (ε, 8300)

MS: FAB mass 356.1 (M^++1), acc. mass found 356.1834, calc. (M^++1) 356.1853

mp: 110-111°C

N^4-benzoyl-5-methoxymethyl-2'-deoxycytidine. (**34**)



The compound 5-methoxymethyl-2'-deoxycytidine (**18**) (135 mg, 0.50 mmol) was dissolved in pyridine (2 mL) at room temperature. TMSCl (190 µL, 1.50 mmol) was added dropwise and the solution stirred. The reaction progress was monitored by TLC (10% MeOH in CH_2Cl_2) for two h during which time all starting material was consumed. To the crude mixture, Et_3N (80 µL, 0.56 mmol) was added followed by BzCl (65 µl, 0.55 mmol) with continuous stirring. After 5 min., a TLC (5% MeOH in CH_2Cl_2) indicated complete reaction. One mL of 50 % MeOH / H_2O was added to the above and stirred for half an hour. The crude mixture was extracted with $CHCl_3$ (100 mL), the organic layer washed with sat. $NaHCO_3$ (25 mL), and then with water (25 mL). The organic fraction was dried with Na_2SO_4, concentrated and purified on a silica gel plug 3 cm in depth using several column volumes of 5% MeOH in CH_2Cl_2. The product, (113 mg, 0.30 mmol, yield 60%) was obtained as a white solid, freeze-dried and submitted for biological testing.

^1H NMR (D_2O): 8.22 (1H, s, H_6), 7.75-7.38 (5H, m, Bz), 6.08 (1H, dd, $H_{1'}$), 4.36 (2H, s, CH_2O), 4.28 (1H, ddd, $H_{3'}$), 3.96 (1H, ddd, $H_{4'}$), 3.75 (1H, dd, $H_{5'}$), 3.62 (1H, dd, $H_{5''}$), 3.25 (3H, s, OCH_3), 2.43 (1H, m, $H_{2''}$), 2.20 (1H, m, $H_{2'}$).

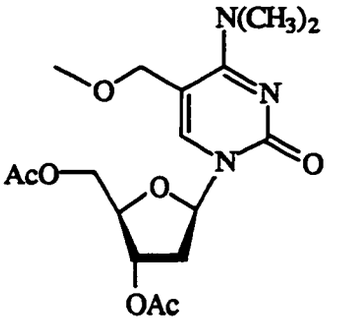
^{13}C NMR (D_2O): 88.1 (d, $C_{1'}$), 42.0 (t, $C_{2'}$), 71.8 (d, $C_{3'}$), 90.1 (d, $C_{4'}$), 63.4 (t, $C_{5'}$), 158.1 (s, C_2), 163.7 (s, C_4), 107.9 (s, C_5), 146.1 (d, C_6), 70.4 (t, CH_2), 59.5 (q, OCH_3), 180.5 (s, benzoyl CO), 130.4 -126.2 (phenyl carbons).

UV: λ_{min}: 238 nm, λ_{max}: 308 nm (ε, 9200)

MS: FAB mass 376.1 (M^++1), acc. mass found 376.1502, calc. (M^++1) 376.1479

mp: 125-126°C

3',5'-diacetyl-N^4,N^4- dimethyl-5-methoxymethyl-2'-deoxycytidine. (**39**)



The compound 3',5'-diacetyl-5-methoxymethyl-2'-deoxyuridine (**37**) (322 mg, 0.90 mmol) in acetonitrile (5 mL), was added to a mixture of Et_3N (3.2 mL, 23 mmol), 1,2,4-triazole (1.68 g, 24.3 mmol) and $POCl_3$ (0.60 mL, 5.4 mmol) at 0°C. The mixture was stirred for 2 h at room temp. The reaction mixture was concentrated and the crude product extracted with ethyl acetate (150 mL) and washed with sat. $NaHCO_3$ (50 mL) then with water (50 mL), and finally with brine (50 mL). The organic layer was dried and concentrated under vacuum. The crude triazolyl intermediate (**38**) was dissolved in dioxane (5 mL), the solution was cooled in an ice water bath, and dimethylamine gas was bubbled into it. After 14 h the crude product 3',5'-diacetyl-N^4,N^4-dimethyl-5-methoxymethyl-2'-deoxycytidine was purified by FCC using 50% acetone/ether to yield a white solid material (208 mg, 0.54 mmol, yield 60%).

^1H NMR ($CDCl_3$): 7.55 (1H, s, H_6), 6.35 (1H, dd, $H_{1'}$), 5.19 (1H, m, $H_{3'}$), 4.35 (2H, d, $H_{5',5''}$), 4.25 (1H, m, $H_{4'}$), 4.12 (2H, d, CH_2), 3.35 (3H, s, OCH_3), 3.3 (6H, s, $N(CH_3)_2$), 2.65 (1H, dd, $H_{2''}$), 2.12-1.93 (7H, m, 2 acetyl CH_3 and $H_{2'}$).

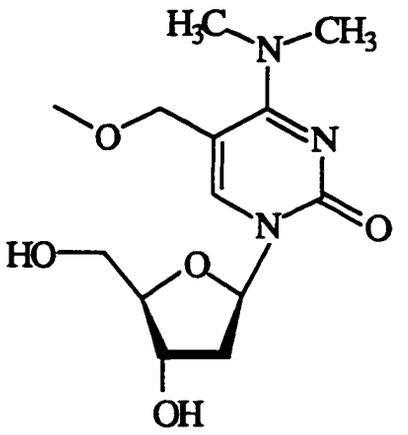
^{13}C NMR ($CDCl_3$): 82.3 (d, $C_{1'}$), 39.9 (t, $C_{2'}$), 74.5 (d, $C_{3'}$), 86.1 (d, $C_{4'}$), 63.9 (t, $C_{5'}$), 154.4 (s, C_2), 164.0 (s, C_4), 103.4 (s, C_5), 142.6 (d, C_6), 70.8 (t, CH_2), 57.4 (q, CH_3), 170.4 and 170.3 (s, 2CO), 38.6 and 38.5 (q, $N(CH_3)_2$), 20.9 and 20.7 (2q, 2 acetyl CH_3).

UV: λ_{min}: 251 nm (ε, 2100), λ_{max}: 280 nm (ε, 9800)

MS: FAB mass 384.1 (M^++1), acc. mass found 384.1795, calc. (M^++1) 384.1757

mp: 144-147°C

N^4,N^4-dimethyl-5-methoxymethyl-2'-deoxycytidine. (**43**)



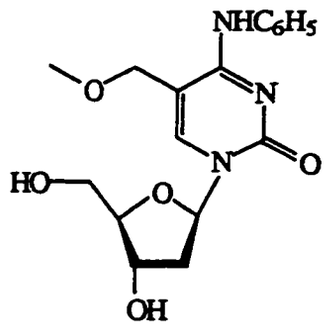
The compound 3',5'-diacetyl-N^4,N^4-dimethyl-5-methoxymethyl-2'-deoxycytidine (**39**) (120 mg, 0.31 mmol) was dissolved in methanol (5 mL) and saturated aq NH_4OH (2 mL) was added to the solution. After 14 h, the crude product was purified by FCC using 10% MeOH in CH_2Cl_2 to yield a white solid (83 mg, 0.28 mmol, yield 90%). The product was freeze-dried and submitted for biological testing.

^1H NMR (D_2O): 7.72 (1H, s, H_6), 6.05 (1H, dd, $H_{1'}$), 4.29 (1H, m, $H_{3'}$), 4.15 (2H, d, CH_2), 3.86 (1H, m, $H_{4'}$), 3.55 (1H, dd, $H_{5'}$), 3.47 (1H, dd, $H_{5''}$), 3.20 (3H, s, OCH_3), 3.05 (6H, s, $N(CH_3)_2$), 2.26 (1H, dd, $H_{2'}$), 2.12 (1H, m, $H_{2'}$).

^{13}C NMR (D_2O): 88.2 (d, $C_{1'}$), 41.8 (t, $C_{2'}$), 72.6 (d, $C_{3'}$), 89.0 (d, $C_{4'}$), 63.4 (t, $C_{5'}$), 158.5 (s, C_2), 165.7 (s, C_4), 107.1 (s, C_5), 147.0 (d, C_6), 72.5 (t, CH_2), 59.0 (q, OCH_3), 42.0 (q, $N(CH_3)_2$).

UV: λ_{min}: 248 nm, λ_{max}: 284 nm (ε, 9400)
MS: FAB mass 300.1 (M^++1), acc. mass found 300.1568, calc. (M^++1) 300.1649
mp: 195-197° C

N^4-phenyl-5-methoxymethyl-2'-deoxycytidine. (**44**)



The crude triazolyl intermediate (**38**) was dissolved in dioxane (5 mL), the solution was cooled in an ice water bath, and aniline (2.0 mL, 22.0 mmol) was added to it. After 24 h the crude product 3',5'-diacetyl-N^4-phenyl-5-methoxymethyl-2'-deoxycytidine (**40**) was dissolved in methanol (5 mL), chilled in ice and ammonia gas was bubbled into the solution. After 14 h, the crude product was purified by FCC using 10% methanol in dichloromethane to yield a white solid (285 mg, 0.82 mmol, yield 73%).

^{1}H NMR (D_2O): 7.70 (1H, s, H_6), 7.30-7.00 (5H, m, C_6H_5), 5.95 (1H, dd, $H_{1'}$), 4.25 (1H, m, $H_{3'}$), 4.15 (2H, s, CH_2), 3.85 (1H, m, $H_{4'}$), 3.65 (1H, dd , $H_{5'}$), 3.55 (1H, dd, $H_{5''}$), 3.18 (3H, s, OCH_3), 2.23 (1H, m, $H_{2''}$), 2.10 (1H, m, $H_{2'}$).

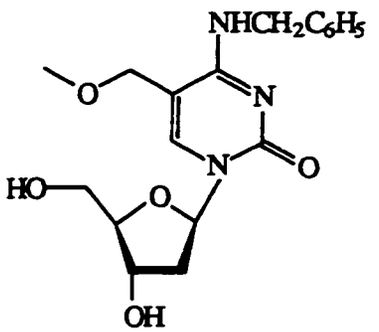
^{13}C NMR (D_2O): 88.6 (d, $C_{1'}$), 42.0 (t, $C_{2'}$), 72.5 (d, $C_{3'}$), 89.1 (d, $C_{4'}$), 63.3 (t, $C_{5'}$), 159.0 (s, C_2), 163.7 (s, C_4), 106.9 (s, C_5), 148.4 (d, C_6), 70.2 (t, CH_2O), 59.5 (q, OCH_3), 139.0, 131.3, 128.2, 126.1 (C_6H_5).

UV: λ_{min}: 253 nm, λ_{max}: 290 nm (ε, 9250)

MS: FAB mass 348.1 (M^++1), acc. mass found 348.1552, calc. (M^++1) 348.1521

mp: 138-140° C

N^4-benzyl-5-methoxymethyl-2'-deoxycytidine. (**45**)



The crude triazolyl intermediate (**38**) was dissolved in dioxane (5 mL), the solution was cooled in an ice water bath, and benzylamine (2.0 mL, 18.3 mmol) was added to it. After 24 h, the crude product 3',5'-diacetyl-N^4-benzyl-5-methoxymethyl-2'-deoxycytidine (**41**) was dissolved in methanol (5 mL), chilled in ice and ammonia gas was bubbled into the solution. After 14 h, the product was purified by FCC using 10% methanol in dichloromethane to yield a white solid (283 mg, 0.78 mmol, yield 70%).

^{1}H NMR (D_2O): 7.55 (1H, s, H_6), 7.10-6.90 (5H, m, C_6H_5), 5.90 (1H, dd, $H_{1'}$), 4.37 (2H, s, NCH_2), 4.15 (1H, m, $H_{3'}$), 4.00 (2H, s, OCH_2), 3.75 (1H, m, $H_{4'}$), 3.60 (1H, dd , $H_{5'}$), 3.50 (1H, dd, $H_{5''}$), 3.05 (3H, s, OCH_3), 2.13 (1H, m, $H_{2''}$), 1.92 (1H, m, $H_{2'}$).

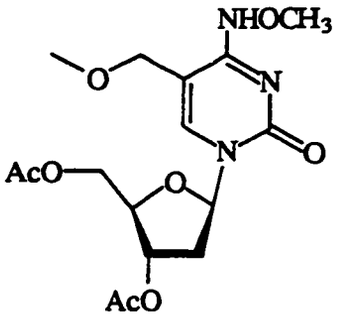
^{13}C NMR (D_2O): 88.3 (d, $C_{1'}$), 41.8 (t, $C_{2'}$), 72.6 (d, $C_{3'}$), 88.9 (d, $C_{4'}$), 63.3 (t, $C_{5'}$), 159.5 (s, C_2), 164.9 (s, C_4), 106.6 (s, C_5), 142.4 (d, C_6), 70.1 (t, CH_2O), 59.2 (q, OCH_3), 46.1 (t, NCH_2), 130.9, 129.6, 129.4, 126.1 (C_6H_5).

UV: λ_{min}: 254 nm, λ_{max}: 276 nm (ε, 8950)

MS: FAB mass 362.1 (M^++1), acc. mass found 362.1712, calc. (M^++1), 362.1681

mp: 133-135 °C

3',5'-diacetyl-N^4-methoxy-5-methoxymethyl-2'-deoxycytidine. (**52**)



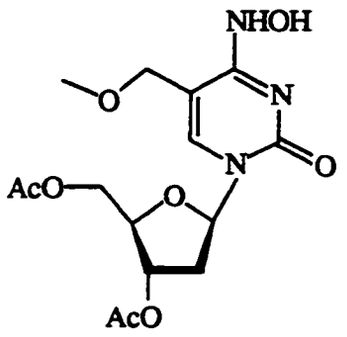
The compound 3',5'-diacetyl-5-methoxymethyl-2'-deoxyuridine (**37**) (200 mg, 0.56 mmol) was dissolved in dry acetonitrile (6 mL). To that was added K_2CO_3 (232 mg, 1.68 mmol) and TsCl (215 mg, 1.12 mmol) at room temperature. The solution was refluxed under a reflux condenser for 3 h, and the solvent was removed under vacuum. To the crude reaction product **51** was added a solution of methoxylamine hydrochloride (1.0 g, 11.2 mmol) in pyridine (25 mL) and the resulting mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated and extracted with ethyl acetate (75 mL), the organic phase was washed with water (2x25 mL) followed by brine (15 mL) and dried over anhydrous sodium sulfate. The solvent was removed and the residue was co-evaporated with absolute ethanol. The crude product was purified by FCC using 10% acetone in ether to yield a white foamy substance (151 mg, 0.39 mmol, yield 70 %).

^{1}H NMR ($CDCl_3$): 8.25 (1H, s, NH), 6.85 (1H, s, H_6), 6.30 (1H, dd, $H_{1'}$), 5.15 (1H, ddd, $H_{3'}$), 4.22 (2H, d, OCH_2), 4.18 (1H, ddd, $H_{4'}$), 4.08 (2H, dd, $H_{5',5''}$), 3.75 (3H, s, $NOCH_3$), 3.31 (3H, s, OCH_3), 2.34-2.16 (2H, m, $H_{2',2''}$), 2.08-2.00 (6H, 2s, 2 acetyl CH_3).

^{13}C NMR ($CDCl_3$): 81.5 (d, $C_{1'}$), 36.5 (t, $C_{2'}$), 74.6 (d, $C_{3'}$), 83.9 (d, $C_{4'}$), 64.1 (t, $C_{5'}$), 142.4 (s, C_2), 148.9 (s, C_4), 108.4 (s, C_5), 126.1 (d, C_6), 66.5 (t, CH_2), 58.6 (q, OCH_3), 61.7 (q, $NOCH_3$), 170.3 (s, 2 acetyl CO), 20.8-20.5 (2q, 2 acetyl CH_3).

UV: λ_{min}: 251 nm, λ_{max}: 273 nm (ε, 8400)
mp: 137-140° C

3',5'-diacetyl-N^4-hydroxy-5-methoxymethyl-2'-deoxycytidine. (**53**)



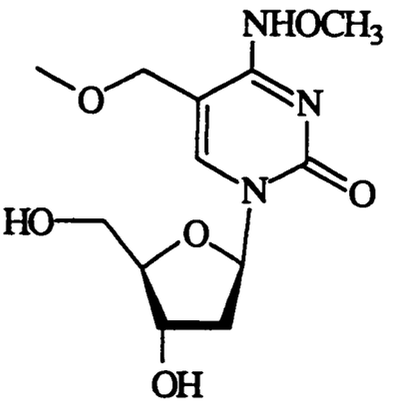
The compound 3',5'-diacetyl-5-methoxymethyl-2'-deoxyuridine (**37**) (341 mg, 0.96 mmol) was dissolved in dry acetonitrile (15 mL). To that was added K_2CO_3 (553 mg, 4.0 mmol) and TsCl (381 mg, 2.0 mmol) at room temperature. The solution was refluxed, after which it was concentrated to dryness on a rotovap. To the crude reaction product **51** was added a solution of hydroxylamine hydrochloride (1.39 g, 20 mmol) in pyridine (20 mL) and the resulting mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated and worked up with ethyl acetate (200 mL) and water (2x50 mL) followed by brine (25 mL). The organic layer was dried over anhydrous sodium sulfate and co-evaporated with absolute ethanol. Purification by FCC using 5% methanol in dichloromethane yielded 3',5'-diacetyl-N^4-hydroxy-5-methoxymethyl-2'-deoxycytidine as a white foamy substance (221 mg, 0.6 mmol, yield 62%) .

^{1}H NMR ($CDCl_3$): 8.00-9.00 (2H, bs, NHOH), 7.90 (1H, s, H6), 6.35 (1H, dd, $H_{1'}$), 5.22 (1H, m, $H_{3'}$), 4.31 (2H, d, $H_{5',5''}$), 4.18 (1H, bs, $H_{4'}$), 4.08 (2H, dd, CH_2O), 3.35 (3H, s, OCH_3), 2.41-2.10 (2H, m, $H_{2',2''}$), 2.05-2.15 (6H, 2s, acetyl CH_3).

^{13}C NMR ($CDCl_3$): 81.6 (d, $C_{1'}$), 36.6 (t, $C_{2'}$), 74.5 (d, $C_{3'}$), 84.2 (d, $C_{4'}$), 64.0 (t, $C_{5'}$), 143.9 (s, C_2), 149.0 (s, C_4), 108.4 (s, C_5), 127.0 (d, C_6), 66.9 (t, CH_2O), 58.5 (q, CH_3), 170.4 (s, 2 acetyl CO), 20.8-20.6 (2q, 2 acetyl CH_3).

UV: λ_{min}: 242 nm, λ_{max}: 273 nm (ε, 8650)
mp: 112-115°C

N^4-methoxy-5-methoxymethyl-2'-deoxycytidine. (**54**)



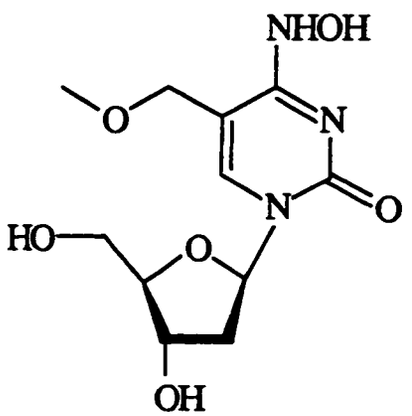
The compound 3',5'-diacetyl-N^4-methoxy-5-methoxymethyl-2'-deoxycytidine (**52**) (100 mg, 0.26 mmol) was dissolved in methanol (5 mL) and the solution was saturated with anhydrous ammonia. After 14 h the crude N^4-methoxy-5-methoxymethyl-2'-deoxycytidine (**54**) was purified by FCC using 10% methanol in dichloromethane. The product, a white solid (63 mg, 0.21 mmol, yield 80%) was freeze-dried and submitted for biological testing.

^{1}H NMR (D_2O): 7.09 (1H, s, H_6), 6.11 (1H, dd, $H_{1'}$), 4.30 (1H, ddd, $H_{3'}$), 4.01 (2H, d, CH_2), 3.83 (1H, ddd, $H_{4'}$), 3.73 (1H, dd, $H_{5'}$), 3.75 (3H, s, $NOCH_3$), 3.60 (1H, dd, $H_{5''}$), 3.21 (3H, s, OCH_3), 2.32-2.11 (2H, m, $H_{2',2''}$).

^{13}C NMR ($CDCl_3$): 85.7 (d, $C_{1'}$), 39.2 (t, $C_{2'}$), 71.5 (d, $C_{3'}$), 86.6 (d, $C_{4'}$), 62.5 (t, $C_{5'}$), 142.7 (s, C_2), 149.4 (s, C_4), 107.9 (s, C_5), 128.9 (d, C_6) 66.7 (t, CH_2O), 58.8 (q, OCH_3), 61.9 (q, $NOCH_3$).

UV: λ_{min}: 253 nm, λ_{max}: 282 nm (ε, 9300)
MS: FAB mass 302.1 (M^++1), acc. mass found 302.1329, calc. (M^++1) 302.1342
mp: 131-133° C

N^4-hydroxy-5-methoxymethyl-2'-deoxycytidine. (**55**)



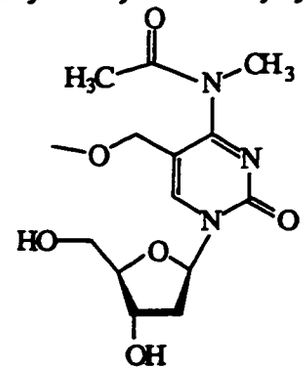
The compound N^4-hydroxy-3',5'-diacetyl-5-methoxymethyl-2'-deoxycytidine (**53**) (90 mg, 0.24 mmol) was dissolved in methanol (10 mL) and the solution saturated with anhydrous ammonia. After 14 h the crude N^4-hydroxy-5-methoxymethyl-2'-deoxycytidine (**55**) was purified over a column of silica gel using 15% methanol in dichloromethane. Evaporation of the solvent yielded a white gummy material (59 mg, 0.20 mmol, yield 85%) which was freeze-dried to give a white powdery solid that was submitted for biological testing.

^{1}H NMR (D_2O): 7.05 (1H, s, H6), 6.12 (1H, dd, $H_{1'}$), 4.31 (1H, ddd, $H_{3'}$), 4.05 (2H, s, OCH_2), 3.83 (1H, ddd, $H_{4'}$), 3.71-3.52 (2H, 2dd, $H_{5'}$,$H_{5''}$), 3.20 (3H, s, OCH_3), 2.18 (2H, m, $H_{2',2''}$).

^{13}C NMR (D_2O): 86.7 (d, $C_{1'}$), 40.2 (t, $C_{2'}$), 73 (d, $C_{3'}$), 88.5 (d, $C_{4'}$), 63.6 (t, $C_{5'}$), 147.7 (s, C_2), 153.2 (s, C_4), 109.4 (s, C_5), 133.8 (d, C_6), 69.4 (t, CH_2), 59.6 (q, CH_3).

UV: λ_{min}: 259 nm, λ_{max}: 283 nm (ε, 9300)
MS: FAB mass 288.1 (M^++1), acc. mass found 288.1191, calc. (M^++1) 288.1176
mp: 147-150° C

N^4-acetyl, N^4-methyl-5-methoxymethyl-2'-deoxycytidine. (**57**)



The analog N^4-methyl-MMdCyd (**24**) (285 mg, 1.0 mmol) was dissolved in 2,6-lutidine (5 mL). TMSCl (395 µL, 4.0 mmol) was added to it dropwise under argon. After 2 h, a TLC (15% methanol in dichloromethane) indicated complete reaction. The reaction flask was cooled to 0°C and acetyl chloride (142 µL, 2.0 mmol) was added dropwise. After 16 h, TLC indicated the reaction was completed. A 1:1 methanol / water mixture (1.0 mL) was added to the solution and after stirring for half an hour, the crude product was extracted from the solution by workup with water (25 mL) and chloroform (25 mL x 3). The combined chloroform fractions were dried over anhydrous sodium sulfate, concentrated and the crude product was purified by FCC using 5% methanol In dichloromethane. The product (87 mg, 0.27 mmol, yield 27%) was isolated as a white solid and submitted for biological testing.

^{1}H NMR (D_2O): 8.55 (1H, s, H_6), 6.05 (1H, dd, $H_{1'}$), 4.35 (1H, m, $H_{3'}$), 4.15 (2H, s, CH_2), 4.00 (1H, m, $H_{4'}$), 3.78 (1H, dd, $H_{5'}$), 3.60 (1H, dd, $H_{5''}$), 3.20 (3H, s, OCH_3), 3.08 (3H, s, NCH_3), 2.48 (1H, m, $H_{2''}$), 2.20 (1H, m, $H_{2'}$), 1.98 (3H, s, acetyl CH_3).

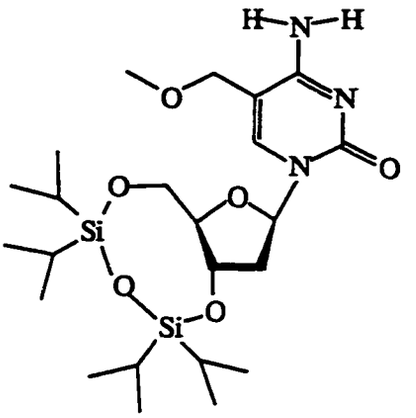
^{13}C NMR (D_2O): 89.3 (d, $C_{1'}$), 41.9 (t, $C_{2'}$), 72.3 (d, $C_{3'}$), 90.0 (d, $C_{4'}$), 63.1 (t, $C_{5'}$), 158.5 (s, C_2), 163.4 (s, C_4), 109.2 (s, C_5), 147.0 (d, C_6), 69.6 (t, CH_2), 59.1 (q, OCH_3), 174.2 (s, acetyl CO), 36.2 (q, NCH_3), 26.9 (q, acetyl CH_3).

UV: λ_{min}: 262 nm, λ_{max}: 296 nm (ε, 8450)

MS: FAB mass 327.1 (M^++1), acc. mass found 327.2311, calc. (M^++1) 327.2378

mp: 129-132°C

3',5'-O-(tetraisopropyldisiloxane)-5-methoxymethyl-2'-deoxycytidine. (**58**)



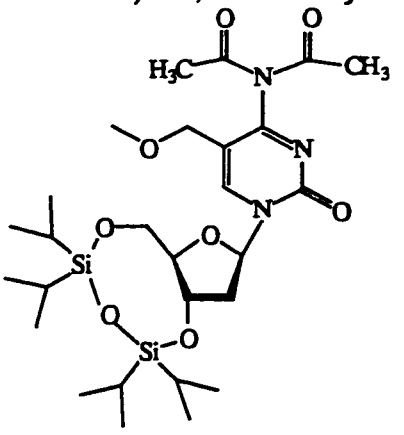
The compound 5-methoxymethyl-2'-deoxycytidine (**18**) (371 mg, 1.4 mmol) was dissolved in pyridine (7.0 mL). To this solution, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (493 µL, 1.54 mmol) was added at room temperature. The reaction was stirred and monitored for 48 h. The crude product was extracted from the mixture by an aqueous workup with water (50 mL) and chloroform (50 mL x 3). The combined organic fractions were dried over anhydrous sodium sulfate, concentrated and co-evaporated with ethanol. The crude product was purified by FCC using 5% methanol in dichloromethane (359 mg, 0.7 mmol, yield 50 %).

^{1}H NMR ($CDCl_3$): 8.50 (2H, bs, NH_2), 7.65 (1H, s, H_6), 5.94 (1H, dd, $H_{1'}$), 4.30 (1H, m, $H_{3'}$), 4.22 (2H, s, CH_2O), 4.10 (1H, dd, $H_{5'}$), 3.95 (1H, dd, $H_{5''}$), 3.72 (1H, m, $H_{4'}$), 3.25 (3H, s, OCH_3), 2.41 (1H, ddd, $H_{2''}$), 2.19 (1H, m, $H_{2'}$), 1.10-0.75 (28 H, m, tetraisopropyl hydrogens).

^{13}C NMR ($CDCl_3$): 84.7 (d, $C_{1'}$), 40.0 (t, $C_{2'}$), 67.1 (d, $C_{3'}$), 84.9 (d, $C_{4'}$), 60.1 (t, $C_{5'}$), 155.4 (s, C_2), 165.4 (s, C_4), 102.0 (s, C_5), 139.1 (d, C_6), 69.6 (t, CH_2), 57.4 (q, OCH_3), 17.4-16.8 (q, 8 CH_3), 13.4-12.4 (d, 4 isopropyl carbons).

UV: λ_{min}: 254 nm, λ_{max}: 278 nm (ε, 8850)
MS: FAB mass 514.1 (M^++1), acc. mass found 514.3025, calc. (M^++1) 514.3042
mp: 61-64°C

3',5'-O-(tetraisopropyldisiloxane)-N^4,N^4-diacetyl-MMdCyd. (**59**)



The base 2,6-lutidine (51 µL, 0.44 mmol) was added to a solution of acetyl chloride (30 µL, 0.4 mmol) in dichloromethane at -40°C under argon. To this solution, 3',5'-O-(tetraisopropyldisiloxane)-5-methoxymethyl-2'-deoxycytidine (**58**) (110 mg, 0.2 mmol) in dichloromethane (2 mL) were added dropwise. The entire solution was warmed up to room temperature and stirred for 18 h. A TLC (5% methanol in dichloromethane) indicated complete reaction. The reaction mixture was worked up with dichloromethane (110 mL) and 1 M HCl (25 mL) and then with saturated $NaHCO_3$ (25 mL). The organic layer was dried over anhydrous sodium sulfate, concentrated and the crude product was purified by FCC using 5% methanol in dichloromethane. The product was isolated as a white solid (94 mg, 0.16 mmol, yield 79%).

^1H NMR ($CDCl_3$): 7.30 (1H, s, H_6), 6.05 (1H, dd, $H_{1'}$), 4.38 (1H, m, $H_{3'}$), 4.20 (1H, dd, $H_{5'}$), 4.05 (1H, dd, $H_{5''}$), 4.01 (2H, s, CH_2O), 3.89 (1H, m, $H_{4'}$), 3.32 (3H, s, CH_3), 2.65 (1H, m, $H_{2''}$), 2.45 (1H, m, $H_{2'}$), 2.30 (6H, s, 2 acetyl CH_3) 1.10-0.75 (28 H, m, tetraisopropyl hydrogens).

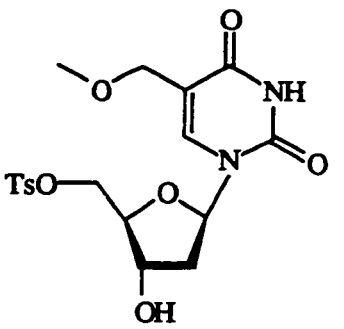
^{13}C NMR ($CDCl_3$): 85.7 (d, $C_{1'}$), 39.5 (t, $C_{2'}$), 66.6 (d, $C_{3'}$), 86.4 (d, $C_{4'}$), 59.8 (t, $C_{5'}$), 154.7 (s, C_2), 166.6 (s, C_4), 112.6 (s, C_5), 144.9 (d, C_6), 68.7 (t, CH_2O), 58.7 (q, OCH_3), 26.2 (q, 2 acetyl CH_3), 17.3-16.8 (q, 8 isopropyl CH_3), 13.4-12.4 (d, 4 isopropyl carbons).

UV: λ_{min}: 264 nm, λ_{max}: 302 nm (ε, 9700)

MS: FAB mass 598.1 (M^++1), acc. mass found 598.3046, calc. (M^++1) 598.3029

mp: 73-76°C

5'-para-toluenesulfonyl-5-methoxymethyl-2'-deoxyuridine. (**64**)



The nucleoside 5-methoxymethyl-2'-deoxyuridine (**6**) (100 mg, 0.37 mmol) was dissolved in pyridine (4 mL) under argon and the solution was cooled to 0° C . To this was added TsCl (76 mg, 0.40 mmol) and the solution was stirred. After 24 h, a TLC in 10% MeOH/ CH_2Cl_2 indicated complete reaction. One mL of water was added, the reaction mixture was stirred for 10 min and worked up with water (25 mL) and chloroform (25 mL x 3). The combined organic layers were washed with saturated $NaHCO_3$ (25 mL), dried with anhydrous sodium sulfate and concentrated. The crude product was purified by FCC using 10% methanol in dichloromethane. The product (110 mg, 0.26 mmol, yield 70%) was isolated as a white solid.

^1H NMR (D_2O): 7.85 and 7.48 (4H, 2d, phenyl protons), 7.52 (1H, s, H_6), 6.27 (1H, dd, $H_{1'}$), 4.41 (1H, m, $H_{3'}$), 4.28-4.20 (2H, 2s, $H_{5',5''}$), 4.12 (2H, s, CH_2O), 4.05 (1H, m, $H_{4'}$), 3.31 (3H, s, OCH_3), 2.45 (3H, s, tosyl CH_3), 2.23 (2H, m, $H_{2'}$, $H_{2''}$).

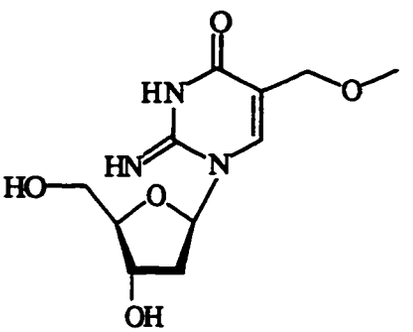
^{13}C NMR (D_2O): 85.5 (d, $C_{1'}$), 38.9 (t, $C_{2'}$), 70.2 (d, $C_{3'}$), 86.8 (d, $C_{4'}$), 67.6 (t, $C_{5'}$), 151.5 (s, C_2), 165.1 (s, C_4), 110.4 (s, C_5), 141.3 (d, C_6) 66.5 (t, CH_2), 57.3 (q, OCH_3), 21.9 (q, tosyl CH_3), 139.5-126.1 (phenyl carbons).

UV: λ_{min}: 249 nm, λ_{max}: 278 nm (ε, 9800)

MS: FAB mass 427.1 (M^++1), acc. mass found 427.1195, calc. (M^++1) 427.1164

mp: 89-94°C

2-imino-5-methoxymethyl-2'-deoxyuridine. (**66**)



The compound 5'-tosyl-MMdUrd (**64**) (90 mg, 0.21 mmol), was dissolved in 95% EtOH (4 mL). One M NaOH (210 μL, 0.21 mmol) was added to the solution and refluxed for 14 h. A TLC in 10% MeOH in CH_2Cl_2 indicated complete reaction. The crude reaction mixture was diluted with water (25 mL) and extracted with chloroform (25 mL x 3). The combined organic fractions were dried over anhydrous sodium sulfate and concentrated. The crude 2-O-5'-anhydro-5-methoxymethyl-2'-deoxyuridine (**65**) was dissolved in MeOH (3 mL) and the solution cooled to 0°C. Anhydrous ammonia gas was bubbled into the solution at a moderate rate for 10 sec.The reaction flask was capped and left at room temperature for 4 days, after which a TLC (20% MeOH in CH_2Cl_2) indicated complete reaction. The reaction mixture was concentrated and the crude product purified by FCC using 20% MeOH in CH_2Cl_2 to yield a white solid (27 mg, 0.10 mmol, yield 47%). This sample was submitted for biological testing.

