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CHIROPTICAL STUDIES OF NUCLEOSIDES AND THEIR
PERIODATE OXIDIZED-BOROHYDRIDE REDUCED PRODUCTS

University — Université

University of Alberta

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

M. Sc

Year this degree conferred — Année d'obtention de ce grade

1982

Name of Supervisor — Nom du directeur de thèse

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CHIROPTICAL STUDIES OF NUCLEOSIDES AND
THEIR PERIODATE OXIDIZED-BOROHYDRIDE REDUCED PRODUCTS

by

(C)

SHIN, SUGIYAMA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

IN

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

SPRING, 1982

THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled CHIROPTICAL STUDIES OF NUCLEOSIDES AND THEIR PERIODATE OXIDIZED-BOROHYDRIDE REDUCED PRODUCTS submitted by Shin Sugiyama in partial fulfilment of the requirements for the degree of Master of Science in Chemistry.

M. J. Roberts

Peter Spohn

David R. Hoyle

Date April 16, 1982

ABSTRACT

Fifty-four pentofuranosyl nucleosides and methyl β -D-ribofuranoside were oxidized with sodium periodate and subsequently reduced with sodium borohydride. After destroying the excess borohydride with aqueous phosphate buffer (pH 6.5), the resulting quantitated solutions were subjected to measurement of their long-wavelength optical rotations.

A previously noted empirical trend related nucleosides having β -D or α -L configurations with positive rotations for their oxidized-reduced products and nucleosides having α -D or β -L configurations with negative rotations for their enantiomeric oxidized-reduced derivatives. Most nucleosides in the present study gave results in harmony with this empirical prediction including adenosine_{ox-red} derivatives with an additional asymmetric center. However, 6-azapyrimidine (1,2,4-triazine) and 6-azapyridine (pyridazine) nucleosides were exceptions with reversed rotations for their oxidized-reduced derivatives. This behavior is suggested to result from the intrinsic optical vector orientation in the chromophore rather than rotameric preferences.

¹⁴C-nucleosides (pseudouridine and formycin) were methylated at the acidic ring nitrogen to circumvent degradation under the oxidation-reduction conditions. Paper electro-

phoresis using a borate buffer (pH 9.3) was found to be a useful analytical method in the present study. The precursor nucleosides and their oxidized-reduced products gave distinct well separated spots. The anodic migrations of β -D-pentofuranosyladenine nucleosides and adenosine_{ox-red} are in the order: lyxo- \geq ribo- $>$ xylo- $>$ ox-red $>$ arabinofuranosyladenine.

Adenosine_{ox-red} (43), tubercidin_{ox-red} (209), inosine_{ox-red} (135) and uridine_{ox-red} (136) were isolated and subjected to solvent and pH effect studies. In aqueous solutions at different pH values, DMF and MeOH, rotation magnitudes were affected but drastic changes (e.g. reversal of the sign) were not observed. However, drastic changes in the rotations of adenosine_{ox-red} (43), tubercidin_{ox-red} (209) and inosine_{ox-red} (135) were observed in pyridine, whereas uridine_{ox-red} (136) behaved normally. This was attributed to the "stacking" effect with "aromatic" purine-type bases.

Qualitative rates of the reduction of periodate by nucleosides (determined spectrophotometrically at a periodate concentration of $\sim 10^{-7}$ M) were found to be: β -D-ribo- $>$ α -D-ribo- $>$ β -D-lyxo \gg β -D-arabino- = β -D-xylo-furanosyladenine. After 1 min, several β -D-ribofuranosyl nucleosides reduced periodate 1.2-1.6 times faster than their corresponding α -D anomers. This difference is presumed to result from the increased steric hindrance

of the α structure. This presents a new method for assigning the configuration of a given anomeric pair of ribofuranosyl nucleosides.

ACKNOWLEDGMENTS

I would like to thank Dr. Robins for his advice and encouragement, Debbie and the children for their patience and lonely evenings, and all my family for their support.

TABLE OF CONTENTS

	<u>Page</u>
<u>INTRODUCTION</u>	1
SURVEY OF METHODS FOR THE DETERMINATION OF ANOMERIC CONFIGURATION	
I. Baker's "trans rule"	2
II. Chiroptical methods	3
1. Hudson's "isorotation rule"	3
2. ORD and CD approaches	4
III. NMR methods	4
1. ¹ H-NMR approaches	5
a. Coupling constant approaches	5
b. Chemical shift approaches	8
c. Nuclear Overhauser effect (NOE) approach	12
d. Relaxation time (T ₁) approach	12
2. ¹³ C-NMR approaches	13
IV. Enzymatic methods	14
V. Chemical methods	14
1. Cyclonucleoside approach	14
2. Degradation approaches combined with optical rotations	16
<u>RESULTS AND DISCUSSION</u>	23
A NOTE ON THE DEFINITION OF α AND β CONFIGURATIONS	35
A NOTE ON THE NOMENCLATURE OF OXIDIZED-REDUCED NUCLEOSIDES (NUCLEOSIDES _{OX-RED}) OF TRIOL TYPES <u>28 AND 29</u>	36

OPTICAL ROTATIONS OF NUCLEOSIDES _{OX-RED} (<u>in situ</u>); AND ELECTROPHORESIS OF NUCLEOSIDES _{OX-RED} (<u>in situ</u>) AND THEIR PRECURSOR NUCLEOSIDES	36
OPTICAL ROTATIONS AND ADENOSINE _{OX-RED} DERIVATIVES	60
pH AND SOLVENT EFFECTS ON THE OPTICAL ROTATIONS OF ADENOSINE _{OX-RED} (43), TUBERCIDIN _{OX-RED} (209), INOSINE _{OX-RED} (135) AND URIDINE _{OX-RED} (136)	63
RATES OF PERIODATE REDUCTION BY NUCLEOSIDES	70
<u>EXPERIMENTAL</u>	75
GENERAL PROCEDURES	75
NUCLEOSIDE MATERIALS	77
PREPARATION OF NUCLEOSIDES _{OX-RED} (<u>in situ</u>), THEIR OPTICAL ROTATION MEASUREMENT AND PAPER ELECTROPHORESIS	77
Preparation of nucleosides _{ox-red} (<u>in situ</u>) ...	77
Optical rotation measurements	78
Paper electrophoresis	79
pH AND SOLVENT EFFECTS ON OPTICAL ROTATIONS OF ADENOSINE _{OX-RED} (43), TUBERCIDIN _{OX-RED} (209), INOSINE _{OX-RED} (135) AND URIDINE _{OX-RED} (136)	80
RATES OF PERIODATE REDUCTION BY NUCLEOSIDES	81
<u>SYNTHESIS</u>	84
<u>REFERENCES</u>	107

LIST OF TABLES

<u>Table</u>	<u>Description</u>	<u>Page</u>
I	Optical rotations of previously reported <u>in situ</u> prepared and isolated nucleosides _{ox-red} .	24
II	Optical rotations of previously reported isolated triols (<u>28</u> , <u>29</u>).	32
III	Optical rotations of nucleosides _{ox-red} (<u>in situ</u>), and electrophoresis of nucleosides _{ox-red} (<u>in situ</u>) and their precursor nucleosides determined in the present study.	38
IV	Optical rotations of a series of modified adenosine _{ox-red} derivatives from our laboratory.	51
V	pH and solvent effects on the optical rotations of adenosine _{ox-red} (<u>43</u>).	65
VI	pH and solvent effects on the optical rotations of tubercidin _{ox-red} (<u>209</u>).	66
VII	pH and solvent effects on the optical rotations of inosine _{ox-red} (<u>135</u>).	67
VIII	pH and solvent effects on the optical rotations of uridine _{ox-red} (<u>136</u>).	68
IX	Rates of periodate reduction by nucleosides.	71

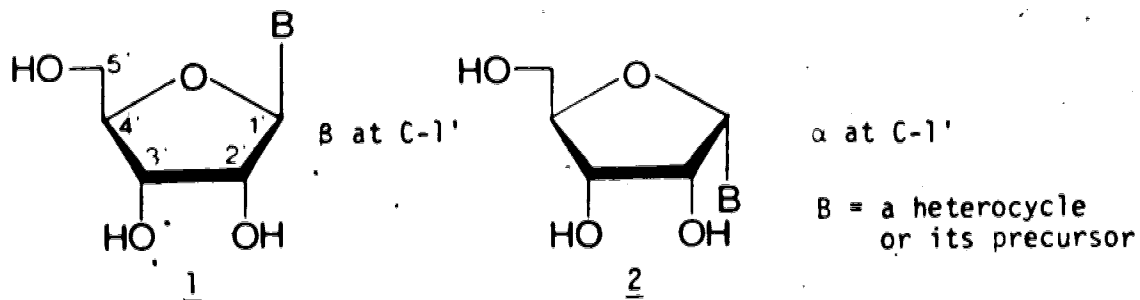
LIST OF SCHEMES

<u>Scheme</u>	<u>Description</u>	<u>Page</u>
I	Formation of purine (14) and pyrimidine (15) cyclonucleosides.	15
II	Proof of anomeric configuration of naturally occurring nucleosides by use of periodate oxidation.	17
III	Equal optical rotations of diastereomeric dialdehydes (22, 23) obtained <u>in situ</u> by periodate oxidation.	18
IV	Formation of hydrated and polymèric forms of nucleoside dialdehydes (18).	19
V	Formation of oxidized-reduced nucleosides of triol types 28 and 29.	20
VI	Formation of nucleosides _{ox-red} (28, 29) from various nucleosides.	21
VII	Order of electrophoretic migrations based upon the migrations of β -D-pentofuranosyl adenine derivatives and adenosine _{ox-red} .	59
VIII	Order of the rates of periodate reduction by nucleosides.	74

INTRODUCTION

The term "nucleoside" was originally introduced by Levene and Jacobs¹ for the carbohydrate derivatives of purines (and later, of pyrimidines) isolated from the alkaline hydrolyzates of yeast nucleic acid. The phosphate esters of nucleosides are called nucleotides. "Nucleosides" now refer to carbohydrate derivatives linked to heterocycle bases, normally at C-1' of the sugar residue (anomeric center), by C-N or C-C bonds. The term includes both naturally occurring and synthetic compounds.

Since there are two possible orientations for attachment of the base at C-1' (namely, α or β as indicated for 1 or 2 in the D-ribose derivatives), the anomeric configuration determination of nucleosides has been a problem of considerable importance. This is especially true in synthetic nucleoside chemistry where coupling of an appropriate sugar and heterocycle (or its precursor) is frequently employed. Although the anomeric configura-



tion of nucleosides found in DNA and RNA are known to

be β , naturally occurring nucleosides having the α -configuration are also known in vitamin B₁₂ coenzymes.² Thus, unknown naturally occurring as well as synthetic nucleosides always have to be subjected to determination of anomeric configuration.

A number of methods for determining the anomeric configuration of nucleosides have been devised. However, most of these, although useful in specific cases, have limitations and/or exceptions. Thus, the combined use of more than one technique is often required. X-ray diffraction provided the first confirmed structural assignments of certain nucleosides obtained from yeast nucleic acid.³ This method gives a rigorous and unambiguous assignment of the anomeric configuration, but requires specialized equipment and skill and is time consuming.

Recently, approaches used for the determination of anomeric configuration of ribofuranosyl nucleosides have been critically reviewed.⁴ In the following discussion, various commonly employed methods and criteria will be surveyed.

I. Baker's "trans rule"⁵

Baker's "trans rule", which states that "condensation of a heavy metal salt of a purine or pyrimidine with an acylated glycosyl halide will form a nucleoside with a

C-1' to C-2' trans configuration in the sugar moiety regardless of the original configuration at C-1'', has provided the initial basis for assignment of the anomeric nature of the products obtained from several coupling methods. The reason for the trans predominance has been attributed to the participation of the acyloxy group at C-2' resulting in formation of an orthoester ion involving C-1'. However, Montgomery and Hewson⁶ have noted an exception to this rule. Fox and co-workers^{7,8} observed that the "trans rule" could not be applied automatically to anomeric assignment of the products obtained from Hilbert-Johnson coupling reactions.

II. Chiroptical methods

1. Hudson's "isorotation rule"⁹

Hudson's "isorotation rule", which was the first attempt to utilize optical rotation data for determining the anomeric configuration of carbohydrates, was used along with Baker's "trans rule" for early assignments of the anomeric configuration of nucleosides. This empirical rule was originally based upon presumed relations between the anomeric configuration and optical rotations of carbohydrates. Although it remains applicable for most methyl glycosides,¹⁰ it was found to fail for certain anomeric pyrimidine nucleosides.^{11,12} Later the noted

exceptions to this rule were found to be general for most pyrimidine nucleosides.¹³

2. ORD and CD approaches

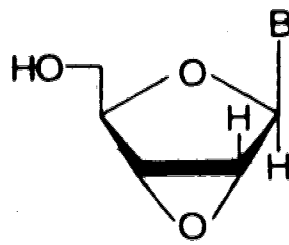
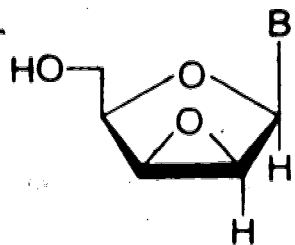
Ulbricht and co-workers¹⁴ measured ORD and CD spectra of series of nucleosides and nucleotides and noted trends correlating the anomeric configuration and sign of the long-wavelength ("B_{2u}") Cotton effects. Purine β-D-nucleosides generally exhibit a negative Cotton effect and purine α-D-nucleosides a positive Cotton effect. Pyrimidine nucleosides generally exhibit reversed Cotton effects (β-D, positive, and α-D, negative). This so-called ORD/CD rule was in general harmony with experiments of Frič et al.¹⁵ and Nishimura et al.¹⁶ However, the sign of the Cotton effect as well as its magnitude is dependent^{17,18} on intrinsic dipole vector orientations within the heterocyclic base, glycosyl rotameric populations, solvents, temperature, etc. Numerous exceptions to this rule have been found.^{14a,c,f,18-20} Thus, it is noted^{15,18b} that ORD and CD stereochemical assignments should be applied to nucleosides only in those cases where the unknown and reference nucleosides have similar transition moment directions, electronic structures in the sugar rings, and glycosyl rotameric populations.

III. NMR methods

1. $^1\text{H-NMR}$ approaches

a. Coupling constant approaches

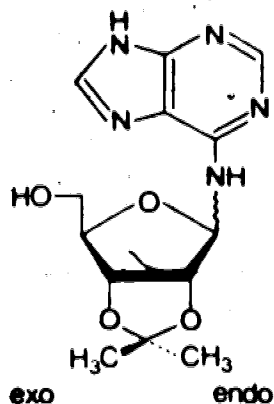
It is now generally recognized^{21,22} that the coupling constant between H-1' and H-2' ($J_{1',2'}$) should be ≤ 1.0 Hz for unequivocal assignment of a 1',2'-trans hydrogen relationship in the case of pentofuranosyl derivatives such as β -D-ribofuranosyl nucleosides (1). This criterion is based upon the Karplus equation,²³ by which the vicinal coupling constant is calculated from the dihedral angle.²⁴ However, $J_{1',2'} > 1.0$ Hz is common for 1',2'-trans hydrogen relationships because of the conformational flexibility of the furanose ring. No assignment is possible for nucleosides having the 1',2'-cis hydrogen relationship of α -D-ribofuranosyl nucleosides (2) owing to the double-angle cosine dependence of the Karplus equation. The only exceptions to this trans $J_{1',2'} < 1.0$ Hz criterion have been observed⁴ in the fused-ring 2',3'-anhydrolyxonucleosides (H-1' and H-2' cis) (3). The anomeric proton signal of (3) appears as a sharp singlet ($J_{1',2'} < 0.7$ Hz) as does the corresponding peak for H-1' of the 2',3'-anhydroribonucleosides (H-1' and H-2' trans) (4).



B = adenin-9-yl,
4-aminopyrrolo-
[2,3-d]pyrimidin-
7-yl

Leonard and Laursen²⁵ employed the 2',3'-O-isopropylidene group for ribonucleosides to restrict the furanose ring conformation and lower the coupling constant ($J_{1',2'}$). Although the β -anomer usually responds to a reduction in $J_{1',2'}$, values in the range of $J_{1',2'} = 2-3$ Hz are common, and unequivocal assignment is precluded.

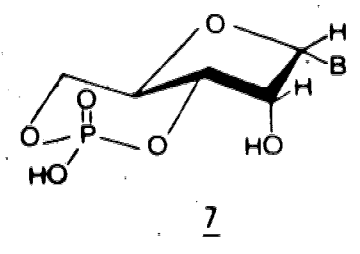
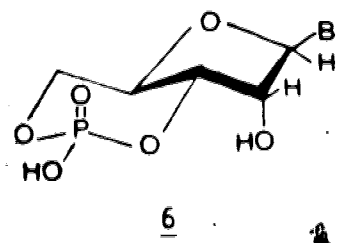
MacCoss et al.²⁶ proposed a "H-4' multiplicity criterion" (H-4' triplet for α -anomers and higher multiplicity for β -anomers). They investigated 12 anomeric pairs of 2',3'-O-isopropylidene ribonucleosides and attributed the H-4' multiplicity (coupling with H-3' as well as H-5', H-5'') in the β -series to the flattened conformation of the 2',3'-O-isopropylidene ribose residue. The H-4' triplet (coupling with H-5' and H-5'' only) in the α -series was related to the conformational change of the sugar residue resulting from interaction between the endo methyl group and the base reducing the dihedral angle between H-3' and H-4' ($\phi_{3',4'}$) to -90° . One observed exception was the anomeric pair of 6-N-(2',3'-



O-isopropylidene-D-ribofuranosyl)adenines (5) which have increased flexibility around the glycosyl bond. Both α and β -anomers gave rise to triplet peaks for H-4'. Thus, a minimum

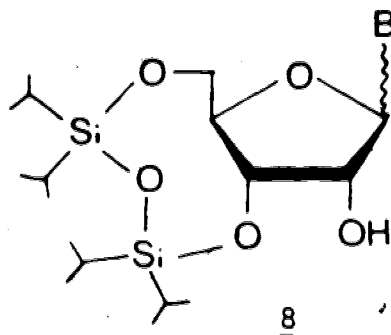
requirement of this rule, as they emphasized, is that the C-1' substituent should be of sufficient bulk and rigidity to ensure that the steric interaction between the 5'-CH₂OH group and the base of the β-anomers will prevent a decrease in $J_{3',4'}$. The foregoing coupling approaches are crucially dependent on the sugar conformation. Conformational rigidity is a predominant factor in their applications.

Jardetzky²⁷ noted that $J_{1',-2'} < 1$ Hz for adenosine 3',5'-cyclic-monophosphate (cAMP) (6, B = adenin-9-yl). However the applicability of this observation to the anomeric assignment of ribonucleosides remained unrecognized until Robins and MacCoss^{4,28} proposed a "geometry-only method" for this determination based upon the coupling of the anomeric proton ($J_{1',-2'}$) of the conformationally rigid anomeric 3',5'-cyclonucleotides (6,7). The proposed criterion was $J_{1',-2'} < 1$ Hz for β-anomers (6) and $J_{1',-2'} \geq 3$ Hz for α-anomers (7). They observed no exceptions in examining over 200 examples and suggested



its general applicability to variously substituted ribo-3',5'-cNMP's (cyclic nucleoside monophosphates) and

β -ara-3',5'-cNMP's: Similar results have been observed²⁹ in our laboratory recently using the 3',5'-O-(1,1,3,3-tetraisopropylididisilox-1,3-diyl) group³⁰ (8) in place of the 3',5'-O-cyclic-monophosphate.

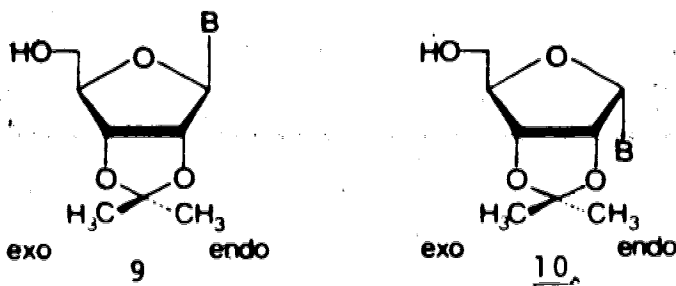


b. Chemical shift approaches

Fox and co-workers³¹ reported a method based on the chemical shift difference of 2'-O-acetyl methyl signals before and after the hydrogenation of a double bond in the heterocyclic base. In pentofuranosyl nucleosides of pyrimidines, they observed a small diamagnetic (upfield) shift with 1',2'-trans and a large paramagnetic (downfield) shift with 1',2'-cis 2'-O-acetyl derivatives upon saturation of the 5,6-double bond. This approach is based upon the anisotropic effect of the heterocycle and appears to be generally applicable to pyrimidine nucleosides. However, it is not readily applicable to purine nucleosides since selective hydrogenation to give 7,8-dihydropurine nucleosides is difficult.

