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A CARBON-13 AND PROTON NMR STUDY OF PYRIMIDINE NUCLEOTIDES IN AQUEOUS SOLUTION

A Thesis

Submitted to

The Faculty of Graduate Studies and Research

at the University of Manitoba

in Partial Fulfillment

of the Requirements for the Degree of

MASTER OF SCIENCE

by

Wayne J. P. Blonski

Winnipeg, Manitoba

September 1983

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read a Master's thesis entitled: A CARBON-13 AND PROTON NMR STUDY OF PYRIMIDINE NUCLEOTIDES IN AQUEOUS SOLUTION.

submitted by Wayne Joseph Paul Blonski

in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

The Thesis Examining Committee certifies that the thesis (and the oral examination, if required) is:

Approved ☑ Not Approved ☐

J. Hruska

Advisor

Norman S. Huston

Peter Loewen

External Examiner

Date Aug 31/83

A CARBON-13 AND PROTON NMR STUDY OF PYRIMIDINE NUCLEOTIDES IN AQUEOUS SOLUTION

by

Wayne J.P. Blonski

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

A series of 3'- and 5'-nucleotides, as well as one 3',5'-nucleosidediphosphate, containing ribose, arabinose, or 2'-deoxyribose sugar were synthesized. Their geminal and vicinal $^{13}C-^{31}P$ coupling constants were then monitored, as a function of pH, in order to extract conformational information about the exocyclic C5'-O5' (ϕ) and C3'-O3' (ϕ') bonds. pH dependent proton spectra were also collected for the 3'-phosphates, in order to determine their sugar ring conformations. These studies allowed an examination of the effect of the 2'-hydroxyl on the exocyclic ϕ and ϕ' bond conformational preferences as a function of pH. The geminal ^{2}J(C5'-P5') and ^{2}J(C3'-P3') couplings showed only a small dependence on the ionization state of the phosphate, decreasing by < 0.5 Hz in the pH 5-7 range. The conformation of the ϕ bond was relatively insensitive to either the nature of the base (C or U), the type of sugar, or the pH of the solution, with all compounds exhibiting t(ϕ) values in the range 71-82%. For the ribose and arabinose 3'-nucleotides, J(C4'-P3') increased (up to 1.5 Hz) in the pH 5-7 range, whereas their J(C2'-P3') couplings decrease (up to 1.5 Hz) over the same pH range. In contrast for the 2'-deoxyribose molecules, both couplings decrease (~0.5 Hz) on phosphate ionization. Sugar-pucker-dependent close contact interactions between the C2'-hydroxyl and the 3'-phosphate are proposed to be responsible for the destabilization of t(ϕ') in the ribose and arabinose derivatives as compared

to the 2'-deoxyribose sugars. Electrostatic attractions, however, are speculated to stabilize the t(ϕ') orientation in basic solution for the 2'-hydroxyl containing sugars.

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TO MY MOTHER

WHO COULD SEE

AND ALWAYS KNEW ...

I. INTRODUCTION

A. General

The structure-function relationship of DNA and RNA, and biological molecules in general, is undisputed. Since the initial report of the fiber structure of DNA by Watson and Crick in 1953[1], the elucidation of the conformational preferences of these polymers has been of prime interest. To understand the basic principles underlying nucleic acid structure many authors have conducted intensive studies of the monomer units of these molecules. The profound differences in the three-dimensional structure adopted by RNA and DNA are well known and are reviewed extensively in the literature[2-5]. This variation can be traced to differences in the nature of the substituent at the C2' position, namely, hydrogen in DNA and hydroxyl in RNA. Thus, much work has been carried out determining the conformational role of the C2' substituent.

The purpose of this work is to investigate some of the effects of the 2'-hydroxyl on the conformational preferences of pyrimidine nucleotides, in particular, its effect on the orientation of the phosphate about the C3'-O bond in 3'-nucleotides and the C5'-O bond in 5'-nucleotides. To examine these effects a ^{13}C and ^{1}H NMR study was carried out on a series of ribose- and 2'-deoxyribose nucleotides incorporating the cytosine and uracil bases. In past studies of the role of the 2'-hydroxyl group, arabinose nucleotides have been used as model compounds[6-8]. These molecules resemble the corresponding ribose derivatives in that they bear a hydroxyl

at C2' but they involve an inversion of configuration at this position (Fig. I). The arabinose nucleotides also mimic the 2'-deoxyribose derivatives in that in both series an exo 2'-hydrogen is located in a vicinal position to the 3'-phosphate. In their own right the arabinose derivatives are an interesting class of compounds as they display potent antiviral activity[9,10]. Since this behaviour has been related to their ability to mimic the 2'-deoxyribose moieties[11-13], this study should also further our understanding of their biological activity.

The remainder of the Introduction outlines some of the history and theoretical background of this problem. Section B presents definitions and nomenclature used for describing nucleotides and their conformation. Section C presents a brief survey of the literature dealing with the role played by C2'-substituents in determining conformation in nucleosides and nucleotides.

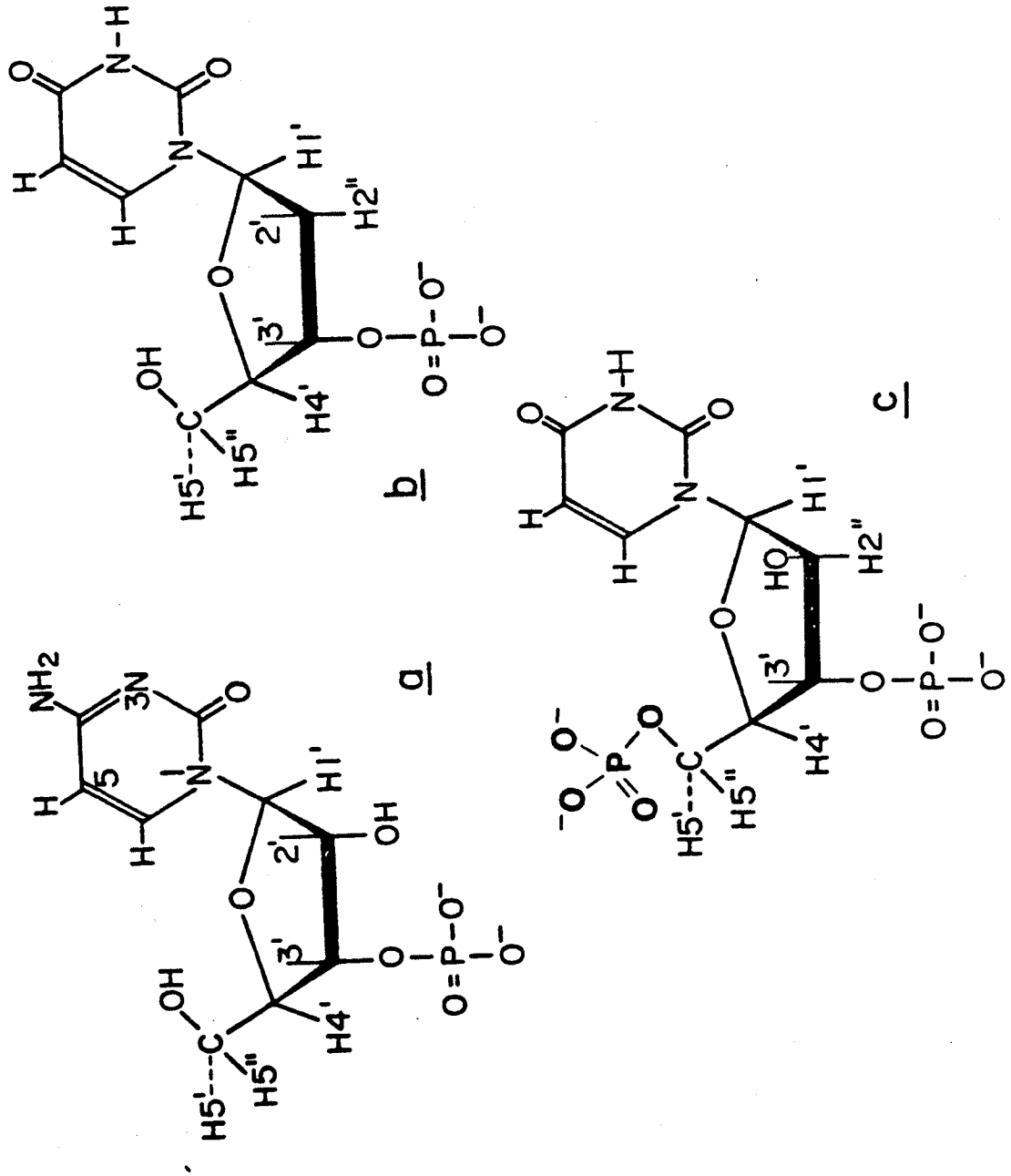
FIGURE 1

The structures of three pyrimidine nucleotides.

a) 3'-CMP

b) 3'-dUMP

c) 3',5'-aUDP


b
c
a

B. Nomenclature

1. Chemical Nomenclature

The chemical nomenclature and abbreviations used in this work will follow the IUPAC-IUB recomendations[14]. For example, the nucleosides: cytidine, 2'-deoxycytidine and arabinocytidine are symbolized as C, dC, and aC respectively. Nucleotides are designated by the site of attachment of the phosphate group; for example, cytidine-5'-monophosphate is designated 5'-CMP while the 3',5'-diphosphate derivative of arabinocytidine is referred to as 3',5'-aCDP.

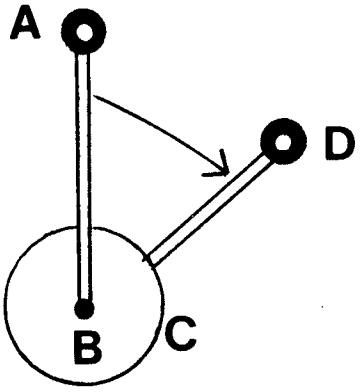
The abbreviations for the protecting groups used will be given as necessary in the text; for example, the benzoyl and monomethoxytrityl groups are written as Bz and MMT, respectively.

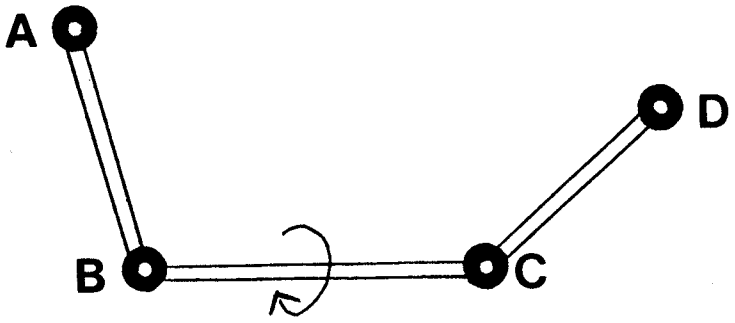
2. Conformational Nomenclature

The conformation of a nucleotidyl unit is described in terms of several dihedral torsion angles[15]. By definition the dihedral angle about a bond B-C, in a continuous segment ABCD, is the angle of rotation of the far bond C-D with respect to the near bond A-B, when viewed along the B-C bond [Fig. 2]. The eclipsed position of the A-B and C-D bonds sets the 0° angle, and positive angles are taken as clockwise rotations of the C-D bond. The angles important in describing the conformation of a nucleotide are

FIGURE 2

A diagramatic representation of a dihedral torsion angle about a bond BC in a continuous segment A-B-C-D. The arrows indicate the direction of rotation which result in positive angles.