^{1}H NMR (D_2O): 7.71 (1H, s, H_6), 5.83 (1H, dd, $H_{1'}$), 4.34 (1H, m, $H_{3'}$), 4.08 (2H, s, CH_2O), 3.83 (1H, m, $H_{4'}$), 3.73 (1H, dd, $H_{5'}$), 3.65 (1H, dd, $H_{5''}$), 3.21 (3H, s, OCH_3), 2.5-2.2 (2H, m, $H_{2',2''}$).

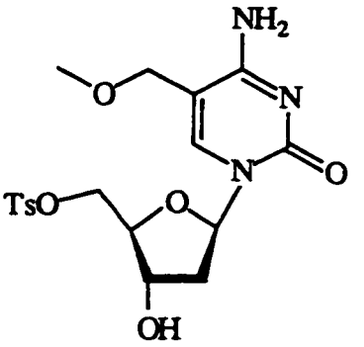
^{13}C NMR (D_2O): 89.3 (d, $C_{1'}$), 40.6 (t, $C_{2'}$), 72.2 (d, $C_{3'}$), 91.0 (d, $C_{4'}$), 62.9 (t, $C_{5'}$), 157.7 (s, C_2), 174.9 (s, C_4), 116.3 (s, C_5), 141.7 (d, C_6), 69.8 (t, CH_2O), 59.8 (q, OCH_3).

UV: λ_{min}: 252 nm, λ_{max}: 280 nm (ε, 9200)

MS: FAB mass 272.1 (M^++1), acc. mass found 272.1250, calc. (M^++1) 272.1239

mp: 105-108° C

5'-para-toluenesulfonyl-5-methoxymethyl-2'-deoxycytidine. (**68**)



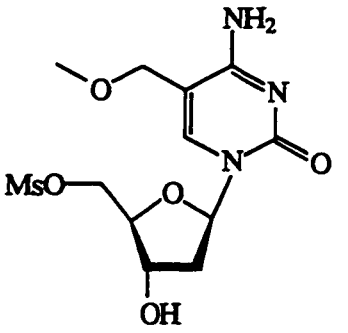
The nucleoside 5-methoxymethyl-2'-deoxycytidine (**18**) (100 mg, 0.37 mmol) was dissolved in pyridine (5 mL) under argon and the solution was cooled to 0° C . To this was added TsCl (78 mg, 0.41 mmol). After stirring for 24 h at 4° C, a TLC in 15% MeOH/ CH_2Cl_2 indicated complete reaction. One mL of water was added to the solution and stirred for 10 min. The reaction mixture was co-evaporated with 95% EtOH followed with toluene. The crude product was purified by FCC using 10% methanol in dichloromethane. The product was isolated as a white solid (100 mg, 0.24 mmol, yield 64%) .

^{1}H NMR (DMSO-d_6): 7.70 and 7.45 (4H, 2d, phenyl protons), 7.55 (1H, s, H6), 6.74 (2H, bs, NH), 6.15 (1H, dd, $H_{1'}$), 4.40-4.30 (2H, m, $H_{5',5''}$), 4.25 (1H, m, $H_{3'}$), 4.12 (2H, s, CH_2O), 3.88 (1H, m, $H_{4'}$), 3.20 (3H, s, OCH_3), 2.41 (3H, s, tosyl, CH_3), 2.10 (2H, m, $H_{2'},H_{2''}$).

^{13}C NMR (DMSO-d_6): 83.2 (d, $C_{1'}$), 39.4 (t, $C_{2'}$), 70.1 (d, $C_{3'}$), 85.0 (d, $C_{4'}$), 67.4 (t, $C_{5'}$), 154.7 (s, C_2), 164.4 (s, C_4), 102.6 (s, C_5), 145.1 (d, C_6) 69.9 (t, CH_2), 56.8 (q, OCH_3), 21.1 (q, tosyl CH_3), 140.7-125.5 (phenyl carbons).

UV: λ_{min}: 245 nm, λ_{max}: 285 nm (ε, 10500)
MS: FAB mass 426.1 (M^++1), acc. mass found 426.1345, calc. (M^++1) 426.1332
mp: 111-114°C

5'-methanesulfonyl-5-methoxymethyl-2'-deoxycytidine. (**69**)



The nucleoside 5-methoxymethyl-2'-deoxycytidine (**18**) (50 mg, 0.19 mmol) was dissolved in pyridine (2 mL) under argon and the solution was cooled to -20° C . To this was added MsCl (17 μL, 0.22 mmol). After 3 h, a TLC in 15% MeOH/ CH_2Cl_2 indicated complete reaction. One mL of water was added and stirred for 10 min and then the reaction mixture was co-evaporated with 95% EtOH followed by toluene. The crude product was purified by FCC using 15% methanol in dichloromethane. The product **69** (38 mg, 0.11 mmol, yield 60%) was isolated as a white solid as well as the disubstituted side product **82**, a white solid (12.2 mg, 0.029 mmol, yield 15%).

^1H NMR (D_2O): 7.65 (1H, s, H6), 6.15 (1H, dd, $H_{1'}$), 4.45 (2H, m, $H_{5',5''}$), 5.45 (1H, m, $H_{3'}$), 4.18 (2H, s, CH_2), 4.10 (1H, m, $H_{4'}$), 3.22 (3H, s, OCH_3), 3.10 (3H, s, Ms), 2.35 (1H, m, $H_{2''}$), 2.18 (1H, m, $H_{2'}$).

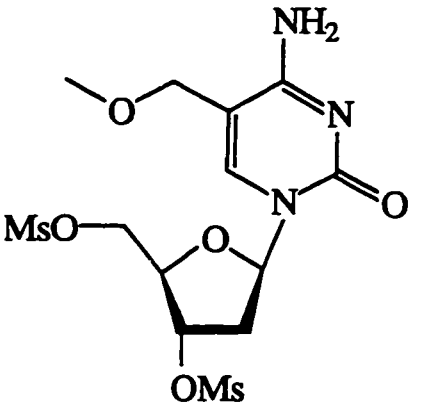
^{13}C NMR (D_2O): 85.9 (d, $C_{1'}$), 41.6 (t, $C_{2'}$), 72.5 (d, $C_{3'}$), 88.8 (d, $C_{4'}$), 71.8 (t, $C_{5'}$), 159.5 (s, C_2), 167.4 (s, C_4), 106.4 (s, C_5), 143.7 (d, C_6) 70.1 (t, CH_2), 59.5 (q, OCH_3), 38.9 (q, Ms).

UV: λ_{min}: 250 nm, λ_{max}: 278 nm (ε, 9400)

MS: FAB mass 350.1 (M^++1), acc. mass found 350.1745, calc. (M^++1) 350.1704

mp: 101-104°C

3',5'-dimethanesulfonyl-5-methoxymethyl-2'-deoxycytidine. (**82**)



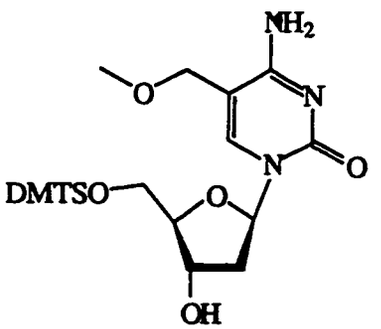
^{1}H NMR (D_2O): 7.65 (1H, s, H6), 6.15 (1H, dd, $H_{1'}$), 5.35 (1H, ddd, $H_{3'}$), 4.50 (3H, m, $H_{4'}$ and $H_{5',5''}$), 4.18 (2H, s, OCH_2), 3.20 (3H, s, OCH_3), 3.15 (3H, s, Ms), 3.11 (3H, s, Ms), 2.67-2.42 (2H, m, $H_{2',2''}$).

UV: λ_{min}: 248 nm, λ_{max}: 279 nm (ε, 8900)

MS: FAB mass 427.1 (M^++1), acc. mass found 427.1852, calc. (M^++1) 427.1795

mp: 109-112 °C

5'-dimethylthexylsilyl-5-methoxymethyl-2'-deoxycytidine. (**71**)



The nucleoside 5-methoxymethyl-2'-deoxycytidine (**18**) (271 mg, 1.0 mmol) was dissolved in DMF (5.0 mL), imidazole (136 mg, 2.0 mmol) was added followed by DMTSCl (215 µL, 1.1 mmol) at 0° C under argon. After 20 h of stirring, a TLC in 10% MeOH / CH_2Cl_2 indicated complete reaction. Saturated $NaHCO_3$ (5 mL) was added to the reaction mixture stirred for 10 min and the reaction mixture was extracted with $CHCl_3$ (50 mL x 3) and water (25 mL). The organic fractions were combined and washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by FCC using 10% MeOH in CH_2Cl_2 to yield a white powder (338 mg, 0.82 mmol, yield 82%) as well as the disubstituted side product (**61**), a white solid, (61 mg, 0.11 mmol, yield 11%).

^1H NMR ($CDCl_3$): 7.70 (1H, s, H_6), 6.27 (1H, dd, $H_{1'}$), 4.37 (1H, m, $H_{3'}$), 4.22 (2H, s, CH_2), 3.95 (1H, m, $H_{4'}$), 3.86 (1H, dd, $H_{5'}$), 3.74 (1H, dd, $H_{5''}$), 3.35 (3H, s, CH_3), 2.47 (1H, m, $H_{2''}$), 2.42 (1H, m, $H_{2'}$), 1.95 (1H, m, SiCCH), 0.85 (12H, m, $C(CH_3)_2C(CH_3)_2$), 0.15 (3H, s, $SiCH_3$), 0.95 (3H, s, $SiCH_3$).

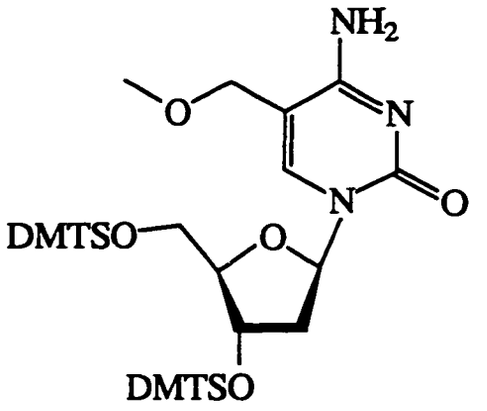
^{13}C NMR ($CDCl_3$): 86.2 (d, $C_{1'}$), 42.2 (t, $C_{2'}$), 71.7 (d, $C_{3'}$), 87.8 (d, $C_{4'}$), 62.6 (t, $C_{5'}$), 154.8 (s, C_2), 164.1 (s, C_4), 102.3 (s, C_5), 139.9 (d, C_6), 69.3 (t, CH_2), 57.6 (q, CH_3), -2.9 (q, $SiCH_3$), -2.5 (q, $SiCH_3$), 18.6 (q, $SiC(\mathbf{C}H_3)_2$), 20.3 (q, $SiCC(\mathbf{C}H_3)_2$), 24.9 (s, Si**C**), 25.4 (s, Si**C**C).

UV: λ_{min}: 242 nm, λ_{max}: 279 nm (ε, 9200)

MS: FAB mass 414.1 (M^++1), acc. mass found 414.1591, calc. (M^++1) 414.1571

mp: 82-84 °C

3',5'-bis(dimethylthexylsilyl)-5-methoxymethyl-2'-deoxycytidine. (**61**)

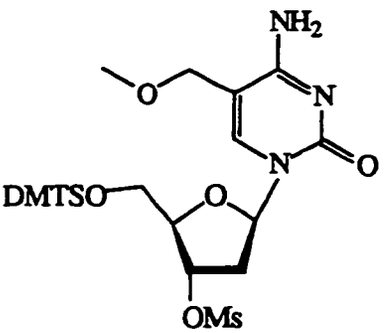


^{1}H NMR ($CDCl_3$): 7.70 (1H, s, H_6), 6.27 (1H, dd, $H_{1'}$), 4.34 (1H, m, $H_{3'}$), 4.22 (2H, d, CH_2), 3.93 (1H, m, $H_{4'}$), 3.86 (1H, dd, $H_{5'}$), 3.74 (1H, dd, $H_{5''}$), 3.32 (3H, s, CH_3), 2.45 (1H, ddd, $H_{2''}$), 1.93 (1H, m, $H_{2'}$), 1.95 (2H, m, SiCCH), 0.85 (24H, m, $C(CH_3)_2C(CH_3)_2$), 0.15 (6H, s, $2SiCH_3$), 0.09 (6H, s, $2SiCH_3$).

^{13}C NMR ($CDCl_3$): 86.2 (d, $C_{1'}$), 42.2 (t, $C_{2'}$), 71.7 (d, $C_{3'}$), 87.7 (d, $C_{4'}$), 62.6 (t, $C_{5'}$), 155.4 (s, C_2), 164.9 (s, C_4), 102.0 (s, C_5), 139.8 (d, C_6), 69.6 (t, CH_2), 57.4 (q, OCH_3), -3.4 (q, $SiCH_3$), -3.3 (q, $SiCH_3$), -2.9 (q, $SiCH_3$), -2.5 (q, $SiCH_3$), 18.6 (q, $SiC(\mathbf{C}H_3)_2$), 18.5 (q, $SiC(\mathbf{C}H_3)_2$), 20.3 (q, $SiCC(\mathbf{C}H_3)_2$), 20.4 (q, $SiCC(\mathbf{C}H_3)_2$), 24.9 (s, SiC), 25.4 (s, SiC**C**).

UV: λ_{min}: 245 nm, λ_{max}: 279 nm (ε, 8500)
MS: FAB mass 556.3 (M^++1), acc. mass found 556.3613, calc. (M^++1) 556.3571
mp: 75-78°C

3'-methanesulfonyl-5'-dimethylthexylsilyl-5-methoxymethyl-2'-deoxycytidine. (**72**)



The compound 5'-dimethylthexylsilyl-5-methoxymethyl-2'-deoxycytidine (**71**) (340 mg, 0.82 mmol) was dissolved in pyridine (4 mL) under argon, cooled to -20° C and MsCl (95 µL, 1.2 mmol) was added. After 20 h, the reaction was complete as indicated by TLC (10% MeOH in CH_2Cl_2). Saturated $NaHCO_3$ (5 mL) was added and after stirring for 10 min the reaction mixture was extracted with $CHCl_3$ (50 mL x 3) and water (25 mL). The organic fractions were combined, washed with brine (25 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum, the residue dissolved in EtOH and evaporated in vacuo. The crude product was purified by FCC using 10% MeOH in CH_2Cl_2 to yield a white amorphous solid (387 mg, 0.79 mmol, yield 96%).

^1H NMR ($CDCl_3$): 7.62 (1H, s, H_6), 6.29 (1H, dd, $H_{1'}$), 5.25 (1H, m, $H_{3'}$), 4.35 (1H, m, $H_{4'}$), 4.22 (2H, d, OCH_2), 3.90 (2H, 2s, $H_{5',5''}$), 3.31 (3H, s, OCH_3), 3.11 (3H, s, Ms), 2.79 (1H, dd, $H_{2''}$), 2.09 (1H, m, $H_{2'}$), 1.62 (1H, m, SiCCH), 0.85 (12H, m, $C(CH_3)_2C(CH_3)_2$), 0.11 (6H, s, $2SiCH_3$), 7.05 and 6.12 (2H, 2bs, NH_2).

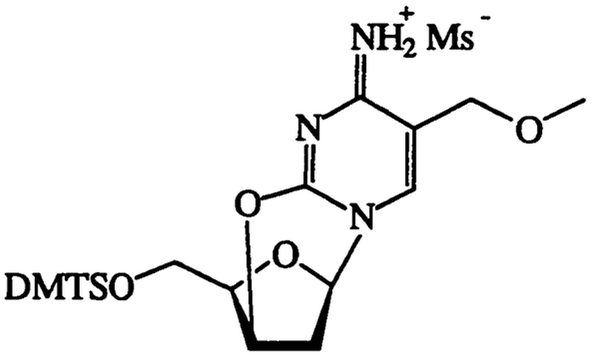
^{13}C NMR ($CDCl_3$): 85.2 (d, $C_{1'}$), 39.5 (t, $C_{2'}$), 80.0 (d, $C_{3'}$), 85.9 (d, $C_{4'}$), 62.6 (t, $C_{5'}$), 155.3 (s, C_2), 165.2 (s, C_4), 102.5 (s, C_5), 139.1 (d, C_6), 69.5 (t, CH_2), 57.5 (q, CH_3), 38.8 (q, Ms), -3.5 (q, $SiCH_3$), -3.4 (q, $SiCH_3$), 18.6 (q, $SiC(\mathbf{C}H_3)_2$), 20.3 (q, $SiCC(\mathbf{C}H_3)_2$), 20.4 (s, SiC), 25.3 (s, Si**C**C).

UV: λ_{min}: 250 nm, λ_{max}: 277 nm (ε, 8600)

MS: FAB mass 492.2 (M^++1), acc. mass found 492.2202, calc. (M^++1) 492.2176

mp: 87-90°C

5'-dimethylthexylsilyl-2-O-3'-anhydro-MMdCyd mesylate. (**73**)



The compound 5'-dimethylthexylsilyl-3'-mesyl-MMdCyd (**72**) (169 mg, 0.34 mmol) was dissolved in a 1:1 ethanol water mixture (10 mL). The solution was refluxed and monitored. After one h, a TLC (10 % methanol / dichloromethane) indicated complete reaction. The reaction mixture was concentrated and purified by FCC using 20% methanol in dichloromethane. The product (mesylate salt) was obtained as a white solid. (121 mg, 0.25 mmol, yield 72 %).

^{1}H NMR (DMSO-d_6): 8.19 (1H, s, H_6), 6.15 (1H, m, $H_{1'}$), 5.45 (1H, m, $H_{3'}$), 4.35 (1H, m, $H_{4'}$), 4.30 (2H, d, CH_2O), 3.74 (2H, m, $H_{5',5''}$), 3.32 (3H, s, OCH_3), 2.73 (1H, d, $H_{2''}$), 2.60 (1H, dd, $H_{2'}$), 2.30 (3H, s, Ms), 1.55 (1H, m, SiCCH), 0.85 (12H, m, $C(CH_3)_2C(CH_3)_2$), 0.11 (6H, s, $2SiCH_3$).

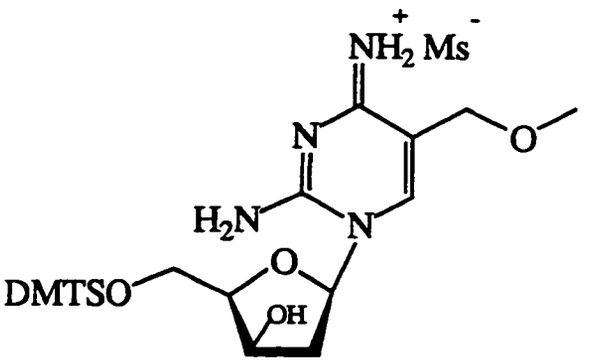
^{13}C NMR (D_2O): 87.8 (d, $C_{1'}$), 36.1 (t, $C_{2'}$), 82.7 (d, $C_{3'}$), 91.8 (d, $C_{4'}$), 62.9 (t, $C_{5'}$), 160.2 (s, C_2), 168.3 (s, C_4), 112.2 (s, C_5), 145.5 (d, C_6), 69.1 (t, CH_2O), 60.1 (q, OCH_3), 40.8 (q, Ms), -1.8 (q, $SiCH_3$), -1.7 (q, $SiCH_3$), 20.2 (q, $SiC(\mathbf{C}H_3)_2$), 22.2 (q, $SiCC(\mathbf{C}H_3)_2$), 22.3 (s, SiC), 27.5 (s, Si**C**).

UV: λ_{min}: 251 nm, λ_{max}: 264 nm (ε, 9600)

MS: FAB mass 492.1 (M^++1), acc. mass found 492.2105, calc. (M^++1) 492.2094

mp: 182-185° C

5'-dimethylthexylsilyl-3'-(S)-hydroxy-2-amino-MMdCyd mesylate. (**74**)



The compound 5'-dimethylthexylsilyl-2-O-3'-anhydro-MMdCyd mesylate (**72**) (120 mg, 0.24 mmol) was dissolved in absolute ethanol (5 mL). The solution was chilled and saturated with anhydrous ammonia gas. After 48 h, a TLC (20 % methanol/dichloromethane) indicated complete reaction. The reaction mixture was concentrated and the crude product purified by FCC using 20% methanol in dichloromethane. The product was obtained as a white solid (102 mg, 0.20 mmol, yield 84%) .

^{1}H NMR (D_2O): 7.95 (1H, s, H_6), 5.70 (1H, d, $H_{1'}$), 4.45 (1H, m, $H_{3'}$), 4.30 (2H, dd, CH_2O), 4.12 (1H, m, $H_{4'}$), 3.90 (2H, m, $H_{5',5''}$), 3.25 (3H, s, OCH_3), 2.70 (3H, s, Ms), 2.60 (1H, m, $H_{2''}$), 2.21 (1H, d, $H_{2'}$), 1.59 (1H, m, SiCCH), 0.95 (12H, m, $C(CH_3)_2C(CH_3)_2$), 0.11 (6H, s, $2SiCH_3$).

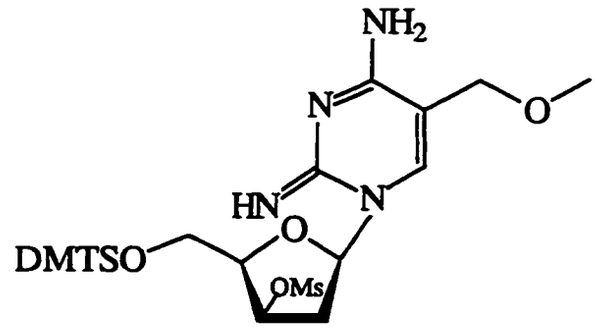
^{13}C NMR (D_2O): 88.5 (d, $C_{1'}$), 42.3 (t, $C_{2'}$), 71.7 (d, $C_{3'}$), 91.1 (d, $C_{4'}$), 62.1 (t, $C_{5'}$), 155.9 (s, C_2), 165.6 (s, C_4), 108.7 (s, C_5), 143.0 (d, C_6), 69.9 (t, CH_2O), 59.5 (q, OCH_3), 40.8 (q, Ms), -1.8 (q, $SiCH_3$), -1.7 (q, $SiCH_3$), 20.2 (q, $SiC(\mathbf{C}H_3)_2$), 22.0 (q, $SiCC(\mathbf{C}H_3)_2$), 22.1 (s, SiC), 26.9 (s, SiC**C**).

UV: λ_{min}: 249 nm, λ_{max}: 284 nm (ε, 9050)

MS: FAB mass 509.1 (M^++1), acc. mass found 509.3162, calc. (M^++1) 509.3105

mp: 190°C d.

5'-dimethylthexylsilyl-3'-(S)-mesyl-2-imino-MMdCyd. (**75**)



The compound 5'-dimethylthexylsilyl-3'-(S)-hydroxy-2-amino-MMdCyd mesylate (**74**) (135 mg, 0.27 mmol) was dissolved in pyridine (3 mL). The solution was cooled to -20°C and mesyl chloride (42 µL, 0.54 mmol) was added. The reaction mixture was kept at 4°C and monitored. After 48 h, a TLC (20% methanol in dichloromethane) indicated complete reaction. One mL of water was added and stirred for 10 min. The reaction mixture was co-evaporated with ethanol and the crude product (**75**) was purified by FCC using 20% methanol in dichloromethane. The free amine was obtained as a white solid (112 mg, 0.22 mmol, yield 82%).

^{1}H NMR (D_2O): 7.71 (1H, s, H_6), 5.95 (1H, d, $H_{1'}$), 5.30 (1H, m, $H_{3'}$), 4.32 (1H, m, $H_{4'}$), 4.20 (2H, d, CH_2O), 3.90 (2H, m, $H_{5',5''}$), 3.25 (3H, s, OCH_3), 3.00 (3H, s, OMs), 2.88 (1H, m, $H_{2''}$), 2.60 (1H, d, $H_{2'}$), 1.51 (1H, m, SiCCH), 0.75 (12H, m, $C(CH_3)_2C(CH_3)_2$), 0.10 (6H, s, $2SiCH_3$).

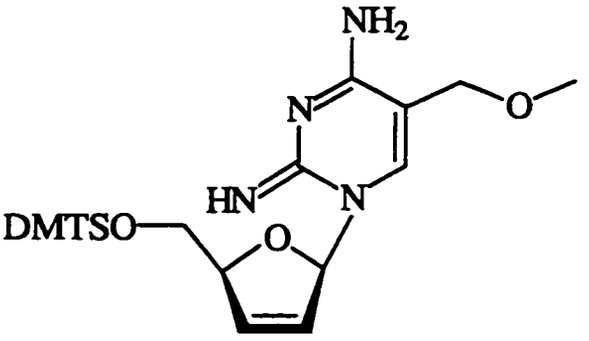
^{13}C NMR (D_2O): 88.3 (d, $C_{1'}$), 44.3 (t, $C_{2'}$), 79.5 (d, $C_{3'}$), 93.2 (d, $C_{4'}$), 62.2 (t, $C_{5'}$), 157.9 (s, C_2), 167.6 (s, C_4), 109.4 (s, C_5), 145.7 (d, C_6), 69.8 (t, CH_2O), 59.7 (q, OCH_3), 42.8 (q, Ms), -2.0 (q, $SiCH_3$), -2.1 (q, $SiCH_3$), 19.4 (q, $SiC(\mathbf{C}H_3)_2$), 21.1 (q, $SiCC(\mathbf{C}H_3)_2$), 21.0 (s, SiC), 26.9 (s, Si**C**).

UV: λ_{min}: 252 nm, λ_{max}: 286 nm (ε, 10100)

MS: FAB mass 507.1 (M^++1), acc. mass found 507.2342, calc. (M^++1) 507.2331

mp: 165-168° C .

5'-dimethylthexylsilyl-2',3'-didehydro-2',3'-dideoxy-2-imino-MMCyd. (**77**)



The compound 5'-dimethylthexylsilyl-3'-(S)-mesyl-2-imino-MMdCyd (**75**) (80 mg, 0.16 mmol), was dissolved in DMF (4 mL). Sodium acetate (26 mg, 0.32 mmol) was added to the solution and the reaction mixture was heated to 80°C. Five drops of water were added in order to render the mixture homogeneous. The reaction mixture was heated for 4 h after which all the starting material was consumed. The reaction mixture was concentrated and the crude product was purified by FCC using 5% methanol in dichloromethane. The pure product was obtained as a white solid (42.0 mg, 0.10 mmol, yield 64%).

^{1}H NMR (D_2O): 7.60 (1H, s, H_6), 6.65 (1H, d, $H_{1'}$), 6.26 (1H, m, $H_{3'}$), 5.90 (1H, d, $H_{2'}$), 4.94 (1H, m, $H_{4'}$), 4.30 (2H, s, CH_2O), 3.80 (1H, dd, $H_{5'}$), 3.58 (1H, dd, $H_{5''}$), 3.32 (3H, s, OCH_3), 1.62 (1H, m, SiCCH), 0.85 (12H, m, $C(CH_3)_2C(CH_3)_2$), 0.15 (6H, s, $2SiCH_3$).

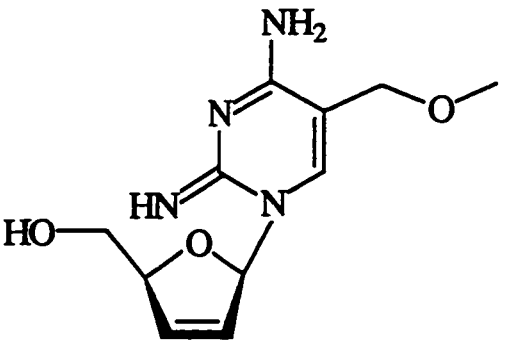
^{13}C NMR (D_2O): 85.8 (d, $C_{1'}$), 130.8 (d, $C_{2'}$), 134.2 (d, $C_{3'}$), 88.3 (d, $C_{4'}$), 63.1 (t, $C_{5'}$), 156.7 (s, C_2), 167.3 (s, C_4), 109.8 (s, C_5), 144.7 (d, C_6), 69.4 (t, CH_2O), 59.6 (q, OCH_3), -1.50 (q, $SiCH_3$), -1.45 (q, $SiCH_3$), 19.5 (q, $SiC(\mathbf{C}H_3)_2$), 20.1 (q, $SiCC(\mathbf{C}H_3)_2$), 20.6 (s, SiC), 26.4 (s, Si**C**C).

UV: λ_{min}: 241 nm, λ_{max}: 284 nm (ε, 11200)

MS: FAB mass 412.1 (M^++1), acc. mass found 412.2731, calc. (M^++1) 412.2695

mp: 146-149° C .

2',3'-didehydro-2',3'-dideoxy-2-imino-MMCyd. (**90**)



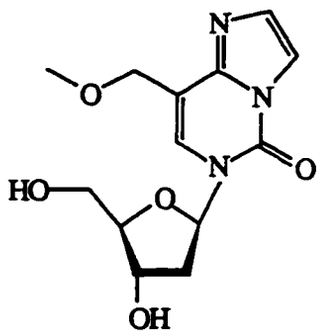
The compound 5'-dimethylthexylsilyl-2',3'-didehydro-2',3'-dideoxy-2-imino-MMCyd (**77**) (40 mg, 0.10 mmol) was dissolved in THF (2 mL) under argon. TBAF (35 mg, 0.14 mmol) was added and the reaction progress was monitored by TLC. After 3 h, the reaction was over and the mixture containing the crude product was concentrated and purified twice on PTLC using 20% methanol in dichloromethane. The pure product was obtained as a white solid (18 mg, 0.07 mmol, yield 70%).

^{1}H NMR (D_2O): 7.63 (1H, s, H_6), 6.60 (1H, d, $H_{1'}$), 6.32 (1H, m, $H_{3'}$), 5.94 (1H, d, $H_{2'}$), 5.00 (1H, m, $H_{4'}$), 4.31 (2H, s, CH_2O), 3.72 (1H, dd, $H_{5'}$), 3.55 (1H, dd, $H_{5''}$), 3.31 (3H, s, OCH_3).

^{13}C NMR (D_2O): 85.2 (d, $C_{1'}$), 130.5 (d, $C_{2'}$), 134.0 (d, $C_{3'}$), 88.9 (d, $C_{4'}$), 63.2 (t, $C_{5'}$), 157.1 (s, C_2), 167.2 (s, C_4), 109.6 (s, C_5), 144.5 (d, C_6), 69.5 (t, CH_2O), 59.7 (q, OCH_3).

UV: λ_{min}: 245 nm, λ_{max}: 282 nm (ε, 10700)
MS: FAB mass 253.1 (M^++1), acc. mass found 253.2718, calc. (M^++1) 253.2702
mp: 177°C d.

3,4-etheno-5-methoxymethyl-2'-deoxycytidine. (**94**)



A 50% w/w solution of chloroacetaldehyde in water (1161 mg, 7.4 mmol) was added dropwise to a flask containing MMdCyd (**18**) (100 mg, 0.37 mmol) at room temperature. After the mixture became homogeneous, the pH was adjusted to 3.5 using 1M HCl. The reaction was left at room temperature for 24 h. A TLC in 20% MeOH in dichloromethane showed a significant amount of starting material. The reaction was left for another 24 h without any apparent progress. The pH of the reaction was raised to 7.0 using saturated methanolic NaOH and the reaction mixture was co-evaporated with ethanol. The crude product was purified by FCC using 15% MeOH in dichloromethane to yield a white solid (47 mg, 0.16 mmol, yield 42%). This product was submitted for biological testing.

^{1}H NMR (D_2O): 7.60 and 7.15 (2H, 2d, ethen), 7.49 (1H, s, H_6), 6.30 (1H, dd, $H_{1'}$), 4.40-4.30 (3H, m, CH_2 and $H_{3'}$), 3.90 (1H, m, $H_{4'}$), 3.70 (1H, dd, $H_{5'}$), 3.62 (1H, dd, $H_{5''}$) , 3.23 (3H, s, OCH_3), 2.42-2.20 (2H, m, $H_{2'}$ and $H_{2''}$).

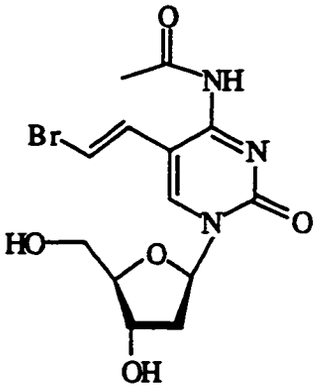
^{13}C NMR (D_2O): 88.5 (d, $C_{1'}$), 41.6 (t, $C_{2'}$), 72.7 (d, $C_{3'}$), 89.2 (d, $C_{4'}$), 63.4 (t, $C_{5'}$), 147.1 (s, C_2), 148.9 (s, C_4), 110.5 (s, C_5), 134.1 (d, C_6), 69.7 (t, CH_2O), 59.9 (q, OCH_3), 130.3 and 115.8 (d, ethen).

UV: λ_{min}: 232 nm, λ_{max}: 271 nm (ε, 9050)

MS: FAB mass 296.1 (M^++1), acc. mass found 296.1249, calc. (M^++1) 296.1227

mp: 165 -167 °C

N^4-acetyl-(E)-5-(2-bromovinyl)-2'-deoxycytidine. (**95**)



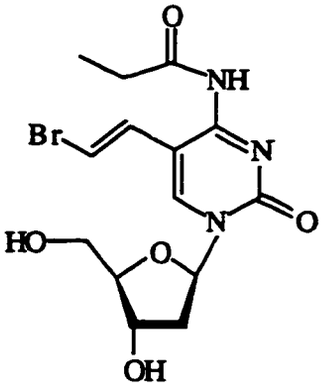
The nucleoside (E)-5-(2-bromovinyl)-2'-deoxycytidine (**17**) (150 mg, 0.45 mmol) was dissolved in DMF (2 mL) at room temperature. The solution was cooled in an ice water bath under argon and acetic anhydride (47 μL, 0.50 mmol) was added. The reaction mixture was left at room temperature for 24 h. A TLC (20% MeOH in CH_2Cl_2) indicated complete reaction. The solution was concentrated to dryness under high vacuum and the crude mixture applied on a silica gel plug three cm in depth and eluted with several column volumes of 5% methanol in dichloromethane to yield a white solid product. This product was freeze dried to obtain the final white solid (104 mg, 0.30 mmol, yield 67%), which was submitted for biological testing.

^{1}H NMR (D_2O): 8.40 (1H, s, H_6), 6.88 and 6.65 (2H, 2d, 13.5 Hz, vinyl hydrogens), 6.09 (1H, dd, $H_{1'}$), 4.32 (1H, m, $H_{3'}$), 4.00 (1H, m, $H_{4'}$), 3.80 (1H, dd, $H_{5'}$), 3.65 (1H, dd, $H_{5''}$), 2.48 (1H, m, $H_{2''}$), 2.25 (1H, m, $H_{2'}$), 2.05 (3H, s, NOAc).