Montgomery^{32a} reported that the 2'-O-acetyl methyl chemical shift per se could be used to determine the anomeric configuration. In a series of acetylated anomeric furanosyl purine pairs, it was observed that 2'-O-acetyl methyl signals of 1',2'-cis nucleosides occur upfield from δ 1.95 ppm while those of 1',2'-trans nucleosides occur downfield from δ 2.05 ppm. Similarly, 2'-O and 2'-S-methyl chemical shifts have been utilized for the anomeric configurational assignment of arabinofuranosyl nucleosides.^{32b} However, the chemical shifts of acetyl methyl groups are solvent dependent³³ and an exception has been noted in the case of 1-(2,3,5-tri-O-acetyl- β -D-ribofuranosyl)benzotriazole.^{34a}

Imbach and co-workers³⁴ proposed a very useful method for the configurational determination of ribofuranosyl nucleosides, which involves the chemical shift difference ($\Delta\delta$) of the two methyl signals (exo and endo) of anomeric 2',3'-O-isopropylidene ribofuranosyl nucleosides (9,10). The proposed $\Delta\delta$ criterion,^{34b} as refined further,^{34a,d} is: $\Delta\delta > 0.15$ ppm for the β -anomer (9) and $\Delta\delta < 0.15$ ppm for the α -anomer (10). This criterion is

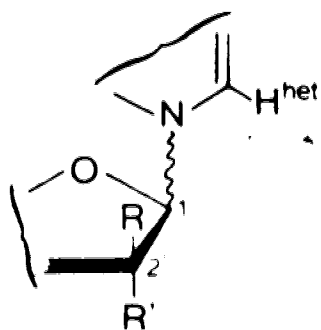


based upon the anisotropic effect of the heterocyclic "aglycon" on the chemical shift of the endo methyl group of the α -anomer. This anisotropy was demonstrated^{4,34a-e} with an α -anomer of 2',3'-O-isopropylidene-5,6-dihydrouridine (10, B = 5,6-dihydrouracil-1-yl), which does not obey the rule. They have noted that the $\Delta\delta$ criterion should be limited to ribofuranosyl compounds having an unsaturated heterocyclic aglycon and no 5'-substituents. These limitations of the criterion are now well documented.³⁵⁻³⁸ However, an exception to this restricted criterion has been reported for 1-(2,3-O-isopropylidene-D-ribofuranosyl)-3-carbamoyl pyridinium salts.³⁹

Nishimura and Shimizu⁴⁰ noted that the anomeric proton of a 1',2'-trans (2'-OH to 1'-heterocycle) nucleoside appears at a higher field than that of the corresponding cis isomer. This chemical shift difference of the anomeric protons of α and β -nucleosides has been considered to result from the diamagnetic shielding⁴¹ of the cis (H-1' to 2'-OH) 2'-OH group on the anomeric proton of 1',2'-trans nucleosides. This method has been used for anomeric configurational assignments and found consistent. However, it has been reported^{37b} that the anomeric proton shifts of isopropylidene derivatives of anomeric mannofuranosylimidazole and mannofuranosyladenines are not in accordance with this rule. Thus,

caution must be exercised with 2'-OH protected nucleosides when this rule is applied. Furthermore, it is not applicable where only one anomer is available since this method requires both anomers for comparison.

Alenin and Domkin⁴² recently proposed a new criterion for establishing the anomeric configuration of furanosyl derivatives of purines, pyrimidines, imidazoles and pyrazoles (11). They observed that when the heterocycle at C-1' is trans to the 2'-hydroxy group the chemical



H^{het} ; H^8 (purines), H^6 (pyrimidines)
 H^2 (imidazoles), H^5 (pyrazoles)

$R = H$, $R' = OH$ in ribo-, xylo- and glucofuranosyl nucleosides

$R = OH$, $R' = H$ in arabinofuranosyl nucleosides

11

shift of the heterocycle proton (H^{het}) appears at lower field than that of H^{het} in a cis orientation. They combined the chemical shift of H^{het} with that of the anomeric proton for their proposed criterion which states that: the chemical shift difference between H^{het} and H-1 ($\Delta\delta = \delta H^{het} - \delta H-1'$) is always greater when the heterocycle and 2'-hydroxy group have a trans orientation. This relation appears to be valid for unprotected nucleosides and their isopropylidene derivatives but not for nucleosides

with acetyl, benzoyl and benzyl protecting groups on the sugar hydroxyls. A similar chemical shift difference of the heterocycle protons has been noted previously with the anomeric C-nucleosides of pseudocytidine.⁴³ This criterion is not confirmed with a large number of examples and it is not applicable to H^{het} substituted nucleosides. Again, both anomers are required.

c. Nuclear Overhauser effect (NOE) approach

Cushley et al.⁴⁴ reported the NOE method for determining the anomeric configuration of purine and pyrimidine nucleosides. They observed signal enhancements of the aglycon protons upon irradiation of H-4' of α -nucleosides, but no enhancement upon irradiation of H-5'. Irradiation of H-5' of β -nucleosides produced enhancements of the aglycon protons while irradiation of H-4' had no effect. However, NOE is dependent on rotameric populations and sugar ring conformations. Overlapping shifts of the sugar protons and coupling between these protons also complicate NOE measurements.

d. Relaxation time (T_1) approach

Hall and Preston⁴⁵ measured the relaxation times (T_1) of the anomeric protons of pyranosyl carbohydrates and observed a difference in the relaxation times (T_1) between α and β -anomers. The applicability of this

method to nucleosides has been demonstrated by Tran-Dinh et al.⁴⁶ They observed that the relaxation times (T_1) of the anomeric protons of the β -anomers of some anomeric C-nucleosides are about two and a half times longer than those of the α -anomers. However, this method requires more sophisticated techniques and calculations and is time consuming. It has not been reported with naturally occurring N-glycosyl nucleoside examples.

2. ^{13}C -NMR approaches

In 1974, Shimizu et al.⁴⁷ reported ^{13}C -NMR spectra of anomeric pyrimidine and purine ribonucleosides. They observed that the C-1' and C-2' chemical shifts of α -anomers appeared at higher fields (2-4 ppm) than those of the β -anomers. Similar chemical shift differences of the anomeric carbon (C-1') have been observed with anomeric 2',3'-O-isopropylidene ribofuranosyl C-nucleosides,³⁸ methyl glycosides⁴⁸ (anomeric ribo, arabino, xylo and lyxofuranosides), 2',3'-O-isopropylidene ribofuranosyl purine nucleosides³⁶ and arabinofuranosylcytosine.^{32b} In all of the above cases, the anomeric carbon (C-1') of the nucleosides and glycosides having a cis relationship between the aglycon and 2'-substituent resonate at a higher field than that of the corresponding 1',2'-trans compounds.

The existence of correlations between chemical shifts

of the isopropylidene methyl groups of 2',3'-O-isopropylidene-D-ribofuranosyl derivatives and the anomeric configuration,^{38,49} and of the central (or quaternary) isopropylidene carbon and the anomeric configuration^{49a} have also been reported. At present, the reliability and generality of these observations in the determination of anomeric configuration has not been fully established and further investigation is needed.

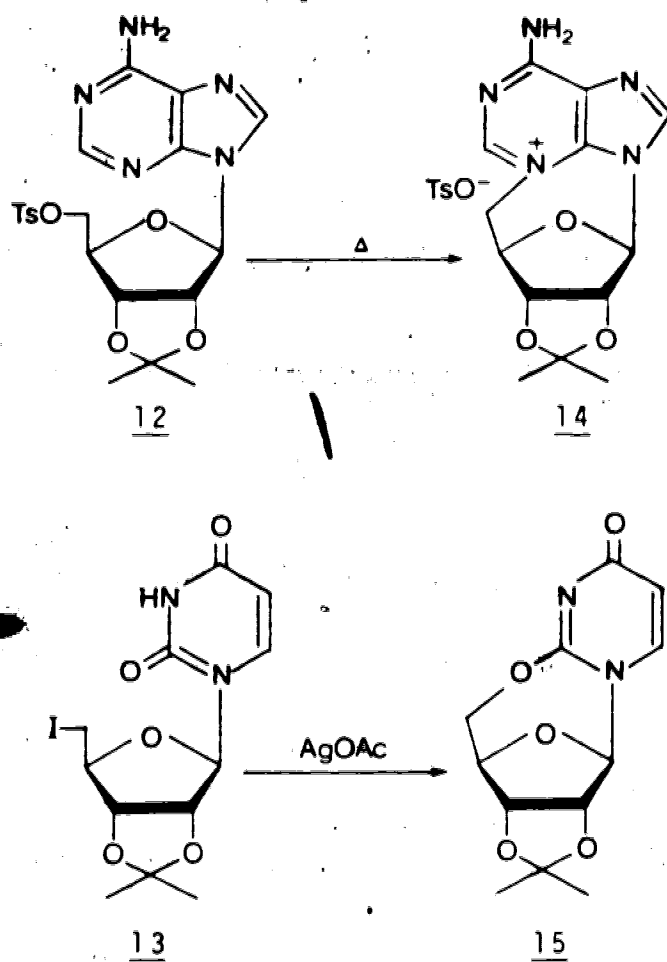
IV. Enzymatic methods

Enzymes such as bacterial nucleoside hydrolase,⁵⁰ pyrimidine nucleoside phosphorylase⁸ and calf intestine deaminase⁵¹ have been used to distinguish certain α and β -nucleosides. However, the general applicability is limited to the substrate specificity of the enzymes.

V. Chemical methods

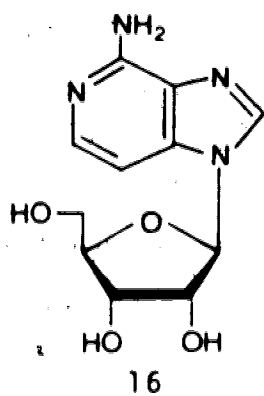
1. Cyclonucleoside approach

Todd and co-workers⁵² discovered a method to assign the glycosyl configuration unequivocally by the formation of cyclonucleosides linking the 5'-position of the sugar residue and the heterocyclic base, such as 14^{52a} and 15.^{52d} Such cyclization is possible only for 1',4'-cis (β -D or β -L) nucleosides (Scheme I). This formation of cyclonucleosides further established^{52b,c} the anomeric



Scheme 1

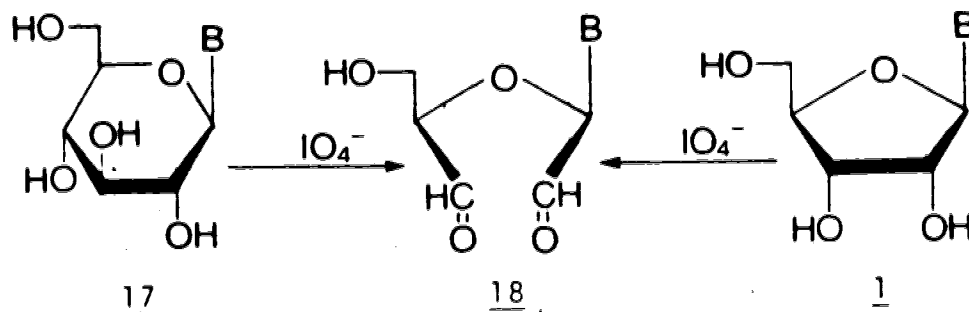
configuration of thymidine, 2'-deoxycytidine and 2'-deoxyadenosine as B. However, synthetic nucleosides with-



out the requisite functionality for the formation of cyclonucleosides, such as 3-deazaadenosine (16),⁵³ are precluded from evaluation by this method.

2. Degradation approaches combined with optical rotations

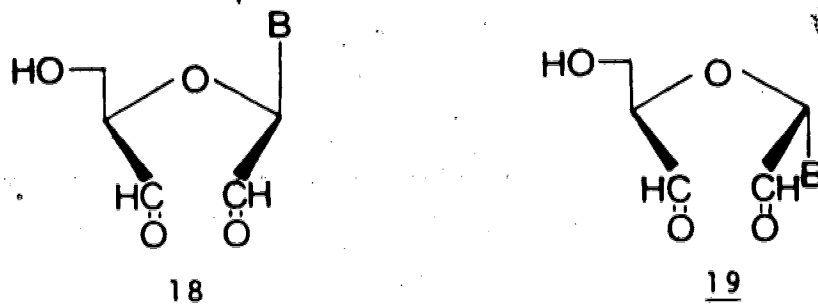
Periodate (or periodic acid) oxidation, which Malaprade⁵⁴ was the first to introduce for selective oxidation of vic-glycols, is a widely used reaction⁵⁵ in the field of carbohydrate chemistry for structural studies.⁵⁶⁻⁵⁹ This oxidation, in combination with enzymatic and chemical reactions, has also been used as a tool for sequence analysis of ribonucleic acid (RNA) and polynucleotides.⁶⁰⁻⁶² Early work on configurational assignments in the carbohydrate field was based on the optical rotations of the oxidation product (the dialdehyde).⁶³ Applications of this oxidation approach in nucleoside chemistry were used to establish the furanose structure of the sugar residue of naturally occurring ribonucleosides and later their anomeric nature.⁶⁴⁻⁶⁷ The first indicative configurational assignment of nucleosides was carried out by Todd and co-workers⁶⁵ using periodate oxidation. They obtained the same dialdehydes (18) for a given pair of synthetic (17) and naturally occurring (1) nucleosides upon oxidation (Scheme II). Since the synthetic glucopyranosyl nucleosides (17) had been considered to have the β -configuration by their method of preparation, they assigned the anomeric configuration of 1 as β . Later the unambiguous confirmation



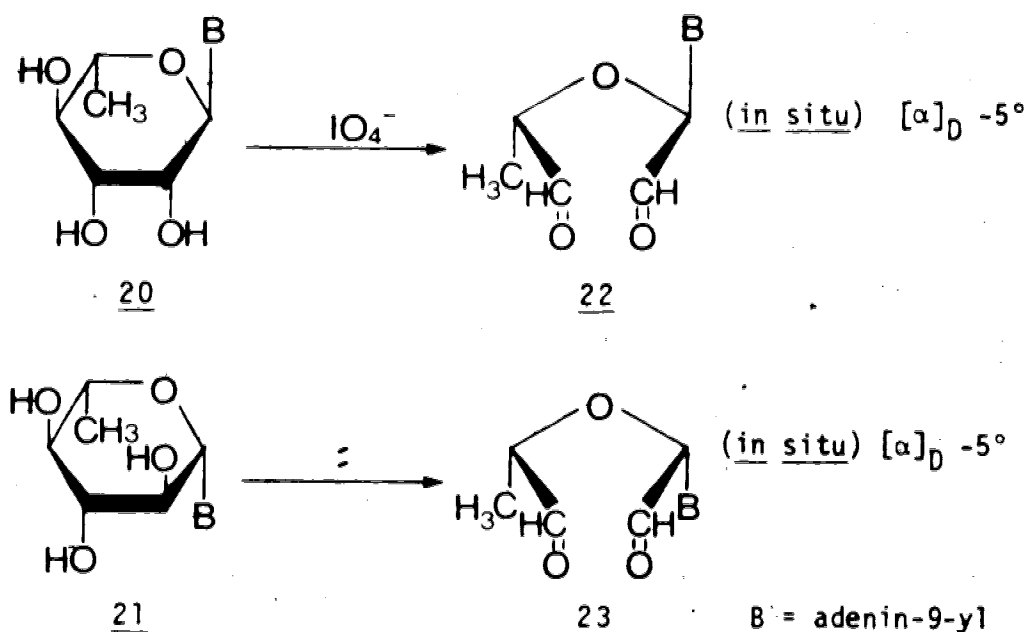
B = adenin-9-yl, uracil-1-yl cytosin-1-yl

Scheme II

of the β -configuration was demonstrated by the formation of cyclonucleosides⁵² and by X-ray diffraction.³ Similar use of optical rotational measurements on the periodate oxidation products (dialdehydes) derived from reference and unknown nucleosides has been made to establish the anomeric nature of other nucleosides^{8,40,67-73} and C-glycosides⁷⁴ and to determine their anomeric purity.⁷⁵ However, there remains some uncertainty in the anomeric configurational assignment based on the optical rotations of these aldehydes. These compounds, such as 18 and 19, contain two asymmetric centers and can exist as diastereomers. Identical rotations have been reported⁷⁶ for

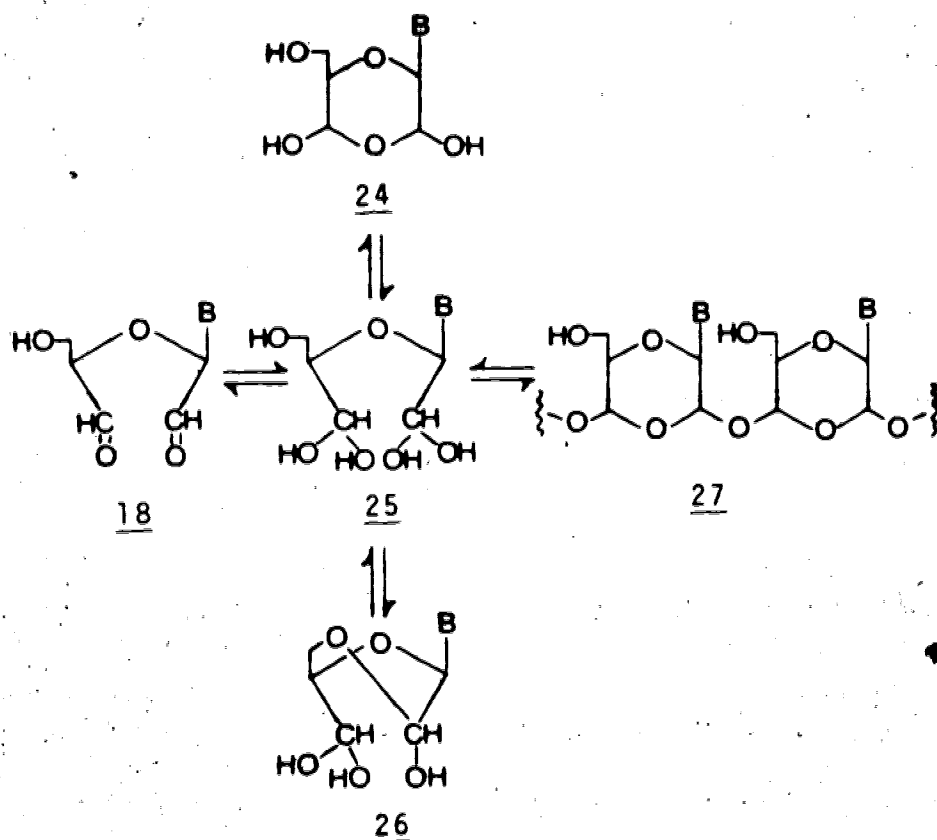


the dialdehydes (22,23) obtained in situ by periodate oxidation of 9-(6-~~deoxy~~-xy- α -L-mannopyranosyl)adenine (20) and 9-(6-deoxy-~~xy~~-glucopyranosyl)adenine (21), even though the dialdehydes 22 and 23 are diastereomers (Scheme III).



Scheme III

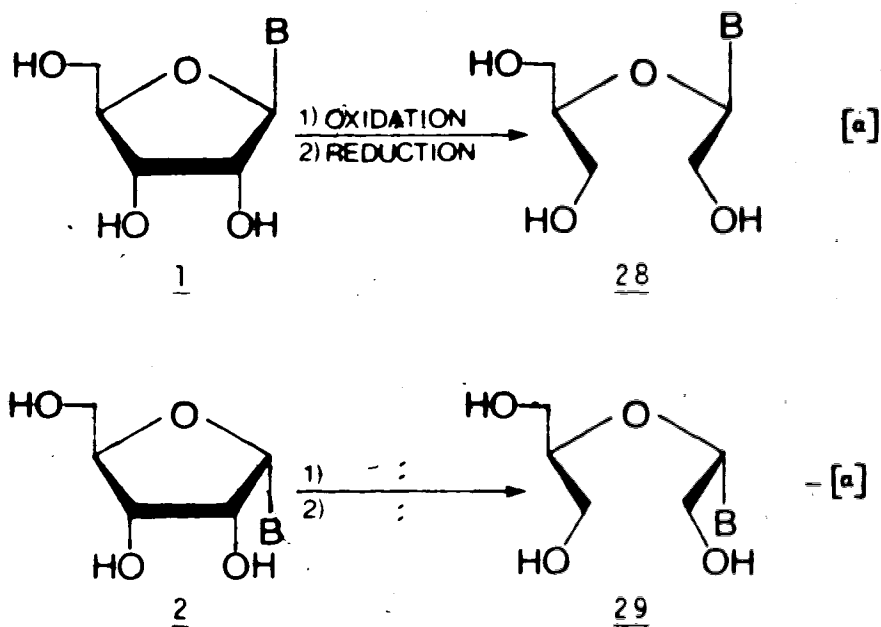
The dialdehydes from common naturally occurring ribonucleosides have been isolated and characterized^{65,66,77-79} usually as derivatives. It is well established^{59,78,79} that dialdehyde such as 18 exists in solution as hydrated species, internal hemiacetals, and hemialdals (24-26). Only a small fraction of these compounds exist as the free aldehydes (Scheme IV). In the solid state, the "dialdehydes" were suggested^{78c} to



Scheme IV

be completely polymerized (27). The internal hemiacetal (26) and hemiacetal (24) forms create additional asymmetric centers. Therefore, cases in which the optical rotations of periodate oxidation products provide sufficient information to allow unequivocal assignment of the anomeric configuration are rare.