A
D
B
C


A
D
B
C

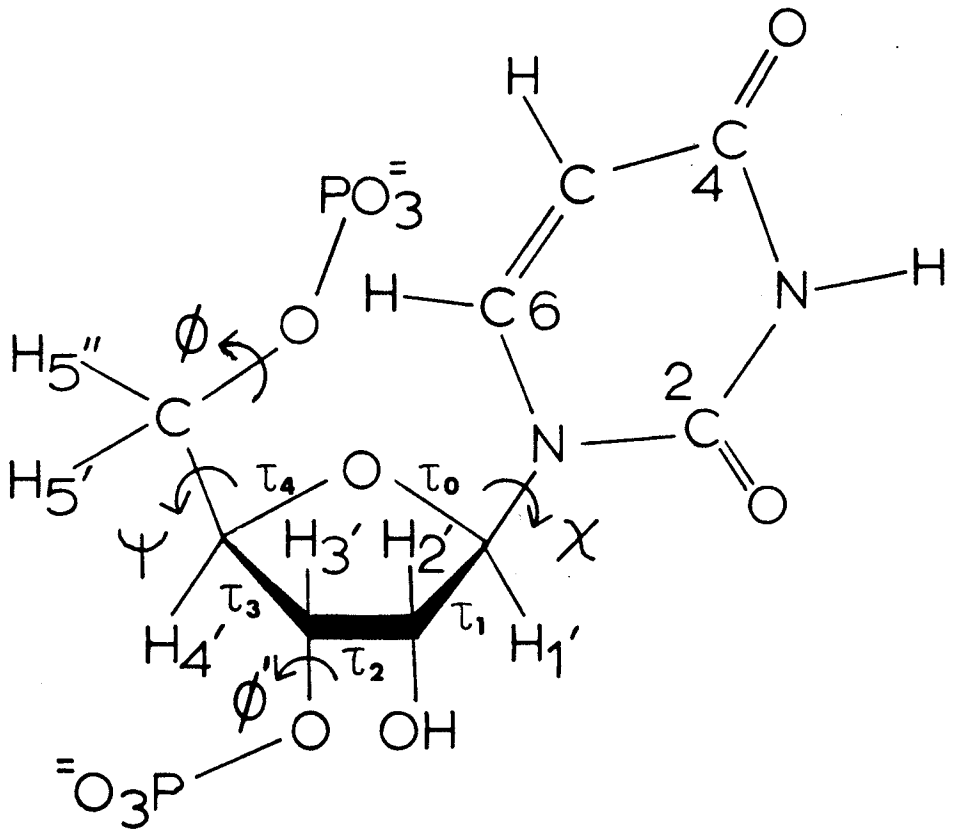
divided into three groups [Fig. 3]: (1) the N-glycosyl bond angle, (2) the sugar ring conformational angles, and (3) the sugar phosphate backbone conformational angles.

(1) The N-glycosyl bond angle, χ, defines the relative orientation of the base and sugar ring planes [Fig. 3]. The zero point of rotation is taken to be the eclipsed position of the N1-C2 and C1'-O4' bonds [Fig. 4a]. The terms syn and anti refer to the broad ranges of χ values which are $0° \pm 90°$ and $180° \pm 90°$, respectively. Fig. 4b shows the side view of a pyrimidine nucleotide in the syn and anti conformations.

(2) The sugar ring conformation is defined by the five dihedral bond angles τ_0, τ_1, τ_2, τ_3, τ_4 [Fig. 3]. Crystal studies have shown that the τ angles adopt values such that the ring is puckered. This puckering can be described in terms of twenty idealized envelope (E) and twist (T) conformations, which can be assembled into a cycle of pseudorotation[12]. The envelope conformations are those having one atom projecting from the plane defined by the remaining four atoms, while the twist conformations are those having two atoms projecting to opposite sides of the plane defined by the remaining three atoms. Projections from the plane on the same or opposite sides as C5' are known as endo or exo, respectively. These idealized conformers are categorized into two families, the 3'endo (or N) type and the 2'endo (or S) type, where N and S refer to the northern and southern hemispheres of the pseudorotation cycle[17]. Perspectives of these two

FIGURE 3

The principle torsion angles used to describe the conformation of the nucleotidyl unit.



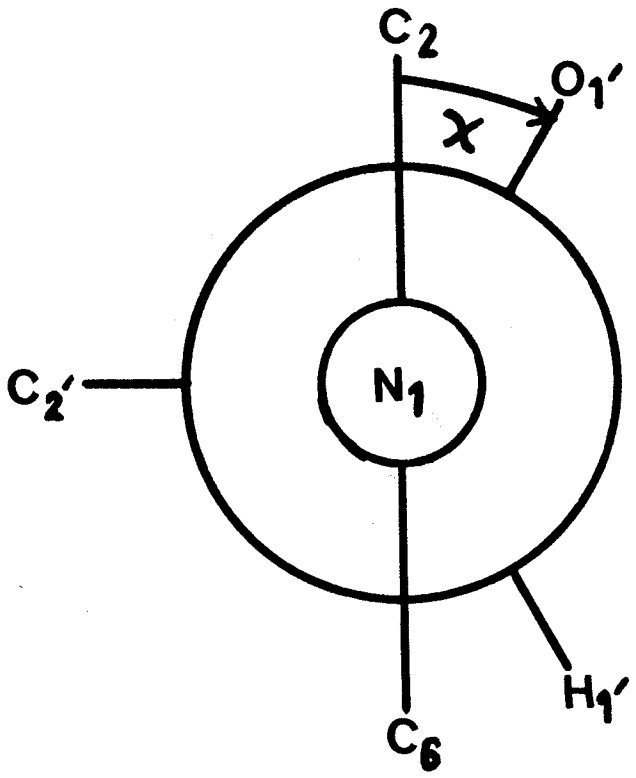
GLYCOSIDIC LINKAGE (χ)

RIBOSE CONFORMATION (τ)

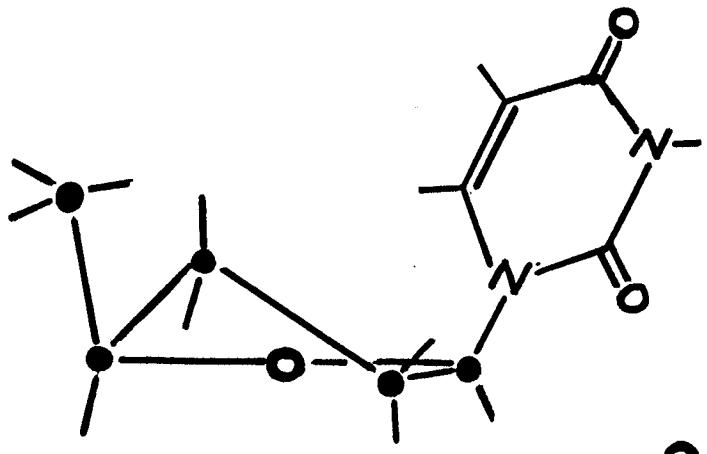
EXOCYCLIC LINKAGES (Ψ, Φ, Φ')

FIGURE 4

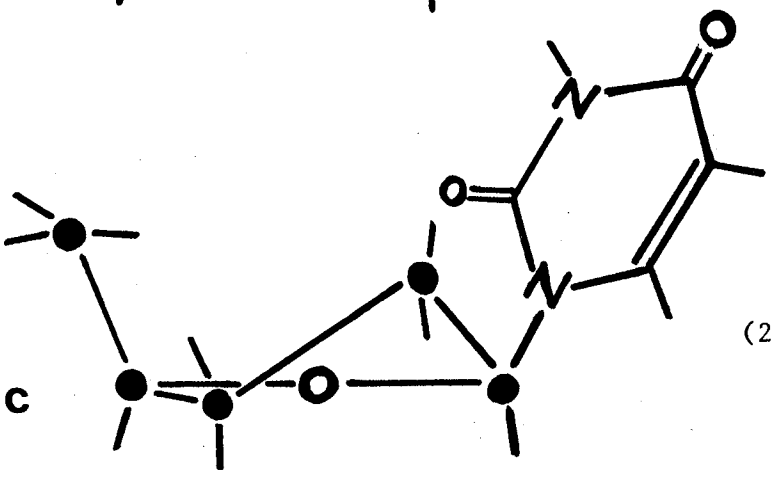
Representations of a nucleoside in the *syn* and *anti* conformation as well as with 2'-*endo* and 3'-*endo* sugar conformations.



a



b (3'-endo , anti)



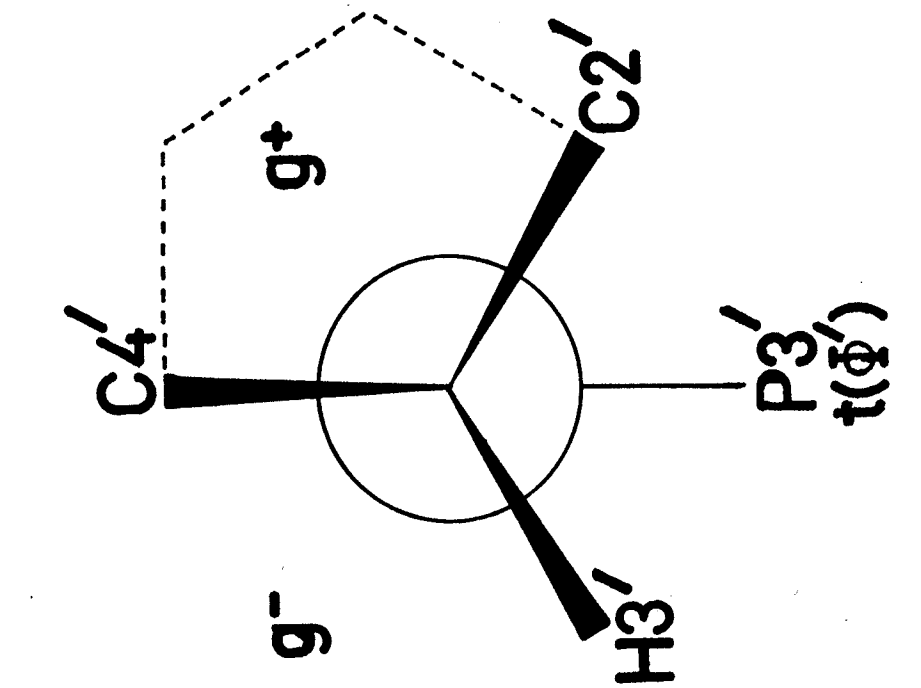
c (2'-endo , syn)