^{13}C NMR (D_2O): 89.5 (d, $C_{1'}$), 42.1 (t, $C_{2'}$), 71.5 (d, $C_{3'}$), 89.9 (d, $C_{4'}$), 63.1 (t, $C_{5'}$), 157.6 (s, C_2), 161.7 (s, C_4), 129.5 (s, C_5), 144.5 (d, C_6), 175.7 (s, acetyl CO), 29.8 (q, acetyl CH_3).

UV: λ_{min}: 216 nm, λ_{max}: 319 nm (ε, 9900)
MS: FAB mass 373.1 and 375.1 (M^++1), acc. mass found 373.0517 and 375.0522, calc. (M^++1) 373.0503 and 375.0486
mp: 128-131°C

N^4-propionyl-(E)-5-(2-bromovinyl)-2'-deoxycytidine. (**96**)



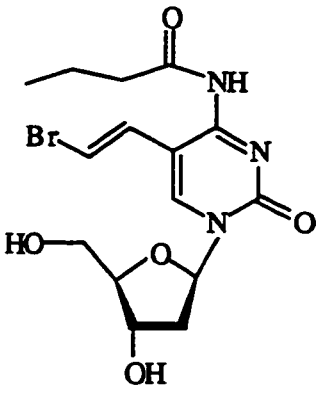
The compound (E)-5-(2-bromovinyl)-2'-deoxycytidine (**17**) (150 mg, 0.45 mmol) was dissolved in DMF (2 mL) at room temperature. The solution was cooled in an ice water bath under argon and propanoic anhydride (87 µL, 0.68 mmol) was added. The reaction mixture was left at room temperature for 24 h. A TLC (10% MeOH in CH_2Cl_2) indicated complete reaction. The solution was concentrated to dryness under high vacuum and the crude mixture applied on a silica gel plug three cm in depth and eluted with several column volumes of 5% methanol in dichloromethane to yield a white solid product. This product was freeze dried to obtain the final white solid (120 mg, 0.31 mmol, yield 69%), which was submitted for biological testing .

^{1}H NMR (D_2O): 8.36 (1H, s, H_6), 6.75 and 6.50 (2H, 2d, 13.8 Hz, vinyl hydrogens), 5.96 (1H, dd, $H_{1'}$), 4.30 (1H, m, $H_{3'}$), 3.95 (1H, m, $H_{4'}$), 3.75 (1H, dd, $H_{5'}$), 3.62 (1H, dd, $H_{5''}$), 2.48 (3H, m, propanoyl CH_2 and $H_{2''}$), 2.18 (1H, m, $H_{2'}$), 1.00 (3H, t, propanoyl CH_3).

^{13}C NMR (D_2O): 89.6 (d, $C_{1'}$), 42.5 (t, $C_{2'}$), 71.7 (d, $C_{3'}$), 90.0 (d, $C_{4'}$), 62.7 (t, $C_{5'}$), 157.7 (s, C_2), 161.8 (s, C_4), 129.7 (s, C_5), 144.7 (d, C_6), 179.3 (s, propanoyl CO), 32.9 (t, propanoyl CH_2), 10.8 (q, propanoyl CH_3).

UV: λ_{min}: 215 nm, λ_{max}: 319 nm (ε, 10500)
MS: FAB mass 388.1 and 390.1 (M^++1), acc. mass found 388.0414 and 390.0477, calc. (M^++1) 388.0371 and 390.0452
mp: 123-125°C

N^4-butanoyl-(E)-5-(2-bromovinyl)-2'-deoxycytidine. (**97**)



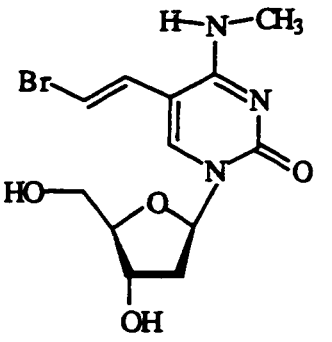
The nucleoside (E)-5-(2-bromovinyl)-2'-deoxycytidine (**17**) (158 mg, 0.48 mmol) was dissolved in DMF (2 mL) at room temperature. The solution was cooled in an ice water bath under argon and butanoic anhydride (120 μL, 0.72 mmol) was added. The reaction mixture was left at room temperature for 24 h. A TLC (10% MeOH in CH_2Cl_2) indicated complete reaction. The entire solution was concentrated to dryness under high vacuum and the crude mixture applied on a silica gel plug three cm in depth and eluted with several column volumes of 5% methanol in dichloromethane to yield a white solid product. This product was freeze dried to obtain the final white solid (125 mg, 0.31 mmol, yield 65%), which was submitted for biological testing .

^{1}H NMR (D_2O): 8.34 (1H, s, H_6), 6.72 and 6.51 (2H, 2d, 13.6 Hz, vinyl hydrogens), 6.05 (1H, dd, $H_{1'}$), 4.35 (1H, m, $H_{3'}$), 3.95 (1H, m, $H_{4'}$), 3.70 (1H, dd, $H_{5'}$), 3.65 (1H, dd, $H_{5''}$), 2.52 (3H, m, butanoyl α CH_2 and $H_{2''}$), 2.19 (1H, m, $H_{2'}$), 1.62 (2H, m, butanoyl β CH_2), 1.00 (3H, t, butanoyl CH_3).

^{13}C NMR (D_2O): 89.3 (d, $C_{1'}$), 42.4 (t, $C_{2'}$), 71.5 (d, $C_{3'}$), 90.1 (d, $C_{4'}$), 62.6 (t, $C_{5'}$), 157.5 (s, C_2), 161.4 (s, C_4), 129.2 (s, C_5), 144.1 (d, C_6), 178.9 (s, butanoyl CO), 32.9 (t, α CH_2), 22.4 (t, β CH_2), 10.8 (q, butanoyl CH_3).

UV: λ_{min}: 218 nm, λ_{max}: 321 nm (ε, 10200)
MS: FAB mass 402.1 and 404.1 (M^++1), acc. mass found 402.0655 and 404.0649, calc. (M^++1) 402.0621 and 404.0628
mp: 119-122°C

N^4-methyl-(E)-5-(2-bromovinyl)-2'-deoxycytidine. (**101**)



The protected nucleoside 3',5'-diacetyl-(E)-5-(2-bromovinyl)-2'-deoxyuridine (**98**) (200 mg, 0.48 mmol) dissolved in acetonitrile (5 mL), was added to a mixture of Et_3N (1.7 mL, 12 mmol), 1,2,4-triazole (890 mg, 13 mmol) and $POCl_3$ (270 µL, 5.4 mmol) at 0°C. The mixture was stirred for 2 h at room temp. The solvent was removed and the crude product extracted with ethyl acetate (150 mL). The organic layer was washed with saturated $NaHCO_3$ (50 mL) then with water (50 mL), and finally with brine (50 mL). The organic layer was dried and concentrated under vacuum. The crude triazolyl intermediate (**99**) was dissolved in dioxane (5 mL), the solution was cooled in an ice water bath, and monomethylamine gas was bubbled. After 14 h the crude product 3',5'-diacetyl-N^4-methyl-5-bromovinyl-2'-deoxycytidine (**100**) was dissolved in methanolic ammonia and after 48 h, the crude N^4-methyl-5-bromovinyl-2'-deoxycytidine was purified by FCC using 15% methanol in dichloromethane to yield a pure white solid (123 mg, 0.36 mmol, yield 74%).

^1H NMR (D_2O): 7.60 (1H, s, H_6), 6.68 and 6.47 (2H, 2d, 12.7 Hz, vinyl hydrogens), 6.05 (1H, dd, $H_{1'}$), 4.35 (1H, m, $H_{3'}$), 3.83 (1H, m, $H_{4'}$), 3.65 (1H, dd, $H_{5'}$), 3.55 (1H, dd, $H_{5''}$), 2.70 (3H, s, NCH_3), 2.25 (1H, ddd, $H_{2''}$), 2.10 (1H, m, $H_{2'}$).

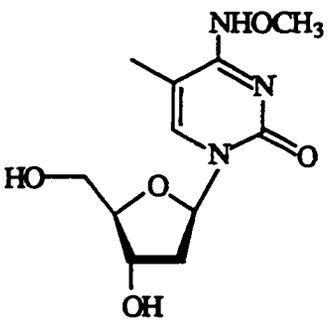
^{13}C NMR (D_2O): 88.5 (d, $C_{1'}$), 41.9 (t, $C_{2'}$), 72.5 (d, $C_{3'}$), 88.9 (d, $C_{4'}$), 63.3 (t, $C_{5'}$), 159.5 (s, C_2), 164.2 (s, C_4), 129.6 (s, C_5), 139.0 (d, C_6), 112.5 and 109.6 (2d, $C_{(5,1)}$ and $C_{(5,2)}$), 30.0 (q, NCH_3).

UV: λ_{min}: 242 nm, λ_{max}: 298 nm (ε, 9550)

MS: FAB mass 346.1 and 348.1 (M^++1), acc. mass found 346.0416 and 348.0382, calc. (M^++1) 346.0403 and 348.0361

mp: 180°C d.

N^4-methoxy-5-methyl-2'-deoxycytidine. (**105**)



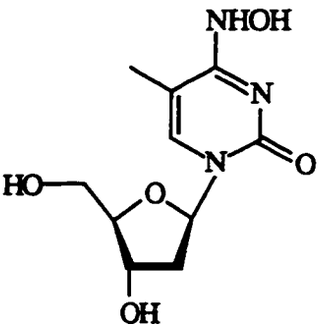
The compound 3',5'-diacetyl-4-triazolyl-2'-deoxythymidine (**102**) (379 mg, 1.0 mmol) was dissolved in pyridine (10 mL). To the solution with stirring methoxylamine hydrochloride (1.67 g, 20.0 mmol) was added. The reaction mixture was stirred for 24 h after which it was worked up with ethyl acetate (200 mL) and water (2x50 mL) followed by brine (25 mL). The organic layer was dried over anhydrous sodium sulfate and co-evaporated with absolute ethanol. The crude 3',5'-diacetyl-N^4-methoxy-5-methyl-2'-deoxycytidine (**103**) was dissolved in methanol (10 mL) and the solution saturated with anhydrous ammonia. After 14 h the deprotected product was further purified by FCC three times (fresh silica each time) using 15% methanol in dichloromethane. The purified product was a white solid (244 mg, 0.9 mmol, yield 90%).

^{1}H NMR (D_2O): 6.78 (1H, s, H_6), 6.09 (1H, dd, $H_{1'}$), 4.29 (1H, m, $H_{3'}$), 3.80 (1H, m, $H_{4'}$), 3.65 (3H, s, $NOCH_3$), 3.65-3.50 (2H, m, $H_{5',5''}$), 2.22-2.05 (2H, m, $H_{2',2''}$), 1.62 (3H, s, CH_3).

^{13}C NMR ($CDCl_3$): 86.4 (d, $C_{1'}$), 40.1 (t, $C_{2'}$), 73.1 (d, $C_{3'}$), 88.3 (d, $C_{4'}$), 63.7 (t, $C_{5'}$), 149.2 (s, C_2), 153.0 (s, C_4), 109.3 (s, C_5), 130.6 (d, C_6), 63.4 (q, $NOCH_3$), 14.6 (q, CH_3).

UV: λ_{min}: 255 nm, λ_{max}: 279 nm (ε, 8350)
MS: FAB mass 272.1 (M^++1), acc. mass found 272.1256, calc. (M^++1) 272.1237
mp: 146-149° C

N^4-hydroxy-5-methyl-2'-deoxycytidine. (**106**)



The compound 3',5'-diacetyl-4-triazolyl-2'-deoxythymidine (**102**) (379 mg, 1.0 mmol) was added to a solution of hydroxylamine hydrochloride (1.39 g, 20.0 mmol) in pyridine (20 mL). The reaction mixture was stirred for 24 h after which it was worked up with ethyl acetate (200 mL) and water (2x50 mL) followed by brine (25 mL). The organic layer was dried over anhydrous sodium sulfate and co-evaporated with absolute ethanol. The crude 3',5'-diacetyl-N^4-hydroxy-5-methyl-2'-deoxycytidine (**104**) was dissolved in methanol (10 mL) and the solution saturated with anhydrous ammonia. After 14 h the deprotected product was purified by FCC using 15% methanol in dichloromethane. The final product was a white solid (231 mg, 0.9 mmol, yield 90%).

^{1}H NMR (D_2O): 6.75 (1H, s, H_6), 6.12 (1H, dd, $H_{1'}$), 4.29 (1H, m, $H_{3'}$), 3.80 (1H, m, $H_{4'}$), 3.65 (1H, dd, $H_{5'}$), 3.55 (1H, dd, $H_{5''}$), 2.28-2.05 (2H, m, $H_{2',2''}$), 1.62 (3H, s, CH_3).

^{13}C NMR ($CDCl_3$): 86.4 (d, $C_{1'}$), 39.9 (t, $C_{2'}$), 73.0 (d, $C_{3'}$), 88.3 (d, $C_{4'}$), 63.7 (t, $C_{5'}$), 149.5 (s, C_2), 153.3 (s, C_4), 109.9 (s, C_5), 129.9 (d, C_6), 14.5 (q, CH_3).

UV: λ_{min}: 249 nm, λ_{max}: 280 nm (ε, 8350)

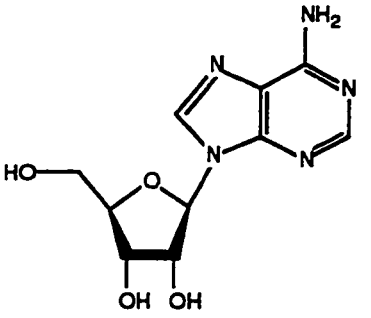
MS: FAB mass 258.1 (M^++1), acc. mass found 258.1090, calc. (M^++1) 258.1069

mp: 153-156° C

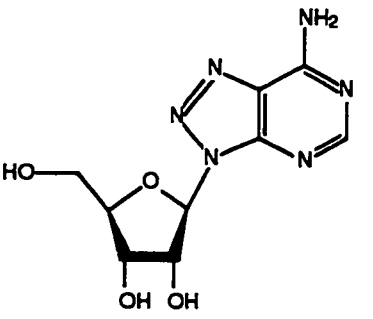
4-CONFORMATIONAL STUDIES

4.1) Introduction

Nucleosides and nucleotides are among the most important molecules in biology. They are found in all living organisms and are responsible for many functions. Knowing the structural features of a nucleoside or a nucleotide is essential to understanding its biological function. The purine nucleoside adenosine (**107**) acts as an energy pool for many enzymatic processes and for muscular work.[66] The analog 8-azaadenosine (**108**) is on the other hand a potent antibiotic even though from a chemical point of view the isoelectronic substitution of 8-CH by an 8-N has only a modest influence on its chemistry.[66] In this particular case the three dimensional structure or conformation of adenosine has changed and this change explains the peculiar properties of 8-azaadenosine.[66]



adenosine (**107**)



8-azaadenosine (**108**)

Another example on the effect of three dimensional structure on biological function is that of MMdCyd (**18**) and N^4-methyl-MMdCyd (**24**). As mentioned before (section 1.4.2), the analog MMdCyd (**18**) is a potent antiherpes agent while its methylated analog N^4-methyl-MMdCyd (**24**) is devoid of antiherpes activity. The explanation of the dramatic change in antiherpes activity has to lie in the structure.[35]

There is growing evidence that conformation plays an important role in

recognition by enzymes.[2,21,33,35,67] To date, considerable data have been accumulated on the parameters which govern the conformational flexibility of nucleosides.[35] The conformations of some nucleoside analogs which are active against herpes simplex virus (HSV) have been determined.[21,34,35] However, more information and correlations with both active and inactive agents are required to understand the structural constraints for recognition by virus-induced and cellular kinases and thereby rationalize the basis of selectivity.

4.2) Materials and Methods

Conformational studies of analogs were performed with high resolution NMR spectroscopy using a Bruker AM-300 spectrometer and related computer software. Second order analysis of spectra were obtained using the Brucker routine PANIC on an Aspect mini-computer of the NMR spectrometer with splitting from observed spectra used as a starting point. Spectra for second order analysis were obtained using NMR samples of 0.1 M concentration in D_2O without adjusting the pD. Experimental coupling constants have a precision of 0.1 Hz.

The software PSEUROT was employed to determine the pseudorotational equilibrium of the 2'-deoxyribose moiety. This software requires vicinal coupling constant values for the endocyclic protons of the 2'-deoxyribose moiety, in our case ($J_{1'2'}$, $J_{1'2''}$, $J_{2'3'}$, $J_{2''3'}$, $J_{3'4'}$). Coupling constants obtained from PANIC were used for input into PSEUROT.

Observed coupling constants were used for the determination of the populations of the exocyclic side chain ($C_{4'}$ - $C_{5'}$) conformations namely g^+, g^-, and t. The populations were calculated using a three equations, three unknowns computer software.[68] The equations are discussed in section 4.4.3.

The *syn/anti* glycosidic torsional preference was deduced from nOe experiments. The enhancements of $H_{1'}$, $H_{2'}$ and $H_{3'}$ on H_6 were recorded and compared. The area under the resonance of the enhanced signal was compared to the area of the same resonance in a control spectrum.

The orientation of N^4-substituents was determined by observing the enhancement (or lack of it) of the N^4-substituent hydrogens on the hydrogens of the 5-methoxymethyl, 5-bromovinyl and 5-methyl side chains.

PSEUROT

Vicinal proton spin-spin coupling constants play an important role in conformational analyses. With the help of Karplus equation-like, the vicinal proton spin-spin coupling constants can be translated into proton-proton torsion angles. In the case of five-membered rings, the proton-proton torsion angles are related to the endocyclic torsion angles which, in turn, are correlated via the laws of pseudorotation. Consequently, the vicinal couplings can be expressed as a function of the pseudorotation parameters: a phase angle of pseudorotation P and a puckering amplitude Φ_m. These parameters define all five endocyclic torsion angles.[69] A minimum two-state equilibrium in solution is assumed, and hence the coupling constants represent time-averaged values of the couplings of the individual conformers. In this case, the coupling constants can be expressed as a function of the five conformational parameters involved: the mole fraction of one conformer and two pseudorotation parameters P and Φ_m for each conformer. The general approach involves corrections for the effects exerted by electronegativity and orientation of substituents on $^3J_{HH}$, as well as corrections for deviations from 120° projection symmetry.[70] The program PSEUROT calculates the best fit of the conformational parameters to the experimental vicinal couplings.

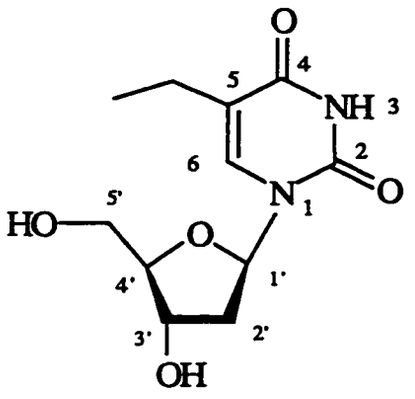
PSEUROT also requires coupling constants obtained at four different

temperatures. The temperatures that were chosen to work with are 278, 298, 310, and 323 K. Four different temperatures are used in order to disturb the equilibrium of the molecule in question and allow the program to calculate the best fit. As well, a large set of input data minimizes experimental errors. All calculations of pseudorotational phase angles and *north/south* population distribution were performed by restraining the maximum puckering amplitude Γ_m to a fixed value of 36.0°. Electronegativities were adjusted where necessary according to PSEUROT specifications.

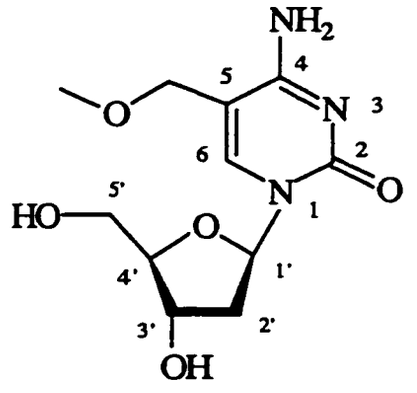
4.3) Pyrimidine Nucleoside Parameters

In structural chemistry, defining the terms used to describe the geometry of molecules is fundamental in understanding basic principles. Definitions of bond angles and torsion angles are somewhat complex because of competing and conflicting terminology. However, the IUPAC-IUB standard definitions[3] will be explained and used in this document.

4.3.1) Atomic Numbering System : figure 4.1 indicates the atomic numbering system adopted for pyrimidine nucleosides. Sugar atoms are distinguished from base atoms by a prime. In a sugar ring, the numbering sequence is C_1'-C_2'-C_3'-C_4'-O_4'-C_1'. The two hydrogens at $C_{5'}$ are labeled $H_{5'}$ and $H_{5''}$ and at deoxyribose $C_{2'}$ are labeled $H_{2'}$ (above the plane of the sugar) and $H_{2''}$ (below the plane of the sugar). In the pyrimidine heterocycle, the numbering sequence is N_1-C_2-N_3-C_4-C_5-C_6. A substituent on any of the pyrimidine atoms bears the number of that atom. For example an ethyl group on position 5 of the pyrimidine ring in 2'-deoxyuridine (dUrd (**22**)) is termed 5-ethyl-2'-deoxyuridine (EtdUrd (**9**)).



5-ethyl-2'-dUrd (**22**)



5-methoxymethyl-2'-dCyd (**18**)

Fig. 4.1: Atomic numbering system of pyrimidine nucleosides

4.3.2) Torsion Angles and Their Ranges: The three-dimensional structure of a molecule is characterized by bond lengths, bond angles, and rotations of groups of atoms about single bonds. These rotations about a central bond B-C are described by torsion angles involving four atoms in sequence, A-B-C-D as seen in figure 4.2.

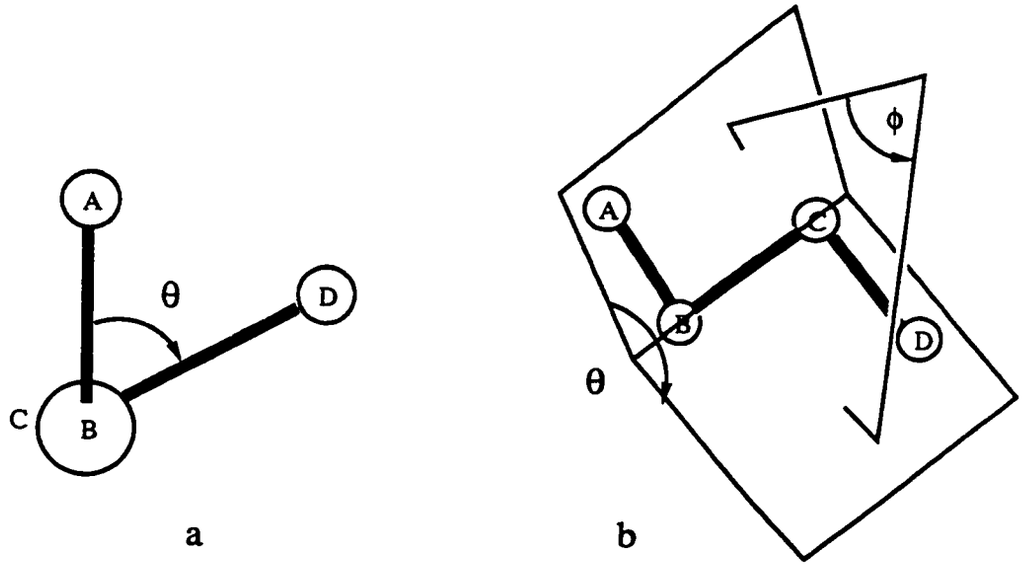


Fig. 4.2
Definition of torsion and dihedral angles. (a) Torsion angle θ describing orientations of bonds A-B and C-D with respect to the central bond B-C. (b) θ is defined as the angle between planes A-B-C and B-C-D while ϕ represents the angle between normals to these planes.

The torsion angle, θ in figure 4.2 is defined as the angle between projected bonds A-B and C-D when looking along the central bond either in direction B-C or in the opposite sense C-B. It is defined as 0° if A-B and C-D are eclipsed (cis and coplanar) and the sign of the angle is positive if the far bond (from the viewer) is rotated clockwise with respect to the near bond. The torsion angle is usually reported either in the range 0° to 360° or -180° to +180°.

Rather than describing a torsion angle in terms of an angle between projected bonds, it can also be described as an angle between the two planes containing atoms A, B, C and B, C, D. Another definition uses the angle between the normals to these planes. The dihedral angle ϕ in figure 1.3 is the complement of the torsion angle. In molecules with rotational freedom about single bonds, certain sterically allowed conformations are preferred. Therefore, it is often convenient to describe a structure with torsion angle ranges. The ranges commonly used in organic chemistry are those proposed by Klyne and Prelog [71]: *syn* (approx 0°), *anti* (approx 180°), ± *synclinal* (approx ± 60°), ± *anticlinal* (approx ±120°). In spectroscopic and crystallographic publications, the notations *cis* (approx 0°), *trans* (approx 180°), ±*gauche* (approx ±60°) are encountered .

4.3.3) Definitions of Torsion Angles of Pyrimidine Nucleosides: The endocyclic torsion angles of the sugar are denoted ν_0 to ν_4. The orientation of the base relative to the sugar is given by χ and the torsion angle describing the exocyclic side chain is given by ψ. Table 4.1 gives the correlation between torsion angles and the corresponding atoms involved.

Table 4.1 Definition of torsion angles in pyrimidine nucleosides

Torsion angle	Atoms involved
χ	O_4-C_1-N_1-C_2
ν_0	C4'-O4'-C1'-C2'
ν_1	O_4-C_1-C_2-C_3'
ν_2	C_1-C_2-C_3-C_4'
ν_3	C_2-C_3-C_4-O_4'
ν_4	C_3-C_4-O_4-C_1'
ψ	C_3-C_4-C_5-O_5'

4.3.4) Geometry of the Pyrimidine Base: Pyrimidine bases have been investigated as individual units and as moieties substituted at the glycosyl nitrogen.[72] In general, pyrimidine bases are planar. Slight deviations of less than 0.1 Angstroms of ring atoms and significant deviations of less than 0.3 Angstroms are not systematic and are attributed to influences of crystal packing forces. In solution, the time-average geometry of base ring atoms will be planar.

4.4) Conformational Parameters

In studying nucleoside conformations, a chemist's main interest revolves around four basic parameters: The puckering of the five membered sugar ring, the rotation of the base above the sugar ring, the orientation of the exocyclic side chain (C4' -C5') above the sugar ring and the orientation of the group or groups attached to the pyrimidine moiety.

4.4.1) Sugar Puckering Modes

The five-membered furanose ring is nonplanar. It can be puckered in an envelope (E) form with four atoms in one plane and the fifth atom is out by 0.5 Angstroms; or in a twist (T) form with two adjacent atoms displaced on opposite sides of a plane passing through the other three atoms. Atoms displaced from these three- or four-atom planes and on the same side as $C_{5'}$ are called *endo*; those atoms on the opposite side are called *exo*.[73] Sugar puckering modes are defined accordingly. It should be noted that because the transition between (E) and (T) forms is facile, usually the atoms defining the four-atom planes are not perfectly coplanar, and displacements from a three-atom plane are rarely symmetrical . Therefore, the larger deviation from planarity of one atom is called major puckering; the lesser deviation is called minor. Furanose rings are puckered because in a planar arrangement, all torsion angles are 0° and the substituents attached to carbon atoms are fully eclipsed which is an energetically unfavorable situation.

In an abbreviated notation, superscripts for *endo* atoms and subscripts for *exo* atoms precede or follow the letter (E) or (T), depending on whether the puckering is major or minor. An unsymmetrical $C_{3'}$-*endo* -$C_{2'}$-*exo* twist with major $C_{3'}$ and minor $C_{2'}$ puckering is represented as $^{3}T_{2}$. The same twist in symmetrical form is designated as $^{3}_{2}T$. A $C_{2'}$-endo envelope is given as ^{2}E, a $C_{3'}$-exo envelope as $_{3}E$ (figure 4.3).

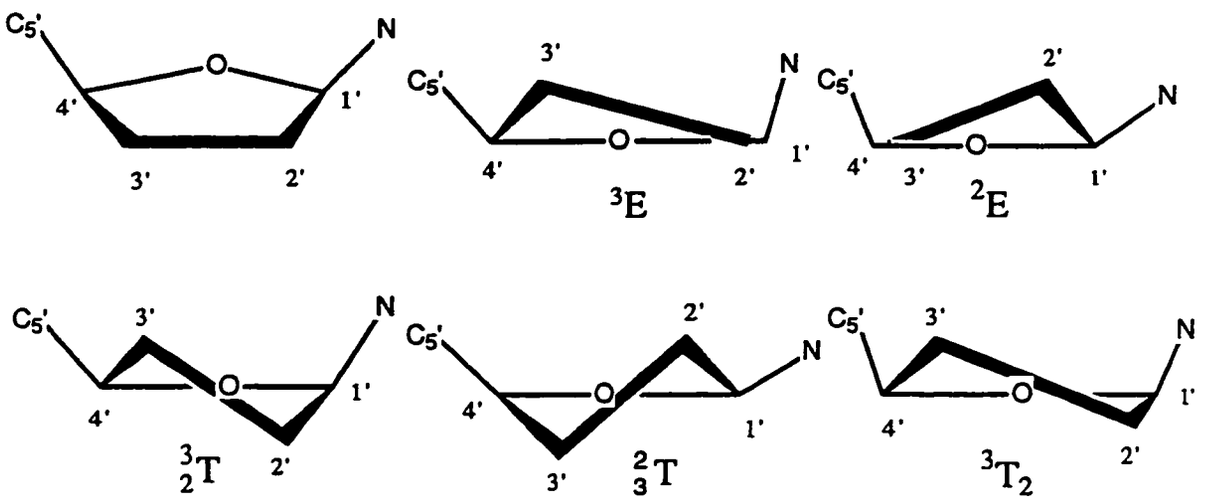


Fig. 4.3
Definition of sugar puckering modes showing the starting position with a flat five-membered ring, two envelope conformations as well as symmetrical and unsymmetrical twists.

4.4.1.1) The Pseudorotation Cycle

The preceding methods for describing sugar puckering are only approximate and are inadequate for describing intermediate twist modes whenever these are considered. Therefore, the puckering of five membered rings has been treated analytically in an elegant manner by the concept of pseudorotation, first introduced for cyclopentane[74] and later applied to substituted furanose rings.[75]

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. These can be described in terms of the maximum torsion angle (degree of pucker), ν_{max}, and the pseudorotation phase angle, **P** (figure 4.4).[76] If the five-membered ring is unsymmetrically substituted as in nucleosides, potential energy thresholds are created which limit the pseudorotation and lead to preferred puckering modes.

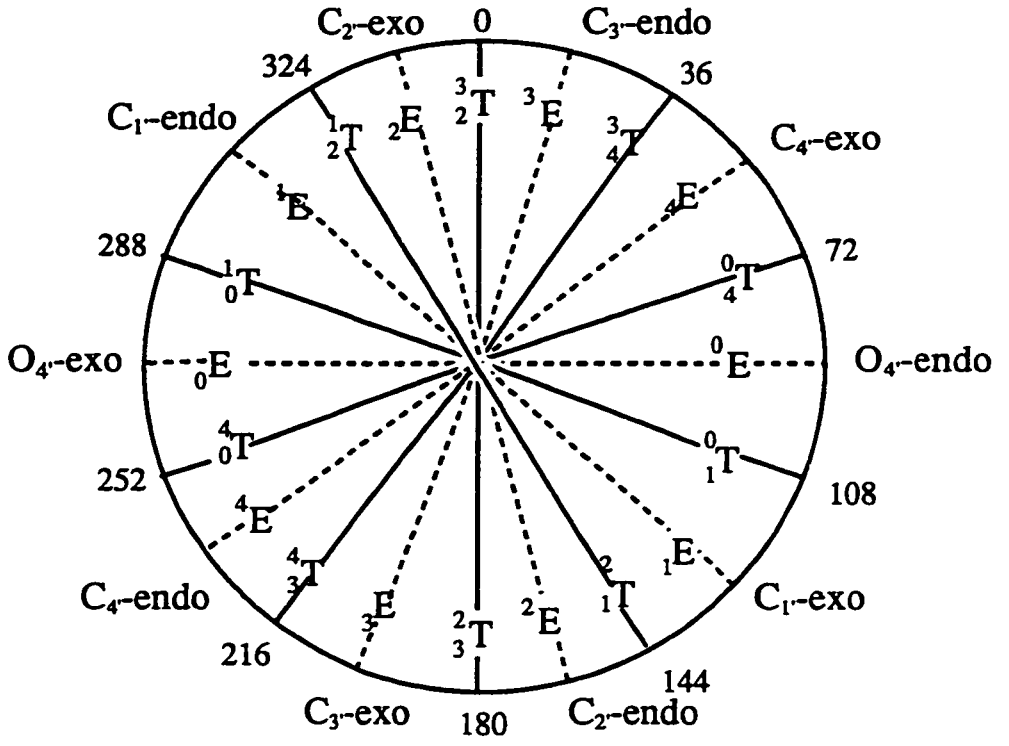


Fig 4.4: Pseudorotation cycle of the furanose ring in nucleosides.Values of phase angles given in multiples of 36°. Envelope E and twist T forms alternate every 18°.