Reduction⁷⁷ of these oxidation products with hydrogen and Raney nickel or borohydride normally gives the corresponding tri-alcohols such as 28 and 29 (Scheme V). Partial reduction of the aldehyde function distal to the aglycon has been noted under controlled conditions.^{77,80b} This

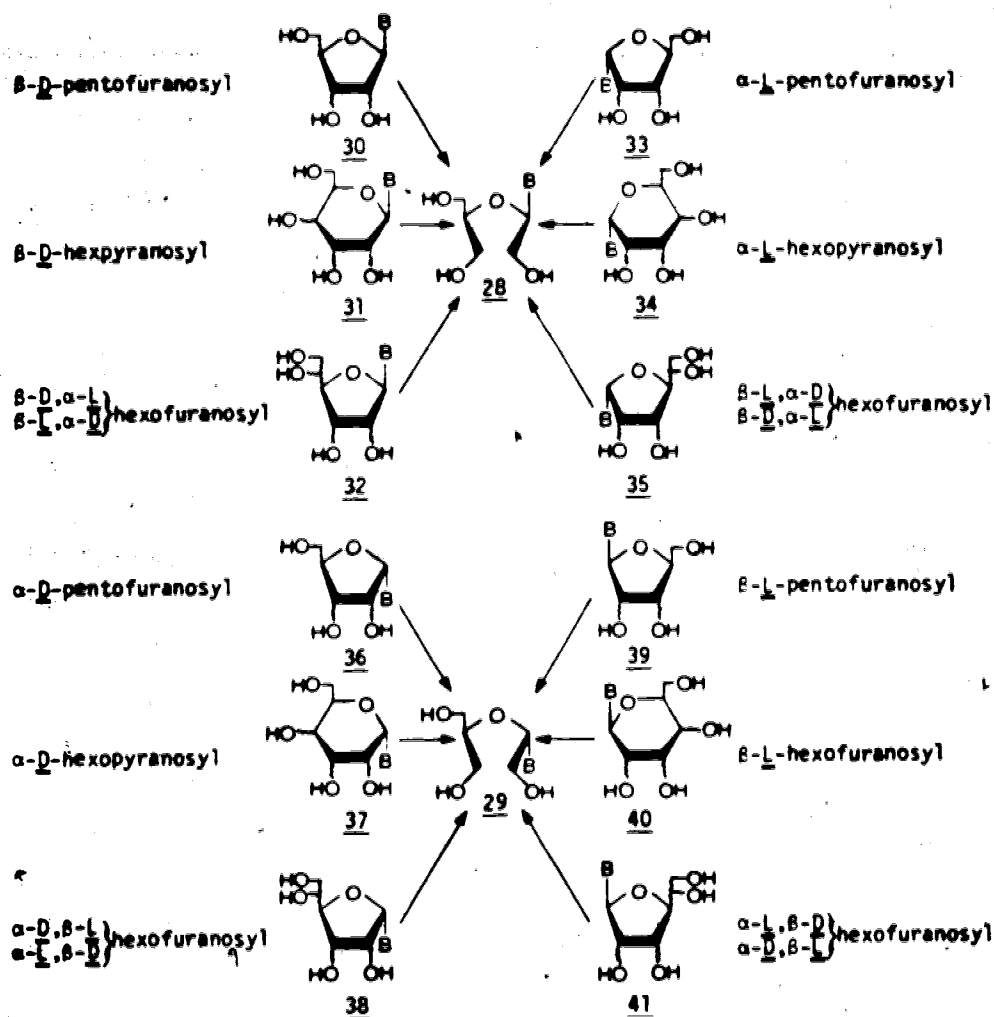


Scheme V

is a superior method for correlating the anomeric configuration of the precursor nucleoside since the only asymmetric center remaining in the reduced compound (28 or 29) is the original anomeric carbon. The triols (28, 29) are now enantiomeric and give equal but opposite rotations so that the anomeric natures of the original nucleosides (1,2) are clearly revealed.

Smith et al.,⁸⁰ who extended the work of Hudson,⁶³ and other workers⁸¹ demonstrated the configurational correlation of methyl glycosides in both the D and L-series based on the rotations of oxidized and reduced products (28,29, B = OMe). This method for determining the anomeric configuration requires a reference compound of known anomeric configuration. However, a principal

advantage is its applicability to a large number of compounds (some of which are shown in Scheme VI). Not only the anomeric configuration but also D and L-series can be correlated and determined^{80,81} from rotation measurements on precursor and triol series.



Scheme VI

In 1958 Khorana and co-workers⁵⁰ introduced the foregoing method to nucleosides in their synthesis of α -adeno-

sine. Since then, a number of examples has appeared. A trend in the optical rotations of oxidized and reduced nucleosides (nucleosides_{ox-red}) has been observed relative to the anomeric configuration of the starting nucleosides (see next section). In the usual procedure a nucleoside_{ox-red} is prepared in situ by the periodate oxidation and subsequent reduction of the nucleoside in question. The optical rotation of the resulting quantitated solution is then measured. When this method is used, caution must be exercised to avoid over-oxidation, and nucleosides that are labile to the degradation conditions also have been reported.⁸²⁻⁸⁹

The various methods described above should be evaluated carefully and exceptions and limitations considered before they are employed to determine the anomeric configuration of nucleosides. In this thesis, the latter degradation method (oxidation and reduction) for nucleosides, which had not been studied systematically, will be investigated.

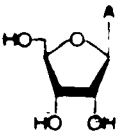
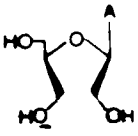

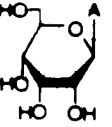
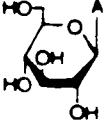
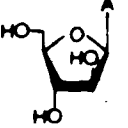
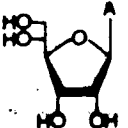
RESULTS AND DISCUSSION

Since Khorana et al.⁵⁰ first employed the furanose degradative procedure (oxidation of the glycol with periodate and reduction of the derived dialdehyde with borohydride) for configurational assignment of α -adenosine, a number of examples has appeared. This method has been used for confirming the anomeric relation of nucleoside pairs as well as for direct anomeric configurational assignment. Examples are summarized in Table I for in situ prepared nucleosides_{ox-red} and isolated "fraudulent" (sugar-modified) nucleosides_{ox-red}. Table II contains data for the isolated nucleosides_{ox-red} of "natural" triol types (28 or 29).

Lerner had observed^{96,97} that nucleosides having a β -D or α -L configuration gave ox-red alcohols with positive optical rotations, and nucleosides having an α -D or β -L configuration gave ox-red alcohols with negative optical rotations. On this basis, assignment of the anomeric configuration of adenine nucleosides such as 70 and 82 in Table I (which presumably give the corresponding nucleosides_{ox-red} 71 and 83, respectively, with more than one asymmetric center) has been proposed.^{96,97} It can be seen from Table I and Table II that Lerner's empirical approach agrees with the examples reported with one exception. The enantiomeric diols (96 and 98) gave opposite relations to those expected. However, optical rotations of the

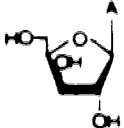
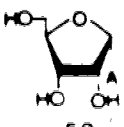
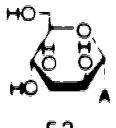
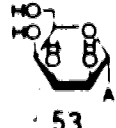
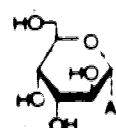

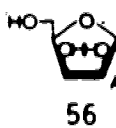
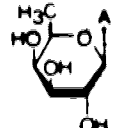
TABLE I

Optical Rotations of Previously Reported in situ
Prepared and Isolated Nucleosides_{ox-red}

Precursor nucleosides	Oxidized-reduced ^{b)} products (nucleosides _{ox-red})	[α] _D ^{c)}	References
 <p>a)</p> <p><u>42</u></p>	 <p><u>43</u></p>	+61-68	37,50,90,91
 <p><u>44</u></p>	<u>43</u>	+57	92
 <p><u>45</u></p>	<u>43</u>	+58	92
 <p><u>46</u></p>	<u>43</u>	+57	92
 <p><u>47</u></p>	<u>43</u>	+66	90
 <p><u>48</u></p>	<u>43</u>	+70	37

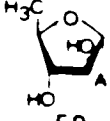
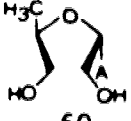

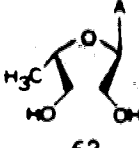
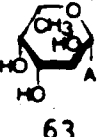
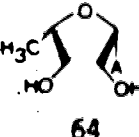

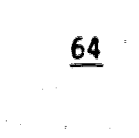

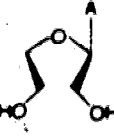
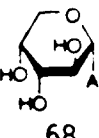
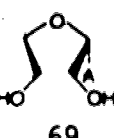
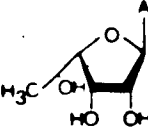
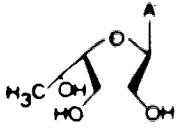
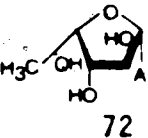
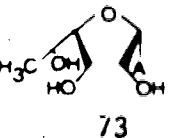
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TABLE I (continued)

	<u>43</u>	+53	93
<u>49</u>			
	<u>51</u>	-66	50
<u>50</u>			
	<u>51</u>	-58	92
<u>52</u>			
	<u>51</u>	-59	92
<u>53</u>			
	<u>51</u>	-57	92
<u>54</u>			
	<u>51</u>	-64	37
<u>55</u>			
	<u>51</u>	-65.0	91
<u>56</u>			
	<u>58</u>	+55 (isolated)	94
<u>57</u>			

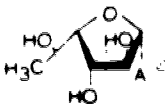
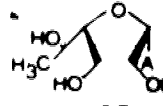
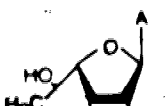
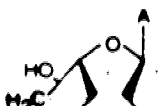
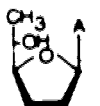
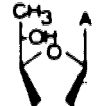


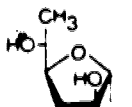
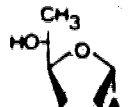




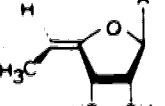
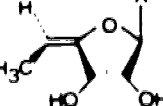
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TABLE I (continued)

 <u>59</u>	 <u>60</u>	-71	95
 <u>61</u>	 <u>62</u>	+74 +80.6 (isolated)	76,95 94
 <u>63</u>	 <u>64</u>	-46 -54 (isolated)	76 94
 <u>65</u>	 <u>64</u>	-50	76
 <u>66</u>	 <u>67</u>	+34.5 (isolated)	94
 <u>68</u>	 <u>69</u>	-36.5 (isolated)	94
 <u>70</u>	 <u>71</u>	+37	96
 <u>72</u>	 <u>73</u>	-64	96

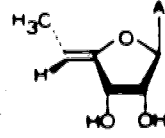
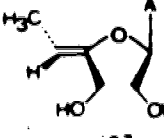
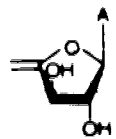
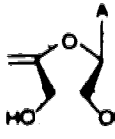


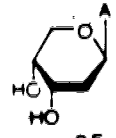
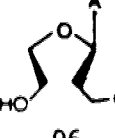
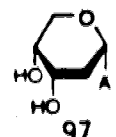
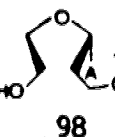
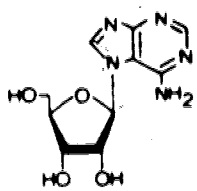
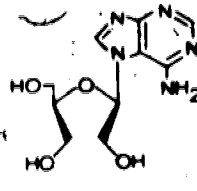
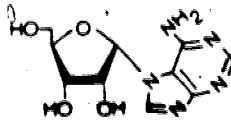
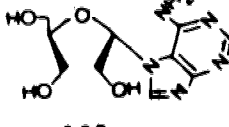
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TABLE I (continued)

 74	 75	-69	96
 76	 77	+65	96
 78	 79	+72	97
 80	 81	-48	97
 82	 83	-62	97
 84	 85	+37	98
 86	 87	-60	98
 88	 89	+18	35

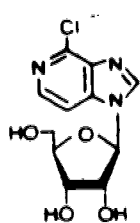
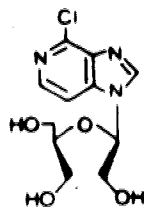
continued.....

TABLE I (continued)

 <u>90</u>	 <u>91</u>	+18	35
 <u>92</u>	 <u>93</u>	+59	99
 <u>94</u>	 <u>93</u>	+55	99
 <u>95</u>	 <u>96</u>	-10.8	100
 <u>97</u>	 <u>98</u>	+10.2	100
 <u>99</u>	 <u>100</u>	+118.1±0.2	101
 <u>101</u>	 <u>102</u>	-117.9±0.2	101

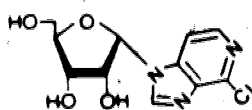
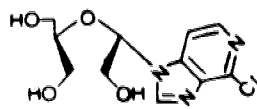
continued.....

TABLE I (continued)

103104

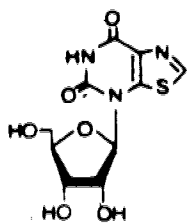
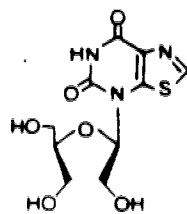
+72.0

53

105106

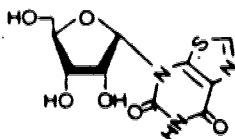
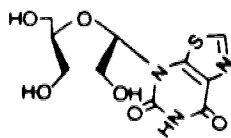
-71.9

53

107108

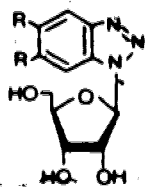
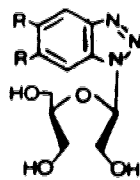
+73.5

102

109110

-75.0

102

111 R=CH₃112 R=Cl113 R=H114 R=CH₃115 R=Cl116 R=H

+65.1

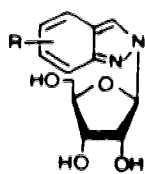
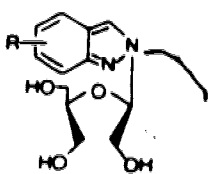
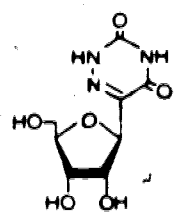
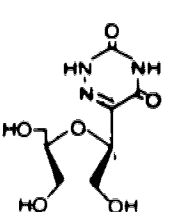
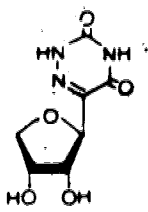
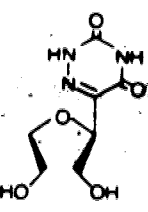
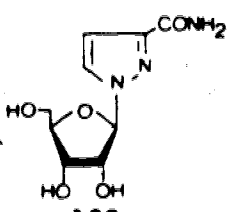
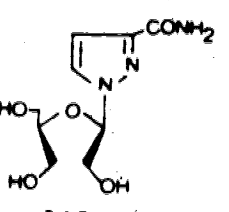
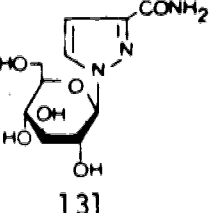

+67.2

+60.7

103

continued.....

TABLE I (continued)

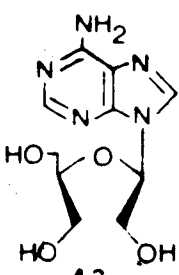
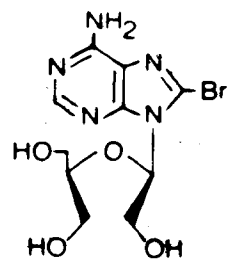
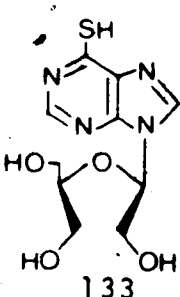
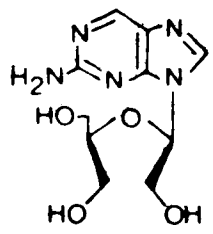
			104
<u>117</u> R=6-NO ₂	<u>121</u> R=6-NO ₂	+14.9	
<u>118</u> R=5-NO ₂	<u>122</u> R=5-NO ₂	+ 7.6	
<u>119</u> R=H	<u>123</u> R=H	+15.2	
<u>120</u> R=3-CN	<u>124</u> R=3-CN	+51.3	
		+46.8	105
<u>125</u>	<u>126</u>		
		+39.2	106
<u>127</u>	<u>128</u>		
		+36.1 ^{d)}	107
<u>129</u>	<u>130</u>		
		+31.8 ^{d)}	107
<u>131</u>	<u>130</u>		

continued.....

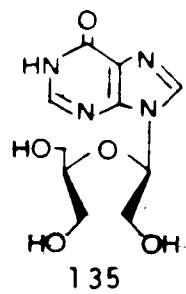
Table 1 (continued)

a) A = adenin-9-yl. b) Structures, if not reported were presumed. c) Rotation values are of in situ prepared nucleosides_{ox-red} unless noted otherwise. Consult the original article for detailed measurement conditions (i.e. temperature etc.). d) The reported $[M]_D$'s were converted to $[\alpha]_D$'s.

TABLE II
Optical Rotations of Previously Reported
Isolated Triols (28, 29)

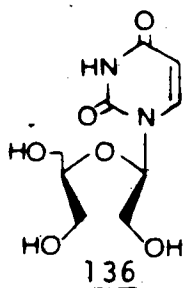
Compounds	$[\alpha]_D^{25}$ ^{a)}	Reference
 <u>43</u>	+58.9	108
 <u>132</u>	+38	109
 <u>133</u>	+73	109
 <u>134</u>	+34	109

continued.....

Table II (continued)

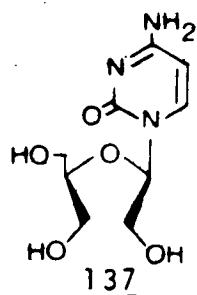
+70.2

109



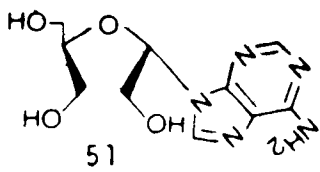
+49±1

108



+62±1

108

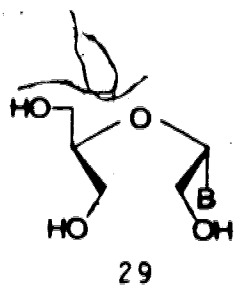
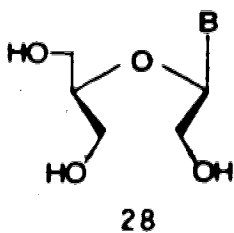


-62.6

94

a) Specific rotation values in H₂O. Consult the original article for detailed conditions (i.e. temperature etc.).

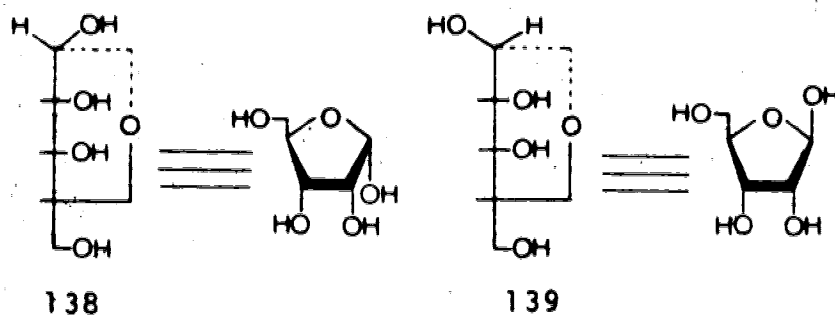
"natural" triols (28 and 29), which can be derived from anomeric pentofuranosyl, hexopyranosyl and hexofuranosyl nucleosides, are in accord with expectations.