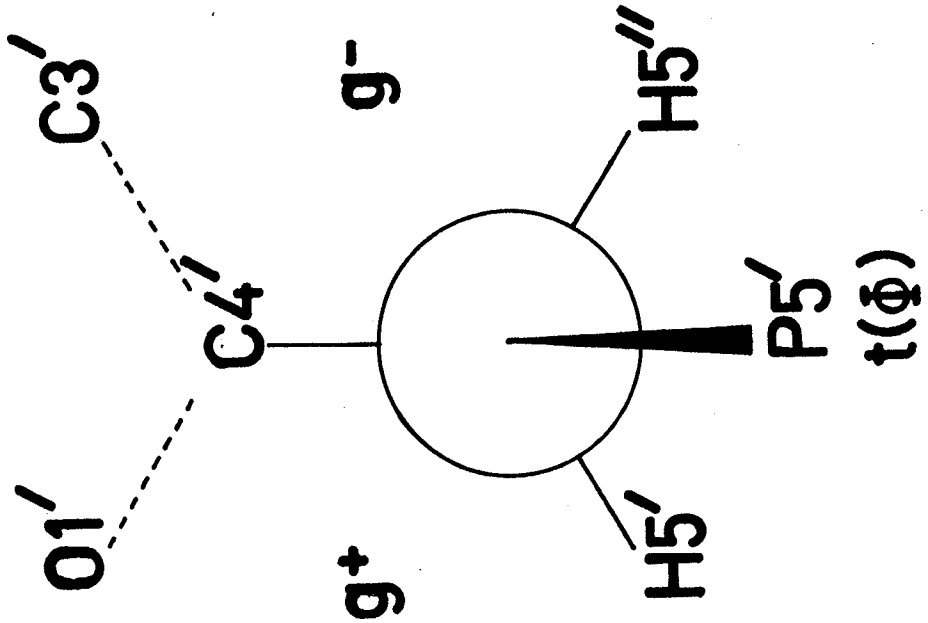
conformations are shown in Fig. 4b & c. The solution conformation can be pictured as a statistical blend of the idealized conformers (3'-endo $\rightleftharpoons$ 2'-endo or N $\rightleftharpoons$ S). Altona and Sundaralingam[17] have presented a pseudorotational analysis (the AS model) which describes a particular conformation in terms of its phase angle of pseudorotation (P) and the amplitude of ring pucker τ_m.

(3) The sugar phosphate backbone is defined, in the Sundaralingam[14] notation by the six torsion angles ω, ϕ, ψ, ψ', ϕ', ω' [Fig. 3]. Alternate notations have been proposed[19,20], but that of Sundaralingam will be used here as it appears most often in the literature. In general, these bonds can be described in solution, as blends of the classical staggered rotamers, gauche$^+$ (g^+), trans (t) and gauche$^-$ (g^-). Fig. 5 shows these rotamers for the ϕ and ϕ' bonds which are of primary interest to this study.

FIGURE 5

Newman projections are shown for the three staggered conformers of the C5'-O5' (ϕ) and C3'-O3' (ϕ') bonds. For both bonds the trans orientation is depicted.


C2′
g+
C4′
P3′
t(Φ′)
g-
H3′


C3′
g-
H5″
C4′
P5′
t(Φ)
O1′
g+
H5′

C. The Role of the 2'-Substituent in Determining Pyrimidine Monomer Conformation

The conformations of a number of nucleosides and nucleotides have been studied by a variety of physical techniques as well as by theoretical calculations. These conformations have proven useful in understanding the forces which stabilize nucleic acid structure, and in particular, the role played by the 2'-hydroxyl in determining conformation. A brief review of some of these studies should be useful at this point. This discussion will focus on the pyrimidine monomers as they are the topic of the present study.

1. Influence on the N-Glycosyl Bond Angle (χ)

Crystal studies[18,21] on pyrimidine ribose and 2'-deoxyribose nucleosides and nucleotides show an overwhelming preference for the anti conformation. However, several syn conformations have been reported to date, for example, 4-thiouridine[22] and 6-substituted pyrimidines[23]. In solution most nucleotides undergo a syn $\rightleftharpoons$ anti interconversion with the barrier to free rotation being due to steric interactions of the base with the endo substituent at C2'[24]. For the arabinose monomers, energy calculations suggest that this interconversion is restricted as compared with its ribose and 2-deoxyribose counterparts[25]. This increased steric hinderance is attributed to the bulky hydroxyl group as compared

with hydrogen for the other sugars.

2. Influence on sugar ring pucker

Results from crystal studies[26] indicate that sugar ring conformations fall into two major modes, type N (~C3'-endo), $-1° < P < 34°$, and type S (~2'-endo), $137° < P < 194°$. In general, the 2'-deoxyribose sugars show a bias toward type S conformations while the ribose derivatives are about equally distributed between N and S. The limited data on arabino derivatives (9 structures reported) demonstrates a slight preference for the S type conformers. All sugars have exhibited conformations outside of the standard P value ranges given above for N and S. In particular, there is a group clustered at the eastern part of the pseudorotational cycle with $84° < P < 106°$ (~O4'-endo).

In solution the sugar ring can be described as a dynamic blend between the two conformational groups $S \rightleftharpoons N$[27,28]. The relative contributions of each conformer can be estimated from ^1H NMR[29]. Results here show that, for pyrimidine monomers, the 2'-deoxyribose sugars favour the S conformers (~70%) while the ribose derivatives show an equal ratio between N and S. It is interesting to note the close relationship between the crystal and NMR results for the 2'-deoxyribose and ribose sugars. A direct comparison of these techniques must be made cautiously, however, as the crystal packing forces, which are important in determining conformations in the

solid state, are not present in solution.

A survey of solution data for seventy-nine arabinosides[30] has suggested that, unlike the interpretation for deoxyribose and ribose sugars, a simple two state model (~2'-endo $\rightleftharpoons$ ~3'-endo) may not describe them sufficiently. Ekiel et al.[30] have postulated that, in some cases, the situation may be more accurately described as a three state equilibrium among ^{2}E, $_{3}^{2}T$ and $_{1}^{0}T$. This view is also supported by theoretical energy calculations[25].

3. Influence on the Ψ, ϕ and ϕ' Bonds of the Backbone

In a survey of crystal data of nucleosides and 5'-nucleotides with ribose, 2'-deoxyribose, and arabinose sugars, Sundaralingam[21] has discussed several aspects of the ψ bond orientation. At the nucleoside level, the most striking feature is that the 2'-deoxynucleotides show a scatter of structures among the three staggered rotamers g^{+}, t and g^{-}, while the ribose sugars dictate primarily the g^{+} conformer with only a few t, and no g^{-} examples observed. At the 5'-nucleotide level there is an almost absolute preference for the g^{+} conformer with only one t conformation being found for a 2'-deoxyribose sugar.

In solution the ψ bond conformation is described as a dynamic equilibrium among the three classical rotamers[29,31] g^{+}, t and g^{-}. ^{1}H studies[31] on a series of 5'-nucleotides show a preference

for g^+ of 72-87% in the ribose derivatives, and 65-70% in the 2'-deoxyribose derivatives.

For arabinose nucleosides, the limited crystal data[21] indicate a preference for the g^+ (ψ) conformer with one example of t (ψ). The only nucleotide crystalized, 5'-aCMP, also demonstrates the preferred g^+ conformation. It should be noted here that unlike the deoxyribose and ribose sugars, some of the arabinose structures are stabilized in the 2'-endo-g^+ conformation via an intramolecular hydrogen bond. In solution, this hydrogen bond does not exist[32]. Indeed, for most pyrimidine arabinosides the 2'-hydroxyl acts to destabilize the g^+ conformer with populations generally less than 50%[30].

The ϕ bond of 5'-nucleotides demonstrates an overwhelming preference for the t conformation in the crystal state. A survey of the data points to ϕ values occurring in a narrow range about 180°[18]. In solution the same situation appears to apply. Assuming the usual three-fold potential Davies and Danyluk[31] have calculated t(ϕ) populations to fall into the range 66-77% for ribose- and 2'-deoxyribose-5'-nucleotides.

The lack of crystal data for 3'-nucleotides precludes a detailed analysis of sugar (i.e. 2'-hydroxyl) effects on the ϕ' bond orientation. To date only eight structures have been reported, all for ribose sugars[18,33,34]. Interestingly, these conformations do not fall into the three classical rotamers, but rather, populate

a continuous range of values centered at the intersection of the g^- and t rotamers. For example, in the crystal structure of 3'-AMP-$2H_2O$, ϕ' is 273°, just 3° from the eclipsed conformation of the $C_{3'}$-$H_{3'}$ and the $O_{3'}$-P bonds. Thus, from the crystal data, the barrier due to this eclipsed conformation appears to be quite small.

In solution, there have been several interpretations proposed for the analysis of the ϕ' bond conformation. At present it appears that a unanimously agreed-upon model has yet to be presented. Therefore, a discussion of the effect of the 2'-hydroxyl on the ϕ' conformational preferences at this point would be nonproductive. Instead, this section will outline past methods of interpreting ^1H and ^{13}C NMR coupling constants in terms of ϕ' bond orientations.

Initial carbon-13 analysis of the ϕ' bond assumed the usual dynamic equilibrium between the three classical staggered rotamers g^+, t, g^-[35]. This led to significant populations of the g^+ conformer and gave poor correlation with later ^1H results[36]. However, X-ray diffraction studies[18], theoretical calculations[37-39], and steric considerations[70] have ruled out $g^+(\phi')$ contributions to ϕ' conformation. This prompted interpretation of ^3J(C-P) and ^3J(H-P) coupling constants in terms of a two state equilibrium, i.e. $\phi'(t)$ $\phi'(g^-)$[40,41,70]. However, the interpretation of NMR vicinal coupling constants in terms of a population equilibrium between two or more fixed states, is only valid if there is a significant barrier

to rotation between them[42-44]. As mentioned above though, crystal structures of 3'-nucleotides[18,34] span a range containing the region in which the barrier should occur (ϕ' = 240°). In addition, some theoretical calculations[45,46] reveal a broad energy minimum in the range of ϕ' values defined by the crystal data. Recognizing this uncertainty, Davies and Danyluk[36] suggested that the C3'-O3' bond of 3'-nucleotides, has a much greater degree of rotational flexibility than is found for the ψ and ϕ bonds of 5'-nucleotides. Nevertheless, studies with both proton[41] and carbon-13 NMR[47] have interpreted the ϕ' bond conformation in terms of the above mentioned two state equilibrium.

The arguments supporting this interpretation from the ^{13}C results[47] are given as follows. Pure gauche and trans conformers (ϕ'= 180° and 300°), for the two ^{3}J(C-P3') couplings, are taken to be 2.1 and 10.1 Hz respectively (as calculated from the Karplus-type equation $^{3}J_{C,P} \simeq 9.5 \cos^{2}\theta - 0.6 \cos\theta$[48]). If there is a rapid equilibrium between these two fixed sites (i.e. ϕ'-180° $\rightleftharpoons$ ϕ'-300°) then their sum (denoted SUM) should be constant, i.e. ^{3}J(C4'-P3') + ^{3}J(C2'-P3') = 12.2 Hz. However, for 3'-UMP measured over a range of pH values, this SUM value was found to be constant at 8.9 $\pm$ 0.1Hz. To accommodate this discrepancy, the non-classical rotamers of 210° and 270° were chosen, which yield calculated values of these couplings such that their SUM is in reasonable agreement with experiment. This constant valued SUM has been given as evidence for the

existence of the non-classical rotamers. However, results from a carbon-13 study on 3'-pyrimidine deoxyribonucleotides[49] as well as the present data on 3'-pyrimidine arabinonucleotides[50] clearly demonstrate the sigmoidal behaviour of the SUM upon titration of the phosphate. This variation of the SUM value in conjunction with the above mentioned crystal data[33,34] and theoretical calculations[45,46] casts serious doubt on the validity of the fixed two site equilibrium model.