In nucleosides, the pseudorotation phase angle **P** is calculated from the endocyclic sugar torsion angles according to [77]:

$$\tan P = \frac{(\nu_4 + \nu_1) - (\nu_3 + \nu_0)}{2\nu_2(\sin 36^\circ + \sin 72^\circ)} \qquad \text{eq. 4.1}$$

The phase angle P = 0° is defined such that torsion angle ν_2 is maximally positive, corresponding to a symmetrical C_2-*exo*-C_3-*endo* twist form, ${}^{3}_{2}T$. The mirror image, C_2-*endo*-C_3-*exo*, ${}^{2}_{3}T$, is represented by P = 180°. The maximum torsion angle ν_{max} which describes the maximum out-of-plane pucker, is given by:

$$\nu_{max} = \frac{\nu_2}{\cos P} \qquad \text{eq. 4.2}$$

In the pseudorotation cycle, envelope (E) and twist (T) conformations

alternate every 18°. (T) Is observed at even multiples of 18° and (E) is observed at odd multiples of 18°. *In nucleotide structure analysis two ranges of pseudorotation phase angles are preferred: C_3-endo at $0° \leq P \leq 36°$ (in the north of the cycle, or N) and C_2-endo at $144° \leq P \leq 190°$ (south, or S).*

4.4.1.2) Preferred Sugar Puckering Modes

As mentioned earlier, the five membered furanose ring in deoxyribonucleosides is never planar (in crystal form or in solution). A detailed analysis of ribose puckering in a large number of nucleosides has shown that the puckering amplitude Γ_m displays unimodal distribution, $\Gamma_m = 38.6 \pm 3°$. This suggests that Γ_m is an intrinsic property of the carbon-oxygen ring structure and is not dependent on type of sugar, base, pseudorotation phase angle **P**, or torsion angles χ and ψ.[78] And since the furanose in nucleosides is unsymmetrical, the pseudorotation phase angles are not evenly distributed over the pseudorotation cycle. *Instead they cluster in two domains centered at C_3-endo, P =-1° to 34° and at C_2-endo, P = 137° to 194°* .[78] Both of these (N) and (S) regions consist of a family of closely related puckering modes. Interconversion between (N) and (S) is possible via an O_4'-*endo* route only since on geometrical grounds in this puckering mode, the two substituents on C_1' and C_4' namely the pyrimidine base and the C_5' are equatorially oriented and thus spatially far apart from one another. On the other hand an O_4'-*exo* puckering mode would give rise to an axial steric interaction between the pyrimidine base and C_5'.[79]

The conformational energy of C_2'-*endo* and C_3'-*endo* furanose puckering modes and the energy barrier separating these two conformational states have been the subject of quantum chemical, extended Huckel and classical potential energy calculations.[80] These show that for unsubstituted ribose and

2'-deoxyribose, $C_{2'}$ and C_3-*endo* puckering modes are almost equivalent energetically. The two conformations are separated by nearly symmetrical potential energy barriers of 2 to 4 Kcal/mole (the energy required to flip from $C_{2'}$-*endo* to C_3-*endo*), which correspond to the unfavorable $O_{4'}$-*exo* and $O_{4'}$-*endo* sugar puckerings. However, if a base is attached to the sugar in a $C_{1'}$-β-N-glycosyl linkage, the energy distribution along the pseudorotation cycle becomes asymmetrical with $C_{2'}$-*endo* and $C_{3'}$-*endo* conformations at energy minima and $O_{4'}$-*endo* at a modest energy barrier of 2.5 to 5 Kcal/mole. $O_{4'}$-*exo* represents a barrier so high that it is practically unsurmountable. In other words, a flip from $C_{2'}$-*endo* to $C_{3'}$-*endo* does not proceed via an $O_{4'}$-*exo* transition state.

The barriers to rotation about C-O bonds are lower than those about C-C bonds. Therefore, the furanose ring adopts conformations with C-O torsion angles υ_o and υ_4 nearly eclipsed (around 0°) and and C-C torsion angles υ_1, υ_2 and υ_3 maximally staggered, resulting in $C_{2'}$-*endo* or -*exo* puckering for $\upsilon_4 = 0°$ and in $C_{3'}$-*endo* or -*exo* puckering for $\upsilon_0 = 0°$. In other words, puckering of $C_{1'}$ out of the plane of the other four atoms causes rotation[81] about bonds $C_{4'}$-$O_{4'}$ and $C_{2'}$-$C_{3'}$. This relieves steric contacts between substituents at $C_{2'}$ and $C_{3'}$ atoms although substituents at $C_{3'}$ and $C_{4'}$ remain eclipsed. The energetic situation is improved (i.e. lowered) if $C_{2'}$ puckers. In such a case rotation occurs about bonds $O_{4'}$-$C_{1'}$ and $C_{3'}$-$C_{4'}$, leaving the torsion angle υ_0 about $C_{4'}$-$O_{4'}$ around 0° and all substituents around the ring in a non eclipsed configuration.

Additionally, C-O-C angles are less flexible than angles with carbon as a vertex[82] and because endocyclic angles at puckered atoms are smaller than angles at in-plane atoms, this angle puckering relationship again counteracts $O_{4'}$ puckering as a stable conformation. The above arguments lead therefore to

C_2-*endo* and C_3-*endo* puckering modes as being preferred modes because nonbonding interactions between furanose ring substituents are at a minimum.

NMR investigations in solution demonstrate that C_2- and C_3-*endo* types of conformations are in rapid equilibrium. This can be evaluated from the ratio of vicinal ^1H coupling constants $J_{1'2'}$ and $J_{3'4'}$ using the equation Keq ~ $J_{1'2'}/J_{3'4'}$.[83] In general terms, pyrimidine deoxyribonucleosides tend to have larger proportions of C_2-*endo* puckered sugars.[84, 85] Finally it is worth mentioning that the barrier to interconversion between these two sugar puckering states C_2-*endo* and C_3-*endo* is so low that it can be measured using $J_{1'2'}$ and $J_{3'4'}$ only under special circumstances in liquid anhydrous deuterated ammonia (ND_3).[83]

4.4.1.3) Factors Affecting Furanose Puckering Modes

NMR spectroscopy shows an influence of the $C_{2'}$ and $C_{3'}$ substituents on the C_3-*endo* (N), C_2-*endo* (S) equilibrium. More electronegative substituents prefer an axial orientation.[86] Systematic surveys of 2'-substituted uridine derivatives indicated that the amount of the C_3-*endo* (N) conformer increases linearly with the electronegativity at the 2'-substituent.[87] This correlation predicts that deoxyribonucleosides would prefer the C_2-*endo* puckering mode because only the slightly electronegative hydrogen is attached at $C_{2'}$.This suggests that the dipole moment of the furanose ring actually determines its conformational characteristics and that a more electronegative substituent favors an axial orientation.

The sugar puckering modes are also influenced by modifications of the base.[88] This is especially true if the pyrimidine C_6 is substituted which will shift the *syn/anti* equilibrium about the glycosyl link toward *syn*. In general, the *syn* conformation correlates with C_2-*endo* (S) sugar pucker. Also cyclizations

between $O_{2'}$ and $O_{3'}$ (in riboses) or between $O_{3'}$ and $O_{5'}$ or intramolecular bond formation between sugar and base (as is the case with $O_{2'},C_2$ or $O_{3'},C_2$ anhydropyrimidines) limit and determine the puckering preferences of furanoses.[84] If the base is removed and replaced by H or by -O-CH_3, NMR studies [89] show that the furanose ring strongly prefers the $C_{3'}$-*endo* form.

More recent studies [90] show that in cytidines the conformational rigidity can be increased by modifying the remote N^4 substituent. It was found that N^4-acetylation and 2'-O-methylation each independently stabilize the $C_{3'}$-*endo* form of the ribose moiety, and that these stabilizations occur additively. The in-solution stabilization of the $C_{3'}$-*endo* form by N^4-acetylation is unexpected because the position of this modification in the base is spatially far from the ribose ring and cannot interact directly with it. In addition, the crystal structure of the N^4-acetyl cytidine has been determined as the $C_{2'}$-*endo* form.[91]

The electron withdrawing group (acetyl) at N^4 enhances the bonding interaction between a lone pair at $O_{4'}$ and the Π^* orbital of the C_5-C_6 double bond,[92,93] and changes the glycosidic torsion angle χ (C_2-N_1-$C_{1'}$-$O_{4'}$) to approximately -180° which may in turn result in stabilization of the $C_{3'}$-*endo* form due to steric repulsion between the 2-carbonyl and the 2'-substituents in the $C_{2'}$-*endo* form. Also a decrease in the electron density of H_6 by the electron attracting N^4-substituent may also provide a probable mechanism for the stabilization of the $C_{3'}$-*endo* form through stabilization of a hydrogen bond-type interaction between H_6 and $O_{5'}$.[94] It should be noted that the N^4-acetyl group was found to be proximal to C_5.[91]

4.4.2) Glycosyl Bond Orientation

Relative to the sugar moiety, the base can adopt two main orientations about the glycosyl $C_{1'}$-N link, called *syn* and *anti* (figure 4.5).[66] They are

defined by torsion angle χ . For pyrimidine nucleosides, χ is defined as torsion angle $O_{4'}$-$C_{1'}$-N_1-C_2. The base is rotated around the glycosidic "axis" relative to the sugar. The rotational position of the base relative to the sugar is sterically restricted and two conformational states are preferred, *syn* and *anti*.[95]

4.4.2.1) *syn-anti* Conformations

In the *anti* conformation of pyrimidine nucleosides there is no particular steric hindrance between sugar and base but in *syn* , the bulky part of the base (i.e. C_2 carbonyl group) is located over the sugar, giving rise to close interatomic contacts. These can be relieved if the sugar adopts the $C_{2'}$-*endo* pucker. In this form ($C_{2'}$-*endo*), the base and $C_{5'}$ atom of the sugar are in equatorial orientation above the 5-membered ring and thus moved apart. In pyrimidine deoxyribonucleosides the anti conformation is preferentially associated with $C_{2'}$-*endo* over $C_{3'}$-*endo* puckering.[96]

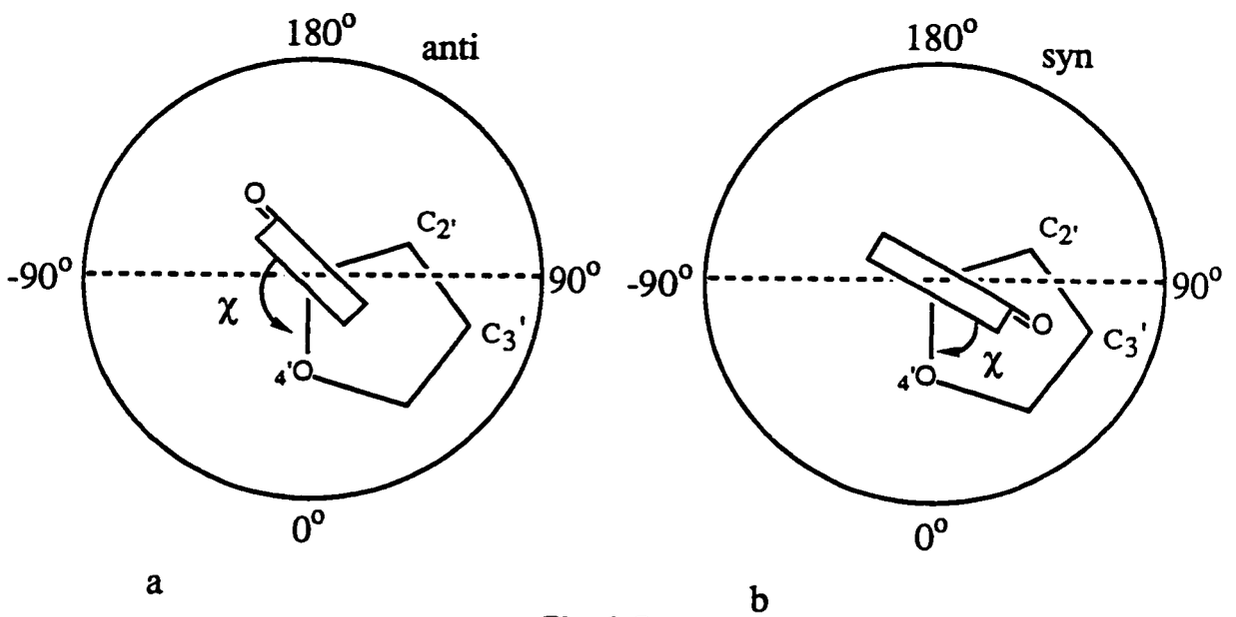


Fig.4.5
Definition of anti and syn conformational ranges. The pyrimidine base is toward the viewer; the base is rotated relative to the sugar. (a) shows the *anti* range from (-90)-180-(+90) °. (b) shows the *syn* range from (+90)-0-(-90) °.

For pyrimidine nucleosides, the *anti* orientation of the base about the

glycosyl bond is finely tuned by the sugar pucker.[97] The torsion angle about the $C_{1'}$-N bond, χ, is correlated with the $C_{2'}$-*endo* or $C_{3'}$-*endo* pucker according to:

$$-180^{o} \leq \chi \leq -138^{o} \text{ for } C_{3'}\text{-}endo,$$

$$-144^{o} \leq \chi \leq -115^{o} \text{ for } C_{2'}\text{-}endo.$$

This angular adjustment with sugar conformation is probably due to steric hindrance. Thus, in $C_{3'}$-*endo* puckering, the hydrogen at $C_{3'}$ ($H_{3'}$) is oriented axially and interacts with C_6-H of the base, leading to χ around 180°. In $C_{2'}$-*endo*, $H_{3'}$ is in an equatorial position and the base can rotate until its O_2 keto oxygen comes in close proximity to $H_{1'}$.[98]

Another structural relation for *syn/anti* conformations is the dependence of the length of the glycosyl $C_{1'}$-N bond on the χ torsion angle. For a number of pyrimidine nucleosides,[99] the $C_{1'}$-N bond length is a maximum of 1.52 angstroms if χ is near -180° and decreases almost linearly to 1.48 angstroms as χ approaches -140°. This finding shows that, at least for pyrimidine nucleosides, hydrogen atoms at $C_{3'}$ and $C_{2'}$ are not the only barrier to free rotation of the base above the sugar.[100] Atom $O_{4'}$ also plays a role; the lengthening of $C_{1'}$-N reflects steric interactions between $O_{4'}$ and the hydrogen atom attached to C_6.

Results of crystallographic studies on the *syn/anti* conformation in nucleosides are supported by a vast body of NMR data which suggests that, in solution, nucleosides undergo rapid *syn/anti* interchange.[84] The ratio of *syn/anti* conformers or the bias in equilibrium can be affected or modified by chemical modifications of both sugar and base moieties. Of particular interest are nucleosides substituted at pyrimidine C_6 because such structural changes, if properly chosen, influence the electronic characteristics of the nucleosides only slightly. On the other hand, the substituents interact directly with the sugar

and shift the equilibrium to *syn*.

Crystallographic and spectroscopic analyses have shown that 6-methyluridine for example occurs mostly in the *syn* conformation,[101] whereas the parent compound uridine occurs mostly in the *anti* conformation. Bulkier substituents such as tert-butyl have been attached to C_6 in order to achieve complete blockage in the *syn* form.[102]

Another factor stabilizing the *syn* conformer in nucleosides is the 5'- OH hydroxyl which can form a hydrogen bond with the O_2 of the pyrimidine. Hydrogen bonds of this type have been detected in many crystal structures of *syn* conformers. However there are also exceptions,[101] indicating that the favorable proximity of pyrimidine O_2 to $O_{5'}$ does not inevitably lead to formation of a hydrogen bond. Hydrogen bonding between C_6-H of the pyrimidine base and sugar $O_{5'}$ stabilizes the *anti* orientation of the base,[103] especially when $O_{5'}$ is phosphorylated.

4.4.3) Exocyclic Orientation about the $C_{4'}$-$C_{5'}$ Bond

Rotation about the exocyclic $C_{4'}$-$C_{5'}$ bond allows $O_{5'}$ to assume different positions relative to the furanose ring. Three main conformations with all substituents in staggered positions are possible as shown in figure 4.6.

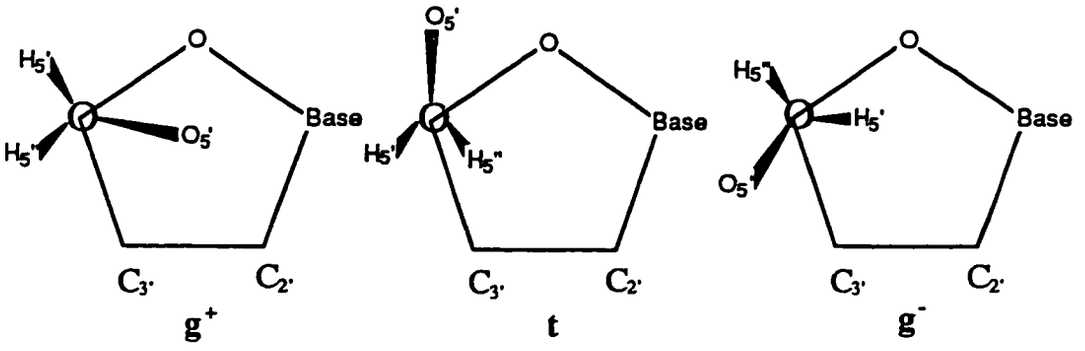


Fig 4.6
Definition of torsion angle ranges about the $C_{4'}$-$C_{5'}$ bond, looking in the direction $C_{5'}$-$C_{4'}$.

The torsion angle ψ : $O_{5'}$-$C_{5'}$-$C_{4'}$-$C_{3'}$ is the most commonly used to define orientation about the $C_{4'}$-$C_{5'}$ bond.[3] The solution conformation of the 5'-CH_2OR-moiety relative to the furanose ring may comprise three types of rotamers which are denoted g^+, g^-, and t. In aqueous solution the rapid interconversion between the three conformers yields weighted time-averaged NMR couplings between $H_{4'}$, $H_{5'}$ and $H_{5''}$ that are related to the couplings of the individual conformers and their relative populations by:

$$^3J_{4',5'} = X_{g+}J'_{g+} + X_{g-}J'_{g-} + X_tJ'_t \qquad \text{eq. 4.3}$$

$$^3J_{4',5''} = X_{g+}J''_{g+} + X_{g-}J''_{g-} + X_tJ''_t \qquad \text{eq. 4.4}$$

where X denotes the mole fraction of each conformer present given that:

$$X_{g+} + X_{g-} + X_t = 1 \qquad \text{eq. 4.5}$$

Knowledge of the individual couplings in these rotamers (J' and J") will allow conformational analysis of the $C_{4'}$-$C_{5'}$ bond in terms of relative rotamer populations. Values for the coupling constants in the individual rotamers were determined from solid-state knowledge of the furanose moiety combined with the use of a generalized Karplus relation.[104] The proton-proton torsion angles for the three different rotamers were deduced from a statistical analysis of 143 crystallographic studies of furanose systems.[78] These torsion angles in combination with the generalized Karplus equation yield the coupling constants shown in table 4.2.

Table 4.2: Torsion angles and coupling constants between protons. a and b are the torsion angles between the indicated protons, J' and J" are the coupling constants between those protons.

ψ	α [$H_{4'}$,$H_{5'}$]	J' (Hz)	β [$H_{4'}$,$H_{5''}$]	J" (Hz)
g⁺(53°):	-64°	2.4	55°	1.3
g⁻(-70°):	174°	10.6	-68°	3.8
t (180°):	64°	2.6	-178°	10.5

Rotation about the exocyclic C_4-$C_{5'}$ bond is very important in positioning the 5'-phosphate group relative to the sugar and base in nucleotides. It has been studied by crystallographic [96] and spectroscopic [85] methods as well as scrutinized by theoretical methods.[105] In contrast to the torsion angle χ about the glycosyl bond with two main populations, the rotation ψ about the C_4-$C_{5'}$ bond follows the classical three fold staggered pattern of ethane. There is one correlation however, or a similarity between the χ and ψ rotations: *syn* and g⁺ position the base (the bulky part of the base) and $O_{5'}$ "over" the ribose frame whereas *anti* and g⁻ or t direct the base and $O_{5'}$ "away" from the ribose.

The three ranges g⁺, g⁻ and t are not uniformly populated. In most crystalline pyrimidine nucleosides, the preferred conformation is g⁺ regardless of sugar pucker, and the few cases exhibiting g⁻ and t are found with C_2-*endo*. In-solution NMR studies on a series of substituted nucleosides are consistent with crystallographic results and suggest a relationship between sugar pucker and rotation about the C_4-$C_{5'}$ bond. The NMR data indicate that g⁻ and t orientations are favoured in C_2-*endo* puckering modes but that in the C_3-*endo*, the g⁺ orientation about the C_4-$C_{5'}$ bond predominates.[88]

4.4.3.1) Factors Influencing the Orientation about the C_4'-C_5' Bond

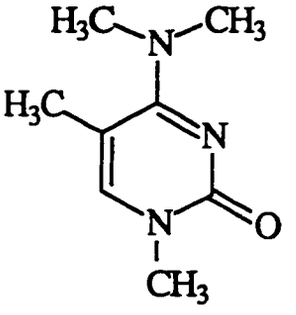
In nucleosides with anti-orientation of the bases, the g^+ conformer is in part stabilized by C_6-H....O_5' hydrogen bonding and therefore g^+ is favored over the other two staggered forms. If the base at C_1' is replaced by a group such as -O-CH_3, (whereby the molecule is not a nucleoside) the g^+ orientation of such a molecule is not preferred as in nucleosides, suggesting that in nucleosides, base····O_5' interactions pull torsion angle ψ into the g^+ orientation range. Such interactions have been observed, and measured.[106] Although H attached to C is not normally considered a hydrogen bond donor, this finding is supported by deuterium exchange NMR experiments [107] which show that pyrimidine protons at C_5 and C_6 appear to be considerably acidic. A quantum chemical account of the energies involved in C-H....O_5' interactions led to the conclusion that between 1.84 and 2.27 kcal/mole stabilize the g^+ and *anti* orientations about C_4'-C_5' and glycosyl bonds of uridine, thymidine and 5-fluorouridine.[106] Analysis of the forces involved in C-H....O_5' hydrogen bonding indicated that it is not only the hydrogen atom that attracts O_5', since if an electron withdrawing group is attached to pyrimidine C_6 instead of H, the resulting electron deficiency at this carbon atom will attract O_5'.

An even greater contribution of C-H...O_5' hydrogen type forces was estimated for 5'-nucleotides because in these, the phosphate attached to O_5' renders this atom more electronegative than the O_5'-H in nucleosides.

4.4.4) The Exocyclic C_4-NR_2 Bond in Cytidines

Several studies have shown that in the cytidine series, the amino group NH_2 is integrated into the resonance system with the pyrimidine ring. The exocyclic C-NH_2 bond length (1.34 Angstroms) is significantly shorter than an

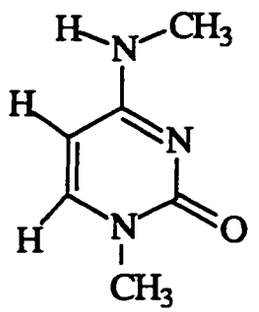
aliphatic C-N single bond (1.472 Angstroms).[108] The exocyclic C-NH_2 bond exhibits partial double bond character, almost halfway between single and double bond lengths.[109] As such, the amidine-like amino group is forced to be coplanar with the pyrimidine heterocycle and rotation about the exocyclic C-NH_2 is hindered. It has also been shown that in overcrowded cytosine derivatives with bulky substituents on the exocyclic nitrogen such as 1,5,N^4,N^4-tetramethylcytosine (**109**), all C and N atoms of the skeleton are coplanar.



1,5,N^4,N^4-tetramethylcytosine (**109**)

The steric repulsion between the two methyl groups on C_5 and N^4 is minimized or relieved by widening of the bond angles rather than by rotation of the N^4,N^4-dimethyl about the C-N exocyclic bond.[110a] The two methyl groups in question move apart from one another while remaining in the same plane.[110b]

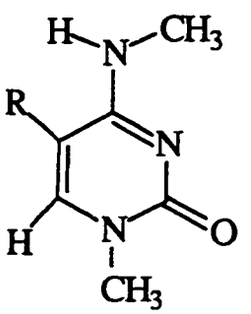
It has also been shown that the solution conformation of mono-N-methyl derivatives of cytosine such as 1,N^4-dimethyl-cytosine (**110**), exist predominantly in a conformation in which the N^4-methyl substituent is distal to C_5, and hence proximal to N_3.[111]



1,N^4-dimethyl-cytosine (**110**)

This observation is due mainly to steric interference between the proton on C_5 and the N-methyl group.[111]

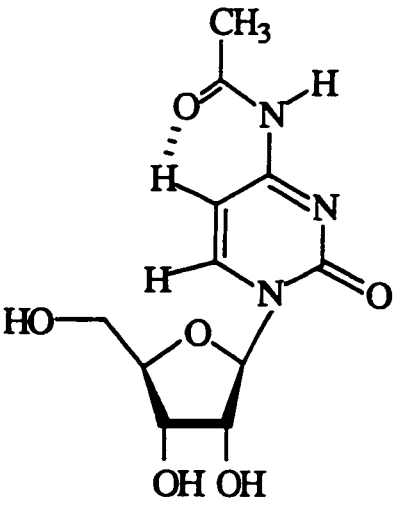
There appears to be no reported literature on the in-solution conformation of mono-N-methyl cytosine derivatives in which C_5 bears a bulky substituent R such as compound **111**.



111

However, it is safe to assume that the presence of such a bulky group as methoxymethyl which is the case with MMdCyd (**18**) increases the steric interaction between the substituents on C_5 and N^4. And hence the orientation of the N^4 substituent would be distal to C_5.

The orientation of the N^4 substituent however is not always distal to C_5. The X-ray crystal structure of the compound N^4-acetyl-cytidine (**112**) has been determined.[91] It was found that the N^4-acetyl group was proximal to C_5 in contrast to its analog N^4-methylcytidine.[91] This proximal orientation of the N^4-acetyl substituent to C_5 is quite unexpected, since the acetyl group is bulkier than a methyl group.



N^4-acetyl-cytidine (**112**)

It is thought in this case that the intramolecular contact between H_5 and the oxygen of the carbonyl substituent is more attractive than it is repulsive giving rise to a stable six-membered ring as is depicted in compound **112**. It is interesting to note that the electron withdrawing acetyl group has polarized H_5 sufficiently to take part in hydrogen bonding. NMR studies have indicated that the H_5 proton which has the δ value of 6 ppm for cytidine moved downfield to over 7 ppm for N^4-acetyl-cytidine (**112**) indicating an increased acidity for this proton.[90] Based on this it is possible that the proximal conformation (acetyl to C_5) in the crystal state is also the preferred conformation in solution.[90]

4.4.5) The C_5 Side Chain on the Pyrimidine Ring

A C_5 side chain with more than one atom in length can be on either side of the plane of the pyrimidine ring, resulting in an additional degree of conformational freedom. Its location is defined as either on the same side of the pyrimidine as the deoxyribose ring oxygen ($O_{4'}$), or on the opposite side. In solution there is evidence of a symmetrical distribution relative to the plane of the heterocyclic ring.[112] In the X-ray crystal structure of MMdCyd (**18**), the two possible conformations of the C_5 side chain are displayed in the two molecules

of the crystals (molecules A and B differ in the conformation of the furanose ring). Molecule A has one conformation of C_5 side chain and molecule B has the other one (fig. 4.7).[21] In molecules where hydrogen bonding plays a more pronounced role such as in 5-hydroxymethyl-2'-deoxyuridine (HMdUrd (**113**)), the X-ray shows that there is only one conformation for the furanose ring while there are two *unequal* occupancy factors for the C_5 side chain.[113]

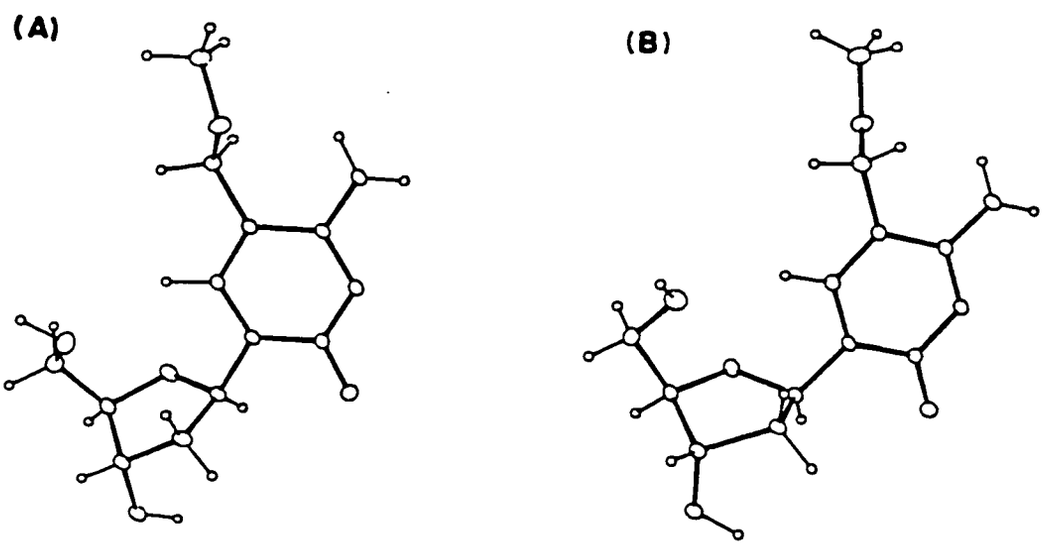


Fig. 4.7: Stereoscopic ORTEP view of MMdCyd. Molecules A and B (taken with permission from Prof. V.S. Gupta)

4.5) NMR Analyses

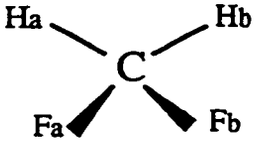
4.5.1) First-Order Spectral Analysis

Spectra arising from coupled nuclei (both individual nuclei and groups of equivalent nuclei) may be treated by first order analysis only when two conditions are satisfied:[114]

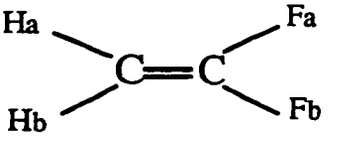
1. The chemical shift difference between nuclei (or groups of nuclei) must be much larger than the spin coupling between them.

2. Coupling must involve groups of nuclei that are magnetically equivalent, not just chemically equivalent. Nuclei are said to be chemically equivalent when they have the same chemical shift, usually as a result of molecular symmetry (e.g., the 2 and 6 protons, or the 3 and 5 protons in phenol) but occasionally as a result of accidental coincidence of shielding effects. Nuclei in a set are magnetically equivalent when they all posses the same chemical shift and all nuclei in the set are coupled equally with any other single nucleus in the molecule.

Thus, in the tetrahedral molecule difluoromethane (**114**), Ha and Hb are magnetically equivalent since they are by symmetry equally coupled to Fa and Fb. On the other hand, in the molecule 1,1-difluoroethene (**115**), Ha and Hb are not magnetically equivalent, since Ha and Fa have a *cis* geometry and are coupled with J (*cis*), while Hb and Fa are coupled with J (*trans*) and in general the two couplings are not equal.



difluoromethane (**114**)



1,1-difluoroethene (**115**)

For sets of magnetically equivalent nuclei, first-order analysis is considered applicable when $(\upsilon_A-\upsilon_B)/J_{AB} > 7$; When $(\upsilon_A-\upsilon_B)/J_{AB} < 7$, second-order analysis should be applied. When $7 < (\upsilon_A-\upsilon_B)/J_{AB} < 20$, there is some bias and /or distortion of intensities from the pattern of the binomial theorem.

4.5.2) Second-Order Spectral Analysis (reprinted with permission from Prof. R.S. Reid)

Second-order spectral analysis is a concept in NMR spectroscopy that

deals with the extraction of spectral parameters from strongly coupled systems. Common attitudes towards spectral analysis are, for instance, that " all real-life spectra are first order at 300 or 400 MHz "and second order analysis does not change the broad picture, just the picky details". Both of these statements are wrong; regardless of field strength, chemical shifts so small as to engender second-order behavior are possible, and incorrect analysis can yield gross errors in the resultant spectral parameters, and in the conclusions drawn.[115]

To dispel such misconceptions, the use of the proton NMR spectrum of ascorbic acid in D_2O as an example is very effective. This is a fine example of an ABMX system, giving a deceptively simple second-order spectrum at 300 MHz. An apparently reasonable first order "analysis" yields a largely erroneous set of coupling constants (the term *splitting* rather than *coupling* would be more appropriate to describe such an analysis).

Figure 4.8 shows the dominant structure for ascorbic acid in acidic D_2O solution. (Keto tautomers are of lesser importance).[116]

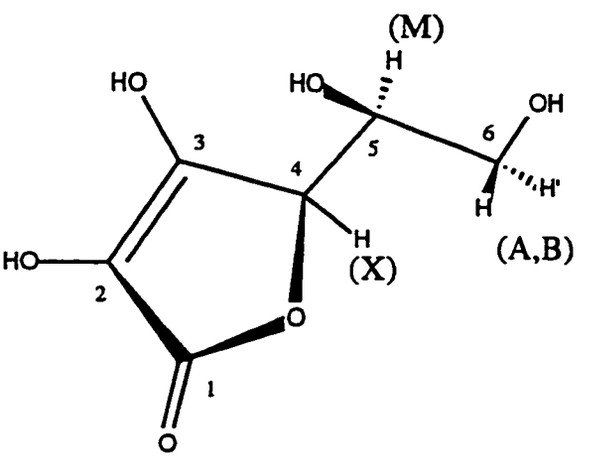


Fig 4.8: Ascorbic acid in acidic D_2O

The hydroxy protons are in rapid magnetic exchange with the solvent and do

not appear as separate resonances. The other four protons form an ABMX system, using the usual convention for spin system nomenclature.[114] The 300-MHz proton NMR spectrum of an acidified D_2O solution is shown in figure 4.9.

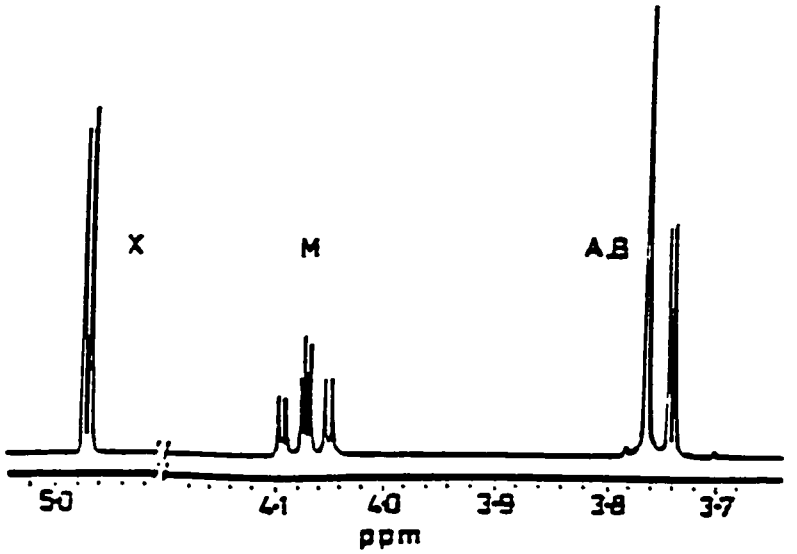


Fig 4.9: The 300-MHz proton NMR spectrum of ascorbic acid in D_2O
(reprinted with permission from Prof. R.S.Reid)

The splitting of 1.8 Hz observed for the X doublet is assigned to J_{mx}. As expected, no splitting ascribable to J_{ax} or J_{bx} is observed. The rest of the spectrum *apparently* also yields readily to first order analysis. The resonance at 4.06 ppm looks very much like a doublet of doublets of doublets, yielding "coupling constants" of 1.8 Hz, 6.1 Hz and 6.9 Hz; the latter values are reasonable for vicinal couplings, and it is concluded that the two methylene protons are nonequivalent. The pattern between 3.7 and 3.8 ppm can then be viewed as two doublets whose high-frequency components overlap, yielding "coupling constants" of 6.9 Hz and 6.1 Hz. These values are summarized in table 4.3.

Table 4.3: Coupling constants (in Hz) from the first and second-order analysis of the proton NMR spectrum of ascorbic acid.