Despite the well-known use of such triols,⁵⁰ this empirical approach has not been investigated systematically. Therefore, we have studied optical rotation relationships of various types of derived triols prepared in situ from pentofuranosyl nucleosides. We have observed that they exhibit a predictable electrophoresis behavior under defined complexing conditions. We have measured optical rotation values for a series of adenosine_{ox-red} derivatives to evaluate substituent effects. The variation in optical rotations of some isolated nucleosides_{ox-red} with pH, and some significant solvent effects have been determined. The rates of periodate reduction with some anomeric pairs of D-ribofuranosyl nucleosides, and with some pentofuranosyl adenine and cytosine nucleosides having a sugar configuration other than ribofuranose have been evaluated qualitatively.

A NOTE ON THE DEFINITION OF α AND β CONFIGURATIONS

In 1909 Hudson⁹ suggested that the more dextrorotatory anomer in the D-series be designated α and the more levorotatory β . In the L-series the more levorotatory would be designated α and the more dextrorotatory β . Freudenberg^{11a} suggested in 1932 that α and β anomers be classified with regard to their configuration rather than the sign and value of their optical rotations. His designation correlated monosaccharides with the same configuration at the anomeric carbon atom (C-1 for aldose) and the highest numbered asymmetric carbon (C-4 for pentoses) as α . Thus, structures (138 and 139) depicted in the Fischer and Haworth projection formulas, represent α -D-ribofuranose and β -D-ribofuranose, respectively. Their mirror images are α -L and β -L-ribofuranose.



In the majority of cases, both the Hudson and Freudenberg conventions concur. However, examples^{11,12} are known of α -D compounds that are less dextrorotatory than their

anomers. Hence, Freudenberg conventions are now universally applied to glycosidic and glycosyl compounds. The pairs of such compounds which differ in configuration at the hemiacetal carbon atom (C-1 for aldoses) were termed "anomers" by Riiber.^{111b}

A NOTE ON THE NOMENCLATURE OF OXIDIZED-REDUCED NUCLEOSIDES
(NUCLEOSIDES_{OX-RED}) OF TRIOL TYPES (28 AND 29)

There are several ways of naming nucleosides_{ox-red} (28 and 29).¹⁰⁸⁻¹¹⁰ For instance oxidized-reduced adenosine (43) has been named as 1-0-(1,3-dihydroxy-2-propyl)-1-(adenin-9-yl)-1(R),2-ethanediol, as 2-0-[1(R)-(9-adeninyl)-2-hydroxyethyl]glycerol and as 2-(R)-(adenin-9-yl)-4-hydroxymethyl-3-oxapentan-1,5-diol. For the sake of clarity in this thesis, oxidized-reduced nucleoside will be informally termed as nucleoside_{ox-red} such as adenosine_{ox-red} for 43. When prepared in situ, nucleoside_{ox-red} (in situ) will be used.

OPTICAL ROTATIONS OF NUCLEOSIDES_{OX-RED} (in situ); AND
ELECTROPHORESIS OF NUCLEOSIDES_{OX-RED} (in situ) AND THEIR
PRECURSOR NUCLEOSIDES

6-Azauridine (182), 6-azacytidine (183), 3-methyl-6-azauridine (184), 3-methyl-6-azauridine_{ox-red} (185), 6-methyl-2-β-D-ribofuranosyl-3-pyridazone (188), 2-β-D-ribofuranosyl-3-pyridazone (189), 6-methyluridine (173).

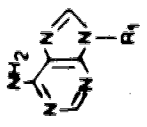
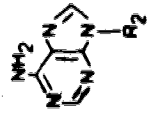
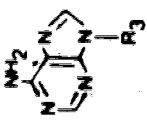
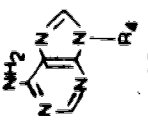
6-methylcytidine (178), 3- β -D-ribofuranosyl-6-methyluracil (171), 1,3-bis(β -D-ribofuranosyl)-6-methyluracil (172), 1,3-dimethylpseudouridine (190), 1-methylformycin (155), 2-methylformycin (156), 2-methylformycin_{ox-red} (157), 5,6-dihydrouridine (187), α -L-uridine (163), β -L-uridine (164) and α -L-cytidine (180) were prepared by reported methods or by the general synthetic procedures developed for the present study (see Experimental Section).

Fifty four pentofuranosyl nucleosides and methyl β -D-ribofuranoside were oxidized with periodate and subsequently reduced with sodium borohydride. Excess sodium borohydride was destroyed with 1 M sodium dihydrogen phosphate buffer (pH 6.5). (Liberation of iodine was observed in several cases when 10% acetic acid was used to destroy the borohydride.) The reduction products were assumed to be the triols 28 or 29, depending upon the precursor nucleosides (see Scheme VI). This appears to be justified based on the quantitative nature of the reactions and the comparison of electrophoretic mobilities of nucleosides_{ox-red} (in situ) with those of the corresponding precursor nucleosides.

Optical rotations of all nucleosides_{ox-red} (in situ) and some isolated nucleosides_{ox-red} were recorded at the wavelength of the sodium D line (589 nm). ORD curves (300-600 nm) and/or optical rotations at wavelengths other than 589 nm (578, 546, 436 and 365 nm bands of mercury)

TABLE III

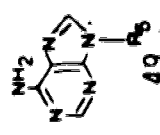
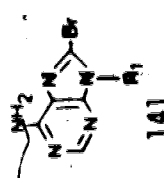
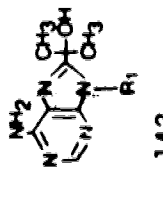

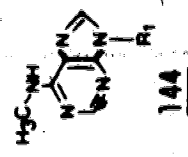
Optical Rotations of Nucleosides_{ox-red} (in situ), and Electrophoresis of Nucleosides_{ox-red} (in situ) and Their Precursor Nucleosides Determined in the Present Study

Precursor Nucleosides	[α] ₃₆₅	[α] ₄₃₆	[α] ₅₄₆	[α] ₅₇₈	[α] ₅₈₉	Anodic migration of nucleosides (in situ) ^{a)}	Anodic migration of precursor nucleosides ^{a)}
 42					+65.6	0.38	0.62
 50	-203.8	-130.6	-77.0	-67.9	-65.2	0.38	0.62
 140	+199.0	+128.2	+75.7	+67.2	+63.9	0.38	0.67
 47	+177.0	+114.2	+67.4	+59.4	+56.8 ^{c)}	0.38	0.12

plain positive curve^{b)}

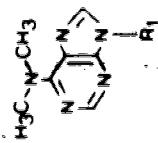
continued.....

Table III (continued)

	+182.4	+116.6	+68.3	+59.7	+57.7 ^{c)}	0.38	0.60	
	positive Cotton effect curve b)						0.36	0.59
	_____ d)						0.40	0.60
	_____ d)						0.39	0.78
	plain positive curve b)						0.52	0.81

continued.....

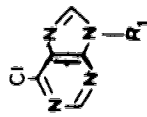
Table III (continued)



145

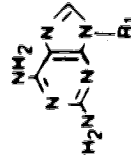
plain positive curve b)

+62.9 0.49 0.78



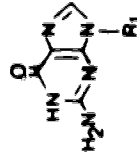
146

+190.3 +121.3 +73.3 +65.0 +61.5 0.57 0.88



147

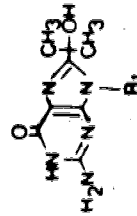
+99.3 +75.8 +48.0 +43.0 +41.4 0.29 0.48



148

positive Cotton curve b)

+40.4 0.64 0.83



149

+468.9 +278.9 +155.9 +137.5 +131.3 0.53 0.74

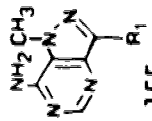
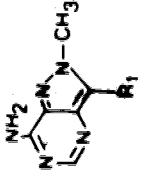
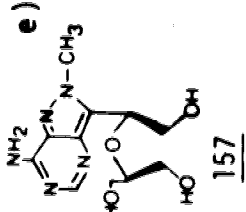
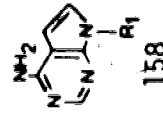
continued.....

Table VII (continued)

Chemical Structure	plain positive curve b)						
 150	+61.6	0.80	0.90				
 151	+3.8	+14.6	+13.4	+11.5	+9.6	0.82	1.10
 152	+87.2	+55.7	+30.4	+26.7	+21.0	0.45	0.74
 153	+273.5	+154.4	+86.8	+76.5	+73.5	0.46	0.83
 154	-269.4	-152.1	-87.6	-76.7	-72.7	0.47	0.84

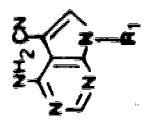
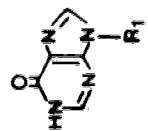
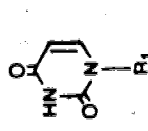
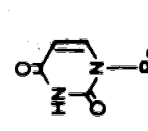
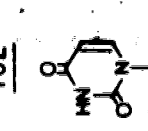
continued.....

Table III (continued)

 <p>155</p>	+173.5	+111.0	+64.1	+56.4	+54.0	0.38	0.64
 <p>156</p>	+115.5	+98.0	+62.0	+55.2	+53.0	0.45	0.67
 <p>e) 157</p>	+130.0	+107.4	+67.6	+59.8	+56.8	0.45	—
 <p>158</p>	positive Cotton effect curve, b) cross over at 302 nm			+32.3	0.41	0.52	

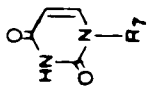
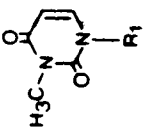
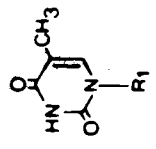
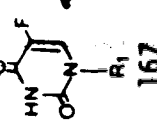
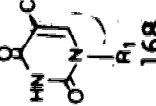
continued.....

Table III (continued)

 <p>159</p>	positive Cotton effect curve b)	+61.0	0.31	0.57			
 <p>160</p>		plain positive curve b)	+70.3	0.81	1.07		
 <p>161</p>	positive Cotton effect curve b)		+50.5	0.71	1.00		
 <p>162</p>		negative Cotton effect curve b)	-51.4	0.71	1.00		
 <p>163</p>	+114.2		+87.0	+56.8	+50.3	+49.1	0.71

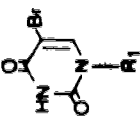
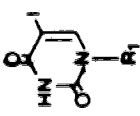
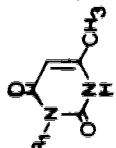
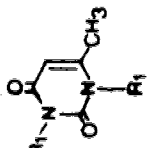
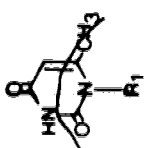
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Table III (continued)

	-117.6	-89.0	-58.2	-52.8	-46.2	0.71	1.00
<u>164</u>							
	+132.4	+94.0	+59.1	+52.2	+49.7	0.55	0.92
<u>165</u>							
positive Cotton effect curve b)							
	+169.5	+107.7	+63.6	+55.2	+51.3	0.98	1.23
<u>166</u>							
	+239.5	+158.2	+96.4	+84.8	+80.4	0.91	1.15
<u>167</u>							
							
<u>168</u>							


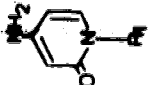


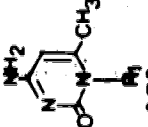
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Table III (continued)

	+196.5	+126.4	+76.9	+68.1	+64.3	0.95	1.10
169							
	+250.6	+158.2	+90.6	+80.6	+74.8	0.86	1.15
170							
	+16.3	+23.8	+18.5	+16.8	+16.3	0.49	0.90
171							
	+102.5	+70.6	+43.5	+39.0	+35.9	0.63	1.15
172							
	+205.9	+125.3	+72.1	+63.3	+60.8	0.63	0.93
173							

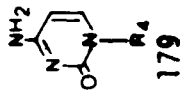
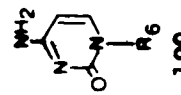
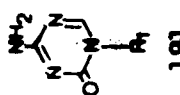
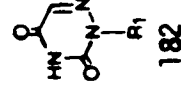
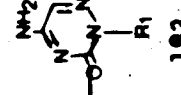
continued.....

Table III (continued)

 174	positive Cotton effect curve, b) cross over at 306 nm	+53.7	0.89	1.12
 175	positive Cotton effect curve, b) cross over at 306 nm	+56.7	0.56	0.79
 176	positive Cotton effect curve b)	+62.2	0.54	0.88
 177	negative Cotton effect curve b)	-61.7	0.54	0.88
 178	+174.2 +107.2 +62.7 +55.4 +53.0	0.50	0.83	

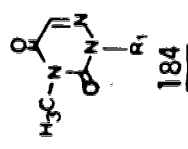
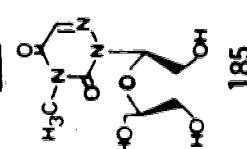
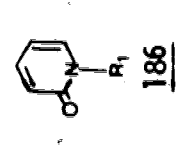
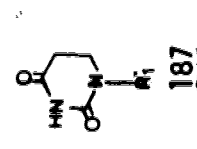
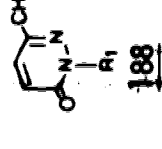
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Table III (continued)

f)									
	179	+157.0	+113.1	+71.1	+63.6	+60.4	0.53	0.17	
	180	+151.1	+109.3	+68.3	+61.0	+58.0	0.53	0.87	
	181	+74.5	+42.1	+25.8	+22.3	+19.9	0.45	0.82	
	182	-148.7	-69.9	-30.7	-25.9	-25.7	1.00	1.40	
	183	-226.7	-92.3	-38.5	-31.9	-30.1	0.41	0.82	

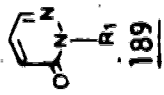
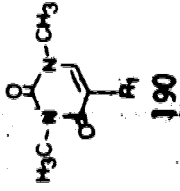
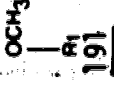

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Table III (continued)

	-131.9	-58.8	-26.1	-22.0	-20.6	0.48	0.93
e) 	-162.3	-73.4	-33.6	-27.8	-26.4	0.48	—
	positive Cotton effect curve, b) cross over at 355 nm						
	+167.9	+109.4	+65.4	+58.2	+55.1	0.45	0.88
	-419.3	-145.3	-53.8	-43.6	-40.6	0.43	0.90

continued.....

Table III (continued)

	-566.3	-225.3	-95.3	-80.9	-74.3	0.51	0.88	
	+317.7	+194.3	+111.9	+98.4	+93.8	0.63	0.94	
	plain positive curve b)						—	—
	+39.1	+105.1	+96.6	+93.4	+93.4	0.46	0.84	

R₁ = β-D-ribofuranosyl; R₂ = α-D-ribofuranosyl; R₃ = β-D-lyxofuranosyl; R₄ = β-D-arabinofuranosyl;
 R₅ = β-D-xylofuranosyl; R₆ = α-L-ribofuranosyl; R₇ = β-L-ribofuranosyl.

continued.....

Table III (continued)

a) Migrations were measured relative to that of uridine (1.00) as reference (absolute anodic migration of uridine ~12 cm). Values are from single determinations. b) Measured between wavelengths of 300 to 600 nm. c) These oxidations were allowed to proceed for 4 days. The lower rotation values observed possibly resulted from partial decomposition. d) Not measured owing to scarcity of samples. e) Isolated nucleoside_{ox-red}. f) HCl salt was used. g) No rotation value was obtained due to the strong absorption of light at this wavelength.

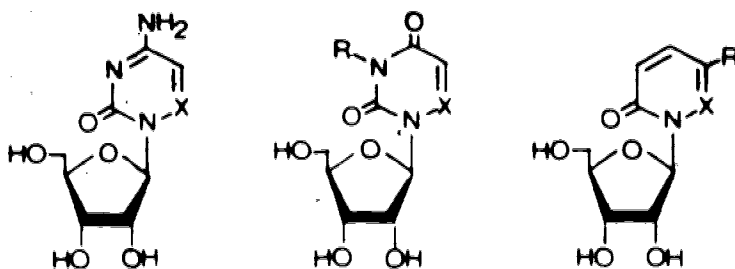
were also obtained for isolated nucleosides_{ox-red} and most nucleosides_{ox-red} (in situ). Electrophoretic mobilities of nucleosides_{ox-red} and precursor nucleosides were recorded. The results are summarized in Table III. All measured nucleosides_{ox-red} (in situ) and isolated_{ox-red} gave plain positive or negative curves, or single positive or negative Cotton effects between 365 and 589 nm.

Lerner's results would predict that the triols 28 should give positive and triols 29 negative rotations at 589 nm. Our results for most of the nucleosides_{ox-red} follow this trend. Thus, the anomeric pairs of nucleosides (42 and 50, 153 and 154, 161 and 162, 163 and 164, 176 and 177) in the D and/or L-series give positive rotations for β -D and α -L-nucleosides_{ox-red} (28) and negative rotations for α -D and β -L-nucleosides_{ox-red} (29). The results of nucleosides_{ox-red} (in situ) derived from 141-150, 165-170, 173, 178 and 187, indicate that substituent effects and a minor change of the aglycon do not alter the sign of the rotation although the magnitudes are affected.

Purine nucleosides and their analogs appear to follow the trend. This is also the case for most of the pyrimidine nucleosides and their analogs and also for azomycin β -D-ribose (192) and methyl β -D-ribofuranoside (191). However, exceptions were found with the 6-azapyrimidine (1,2,4-triazene) and pyridazine nucleosides_{ox-red}. The ox-red products from 6-azauridine (182), 6-azacytidine

(183), 3-methyl-6-azauridine (184), 6-methyl-2- β -D-ribofuranosyl-3-pyridazone (188) and 2- β -D-ribofuranosyl-3-pyridazone (189) gave negative $[\alpha]_D$ values and plain negative curves between 365 and 589 nm, although these compounds were derived from β -D-ribofuranosyl nucleosides. This reversed trend was also reported with simple ox-red N-acetylglycosylamines¹¹² (28 and 29, B = N-acetylamino), whereas the methyl glycosides^{80,81} (28 and 29, B = OMe) behaved normally.

Initially a commercial sample of 6-azauridine was used for our study. However, the observation of a reversed sign for 6-azauridine_{ox-red} led us to synthesize several 6-azapyrimidine and pyridazine nucleosides (182-184, 188 and 189). These compounds were reported to have CD and ORD spectra with reversed Cotton effects relative to their pyrimidine and pyridine counterparts.^{14a-c,f,18e,113} The same structural relationship exists between pyrimidine and 6-azapyrimidine nucleosides as between pyridine and pyridazine nucleosides. Likewise, the rotations observed for the ox-red pyrimidine and pyridine nucleosides (161, 176, 165 and 186) were positive whereas those from their aza analogs (182, 183, 184 and 189, respectively) were negative. Isolated 3-methyl-6-azauridine_{ox-red} (185) showed a parallel negative rotational pattern with the in situ result. This corroborated the structure of 3-methyl-6-azauridine_{ox-red} (in situ) as the triol and



176; X = CH 161; X = CH, R = H 186; X = CH, R = H

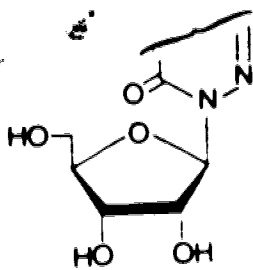
183; X = N 165; X = CH, R = CH₃ 188; X = N, R = CH₃

182; X = N, R = H 189; X = N, R = H

184; X = N, R = CH₃

confirmed its negative rotation. Thus, the empirical trend clearly is not followed by 6-azapyrimidine and pyridazine nucleosides.

The common structural feature of the 6-azapyrimidine and pyridazine nucleosides (182-184, 188 and 189) consists of a six membered ring aglycon in which a second ring nitrogen atom and a carbonyl group are bonded to the ring nitrogen bearing the glycosyl moiety (193). The



193

reversed sign of the Cotton effect of 6-azapyrimidine nucleosides was rationalized^{18e} by suggesting a preferred conformation of the base. This was presumed to result from the interaction of the lone electron

pair on N-6 and the lone pair on the ether oxygen of the ribose ring. If this were valid for the open-chain nucleosides^{ox-red}, it might also explain the abnormal

behavior of 6-azapyrimidine and pyridazine nucleosides¹¹⁴_{ox-red}.