Due to the uncertainties expressed in the above discussion the coupling constants determined in this work shall be interpreted in a qualitative manner only. The sole assumption will be that the two ^{3}J(C-P3') vicinal coupling constants vary in a Karplus manner[51,52,35] with the ϕ' torsion angle. Thus, ^{3}J(C2'-P3') and ^{3}J(C4'-P3') should be relatively large in the g^- and trans domains respectively, and relatively small in the t and g^- domains, respectively.

Recently Pattabiraman *et al.*[53] have presented energy calculations comparing ribose and 2'-deoxyribose 3'-nucleotides. P-ϕ' contour maps were presented allowing an examination of the interdependence of the ϕ' bond and the sugar ring conformation. The following table lists the ϕ' angles for which energy minima were found for given conformations of the sugar ring:

	2'-endo(S)		3'-endo(N)	
Ribose	280°(g^-)	not allowed	270°(g^-)	200°(t)
2'-deoxyribose	280°(g^-)	190°(t)	270°(g^-)	180°(t)

These results predict the probable existence of all pairings except for the 2'-endo t(ϕ') combination for the ribose sugar. This is presumably due to strong short contacts between the 2'-hydroxyl and the atoms of the phosphate group. These interactions are diminished in 2'-deoxyribose, due to the smaller size of the hydrogen. The N$\rightleftharpoons$S interconversion is also shown to be affected by the ϕ' orientation. That is, transitions between 3'-endo and 2'-endo are restricted to high values of ϕ' for ribose (~250° < ϕ' < 280°), while both low (140° < ϕ' < 200°) and high (260° < ϕ' < 280°) ϕ' values are allowed for 2'-deoxyribose.

As well, the N$\rightleftharpoons$S transitions are possible through both 04'-endo and 04'-exo for the deoxyribose sugars (energy barrier ~ 2 Kcal $mole^{-1}$) but are only possible through 04'-exo for ribose sugars (energy barrier ~ 1 Kcal $mole^{-1}$). These results point to the role the 2'-hydroxyl plays in restricting the relative flexibility of the ribose as compared with the 2'-deoxyribose 3'-nucleotides.

It is useful at this point to recap the intent of this work and outline the approach used in its investigation. This study was undertaken to determine the effect of the 2'-hydroxyl on the conformational preferences of the exocyclic ϕ and ϕ' bonds in pyrimidine 3'- and 5'-mononucleotides. To this end, a series of uracil and cytosine 3'- and 5'-nucleotides were synthesized for each of the ribose, arabinose and 2'-deoxyribose sugars. The above sugars exemplify the exo or endo configurations of the 2'-hydroxyl, as well as the situation in which it is not present. The molecules were then examined by carbon-13 NMR to determine their vicinal ^{3}J (C-P) couplings, from which the conformational preferences of the ϕ and ϕ' bonds may be elucidated. In addition, each molecule was examined at several pH values in order to determine conformational changes which may arise upon phosphate ionization. Finally, the data for the 3'-phosphates were examined in terms of the nature of the equilibrium which exists for the ϕ' bond conformers.

II. EXPERIMENTAL

A. Synthetic Method

1. Apparatus and Materials

The compounds used in this work were either purchased from commercial sources or chemically synthesized. The synthetic procedures were obtained from the literature and any modifications to them will be described as necessary.

The following nucleosides and nucleotides were purchased from the Sigma Chemical Co. (P.O. Box 14508, St. Louis, MO, 63178, U.S.A.): dU, aU, dC, aC, 3'-UMP, 5'-UMP, 3'-CMP, 5'-CMP, 5'-aCMP, 2',2-O-anhydroU and 2',2-O-anhydroC. The commercial sources of specialty reagents will be identified as necessary, although the origin of common solvents will not be specified.

Several chromatographic techniques were employed during the course of the synthesis. Analytical thin layer chromatography (tlc), performed on Brinkman Polygram SilG/UV_{254} sheets (Macharey Nagel and Co.), was used to monitor reaction progress and to check the purity of the final products. Preparative thin layer chromatography was used to separate reaction intermediates and for final purification of the protected nucleotides. This was carried out on 20 x 20 cm glass plates coated with 1-2 mm of MN-Kieselgel P/UV_{254} (Macharey Nagel and Co., Germany, distributed by Brinkman Instruments, Ontario, Canada). Paper chromatography was performed by the descending technique on Watman 3 MM paper utilizing several solvent systems which are listed at the end of

this section. Analytical samples were run on cellulose tlc strips (Eastman Kodak Co. Rochester, N.Y) in the same solvent systems. Paper chromatography was used as the final purification technique for the deprotected nucleotides.

High voltage electrophoresis was used occasionally to check the charge on the synthetic nucleoside diphosphates. These trials were performed in TEAB buffer untilizing a Savant flat bed high voltage electrophoresis apparatus.

High grade solvents were used without further purification except for methanol and pyridine which were freshly dried for some applications (see procedures section). UV spectra were recorded to determine changes to the base. These were obtained on a Unicam SP800B UV recording spectrophotometer.

Paper chromatography solvent systems.

Solvent	Composition	Volume ratio
A	isopropanol:water:ammonia	(7:2:1)
F	n-propanol:conc.ammonia:water	(11:2:7)
U	ethylacetate:n-propanol:water	(4:1:2)

2. 2'-Deoxyribonucleotide Synthesis

The synthesis of the 2'-deoxyribonucleotides involved the site-specific addition of a phosphate group to a hydroxyl of the sugar ring. To ensure specificity of attachment the remaining hydroxyl must be blocked with a suitable protecting group. In the case of some bases (A, G and C) the exocyclic amino function must also be protected. After suitably protecting the molecule the free hydroxyl site may be then phosphorylated. Finally, removal of all protecting groups will yield the desired nucleotide.

The following mononucleotide syntheses employ this basic scheme, and so, the discussion will follow the same format, i.e. blocking reactions, phosphorylation, and deprotection. The reactions themselves are standard and are discussed in an excellent review of polynucleotide synthesis by Amarnath and Broom[54].

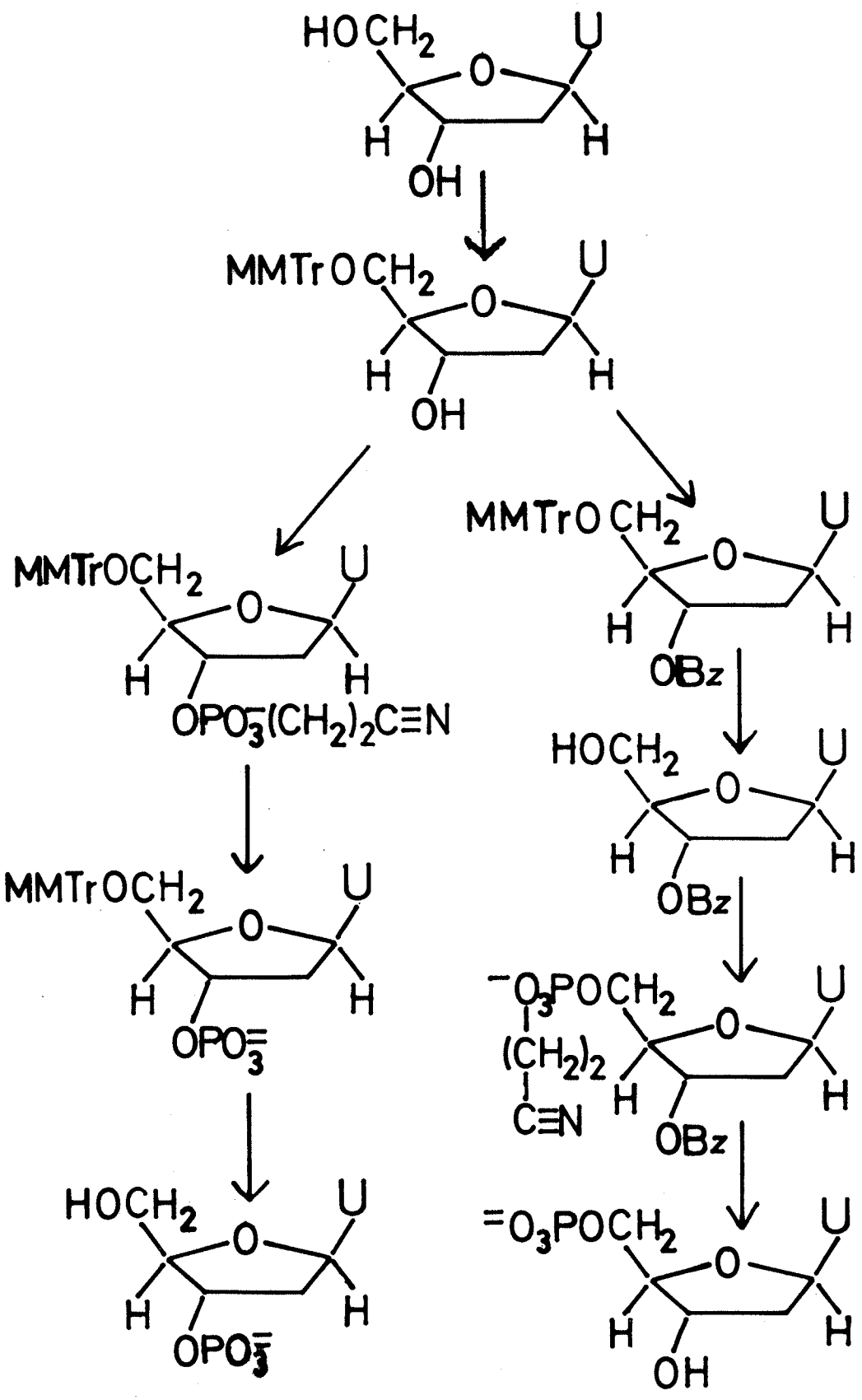
a. Synthesis of 3'-dUMP and 5'-dUMP

i) Discussion

The synthesis of these two molecules demonstrates the simplest type of nucleotide synthesis. Several reasons for this are as follows. (1) The deoxy sugar allows for simply preferentially protecting either a primary (5') or secondary (3') hydroxyl while having the other site free for phosphorylation. (2) The uracil base needs no protection prior to phosphorylation. (3) The

FIGURE 6

The reaction sequence used in the synthesis of 3'-dUMP and 5'-dUMP.


HOCH2
U
O
H
H
OH
MMTrOCH2
U
O
H
H
OH
MMTrOCH2
U
O
H
H
OPO3(CH2)2C≡N
MMTrOCH2
U
O
H
H
OPO3
HOCH2
U
O
H
H
OPO3
MMTrOCH2
U
O
H
H
OBz
HOCH2
U
O
H
H
OBz
-O3POCH2
(CH2)2
C≡N
U
O
H
H
OBz
=O3POCH2
U
O
H
H
OH

glycosyl C(1')-N(1) bond is quite stable as compared with the purine derivatives. Fig. (6) shows the reaction scheme used to synthesize both 3'- and 5'-dUMP.