Analysis	J_{mx}	J_{am}	J_{bm}	J_{ab}
First order:	1.8	6.9	6.1	?!
Second-order:	1.8	7.5	5.7	-11.6

This "analysis", although quite plausible, is basically wrong. It completely ignores the presence of the small peaks in the 3.7-3.8 ppm pattern, which cannot be ascribed to spinning sidebands or to ^{13}C satellites. Most importantly, although it has been concluded that the methylene protons are nonequivalent, the "analysis" completely ignores the possibility of geminal coupling, for which a constant in the -10 to -20 Hz range would be expected.[117]

The moral is, that one cannot meaningfully apply first-order analysis to a second-order spin system. Second-order analysis using the Bruker routine PANIC yields the parameters shown in table 4.3. The substantial differences between values obtained by the two methods are of more than theoretical significance; the conformational behavior of ascorbic acid in solution around the C_5-C_6 bond has been elucidated using these values as follows.[118]

It is assumed that the molecule exists only as the three low-energy conformers shown in Newman projection in figure 4.10.

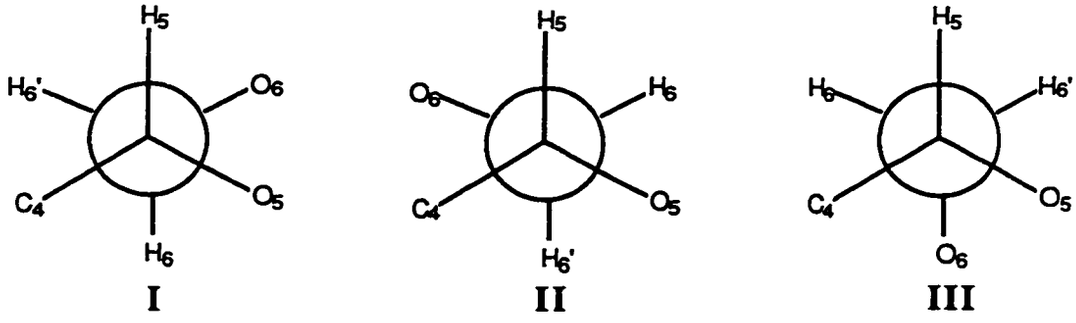


Fig. 4.10: Newman projections for the three low-energy conformations around the C_5-C_6 bond of ascorbic acid.

Using the generalized Karplus equation [104], $J_{5,6}$ is predicted for each of these. The observed J value is the population-weighted average of those of the different conformers. Since the three populations sum to unity, we have

$$J_{5,6} = P_I . J_{5,6,I} + P_{II} . J_{5.6.II} + (1 - P_I - P_{II}) . J_{5,6,III} \qquad \text{eq. 4.6}$$

where P_i refers to the population of conformer i and $J_{5,6,i}$ to its coupling constant. A similar equation can be written for $J_{5,6'}$, and the two equations then solved simultaneously for P_I, P_{II} and hence P_{III}.

Such an analyses is summarized in table 4.4, using both the true and "apparent" J values. Note that whereas the former demonstrate that conformer I is dominant, the latter suggest that the population of I and II are almost equal.

It should be noted here that there are factors other than angle dependence that influence coupling constants. Reading the "angle" directly from the value of the coupling constant or vice-versa can lead to misinterpretations. The values of $J_{5,6}$ for conformers II and III in table 4.4 are a good example. Jackman and Sternhell provide a good discussion on the limitations of Karplus correlations.[119]

Table 4.4 Predicted Vicinal Proton Coupling Constants across the C_5-C_6 Bond for the Three Low-Energy Conformers, and Calculated Conformer Populations

Conformer	I	II	III
$J_{5,6}$ (Hz)	10.8	5.0	0.8
$J_{5,6'}$ (Hz)	3.0	10.8	2.9
Population [a], %	53	34	13
Population [b], %	43	40	17

[a] Based on observed J's taken from second-order analysis (table 4.3)
[b] Based on observed J's taken from first-order "analysis".

4.5.3) The Nuclear Overhauser Effect

The nuclear Overhauser effect (nOe) is a very important phenomenon in modern NMR spectroscopy. The nOe experiment is radically different from all others in that it does not depend on the presence of scalar coupling between nuclei for its operation. The interaction involved in nOe is the direct magnetic coupling between nuclei (non-bonded dipolar coupling). The nOe provides an indirect way to obtain information about dipolar coupling and this coupling is related to internuclear distances and molecular motion in solution.[120]

The nOe is dependant upon $1/r^6$ where r is the internuclear distance.[121] The common notation used for nOe is $\eta_{I}\{s\}$ which indicates that this is the nOe of nucleus **I** when nucleus **(s)** is saturated.

4.5.3.1) The Origin of nOe

The nOe is the change in the intensity or area of an NMR resonance when the transitions of another resonance are perturbed. The perturbation of interest is saturation. In essence, an nOe experiment consists of eliminating the population difference across transitions of a resonance peak by irradiating

them with a weak radio-frequency field, to observe the effect on other resonances in the same system. Therefore in an nOe, a spectroscopist would forcibly change the population differences of a part of a spin system so other parts of the same system would change in compensation. In other words the total system is attempting to stay at thermal equilibrium.[120]

4.5.3.2) Practical Uses of nOe

A simple example of the use of nOe in practical structural investigations would be the elucidation of geometrical isomerism in olefinic compounds. The trans:cis internuclear distance ratio is about 1.3:1. If we consider two hypothetical olefinic molecules, one is *cis* and the other is *trans* such as in figure 4.11. (assuming R and R are non protic groups and considering only H and CH_3 as the basis for our *cis/trans* definition)

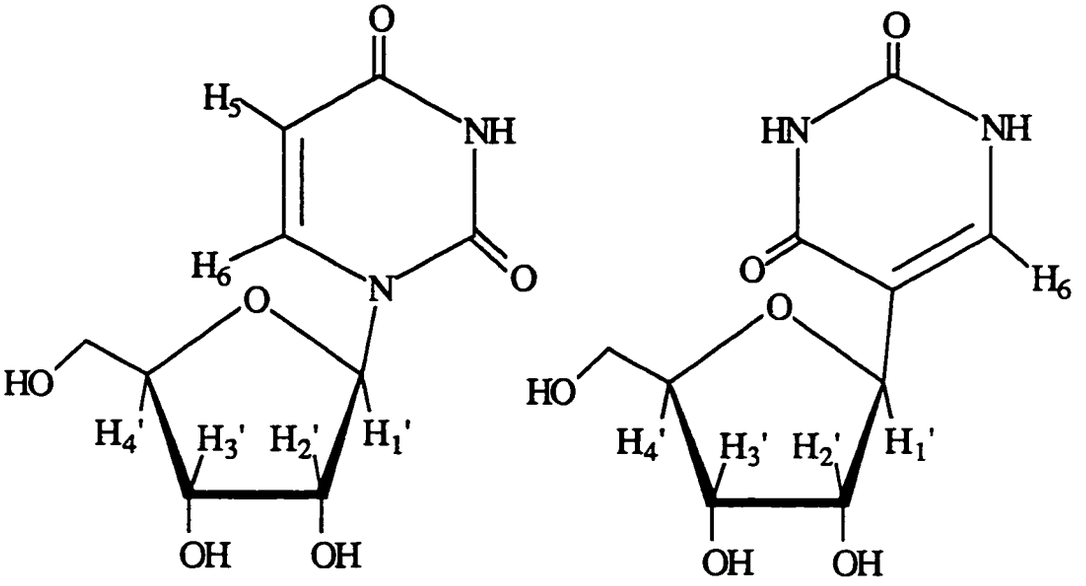
Cis isomer Trans isomer

Fig. 4.11: Cis and trans olefinic molecules

An nOe experiment on each separate molecule in solution would be useful to determine which solution contains the *cis* isomer as well as the solution that contains the other isomer. If the resonance for the H was saturated in both molecules then the area under the CH_3 resonance would be larger for the *cis* isomer than for the *trans* since in the *cis* isomer the internuclear distance or the distance through space is closer.

4.5.3.3) nOe and Nucleoside Conformations

As discussed previously, a pyrimidine nucleoside consists of two units, the sugar and the base linked by the glycosidic bond about which rotation can occur. The glycosidic torsion angle has been defined by O_4'-C_1'-N_1-C_2. If we consider the example of the two molecules (uridine (**116**) and pseudo-uridine (**117**) shown in fig. 4.12, the use of nOe in nucleoside conformational studies becomes clearer.



uridine (**116**) pseudo-uridine (**117**)

Fig. 4.12: Uridine and pseudo-uridine glycosidic conformations

In reality, uridine (**116**) and pseudo-uridine (**117**) as well as all such nucleosides and their analogs can exist in two glycosidic conformational regions in solution. One is called *syn*, in which H_6 is closer to H_1', and the other is termed *anti* whereby the pyrimidine ring is rotated making H_6 closer to H_2' and H_3'. Some experimentally determined steady-state enhancements for these two compounds are given in table 4.5.[122]

(in fig. 4.12, uridine (**116**) is drawn in an *anti* mode and pseudo-uridine (**117**)

in a *syn* mode).

Table 4.5: nOe Enhancements observed in uridine and pseudo-uridine

Compound	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$
uridine	8	23	8
pseudo-uridine	26	10	9

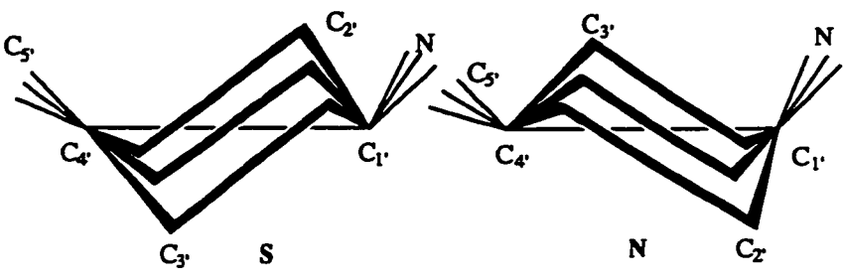
This pattern of enhancements is not consistent with any *one conformation* for either nucleoside, the data does show however that the above two molecules must exist in two conformations in which one conformation is predominant. The conformations are in biased dynamic equilibrium and are in fast exchange on the chemical shift time scale and on the T_1 time scale also. With such a study, one will be able to state that the population distribution prefers one conformer over the other; that is, in solution one of the two defined conformations is predominant.[121]

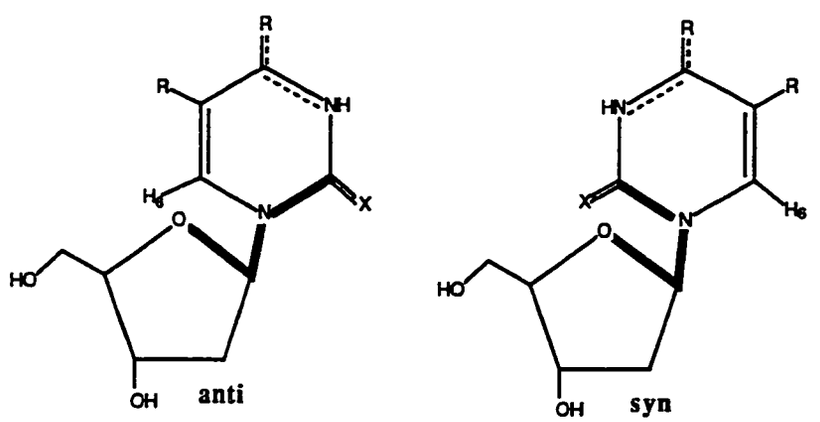
4.6) Results and Discussion

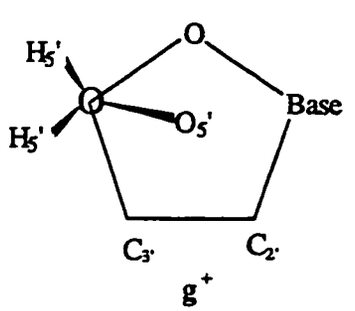
As mentioned earlier, the program PSEUROT required data input at 4 different temperatures. In this section, the furanose coupling constants and C_5 exocyclic conformational populations at 310 K (37° C) will be presented. The other conformational parameters namely S, P_S, N and P_N represent the best fit obtained from the four acquisition temperatures. A tabular form for each group of related molecules is used, followed by a discussion of the parameters. The entire data at the four temperatures for each compound is presented in the experimental subsection of this chapter.

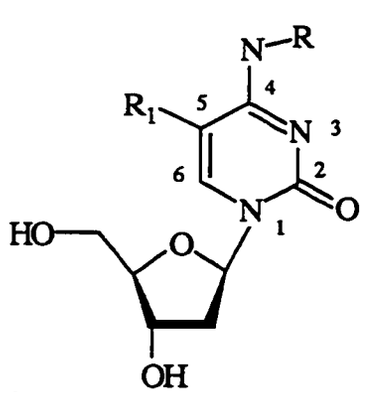
The diagrams in fig. 4.13 provide a graphic description of the conformations

discussed with each group of molecules.

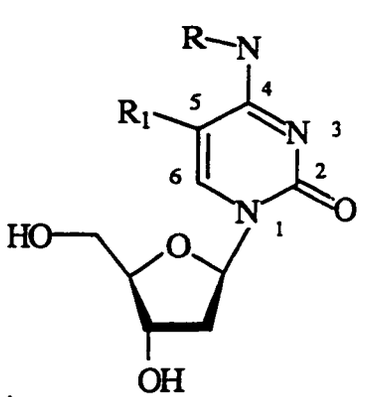








N^4 substituent proximal to N_3



N^4 substituent proximal to C_5

Fig. 4.13: Representative conformations

4.6.1) MMdCyd Analogs (N^4-substitution)

4.6.1.1) N^4-acyl and N^4-aroyl MMdCyd

The NMR data for the N^4 analogs of MMdCyd: N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**), N^4-butanoyl-MMdCyd (**32**), N^4-trimethylacetyl (pivaloyl)-MMdCyd (**33**), N^4-benzoyl-MMdCyd (**34**) and N^4-acetyl-N^4-methyl-MMdCyd (**57**) are summarized in tables 4.6.1.1.1, 4.6.1.1.2 and 4.6.1.1.3. The data presented show similar features.

TABLE 4.6.1.1.1

Furanose coupling constants (Hz) of N^4-acyl and N^4-aroyl-MMdCyd analogs.

310 K N^4:	acetyl		propanoyl		butanoyl		pivaloyl		benzoyl		acetyl- methyl	
37° C	Jexp	Jcal.	Jexp	Jcal.	Jexp	Jcal.	Jexp	Jcal.	Jexp	Jcal.	Jexp	Jcal.
$J_{1'2'}$	6.2	6.3	6.3	6.2	6.3	6.2	6.3	6.4	6.1	6.0	6.8	7.0
$J_{1'2''}$	6.2	6.4	6.1	6.3	6.0	6.2	6.3	6.4	6.5	6.3	6.6	6.5
$J_{2'2''}$	- 13.8		- 13.7		- 12.9		- 13.8		- 13.1		- 13.1	
$J_{2'3'}$	6.4	6.6	6.2	6.4	6.2	6.4	6.3	6.6	6.2	6.3	6.5	6.2
$J_{2''3'}$	5.0	5.1	5.2	5.2	5.1	5.1	4.9	5.0	5.2	5.3	4.4	4.2
$J_{3'4'}$	4.8	4.7	4.5	4.3	4.4	4.2	4.8	4.6	4.3	4.1	4.2	4.0
$J_{4'5'}$	3.4		3.4		3.7		3.5		3.7		4.0	
$J_{4'5''}$	4.7		4.5		4.8		4.8		4.4		4.5	
$J_{5'5''}$	- 12.3		- 12.5		- 12.3		- 12.1		- 12.1		- 12.3	

J experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.1.1.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of N^4-acyl and N^4-aroyl-MMdCyd analogs.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
N^4-acetyl (**30**):	55 (148°)[c]	45 (-3.0°)	55	11	34
N^4-propanoyl (**31**):	53 (157°)	47 (-7.0°)	57	11	32
N^4-butanoyl (**32**):	54 (158°)	46 (-8.0°)	51	15	34
N^4-pivaloyl (**33**):	56 (148°)	44 (-0.0°)	53	13	34
N^4-benzoyl (**34**):	53 (164°)	47 (-9.0°)	55	15	30
N^4-acetyl-N^4-methyl (**57**):	58 (164°)	42 (1.0°)	50	23	27

[a] In the PSEUROT calculations, τ m was constrained to 36.0°. The rms deviation for each calculation was: N^4: acetyl: 0.204 E; propanoyl: 0.166 E; butanoyl: 0.181 E; pivaloyl: 0.256 E, benzoyl: 0.146 E and N^4-acetyl,N^4-methyl: 0.208 .

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.1.1.3

Syn/anti glycosidic preference of N^4-acyl and N^4-aroyl-MMdCyd analogs.

Conformer:	$\eta_{6\{1'\}}$	$\eta_{6\{2'\}}$	$\eta_{6\{3'\}}$	glycosidic preference [a]
N^4-acetyl (**30**):	4	9	5	anti
N^4-propanoyl (**31**):	5	11	6	anti
N^4-butanoyl (**32**):	3	7	4	anti
N^4-pivaloyl (**33**):	4	8	5	anti
N^4-benzoyl (**34**):	4	9	5	anti
N^4-acetyl-N^4-methyl (**57**):	5	10	7	anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

All of these analogs display an approximately 55/45 *south/north* deoxyribose distribution. This equilibrium differs slightly from the usual 60/40 ratio for deoxyriboses in nucleosides that are not substituted with an N^4-acyl or N^4-aroyl group. All the above analogs as well have a predominant **g+** rotamer for the $C_{5'}$ exocyclic side chain. The glycosidic *syn/anti* equilibrium is biased towards the *anti* position. In all of the above analogs, the α protons (β protons in N^4-pivaloyl) of the N^4-acyl or N^4-aroyl group did show a significant nOe on the $C_{(5,1)}$ and $C_{(5,3)}$ protons. With the analog N^4-acetyl-N^4-methyl-MMdCyd (**56**), the acetyl protons did have an nOe on $C_{(5,1)}$ and $C_{(5,3)}$ protons, however the methyl protons did not.

It was mentioned in section 4.4.4. that the N^4-acetyl group in N^4-acetyl-cytidine (**112**) was proximal to C_5 due to the stability of the six-membered ring formed. It was also mentioned that with N^4-methyl analogs of cytosine, the N^4-methyl is proximal to N_3. In this case with N^4-acyl and aroyl analogs of MMdCyd, the ring that can possibly form is a seven-membered ring if one of the hydrogens on $C_{(5,1)}$ forms a hydrogen bond with the oxygen of the carbonyl. Another probable reason that would explain the fact that the N^4-acyl and N^4-aroyl groups are distal to N_3 (proximal to C_5), is the repulsion that would take place between the partial negative charges on the oxygen of the carbonyl and on N_3 (non-bonded dipolar interaction), since in such an arrangement, N_3 and the oxygen would be in the same plane (due to conjugation) and close to one another.

In the case of N^4-acetyl-N^4-methyl-MMdCyd (**57**), the acetyl group is proximal to C_5, while the methyl group is proximal to N_3. In other words the two literature cases reporting that N^4-acetyl groups are proximal to C_5 and N^4-methyl groups are proximal to N_3 are observed in the compound N^4-acetyl-N^4-methyl-MMdCyd (**57**).

4.6.1.2) N^4-alkyl and N^4-alkenyl analogs of MMdCyd

The NMR data for the analogs N^4-methyl-MMdCyd (**24**), N^4,N^4-dimethyl-MMdCyd (**43**), 3,4-etheno-MMdCyd (**94**) and MMdCyd (**18**) are summarized in tables 4.6.1.2.1, 4.6.1.2.2, and 4.6.1.2.3. These analogs show similar features.

TABLE 4.6.1.2.1

Furanose coupling constants (Hz) of N^4-alkyl and alkenyl MMdCyd analogs.

310 K 37° C	MMdCyd		N^4-methyl		N^4,N^4-dimethyl		3,4-etheno	
	Jexp.	Jcal.	Jexp.	Jcal.	Jexp.	Jcal.	Jexp.	Jcal.
$J_{1'2'}$	7.1	7.2	7.2	7.3	6.7	7.0	7.0	6.8
$J_{1'2''}$	6.7	6.8	6.8	6.8	6.3	6.4	6.3	6.4
$J_{2'2''}$	-14.1		-14.7		-13.8		-13.2	
$J_{2'3'}$	6.7	6.4	6.7	6.5	6.8	6.5	5.8	6.2
$J_{2''3'}$	4.3	4.4	4.1	4.3	4.2	4.5	4.8	4.7
$J_{3'4'}$	4.0	4.1	4.1	4.1	4.2	4.2	4.2	
$J_{4'5'}$	4.7		4.7		3.7		3.7	
$J_{4'5''}$	4.6		3.9		4.8		4.6	
$J_{5'5''}$	-12.3		-12.6		-11.9		-12.6	

J experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.1.2.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of N^4-alkyl and alkenyl MMdCyd analogs.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
MMdCyd (**18**):	60 (162°) [c]	40 (27°)	45	27	28
N^4-methyl (**24**):	60 (161°)	40 (30°)	52	27	21
N^4,N^4-dimethyl (**43**):	61 (151°)	39 (6.0°)	51	15	34
3,4-etheno (**94**):	61 (161°)	39 (2.0°)	52	16	32

[a] In the PSEUROT calculations, τ_m was constrained to 36.0°. The rms deviation for each calculation was: N^4-methyl: 0.112 E; N^4,N^4-dimethyl: 0.242 E; 3,4-etheno: 0.261 E

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.1.2.3

Syn/anti glycosidic preference of N^4-alkyl and alkenyl MMdCyd analogs

Conformer:	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$	glycosidic preference [a]
MMdCyd (**18**):	4	8	5	anti
N^4-methyl (**24**):	3	7	4	anti
N^4,N^4-dimethyl (**43**):	8	14	10	anti
3,4-etheno (**94**):	8	15	11	anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

These four analogs display an approximately 60/40 *south/north* equilibrium for the deoxyribose conformation. This equilibrium is classical for deoxyriboses in nucleosides (ordinary deoxyuridines and deoxycytidines). All the above analogs have a predominant **g+** rotamer for the $C_{5'}$ exocyclic side chain. The population of **g+** however is lower than in N^4-acyl or N^4-aroyl analogs. The glycosidic *syn/anti* equilibrium is biased towards the *anti* position.

The N^4-substituent protons in N^4-methyl-MMdCyd (**24**) and 3,4-etheno-MMdCyd (**94**) did not show any nOe on the $C_{(5,1)}$ and $C_{(5,3)}$ protons. The two N^4-methyl substituents in the compound N^4,N^4-dimethyl-MMdCyd (**43**) have the same chemical shift (coincidence). Attempts to separate the two signals by changing the solvent and/or lowering the temperature were without success. Instead of perturbing the resonance of the two N^4 methyl groups, the resonance of the C_5 methylene signal was irradiated. It showed almost equal nOe on the methoxy of the C_5 side chain and on the resonance containing the N^4 methyl groups leading to the conclusion that only *one* of the two N^4 methyl groups experienced enhancement. This and the discussion in section (4.4.4) about the rigidity of the exocyclic C-N bond are sufficient evidence that the N^4,N^4-dimethyl substituents of N^4,N^4-dimethyl-MMdCyd (**43**) are coplanar with the pyrimidine base and thus one methyl is proximal to C_5 and the other methyl is proximal to N_3.

4.6.1.3) N^4-aryl analogs of MMdCyd

The NMR data for the analogs N^4-phenyl-MMdCyd (**44**) and N^4-benzyl-MMdCyd (**45**) as well as MMdCyd (**18**) (for comparison) are presented in tables 4.6.1.3.1, 4.6.1.3.2, and 4.6.1.3.3. The data show similar features between the three analogs and similarities to the data for N^4-alkyl analogs of MMdCyd (section 4.6.1.2).

TABLE 4.6.1.3.1

Furanose coupling constants (Hz) of N^4-phenyl and N^4-benzyl-MMdCyd analogs.

310 K 37° C	N^4-phenyl		N^4-benzyl		MMdCyd	
	Jexp.	Jcal.	Jexp.	Jcal.	Jexp.	Jcal.
$J_{1'2'}$	7.2	7.0	7.0	7.2	7.1	7.2
$J_{1'2''}$	6.7	6.9	6.7	6.8	6.7	6.8
$J_{2'2''}$	-13.1		-13.1		-14.1	
$J_{2'3'}$	6.8	6.4	6.8	6.3	6.7	6.4
$J_{2''3'}$	4.1	4.4	4.1	4.3	4.3	4.4
$J_{3'4'}$	4.0	4.1	4.0	4.0	4.0	4.1
$J_{4'5'}$	3.5		3.5		4.7	
$J_{4'5''}$	4.5		4.4		4.6	
$J_{5'5''}$	-11.8		-11.9		-12.3	

J experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.1.3.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of N^4-phenyl and N^4-benzyl-MMdCyd analogs.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
N^4-phenyl (**44**):	63 (157°) [c]	37 (9.0°)	50	16	34
N^4-benzyl (**45**):	62 (161°)	38 (11°)	51	17	32
MMdCyd (**18**):	60 (162°)	40 (27°)	45	27	28

[a] In the PSEUROT calculations, τ m was constrained to 36.0°. The rms deviation for each calculation was: N^4-phenyl: 0.202 E and N^4-benzyl: 0.192 E.

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.1.3.3

Syn/anti glycosidic preference of N^4-phenyl-MMdCyd and N^4-benzyl-MMdCyd.

Conformer	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$	glycosidic preference [a]
N^4-phenyl (**44**):	8	13	10	anti
N^4-benzyl (**45**):	7	13	9	anti
MMdCyd (**18**):	4	8	5	anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

The two analogs N^4-phenyl-MMdCyd (**44**) and N^4-benzyl-MMdCyd (**45**) display an approximate 60/40 *south/north* equilibrium for the deoxyribose conformations. They have a predominant **g+** rotamer for the $C_{5'}$ exocyclic side chain. The **g+** conformer has a higher population than that of MMdCyd (**18**). The glycosidic *syn/anti* equilibrium is biased towards the *anti* position. The N^4-substituent aromatic protons in N^4-phenyl and the benzylic (aliphatic) as well as the aromatic protons in N^4-benzyl did not show any nOe on the $C_{(5,1)}$ and $C_{(5,3)}$ protons. Therefore it is concluded that the N^4-substituents in both analogs are proximal to N_3.

4.6.1.4) N^4-methoxy and N^4-hydroxy analogs of MMdCyd.

The NMR data for N^4-methoxy-MMdCyd (**54**) and N^4-hydroxy-MMdCyd (**55**) and MMdCyd (**18**) (for comparison) are presented in tables 4.6.1.4.1, 4.6.1.4.2 and 4.6.1.4.3. The data show similar features to N^4-alkyl and N^4-aryl analogs of MMdCyd presented in section 4.6.1.3.

TABLE 4.6.1.4.1

Furanose coupling constants (Hz) of N^4-methoxy and N^4-hydroxy and MMdCyd analogs.

310 K 37° C	N^4-methoxy		N^4-hydroxy		MMdCyd	
	Jexp.	Jcal.	Jexp.	Jcal.	Jexp.	Jcal.
$J_{1'2'}$	7.0	6.8	7.0	6.8	7.1	7.2
$J_{1'2''}$	6.0	6.3	6.2	6.4	6.7	6.8
$J_{2'2''}$	-14.2		-14.2		-14.1	
$J_{2'3'}$	6.4	6.4	6.1	6.3	6.7	6.4
$J_{2''3'}$	4.8	4.6	4.8	4.6	4.3	4.4
$J_{3'4'}$	4.1	4.0	4.2	4.1	4.0	4.1
$J_{4'5'}$	3.9		4.0		4.7	
$J_{4'5''}$	4.4		4.8		4.6	
$J_{5'5''}$	-12.2		-12.4		-12.3	

J experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.1.4.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of N^4-methoxy and N^4-hydroxy and MMdCyd analogs.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
N^4-methoxy (**54**):	62 (154°) [c]	38 (-3.0°)	53	18	29
N^4-hydroxy (**55**):	61 (161°)	39 (3.0°)	48	19	33
MMdCyd (**18**):	60 (162°)	40 (27°)	45	27	28

[a] In the PSEUROT calculations, τ_m was constrained to 36.0°. The rms deviation for each calculation was: N^4-methoxy: 0.145 E and N^4-hydroxy: 0.150 E.

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.1.4.3

Syn/anti glycosidic preference of N^4-methoxy-MMdCyd and N^4-hydroxy-MMdCyd.

Conformer:	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$	glycosidic preference [a]
N^4-methoxy (**54**):	5	11	7	anti
N^4-hydroxy (**55**):	4	10	6	anti
MMdCyd (**18**):	4	8	5	anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

The two analogs display an approximate 60/40 *south/north* equilibrium for the deoxyribose conformations. They have a predominant **g+** rotamer for the $C_{5'}$ exocyclic side chain. The glycosidic *syn/anti* equilibrium is biased towards the *anti* position. The N^4-substituent protons in N^4-methoxy-MMdCyd (**54**) did not show any nOe on the $C_{(5,1)}$ and $C_{(5,3)}$ protons. Likewise the hydroxyl proton in N^4-hydroxy-MMdCyd (**55**) (when this analog is dissolved in chloroform-d) had no nOe on $C_{(5,1)}$ and $C_{(5,3)}$ protons. Once again the N^4-substituents in both analogs are proximal to N_3.

4.6.2) MMdUrd and MMdCyd analogs (C_2-substitution)

4.6.2.1) 2-imino-MMdUrd

The NMR data for 2-imino-MMdUrd (**66**) is presented with MMdUrd (**6**) [123] and MMdCyd (**18**) (for comparison) in tables 4.6.2.1.1, 4.6.2.1.2 and 4.6.2.1.3.

TABLE 4.6.2.1.1

Furanose coupling constants (Hz) of MMdUrd, MMdCyd and 2-imino-MMdUrd.

310 K 37° C	MMdUrd		MMdCyd		2-imino MMdUrd	
	Jexp.	Jcal.	Jexp.	Jcal.	Jexp.	Jcal.
$J_{1'2'}$	6.2	7.1	7.1	7.2	7.0	7.1
$J_{1'2''}$	6.5	6.4	6.7	6.8	6.7	6.7
$J_{2'2''}$	-14.0		-14.1		-14.2	
$J_{2'3'}$	6.1	5.8	6.7	6.4	6.6	6.4
$J_{2''3'}$	4.4	4.9	4.3	4.4	4.3	4.4
$J_{3'4'}$	4.0	4.2	4.0	4.1	4.1	4.2
$J_{4'5'}$	3.1		4.7		4.4	
$J_{4'5''}$	4.6		4.6		3.3	
$J_{5'5''}$	-12.2		-12.3		-12.6	

J experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.2.1.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of MMdUrd, MMdCyd and 2-imino-MMdUrd.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
MMdUrd (**6**):	60 (162°) [c]	40 (18°)	61	8	31
MMdCyd (**18**):	60 (162°) [d]	40 (27°)	45	27	28
2-imino-MMdUrd (**66**):	60 (160°) [d]	40 (24°)	72	13	15

[a] In the PSEUROT calculations, τ_m was constrained to 36.0°. The rms deviation for the calculation of MMdCyd: 0.125 E; 2-imino-MMdUrd: 0.110 E.
[b] $C_{5'}$ Exocyclic orientation at 37°C.
[c] P_S and P_N were assumed not calculated
[d] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.2.1.3

Syn/anti glycosidic preference of MMdCyd, MMdUrd and its analog 2-imino-MMdUrd.

Conformer:	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$	glycosidic preference [a]
MMdUrd (**6**):	4	10	7	anti
MMdCyd (**18**):	4	8	5	anti
2-imino-MMdUrd (**66**):	12	9	2	syn

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

The conformational features of 2-imino-MMdUrd (**66**) are significantly different than those observed for MMdUrd (**6**), 4-amino-MMdUrd (MMdCyd) (**18**) and other analogs of MMdCyd (**18**) presented in previous sections. It does display a 60/40 *south/north* deoxyribose equilibrium. The population of the **g**$^+$ rotamer is not only predominant but rather unusually high due to increased hydrogen bonding between the NH on C_2 and $C_{5'}$-OH. The glycosidic preference in this case is mainly *syn* again due to increased hydrogen bonding between the NH on C_2 and $C_{5'}$-OH.

4.6.2.2) 2-imino-5-methoxymethyl-2',3'-didehydro-2',3'-dideoxycytidine (2-imino-MMddCyd)

The program PSEUROT does not analyze the conformation of didehydro-dideoxy furanoses. Therefore, calculated coupling constants as well as *south* and *north* parameters are not available. The available NMR data for the compound 2-imino-MMddCyd (**90**) is presented in tables 4.6.2.2.1 and 4.6.2.2.2 along with data for 2-imino-MMdUrd (**66**) and MMdCyd (**18**) (for comparison).

TABLE 4.6.2.2.1

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of MMdCyd, 2-imino-MMdUrd and 2-imino-MMddCyd.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
2-imino-MMdUrd (**66**):	60 (160°) [c]	40 (24°)	72	13	15
MMdCyd (**18**):	60 (162°) [c]	40 (27°)	45	27	28
2-imino-MMddCyd (**90**):			68	14	18

[a] In the PSEUROT calculations, τ_m was constrained to 36.0°. The rms deviation for the calculation of MMdCyd: 0.125 E, for 2-imino-MMdUrd: 0.110 E.

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.2.2.2

Syn/anti glycosidic preference of MMdCyd, 2-imino-MMdUrd and 2-imino-MMddCyd.

Conformer:	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$	glycosidic preference [a]
2-imino-MMdUrd (**66**):	12	9	2	syn
MMdCyd (**18**):	4	8	5	anti
2-imino-MMddCyd (**90**):	10	5	3	syn

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

The available conformational features of 2-imino-MMddCyd (**90**) are similar to those of 2-imino-MMdUrd (**66**) and are significantly different than those observed for MMdUrd (**6**), 4-amino-MMdUrd (MMdCyd) (**18**) and other analogs of MMdCyd (**18**) presented in previous sections. The population of the **g+** rotamer is unusually high due to increased hydrogen bonding between the NH on C_2 and $C_{5'}$-OH. The glycosidic preference in this case is mainly *syn* again due to increased hydrogen bonding between the NH on C_2 and $C_{5'}$-OH.

4.6.3) BrVdCyd analogs (N^4-substitution)

4.6.3.1) N^4-acyl BrVdCyd

The NMR data for the three N^4-acyl analogs of BrVdCyd (**17**) namely N^4-acetyl-BrVdCyd (**95**), N^4-propanoyl-BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**) are presented in tables 4.6.3.1.1, 4.6.3.1.2 and 4.6.3.1.3. The data shows that these compounds are similar to one another and to the N^4-acyl analogs of MMdCyd (**18**) presented in section 4.6.1.1.

TABLE 4.6.3.1.1

Furanose coupling constants (Hz) of N^4-acyl-BrVdCyd analogs.