However, 5-β-D-ribofuranosyl-6-azauridine (125, Table I), which has an adjacent carbonyl function and a nitrogen atom at the same ring position relative to the atom bearing the ribosyl moiety as 6-azapyrimidine nucleosides, has been reported to give the ox-red product (presumably 126) with a positive rotation.¹⁰⁵ Furthermore, a much greater conformational flexibility around the glycosyl bond of a nucleoside_{ox-red} would be expected. This is supported by the "normal" positive optical rotations of nucleosides_{ox-red} (*in situ*) derived from 6-methyluridine (173), 6-methylcytidine (178), 3-β-D-ribofuranosyl-6-methyluracil (171), and 8-(α-hydroxyisopropyl)guanosine (149). In contrast, the latter intact nucleosides represent exceptions from the ORD/CD rule (see the Introduction) and give a reversed Cotton effect relative to their unsubstituted pyrimidine and purine analogs.^{14c,18b,f,e} These reversed Cotton effects have been attributed to preferred base conformations (restricted ranges of the torsion angles, ϕ_{CN} ¹¹⁵) resulting from coulombic repulsion between base carbonyl and the intact-ring oxygens of 171,^{18e} steric effects of the bulky substituent at the 6-position of 173 and 178^{18b} and at the 8-position of 142 and 149.^{18f,116} After oxidation and reduction, their open-chain nucleoside analogs gave the usual positive $[\alpha]_D$ values and/or plain

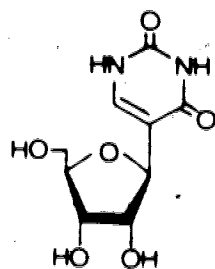
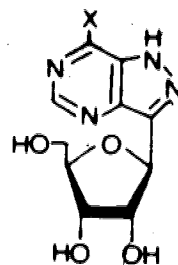
positive curves or positive Cotton effects between 365 and 589 nm. This would imply the existence of increased rotational flexibility around the glycosyl bond of the nucleosides_{ox-red}.

In contrast to these results, 6-azapyrimidine and pyridazine nucleosides_{ox-red} retain the abnormal (reversed sign) optical rotation behavior of their precursor nucleosides. This suggests that the reversed rotation behavior of 6-azapyrimidine and pyridazine nucleosides arises mainly from the chromophoric effects (intrinsic optical vector orientation in the chromophore) rather than rotational preferences^{18e} about the glycosyl bond. Similarly, the reversed chiroptical behavior of 6-azapyrimidine and pyridazine nucleosides_{ox-red} may be attributed to the same phenomenon.

Our results with ox-red products provide additional support for the glycosyl conformational preference rationale for the reversed ORD and CD Cotton effects of the precursor 6-substituted pyrimidine nucleosides (173, 178), 8-substituted purine nucleosides (142, 149) and 3- β -D-ribofuranosyl-6-methyluracil (171). The combined observations suggest that the long wavelength rotation behavior of nucleosides_{ox-red} may be useful for the study of nucleoside glycosyl rotameric conformational preferences. Comparison of the CD and ORD spectral properties of the precursor nucleosides and the rotations of

the ox-red products with enhanced glycosyl bond flexibility will provide an additional parameter to evaluate steric conformational vs. intrinsic optical vector effects with the base chromophore.

Pseudouridine (5-B-D-ribofuranosyluracil) (194) was known to be labile under the periodate oxidation and borohydride reduction conditions.⁸⁸

194

195; X = NH₂
196; X = OH

An elimination mechanism has been proposed by Chambers.¹¹⁷ Formycin (195) and formycin B (196) have been reported to give 3-formylpyrazolo[4,3-d]pyrimidine derivatives upon prolonged oxidation with periodate.⁸⁹ A similar over-oxidation has been reported for a benzimidazole C-nucleoside derivative.⁸⁴ Thus, it appears that C-nucleosides can not be subjected directly to the present procedure of periodate oxidation and borohydride reduction. However, this procedure was employed with formycin at the 3'-terminal of a tRNA during a study of the specificity of aminoacyl-tRNA synthetases.¹¹⁸ The negative results

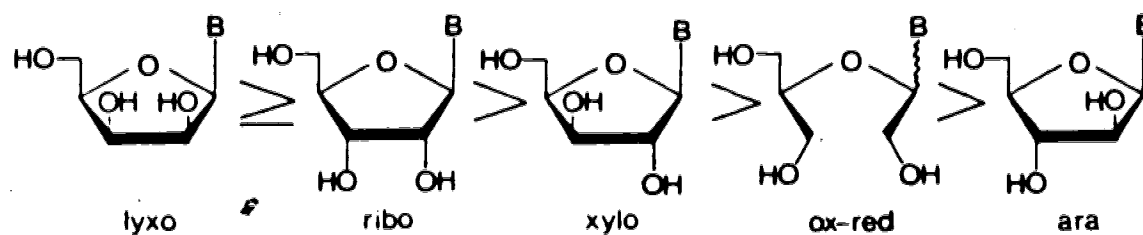
observed in the aminoacylation with the 3'-terminal modified tRNA (formycin_{ox-red} at 3'-terminal) now would appear to result from degradation of the terminal formycin and not necessarily to the reported specificity effects of the supposed triol product.

In order to circumvent the degradation of these labile C-nucleoside intermediates by the reaction conditions, methylation of the acidic NH functions on the bases was examined. Treatment of pseudouridine (194) with N,N-dimethylformamide dimethyl acetal gave 1,3-dimethylpseudouridine (190) which was subjected to the usual periodate oxidation and borohydride reduction conditions. The resulting 1,3-dimethylpseudouridine_{ox-red} (in situ) gave positive rotation values and a single anodic migrating spot upon electrophoresis. The anodic mobilities of 0.63 for 1,3-dimethylpseudouridine_{ox-red} (in situ) and 0.94 for its precursor 1,3-dimethylpseudouridine indicate that the product is the expected triol (28, B = 1,3-dimethyluracil-5-yl). After oxidation and reduction, synthetic 1-methylformycin (155) and 2-methylformycin (156) gave ox-red products with positive rotations and homogeneous electrophoretic spots corresponding to triol species. The stability of these N-methyl nucleosides toward the reaction conditions was further substantiated by the isolation of 2-methylformycin_{ox-red} (157). The electrophoretic mobilities and optical rotations of the isolated

and in situ prepared 2-methylformycin_{ox-red} were identical. The stability of the N-methylated formycins (156, 157) and N¹,N³-dimethylated pseudouridine (190) suggests that the NH group is the key factor in the labilities of pseudouridine and formycin oxidation intermediates. The rotations of these methylated nucleosides_{ox-red} agree with the usual β (positive) trend.

In most reported cases, no attempt was made to isolate or characterize the ox-red products from nucleosides except for measurement of the optical rotation. The structures of most nucleosides_{ox-red} (in situ) have been presumed on the basis of the quantitative nature of the reactions. Paper electrophoresis was found to be a very useful tool. Electrophoretic examination of nucleosides_{ox-red} and their intact precursors using a borate buffer at pH 9.3 revealed well separated spots for the two classes and consistent trends were observed in their anodic migrations.

Nucleosides_{ox-red} migrated faster than arabinofuranosyl nucleosides, but slower than other pentofuranosyl nucleosides (xylo-, lyxo- and ribofuranosyl compounds). The following order of anodic migrations is based on adenine derivatives that were available in the complete set of β -D-pentofuranosyl derivatives. (Scheme VII). This order of migration can be attributed to the ease of formation of ionic, cyclic borate complexes with the



B = adenin-9-yl

Scheme VII

hydroxyl groups.¹¹⁹ Differences in the electrophoretic migration of anomeric pairs under our conditions was not observed, although the anomeric pair of pseudouridines was reported to separate using a borate buffer at pH 9.6.¹²⁰ The electrophoresis of methyl β -D-ribofuranoside_{ox-red} (*in situ*) was not determined but its structure (as 28, B = OMe) was supported by the correspondence of the reported value of $[\alpha]_D +12-15^\circ$.⁸¹

In summary, the previously known trend of $\beta(+)$ and $\alpha(-)$ rotation signs for nucleosides_{ox-red} is in accord with most of the nucleosides presently studied. However, 6-azapyrimidine and pyridazine nucleosides are exceptions. This reversed behavior probably results from the intrinsic optical properties of the aglycons. The results suggest that enhanced flexibility exists about the glycosyl bond of nucleosides_{ox-red}. This information can be used in conjunction with ORD/CD studies on the precursor nucleosides for conformational studies of the latter. Formycin and pseudouridine are labile to the

reaction conditions, but their N-methylated derivatives are stable. Paper electrophoresis with a borate buffer at pH 9.3 appears to be reliably useful for qualitative characterization of nucleosides_{ox-red}.

OPTICAL ROTATIONS OF ADENOSINE_{OX-RED} DERIVATIVES

Lerner^{96,97} has employed the empirical trend in the signs of optical rotations of nucleosides_{ox-red} for assigning the anomeric configuration of adenine nucleosides such as 70 and 82 in Table I. Such compounds give ox-red products with three chiral centers. We have determined the optical rotations of a series of modified adenosine_{ox-red}¹²¹ derivatives that were available in our laboratory. The results are tabulated in Table IV. It can be seen that these modified adenosine_{ox-red} products (58, 197-208) have positive rotations in agreement with the "normal" β enantiomeric ox-red products. The rationalization for application of the empirical trend to such nucleosides (70, 82)^{96,97} is based upon the observation that chiral centers other than the anomeric center do not appear to contribute significantly to the rotation. This is in harmony with ORD and CD studies of pentofuranosyl nucleosides which have shown that the anomeric center is the major contributor to the optical activities.^{14,16,18e,20} The configurations of other centers normally are of minor consequence.

TABLE IV

Optical Rotations of a Series of Modified Adenosine_{ox-red} Derivatives from our Laboratory¹²¹

Compound	R	R'	[α] ₃₆₅	[α] ₄₃₆	[α] ₅₄₆	[α] ₅₇₈	[α] ₅₈₉	Concentration and solvent a)
197	OH	TsO	+149.0	+87.0	+49.0	+42.8	+40.4	c 0.53, 20% MeOH
198	TsO	OH	+35.9	+35.6	+25.4	+23.2	+22.2	c 0.39, 20% MeOH
58	OH	H	+188.7	+120.1	+71.1	+59.1	+50.3	c 0.16, H ₂ O
199	H	OH	+149.8	+98.9	+58.9	+52.1	+49.8	c 0.52, H ₂ O
200	OH	Cl	+209.6	+132.2	+76.3	+67.4	+64.5	c 0.58, H ₂ O
201	Cl	OH	+188.4	+124.1	+73.9	+65.5	+62.5	c 0.54, H ₂ O
202	OH	NH ₂	+275.3	+176.8	+104.7	+92.2	+88.5	c 0.49, H ₂ O
203	NH ₂	OH	+176.3	+116.8	+69.7	+61.7	+58.5	c 0.47, H ₂ O
204	OH	N ₃	+37.4	+27.4	+17.5	+15.5	+15.0	c 0.52, H ₂ O
205	N ₃	OH	+256.6	+161.4	+94.4	+83.4	+78.9	c 0.36, H ₂ O
206	I	OH	+99.4	+75.5	+48.3	+44.7	+41.5	c 0.36, H ₂ O

continued.....

Table IV (continued)

<u>207</u>	OH	OP ₃ H ₂	+111.1	+68.3	+42.3	+34.5	+33.4	c 0.28, H ₂ O
<u>208</u>	Br	OH	+146.8	+100.0	+60.8	+54.0	+51.8	c 0.25, H ₂ O

a) All optical rotations were measured at room temperature (23-24°C).

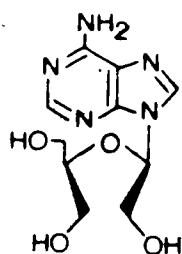
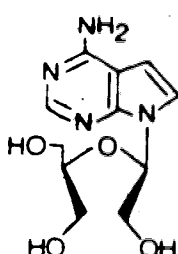
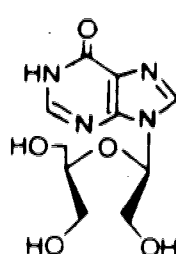
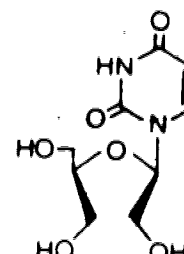
However, a comparison of the optical rotation magnitudes of certain isomer pairs (197 and 198; 204 and 205) undoubtedly reveals the marked influence of the substituent groups. Thus, the contribution from chiral centers other than the anomeric center to the composite optical rotation may be significant in certain cases. Therefore, application of the empirical trend to nucleosides that give ox-red products with more than one chiral center cannot be assumed to provide a secure assignment of the configuration at C-1'. This is especially critical in cases of a small composite rotation magnitude or with structures that have polarizable substituents on chiral centers.

pH AND SOLVENT EFFECTS ON THE OPTICAL ROTATIONS OF
ADENOSINE_{OX-RED} (43), TUBERCIDIN_{OX-RED} (209), INOSINE_{OX-RED}
(135) AND URIDINE_{OX-RED} (136)

It is well known¹²² that solvent modification can effect the chiroptical properties of a compound. Factors to be considered include solute-solvent interactions, dipole-dipole influences, hydrogen bonding, conformational equilibria changes, effects on ionization, etc. Solvent effects have been reported^{18b} to cause sign reversals of CD Cotton effects with certain nucleosides. Therefore, the influence of changes in pH and solvents on the optical rotations of two acidic and two basic ionizable nucleo-

sides_{ox-red} were examined.

Adenosine_{ox-red} (43), tubercidin_{ox-red} (209), inosine_{ox-red} (135) and uridine_{ox-red} (136) were synthesized and characterized. The proton NMR spectra of these nucleosides_{ox-red} had characteristic triplets for the "anomeric" proton signals ($J = 6$ Hz) due to splitting by the "2'-CH₂OH" group. Solvents examined were MeOH, H₂O,

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DMF and pyridine. Aqueous (~ 0.1 M) solutions were measured at pH 1, 3, 5, 7, 9, 11 and 13. Inosine_{ox-red} was not examined in MeOH because of its insolubility. The rotations were determined at 365, 436, 546, 578 and 589 nm. The results are presented in Tables V, VI, VII and VIII.

In the aqueous solutions, adenosine_{ox-red} (43), tubercidin_{ox-red} (209) and inosine_{ox-red} (135) showed a significant decrease in the rotation magnitude with increased pH. In contrast, uridine_{ox-red} (136) had a slight increase in magnitude with increased pH, although the rotations at higher wavelengths remained relatively unchanged. All of these nucleosides_{ox-red} had plain positive

TABLE V

pH and Solvent Effects on the Optical Rotations
of Adenosine_{ox-red} (43)

A. pH effect^{a),b)}

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
pH 1	+266.1	+166.8	+97.1	+85.7	+82.0
2	+236.7	+150.8	+88.4	+77.8	+74.6
5	+221.4	+141.7	+83.0	+73.1	+70.1
7	+226.3	+144.3	+85.2	+75.0	+71.6
9	+217.6	+138.6	+81.4	+71.6	+66.7
11	+213.4	+137.2	+80.4	+71.0	+67.7
13	+161.6	+106.5	+85.7	+55.5	+53.2

B. Solvent effect^{a),b)}

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
MeOH	+228.3	+146.2	+85.6	+75.3	+71.5
H ₂ O	+226.7	+145.0	+84.9	+74.6	+71.1
pyridine	+55.7	+55.2	+38.4	+34.7	+33.2
DMF	+175.3	+117.2	+70.7	+62.5	+59.9

a) Concentration; 11.16 mg/2 ml. b) Each value obtained is an average of two determinations.

TABLE VI
pH and Solvent Effects on the Optical Rotations
of Tubercidin_{ox-red} (209)

A. pH effect^{a), b)}

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
pH 1	+144.2	+95.6	+57.2	+50.9	+48.4
3	+101.8	+69.8	+42.6	+37.8	+36.0
5	+100.1	+69.0	+44.6	+37.1	+36.0
7	+86.1	+60.5	+37.3	+33.3	+31.9
9	+85.0	+59.0	+37.0	+32.9	+31.3
11	+83.8	+59.1	+36.4	+32.4	+31.2
13	+51.3	+41.0	+26.9	+24.0	+23.1

B. Solvent effect^{a), b)}

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
MeOH	+90.9	+64.4	+40.3	+35.7	+34.2
H ₂ O	+85.8	+59.8	+37.0	+32.9	+31.9
pyridine	-85.3	-17.5	+0.4	+2.0	+2.3
DMF	+57.9	+51.2	+34.3	+31.0	+29.7

a) Concentration; 13.05 mg/2 ml. b) Each value obtained is an average of two determinations.

TABLE VII
pH and Solvent Effects on the Optical Rotations
of Inosine_{ox-red} (135)

A. pH effect^{a), b)}

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
pH 1	+275.3	+174.0	+101.6	+99.6	+85.9
3	+221.5	+140.5	+82.3	+72.1	+69.5
5	+224.3	+142.5	+83.6	+73.4	+70.4
7	+224.1	+142.3	+83.2	+73.3	+70.0
9	+218.2	+138.9	+81.6	+71.6	+68.8
11	+197.5	+127.3	+75.2	+66.1	+63.4
13	+154.8	+102.3	+61.1	+54.0	+51.6

B. Solvent effect^{a), b)}

	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
H ₂ O	+223.9	+142.3	+83.5	+73.6	+70.5
pyridine	+16.7	+31.3	+25.2	+22.6	+22.4
DMF	+196.7	+127.8	+75.5	+66.8	+64.0

a) Concentration; 10-14 mg/2 ml. b) Each value obtained is an average of two determinations.

TABLE VIII
pH and Solvent Effects on the Optical Rotations
of Uridine_{ox-red} (136)

<u>A. pH effect^{a),b)}</u>					
	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
pH 1	+112.0	+86.5	+56.4	+50.6	+48.8
3	+113.9	+88.0	+57.4	+51.3	+49.2
5	+114.9	+88.7	+58.1	+52.1	+50.1
7	+110.6	+85.3	+55.7	+50.1	+47.8
9	+115.2	+88.4	+57.5	+51.5	+49.5
11	+124.8	+89.9	+57.2	+51.1	+48.8
13	+138.1	+92.7	+57.3	+49.0	+48.6
<u>B. Solvent effect^{a),b)}</u>					
	$[\alpha]_{365}$	$[\alpha]_{436}$	$[\alpha]_{546}$	$[\alpha]_{578}$	$[\alpha]_{589}$
MeOH	+153.3	+110.9	+69.7	+62.1	+59.3
H ₂ O	+117.3	+91.0	+59.3	+53.1	+51.2
pyridine	+211.3	+126.5	+73.2	+64.7	+62.0
DMF	+221.8	+139.2	+82.2	+72.7	+69.9

a) Concentration; 11.21 mg/2 ml. b) Each value obtained is an average of two determinations.

curves between 365 and 589 nm. This suggests that ionization of the chromophoric bases does not drastically affect the long wavelength chiroptical properties of these nucleosides_{ox-red}. Thus, these longer wavelength optical rotations are less sensitive to ionization of the chromophore than the ultraviolet-CD measurements, an experimentally useful fact.

In the protic solvents H₂O and MeOH, and the aprotic solvent DMF, these four nucleosides_{ox-red} showed plain positive rotation curves analogous to those obtained in aqueous solutions. This indicates that the hydrogen bonding and dipole-dipole interactions between solvents and nucleosides_{ox-red} exert a minor effect on their rotations at long wavelength (365-589 nm). In pyridine, however, drastic changes in the rotation values were observed with adenosine_{ox-red} (43), inosine_{ox-red} (135) and tubercidin_{ox-red} (209). Tubercidin_{ox-red} in this solvent exhibited a cross over in the sign of rotation between 436 and 546 nm. Uridine_{ox-red} (136) was not affected drastically. It is obvious that pyridine is not functioning merely as a base since parallel changes were not observed at increasing pH values. The normal behavior of uridine_{ox-red} (136) in pyridine suggests that this effect does not result from interactions between solvent and the acyclic side chains. The strong interaction with the purine-type compounds is in harmony with

the known base-stacking²⁰ (i.e. π - π complex formation with pyridine) effects relative to the weak interactions noted with pyrimidines. Thus, caution must be exercised if chiroptical studies of nucleosides_{ox-red} are attempted using pyridine or other aromatic "stacking" solvents.