For the 3'-nucleotide, dU is first blocked at the 5'-position with the p-monomethoxytrityl group. This is a highly selective reagent toward primary hydroxyls with excellent yields (~90%). The 5'-protected compound may then be isolated on preparative tlc plates. Phosphorylation of the 3'-OH follows by treatment with β-cyanoethylphosphate in the presence of 2,4,6-tri-isopropyl-benzenesulphonyl chloride (TPS). Deprotection of the 3'-nucleotide involves alkali hydrolysis of the cyanoethyl group followed by acid cleavage of the monomethoxytrityl group.

The 5'-nucleotide synthesis also begins with monomethoxytritylation at C5', followed in the same reaction vessel by benzoylation at C3'. Although the tlc of this reaction mixture is complex, this two reaction sequence eliminates an isolation step thus avoiding product losses on the preparative tlc plates. The monomethoxy trityl group is then hydroxylzed with acid, leaving a 3'-protected nucleoside. Phosphorylation as described above followed by deprotection in base yields the desired 5'-dUMP.

ii. Procedures

5'-MMT-2'-deoxyuridine

2'-dU (1 mmole, 0.228g) is dried by evaporation with dry pyridine (3 x 5 mls). This material is then dissolved in dry pyridine

(10 ml) to which p-monomethoxytritylchloride (1.1 mmole, 0.340g) is added. The mixture is stirred o/n and checked for completion by tlc. The reaction is quenched by the addition of ice (~10 g) and stirred for approximately 2 hrs. CH_2Cl_2 (~25 mls) is added and the organic layer is washed (3 x 10 mls) with water. The organic layer is separated and the solvents removed on a Roto-vap. Residual traces of pyridine are removed by repeated co-evaporations with toluene. The resulting yellow solid is dissolved in minimal CH_2Cl_2:MeOH (~10:1) and applied to preparative tlc plates and developed in CH_2Cl_2:MeOH (12:1). The major product (rf ~0.3) was scraped from the plates and eluted from the silica gel with CH_2Cl_2:MeOH (1:1). The product was compared with authentic sample by tlc. Typical yields for this reaction are >90%.

Preparation of 3'-benzoyl-5'-monomethoxytrityl-2'-deoxyuridine

dU is treated analogously as above up to the end of the tritylation reaction. At this point the solution is cooled to 0°C and benzoyl chloride (1.1 equi, 0.154g) is added dropwise with stirring over a 30 min period. The temperature is allowed to rise slowly to ~20°C and the mixture stirred for 3-4 hours. After the reaction is complete, as determined by tlc, the reaction is quenched and worked up as described above. The product is isolated by preparative tlc (solvent 20:1 CH_2Cl_2:MeOH). The product was

eluted with CH_2Cl_2:MeOH (1:1) (overall yield ≃ 70-80 %).

3'-O-Benzoyl-2'-deoxyuridine

3'-Benzoyl-5'-MMT-2'-deoxyuridine (1 mmole) is dissolved in 80% acetic acid (30 mls) and stirred at 100°C for 20 min or o/n at room temperature. After complete deprotection the solution is diluted with water (~20 mls) and the acetic acid removed on a roto vap, followed by several codistillations with toluene. The resultant dry powder is triturated several times with diethylether. The resultant 3'-O-benzoyldU was suitable for further reactions. Overall yield from dU is ~60-70%.

b. Synthesis of 3'-dCMP and 5'-dCMP

i. Discussion

The synthesis of the 2'-deoxycytidine nucleotides differs from that for uridine in that the amine function of the base must also be protected. The anisoyl group is most adequate for this use[54].

In the synthesis of 5'-dCMP the first step is the monomethoxy tritylation of the 5'-hydroxyl. This is followed by the simultaneous protection of the 3'-hydroxyl as well as the exocyclic amine function of the base with anisoyl chloride. Acid hydrolysis of the MMT group followed by phosphorylation, as is described for

the uridine derivatives, yields the protected nucleotide. The remaining protecting groups may be removed by base hydrolysis yielding the desired 5'-dCMP.

The synthesis of the 3'-nucleotide begins with the same protection steps of 5'-monomethoxy tritylation and C3'-,N4-anisoylation. At this point it is possible to take advantage of the increased susceptibility to alkali of the anisoyl group at C3' over that at N4. A suitable base for this is 0.1 M NaOMe/MeOH. After neutralization, the free 3'-hydroxyl may then be phosphorylated as described for its uracil counterpart. Acid hydrolysis followed by basic hydrolysis yields the desired 3'-dCMP.

ii. Procedures

5'-Monomethoxytrityl-2'-deoxycytidine

The procedure, reaction conditions and work-up of this synthesis are identical to those for the corresponding uracil derivative. Reaction times and yields are similar.

5'-Monomethoxytrityl-3'-N-dianisoyl-2'-deoxycytidine

Anisoyl chloride (2.2 mmoles, 0.374g) is added dropwise over 30 min to a stirred solution of 5'-MMTdC (1 mmole) at 0°C in pyridine (5-10 mls). The reaction is monitored by tlc (CH_2Cl_2: CH_3OH, 24:1), quenched with ice after about 4 hrs and stirred for 1 hr. The reaction mixture is diluted with $CHCl_3$ and separated

from the water. The solvents are removed and the remaining gum again taken up in $CHCl_3$ and washed (3 x 10 ml) with saturated sodium bicarbonate and (1 x 10 ml) with water. The organic layer is concentrated and final traces of pyridine are removed by several co-evaporations with toluene. The final crude product was checked by tlc and found to be sufficiently pure for further reactions.

Detritylation of 5'-MMT-3'-N-anisoyldC

The reaction conditions, work up procedures and yield of this reaction are identical to those for the uracil derivatives.

Preparation of 5'-MMT-N-anisoyldC from 5'-MMT-3'-N-dianisoyldC

Freshly prepared NaOME/MeOH (1.0M, 18.2 ml) was added to a stirred solution of 5'-MMT-3'-N-dianisoyl dC (1 mmole) in dry methanol (23.4 ml) at room temperature. After 15 min an excess of Dowex -X8 (pyridinium form) was added to the solution and stirred until neutral (~15 min). The solution was filtered and the resin washed well with dry methanol. The solvents were removed and the resultant powder triturated with diethylether. The final product was suitable for phosphorylation.

C. General Phosphorylation Reaction Conditions

i. Discussion

The reaction conditions for phosphorylation follow the same procedure regardless of the position. In general, 3'-phosphorylation took slightly longer (5-6 hrs) than 5'-phosphorylation (3-4 hrs). The phosphorylating agent used was β-cyanoethylphosphate.

ii. Procedures

The protected nucleoside, 1 equiv., was mixed with two equivalents of β-cyanoethylphosphate (pyridinium salt as a 0.4 mmole/ml solution in pyridine). The pyridine was removed under vacuum and dried by five coevaporations with pyridine (5 ml). The resulting product was dissolved in pyridine (10 ml/equiv) and four equivalents TPS were added. The reaction was stirred at room temperature until completion (~3-6 hrs). The flask was cooled on ice and about 5 g crushed ice added to quench the reaction. The resulting solution was stirred for one hour. $CHCL_3$ (~50 ml) was then added and the organic layer separated. The organic layer was washed 3 times with water and finally the solvents were removed under reduced pressure.

3. Arabino Nucleotide Synthesis

a. Hydrolysis of 2'-2-O-anhydro-cyclonucleosides

i. Discussion

A convenient method of producing pyrimidine arabinonucleosides is through the alkaline or acid hydrolysis of their corresponding 2'-2-O-anhydro derivative. It should be noted that while the anhydro bond of the uracil derivative is labile to both base and acid, this linkage in cytosine derivatives showed virtually no hydrolysis in the presence of the several acids tested (80% acetic acid, 0.5 M HCl, 0.5 M H_2SO_4). Higher concentrations of acid resulted in glycosidic bond cleavage.

The anhydro aU was best hydrolyzed to the aU derivative with 80% acetic acid. This gave the cleanest reaction, and remaining acetic acid was easily removed on a Roto-vap by co-evaporation with water and toluene. The anhydro aC was hydrolyzed with 0.5 M KOH, followed by neutralization with Dowex-H (Bio-Rad). Filtration and removal of water yielded aC. Both these methods afford very clean products in high yields (>95%) with little or no evidence of glycosidic bond cleavage.

ii. Procedures

aU from 2',2-O-anhydroU

2',2-O-Anhydrouridine (1 mmole) was dissolved in 80% acetic

acid (15 mls) and stirred at 50°C for four hours followed by evaporation of the solvents. Co-evaporation with toluene helped to remove the last traces of acetic acid. The final product was compared with authentic aU by cellulose tlc [solvent U] and UV showing identical characteristics.

aC from 2',2-O-anhydro C

2',2-O-Anhydrocytidine (1 mmole) was dissolved in 10 mls of an aqueous KOH (0.5 M) solution. The mixture was stirred for four hours at room temperature. Dowex-H was added until the solution pH was 7 (pH meter). The Dowex was filtered off and washed several times with water then methanol. The solvents were removed on a rotovap and the product dried by coevaporation with heptane. The product was compared with authentic aC by tlc [solvent U] and UV showing identical results.

b. 3'-aUMP Synthesis

i. Discussion

The major difficulty in this synthesis comes in the selective protection of the 2'-hydroxyl. Methods for 2'-hydroxyl protection have been suggested for ribonucleotides[54], but generally, they suffer from low yields and are quite time consuming. Furthermore, it is not known how well these methods would work

on the arabinose 2'-hydroxyl, with its endo configuration. For these reasons, 2'-2-O-anhydroarabinouridine was chosen as the starting material in order to utilize the anhydro linkage as the 2'-hydroxyl protecting group[55]. The procedures of protection, phosphorylation and deprotection are then identical to those for 3'-dUMP (see section II.A.2.a). As expected in the deprotection stage, the acid hydrolysis of the MMT group at high temperatures also resulted in the cleavage of the 2'-2-O-anhydro linkage yielding an arabinose sugar.

c. Synthesis of 5'-aUMP

i. Discussion

The synthesis of 5'-ara UMP was attempted via two strategies. The first was completely analogous to the previously described synthesis for 5'-dUMP, except in this case an additional equivalent of benzoyl chloride must be added during the protection stage.

The second method involved a direct phosphorylation of the 5'-hydroxyl of either aU[56] or its 2'-2-O-anhydro analogue. The synthesis, according to Yoshikawa et. al.[56], involved the one step addition of phosphorylchloride to the nucleoside in a trialkylphosphate. The conditions for the reaction are mild (0°C) and the reaction time is reasonable (o/n). Quenching of the reaction with water hydrolyzed the phosphorylated mixture

to the phosphate. The 5'-nucleotides were the major product >80% and may be isolated with paper chromatography.

ii. Procedures

Arabinouridine (1 mmole,0.24g) was added to a stirred solution of $POCl_3$ (2 mmole 0.307 g) in 5 mls triethyl phosphate at 0°C (caution: the fumes of $POCl_3$ are very corrosive to the lungs, use only in a fume hood). The reaction was left to stir o/n (12-15 hrs) at this temperature and was quenched with the addition of crushed ice (~2-3 g). The mixture was neutralized with 0.01 N NaOH solution and the solvents removed on a rotovap. The remaining liquid was loaded onto chromatography papers and developed in solvent A. The largest band (Rf. ~0.5) was eluted and lyophylized. The resultant product was the desired 5'-aUMP.

d. 3'-aCMP Synthesis

i. Discussion

The synthesis of this compound was attempted by two procedures, with one being successful. Both methods are discussed below.