310 K 37° C	N^4-acetyl		N^4-propanoyl		N^4-butanoyl	
	Jexp.	Jcal.	Jexp.	Jcal.	Jexp.	Jcal.
$J_{1'2'}$	6.0	6.2	6.1	6.2	6.3	6.1
$J_{1'2''}$	6.7	6.6	6.7	6.7	6.0	6.6
$J_{2'2''}$	-12.9		-12.9		-12.9	
$J_{2'3'}$	6.0	6.1	6.0	6.1	6.2	6.2
$J_{2''3'}$	4.6	5.0	4.7	5.1	5.1	5.2
$J_{3'4'}$	4.2	4.0	4.5	4.2	4.4	4.3
$J_{4'5'}$	3.5		3.6		3.7	
$J_{4'5''}$	4.7		4.7		4.8	
$J_{5'5''}$	-12.4		-12.2		-12.3	

J experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.3.1.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of N^4-acyl-BrVdCyd analogs.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
N^4-acetyl (**95**):	56 (178°) [c]	44 (6.0°)	55	13	32
N^4-propanoyl (**96**):	55 (175°)	45 (9.0°)	55	15	30
N^4-butanoyl (**97**):	55 (171°)	45 (5.0°)	52	17	31

[a] In the PSEUROT calculations, τ m was constrained to 36.0°. The rms deviation for each calculation was: N^4: acetyl: 0.250 E; propanoyl: 0.227 E; butanoyl: 0.179 E.
[b] $C_{5'}$ Exocyclic orientation at 37°C.
[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.3.1.3

Syn/anti glycosidic preference of N^4-acyl-BrVdCyd analogs.

Conformer:	$\eta_{6\{1'\}}$	$\eta_{6\{2'\}}$	$\eta_{6\{3'\}}$	glycosidic preference [a]
N^4-acetyl (**95**):	8	15	11	anti
N^4-propanoyl (**96**):	9	14	13	anti
N^4-butanoyl (**97**):	5	10	8	anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

All of these N^4-acyl-BrVdCyd analogs display an approximately 55/45 *south/north* equilibrium for the deoxyribose conformation. This equilibrium differs slightly from the usual 60/40 ratio for deoxyriboses in nucleosides that are not substituted with an N^4-acyl or N^4-aroyl group. All the above analogs as well have a predominant **g+** rotamer for the $C_{5'}$ exocyclic side chain. The glycosidic *syn/anti* equilibrium is biased towards the *anti* position. In all of the above analogs, the α protons of the N^4-acyl group did show a significant nOe on the $C_{(5,1)}$ and $C_{(5,2)}$ olefinic protons of the bromovinyl side chain. It was mentioned in section 4.4.4. that the N^4-acetyl group in N^4-acetyl-cytidine (**112**) was proximal to C_5 due to the stability of the six membered ring formed. In this case with N^4-acyl analogs of BrVdCyd, the ring that can possibly form is a seven-membered ring if the olefinic hydrogen on $C_{(5,1)}$ forms a hydrogen bond with the oxygen of the carbonyl. Another probable reason that would explain the fact that the N^4-acyl groups are distal to N_3 (proximal to C_5), is the repulsion that would take place between the partial negative charges on the oxygen of the carbonyl and on N_3 (non-bonded dipolar interaction), since in such an arrangement, N_3 and the oxygen would be in the same plane (due to conjugation) and close to one another.

4.6.3.1) BrVdCyd and N^4-methyl-BrVdCyd

The NMR data for BrVdCyd (**17**) and N^4-methyl-BrVdCyd (**101**) are presented in tables 4.6.3.2.1, 4.6.3.2.2 and 4.6.3.2.3. These data show similar features for the two compounds as well as similar features to MMdCyd (**18**) and its N^4-alkyl substituents presented in section 4.6.1.2.

TABLE 4.6.3.2.1

Furanose coupling constants (Hz) of BrVdCyd and N^4-methyl-BrVdCyd .

310 K 37° C	BrVdCyd		N^4-methyl-BrVdCyd	
	Jexp.	Jcal.	Jexp.	Jcal.
$J_{1'2'}$	6.9	7.1	7.0	7.0
$J_{1'2''}$	6.8	6.4	6.7	6.6
$J_{2'2''}$	-13.6		-14.2	
$J_{2'3'}$	6.0	6.1	5.8	6.2
$J_{2''3'}$	3.9	4.5	4.1	4.4
$J_{3'4'}$	4.0	3.6	4.2	3.8
$J_{4'5'}$	3.9		3.7	
$J_{4'5''}$	4.4		4.5	
$J_{5'5''}$	-12.1		-12.4	

J experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.3.2.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of N^4-methyl-BrVdCyd.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
BrVdCyd (**17**):	60 (161°) [c]	40 (22°)	57	18	25
N^4-methyl (**101**):	62 (166°) [c]	38 (16°)	54	15	31

[a] In the PSEUROT calculations, τ m was constrained to 36.0°. The rms deviation for each calculation was: BrVdCyd: 0.193; N^4-methyl-BrVdCyd: 0.175 E.

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.3.2.3

Syn/anti glycosidic preference of BrVdCyd and N^4-methyl-BrVdCyd.

Conformer:	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$	glycosidic preference [a]
BrVdCyd (**17**):	4	9	6	anti
N^4-methyl (**101**):	5	9	7	anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

For BrVdCyd (**17**) and its analog N^4-methyl-BrVdCyd (**101**), there is a 60/40 *south/north* equilibrium for the deoxyribose, a predominant **g+** conformer for the $C_{5'}$ exocyclic side chain and an *anti* preference for the glycosidic torsional parameter. The N^4-methyl group shows no nOe on the olefinic $C_{(5,1)}$ and $C_{(5,2)}$ protons. It was mentioned in section (4.4.4), that with N^4-methyl analogs of cytosine, the N^4-methyl was proximal to N_3. With N^4-methyl-BrVdCyd (**101**), the steric interaction between the N^4-methyl group and the olefinic C_5 chain is expected to be more pronounced than that of N^4-methyl-cytosine. Hence, it is concluded that the absence of nOe on the olefinic protons denotes that the N^4-methyl group is proximal to N_3.

4.6.4) 5-Methyl-2'-deoxycytidine analogs (N^4-substitution)

4.6.4.1) N^4-methoxy and N^4-hydroxy-5-Me-dCyd

The NMR data for the two analogs N^4-methoxy-5-Me-dCyd (**105**) and N^4-hydroxy-5-Me-dCyd (**106**) are presented in tables 4.6.4.1.1, 4.6.4.1.2 and 4.6.4.1.3. These data show similar features between the two analogs and similar features with N^4-methoxy-MMdCyd (**54**) and N^4-hydroxy-MMdCyd (**55**) analogs presented in section 4.6.1.4.

TABLE 4.6.4.1.1

Furanose coupling constants (Hz) of N^4-methoxy and N^4-hydroxy-5-Me-dCyd analogs.

310 K 37° C	N^4-methoxy		N^4-hydroxy	
	Jexp.	Jcal.	Jexp.	Jcal.
$J_{1'2'}$	6.9	6.8	7.1	6.9
$J_{1'2''}$	6.4	6.3	6.5	6.4
$J_{2'2''}$	-14.0		-14.1	
$J_{2'3'}$	6.0	6.4	6.0	6.1
$J_{2''3'}$	5.1	4.6	4.9	4.7
$J_{3'4'}$	4.5	4.0	4.0	4.1
$J_{4'5'}$	3.6		3.7	
$J_{4'5''}$	4.4		4.5	
$J_{5'5''}$	-12.3		-11.9	

experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.4.1.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of N^4-methoxy and N^4-hydroxy-5-Me-dCyd analogs.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
N^4-methoxy (**105**):	60 (161°) [c]	40 (12.0°)	53	14	33
N^4-hydroxy (**106**):	59 (167°)	41 (3.0°)	48	20	32

[a] In the PSEUROT calculations, τ_m was constrained to 36.0°. The rms deviation for each calculation was: N^4-methoxy: 0.237 E and N^4-hydroxy: 0.213 E.

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.4.1.3

Syn/anti glycosidic preference of N^4-methoxy -5-Me-dCyd and N^4-hydroxy-5-Me-dCyd.

Conformer:	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$	glycosidic preference [a]
N^4-methoxy (**105**):	7	14	9	anti
N^4-hydroxy (**106**):	5	10	7	anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

The above two analogs **105** and **106** display an approximately 60/40 *south/north* equilibrium for the deoxyribose conformation. They have a predominant **g+** rotamer for the $C_{5'}$ exocyclic side chain. The glycosidic *syn/anti* equilibrium is biased towards the *anti* position. The N^4-substituent protons in N^4-methoxy-5-Me-dCyd (**105**) did not show any nOe on the methyl protons of the C_5 side chain. Measurement for an nOe of the N^4-hydroxyl proton in **106** on the methyl protons of the C_5 side chain was not performed. It is clear at this stage that the N^4-hydroxyl proton should not exert any nOe on the methyl protons similar to the N^4-hydroxyl proton of the analog N^4-hydroxy-MMdCyd (**54**) in section 4.6.1.4. It is concluded therefore that the N^4-substituents in these two analogs are proximal to N_3.

4.6.5) Miscellaneous agents

4.6.5.1) EtdUrd [112] and EtdCyd [112]

The NMR data for the two analogs EtdUrd (**9**) and EtdCyd (**118**) are presented in tables 4.6.5.1.1, 4.6.5.1.2 and 4.6.5.1.3. These data show similar features between the two analogs.

TABLE 4.6.5.1.1

Furanose coupling constants (Hz) of EtdUrd and EtdCyd.

310 K 37° C	EtdUrd		EtdCyd	
	Jexp.	Jcal.	Jexp.	Jcal.
$J_{1'2'}$	6.9	7.0	6.9	7.0
$J_{1'2''}$	6.2	6.3	6.5	6.5
$J_{2'2''}$	-13.8		-14.3	
$J_{2'3'}$	5.9	6.2	6.1	6.3
$J_{2''3'}$	4.4	4.5	4.2	4.5
$J_{3'4'}$	4.0	3.9	4.2	4.0
$J_{4'5'}$	4.1		3.7	
$J_{4'5''}$	3.6		4.4	
$J_{5'5''}$	-11.6		-11.7	

experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.5.1.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of EtdUrd and EtdCyd.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
EtdUrd (**9**):	62 (160°) [c]	38 (3.0°)	60	20	20
EtdCyd (**118**):	63 (159°)	37 (10°)	55	15	30

[a] In the PSEUROT calculations, τ_m was constrained to 36.0°. The rms deviation for each calculation was: EtdUrd: 0.144 E and EtdCyd: 0.143 E.

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.5.1.3

Syn/anti glycosidic preference of EtdUrd and EtdCyd.

Conformer:	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$	glycosidic preference [a]
EtdUrd (**9**):	6	12	5	anti
EtdCyd (**118**):	5	7	4	anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

The two compounds EtdUrd (**9**) and EtdCyd (**118**) display an approximate 60/40 *south/north* equilibrium for the deoxyribose conformation. They have a predominant **g+** rotamer for the $C_{5'}$ exocyclic side chain. The glycosidic *syn/anti* equilibrium is biased towards the *anti* position in both compounds. However, it is noticeable that the *anti* glycosidic conformer in EtdCyd (**118**) has a lower contribution than in all *anti* compounds presented in this study. Implications of such an observation are discussed in the chapter on Discussions and Conclusions.

4.6.5.2) 5-hydroxymethyl-2'-deoxyuridine (HMdUrd) [124], 3'-deoxy-3'-azidothymidine (AZT) [124] and 3'-deoxy-3'-azido-5-hydroxymethyl-2'-deoxyuridine (AzHMddUrd) [124].

The NMR data for the three analogs HMdUrd (**113**), AZT (**28**) and AzHMddUrd (**119**) are presented in tables 4.6.5.2.1, 4.6.5.2.2 and 4.6.5.2.3. AZT (**28**) and AzHMddUrd (**119**) show similar features to one another while HMdUrd (**113**) shows similar features to MMdUrd (**6**) and MMdCyd (**18**) discussed in sections 4.6.1.2 and 4.6.2.

TABLE 4.6.5.2.1

Furanose coupling constants (Hz) of HMdUrd, AZT and AzHMddUrd.

310 K 37° C	HMdUrd		AZT		AzHMddUrd	
	Jexp.	Jcal.	Jexp.	Jcal.	Jexp.	Jcal.
$J_{1'2'}$	6.8	7.1	6.3	6.0	6.1	5.9
$J_{1'2''}$	6.5	6.5	6.6	6.8	6.6	6.7
$J_{2'2''}$	-14.4		0.0		0.0	
$J_{2'3'}$	6.7	6.5	6.6	6.8	6.6	6.8
$J_{2''3'}$	4.3	4.5	6.1	5.8	6.1	6.0
$J_{3'4'}$	4.0	4.2	5.0	5.0	5.1	5.0
$J_{4'5'}$	4.7		4.8		4.8	
$J_{4'5''}$	3.5		3.4		3.4	
$J_{5'5''}$	-12.3		-12.9		-12.9	

J experimental; precision 0.1 Hz

J calculated using PSEUROT with $\tau_m = 36.0°$

TABLE 4.6.5.2.2

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of HMdUrd, AZT and AzHMddUrd.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
HMdUrd (**113**):	63 (152°)[c]	37 (11°)	56	28	16
AZT (**28**):	50 (153°)	50 (10°)	60	22	18
AzHMddUrd (**119**):	49 (152°)	51 (8.0°)	57	29	14

[a] In the PSEUROT calculations, τ_m was constrained to 36.0°. The rms deviation for each calculation was: HMdUrd: 0.138 E; AZT: 0.193 E; AzHMddUrd: 0.146 E.

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 4.6.5.2.3

Syn/anti glycosidic preference of HMdUrd, AZT and AzHMddUrd.

Conformer:	$\eta_{6\{1'\}}$	$\eta_{6\{2'\}}$	$\eta_{6\{3'\}}$	glycosidic preference [a]
HMdUrd (**113**):	4	11	5	anti
AZT (**28**):	6	10	5	anti
AzHMddUrd (**119**):	7	16		anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

The analog HMdUrd (**113**) displays a 60/40 *south/north* deoxyribose equilibrium, AZT (**28**) and AzHMddUrd (**119**) have an approximately 50/50 *south/north* equilibrium. The major conformer in all three compounds is **g**$^+$ and all three have an *anti* glycosidic preference (H_3· for AzHMddUrd (**119**) is hidden under another peak therefore its individual resonances could not be irradiated).

TABLE 4.6.5.2.4

Summary of conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain for all compounds studied.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
MMdCyd (**18**):	60 (162°) [c]	40 (27°)	45	27	28
N^4-acetyl (**30**):	55 (148°)	45 (-3.0°)	55	11	34
N^4-propanoyl (**31**):	53 (157°)	47 (-7.0°)	57	11	32
N^4-butanoyl (**32**):	54 (158°)	46 (-8.0°)	51	15	34
N^4-pivaloyl (**33**):	56 (148°)	44 (-0.0°)	53	13	34
N^4-benzoyl (**34**):	53 (164°)	47 (-9.0°)	55	15	30
N^4-acetyl-N^4-methyl (**57**):	58 (164°)	42 (1.0°)	50	23	27
N^4-methyl (**24**):	60 (161°)	40 (30°)	52	27	21
N^4,N^4-dimethyl (**43**):	61 (151°)	39(6.0°)	51	15	34
3,4-etheno (**94**):	61 (161°)	39 (2.0°)	52	16	32
N^4-phenyl (**44**):	63 (157°)	37 (9.0°)	50	16	34
N^4-benzyl (**45**):	62 (161°)	38 (11°)	51	17	32
N^4-methoxy (**54**):	62 (154°)	38 (-3.0°)	53	18	29
N^4-hydroxy (**55**):	61 (161°)	39 (3.0°)	48	19	33
MMdUrd (**6**):	60 (162°) [d]	40 (18°)	61	8	31
2-imino-MMdUrd (**66**):	60 (160°)	40 (24°)	72	13	15
2-imino-MMddCyd: (**90**)			68	14	18

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
BrVdCyd (**17**):	60 (161°)	40 (22°)	57	18	25
N^4-acetyl (**95**):	56 (178°)	44 (6.0°)	55	13	32
N^4-propanoyl (**96**):	55 (175°)	45 (9.0°)	55	15	30
N^4-butanoyl (**97**):	55 (171°)	45 (5.0°)	52	17	31
N^4-methyl (**101**):	62 (166°)	38 (16°)	54	15	31
N^4-methoxy-5-Me-dCyd (**105**):	60 (161°)	40 (12.0°)	53	14	33
N^4-hydroxy 5-Me-dCyd (**106**):	59 (167°)	41 (3.0°)	48	20	32
EtdUrd (**9**):	62 (160°)	38 (3.0°)	60	20	20
EtdCyd (**118**):	63 (159°)	37 (10°)	55	15	30
HMdUrd (**113**):	63 (152°)	37 (11°)	56	28	16
AZT (**28**):	50 (153°)	50 (10°)	60	22	18
AzHMddUrd (**119**):	49 (152°)	51 (8.0°)	57	29	14

[a] In the PSEUROT calculations, τ_m was constrained to 36.0°.

[b] C_5· Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

[d] P_S and P_N were assumed not calculated

TABLE 4.6.5.2.5

Summary of syn/anti glycosidic preference for all compounds studied.

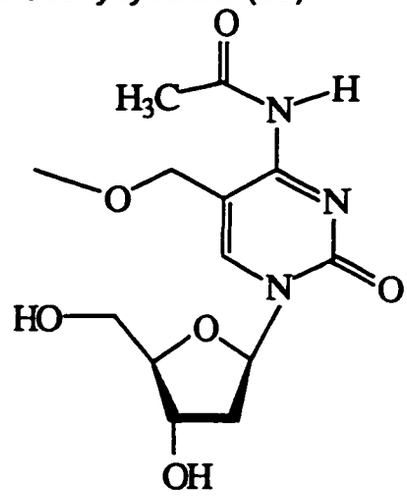
Conformer:	$\eta_{6\{1'\}}$	$\eta_{6\{2'\}}$	$\eta_{6\{3'\}}$	glycosidic preference [a]
MMdCyd (**18**):	4	8	5	anti
N^4-acetyl (**30**):	4	9	5	anti
N^4-propanoyl (**31**):	5	11	6	anti
N^4-butanoyl (**32**):	3	7	4	anti
N^4-pivaloyl (**33**):	4	8	5	anti
N^4-benzoyl (**34**):	4	9	5	anti
N^4-acetyl-N^4-methyl (**57**):	5	10	7	anti
N^4-methyl (**24**):	3	7	4	anti
N^4,N^4-dimethyl (**43**):	8	14	10	anti
3,4-etheno (**94**):	8	15	11	anti
N^4-phenyl (**44**):	8	13	10	anti
N^4-benzyl (**45**):	7	13	9	anti
N^4-methoxy (**54**):	5	11	7	anti
N^4-hydroxy (**55**):	4	10	6	anti
MMdUrd (**6**):	4	10	7	anti
2-imino-MMdUrd (**66**):	12	9	2	syn
2-imino-MMddCyd (**90**):	10	5	3	syn
BrVdCyd (**17**):	4	9	6	anti
N^4-acetyl (**95**):	8	15	11	anti
N^4-propanoyl (**96**):	9	14	13	anti
N^4-butanoyl (**97**):	5	10	8	anti

Conformer:	$\eta_{6\{1'\}}$	$\eta_{6\{2'\}}$	$\eta_{6\{3'\}}$	glycosidic preference [a]
N^4-Me-BrVdCyd (**101**):	5	9	7	anti
N^4-methoxy-5-Me-dCyd (**105**):	7	14	9	anti
N^4-hydroxy-5-Me-dCyd (**106**):	5	10	7	anti
EtdUrd (**9**):	6	12	5	anti
EtdCyd (**118**):	5	7	4	anti
HMdUrd (**113**):	4	11	5	anti
AZT (**28**):	6	10	5	anti
AzHMddUrd (**119**):	7	16		anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

4.7) Experimental

N^4-acetyl-5-methoxymethyl-2'-deoxycytidine. (**30**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 5.9	6.0	-13.9	6.4	5.4	4.7	3.2	4.8	-12.4
298 K: 6.1	6.2	-14.2	6.3	4.8	4.8	3.3	4.5	-12.2
310 K: 6.2	6.2	-13.8	6.4	5.0	4.8	3.4	4.7	-12.3
323 K: 6.6	6.4	-13.7	6.4	4.7	4.6	3.6	4.7	-12.1

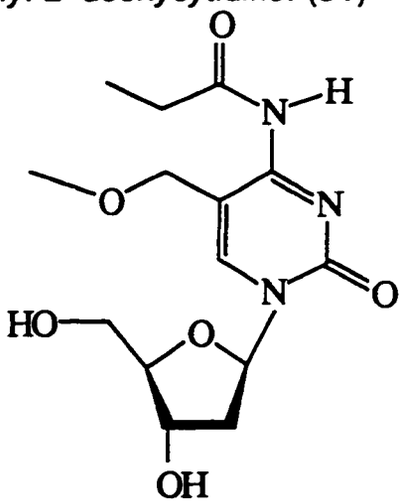
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.0	6.4	6.6	5.4	4.7
298 K: 6.3	6.4	6.6	5.0	4.6
310 K: 6.3	6.4	6.6	5.1	4.7
323 K: 6.6	6.3	6.6	4.8	4.4

Exocyclic sidechain population (%).

	χ_{g+}	χ_{g-}	χ_t
278 K:	55	9	36
298 K:	58	10	32
310 K:	55	11	34
323 K:	53	14	33

N^4-propanoyl-5-methoxymethyl-2'-deoxycytidine. (**31**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 5.7	6.1	-13.6	6.3	5.3	4.4	3.4	4.7	-12.5
298 K: 6.0	6.2	-13.5	6.2	5.4	4.3	3.5	4.6	-12.8
310 K: 6.3	6.1	-13.7	6.2	5.2	4.5	3.4	4.5	-12.5
323 K: 6.2	6.3	-13.3	6.1	4.9	4.3	3.3	4.4	-12.6

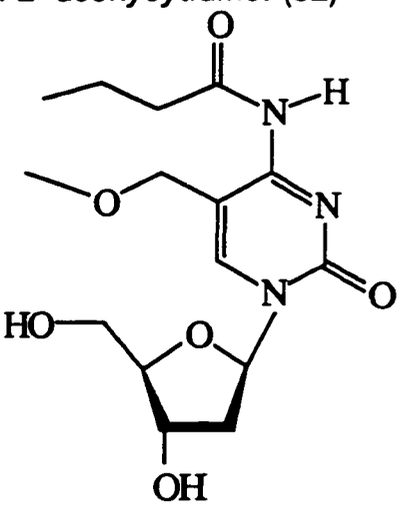
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 5.9	6.4	6.4	5.4	4.4
298 K: 6.0	6.4	6.4	5.3	4.4
310 K: 6.2	6.3	6.4	5.2	4.3
323 K: 6.3	6.3	6.4	4.0	4.2

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	55	11	34
298 K:	55	13	32
310 K:	57	11	32
323 K:	59	10	31

N^4-butanoyl-5-methoxymethyl-2'-deoxycytidine. (**32**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
278 K: 5.9	6.3	-13.0	6.1	5.2	4.2	3.6	4.4	-12.1
298 K: 6.2	6.1	-13.1	6.3	5.3	4.4	3.4	4.6	-12.5
310 K: 6.3	6.0	-12.9	6.2	5.1	4.4	3.7	4.8	-12.3
323 K: 6.0	6.2	-13.3	6.0	4.9	4.1	3.8	4.9	-12.4

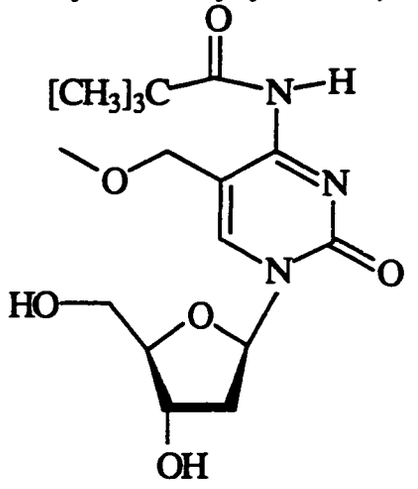
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2''	2',3'	2'',3'	3',4'
278 K: 6.1	6.3	6.4	5.3	4.3
298 K: 6.1	6.3	6.4	5.2	4.3
310 K: 6.2	6.3	6.4	5.1	4.2
323 K: 6.3	6.3	6.4	5.1	4.1

Exocyclic sidechain population (%).

	χ_{g+}	χ_{g-}	χ_t
278 K:	56	14	30
298 K:	56	11	33
310 K:	51	15	34
323 K:	49	16	35

N^4-trimethylacetyl-5-methoxymethyl-2'-deoxycytidine. (**33**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
278 K: 6.1	6.3	-13.8	6.3	4.8	4.7	3.4	4.9	-12.2
298 K: 6.2	6.4	-14.2	6.3	4.7	4.6	3.4	5.0	-12.3
310 K: 6.3	6.3	-13.8	6.3	4.9	4.8	3.5	4.8	-12.1
323 K: 6.2	6.2	-13.7	6.7	4.2	4.7	3.8	4.8	-12.1

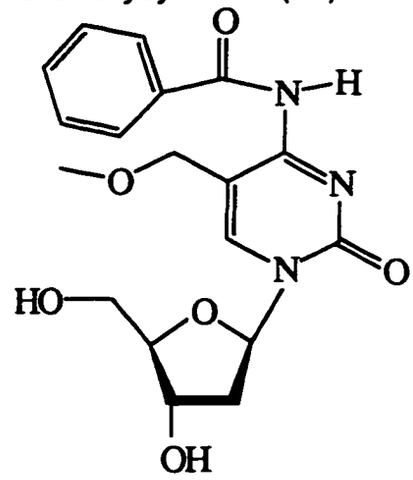
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2''	2',3'	2'',3'	3',4'
278 K: 6.3	6.4	6.6	5.0	4.6
298 K: 6.4	6.4	6.6	5.0	4.5
310 K: 6.4	6.4	6.6	5.0	4.6
323 K: 6.6	6.3	6.6	4.8	4.4

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	53	11	36
298 K:	52	11	37
310 K:	53	13	34
323 K:	50	16	34

N^4-benzoyl-5-methoxymethyl-2'-deoxycytidine. (**34**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 5.7	6.1	-13.2	6.3	5.3	4.0	3.5	4.6	-12.1
298 K: 5.9	6.3	-13.0	6.2	5.4	4.1	3.6	4.7	-12.3
310 K: 6.1	6.5	-13.1	6.2	5.2	4.3	3.7	4.4	-12.1
323 K: 5.9	6.2	-13.1	6.1	4.9	4.2	3.6	4.6	-12.2

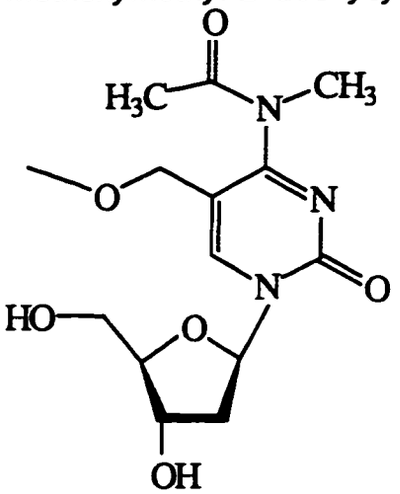
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 5.9	6.4	6.3	5.4	4.2
298 K: 5.9	6.4	6.3	5.3	4.2
310 K: 6.0	6.3	6.3	5.3	4.1
323 K: 6.1	6.3	6.3	5.2	4.0

Exocyclic sidechain population (%).

	χ_{g+}	χ_{g-}	χ_t
278 K:	55	13	32
298 K:	53	14	33
310 K:	55	15	30
323 K:	54	14	32

N^4-acetyl, N^4-methyl-5-methoxymethyl-2'-deoxycytidine. (**57**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 6.5	6.3	-13.3	6.4	4.9	4.3	3.7	4.8	-12.4
298 K: 6.4	6.2	-14.0	6.2	4.5	4.5	3.6	4.9	-12.3
310 K: 6.8	6.6	-13.1	6.5	4.4	4.2	4.0	4.5	-12.3
323 K: 6.7	6.5	-13.5	6.4	4.6	4.4	3.9	4.7	-12.1

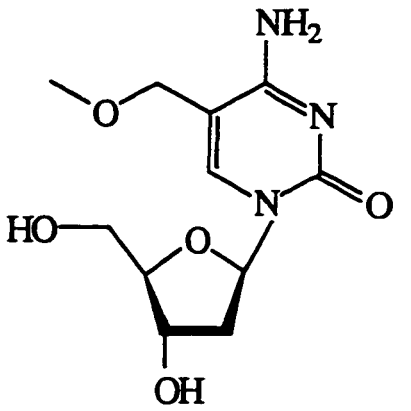
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.8	6.2	6.3	4.2	4.1
298 K: 6.9	6.4	6.2	4.3	4.3
310 K: 7.0	6.5	6.2	4.2	4.0
323 K: 6.8	6.3	6.5	4.5	4.1

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	51	26	23
298 K:	53	24	23
310 K:	50	23	27
323 K:	52	24	24

5-methoxymethyl-2'-deoxycytidine. (18)



observed coupling constants (splitting) (Hz):

J :1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'
278 K: 4.2	4.2	-14.2	6.5	4.5	3.8
298 K: 4.7	4.6	-14.6	6.6	4.7	4.0
310 K: 4.5	4.3	-14.8	6.6	4.2	4.0
323 K: 4.5	4.6	-15.0	6.7	4.5	4.0

experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
278 K: 7.0	6.9	-14.2	6.3	4.3	4.2	4.0	4.0	-12.1
298 K: 7.0	6.8	-14.1	6.3	4.3	4.2	3.6	3.6	-12.0
310 K: 7.1	6.7	-14.1	6.7	4.3	4.0	4.7	4.6	-12.4
323 K: 7.0	6.7	-13.9	6.5	4.4	4.4	4.2	4.1	-12.3

calculated coupling constants (Hz) (PSEUOROT):

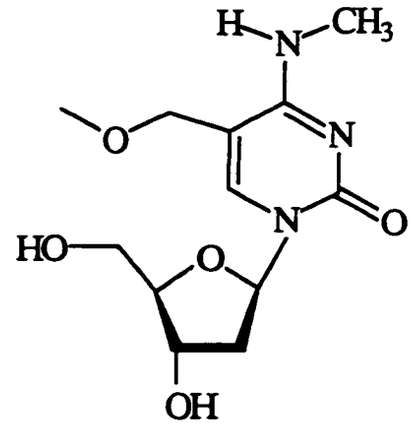
J :1',2'	1',2''	2',3'	2'',3'	3',4'
278 K: 7.1	6.8	6.4	4.5	4.2
278 K: 7.1	6.8	6.4	4.5	4.2
310 K: 7.2	6.7	6.4	4.4	4.1

323 K: 7.1 6.7 6.5 4.6 4.3

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	57	19	24
298 K:	65	14	21
310 K:	45	27	28
323 K:	55	21	24

N^4-methyl-5-methoxymethyl-2'-deoxycytidine. (**24**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 7.1	6.8	-14.0	6.5	4.4	4.1	4.2	4.0	-12.7
298 K: 7.2	6.8	-14.0	6.5	4.0	4.1	4.1	3.6	-12.3
310 K: 7.2	6.8	-14.7	6.7	4.1	4.1	4.7	3.9	-12.6
323 K: 7.2	6.7	-14.2	6.6	4.4	4.1	5.0	4.2	-12.4

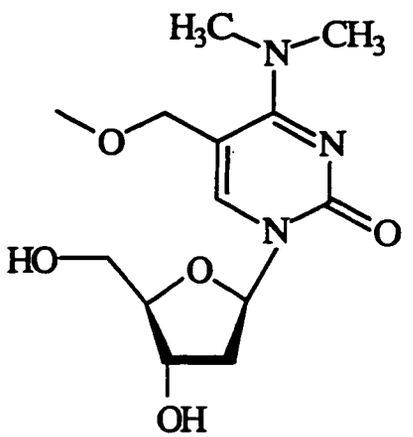
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 7.2	6.8	6.5	4.4	4.2
298 K: 7.4	6.8	6.5	4.2	4.1
310 K: 7.3	6.8	6.5	4.3	4.1
323 K: 7.3	6.8	6.5	4.4	4.2

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	55	21	24
298 K:	60	20	20
310 K:	52	27	21
323 K:	46	31	23

N^4,N^4-dimethyl-5-methoxymethyl-2'-deoxycytidine. (**43**)



experimental coupling constants (Hz) (PANIC):

	J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K:	6.5	6.4	-13.9	6.3	4.4	4.3	3.5	5.1	-12.1
298 K:	6.7	6.6	-13.8	6.0	4.1	4.6	3.5	4.7	-12.0
310 K:	6.7	6.3	-13.8	6.8	4.2	4.2	3.7	4.8	-11.9
323 K:	6.8	6.4	-13.7	6.6	4.4	4.2	3.6	4.8	-11.7

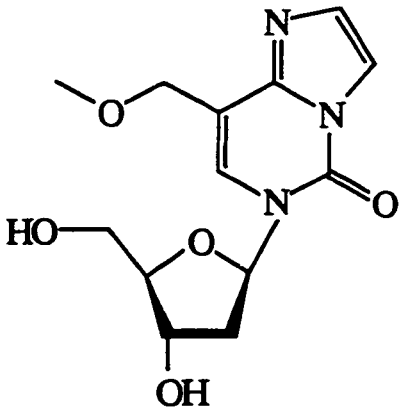
calculated coupling constants (Hz) (PSEUOROT):

	J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K:	6.8	6.4	6.5	4.7	4.3
298 K:	6.9	6.4	6.5	4.6	4.2
310 K:	7.0	6.4	6.5	4.5	4.2
323 K:	6.9	6.4	6.5	4.5	4.2

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	50	12	38
298 K:	54	13	33
310 K:	51	15	34
323 K:	52	14	34

3,4-etheno-5-methoxymethyl-2'-deoxycytidine. (**94**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
278 K: 6.9	6.1	-13.5	5.7	4.8	4.1	3.5	4.5	-12.5
298 K: 7.0	6.2	-13.9	5.5	4.5	4.0	3.2	4.3	-12.1
310 K: 7.0	6.3	-13.2	5.8	4.8	4.2	3.7	4.6	-12.6
323 K: 6.8	6.2	-13.6	6.0	4.7	4.0	3.8	4.7	-12.2

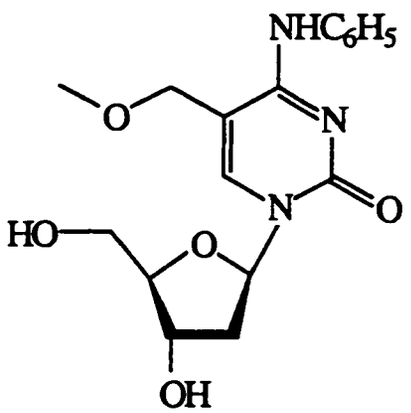
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2''	2',3'	2'',3'	3',4'
278 K: 6.8	6.4	6.2	4.6	3.9
298 K: 7.0	6.3	6.2	4.5	3.8
310 K: 6.8	6.4	6.2	4.7	4.0
323 K: 6.8	6.4	6.2	4.6	3.9

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	50	15	35
298 K:	53	14	33
310 K:	52	16	32
323 K:	50	16	34

N^4-phenyl-5-methoxymethyl-2'-deoxycytidine. (**44**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 7.0	6.7	-13.9	6.3	4.4	4.3	3.8	5.1	-12.1
298 K: 7.1	6.9	-13.8	6.2	4.1	4.5	3.5	4.7	-12.0
310 K: 7.2	6.8	-13.8	6.8	4.0	4.1	3.7	4.8	-11.8
323 K: 7.0	6.8	-13.7	6.6	4.4	4.2	3.6	4.8	-11.7

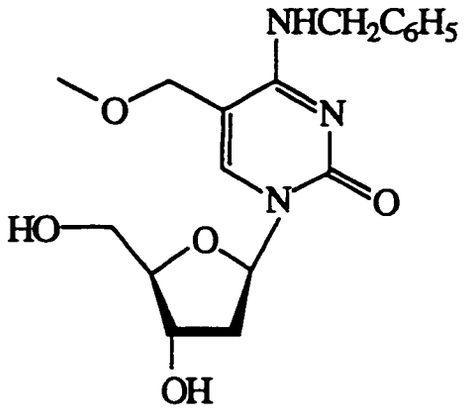
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 7.2	6.9	6.3	4.7	4.3
298 K: 7.1	6.8	6.5	4.6	4.3
310 K: 7.0	6.9	6.4	4.4	4.1
323 K: 7.3	6.6	6.5	4.5	4.2

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	51	11	38
298 K:	53	13	34
310 K:	50	16	34
323 K:	52	16	32

N^4-benzyl-5-methoxymethyl-2'-deoxycytidine. (**45**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 6.9	6.6	-13.5	6.4	4.2	4.2	3.9	4.9	-12.3
298 K: 7.2	7.0	-13.2	6.2	4.0	4.4	3.4	4.6	-12.0
310 K: 7.0	6.7	-13.1	6.8	4.1	4.0	3.5	4.4	-11.9
323 K: 7.3	6.9	-13.4	6.5	4.3	4.1	3.5	4.7	-11.8

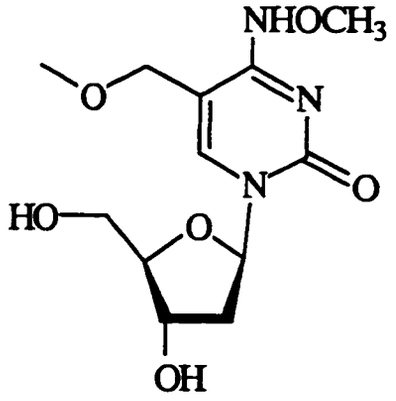
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 7.3	7.0	6.4	4.5	4.2
298 K: 7.0	6.8	6.6	4.4	4.1
310 K: 7.2	6.8	6.3	4.3	4.0
323 K: 7.1	6.9	6.4	4.5	4.2

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	54	10	36
298 K:	51	15	34
310 K:	51	17	32
323 K:	54	16	30

N^4-methoxy-5-methoxymethyl-2'-deoxycytidine. (**54**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
278 K: 7.0	6.1	-14.1	6.3	4.7	4.0	3.8	4.4	-12.5
298 K: 7.1	6.2	-14.3	6.2	4.5	4.0	3.6	4.5	-12.3
310 K: 7.0	6.0	-14.2	6.4	4.8	4.1	3.9	4.4	-12.2
323 K: 6.9	6.3	-14.0	6.3	4.9	3.9	3.8	4.7	-12.5

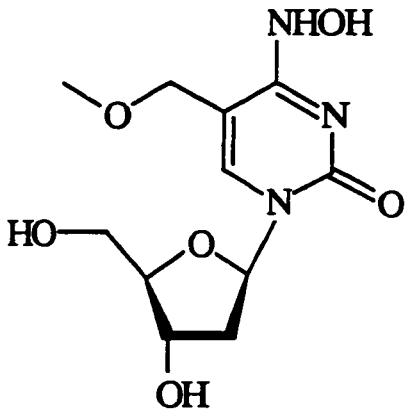
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2''	2',3'	2'',3'	3',4'
278 K: 6.9	6.2	6.4	4.5	4.0
298 K: 7.0	6.2	6.4	4.4	3.9
310 K: 6.8	6.3	6.4	4.6	4.0
323 K: 6.8	6.3	6.4	4.7	4.0

Exocyclic sidechain population (%).