RATES OF PERIODATE REDUCTION BY NUCLEOSIDES

The rate of consumption of periodate by anomeric pairs of D-ribofuranosyl nucleosides (42 and 50; 176 and 177; 161 and 162; 153 and 154), lyxo-A (140), and pentofuranosyl nucleosides having a 2',3'-trans glycol unit (47, 49, and 179) were examined spectrophotometrically by the method of Dixon and Lipkin.¹²³ The results are summarized in Table IX. Nucleosides having a 2',3'-cis glycol structure were oxidized completely in ~20 min. Nucleosides having a 2',3'-trans diol configuration were oxidized much more slowly. This is consistent with the general rule that cis-diols are more rapidly oxidized than trans-diols.¹²⁴ Under the conditions used in this study for preparation of nucleosides_{ox-red} (in situ), ara-A (47), xylo-A (49) and ara-C (179) were oxidized completely in ~4 days (monitored by electrophoresis). However, the concentrations employed in the spectrophotometric assay are much lower resulting in a longer oxidation time owing to the dilution effect. An alternative procedure employing higher concentrations of periodate was noted for compound

TABLE IX
Rates of Periodate Reduction by Nucleosides^{e)}

Nucleosides	Nucleoside ^{a)} added (mole x 10 ⁷)	Periodate added (mole x 10 ⁷)	Wavelength ^{b)} (nm)	1 min ^{c)}	1.5 min	2.5 min	5.5 min	10.5 min	15.5 min
adenosine (42)	0.929	2.468	227	0.859	0.926	0.959	0.968	0.968	0.968
α -adenosine (50)	0.985	2.468	227	0.696	0.823	0.944	1.025	1.034	1.036
lyxo-A (140)	1.198	2.468	227	0.614	0.785	0.951	1.032	1.032	1.032
cytidine (176)	0.985	2.468	227	0.774	0.892	0.986	1.025	1.026	1.026
α -cytidine (177)	0.611	2.468	227	0.544	0.667	0.845	1.009	1.036	1.037
uridine (161)	0.970	2.468	230	0.899	1.000	1.055	1.069	1.071	1.071
cf-uridine (162)	1.134	2.468	230	0.572	0.717	0.866	0.972	0.984	0.985
7- β -D-ribofuranosyl-theophylline (153)	1.015	2.468	230	0.981	1.041	1.054	1.055	1.056	1.056
7- α -D-ribofuranosyl-theophylline (154)	0.915	2.468	230	0.631	0.794	0.943	1.024	1.027	1.027
ara-A (47)	9.142	15.896	227	-60% of the periodate per nucleoside was reduced in 9 days					
xyle-A (49)	7.318	15.896	227	-45% of the periodate per nucleoside was reduced in 9 days					
ara-C (179) ^{d)}	3.677	15.896	227	-55% of the periodate per nucleoside was reduced in 6 days					

continued.....

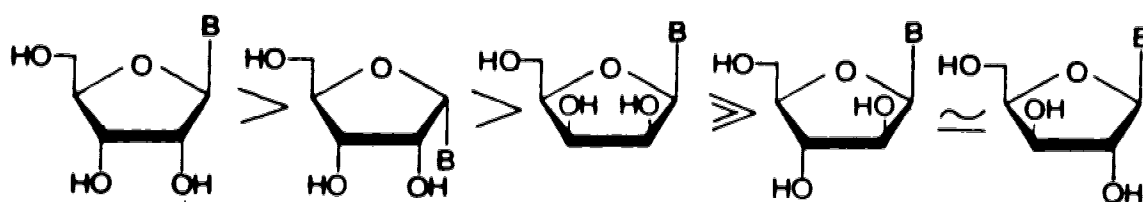
TABLE IX (continued)

a) The concentrations of nucleosides in H₂O were determined using the following extinction coefficients; adenosine and its anomer (ϵ 15,100 at 259 nm), lyxo-A (ϵ 14,900 at 259 nm),¹²⁵ cytidine (ϵ 9,100 at 270 nm), α -cytidine (ϵ 9,100 at 271 nm), uridine (ϵ 10,300 at 262 nm), α -uridine (ϵ 10,050 at 264 nm),¹²⁶ 7- β -D-ribofuranosyltheophylline (ϵ 8,700 at 272 nm),¹²⁷ 7- α -D-ribofuranosyltheophylline (ϵ 8,700 at 274 nm), ara-A (ϵ 13,400 at 259 nm), xylo-A (ϵ 15,100 at 258 nm),¹²⁸ ara-C·HCl salt (ϵ 9,900 at 273 nm). b) Wavelength at which the decrease in absorbance was followed. c) Each value is an average of three determinations, except two for α -uridine (162). Deviations from the mean values were $\pm 10\%$. Values are of moles of periodate reduced per mole of nucleoside. d) HCl salt was used. e) Measurements were performed at room temperature (24 \pm 2°C).

that are oxidized at inconveniently slow rates.¹²³

Comparison of the spectrophotometric rates of periodate reduction clearly demonstrates the possibility of assignment of anomeric configuration of ribofuranosyl nucleosides. During the initial few minutes, ribofuranosyl nucleosides with the β -configuration (42, 176, 161, 153) were oxidized faster than α -anomers (50, 177, 162, 154). The rate of periodate consumption by these β -nucleosides after 1 min is ~1.2 to ~1.6 times faster than for the α -anomers. The formation of a covalently linked, cyclic bidendate complex between the oxidant and a glycol gives the obligatory intermediate.^{55,56} Any steric effect that would hinder the formation of this intermediate or inhibit the approach of periodate would reduce the initial rate.⁵⁶ Hence, the observed difference in the velocity of periodate consumption by anomeric pairs of ribonucleosides can be explained by the steric hindrance of the α structure. This also accounts for the slower oxidation of lyxo-A (140) relative to α -adenosine (50) since in the former, both 4'-CH₂OH and the chromophore are on the approach-side for the attack of periodate.

The results of the present study give the following order for the oxidation rate (Scheme VIII). However, the rapid oxidation of all nucleosides having a cis 2',3'-diol structure (~20 min. completion) suggests that α and β -anomers may not be distinguishable with higher con-



Scheme VIII

centrations of periodate. These results demonstrate a new method of assigning the anomeric configurations of a given anomeric pair of ribonucleosides using spectrophotometric measurement of the rate of periodate reduction at a concentration of $\sim 10^{-7}$ M IO_4^- .

EXPERIMENTAL

GENERAL PROCEDURES

Melting points were taken on a Reichert microstage apparatus and are uncorrected. A Cary 15 spectrophotometer was used for the determination of UV spectra and reduction rates of periodate by nucleosides. CD and ORD data were obtained on a Jasco ORD-UV-5 (CD ss-20) spectropolarimeter at room temperature (23-25°C) using a 1 cm cell. Optical rotations were taken on a Perkin Elmer Model 141 polarimeter using a 1 dm, 1 ml microcell. Proton NMR spectra were recorded on Varian HA-100 (100 MHz) or Perkin Elmer R32 (90 MHz) instruments in DMSO-d₆ with TMS as an internal standard or D₂O with DOH as standard (δ 4.8 ppm). Electron impact mass spectra were obtained by the mass spectrometry laboratory of this department on an AEI MS-50 instrument using direct probe sample introduction at 150-200°C. Chemical ionization mass spectra were obtained on an AEI MS-12 instrument using ammonia as the ionizing gas. Elemental analyses were determined by the microanalytical laboratory of this department.

Electrophoresis was performed on Whatman No. 1 paper using a Savant flatplate apparatus (HV-3000 A). Evaporations were effected using a Buchler rotary evaporator equipped with a dry-ice condenser under water aspirator

or oil pump vacuum. Hydrogenations were effected using a Parr shaking apparatus. Diffusion crystallization¹²⁹ was carried out using alcohol with ether diffusion. Silica gel column chromatography was performed on JT Baker No. 3405 silica gel (60-200 mesh), and alumina column chromatography on Merck Aluminum oxide (acid-washed). Anion and cation exchange chromatography were carried out using Dow chemical Dowex 1x2 (OH⁻) and Dowex 50Wx8 (H⁺) resins, respectively. Barneby-Cheney AU-4 charcoal for absorption of nucleosides and column chromatography was conditioned prior to use by heating at reflux with 1N HCl for several hours, washing with water to neutrality, refluxing with 1N NaOH for several hours, again washing with water to neutrality, and then washing with methanol, chloroform, and air drying. All solvents used were of reagent grade. Solvents (water, methanol, DMF, pyridine) used for spectrophotometric and polarimetric study were further purified. Water was refluxed over potassium permanganate and distilled twice. Aqueous solutions of specified pH and buffer solutions (pH meter) were prepared using this water. Methanol was refluxed over and distilled from magnesium metal turnings. DMF was redistilled. Pyridine was dried over potassium hydroxide pellets for 18 hours and then distilled. Thin layer chromatography (TLC) was performed on Merck precoated plastic sheets (0.2 mm) or glass plates (0.25 mm, silica gel 60 F-254).

Developed chromatograms were evaluated under UV light (254 or 366 nm) or by spraying with a 5% solution of H_2SO_4 in ethanol and heating the plate to 100-200°C. Solvent systems used for TLC were Solvent S_1 : the upper phase of ethyl acetate/n-propanol/ H_2O (4:1:2), solvent S_2 : isopropanol/chloroform/conc. aqueous ammonia (10:10:1), solvent S_3 : methanol/chloroform (3:7). Sodium metaperiodate (BDH Chemicals, LTD., England) was used for all periodate oxidations, and sodium borohydride (Fischer Scientific Company, U.S.A.) was used for all reductions of the periodate oxidation products.

NUCLEOSIDE MATERIALS

5-Azacytidine was purchased from Aldrich Chemical Company, Inc. 8-Azaguanosine and 8-azaadenosine were kindly provided by Dr. J.A. Montgomery, Southern Research Institute. α -Adenosine, 7- β -D-ribofuranosyltheophylline, 7- α -D-ribofuranosyltheophylline, α -L-uridine-5'-phosphate dilithium salt, β -L-uridine-5'-phosphate dilithium salt, and α -L-cytidine-5'-phosphate dilithium salt were kindly provided by Dr. B. Shimizu and Dr. M. Kaneko, Sankyo Co., LTD., Japan. Other compounds were available in our laboratory or were synthesized as described (vide infra).

PREPARATION OF NUCLEOSIDES_{OX-RED} (in situ), THEIR OPTICAL ROTATION MEASUREMENT AND PAPER ELECTROPHORESIS

Preparation of nucleosides_{OX-red} (in situ)

Method 1. To a nucleoside ($\leq 80 \mu\text{mol}$) in a 2 ml volumetric test tube was added a sodium periodate solution (1.2 ml, 1.2-1.5 eq.), and the mixture was stirred magnetically at room temperature while protected from light for 1.5 hr. Sodium borohydride (6-8 mg) was added and the solution was stirred for 30 min. Excess sodium borohydride was destroyed by the slow addition of 1M sodium dihydrogen phosphate buffer (pH 6.5) (~0.5 ml), and the solution was stirred for 30 min. The volume was then adjusted to exactly 2 ml by addition of the buffer solution and stirred for an additional 30 min before subjection to the optical rotation measurement. Nucleosides_{ox-red} (in situ) for ORD measurement were prepared in the same manner but eight to ten times less nucleoside was used. A reference solution for the ORD measurement was made following the same procedure with omission of the nucleoside.

Method 2. Ara-A, xylo-A and ara-C (as its HCl salt) were treated in the same manner except the oxidation was continued for 4 days using ~1.5 equivalent of the periodate solution.

Optical rotation measurements

Optical rotations of all nucleosides_{ox-red} (in situ) were measured at room temperature (23-26°C). The

reference solution showed no optical rotation and was used as a base line for the ORD measurement. Specific rotations were calculated based upon the theoretical weight of nucleosides_{ox-red} produced. Specific rotations were obtained for all nucleosides_{ox-red} (in situ) at the wavelength of the sodium D line (589 nm). ORD curves (300-600 nm) and/or specific rotations at wavelengths other than 589 nm (365, 436, 546 and 578 nm bands of mercury) were recorded to examine whether nucleosides_{ox-red} (in situ) gave negative or positive Cotton effects (or plain curves) in these regions.

Paper electrophoresis

Paper electrophoresis of all nucleosides_{ox-red} (in situ) and their precursor nucleosides was performed on Whatman No. 1 paper (25.5 x 56.5 cm) using 0.1M sodium borate at pH 9.3, at 1.2-1.5 KV for 1.5 hr. Solutions of the compounds were applied as spots 10 cm from the cathodic end of a paper. Migrations were measured relative to that of uridine as a standard (which migrated ~12 cm toward the anode). Each nucleoside_{ox-red} (in situ) migrated as a single spot. All ox-red products were observed under UV light (254 nm) except 5,6-dihydrouridine_{ox-red} (in situ) and its precursor nucleoside, which were detected by the method of Fink et al,¹³⁰ and methyl B-D-ribofuranoside_{ox-red} (in situ) and its precursor

glycoside.

A compilation of the above results are summarized in Table III.

PH AND SOLVENT EFFECTS ON OPTICAL ROTATIONS OF
ADENOSINE_{OX-RED} (43), TUBERCIDIN_{OX-RED} (209), INOSINE_{OX-RED}
(135) AND URIDINE_{OX-RED} (136)

Aqueous solutions used were: pH 1 (0.1N HCl), pH 3 (0.1M NaH₂PO₄ adjusted with 0.1N HCl), pH 5 (0.1M NaH₂PO₄ adjusted with 0.1N NaOH), pH 7 (0.1M NaH₂PO₄ adjusted with 0.1N NaOH), pH 9 (0.1M Na₂HPO₄ adjusted with 0.1N NaOH), pH 11 (0.1M Na₂HPO₄ adjusted with 0.1N NaOH) and pH 13 (0.1N NaOH adjusted with 1N NaOH).

Water, methanol, N,N-dimethylformamide (DMF), and pyridine were used in the solvent effect study on optical rotations of the title compounds. Since the quantity of crystalline inosine_{ox-red} (10-14 mg/2 ml) used was not readily soluble in methanol (other than aqueous methanol), only water, DMF and pyridine were used. Each sample was evaluated in the following way: Methanol solutions of adenosine_{ox-red}, tubercidin_{ox-red} and uridine_{ox-red} were prepared. Exact aliquots of each solution were pipetted into 2 ml volumetric test tubes and the methanol was evaporated at room temperature. The test tubes containing the residue were dried over P₂O₅ under vacuum at room temperature overnight. The concentration of the aliquot

taken was redetermined by the extinction coefficient of nucleoside_{ox-red} (adenosine_{ox-red} ϵ 15,200 at 260 nm in H₂O, tubercidin_{ox-red} ϵ 11,500 at 270 nm in H₂O, and uridine_{ox-red} ϵ 10,100 at 262 nm in H₂O). Inosine_{ox-red} was weighed directly into the 2 ml volumetric test tubes. Each sample was dissolved in 2 ml of the appropriate pH solution or solvent. Optical rotations were measured at room temperature (23-24°C) at 365, 436, 546, 578 and 589 nm. After the measurements, paper electrophoresis was performed to confirm that there was no decomposition or hydrolysis of the compounds in the pH solutions and solvents used.

The results are summarized in Table V-VIII.

RATES OF PERIODATE REDUCTION BY NUCLEOSIDES

The rate of reduction of periodate by nucleosides (adenosine and its α -anomer, lyxo-A, uridine and its α -anomer, cytidine and its α -anomer, 7- β -D-ribofuranosyltheophylline and its α -anomer, ara-A, xylo-A, ara-C) was estimated spectrophotometrically by the method of Dixon and Lipkin.¹²³

Method 1. Nucleosides with the readily oxidized 2',3'-cis glycol unit (adenosine and its α -anomer, lyxo-A, uridine and its α -anomer, cytidine and its α -anomer, 7- β -D-ribofuranosyltheophylline and its α -anomer) were followed over a 20 minutes time course.

Four quartz cells (1-cm path length, ~1.5 ml cell capacity) were used. Cell₁ was filled with water and used as a blank. A 0.6 ml aliquot of aqueous nucleoside solution (containing 10^{-7} mole) was added to both cell₂ and cell₄. Water (0.6 ml) was added to both cell₂ and cell₃. A 0.6 ml aliquot of 4.113×10^{-4} M NaIO₄/H₂O (2.468×10^{-7} mole) was added to cell₃. The solutions in cell₂ and cell₃ were mixed (independently) and their absorbances were recorded. Then, 0.6 ml of the same periodate solution was added to cell₄, the contents was mixed rapidly and the absorbance was taken immediately and at regular time intervals (min). An appropriate wavelength was chosen where the absorbance of the nucleoside was minimal and the absorption of periodate anion was close to its maximum value (the periodate anion has a maximum absorption at $\sim 223 \text{ nm}^{131}$). When the absorbance of cell₄ had declined to a constant value, the oxidation was considered to be complete. Reduction of the equivalents of periodate per nucleoside at a given time was calculated as follows;

$$A_{\text{red}} = (A_{\text{cell}_2} + A_{\text{cell}_3}) - A_{\text{cell}_4} \text{ (at the constant wavelength)}$$

where A_{red} ; the decrease in absorbance by reduction of periodate

A_{cell_2} ; the absorbance of cell₂

A_{cell_3} ; the absorbance of cell₃

A_{cell_4} ; the absorbance of cell₄

$\frac{A_{red}}{A_{cell_3 \text{ at zero time}}} = \text{the periodate fraction which was reduced}$

Thus,

$\frac{A_{red}}{A_{cell_3 \text{ at zero time}}} \times \frac{\text{added periodate (mole)}}{\text{added nucleoside (mole)}} = \frac{\text{the ratio of equivalents of reduced periodate}}{\text{per nucleoside}}$

The absorbance of $cell_3$ was monitored over a period of 25 min to ascertain any change in absorption of periodate anion due to changes in temperature.^{123,131} However, there was but a negligible change.

Method 2. Nucleosides with a slowly oxidized 2',3'-trans glycol unit (xylo-A, ara-A, ara-C) were studied by an analogous method over a period of several days. Aliquots (5 ml) of a nucleoside solution ($\sim 10^{-6}$ mole) were added to 10 ml volumetric flask₁ and flask₃. Water (5 ml) was added to 10 ml volumetric flask₁ and flask₂. A 5 ml portion of periodate solution ($\sim 10^{-6}$ mole) was then added to flask₂ and flask₃. All the solutions were mixed well and protected from light except while rotations were being measured. The absorbances of all the solutions were recorded every 24 hr using water as a blank. The periodate reduction was calculated as in method 1.

The results are summarized in Table IX.

SYNTHESESPreparation of 6-methylcytidine (178)

This compound was prepared by the general Hibert-Johnson silyl ether method of Niedballa and Vorbrüggen.¹³²

6-Methylcytosine¹³³ (1.25 g, 10 mmole) was silylated by refluxing for 3 hr with hexamethyldisilazane (HMDS) (6 ml) and trimethylchlorosilane (TMCS) (1 ml) with exclusion of moisture. Excess HMDS and TMCS were removed under reduced pressure to leave the silylated 6-methylcytosine as a crystalline solid. A solution of 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose (4 g, 8 mmole) in dry CH_3CN (60 ml) was added to the silylated 6-methylcytosine, and the mixture was stirred. Then, the mixture was cooled in an ice bath, and stannic chloride (1.3 ml) in dry CH_3CN (8 ml) was added. An additional portion of stannic chloride (1 ml) in dry CH_3CN (8 ml) was added after 1.5 hr. The reaction mixture was stirred at -25°C for 12 hr and evaporated under reduced pressure at -25°C . To this residue was added 1,2-dichloroethane (200 ml) and the solution was treated with saturated aqueous sodium bicarbonate solution (300 ml). The resulting emulsion was filtered through a Celite pad. The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on a column of alumina (18 x 4.5 cm) which was eluted with ethyl acetate/

n-hexane (2:1) (700 ml), followed by ethyl acetate/
 methanol (9:1) to give 2.93 g of 1-(2,3,5-tri-O-benzoyl-
 β -D-ribofuranosyl)-6-methylcytosine as a solid foam. To
 1.12 g of this foam was added methanol (50 ml) saturated
 with ammonia at 0°C. The solution was sealed in a steel
 bomb and heated at 60°C overnight. The solution was
 evaporated to dryness and the residue was partitioned
 between water (10 ml) and 1,2-dichloroethane (20 ml).
 The aqueous layer was concentrated to a small volume and
 applied to a column (21 x 2.5 cm) of Dowex 1x2 (OH⁻).
 The column was eluted with water. The appropriate frac-
 tions were collected and evaporated to dryness. The
 residue was crystallized from methanol/water to give 319
 mg of 178. The remainder of the 6-methylcytidine tri-
 benzoate was treated in the same manner to give 725 mg
 of 178 (overall yield, 1.044 g, 51% from 1-O-acetyl-2,
 3,5-tri-O-benzoyl- β -D-ribofuranose): mp 233-235°C;
 $[\alpha]_D^{23}$ -47.4° (c 0.18, H₂O) [lit.¹³³ mp 230-232°C dec.,
 $[\alpha]_D^{30}$ -41.5° (c 1.55, H₂O)]; CD (0.0739 mmole/l, H₂O,
 room temperature) $[\theta]_{270}$ -0.5 x 10³, $[\theta]_{250}$ 1.2 x 10³,
 $[\theta]_{220}$ -13.5 x 10³ [lit.^{18e} CD (H₂O) $[\theta]_{270}$ -1.2 x 10³,
 $[\theta]_{247}$ 0.8 x 10³, $[\theta]_{214}$ -15.0 x 10³]; UV (H₂O)_{max} 270 nm
 (ε 9,600), 233 nm (ε 9,800), (0.1N HCl)_{max} 277 nm
 (ε 14,500), (0.1N NaOH)_{max} 272 nm (ε 9,700), 231 nm
 (ε 9,300); NMR (DMSO-d₆) δ 2.23 (s, 3, 6-CH₃), 3.52 (m,
 2, two 5'-H's), 3.72 (m, 1, 4'-H), 4.09 (m, 1, 3'-H), 4.60

(m, 1, 2'-H), 4.85 (m, 2, two OH's), 5.03 (d, 1, $J = 6$ Hz, OH), 5.47 (d, 1, $J_{1'-2'} = 5$ Hz, 1'-H), 5.57 (s, 1, 5-H), 7.10 (s, 2, NH_2); MS m/e 240.0747 ($\text{M}^+ - \text{NH}_3$), calcd for $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_5$: 240.0746. Anal. Calcd. for $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_5$: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.90; H, 5.99; N, 16.29.