The first method was analogous to the successful synthesis of 3'-aUMP. A difficulty in this case arose from the insoluble nature of the 2'-2-O-anhydrocytidine nucleoside in solvents

suitable for the blocking reaction (pyridine, collidine, DMF, etc.). This was most likely due to the charged imino function on the base. For example, a tritylation reaction under standard conditions was left for three weeks followed by two days at 80°C with no change.

The second attempt was performed in two parts according to the literature[57,58]. The first part involve the preparation of tri-n-butyl ammonium cytidine 2',3'-cyclic phosphate. This was carried out as reported[57], except for the final isolation in which the product was precipitated from absolute ethanol solution at 0°C with anhydrous ether. This intermediate was dried by several coevaporations with dry pyridine and converted to 3'-aCMP following Nagyvary[58]. The final crude product was dissolved in water (with a few drops of ethanol), loaded onto chromatography papers and developed in solvent F for twenty-four hours. Elution of the major band yielded 2'-2-O-anhydrocytidine-3'-phosphate. Hydrolysis in base yielded the desired 3'-aCMP.

ii. Procedures

Preparation of 3'-aCMP from cytidine-2'(3')monophosphate

To a solution of cytidine-2'(3')monophosphate[57] (1 mmole) in water (3 ml) was added tri-n-butylamine (5 mmole, 0.72 ml), followed by ethyl chloroformate (2 mmoles, 0.19 ml). The mixture was stirred vigorously for 5-10 min. The solvents were removed on a rotovap and the residue dried by several co-evaporations with absolute

ethanol. The final residue was taken up in a minimum of ethanol and the solution cooled to 0°C on an ice bath. The tri-n-butylammonium cytidine 2',3'-cyclic phosphate was precipitated out by the slow addition of ether. The precipitate was collected by filtration and used without further purification for the following reaction.

The tri-n-butylammonium cytidine 2',3'-cyclic phosphate (1 mmole) was dried by several (~3 x 5 mls) coevaporations with dry pyridine followed by the addition of 50 mmoles dry pyridine. To this suspension was added tri-n-butylamine followed by trimethylsilyl chloride (~10 mmole) which was added dropwise at room temperature. The reaction mixture was then stirred at 80°C for 1 hr, after which time the solvents were removed on a Roto-vap. The resulting gum was dissolved in cold 95% ethanol at a concentration of 0.2 M and allowed to stand for 15 min at 0°C. The crude nucleotide was precipitated by addition of cold ether. This was dissolved in water (with a few drops of ethanol), loaded onto chromatography papers and developed in solvent F. The major band was eluted, yielding 2'-2-O-anhydrocytidine-3'-phosphate. The anhydro was then hydrolyzed in basic solution as described previously, resulting in 3'-aCMP.

e. 3',5'-aCDP Synthesis

i. Discussion

This synthesis was carried out according to Roberts and Dekker[59] with modifications which are outlined as follows.

After the neutralization step, the lithium phosphate was filtered off and the filtrate volume reduced by evaporation. When the filtrate volume reached about 10 ml a precipitate formed again, but showed no evidence of containing nucleoside and was therefore discarded. The remaining solution was loaded onto chromatography papers and developed in solvent F. The slowest band (~60%) was eluted with water and then lyophylized to yield the desired 3',5'-aCDP.

ii. Procedure

Cytidine (0.4864 g, 2 mmoles) was suspended in 9.73 g polyphosphoric acid and stirred in a stoppered flask at 80°C for 30 hours. The resultant dark brown viscous mass was dissolved in 20 ml water and stirred just below reflux for 1 hour to break down pyrophosphates. A 10% LiOH solution was slowly added to bring the solution to pH 9. The mixture was filtered to remove lithium phosphate and the solution concentrated to about 10 mls and refiltered. The filtrate was loaded onto eight Watman 3 MM papers and developed for 20 hrs in solvent F. The

major product (Rf.~0.3) was eluted with water and the solution freeze dried. The compound was identified by ^{13}C NMR and the charge number confirmed by electrophoresis.

B. NMR Method

1. Sample Preparation

All samples were treated for the removal of paramagnetic metal ions following recommendations in the literature[60]. Samples from commercial sources were dissolved in 99.7% D_2O, (Stohler Isotope Chemicals, Rutherford, N.J. U.S.A.), pH was adjusted to 6.5, and extracted twice with a 0.1% (wt/wt) solution of dithizone (BDH Chemicals Ltd. England) in CCl_4, and then once with pure CCl_4. The synthesized samples contained considerably more paramagnetic impurities and were treated slightly more vigorously, as follows. The samples were first dissolved in about 2 ml deionized distilled water and passed through a Chelex 100 disposable column (Bio-Rad Laboratories), at a flow rate of about 30-40 drops/min. The collected solution was extracted with dithizone as above. The samples were then freeze-dried to remove water. Finally, each sample was redissolved in 1 ml D_2O (99.7%) and filtered directly into a 10 mm (OD) NMR tube for carbon-13 analysis. In the case of the nucleoside diphosphates a small amount (2.0 mM total concentration) of EDTA was added directly to the NMR tube to ensure total paramagnetic ion removal.

pH measurements were made with a Beckman Expandomatic Model SS-2 pH meter (Beckman Instruments Ltd., Ontario) at room temperature using a Beckman combination glass electrode. The meter was calibrated within a range of $\pm$ 1 pH unit of each reading using standard buffers. pH adjustments of the sample were carried out

by the careful addition of dilute solutions(~0.5 M) of DCl or NaOD(Merck Sharp and Dohme, Montreal, Canada).

Samples for ^{1}H NMR analysis were treated, as above, for the removal of metal ions, followed by lyophylization three times from 99.7% D_2O to reduce the size of the residual HOD peak. Final sample preparation consisted of dissolving 2-10 mg of the nucleotide in ~0.6 ml of 99.996 D_2O (Aldrich Chem. Co.),followed by direct filtration into a 5 mm NMR tube. This procedure is most effective when performed in a dry atmosphere.

2. Spectral Acquisition

The carbon-13 spectra were recorded on a Bruker WH-90 DS spectrometer (22.628 MHz) at the University of Manitoba, equipped with a Nicolet-1180 computer, a 293 A pulse programmer and quadrature phase detection. Nicolet spectrometer operation was controlled via the NTCFT-1180 software package. Spectral widths were typically 4000 Hz acquired into 16 K data points, zero filled to 32 K yielding a digital resolution of 0.125 Hz per point. The number of acquisitions depended on the signal strength and varied from 100-10,000. The final free induction decay (FID) was base line corrected to remove any residual DC bias and then apodized by exponential multiplication with a line broadening value equal to the reciprocal of the acquisition time (~0.5 Hz). Normally, spectra

were collected with complete proton noise decoupling, although several proton-coupled spectra were obtained to facilitate peak assignments. The probe temperature was regulated at 300 $\pm$ 1 K.

The proton spectra were recorded on either the Bruker WH-90 described above, operating at 90 MHz, or on a Bruker HX 270 equipped with a Nicolet 1180 computer and a 293 B pulse programmer, operating at 270 MHz. The raw data were massaged in a similar fashion to the ^{13}C data above, except the amount of line broadening was generally smaller (0.2 Hz).

III. DATA

A. Carbon-13 NMR Data

Tables 1-13 contain the chemical shift and coupling constant data for the 3'- and 5'-nucleotides investigated in this study (also included is the data for 3',5'-aCDP).

The assignment of the ^{13}C resonances of the uracil and cytosine 2'-deoxyribonucleotides followed from the work on 2'-deoxythymidine and its mono- and diphosphates[49]. The assignments of the uracil and cytosine ribonucleotides followed from the work on 3'-UMP and 5'-UMP by Alderfer and Ts'o[47] who considered the effects of phosphorylation and phosphate ionization on the chemical shifts.

In addition to considering such effects, an absolute assignment of all furanose carbons from ^{1}H-^{13}C chemical shift correlation spectra has been obtained, following Freeman and Morris[61]. This method requires the knowledge of the ^{1}H spectrum of each of these molecules to assign the correlations. ^{1}H data for this purpose was either collected (for the 3'-nucleotide) or taken from the literature[31] as needed. Assignments made by this method are in agreement with those of Alderfer and Ts'o[47].

The assignment of the uracil and cytosine arabinonucleotides was based on the published data for the nucleosides, aU and aC[62]. As in the 3'-ribonucleotides the chemical shifts of C2' and C3', and their ^{13}C-^{31}P coupling constants, were similar, and thus their assignment is not obvious from the ^{13}C spectrum. However, an absolute assignment could again be obtained in all cases from

^{1}H-^{13}C chemical shift correlation spectra. The effects of phosphorylation and phosphate ionization on the ^{13}C chemical shifts supported the assignments.

B. Proton NMR Data

Tables 14-16 contain the chemical shifts and coupling constant data for the 3'-nucleotides investigated in this study.

Proton NMR data was collected for each of the 3'-nucleotides in order to determine the conformation of some bonds of the nucleotidyl unit which are not accessible via the carbon-13 technique. This data is used primarily to estimate the sugar ring conformation which is necessary when discussing correlations between sugar pucker and the ϕ' bond conformation.

^{1}H NMR spectra were collected for each nucleotide at two pH values (pH $\leq$ 5 and pH $\geq$ 7), thus allowing an examination of the phosphate ionization effects.

The first order spectral parameters were determined from the observed data by computer simulation and iteration with the program LAME 8[63]. The computer generated simulation was compared with the original spectrum in each case as the final check.

Chemical shift assignments were made in comparison with the literature[36], as well, some decoupling experiments were used to differentiate between H2' and H3' in the arabinose monophosphates. Tables 14-16 contain the ^{1}H NMR shifts and couplings for the six 3'-nucleotides used in this study.

TABLE 1

CARBON CHEMICAL SHIFTS AND CARBON-PHORPHORUS COUPLING CONSTANTS FOR 3'-UMP AT 300 K[a].

δ[b] / pH[c]	3.80	5.4	7.30	9.10
δ(4)	167.27	167.44	167.57	171.80
δ(2)	152.95	152.96	153.05	156.16
δ(6)	143.08	143.12	143.18	142.68
δ(5)	103.87	103.69	103.67	103.91
δ(1')	90.06	90.27	90.46	90.57
δ(4')	85.00	85.00	85.07	84.89
δ(2')	74.25	74.42	74.85	74.77
δ(3')	74.14	73.91	73.28	73.39
δ(5')	62.00	61.91	62.03	62.17
^3J(C4'-P3')[d]	4.5	4.8	5.9	5.8
^3J(C2'-P3')	4.3	4.0	2.9	3.0
^2J(C3'-P3')	4.9	4.9	4.6	4.5
SUM	8.8	8.8	8.8	8.8

a) Sample concentration was 75 mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane

CONTINUATION OF FOOTNOTES FOR TABLE 1

reference equal to 67.859 ppm. Estimated accuracy ± 0.01 ppm.