	χ_{g+}	χ_{g-}	χ_t
278 K:	54	17	29
298 K:	55	14	31
310 K:	53	18	29
323 K:	51	16	33

N^4-hydroxy-5-methoxymethyl-2'-deoxycytidine. (**55**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 6.9	6.3	-13.9	6.2	4.9	4.1	3.9	4.5	-12.5
298 K: 7.0	6.4	-13.8	6.2	4.5	4.0	4.0	4.6	-12.2
310 K: 7.0	6.2	-14.2	6.1	4.8	4.2	4.0	4.8	-12.4
323 K: 7.1	6.2	-14.1	6.0	4.7	4.0	4.1	4.5	-12.1

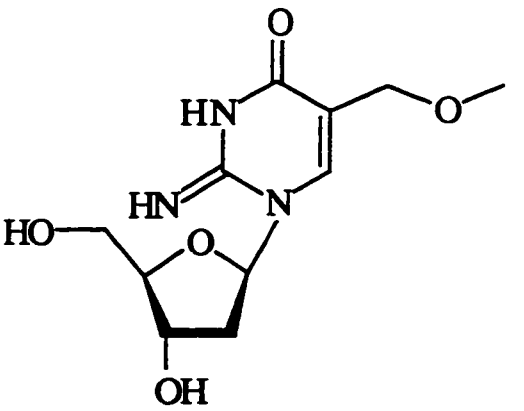
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.7	6.4	6.3	4.7	4.1
298 K: 6.9	6.3	6.3	4.5	4.0
310 K: 6.8	6.4	6.3	4.6	4.0
323 K: 6.9	6.3	6.3	4.5	4.0

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	52	18	30
298 K:	50	19	31
310 K:	48	19	33
323 K:	51	20	29

2-imino-5-methoxymethyl-2'-deoxyuridine. (**66**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 6.9	6.7	-14.0	6.5	4.8	4.3	3.5	3.0	-12.5
298 K: 7.0	6.8	-14.0	6.6	4.4	4.1	3.7	3.6	-12.7
310 K: 7.0	6.7	-14.2	6.6	4.3	4.1	4.4	3.3	-12.6
323 K: 6.9	6.7	-14.6	6.5	4.3	4.1	3.6	3.5	-12.4

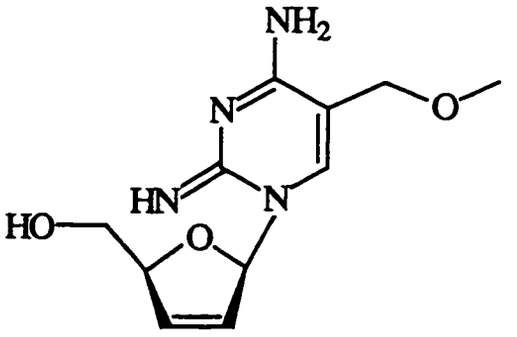
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.9	6.8	6.5	4.7	4.4
298 K: 7.1	6.7	6.4	4.5	4.2
310 K: 7.1	6.7	6.4	4.4	4.2
323 K: 7.1	6.7	6.4	4.5	4.2

Exocyclic sidechain population (%).

	χ_{g+}	χ_{g-}	χ_t
278 K:	72	13	15
298 K:	63	16	21
310 K:	61	23	16
323 K:	65	15	20

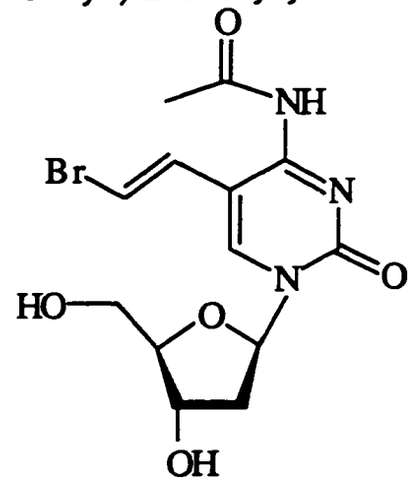
2-imino-5-methoxymethyl-2',3'-didehydro-2',3'-dideoxycytidine. (**90**)



Exocyclic sidechain population (%).

	χ_{g+}	χ_{g-}	χ_t
310 K:	68	14	18

N^4-acetyl-(E)-5-(2-bromovinyl)-2'-deoxycytidine. (**95**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 5.7	6.3	-12.8	6.2	4.9	4.3	3.7	4.6	-11.9
298 K: 5.9	6.6	-13.0	5.9	4.7	4.4	3.9	4.8	-12.0
310 K: 6.0	6.7	-12.9	6.0	4.6	4.2	3.5	4.7	-12.4
323 K: 6.0	6.5	-13.2	6.1	4.5	4.2	3.6	4.5	-12.1

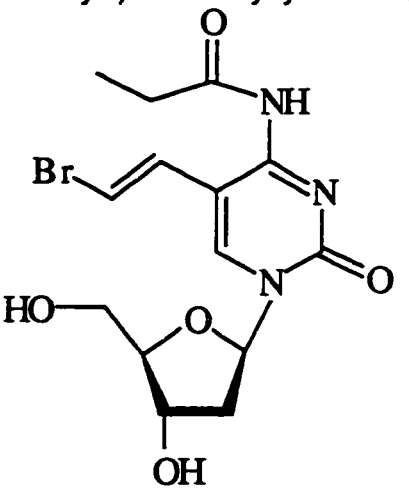
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.0	6.7	6.1	5.2	4.1
298 K: 6.1	6.6	6.1	5.1	4.1
310 K: 6.2	6.6	6.1	5.0	4.0
323 K: 6.3	6.6	6.1	5.0	4.0

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	54	10	36
298 K:	56	12	32
310 K:	55	13	32
323 K:	53	14	33

N^4-propanoyl-(E)-5-(2-bromovinyl)-2'-deoxycytidine. (**96**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 5.9	6.5	-12.5	6.0	5.1	4.5	3.9	4.8	-11.8
298 K: 5.9	6.6	-13.0	5.9	4.8	4.4	3.7	4.9	-12.2
310 K: 6.1	6.7	-12.9	6.0	4.7	4.5	3.6	4.7	-12.2
323 K: 6.0	6.7	-13.2	6.1	4.6	4.3	3.6	4.7	-12.3

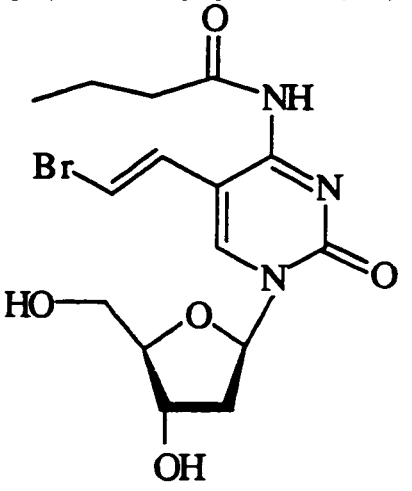
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.0	6.7	6.2	5.3	4.3
298 K: 6.2	6.7	6.2	5.1	4.2
310 K: 6.2	6.7	6.1	5.1	4.2
323 K: 6.3	6.7	6.1	5.0	4.1

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	54	12	34
298 K:	55	11	34
310 K:	55	15	30
323 K:	54	14	32

N^4-butanoyl-(E)-5-(2-bromovinyl)-2'-deoxycytidine. (**97**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 6.2	6.6	-12.7	5.8	5.0	4.3	3.5	4.7	-11.5
298 K: 5.9	6.5	-13.1	5.9	4.9	4.4	3.6	4.8	-12.1
310 K: 6.1	6.4	-12.5	6.0	5.1	4.5	3.4	4.6	-12.0
323 K: 6.0	6.7	-13.0	6.1	5.0	4.3	3.5	4.8	-11.7

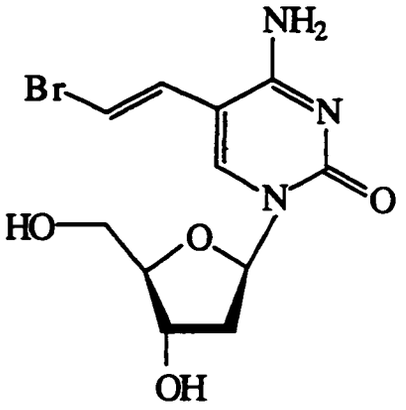
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.2	6.6	6.2	5.1	4.2
298 K: 6.1	6.6	6.2	5.2	4.2
310 K: 6.1	6.6	6.2	5.2	4.3
323 K: 6.1	6.6	6.2	5.2	4.2

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	56	11	33
298 K:	54	11	35
310 K:	52	17	31
323 K:	53	16	31

(E)-5-(2-bromovinyl)-2'-deoxycytidine. (**17**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
278 K: 6.8	6.7	-13.4	6.1	4.3	4.0	3.4	4.5	-12.3
298 K: 7.1	6.8	-13.5	5.8	4.1	3.9	3.3	4.7	-12.5
310 K: 6.9	6.8	-13.6	6.0	3.9	4.0	3.9	4.4	-12.1
323 K: 6.8	6.5	-13.9	5.9	4.4	4.1	3.4	4.3	-12.2

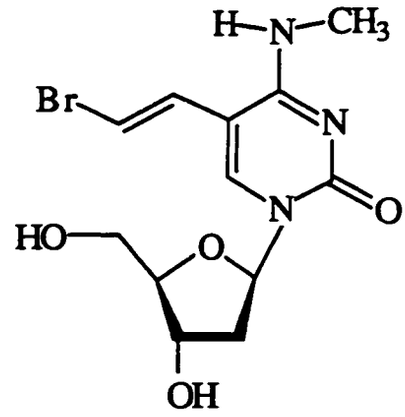
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2''	2',3'	2'',3'	3',4'
278 K: 7.0	6.6	6.0	4.5	4.1
298 K: 7.1	6.6	6.1	4.4	4.0
310 K: 7.1	6.4	6.1	4.5	3.6
323 K: 6.9	6.8	5.9	4.3	3.8

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	55	15	30
298 K:	58	14	28
310 K:	57	18	25
323 K:	58	16	26

N^4-methyl-(E)-5-(2-bromovinyl)-2'-deoxycytidine. (**101**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
278 K: 6.9	6.5	-13.7	6.2	4.5	4.1	3.5	4.6	-12.1
298 K: 7.0	6.6	-13.9	5.9	4.2	4.0	3.4	4.4	-12.5
310 K: 7.0	6.7	-14.2	5.8	4.1	4.2	3.7	4.5	-12.4
323 K: 6.8	6.5	-14.1	6.1	4.4	4.0	3.5	4.3	-12.4

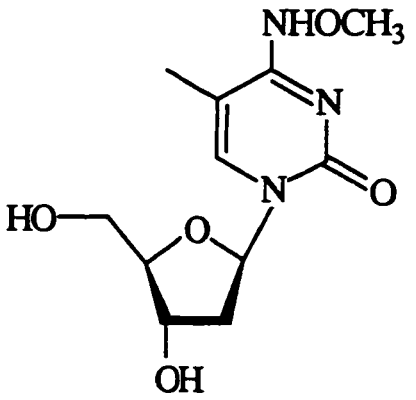
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2''	2',3'	2'',3'	3',4'
278 K: 6.9	6.6	6.2	4.6	4.0
298 K: 7.1	6.5	6.1	4.4	3.8
310 K: 7.0	6.6	6.2	4.4	3.8
323 K: 6.9	6.6	6.2	4.5	3.9

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	55	13	32
298 K:	58	11	31
310 K:	54	15	31
323 K:	58	13	29

N^4-methoxy-5-methyl-2'-deoxycytidine. (**105**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 7.2	6.6	-13.8	5.9	4.8	4.1	3.7	4.5	-12.0
298 K: 7.0	6.5	-14.1	5.8	4.7	4.2	3.7	4.6	-12.1
310 K: 6.9	6.4	-14.0	6.0	5.1	4.5	3.6	4.4	-12.3
323 K: 7.1	6.5	-13.9	6.0	4.9	4.3	3.8	4.7	-12.1

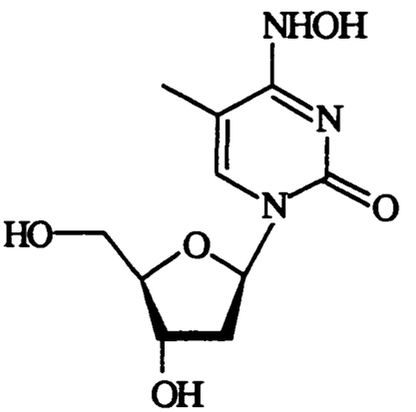
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.9	6.5	6.3	4.6	4.0
298 K: 6.9	6.5	6.3	4.6	3.9
310 K: 6.6	6.6	6.3	4.9	4.3
323 K: 6.8	6.6	6.3	4.7	4.1

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	50	15	35
298 K:	51	19	30
310 K:	53	14	33
323 K:	53	16	31

N^4-hydroxy-5-methyl-2'-deoxycytidine. (**106**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 6.9	6.4	-13.9	6.1	4.5	4.1	3.7	4.4	-12.3
298 K: 7.0	6.3	-13.8	6.0	4.4	3.9	4.0	4.6	-12.1
310 K: 7.1	6.5	-14.1	6.0	4.9	4.0	3.7	4.5	-11.9
323 K: 7.0	6.6	-14.0	6.2	4.8	4.2	3.9	4.6	-12.0

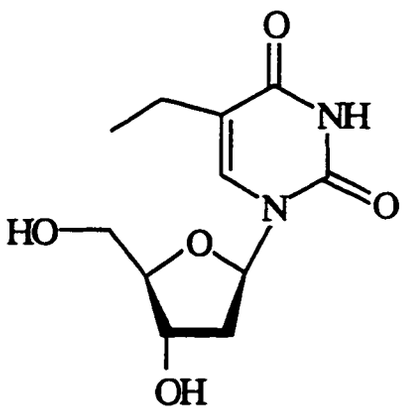
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.9	6.5	6.4	4.6	4.1
298 K: 7.0	6.4	6.3	4.5	3.9
310 K: 6.9	6.4	6.1	4.7	4.1
323 K: 7.1	6.3	6.2	4.4	4.0

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	49	17	34
298 K:	50	20	30
310 K:	48	20	32
323 K:	51	22	27

5-ethyl-2'-deoxyuridine. (**9**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
278 K: 6.8	6.2	-13.9	6.0	4.6	4.0	3.3	4.0	-11.5
298 K: 6.9	6.3	-11.2	6.0	4.5	4.1	4.4	3.8	-11.6
310 K: 6.9	6.2	-13.8	5.9	4.4	4.0	4.1	3.6	-11.6
323 K: 6.9	6.4	-13.8	6.0	4.4	4.0	4.1	2.9	-11.0

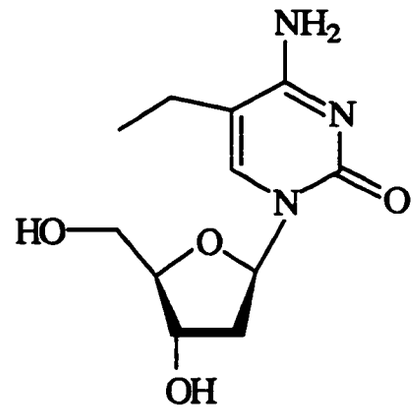
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2''	2',3'	2'',3'	3',4'
278 K: 6.8	6.4	6.2	4.6	4.0
298 K: 6.9	6.4	6.2	4.6	3.9
310 K:7.0	6.3	6.2	4.5	3.9
323 K: 6.9	6.4	6.2	4.5	3.9

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	63	10	27
298 K:	55	24	21
310 K:	60	20	20
323 K:	68	20	12

5-ethyl-2'-deoxycytidine. **(118)**



experimental coupling constants (Hz) (PANIC):

J	:1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
278 K:	6.9	6.4	-13.3	6.1	4.4	4.0	3.6	4.1	-11.8
298 K:	7.0	6.4	-14.1	6.2	4.3	4.1	3.5	4.5	-11.8
310 K:	6.9	6.5	-14.3	6.1	4.2	4.2	3.7	4.4	-11.7
323 K:	6.9	6.4	-14.2	6.1	4.1	4.1	3.6	4.3	-11.9

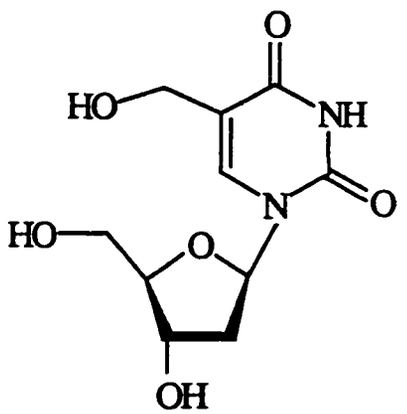
calculated coupling constants (Hz) (PSEUOROT):

J	:1',2'	1',2''	2',3'	2'',3'	3',4'
278 K:	7.0	6.5	6.3	4.5	4.0
298 K:	7.0	6.4	6.3	4.4	3.9
310 K:	7.0	6.5	6.3	4.5	4.0
323 K:	7.1	6.4	6.3	4.4	3.9

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	59	14	27
298 K:	56	13	31
310 K:	55	15	30
323 K:	57	14	29

5-hydroxymethyl-2'-deoxyuridine. (**113**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 7.3	6.4	-14.6	6.4	4.2	4.0	4.1	3.6	-12.3
298 K: 7.3	6.4	-14.0	6.7	4.2	4.1	5.3	3.4	-12.9
310 K: 6.8	6.5	-14.4	6.7	4.3	4.0	4.7	3.5	-12.3
323 K: 6.8	6.5	-14.1	6.2	4.4	4.3	4.7	3.5	-12.3

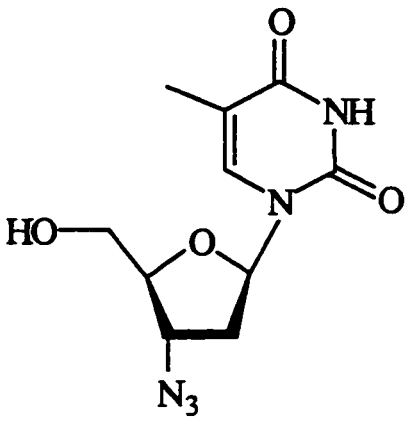
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 7.3	6.4	6.4	4.2	4.0
298 K: 7.3	6.4	6.4	4.2	4.0
310 K: 7.1	6.5	6.5	4.5	4.2
323 K: 7.0	6.5	6.5	4.6	4.3

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
temp 1:	60	20	20
temp 2:	52	35	13
temp 3:	56	28	16
temp 4:	56	28	16

3'-deoxy-3'-azidothymidine. **(28)**



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 6.3	6.8	0.0	6.6	6.1	5.0	4.4	3.3	-12.6
298 K: 6.3	6.7	0.0	6.6	6.1	5.0	4.4	3.4	-12.6
310 K: 6.3	6.6	0.0	6.6	6.1	5.0	4.8	3.4	-12.9
323 K: 6.3	6.6	0.0	6.6	6.1	5.0	4.4	4.2	-12.1

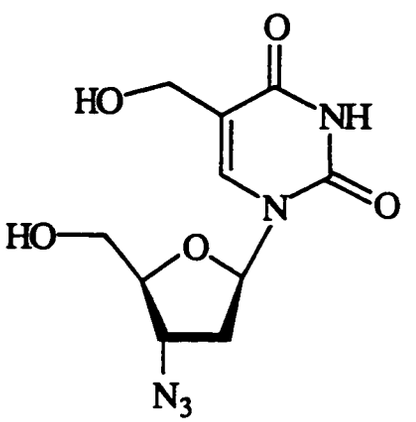
calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 6.0	6.8	6.8	5.9	5.0
298 K: 6.0	6.8	6.8	5.9	5.0
310 K: 6.0	6.8	6.8	5.8	5.0
323 K: 6.0	6.8	6.8	5.8	5.0

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	52	23	25
298 K:	52	23	25
310 K:	60	22	18
323 K:	60	22	18

3'-deoxy-3'-azido-5-hydroxymethyl-2'-deoxyuridine. (**119**)



experimental coupling constants (Hz) (PANIC):

J :1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"	5',5"
278 K: 6.1	6.7	0.0	6.6	6.1	5.1	4.4	3.3	-12.6
298 K: 6.1	6.6	0.0	6.6	6.1	5.1	4.4	3.4	-12.6
310 K: 6.1	6.6	0.0	6.6	6.1	5.1	4.8	3.4	-12.9
323 K: 6.1	6.6	0.0	6.6	6.1	5.0	4.4	4.2	-12.1

calculated coupling constants (Hz) (PSEUOROT):

J :1',2'	1',2"	2',3'	2",3'	3',4'
278 K: 5.9	6.7	6.8	6.0	5.0
298 K: 5.9	6.7	6.8	6.0	5.0
310 K: 5.9	6.7	6.8	6.0	5.0
323 K: 5.9	6.7	6.8	5.9	5.0

Exocyclic sidechain population (%).

	X_{g+}	X_{g-}	X_t
278 K:	61	23	16
298 K:	61	23	16
310 K:	57	29	14
323 K:	52	23	25

5-BIOLOGICAL STUDIES

5.1) Introduction

The antiviral activity of analogs of MMdCyd (**18**), MMdUrd (**6**), BrVdCyd (**17**) and 5-Me-dCyd (**29**) against HSV-1 was determined by plaque reduction assay. The cytotoxicity of all compounds was also determined. The stability of active compounds at different pH values was also investigated. The antiviral assays were performed by Dr. Homayoon Z. Kamaly, Dept. of Veterinary Physiological Sciences, WCVM, University of Saskatchewan. Results of these studies are presented in this chapter.

5.2) Materials and Methods

Synthesis of nucleoside analogs was described in chapter 3. The biological activity of the following compounds was determined:

(i) **MMdCyd analogs**: N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**), N^4-butanoyl-MMdCyd (**32**), N^4-pivaloyl-MMdCyd (**33**), N^4-benzoyl-MMdCyd (**34**), N^4,N^4-dimethyl-MMdCyd (**43**), N^4-phenyl-MMdCyd (**44**), N^4-benzyl-MMdCyd (**45**), N^4-methoxy-MMdCyd (**54**), N^4-hydroxy-MMdCyd (**55**), 3,4-etheno-MMdCyd (**94**) and 2-imino MMddCyd (**90**).

(ii) **MMdUrd analog**: 2-imino-MMdUrd (**66**).

(iii) **BrVdCyd analogs**: N^4-methyl-BrVdCyd (**101**), N^4-acetyl-BrVdCyd (**95**), N^4-propanoyl-BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**).

(iv) **5-MedCyd analogs**: N^4-methoxy-5-Me-dCyd (**105**) and N^4-hydroxy-5-Me-dCyd (**106**).

For the stability studies (section 5.4), a Kontron Uvikon 860 ultra-violet spectrophotometer was used. Solutions pH (1-13) were prepared and the pH

was checked using a pH meter (Fisher model 230). Ready-mix buffers were supplied by Sargent-Welch and are certified at the specified pH ±0.02 at 25°C.

- pH 1.00 (0.1 M HCl solution);
- pH 3.00 buffer was prepared from glycine - HCl ;[125]
- pH 5.00 buffer was prepared using a Metrepak ready-mix sodium and potassium phosphates mixture, control No. 71707;
- pH 7.00 buffer was prepared using sodium dihydrogen orthophosphate (monobasic) - NaOH mixture;[125]
- pH 9.00 buffer was prepared using a Metrepak ready-mix potassium phosphate and sodium borate mixture, control No. 73509;
- pH 11.00 buffer was prepared using a Metrepak ready-mix sodium borate and sodium carbonate mixture, control No. 71957 and
- pH 13.00 solution was prepared using 0.1 M NaOH.

5.3) Antiviral Activity

5.3.1) Cell Culture

African green monkey kidney (VERO) cells were used. The cell line was regularly tested for mycoplasma contamination. The cells were cultured in a 1:10 diluted Eagle's minimum essential medium (10 x MEM, No. 14-100-54, Flow Laboratories, Mississauga, Ontario). Each liter of medium was supplemented with 2 mmol of L-glutamine (No. 16-801-49, Flow lab.), 10 mL of nonessential amino acids (200 x MEM nonessential amino acids, No. 16-810-49, Flow Lab.), 54 mL of 4.4% sodium bicarbonate with phenol red, 2.5 µg/mL fungizone, 100 µg/mL penicillin, 100 µg/mL streptomycin and 10% fetal bovine serum (FBS, heat inactivated, sterile, mycoplasma and virus free, No. 29-102-54, Flow Lab.) for growth of the cells or 4% FBS for maintenance. Cells were grown and maintained in sterile 75 and 175 cc tissue culture flasks (Miles

Scientific, Naperville, IL). The cultures were incubated at 37° C in humidified CO_2 (5%) atmosphere. The monolayers were trypsin-versenized and transferred to other flasks twice a week. The trypsin and versene were obtained from the media preparation laboratory, Dept. of Veterinary Microbiology, University of Saskatchewan. For antiviral assays, monolayers were prepared in 16 or 36-well microtitre tissue culture plates (Flow Lab).

5.3.2) Viruses

HSV-1 strain KOS was used. The strain was originally obtained from Dr. V. Misra (Dept. of veterinary Microbiology, WCVM, University of Saskatchewan) and has been maintained in the laboratories of the Dept. of Veterinary Physiological Sciences. VERO cells were grown to confluent monolayers in 175 cc flasks. Cells seeded in flasks were counted using a hemocytometer to give an estimate of cell number. The media in the flasks was aspirated and 4 mL of dilute virus were added. The stock virus was diluted so as to achieve a low multiplicity of infection (0.001 plaque forming unit, PFU, per cell). The flasks were incubated for one hour to allow for virus adsorption, after which unadsorbed virus was removed by washing with MEM then MEM containing 4% FBS was added. At the end of 48 hours, the cells were examined for infection. If the infection was not 100%, the media overlaying the cells was removed and centrifuged at 1000 x g at 4° C for 5 minutes to pellet detached cells. The supernatant was sucked, discarded, the cell pellet resuspended in fresh MEM, and overlaid on cells in the same flask. Additional MEM with 4% FBS was added and incubated for a further 24-48 hours until infection was 100% complete. At the end of this period, the cell culture fluid and cell debris were subjected to four 30 second pulses of sonar boom in order to lyse the cells and release intracellular virus. The fluid and debris was centrifuged at

1000 x g and 4° C for 10 minutes. The supernatant was dispensed into vials (0.5 mL/vial) and stored at -70° C until used. The virus titer was determined by the Karber method of 50% end point determination. Monolayer cells in microtiter plates were infected with 0.1 mL serial dilution of the stock virus. At the end of 72 hours the cultures were examined for infectivity.

Antibodies specific to the HSV-1 were prepared as follows: 0.5 mL of virus titer 10^6 $TCID_{50}$/mL was injected intravenously into rabbits. After two weeks, animals were challenged with a second dose of the same herpes virus. Four weeks after the initial injection of virus, blood was collected, serum filtered-sterilized, heat inactivated at 56° C and titrated by virus neutralization.

5.3.3) Antiviral Assays

The antiviral assays were carried out by infecting confluent cell monolayers with 50 to 300 PFU per well in a microtiter plate as described previously. Virus dilutions were made in serum-free MEM. A 0.10 mL aliquots were added to the respective wells and the microtiter plate was incubated at 37° C and 5% CO_2 for one hour to allow for virus adsorption. Unadsorbed virus was removed by washing with MEM. Each antiviral compound in maintenance medium was added at the appropriate concentration along with one or two neutralizing units of antibody specific for HSV-1. In each experiment, toxicity controls (containing the test compound and medium only), cell controls (containing medium only), and virus controls (containing virus and medium only) were run concurrently. Culture plates were incubated at 37° C and 5% CO_2 and plaques allowed to develop for 48 to 72 hours. After this period the culture fluid was removed, the monolayer fixed and stained with 1% crystal violet in 70% ethanol, air dried and examined for virus plaques. The percentage of inhibition (calculated from reduction in the number of plaques) at each concentration of the compound

was determined. The 50% inhibition (ED_{50}) value was calculated from dose response curves.

5.3.4) Results and Discussion

5.3.4.1) The data for the antiviral assays of MMdCyd (**18**) and its analogs are presented in table 5.3.4.1. N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**) and N^4-butanoyl-MMdCyd (**32**), inhibited replication of HSV-1. All other derivatives of MMdCyd (**18**) were devoid of antiviral activity. Further work on ED_{50} values of active compounds is now in progress.

TABLE 5.3.4.1

Antiviral activity of MMdCyd and its analogs.

Compound	Virus Strain	PFU	ED_{50} [a] (μM)
MMdCyd (**18**):	KOS	50	26 [1]
MMdCyd (**18**):	X-265 (HSV-2)	50	130 [1]
N^4-acetyl (**30**):	KOS	50	< 26
N^4-propanoyl (**31**):	KOS	50	< 26
N^4-butanyol (**32**):	KOS	50	< 26
N^4-pivaloyl (**33**):	KOS	50	> 512 [b]
N^4-benzoyl (**34**):	KOS	50	> 512 [b]
N^4,N^4-dimethyl (**43**):	KOS	50	> 512 [b]
N^4-phenyl (**44**):	KOS	50	> 512 [b]
N^4-benzyl (**45**):	KOS	50	> 512 [b]
N^4-methoxy (**54**):	KOS	50	> 512 [b]
N^4-hydroxy (**55**):	KOS	50	> 512 [b]
2-imino-MMddCyd (**90**):	KOS	50	> 512 [b]
3,4-etheno (**94**):	KOS	50	> 512 [b]

[a] ED_{50}: concentration required to reduce viral plaque formation by 50%.
[b] :highest concentration tested. No reduction in virus plaques was observed.
PFU: virus input 50 plaque forming unit.

The activity of N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**) and N^4-butanoyl-MMdCyd (**32**) is higher than that of the parent compound MMdCyd (**18**). It is interesting to note that these analogs of MMdCyd (**18**) were non cytotoxic to VERO cells up to 512 μM.

5.3.4.2) The data for the antiviral assays of MMdUrd (**6**) and its analog 2-imino-MMdUrd (**66**) against HSV-1 in VERO cells are presented in table 5.3.4.2. The data shows that MMdUrd (**6**) is active while 2-imino-MMdUrd (**66**) is devoid of antiviral activity. The analog 2-imino-MMdUrd (**66**) is also non toxic to VERO cells up to 512 μM.

TABLE 5.3.4.2

Antiviral activity of MMdUrd and 2-imino-MMdUrd.

Compound	Virus	PFU	ED_{50} [a] (μM)
MMdUrd (**6**):	KOS	50	59
2-imino-MMdUrd (**66**):	KOS	50	> 512 [b]

[a] ED_{50}: concentration required to reduce viral plaque formation by 50%.

[b] :highest concentration tested. No reduction in virus plaques was observed.

PFU: virus input 50 plaque forming unit.