Preparation of 3- β -D-ribofuranosyl-6-methyluracil (171)
and 1,3-bis(β -D-ribofuranosyl)-6-methyluracil (172)

An attempt to prepare 6-methyluridine by a reported method^{132b} gave only the title compounds.

6-Methyluracil¹³⁴ (1.28 g, 10 mmole) was silylated with HMDS (6 ml) and TMCS (1 ml) by refluxing the mixture for 2 hr with exclusion of moisture. After removal of excess HMDS and TMCS, the silylated 6-methyluracil was distilled to obtain 2.5 g of an oil. To this oil in an ice bath was added 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose (4 g, 8 mmole) in dry CH_3CN (120 ml) and stannic chloride (1.3 ml) in dry CH_3CN (60 ml) with stirring. The solution was then stirred at 22°C for 4 hr. After work up,^{132b} the oily residue was chromatographed on an alumina column (22 x 4.5 cm). Elution was effected with *n*-hexane/ethyl acetate (9:1) (600 ml), ethyl acetate/*n*-hexane (2:1) (700 ml) and finally ethyl acetate/methanol (9:1), and 18 ml fractions were collected. Fractions 47-60 were combined and evaporated to give 0.88 g of 1,3-bis-

(2,3,5-tri-0-benzoyl- β -D-ribofuranosyl)-6-methyluracil
as a foam.

This foam was treated with absolute methanol (25 ml) and sodium (0.12 g) was added. The mixture was sealed and kept overnight at room temperature. The resulting solution was evaporated to a small volume under reduced pressure at room temperature. Water (30 ml) was added and the solution was neutralized with Dowex 50W x 8 (H^+) resin. The resin was filtered and the solution was extracted with CH_2Cl_2 (20 ml x 2). The aqueous layer was evaporated to give 295 mg of 172 as a foam (19% from 1-0-acetyl-2,3,5-tri-0-benzoyl- β -D-ribofuranose): TLC R_f = 0.18 (solvent S_1), R_f = 0.48 (solvent S_3); UV (H_2O)_{max} 265 nm (ϵ 11,200), 270 nm (sh) (ϵ 8,900); ORD (c 0.022, H_2O , room temperature) $[\alpha]_{283} -2,600$, $[\alpha]_{258} 2,300$, $[\alpha]_{227} -7,700$ [lit. ^{135a} UV (pH 6.7)_{max} 265 nm ($\log \epsilon$ 4.07), 2.08 nm ($\log \epsilon$ 3.96), ORD (c 0.075) $[\alpha]_{283} -5,300$, $[\alpha]_{258} 5,100$, $[\alpha]_{227} -12,900$]; NMR ($DMSO-d_6$) δ 2.27 (s, 3, 6- CH_3), 3.30-5.20 (m, 16, sugar protons), 5.46 (d, 1, $J_{1'-2'} = 4$ Hz, 1'-H of N_1 -ribo-syl), 5.66 (s, 1, 5-H), 6.02 (d, 1, $J_{1'-2'} = 4$ Hz, 1'-H of N_3 -ribosyl).

Fractions 80-105 were combined and evaporated to give 2.54 g of 3-(2,3,5-tri-0-benzoyl- β -D-ribofuranosyl)-6-methyluracil as a foam. This was debenzoylated using sodium in methanol, as described above (for 172), to give 980 mg of 171 as a foam (48% from 1-0-acetyl-2,3,5-tri-

O-benzoyl-B-D-ribofuranose): TLC $R_f = 0.36$ (solvent S_1),
 $R_f = 0.61$ (solvent S_3); $[\alpha]_D^{23} -26.6^\circ$ (c 0.12, H_2O); UV
 $(H_2O)_{max}$ 264 nm (ϵ 7,600), $(0.1N\ HCl)_{max}$ 264 nm (ϵ 7,400),
 $(0.1N\ NaOH)_{max}$ 289 nm (ϵ 7,900), 224 nm (ϵ 5,900) [lit.¹³³
 $[\alpha]_D^{27} -27.6^\circ$ (c 1, H_2O), UV (pH 1 and pH 4) $_{max}$ 265 nm
(ϵ 8,700), (pH 11) $_{max}$ 289 nm (ϵ 9,800); (pH 14) $_{max}$ 289 nm
(ϵ 11,300)]; CD (c 0.0724 mmole/l, H_2O , room temperature)
 $[e]_{270} -1.7 \times 10^3$, $[\theta]_{244} 2.6 \times 10^3$ [lit.^{18b} $[\theta]_{268} -4.3 \times$
 10^3 , $[e]_{242} 6.2 \times 10^3$]; NMR (DMSO- d_6) δ 2.00 (d, 3, $J <$
1 Hz, 6- CH_3), 3.30-3.80 (m, 3, 4-H and two 5'-H's), 4.08
(t, 1, $J = 6$ Hz, 3'-H), 4.43 (dd, 1, $J_{1,-2} = 4$ Hz,
 $J_{2,-3} = 6$ Hz, 2'-H), 5.37 (d, 1, $J < 1$ Hz, 5-H), 6.04
(d, 1, $J_{1,-2} = 4$ Hz, 1'-H), 11.0 (bs, 1, NH); MS m/e
240.0738 ($M^+ - H_2O$), calcd for $C_{10}H_{12}N_2O_5$: 240.0746.

Preparation of 6-methyluridine (173)

This compound was prepared by the method of Winkley and Robins.¹³³

To 6-methylcytidine (220 mg, 0.86 mmole) in 2N acetic acid (35 ml) was added sodium nitrate (593 mg, 8.6 mmole), and the reaction mixture was sealed and stirred at room temperature for 2 days. The solution was neutralized with 1N sodium hydroxide solution and the volume was reduced by evaporation at 35°C. AU-4 charcoal (2.5 g) was added and the mixture was stirred for 4 hr. The charcoal was collected, washed with water, and

extracted for 15 hr with ethanol/conc. aqueous ammonia (9:1) (150 ml) in a soxhlet apparatus. The extract was evaporated to dryness. The residue was dissolved in water and applied to a column (18 x 2.5 cm) of Dowex 50Wx8 (H⁺). The column was eluted with water and the appropriate fractions were collected and evaporated. The residue was crystallized from methanol with ether diffusion to give 50 mg (22%) of 173: mp 178-180°C; $[\alpha]_D^{23}$ -45.8° (c 0.07, H₂O) [lit. ¹³³ mp 177-178°C, $[\alpha]_D^{30}$ -28.6° (c 1.52, H₂O), lit. ^{135a} mp 179-181°C]; CD (c 0.0759 mmole/l, H₂O, room temperature) $[\theta]_{265} -0.3 \times 10^3$, $[\theta]_{250} 0.8 \times 10^3$, $[\theta]_{214} -9.9 \times 10^3$ [lit. ^{18e} $[\theta]_{265} -0.3 \times 10^3$, $[\theta]_{250} 1.0 \times 10^3$, $[\theta]_{214} -10.0 \times 10^3$]; NMR (DMSO-d₆) δ 2.26 (s, 3, 6-CH₃), 3.40-3.80 (m, 3, 4'-H and two 5'-H's), 4.06 (q, 1, J = 6 Hz, 3'-H), 4.58 (m, 2, 2'-H was overlapped with OH), 4.90 (d, 1, J = 6 Hz, OH), 5.17 (d, 1, J = 6 Hz, OH), 5.44 (d, 1, J_{1'-2'} = 4 Hz, 1'-H), 5.54 (s, 1, 5-H), 11.20 (s, 1, NH); MS m/e 227.0672 (M⁺-CH₂OH), calcd for C₉H₁₁N₂O₅: 227.0668: Anal. Calcd for C₁₀H₁₄N₂O₆·1/4 H₂O; C, 45.72; H, 5.56; N, 10.66. Found: C, 45.87; H, 5.50; N, 10.42.

Preparation of 6-azauridine (182)

The method of Niedballa and Vorbrüggen^{132a} gave 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-azauridine in 80% yield. Methanolysis of this 6-azauridine tribenzoate (1.12 g, 2 mmole) using sodium methoxide (6 mmole) in

absolute methanol (30 ml) followed by crystallization of the product from methanol gave 420 mg (85%) of 182: mp 159-160°C; $[\alpha]_D^{23}$ -134.7° (c 0.13, pyridine) [lit. ^{135b} mp 158°C, $[\alpha]_D^{25}$ -132° (c 0.5, pyridine)]; UV (H₂O)_{max} 262 nm (ε 6,400); CD (c 0.127 mmole/l, H₂O, room temperature) $[\theta]_{260} -12.0 \times 10^3$ [lit. ^{18e} $[\theta]_{257} -10.0 \times 10^3$]; NMR (DMSO-d₆) δ 3.44 (m, 2, two 5'-H's), 3.78 (m, 1, 4'-H), 4.00 (m, 1, 3'-H), 4.20 (m, 1, 2'-H), 4.60 (m, 1, OH), 4.98 (bs, 1, OH), 5.20 (bs, 1, OH), 5.90 (d, 1, J_{1'-2'} = 4 Hz, 1'-H), 7.56 (s, 1, 5-H); MS m/e 246.0723 (M⁺+1), calcd for C₈H₁₂N₃O₆: 246.0726. Anal. Calcd for C₈H₁₁N₃O₈: C, 39.19; H, 4.52; N, 17.14. Found: C, 39.06; H, 4.63; N, 17.09.

Preparation of 3-methyl-6-azauridine (184).

A mixture of 6-azauridine (247 mg, 1 mmole), N,N'-dimethylformamide dimethyl acetal (0.96 g, 8 mmole) and dry chloroform (5 ml) was gently refluxed for 7 hr while protected from moisture. The resulting solution was evaporated to a syrup, which was dissolved in water (6 ml) and kept overnight. The aqueous solution was evaporated and the product was purified on a preparative TLC plate (Whatman PLK5F, silica gel, 20 x 20 cm) using solvent system S₁. The main UV absorbing zone (R_f = 0.65) was collected and the product eluted with methanol. Evaporation of the methanol extract and crystallization of the

residue from methanol with ether diffusion gave 209 mg (80%) of 184: mp 125-127°C; $[\alpha]_D^{23}$ -125.6° (c 0.11, pyridine) [lit. ¹³⁶ mp 124°C, $[\alpha]_D^{25}$ -128° (c 1.3, pyridine)]; UV (MeOH)_{max} 263 nm (ε 6,500); NMR (DMSO-d₆) δ 3.18 (s, 3, CH₃), 3.47 (m, 2, two 5'-H's), 3.82 (q, 1, J = 5 Hz, 4'-H), 4.04 (t, 1, J = 5 Hz, 3'-H), 4.27 (dd, 1, J_{1'-2'} = 3.5 Hz, J_{2'-3'} = 5 Hz, 2'-H), 4.96 (bs, 3, three OH's), 5.97 (d, 1, J_{1'-2'} = 3.5 Hz, 1'-H), 7.66 (s, 1, 5-H); MS m/e 241.0701 (M⁺-H₂O), calcd for C₉H₁₁N₃O₅: 241.0698. Anal. Calcd for C₉H₁₃N₃O₆: C, 41.70; H, 5.06; N, 16.21. Found: C, 41.62; H, 5.14; N, 16.24.

Preparation of 6-azacytidine (183)

6-Azacytidine was prepared by the method described above for 6-methylcytidine (178).

6-Azacytosine ¹³⁷ (300 mg, 2.7 mmole) was silylated as described in the above preparation of 178. After removal of excess silylating reagents, the product was suspended in dry 1,2-dichloroethane (3 ml) and treated with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (1.21 g, 2.4 mmole) in dry 1,2-dichloroethane (15 ml) in the presence of stannic chloride (0.3 ml), followed by additional stannic chloride (0.3 ml) after 2 hr. The reaction mixture was stirred at room temperature for a total of 20 hr. The reaction mixture was diluted with 1,2-dichloroethane (20 ml) and treated with aqueous saturated sodium

bicarbonate solution (40 ml). The resulting emulsion was filtered through a Celite pad and the organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The residue was applied to an alumina column (1.5 x 2.5 cm) and the column was eluted with ethyl acetate/*n*-hexane (2:1) (200 ml) followed by chloroform/methanol (9:1). Appropriate fractions were collected and evaporated to dryness to give a crystalline solid. This was recrystallized from 95% ethanol to give 1.18 g (88%) of 1-(2,3,5-tri-*O*-benzoyl- β -*D*-ribofuranosyl)-6-azacytosine (mp 219-220°C). A sample of this 6-azacytidine tribenzoate (550 mg, 0.99 mmole) in methanol (60 ml) saturated with ammonia at 0°C was sealed and kept at room temperature for 3 days. The solvent was evaporated, and the residue was dissolved in water (10 ml). The aqueous solution was extracted with CH₂Cl₂ (10 ml x 3) and evaporated. The residue was crystallized from methanol to give 217 mg (90%) of 183: mp 222-224°C [lit.¹³⁸ mp 222-224°C (dec)]; $[\alpha]_D^{23}$ -127.8° (c 0.08, H₂O); UV (H₂O)_{max} 263 nm (ϵ 7,800), (0.1N HCl)_{max} 279 nm (ϵ 6,800), (0.1N NaOH)_{max} 275 nm (ϵ 5,600), 225 nm (ϵ 9,300); CD (c 0.1049 mmole/l, H₂O, room temperature) $[\theta]_{270}$ -6.5 x 10³; NMR (DMSO-d₆) δ 3.40 (m, 2, two 5'-H's), 3.76 (q, 1, J = 5 Hz, 4'-H), 3.97 (t, 1, J = 5 Hz, 3'-H), 4.20 (t, 1, J = 5 Hz, 2'-H), 4.62 (bs, 1, OH), 4.96 (bs, 2, two OH's), 5.98 (d, 1, J_{1'-2'} = 5 Hz, 1'-H), 7.50 (s, 1, 5-H), 7.90 (bs, 2, NH₂); MS m/e 226.0697 (M⁺-H₂O),

calcd for $C_8H_{10}N_4O_4$: 226.0702. Anal. Calcd for $C_8H_{12}N_4O_5$:
C, 39.35; H, 4.95; N, 22.94. Found: C, 39.14; H, 4.82;
N, 23.24.

Preparation of 6-methyl-2-β-D-ribofuranosyl-3-
pyridazone (188)

To a solution of 6-methyl-3-(2H)-pyridazone¹⁴⁴ (300 mg, 2.7 mmole) and 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (1.36 g, 2.7 mmole) in dry CH_3CN (30 ml) was added stannic chloride (0.4 ml) and the solution was stirred at room temperature for 24 hr with exclusion of moisture. Additional stannic chloride (0.4 ml) was added and the solution was stirred at room temperature for 24 hr. The solution was evaporated at 30°C under reduced pressure. The residue was dissolved in methylene chloride (40 ml) and treated with saturated aqueous sodium bicarbonate solution (40 ml). The resulting emulsion was filtered through a Celite pad. The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on a silica gel column (18 x 2.5 cm). Elution with ethyl acetate/n-hexane (2:1) and crystallization from 95% ethanol gave 700 mg (47%) of 6-methyl-2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-3-pyridazone: mp 120-121°C; Anal. Calcd for $C_{31}H_{26}N_2O_8$: C, 67.14; H, 4.73; N, 5.05. Found: C, 67.42; H, 4.86; N, 4.97.

The above compound (320 mg, 0.58 mmole) was de-benzoylated as described in the above preparation of 6-azacytidine (183). Crystallization of the product from methanol with ether diffusion gave 112 mg (79%) of 188: mp 103-105°C; $[\alpha]_D^{23} -105.9^\circ$ (c 0.09, H₂O); UV (H₂O)_{max} 290 nm (ε 3,500), (0.1N HCl)_{max} 290 nm (ε 3,500), (0.1N NaOH)_{max} 293 nm (ε 3,500); CD (c 0.1680 mmole/L, H₂O, room temperature) $[\theta]_{288} -4.2 \times 10^3$, $[\theta]_{236} -6.3 \times 10^3$, $[\theta]_{216} -10.7 \times 10^3$; NMR (D₂O) δ 2.43 (s, 3, CH₃), 3.89 (m, two 5'-H's), 4.24 (m, 1, 4'-H), 4.55 (t, 1, J_{2'-3'} = 5.5 Hz, 3'-H), 4.67 (dd, 1, J_{1'-2'} = 3 Hz, J_{2'-3'} = 5.5 Hz, 2'-H), 6.43 (d, 1, J_{1'-2'} = 3 Hz, 1'-H), 7.04 (d, 1, J₄₋₅ = 10 Hz, 4-H), 7.49 (d, 1, J₄₋₅ = 10 Hz, 5-H); M_s m/e 243.0976 (M⁺+1), calcd for C₁₀H₁₅N₂O₅: 243.0980; 242.0897 (M⁺), calcd. for C₁₀H₁₄N₂O₅: 242.0902. Anal. Calcd for C₁₀H₁₄N₂O₅ · 1/3 H₂O: C, 48.39; H, 5.96; N, 11.28. Found: C, 48.42; H, 5.80; N, 11.46.

Preparation of 2-β-D-ribofuranosyl-3-pyridazone (189)

This compound was prepared in an analogous manner to the above preparation of 6-methyl-2-β-D-ribofuranosyl-3-pyridazone (188).

To a solution of 3(2H)-pyridazone¹⁴⁵ (255 mg, 2.7 mmole) and 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (1.31 g, 2.6 mmole) in dry CH₃CN (30 ml) was added stannic chloride (0.4 ml), and the solution was stirred at room temperature

for 24 hr with exclusion of moisture. Additional stannic chloride (0.4 ml) was added and the solution was stirred at room temperature for 48 hr. After work up, the residue was chromatographed on a silica gel column (19 x 2.5 cm). Elution with ethyl acetate/n-hexane (2:1) and crystallization of the product from ethyl acetate gave 165 mg (12%) of 2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-3-pyridazine: mp 110-112°C (lit.¹¹³ mp 117-118°C); Anal. Calcd for C₃₀H₂₄N₂O₈: C, 66.66; H, 4.48; N, 5.18. Found: C, 66.36; H, 4.58; N, 5.08.

The above compound (160 mg, 0.30 mmole) in methanol (30 ml) saturated with ammonia at 0°C was sealed and kept at room temperature for 3 days. After work up, crystallization of the product from methanol with ether diffusion gave 52 mg (77%) of 189: mp 168-170°C; $[\alpha]_D^{23}$ -132.1° (c 0.08, H₂O) [lit.¹³⁹ mp 171.5-173.5°C; $[\alpha]_D^{20}$ = -132.3° (c 2.0, H₂O)]; UV (H₂O)_{max} 284 nm (ε 4,000), (0.1N HCl)_{max} 285 nm (ε 4,200), (0.1N NaOH)_{max} 286 nm (ε 3,900); CD (c 0.1709 mmole/l, H₂O, room temperature) $[\theta]_{283}$ -5.4 x 10³, $[\theta]_{238}$ -6.3 x 10³, $[\theta]_{216}$ -10.4 x 10³ [lit.¹¹³ CD (H₂O) $[\theta]_{290}$ -4.9 x 10³, $[\theta]_{245}$ -6.2 x 10³, $[\theta]_{223}$ -10.1 x 10³]; NMR (D₂O) δ 3.85 (m, 2, two 5'-H's), 4.22 (m, 1, 4'-H), 4.51 (t, 1, J_{2'-3'} = 5.5 Hz, 3'-H), 4.69 (dd, 1, J_{1'-2'} = 3 Hz, J_{2'-3'} = 5.5 Hz, 2'-H), 6.43 (d, 1, J_{1'-2'} = 3 Hz, 1'-H), 7.13 (dd, 1, J₄₋₆ = 2 Hz, J₄₋₅ = 10 Hz, 4-H), 7.57 (dd, 1, J₄₋₅ = 10 Hz, J₅₋₆ = 4 Hz, 5-H), 8.15 (dd,

1, $J_{4-6} = 2$ Hz, $J_{5-6} = 4$ Hz, 6-H); MS m/e 229.0830 ($M^+ + 1$),
 calcd for $C_9H_{13}N_2O_5$: 229.0824; 228.0748 (M^+), calcd for
 $C_9H_{12}N_2O_5$: 228.0746. Anal. Calcd for $C_9H_{17}N_2O_5$: C,
 47.37; H, 5.30; N, 12.28. Found: C, 47.17; H, 5.15; N,
 12.17.