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy ± 0.2 Hz.

TABLE 2

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 3'-CMP AT 300 K[a]

δ^b / pH[c]	4.5	5.4	7.0	8.4	9.9	7.0
δ(4)	164.28	166.59	167.40	167.40	167.40	167.41
δ(2)	154.72	157.78	158.88	158.95	158.95	158.89
δ(6)	144.06	143.19	143.02	142.98	142.97	143.02
δ(5)	97.09	97.47	97.65	97.69	97.69	97.65
δ(1')	91.16	91.17	91.21	91.17	91.17	91.20
δ(4')	84.69	84.51	84.62	84.65	84.66	84.62
δ(2')	74.64	74.70	75.04	75.06	75.07	75.04
δ(3')	73.72	73.63	73.26	73.26	73.26	73.26
δ(5')	61.62	61.77	62.02	62.10	62.10	62.02
^2J(C3'-P3')[d]	4.9	4.8	4.6	4.5	4.4	4.6
^3J(C4'-P3')	5.1	5.3	6.0	6.0	6.1	6.0
^3J(C2'-P3')	3.8	3.7	2.8	2.7	2.7	2.8
SUM	8.9	9.0	8.8	8.7	8.8	8.8

a) Sample concentration was 75mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = +/- 0.01 ppm.

CONTINUATION OF FOOTNOTES FOR TABLE 2

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy +/- 0.2 Hz.

TABLE 3

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 3'-dUMP AT 300 K[a]

δ^b / pH[c]	2.8	5.0	7.0	9.0
δ(4)	167.46	167.48	167.58	169.26
δ(2)	152.83	152.85	152.95	154.16
δ(6)	143.31	143.33	143.42	143.20
δ(5)	103.58	103.57	103.52	103.60
δ(1')	86.79	86.77	87.51	87.47
δ(4')	87.25	87.27	86.75	86.76
δ(2')	39.13	39.17	39.46	39.53
δ(3')	75.79	75.69	74.45	74.29
δ(5')	62.45	62.45	62.64	62.69
^3J(C4'-P3') [d]	6.2	6.3	5.8	5.7
^3J(C2'-P3')	3.5	3.3	2.8	3.0
^2J(C3'-P3')	5.0	5.1	4.5	4.5
SUM	9.7	9.6	8.6	8.7

a) Sample concentration was 75mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = +/- 0.01 ppm.

CONTINUATION OF FOOTNOTES FOR TABLE 3

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy +/- 0.2 Hz.

TABLE 4

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 3'-dCMP AT 300 K[a].

δ^b / pH[c]	1.4	3.8	5.0	6.1	8.5	10.6	11.5
δ(4)	160.42	161.83	165.94	167.18	167.42	167.43	167.44
δ(2)	149.51	151.40	156.85	158.81	158.92	158.93	158.95
δ(6)	145.88	145.27	143.52	143.05	143.00	142.99	142.99
δ(5)	96.29	96.55	97.33	97.56	97.61	97.61	97.62
δ(4')	87.71	87.64	87.31	87.27	87.42	87.43	87.44
δ(1')	87.89	87.78	87.50	87.41	87.36	87.36	87.36
δ(3')	75.85	75.57	75.65	75.23	74.45	74.40	74.39
δ(5')	62.20	62.28	62.45	62.57	62.75	62.76	62.77
δ(2')	39.58	39.65	39.68	39.78	39.97	39.99	39.99
^2J(C3'-P3')[d]	5.0	5.0	5.1	4.8	4.5	4.5	4.6
^3J(C2'-P3')	3.4	3.4	3.6	3.0	3.0	3.0	3.1
^3J(C4'-P3')	6.6	6.4	6.5	6.0	5.6	5.6	5.5
SUM	10.0	9.8	10.1	9.0	8.3	8.6	8.6

a) Sample concentration was 75 mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = ± 0.01 ppm.

CONTINUATION OF FOOTNOTES FOR TABLE 4

c) pH values are the readings of pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy $\pm$ 0.2 Hz.

TABLE 5

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 3'-aUMP AT 300 K[a]

δ^{b} / pH[c]	3.8	5.7	6.8	7.5	9.5
δ(4)	167.52	167.52	167.56	167.68	171.37
δ(2)	152.58	152.63	152.73	152.83	155.52
δ(6)	144.48	144.50	144.51	144.49	144.02
δ(5)	102.28	102.32	102.39	102.40	102.56
δ(1')	87.11	86.91	86.65	86.64	86.76
δ(4')	84.94	84.86	84.74	84.73	84.61
δ(3')	80.19	79.65	78.87	78.81	79.00
δ(2')	75.66	75.90	76.21	76.23	76.27
δ(5')	62.12	62.10	62.09	62.09	62.21
^{2}J(C3'-P3')[d]	5.0	4.9	4.5	4.5	4.5
^{3}J(C2'-P3')	4.4	3.9	3.2	3.2	3.2
^{3}J(C4'-P3')	5.5	5.6	6.0	6.1	6.0
SUM	9.9	9.5	9.2	9.2	9.2

a) Sample concentration was 75mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = +/- 0.01 ppm.

CONTINUATION OF FOOTNOTES FOR TABLE 5

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy +/- 0.2 Hz.

TABLE 6

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 3'-aCMP AT 300 K [a]

δ[b] pH[c]	4.8	5.9	7.5	8.6
δ(4)	165.87	167.12	167.33	167.38
δ(2)	156.53	158.26	158.59	158.63
δ(6)	144.74	144.26	144.18	144.18
δ(5)	96.31	96.53	96.57	96.58
δ(1')	87.75	87.56	87.49	87.46
δ(4')	85.01	84.91	84.92	84.91
δ(3')	80.23	79.68	79.36	79.31
δ(2')	75.59	75.88	76.01	76.05
δ(5')	62.27	62.39	62.39	62.39
^3J(C4'-P3') [d]	5.5	5.5	5.6	5.7
^2J(C3'-P3')	5.1	4.8	4.5	4.5
^3J(C2'-P3')	4.1	3.5	3.2	3.5
SUM	9.6	9.0	8.8	9.2

a) Sample concentration was 75mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. estimated accuracy = +/- 0.01 ppm.

CONTINUATION OF FOOTNOTES FOR TABLE 6

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy +/- 0.2 Hz

TABLE 7

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 3',5'-aCDP AT 300 K[a]

δ^b / pH[c]	3.1	4.5	5.3	6.0	7.3	9.7
δ(4)	161.22	163.97	166.48	166.89	167.37	167.47
δ(2)	150.43	154.08	157.43	157.94	158.56	158.68
δ(6)	146.50	145.43	144.55	144.47	144.51	144.57
δ(5)	95.67	96.16	96.58	96.64	96.68	96.64
δ(1')	87.73	87.71	87.58	87.59	87.89	88.09
δ(4')	83.47	83.51	83.43	83.61	94.50	84.96
δ(3')	79.52	79.62	79.39	79.35	79.48	79.65
δ(2')	75.55	75.64	75.82	75.92	75.94	75.91
δ(5')	65.09	65.13	65.01	64.96	64.98	65.03
^3J(C4'-P3')[d]	5.7	5.8	5.8	5.7	6.3	6.1
^3J(C4'-P5')	8.1	7.7	7.9	7.9	7.8	8.6
^2J(C3'-P3')	5.1	5.1	4.9	4.7	4.4	4.6
^3J(C2'-P3')	4.2	4.2	3.8	3.8	3.0	2.8
^2J(C5'-P5')	4.8	4.9	4.8	4.7	3.9	4.1
SUM	9.9	10.0	9.6	9.5	9.3	8.9

a) Sample concentration was 75 mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane

CONTINUATION OF FOOTNOTES FOR TABLE 7

reference equal to 67.859 ppm. Estimated accuracy = ± 0.01 ppm.

c) pH values are the readings of pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy ± 0.2 Hz.

TABLE 8

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 5'-UMP AT 300 K [a]

δ [b] / pH [c]	3.3	4.4	5.4	6.5	9.1
δ(4)	167.46	167.46	167.48	167.54	170.47
δ(2)	153.05	153.05	153.07	153.13	153.30
δ(6)	142.89	142.90	142.95	143.25	143.03
δ(5)	103.82	103.82	103.84	103.93	104.10
δ(1')	89.81	89.80	89.78	89.68	89.70
δ(4')	84.62	84.64	84.73	85.22	85.29
δ(2')	75.14	75.15	75.15	75.20	75.18
δ(3')	71.00	71.01	71.05	71.25	71.38
δ(5')	65.43	65.41	65.30	64.76	64.47
^2J(C5'-P5') [d]	5.3	4.9	5.1	4.7	4.8
^3J(C4'-P5')	8.7	8.8	8.8	8.7	8.7

a) Sample concentration was 75mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = +/- 0.01 ppm.

CONTINUATION OF FOOTNOTES FOR TABLE 8

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy +/- 0.2 Hz.

TABLE 9

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 5'-CMP AT 300 K[a]

δ^{b} / pH[c]	3.3	4.7	5.8	6.6	9.2
δ(4)	160.95	164.72	167.02	167.37	167.41
δ(2)	150.41	155.42	158.45	159.01	159.05
δ(6)	145.17	143.71	142.95	143.05	143.14
δ(5)	96.55	97.25	97.70	97.85	97.90
δ(1')	90.92	90.75	90.61	90.50	90.51
δ(4')	84.62	84.30	84.25	84.58	84.75
δ(2')	75.63	75.58	75.57	75.59	75.55
δ(3')	70.43	70.56	70.69	70.85	70.98
δ(5')	64.91	65.05	64.93	64.50	64.30
^{3}J(C4'-P5')[d]	8.7	8.8	8.8	8.8	8.7
^{2}J(C5'-P5')	5.1	4.6	4.8	4.6	4.5

a) Sample concentration was 75mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = +/- 0.01 ppm.

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy +/- 0.2 Hz.

TABLE 10

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 5'-dCMP AT 300 K [a]

δ [b] / pH [c]	2.8	4.6	5.7	6.5	7.8	9.6
δ(4)	160.51	163.64	166.72	167.32	167.44	167.38
δ(2)	149.73	153.85	157.90	158.73	158.91	158.88
δ(6)	145.68	144.36	143.16	143.14	143.22	143.17
δ(5)	96.29	96.93	97.59	97.80	97.86	97.86
δ(1')	87.97	87.65	87.33	87.16	87.09	87.06
δ(4')	87.51	87.21	86.97	87.18	87 e	87 e
δ(2')	40.87	40.86	40.80	40.60	40.53	40.41
δ(3')	71.98	72.07	72.17	72.24	72.28	72.25
δ(5')	65.70	65.74	65.70	65.14	64.87	64.92
^2J(C5'-P5') [d]	5.0	5.1	4.9	4.6	4.0	4.6
^3J(C4'-P5')	8.7	8.8	8.7	8.5	-----e	-----e

a) Sample concentration was 75mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = +/- 0.01 ppm.