5.3.4.3) The data for the antiviral activity of BrVdCyd (**17**) and its analogs against HSV-1 in VERO cells is presented in table 5.3.4.3. The amide derivatives of BrVdCyd (**17**) are more active than the parent compound whereas N^4-methyl-BrVdCyd (**101**) was inactive. All analogs of BrVdCyd (**17**) were non toxic at 512 μM to VERO cells.

TABLE 5.3.4.3

Antiviral activity of BrVdCyd and its analogs.

Compound	Virus	PFU	ED_{50} [a] (μM)
BrVdCyd (**17**):	KOS	50	1.08 [1]
N^4-acetyl (**95**):	KOS	50	< 1.08
N^4-propanoyl (**96**):	KOS	50	< 1.08
N^4-butanyol (**97**):	KOS	50	< 1.08
N^4-methyl (**101**):	KOS	50	> 512 [b]

[a] ED_{50}: concentration required to reduce viral plaque formation by 50%.

[b]: highest concentration tested. No reduction in virus plaques was observed.

PFU: virus input 50 plaque forming unit.

5.3.4.4) The data for the antiviral assays of N^4-methoxy-5-Me-dCyd (**105**) and N^4-hydroxy-5-Me-dCyd (**106**) against HSV-1 in VERO cells is shown in table 5.3.4.4.. The analogs N^4-methoxy-5-Me-dCyd (**105**) and N^4-hydroxy-5-Me-dCyd (**106**) did not inhibit HSV-1 replication and were non toxic to VERO cells.

TABLE 5.3.4.4

Antiviral activity of N^4-methoxy-5-Me-dCyd. and N^4-hydroxy-5-Me-dCyd.

Compound	Virus	PFU	ED_{50} µM [a]
N^4-methoxy-5-Me-dCyd (**105**):	KOS	50	> 512 [b]
N^4-hydroxy-5-Me-dCyd (**106**):	KOS	50	> 512 [b]

[a] ED_{50}: concentration required to reduce viral plaque formation by 50%.

[b]: highest concentration tested. No reduction in virus plaques was observed.

PFU: virus input 50 plaque forming unit.

5.4) Stability Studies

The stability of the N^4-bond of biologically active N^4-acyl analogs of MMdCyd (**18**) and BrVdCyd (**17**) in pH range 1 to 13 was determined. The change in *absorption at the* λ_{max} for each compound was monitored by ultra-violet spectrophotometry. A reading was taken every minute for a thirty minute period. In addition, N^4-butanoyl-MMdCyd (**32**), N^4-benzoyl-MMdCyd (**34**) and N^4-butanoyl-BrVdCyd (**97**) in phosphate buffer, pH 7 were incubated at 37°C for 24 and 48 h. At the end of the incubation period, absorption spectra were recorded.

5.4.1) N^4-acyl MMdCyd analogs

The stability data for N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**) and N^4-butanoyl-MMdCyd (**32**) are presented in table 5.4.1.1.

TABLE 5.4.1.1

pH stability of N^4-acyl analogs of MMdCyd over a 30 min period.

	N^4-acetyl	N^4-propanoyl	N^4-butanoyl
pH 1	partially [a]	partially	partially
pH 3	stable [b]	stable	stable
pH 5	stable	stable	stable
pH 7	stable	stable	stable
pH 9	stable	stable	stable
pH11	slightly [c]	slightly	slightly
pH13	slightly	slightly	slightly

[a] partially stable: significant drop in absorbance(35-40%) and appearance of MMdCyd λ_{max}.

[b] stable: no change or drop in absorbance λ_{max}.

[c] slightly unstable: small drop in absorbance(< 10%) with no appearance of MMdCyd λ_{max}.

The amide derivatives of MMdCyd (**18**) appear to be stable in the pH range 3 to 9. Significant loss in absorption (λ_{max}) occurred for all compounds at pH 1. Partial loss (< 10%) of absorption was also observed at pH 11 and 13.

5.4.2) N^4-acyl BrVdCyd analogs

The results of stability studies for N^4-acetyl-BrVdCyd (**95**), N^4-propanoyl-BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**) are summarized in table 5.4.2.1.

TABLE 5.4.2.1

pH stability of N^4-acyl analogs of BrVdCyd over a 30 min period.

	N^4-acetyl	N^4-propanoyl	N^4-butanoyl
pH 1	unstable [a]	unstable	unstable
pH 3	slightly [b]	slightly	slightly
pH 5	stable [c]	stable	stable
pH 7	stable	stable	stable
pH 9	stable	stable	stable
pH11	stable	stable	stable
pH13	stable	stable	stable

[a] unstable: λ_{max} of acyl derivative changed to BrVdCyd λ_{max}.

[b] slightly unstable: small drop in absorbance(< 10 %) in λ_{max} value of acyl analog.

[c]: no drop in absorbance at λ_{max}.

The acyl analogs of BrVdCyd (**17**) appear to be stable in the pH range 5 to 13. However, at pH 1 there appears to be complete hydrolysis of the amide bond as indicated by the change in the λ_{max} value.

5.4.3) N^4-butanoyl-MMdCyd, N^4-benzoyl-MMdCyd and N^4-butanoyl-BrVdCyd

The results of stability studies for N^4-butanoyl-MMdCyd (**32**), N^4-benzoyl-MMdCyd (**34**) and N^4-butanoyl-BrVdCyd (**97**) after incubation in pH 7 buffer solution for 24 and 48 h at 37° C are shown in table 5.4.3.1.

TABLE 5.4.3.1

Stability of N^4-butanoyl-MMdCyd, N^4-benzoyl-MMdCyd and N^4-butanoyl-BrVdCyd at 37°C in pH 7.

Compound:	after 24 h	after 48 h
N^4-butanoyl-MMdCyd (**32**):	slightly [a]	partially [b]
N^4-benzoyl-MMdCyd (**34**):	slightly	partially
N^4-butanoyl-BrVdCyd (**97**):	stable [c]	stable

[a]: slightly unstable: small drop in absorbance (5 -10 %) with no appearance of MMdCyd λ_{max}.

[b]: partially stable: significant drop in absorbance (15 - 45 %) at analog λ_{max} and appearance of MMdCyd λ_{max}.

[c]: no drop in absorbance at analog λ_{max}.

5.4.4) Discussion

On the basis of stability data, it is logical to conclude that N^4-acyl analogs of MMdCyd (**18**) have a higher tolerance for acidic media than the corresponding BrVdCyd (**17**) counterparts over a short incubation period. This is evident from the observations that only partial hydrolysis of the amide bond of N^4-acyl analogs of MMdCyd (**18**) occured; whereas complete hydrolysis of the amide bond of N^4-acyl analogs of BrVdCyd (**17**) was observed. Interestingly, in contrast, N^4-acyl analogs of BrVdCyd (**17**) have a higher

stability profile in basic media (pH 11 and 13) compared to N^4-acyl derivatives of MMdCyd (**18**).

N^4-butanoyl-MMdCyd (**32**) and N^4-benzoyl-MMdCyd (**34**) were less stable than N^4-butanoyl-BrVdCyd (**97**). The absorbance decreased slightly (~10 %) for acyl analogs of MMdCyd (**18**) after a 24 h incubation period. However, with prolonged incubation (48 h), a significant proportion of N^4-butanoyl-MMdCyd (**32**) seems to be hydrolyzed (~ 45% decrease in absorbance). Under similar conditions, N^4-benzoyl-MMdCyd (**34**) appears to be less susceptible to hydrolytic cleavage (15 % decrease in absorbance). N^4-butanoyl-BrVdCyd (**97**) appears to be a very stable compound as indicated by the fact that there was no change in absorbance after incubation for 48 h at 37° C. Under similar conditions, N^4-butanoyl-MMdCyd (**32**) appears to undergo gradual hydrolytic cleavage as indicated by substantial decrease in absorbance at λ_{max} after 48 h with concomitant appearance of the new absorption peak (λ_{max}) due to the formation of MMdCyd (**18**). The stability profile of N^4-benzoyl-MMdCyd (**34**) was similar to N^4-butanoyl-MMdCyd (**32**), however, the rate of hydrolysis of the amide bond seems to occur at a slower rate. The identity of the hydrolysis product, MMdCyd (**18**), arising from hydrolysis of the N^4-acyl bond of amide derivatives of MMdCyd (**18**) was confirmed by concentrating the buffer solution and co-spoting the residue with MMdCyd (**18**) (20% MeOH/CH_2Cl_2).

6-CONCLUSION

6.1) Structure/Activity Discussions and Conclusions

In order to understand the molecular basis for selectivity of action of antiherpes drugs, studies on structural, conformational, stereochemical and electronic requirements have been undertaken.[2,21,34,126,127] Interestingly, for 5-halogenated derivatives of 2'-deoxyuridine (**22**), for example, IdUrd (**1**) and F_3dThd (**2**), which exhibit very little selectivity in their antiviral action, Yates and Patel concluded that electronic effects are more important than the geometry of a molecule in determining the antiviral activity.[127] In contrast, studies on 5-substituted-2'-deoxyuridine analogs with selectivity of action against HSV, revealed no systematic relationship between the electronic effect of the C_5-substituent and the biological activity of the molecule. However, it was concluded that the selectivity of action against HSV was related to the length of the C_5 side chain of the pyrimidine ring.[113,129] The optimum chain length of the C_5-substituent seems to be three non hydrogen atoms as is the case with MMdUrd (**6**) and BrVdUrd (**8**).[122] Furthermore, it was concluded from structure activity relation correlations of 5-substituted halovinyl compounds, that the maximum potency was reached when the C_5-substituent was an unsaturated moiety with an *E* stereochemistry, conjugated with the pyrimidine ring, having no more than four carbon atoms in a non branching arrangement and containing a hydrophobic electronegative function. All of these features are present in BrVdUrd (**8**) which is one of the most active compounds against HSV.[126]

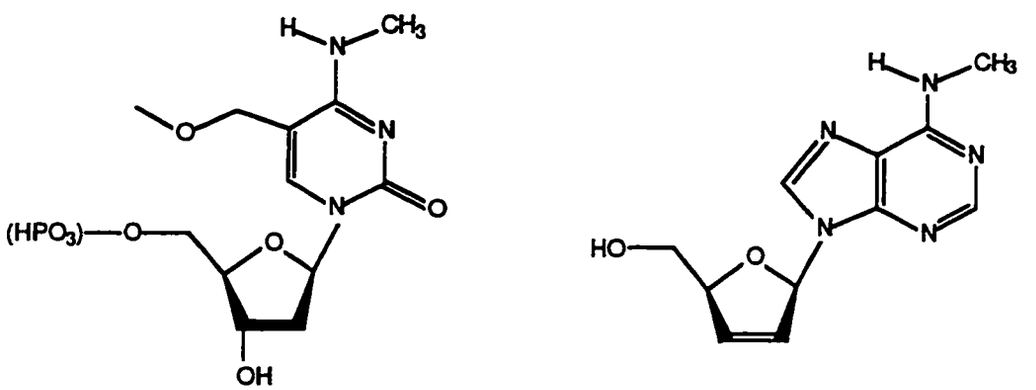
Thus, structure activity relation correlations on 5-substituted pyrimidine analogs have been useful for understanding electronic and stereochemical requirements of the C_5 moiety of pyrimidine nucleosides in relation to

selectivity of antiherpes compounds. However, the major limitation of this approach is that it does not provide information regarding the stereochemical requirements of the entire molecule for biological activity.

Receptors recognize and discriminate molecules by a process of highly specific binding. The basic tenet of pharmacological response hypothesis is that a drug interacts with the same active site on the receptor. Therefore, correlation of biological activity to molecular structure is indispensable to the systematic design of drugs. The selective antiviral activity of antiherpes drugs results from phosphorylation by the virus induced pyrimidine deoxyribonucleoside kinase. Information on conformational requirements for preferential phosphorylation by the viral induced kinase is obtained by X-ray crystallography for the solid state and NMR spectroscopy in solution. The correlation between the molecular conformation of nucleosides and their biological activity should provide information concerning the conformation required for binding to the active center of the viral kinase. The collaborative studies on structure-activity relationships for anti-HSV activity have been in progress at the University of Saskatchewan as a part of the program to define conformational parameters essential for antiherpes activity.[123] The rationale for these investigations is that by correlating conformational parameters with antiviral activity and cytotoxicity, it should be possible to identify the pharmacophoric pattern essential for binding to the virus-induced dThd/dCyd kinase.

In order to confer resistance to deamination by deaminating enzymes, N^4-Me-MMdCyd (**24**) and its corresponding monophosphate nucleotide N^4-Me-MMdCydMP (**120**) were synthesized.[1] Unfortunately, N^4-Me-MMdCyd (**24**) and N^4-Me-MMdCydMP (**120**) (fig. 6.1) were found to be devoid of anti-HSV activity. The molecular conformations of MMdCyd (**18**) and N^4-Me-MMdCyd

(**24**) as determined by X-ray crystallography, revealed that the conformation of the deoxyribose ring in MMdCyd (**18**) (active) and N^4-Me-MMdCyd (**24**) (inactive) are different (**g**$^+$ in MMdCyd (**18**), **t** in N^4-Me-MMdCyd (**24**)).[21,35] Interestingly, however when these studies were in progress, Chu *et al.* reported that 2',3'-dideoxy-N^6-methyl-adenosine (N^6-Me-ddA (**121**)) (fig. 6.1) was resistant to deamination and active against HIV.[128]



N^4-methyl-MMdCyd-MP (**120**) N^6-Me-ddA (**121**)

Fig. 6.1: Chemical structure of N^4-Me-MMdCyd-MP and N^6-Me-ddA

This was a significant finding because methylation of the amino group of the purine moiety did not result in loss of antiviral activity. On the basis of these reports, it was reasoned that a detailed systematic study of N^4-substituted analogs of 5-substituted deoxycytidines should be undertaken to correlate biological activity with molecular structure.

N^4,N^4-Dimethyl-MMdCyd (**43**), N^4-phenyl-MMdCyd (**44**), N^4-benzyl-MMdCyd (**45**), N^4-methoxy-MMdCyd (**54**), N^4-hydroxy-MMdCyd (**55**), 3,4-etheno-MMdCyd (**94**), N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**), N^4-butanoyl-MMdCyd (**32**), N^4-pivaloyl-MMdCyd (**33**) and N^4-benzoyl-MMdCyd (**34**) were synthesized and evaluated for biological activity. The results of preliminary screening for anti-HSV activity, revealed that N^4-acetyl-

MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**) and N^4-butanoyl-MMdCyd (**32**) inhibited replication of HSV-1; whereas N^4-pivaloyl-MMdCyd (**33**), N^4-benzoyl-MMdCyd (**34**) and all other N^4-substituted analogs of MMdCyd (**18**) were devoid of antiherpes activity and cytotoxicity up to 512 μM (highest concentration tested). The studies were extended and synthesis of N^4-methyl-BrVdCyd (**101**), N^4-acetyl-BrVdCyd (**95**), N^4-propanoyl-BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**) was accomplished. Analogs of BrVdCyd (**17**) were screened for activity against HSV-1. The acyl analogs of BrVdCyd (**17**), namely, N^4-acetyl-BrVdCyd (**101**), N^4-propanoyl-BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**) inhibited replication of the virus. However, N^4-methyl-BrVdCyd (**101**) was found to be inactive. On the basis of stability studies (section 5.4), it appears that the antiherpes activity of N^4-acyl derivatives of MMdCyd (**18**) and BrVdCyd (**17**) is intrinsic i.e. due to the intact molecule. To correlate biological activity with structure, studies on conformation were undertaken.

The conformational studies indicate that the populations of the deoxyribose conformers were on average in the ratio of 60/40 *south* to *north* for N^4,N^4-dimethyl-MMdCyd (**43**), N^4-phenyl-MMdCyd (**44**), N^4-benzyl-MMdCyd (**45**), N^4-methoxy-MMdCyd (**54**), N^4-hydroxy-MMdCyd (**55**), 3,4-etheno-MMdCyd (**94**) and N^4-methyl BrVdCyd (**101**). The populations of the deoxyribose conformers in N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**), N^4-butanoyl-MMdCyd (**32**), N^4-pivaloyl-MMdCyd (**33**), N^4-benzoyl-MMdCyd (**34**), N^4-acetyl-BrVdCyd (**95**), N^4-propanoyl-BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**) analogs were in the ratio of 55/45 *south* to *north*. (table 4.6.5.2.4).

For all N^4-substituted analogs, the population of the **g⁺** rotamer for the C_5· exocyclic side chain was higher (45-57 %) compared to the other two possible

rotamers **g⁻** (11-23 %) and **t** (21-34 %) in all of the analogs prepared. (table 4.6.5.2.4)

The glycosidic torsional preference was predominantly *anti* for all N^4-substituted analogs of BrVdCyd (**17**) and MMdCyd (**18**). (table 4.6.5.2.5)

The position of the N^4-substituent was *proximal* to pyrimidine N_3 in N^4-phenyl-MMdCyd (**44**), N^4-benzyl-MMdCyd (**45**), N^4-methoxy-MMdCyd (**54**), N^4-hydroxy-MMdCyd (**55**), 3,4-etheno-MMdCyd (**94**) and N^4-methyl-BrVdCyd (**101**). For N^4,N^4-dimethyl-MMdCyd (**43**), one methyl substituent was proximal to N_3 and the second methyl substituent was proximal to C_5. The N^4-substituent was *proximal* to pyrimidine C_5 in N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**), N^4-butanoyl-MMdCyd (**32**), N^4-pivaloyl-MMdCyd (**33**), N^4-benzoyl-MMdCyd (**34**), N^4-acetyl-BrVdCyd (**95**), N^4-propanoyl-BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**) analogs.

The population of the deoxyribose conformers for 2-imino-MMdUrd (**66**) was in the ratio of 60/40 *south/north*. (table 4.6.5.2.4)

For the two analogs 2-imino-MMdUrd (**66**) and 2-imino-MMddCyd (**90**), the population of the **g⁺** rotamer for the $C_{5'}$ exocyclic side chain was higher (72 and 68 respectively) compared to all other compounds studied, most likely due to stronger hydrogen bonding. The glycosidic torsional preference for 2-imino-MMdUrd (**66**) and 2-imino-MMddCyd (**90**) was predominantly *syn*.

From conformational studies, the following conclusions can be drawn: conformation appears to play a vital role in determining antiviral (antiherpes) activity. The compounds that were active, namely N^4-acetyl-MMdCyd (**30**), N^4-propanoyl-MMdCyd (**31**), N^4-butanoyl-MMdCyd (**32**), N^4-acetyl-BrVdCyd (**95**), N^4-propanoyl-BrVdCyd (**96**) and N^4-butanoyl-BrVdCyd (**97**) had *optimal* features for two of the four conformational parameters that were investigated, namely, glycosidic torsional preference and orientation of N^4-substituent. The

glycosidic torsional preference was mainly *anti* and the N^4-substituent was *proximal* to pyrimidine C_5. No direct correlation could be drawn for the remaining two conformational parameters, namely $C_{5'}$ exocyclic orientation and furanose conformation since in all compounds studied, **g+** was the predominant exocyclic rotamer and the furanose conformation was in the range of 40/60 or 45/55 north to south distribution.

The analogs that have activity against HSV and their conformational features are summarized in tables 6.1.1 and 6.1.2.

TABLE 6.1.1

Conformation populations (%): S- and N- parameters of the furanose ring and the three rotamers of the exocyclic $C_{5'}$ side chain of N^4-acyl analogs active against HSV-1.

Conformer [a]:	S	N	g+ [b]	g- [b]	t [b]
N^4-acetyl-MMdCyd (**30**):	55 (148°)[c]	45 (-3.0°)	55	11	34
N^4-propanoyl-MMdCyd (**31**):	53 (157°)	47 (-7.0°)	57	11	32
N^4-butanoyl-MMdCyd (**32**):	54 (158°)	46 (-8.0°)	51	15	34
N^4-acetyl-BrVdCyd (**95**):	56 (178°) [c]	44 (6.0°)	55	13	32
N^4-propanoyl-BrVdCyd (**96**):	55 (175°)	45 (9.0°)	55	15	30
N^4-butanoyl-BrVdCyd (**97**):	55 (171°)	45 (5.0°)	52	17	31

[a] In the PSEUROT calculations, τ_m was constrained to 36.0°.

[b] $C_{5'}$ Exocyclic orientation at 37°C.

[c] Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N

TABLE 6.1.2.

Syn/anti glycosidic preference of N^4-acyl analogs active against HSV-1.

Conformer:	$\eta_6\{1'\}$	$\eta_6\{2'\}$	$\eta_6\{3'\}$	glycosidic preference [a]
N^4-acetyl-MMdCyd (**30**):	4	9	5	anti
N^4-propanoyl-MMdCyd (**31**):	5	11	6	anti
N^4-butanoyl-MMdCyd (**32**):	3	7	4	anti
N^4-acetyl-BrVdCyd (**95**):	8	15	11	anti
N^4-propanoyl-BrVdCyd (**96**):	9	14	13	anti
N^4-butanoyl-BrVdCyd (**97**):	5	10	8	anti

[a] Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either syn or anti. For syn orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For anti orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

The conformational parameters for analogs that showed no activity against HSV-1 were similar to compounds endowed with antiviral activity with the exception of one strikingly different feature. The N^4-substituent was *proximal* to pyrimidine N_3 in N^4,N^4-dimethyl-MMdCyd (**43**), N^4-phenyl-MMdCyd (**44**), N^4-benzyl-MMdCyd (**45**), N^4-methoxy-MMdCyd (**54**), N^4-hydroxy-MMdCyd (**55**), N^4-acetyl-N^4-methyl-MMdCyd (**57**) 3,4-etheno-MMdCyd (**94**) and N^4-methyl-BrVdCyd (**101**). The comparable features are: 60/40 *south* to *north* furanose conformation, predominant **g+** rotamer for the exocyclic side chain and a mainly *anti* glycosidic torsional preference. Thus, it appears that when the N^4-substituent (or one of the N^4-substituents as in **43** and **57**) is *proximal* to pyrimidine N_3, the ability of the analog to bind to HSV-induced pyrimidine kinase is hindered due to steric factors and the initial step of phosphorylation by the viral-induced kinase is prevented, therefore, such a compound does not

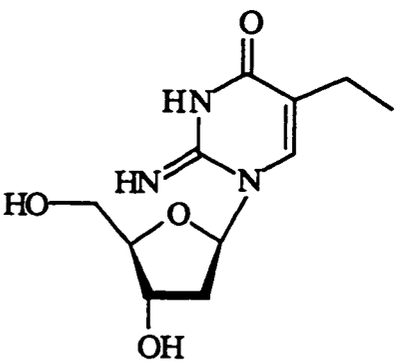
inhibit HSV replication. Alternatively, if this compound is "activated" by initial phosphorylation to the monophosphate, it is possible that it is not converted to the triphosphate stage and therefore does not act as an alternate substrate or inhibitor of DNA polymerase. However, when the N^4-substituent is *proximal* to pyrimidine C_5, the ability of the analog to bind to the viral-induced kinase does not appear to be hindered and hence phosphorylation to the monophosphate is possible and therefore the analog does inhibit HSV replication. Studies using cellular dThd kinase and viral dThd/dCyd kinase are planned to determine the substrate specificity of N^4-acyl analogs of MMdCyd (**18**) and BrVdCyd (**17**). Dr. Kamaly is also investigating the effects of deaminase inhibitors on the antiviral potency of these N^4-acyl analogs. When completed, these studies should provide a deeper understanding of the relationship between conformation and antiviral activity.

In the case of N^4-pivaloyl-MMdCyd (**33**) and N^4-benzoyl-MMdCyd (**34**), all the features of the related N^4-acyl analogs were displayed, however these two analogs are biologically inactive. The size of the two substituents N^4-pivaloyl and N^4-benzoyl might be the reason for the lack of activity. It is possible that these bulky groups prohibit approach of the analogs to the binding pocket of the viral kinase.

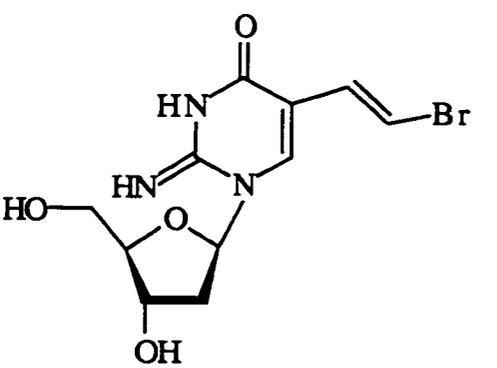
Thus it appears from the above four conformational parameters (furanose conformation, $C_{5'}$ exocyclic orientation, glycosidic torsional preference and orientation of N^4-substituent) that two parameters have a direct correlation with antiviral activity. These two are glycosidic torsional preference and orientation of N^4-substituent. If one of these two conformational features is not "optimal", then the analog has no anti-HSV activity. A good example are the analogs N^4-methyl-MMdCyd (**24**) (inactive) which resembles N^4-acetyl-MMdCyd (**30**) (active) in all its conformational features with one exception. In **24**, the N^4-

substituent is proximal to proximal to N_3 while, in **30** it is proximal to C_5.

In the case of 2-imino-MMdUrd (**66**), the probable reason for its lack of activity appears to be the mainly *syn* glycosidic torsional preference.The parent active compound MMdUrd (**6**) has an *anti* glycosidic conformation.[123] The furanose conformation follows a 60/40 *south* to *north* distribution and the g^+ rotamer population is quite high. Recently, 2-imino-EtdUrd (**122**) and 2-imino-BrVdUrd (**123**) were synthesized (fig. 6.2). Both of these analogs were devoid of activity against herpes virus.[33] Although information on conformation of 2-imino-EtdUrd (**122**) and 2-imino-BrVdUrd (**123**) is not available, it is reasonable to assume that the conformation of the glycosidic bond for the compounds is most likely *syn* (similar to 2-imino-MMdUrd (**66**)) and this might be the reason for lack of activity.



2-imino-EtdUrd (**122**)



2-imino-BrVdUrd (**123**)

Fig. 6.2: Chemical structures of 2-imino-EtdUrd and 2-imino-BrVdUrd

In the case of 2-imino-MMddCyd (**90**), the lack of activity against HSV-1 is not surprising. The structure of the furanose in this molecule (2',3'-didehydro-2',3'-dideoxy ring) is similar to other 2',3'-dideoxynucleosides. These compounds are active against HIV and are anabolized by cellular thymidine kinase. Dideoxydidehydro nucleosides are devoid of antiherpes activity and

the most likely reason is that they do not serve as substrates for HSV-induced kinase. Furthermore, previous studies have shown that the presence of a 3' hydroxy group below the surface of the deoxyribose is necessary for antiherpes activity in pyrimidine 2'-deoxynucleoside analogs.[2] Moreover the conformation of the glycosidic bond is mainly *syn* and this also could be the reason for the lack of antiherpes activity.

The two analogs N^4-methoxy-5-Me-dCyd (**105**) and N^4-hydroxy-5-Me-dCyd (**106**) were prepared to complete studies on this series. Both analogs are inactive like the parent compound 5-Me-dCyd (**29**).

The studies on EtdUrd (**9**) and EtdCyd (**118**) also suggest that conformation determines activity. EtdUrd (**9**) is active while EtdCyd (**118**) is inactive. It was noticed that while the glycosidic conformation is *anti* in EtdCyd (**118**), the *anti* contribution is only slightly higher than the *syn* contribution (table 4.6.5.1.3). The *anti* contribution (to the dynamic *syn/anti* equilibrium) is lower in EtdCyd (**118**) than it is in EtdUrd (**9**) and in all the other analogs presented in this study. This conformational feature might be the reason behind the lack of activity of EtdCyd (**118**).

The features of the remaining analogs, HMdUrd (**113**), AZT (**28**) and AzHMddUrd (**119**) are as follows. HMdUrd (**113**) (active) has conformational features similar to MMdUrd (**6**) and MMdCyd (**18**) i.e. an approximately 60/40 *south* to *north* furanose distribution, a predominant **g$^+$** rotamer and a mainly *anti* glycosidic preference. However, AZT (**28**) and AzHMddUrd (**119**) which have a 50/50 *south* to *north* furanose distribution, a predominant **g$^+$** and a mainly *anti* glycosidic preference are both inactive against HSV-1.

In summary, analogs of MMdCyd (**18**), MMdUrd (**6**) and BrVdCyd (**17**) were synthesized and a correlation between molecular conformation and antiviral activity was determined.

The elucidation of the three dimensional structures of the enzyme-substrate complex[130] and the enzyme-inhibitor (ganciclovir (**5**)) complex[131] of HSV-1 thymidine kinase should prove to be an important breakthrough to understand the molecular basis of antiherpes drugs. These findings provide information about the active center or the binding pocket in the enzyme. The authors of the latter publication[131] conclude that the binding pocket of HSV-1 TK is large enough to accommodate a variety of nucleoside analogs and it displays the flexibility to optimize potential interactions in order to achieve phosphorylation of the nucleoside analog.

The exploitation of differences between the HSV-1 TK and its corresponding host cellular enzyme (upon determination of its structure) should aid in the design of more effective and less toxic antiherpes drugs by molecular modeling techniques. The knowledge gained on conformational parameters of nucleoside analogs, the resonance and field effects (electronic effects) as well as the steric size of substituents (molar refractivity) should prove useful in molecular modeling studies.

Selective antiherpes drugs are also being considered for the treatment of tumors which fail to respond favorably to anticancer drugs.[132] Thus from a therapeutic point unrelated to HSV infections, it is interesting to note that nucleoside analogs selectively phosphorylated by HSV-1 TK can be used for the treatment of cancer by using the novel therapeutic approach of "Gene Therapy". Gene therapy is defined as the alteration of a genetic material of a cell with resultant benefit to a patient.[132] One of the first trials was by the use of modified tumor cells as a drug delivery system. In this system, cells are genetically modified in order to alter their phenotype so that they would become sensitive to an agent that would otherwise be nontoxic. The cancer cells are transfected with the gene that codes for HSV-1 TK so these cells will

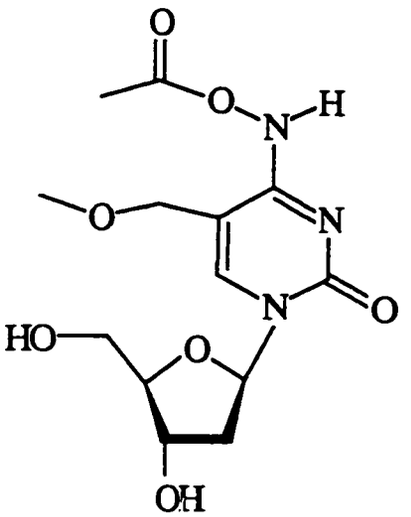
express the HSV-1 TK gene.[133]

Preclinical studies have shown that tumor cells which have been modified with a retroviral vector containing the HSV-1 TK gene are sensitive to ganciclovir (**5**). The rationale is that tumor cells containing the HSV-1 TK gene will be able to anabolize the antiherpes compound to its corresponding triphosphate (active moiety) and thus be killed. Even if only a fraction of the tumor contained the HSV-1 TK gene, the entire tumor would be eliminated.[133]

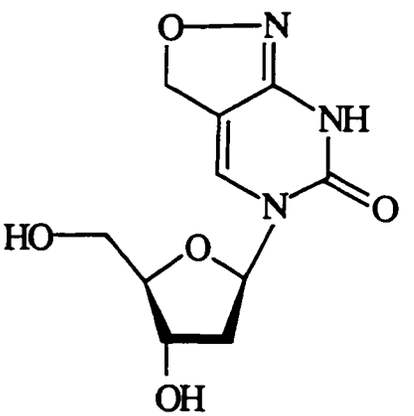
This is a novel approach for the treatment of neoplastic disorders for which alternative treatments do not exist. The amide derivatives of MMdCyd (**18**) may prove effective using the technique of "Gene Therapy" for the treatment of cancer because these compounds are activated only in HSV infected cells.

6.2) Suggestions for further work

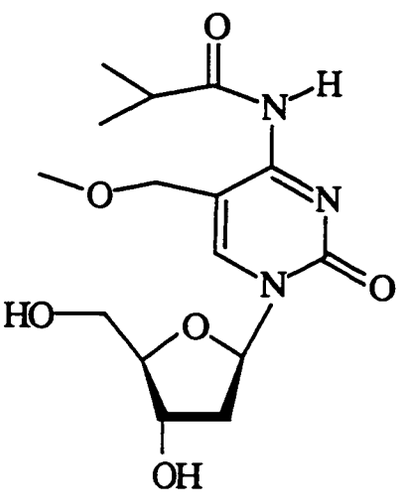
1) The compounds whose structures are shown in fig. 6.3 are of interest to prepare and test for biological activity.



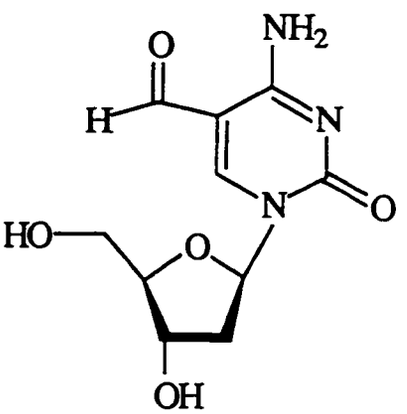
N^4-Acetoxy-MMdCyd (**124**)



1-β-D-2'-deoxyribofuranosyl 3H,5H,7H-pyrimido [4,5-c] [1,2] oxazol-6-one (**125**)



N^4-Isobutyl-MMdCyd (**126**)



5-Formyl-2'-dCyd (**127**)

Fig. 6.3: Chemical structures of compounds of interest to prepare

2) Of interest also is to determine the partition coefficient of N^4-acyl analogs of MMdCyd (**18**) in isooctane/water to determine what role does lipophilicity play in the observed potentiation of activity of these analogs over the parent compound MMdCyd (**18**) .

3) Substrate specificity studies using cellular dThd kinase and viral kinase

(dThd/dCyd kinase) should be undertaken to gain more insight into the remarkable activity of N^4-acyl analogs of MMdCyd (**18**) and BrVdCyd (**17**).

4) Studies with radiolabelled compounds, for example, tritium labelled (H_6) N^4-acyl analogs of MMdCyd (**18**) and BrVdCyd (**17**) would serve to ascertain whether the analogs are intrinsically active or serve as pro-drugs for the release of MMdCyd (**18**) and BrVdCyd (**17**).

5) Stability of of N^4-acyl analogs of MMdCyd (**18**) and BrVdCyd (**17**) which are active against HSV-1 in serum, plasma or blood is important to determine.

6) An urgent need exists now for the development of antiviral agents that are active against one of the more notorious members of the herpes virus family, namely the cytomegalovirus (CMV). This virus does not exhibit a TK and thus nucleoside analogs that require initial phosphorylation by the viral induced TK are not effective against CMV.[134] The analog ganciclovir (**5**) has been used, in the treatment of CMV infections intravenously.[135] Phosphorylation of ganciclovir (**5**) in CMV infected cells is achieved through a viral specific phosphotransferase,[134] while in HSV-1 and HSV-2, **5** is phosphorylated by the viral induced TK.

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