Preparation of 1,3-dimethylpseudouridine (190)

A mixture of pseudouridine (73 mg, 0.3 mmole) and N,N'-dimethylformamide dimethyl acetal (0.42 g, 3.5 mmole) in absolute methanol (5 ml) was refluxed for 24 hr while protected from moisture. The solution was evaporated and the residue was applied to a Dowex 1x2 (OH^-) column (9 x 2.5 cm). The column was eluted with water. Appropriate fractions were collected and evaporated to give a solid. Recrystallization of this product from methanol with ether diffusion gave 45 mg (56%) of 190: mp 177-179°C (lit.¹⁴⁰ 174°C); UV (MeOH)_{max} 270 nm (ϵ 8,800); NMR (DMSO- d_6) δ 3.17 & δ 3.30 (s & s, 3 & 3, N_1-CH_3 and N_3-CH_3), 3.40-3.80 (m, 3, two 5'-H's and 4'-H), 3.90 (m, 2, 2'-H and 3'-H), 4.51 (d, 1, $J_{1'-2'} = 4$ Hz, 1'-H), 4.60-5.00 (m, 3, three OH's), 7.78 (s, 1, 6-H); MS m/e 273.1075 ($M^+ + 1$), calcd for $C_{11}H_{17}N_2O_6$: 273.1084; 272.1006 (M^+), calcd for $C_{11}H_{16}N_2O_6$: 272.1008. Anal. Calcd for $C_{11}H_{16}N_2O_6$: C, 48.53; H, 5.92; N, 10.29. Found: C, 48.52; H, 6.00; N, 10.22.

Preparation of 5,6-dihydrouridine (187)

This compound was prepared by the reported method.¹⁴¹ Uridine (161) (488 mg, 2 mmole) in water (50 ml) was hydrogenated over 5% Rh-Al₂O₃ (100 mg) at 25 psi for 2 hr. The catalyst was filtered and the aqueous solution was evaporated to give an oil. The oily product was applied to a silica gel column (20 x 2 cm) and the column was eluted with methanol/chloroform (3:7). Fractions (10 ml) 4-16 were combined and evaporated to dryness under reduced pressure to leave a yellow foam. The foam was dissolved in aqueous methanol and decolorized with charcoal. The charcoal was filtered and the solvent was evaporated to give 473 mg (97%) of 187: TLC R_f = 0.38 (solvent S₃); [α]_D²³ -38.3° (c 0.05, H₂O) [lit.¹⁴¹ [α]_D²⁰ -36.8 ± 0.4° (c 2.12, H₂O)]; NMR (D₂O) δ 2.80 & 3.62 (t & t, 2 & 2, J₅₋₆ = 6.5 Hz, 5-CH₂ & 6-CH₂), 3.80 (m, 2, two 5'-H's), δ 4.00-4.44 (m, 3, 2'-H, 3'-H and 4'-H), 5.89 (d, 1, J_{1'-2'} = 6 Hz, 1'-H); MS m/e 228.0746 (M⁺-H₂O), calcd for C₉H₁₂N₂O₅: 228.0747. Anal. Calcd for C₉H₁₄N₂O₆·1/5 H₂O: C, 43.27; H, 5.81; N, 11.21. Found: C, 43.37; H, 5.69; N, 11.18.

Preparations of 1-methylformycin (155) and 2-methylformycin (156)

These compounds were prepared by the method of

Townsend et al.¹⁴² Better separation of the two compounds than that reported was effected on a Dowex 1x2 (OH⁻) column using stepwise elution with aqueous methanol as noted below.

A mixture of formycin (508 mg, 1.9 mmole) and sodium (56 mg, 2.4 mmole) in dry ethanol (12 ml) was stirred at room temperature for 30 min. Methyl iodide (270 mg, 1.9 mmole) was added to the resulting solution, followed by an additional portion of methyl iodide (270 mg, 1.9 mmole) after 1.5 hr. The solution was stirred overnight at room temperature and evaporated to dryness. The residue was dissolved in water and applied to a column of Dowex 1x2 (OH⁻) (20 x 4.5 cm). The column was eluted with water (600 ml), 10% aq. methanol (1200 ml) 30% aq. methanol (1200 ml) and finally 50% aq. methanol, and 6 ml fractions were collected. Fractions 390-468 were combined and evaporated to dryness. The residue was crystallized from methanol with ether diffusion to give 292 mg (55%) of 156; TLC R_f = 0.45 (solvent S₂); mp 206-207°C (lit.¹⁴² 205-206°C); $[\alpha]_D^{23}$ -93.0° (c 0.20, MeOH); UV (H₂O)_{max} 317 nm (sh) (ε 8,000), 305 nm (ε 12,200), 295 nm (sh) (ε 10,600), 234 nm (ε 5,600), (0.1N HCl)_{max} 306 nm (ε 10,000), 270 nm (ε 4,600), 260 nm (ε 4,700), 233 nm (ε 9,200), (0.1N NaOH)_{max} 318 (sh) (ε 8,100), 306 nm (ε 12,100), 296 (sh) (ε 10,400), 234 nm (sh) (ε 5,600); NMR (DMSO-d₆) δ 3.58 (m, 2, two 5'-H's), 3.90-4.30 (m, 2,

3'-H and 4'-H), 4.13 (s, 3, N-CH₃), 4.61 (m, 1, 2'-H), 4.99 (d, 1, J = 5 Hz, OH), 5.14 (m, 2, 1'-H was overlapped with OH, J_{1'-2'} = 7.5 Hz), 5.91 (m, 1, OH), 7.58 (s, 2, NH₂), 8.02 (s, 1, 5-H); MS m/e 281.1127 (M⁺), calcd for C₁₁H₁₅N₅O₄: 281.1124). Anal. Calcd for C₁₁H₁₅N₅O₄: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.79; H, 5.41; N, 25.05.

Fractions 553-732 were combined and evaporated to dryness. Crystallization from ethanol-water gave 81 mg (14%) of 155: TLC R_f = 0.25 (solvent S₂); mp 156°C (foaming), 218-219°C (completely melted) (lit.¹⁴² mp 170-173°C foams, dec. > 200°C, lit.¹⁴³ mp 96-98°C transition point, dec. > 200°C); [α]_D²³ -65.6° (c 0.12, MeOH); UV (H₂O)_{max} 315 nm (sh) (ε 6,600), 302 nm (ε 11,000), 294 nm (sh) (ε 10,500), 233 nm (ε 8,600), (0.1N HCl)_{max} 302 nm (ε 11,300), 239 nm (ε 10,100), (0.1N NaOH)_{max} 314 nm (sh) (ε 8,300), 302 nm (ε 11,500), 294 nm (sh) (ε 10,700), 232 nm (ε 8,000); NMR (DMSO-d₆) δ 3.57 (m, 2, two 5'-H's), 3.90-4.20 (m, 2; 3'-H and 4'-H), 4.19 (s, 3, N-CH₃), 4.48 (m, 1, 2'-H), 4.87 (m, 3, 1'-H was overlapped with two OH's, J_{1'-2'} = 7.5 Hz), 5.84 (m, 1, OH), 7.40 (s, 2, NH₂), 8.09 (s, 1, 5-H); MS m/e 281.1118 (M⁺), calcd for C₁₁H₁₅N₅O₄: 281.1124. Anal. Calcd for C₁₁H₁₅N₅O₄·H₂O: C, 44.15; H, 5.73; N, 23.40. Found: C, 43.91; H, 5.46; N, 23.26.

Preparation of α-L-uridine (163), β-L-uridine (164)
and α-L-cytidine (180) from their 5'-phosphate salts.

Each nucleoside-5'-phosphate dilithium salt (~10 mg) was dephosphorylated with bacterial alkaline phosphatase (0.1 ml, solution of enzyme supplied by Sigma Chemical Co.) in 0.5M tris(hydroxymethyl)aminomethane buffer (pH 8.0) (1.5 ml) at 37°C for 5 hr. The free nucleoside was purified by descending paper chromatography (Whatman No. 1 paper) using isopropanol/conc. aqueous ammonia/water (7:1:2) as developing solvent. The product was detected by UV light (254 nm); the zone that contained the main UV absorption was cut out (R_f of α -L-uridine 0.30, R_f of β -L-uridine 0.31, R_f of α -L-cytidine 0.29). Each free nucleoside was eluted from the paper strip with water to give α -L-uridine (4.47 mg), β -L-uridine (2.66 mg) and α -L-cytidine (5.95 mg). The above yields were estimated by using the UV extinction values of α -D-uridine¹²⁶ (ϵ 10,050 at 264 nm in H₂O) and uridine¹²⁶ (ϵ 10,300 at 262 nm in H₂O), respectively, and cytidine (ϵ 9,100 at 270 nm in H₂O).

α -L-uridine: $[\alpha]_D^{24} +58.0^\circ$ (c 0.22, H₂O) [lit.¹²⁶ α -D-uridine $[\alpha]_D -68^\circ$ (c 1.0, H₂O)]. β -L-uridine: $[\alpha]_D^{24} -8.3^\circ$ (c 0.13, H₂O) [lit.¹²⁶ β -D-uridine $[\alpha]_D +4.6^\circ$ (c 5.2, H₂O)]. α -L-cytidine $[\alpha]_D^{24} +94.1^\circ$ (c 0.30, H₂O).

Preparation of 2-methylformycin_{ox-red} (157)

To 2-methylformycin (156) (140 mg, 0.5 mmole) was added sodium periodate (128 mg, 0.6 mmole) dissolved in

water (10 ml). The mixture was stirred at room temperature while protected from light for 1.5 hr. Sodium borohydride (40 mg, 1 mmole) was added to the solution and stirring was continued for 30 min at room temperature. Excess sodium borohydride was destroyed with 1M sodium dihydrogen phosphate buffer (pH 6.5) (4 ml), and the solution was stirred for 1 hr. AU-4 charcoal (3 g) was added to the solution and the mixture was stirred for 4 hr. The charcoal was collected by filtration, washed with water and packed into a column (20 x 2.5 cm).

The column was eluted with water (200 ml), followed by 50% aq. CH₃CN. The eluate was evaporated. The residue was dissolved in water and decolorized with charcoal. Evaporation of the water and crystallization of the residue from methanol with ether diffusion gave 103 mg (72%) of 157. Paper electrophoresis of this compound showed an identical anodic mobility (0.45, uridine as a standard) as 2-methylformycin_{ox-red} (in situ): TLC R_f = 0.18 (solvent S₁); mp 140-143°C; specific rotations (c 0.50, H₂O) $[\alpha]_{589}^{24} +56.8^\circ$, $[\alpha]_{578}^{24} +59.8^\circ$, $[\alpha]_{546}^{24} +67.6^\circ$, $[\alpha]_{436}^{24} +107.4^\circ$, $[\alpha]_{365}^{24} +130.0^\circ$; UV (H₂O)_{max} 317 (sh). (ϵ 7,900), 305 nm (ϵ 12,300), 295 nm (sh) (ϵ 10,800), 234 nm (sh) (ϵ 6,200), (0.1N HCl)_{max} 306 nm (ϵ 10,700), 270 nm (ϵ 5,000), 260 nm (ϵ 5,200), 233 nm (ϵ 9,500), (0.1N NaOH)_{max} 318 (sh) (ϵ 8,000) 306 nm (ϵ 12,100), 296 nm (sh) (ϵ 10,500), 234 nm (sh) (ϵ 6,400); NMR (DMSO-d₆)

δ 3.38 (m, 6, 2'-H₂, 3'-H₂, 5'-H₂), 4.19 (s, 3, N-CH₃),
 3.87 (t, 1, J = 5 Hz, 4'-H), 5.16 (t, 1, J = 6 Hz, 1'-H),
 7.50 (s, 2, NH₂), 8.04 (s, 1, 5-H); MS m/e 284.1356
 (M⁺+1), calcd for C₁₁H₁₈N₅O₄: 284.1359. Anal. Calcd
 for C₁₁H₁₇N₅O₄·1/3 H₂O: C, 45.67; H, 6.16; N, 24.21.
 Found: C, 45.68; H, 6.07; N, 24.13.

Preparation of 3-methyl-6-azauridine_{ox-red} (185)

3-Methyl-6-azauridine (184) (100 mg, 0.39 mmole)
 was treated in the same manner as described above for the
 preparation of 2-methylformycin_{ox-red} (157) to obtain
 89 mg of 185 as a syrup (85% yield estimated from the
 extinction coefficient of 3-methyl-6-azauridine (184);
 ϵ 6,500 at 263 nm in MeOH). Paper electrophoresis of
 this compound showed an identical anodic mobility (0.48,
 uridine as a standard) as 3-methyl-6-azauridine_{ox-red}
 (in situ); TLC R_f = 0.60 (solvent S₁), R_f = 0.68 (solvent
 S₃); UV (MeOH)_{max} 263 nm, specific rotations (c 0.52,
 H₂O) (concentration estimated using the extinction coef-
 ficient of 184) $[\alpha]_{589}^{23}$ -26.4°, $[\alpha]_{578}^{23}$ -27.8°, $[\alpha]_{546}^{23}$
 -33.6°, $[\alpha]_{436}^{23}$ -73.4°, $[\alpha]_{365}^{23}$ -162.3°; NMR (DMSO-d₆)
 δ 3.18 (s, 3, CH₃ overlapped with sugar proton), 3.10-3.80
 (m, 7, 2'-H₂, 3'-H₂, 5'-H₂ and 4'-H), 4.30-5.10 (bs, 3,
 three OH's), 5.89 (t, 1, J = 6 Hz, 1'-H), 7.64 (s, 1,
 5-H).

Preparation of adenosine_{ox-red} (43)^{108,114,121}

To adenosine (42) (1 g, 3.7 mmole) was added an 80 mM of solution of sodium periodate (50 ml, 4 mmole), and the mixture was stirred at room temperature for 2 hr while protected from light. The resulting solution was cooled in an ice bath and sodium borohydride (282 mg, 7.5 mmole) dissolved in water (10 ml) was added. The solution was stirred for 15 min in the ice bath followed by 45 min at room temperature. The solution was neutralized with acetic acid and stirred with AU-4 charcoal (7 g) until UV absorption indicated <5% of the nucleoside remained in solution. The charcoal was collected by filtration and washed with water (~200 ml). The crude product was eluted from the charcoal by continuous extraction for 2 days in a soxhlet apparatus with ethanol/conc. aqueous ammonia (9:1) (300 ml). After evaporation of the extract, the residue was dissolved in water and applied to a column of Dowex 1x2 (OH⁻) resin (35 x 2.5 cm). The column was washed with water (150 ml), and the product was eluted with 20% aq. methanol. Appropriate fractions were combined and evaporated. The residue was dissolved in aqueous ethanol and decolorized by treatment with charcoal. Evaporation of the solvent and crystallization of the residue from ethanol with ether diffusion gave 630 mg (63%) of 43: TLC R_f = 0.17 (solvent S₁);

mp 139-141°C; [lit.¹²¹ mp 143-145°C]; UV. (H₂O)_{max} 260 nm (ε 15,200), (0.1N HCl)_{max} 258 nm (ε 14,600), (0.1N NaOH)_{max} 261 nm (ε 15,000); NMR (DMSO-d₆) δ 3.18 (m, 2, 3'-H₂), 3.48 (m, 3, 5'-H₂ and 4'-H), 3.86 (t, 2, J = 6 Hz, 2'-H₂), 4.42 (t, 1, J = 6 Hz, 5'-OH), 4.66 (t, 1, J = 5 Hz, 3'-OH), 5.11 (t, 1, J = 6 Hz, 2'-OH), 5.87 (t, 1, J = 6 Hz, 1'-H), 7.19 (s, 2, NH₂), 8.14 (s, 1, 2-H), 8.25 (s, 1, 8-H); MS m/e 269.1121 (M⁺), calcd for C₁₀H₁₅N₅O₄: 269.1124. Anal. Calcd for C₁₀H₁₅N₅O₄: C, 44.61; H, 5.62; N, 26.01. Found: C, 44.38; 5.45; N, 26.28.

Preparation of tubercidin_{ox-red} (209)

Tubercidin_{ox-red} was prepared from tubercidin (158) (1 g, 3.7 mmole) by the same procedure used for the preparation of adenosine_{ox-red} (43). The product, 580 mg (58%) of 209, was obtained as a hygroscopic foam after co-evaporation with toluene (2 ml x 2), followed by drying at 50°C over P₂O₅ in vacuo overnight: TLC R_f = 0.26 (solvent S₁); UV (H₂O)_{max} 270 nm (ε 11,500), (0.1N HCl)_{max} 272 nm (ε 10,800), (0.1N NaOH)_{max} 270 nm (ε 10,900); NMR (DMSO-d₆) δ 3.00-4.00 (m, 7, 2'-H₂, 3'-H₂, 5'-H₂ and 4'-H), 4.38 (t, 1, J = 6 Hz, OH), 4.57 (t, 1, J = 5 Hz, OH), 4.98 (t, 1, J = 6 Hz, OH), 6.00 (t, 1, J = 6 Hz, 1'-H), 6.56 (d, 1, J = 4 Hz, 5-H), 6.95 (s, 1, NH₂), 7.24 (d, 1, J = 4 Hz, 6-H), 8.04 (s, 1, 2-H); MS (CI/NH₃) 269 (M⁺+1). Anal. Calcd for C₁₁H₁₆N₄O₄·1/4 H₂O: C, 48.44;

H, 6.10; N, 20.54. Found: C, 48.30; H, 6.15; N, 20.65.

Preparation of inosine_{ox-red} (135)¹⁰⁹

Inosine (160) (1 g, 3.7 mmole) was treated in the same manner as described for the preparation of adenosine_{ox-red} (43). The crude product obtained from evaporation of the extract was dissolved in water and applied to a column of Dowex 1x2 (OH⁻) resin (35 x 2.5 cm). The column was washed with water (150 ml), and the product was eluted with a 200 mM aqueous solution of triethylammonium bicarbonate (pH 7.5). Evaporation of the eluate, decolorization of the solution with charcoal and crystallization from ethanol (with small amount of water) with ether diffusion gave 743 mg of 135: TLC R_f = 0.11 (solvent S₁); mp 207-209°C; [lit.¹⁰⁹ mp 207°C, [α]_D²⁵ +70.2° (c, 1.61, H₂O)]; UV (H₂O)_{max} 250 nm (ε 12,500), (0.1N HCl)_{max} 249 nm (ε 11,400), (0.1N NaOH)_{max} 254 nm (ε 13,000); NMR (DMSO-d₆) δ: 3.00-4.00 (m, sugar protons), 3.83 (d, 2, J = 6 Hz, sugar CH₂) 4.40 (bs, 1, OH), 4.70 (bs, 1, OH), 5.10 (bs, 1, OH), 5.84 (t, J = 6 Hz, 1'-H), 8.04 (s, 1, 2-H), 8.20 (s, 1, 8-H); MS m/e 270.0966 (M⁺), calcd for C₁₀H₁₄N₄O₅: 270.0964. Anal. Calcd for C₁₀H₁₄N₄O₅: C, 44.44; H, 5.22; N, 20.73. Found: C, 44.51; H, 5.17; N, 20.83.

Preparation of uridine_{ox-red} (136)^{108,114}

Uridine (161) (1 g, 4 mmole) was treated in the same manner as described for the preparation of adenosine_{ox-red} (43). The crude product obtained from evaporation of the extract was dissolved in water and applied to a Dowex 1x2 (OH⁻) column (35 x 2.5 cm). The column was washed with water (150 ml), and eluted with a 200 mM aqueous solution of triethylammonium bicarbonate (pH ~ 7.5). Appropriate fractions were collected and evaporated. The residue was dissolved in water, decolorized with charcoal and lyophilized. Further drying over P₂O₅ at 100°C overnight gave 560 mg of 136 as an extremely hygroscopic solid glass: TLC R_f = 0.31 (solvent S₁); UV (H₂O)_{max} 262 nm (ε 10,100), (0.1N HCl)_{max} 262 nm (ε 10,100), (0.1N NaOH)_{max} 262 nm (ε 7,500); NMR (DMSO-d₆) δ 3.20-3.70 (m, sugar protons), 4.58 (m, 2, two OH's), 5.03 (bs, 1, OH), 5.56 (d, 1, J = 8 Hz, 5-H), 5.77 (t, 1, J = 6 Hz, 1'-H), 7.61 (t, 1, J = 8 Hz, 6-H), 11.14 (bs, 1, NH); MS m/e 215.0671 (M⁺-CH₂OH), calcd for C₈H₁₁N₂O₅: 215.0668.

Anal. Calcd for C₉H₁₄N₂O₆: C, 43.90; H, 5.73; N, 11.38.
 Found: C, 43.67; H, 5.88; N, 11.08.

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