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

CONTINUATION OF FOOTNOTES FOR TABLE 10

d) Estimated accuracy +/- 0.2 Hz.

e) Due to overlap with the 1' resonance the error in the shift is large, and the coupling cannot be determined.

TABLE 11

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 5'-dUMP AT 300 K [a]

δ [b] / pH [c]	2.2	4.6	6.0	7.5	8.7	10.0
$\delta(4)$	167.54	167.55	167.57	167.68	167.99	173.14
$\delta(2)$	152.89	152.89	152.91	153.00	153.26	157.02
$\delta(6)$	143.18	143.20	143.34	143.62	143.58	142.72
$\delta(5)$	103.68	103.69	103.72	103.81	103.83	104.09
$\delta(1')$	86.74	86.74	86.67	86.56	86.54	86.55
$\delta(4')$	86 e	86 e	87.16	87.56	87.59	87.25
$\delta(2')$	40.25	40.25	40.20	40.12	40.11	40.07
$\delta(3')$	72.24	72.25	72.31	72.43	72.45	72.48
$\delta(5')$	65.98	65.93	65.57	64.92	64.84	64.94
$^2J(C5'-P5')$ [d]	5.0	5.0	4.9	4.6	4.2	4.6
$^3J(C4'-P5')$	-----e	-----e	8.7	8.6	8.6	8.4

a) Sample concentration was 75mg/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = +/- 0.01 ppm.

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

CONTINUATION OF FOOTNOTES FOR TABLE 11

d) Estimated accuracy +/- 0.2 Hz.

e) Due to overlap with the 1' resonance the error in the shift is large, and the coupling cannot be determined.

TABLE 12

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 5'-aUMP AT 300K[a].

δ[b] / pH[c]	3.8	5.8	6.3	7.5	9.0
δ(4)	167.54	167.56	167.58	167.69	169.77
δ(2)	152.75	152.76	152.78	152.87	154.39
δ(6)	144.20	144.27	144.36	144.47	144.23
δ(5)	102.67	102.68	102.70	102.74	102.83
δ(1')	86.22	86.20	86.17	86.12	86.19
δ(4')	82.60	82.77	82.96	83.23	83.21
δ(3')	76.71	76.70	76.69	76.67	76.29
δ(2')	75.22	75.23	75.25	75.30	75.49
δ(5')	64.63	64.37	64.09	63.68	63.72
$^2J_{(P-5')}$[d]	4.9	4.8	4.7	4.5	4.4
$^3J_{(P-4')}$	8.4	8.4	8.4	8.4	8.3

a) Sample concentration was 75 ml/ml in D_2O, prepared as described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from extrenal TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = $\pm$ 0.01 ppm.

CONTINUATION OF FOOTNOTES FOR TABLE 12

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy $\pm$ 0.2 Hz.

TABLE 13

CARBON CHEMICAL SHIFTS AND CARBON-PHOSPHORUS COUPLING CONSTANTS FOR 5'-aCMP AT 300 K[a]

δ[b] / pH[c]	4.8	5.7	6.2	6.9	7.5	8.0	10.0
$\delta(4)$	165.42	166.71	167.20	167.34	167.38	167.40	167.40
$\delta(2)$	156.14	157.85	158.50	158.67	158.74	158.77	158.77
$\delta(6)$	144.68	144.25	144.20	144.22	144.22	144.21	144.24
$\delta(5)$	96.54	96.76	96.85	96.89	96.90	96.90	96.91
$\delta(1')$	86.90	86.86	86.82	86.80	86.79	86.80	86.79
$\delta(4')$	82.82	82.89	83.11	83.26	83.31	83.33	83.33
$\delta(2')$	76.63	76.66	76.66	76.67	76.64	76.64	76.64
$\delta(3')$	75.58	75.72	75.88	75.87	75.98	76.00	75.99
$\delta(5')$	64.77	64.57	64.31	64.04	64.02	64.01	64.00
$^3J_{(C4'-P5')}$[d]	8.5	8.3	8.3	8.3	8.2	8.3	8.3
$^2J_{(C5'-P5')}$	4.7	4.7	4.6	4.5	4.3	4.3	4.4

a) Sample concentration was 75mg/ml in D_2O, prepared as described described in Chapter II, and contained 1% dioxane as an internal reference.

b) Chemical shifts are reported in ppm downfield from external TMS by setting the chemical shift of the internal dioxane reference equal to 67.859 ppm. Estimated accuracy = +/- 0.01 ppm.

CONTINUATION OF FOOTNOTES FOR TABLE 13

c) pH values are the readings of the pH meter and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy +/- 0.2 Hz.

TABLE 14

PROTON CHEMICAL SHIFTS (δ) AND COUPLING CONSTANTS (J) FOR A SERIES OF 3'-RIBONUCLEOTIDES[a]

Parameter[b]	3'-UMP pH 5.2[c]	3'-UMP pH 7.2	3'-CMP pH 5.2	3'-CMP pH 7.2
δ6	7.889	7.888	7.896	7.850
δ5	5.912	5.911	6.096	6.068
δ1'	5.945	5.938	5.940	5.945
δ2'	4.428	4.410	4.417	4.378
δ2"	-	-	-	-
δ3'	4.531	4.513	4.555	4.592
δ4'	4.253	4.235	4.285	4.226
δ5'	3.912	3.911	3.937	3.920
δ5"	3.863	3.862	3.849	3.874
J(5,6)[d]	8.15	8.17	7.63	7.54
J(1',2')	4.35	4.10	4.18	3.97
J(1',2")	-	-	-	-
J(2',2")	-	-	-	-
J(2',3')	5.02	4.96	4.74	5.44
J(2",3')	-	-	-	-
J(3',4')	5.59	5.59	5.41	5.71
J(3',P)	7.89	7.46	7.70	7.71
J(4',5')	2.39	3.10	2.77	2.59
J(4',5")	4.84	4.10	4.23	4.96
J(5',5")	-12.96	-12.80	-12.91	-13.00

FOOTNOTES FOR TABLE 14

a) Sample concentrations were 10 mg/ml in D_2O, prepared as described in Chapter II. Probe sample temperature 300 K.

b) ^1H frequency = 90 MHz, chemical shifts are reported in ppm downfield from internal TSP. Estimated accuracy $\pm$ .0015 ppm.

c) pH values are the readings of the pH meter, and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy $\pm$ .15 Hz.

TABLE 15

PROTON CHEMICAL SHIFTS (δ) AND COUPLING CONSTANTS (J) FOR A SERIES OF 3'-(2'-DEOXYRIBONUCLEOTIDES)[a]

Parameter[b]	3'-dUMP pH 5.2[c]	3'-dUMP pH 7.2	3'-dCMP pH 5.2	3'-dCMP pH 7.2
6	7.869	7.889	7.885	7.860
5	5.897	5.900	6.093	6.067
1'	6.288	6.312	6.278	6.287
2'	2.420	2.382	2.352	2.315
2"	2.589	2.569	2.602	2.572
3'	4.768	4.682	4.755	4.658
4'	4.207	4.174	4.218	4.166
5'	3.859	3.862	3.860	3.862
5"	3.790	3.802	3.787	3.795
J(5,6)[d]	8.10	8.09	7.61	7.55
J(1',2')	6.62	6.79	6.89	6.67
J(1',2")	6.65	6.65	6.52	6.62
J(2',2")	-14.41	-14.13	-14.21	-14.06
J(2',3')	7.91	6.79	7.41	6.92
J(2",3')	3.29	3.88	3.46	4.06
J(3',4')	3.66	3.77	3.63	3.94
J(3',P)	7.53	7.57	7.55	7.53
J(4',5')	3.46	3.67	3.42	3.75
J(4',5")	4.99	4.84	5.12	5.18
J(5',5")	-12.55	-12.57	-12.48	-12.42

FOOTNOTES FOR TABLE 15

a) Sample concentrations were 10 mg/ml in D_2O, prepared as described in Chapter II. Probe sample temperature 300 K.

b) ^1H frequency = 90 MHz, chemical shifts are reported in ppm downfield from internal TSP. Estimated accuracy $\pm$ .0015 ppm.

c) pH values are the readings of the pH meter, and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy $\pm$ .15 Hz.

TABLE 16

PROTON CHEMICAL SHIFTS (δ) AND COUPLING CONSTANTS (J) FOR A SERIES 3'-ARABINONUCLEOTIDES[a]

Parameter[b]	3'-aUMP pH 5.0[c]	3'-aUMP pH 8.0	3'-aCMP pH 5.0	3'-aCMP pH 8.0
δ6	7.877	7.895	7.857	7.848
δ5	5.879	5.833	6.066	6.049
δ1'	6.212	6.248	6.220	6.246
δ2'	-	-	-	-
δ2"	4.531	4.529	4.520	4.512
δ3'	4.471	4.411	4.461	4.397
δ4	4.191	4.135	4.209	4.150
δ5	3.937	3.935	3.934	3.935
δ5"	3.874	3.879	3.868	3.874
J(5,6)[d]	8.13	8.10	7.65	7.55
J(1',2')	4.46	5.02	4.25	4.73
J(1',2")	-	-	-	-
J(2',2")	-	-	-	-
J(2',3')	-	-	-	-
J(2",3')	3.13	4.15	2.71	3.60
J(3',4')	4.11	5.06	3.76	4.70
J(3',P)	8.35	7.72	8.34	8.09
J(4',5')	3.50	3.28	3.51	3.33
J(4',5")	6.18	5.77	6.46	5.84
J(5',5")	-12.46	-12.59	-12.39	-12.49

FOOTNOTES FOR TABLE 16

a) Sample concentrations were 4 mg/ml in D_2O, prepared as described in Chapter II. Probe sample temperature = 300 K.

b) ^1H frequency = 270 MHz, chemical shifts are reported downfield from internal tSP. Estimated accuracy $\pm$ 0.0005 ppm.

c) pH values are the readings of the pH meter, and are uncorrected for the deuterium isotope effect.

d) Estimated accuracy $\pm$ .15 Hz.

IV. RESULTS AND DISCUSSION

A. Geminal ^{13}C-^{31}P Coupling Constants

The pH dependence of the geminal couplings, J(C3'-P3') and J(C5'-P5') are shown in Figure 7 . Though the C5' and C3' are primary and secondary carbons, respectively, the two sets of data are similar (overall range: 4.3 - 5.1 Hz for J(C3'-P3'); 3.9 - 5.3 Hz for J(C5'-P5'). No significant dependence on the type of sugar or base is apparent. Decreases of about 0.5 Hz occur upon ionization of the phosphate in the pH 5-7 range, in line with the secondary pKa values (5.7 - 6.3) reported for 3'- and 5'-ribo- and 2'-deoxyribonucleotides[64]. (No determinations of phosphate pKa values for arabinonucleotides are known at this time; the titration curves in Figure 7 suggest that these are, to within 0.5 pH units, identical to those of the ribo- and deoxyribonucleotides.)

These results are in reasonable agreement with those of other authors[47,49], although data for 3'-UMP showed no change in the coupling over the pH range 4.2 - 8.4. An interesting point noted in this and other[49] data is the slight increase (0.2 - 0.5 Hz) in ^{2}J(C5'-P5') at pH > 9. However, many more data points must be accumulated at these pH values before a more concrete trend may be stated.

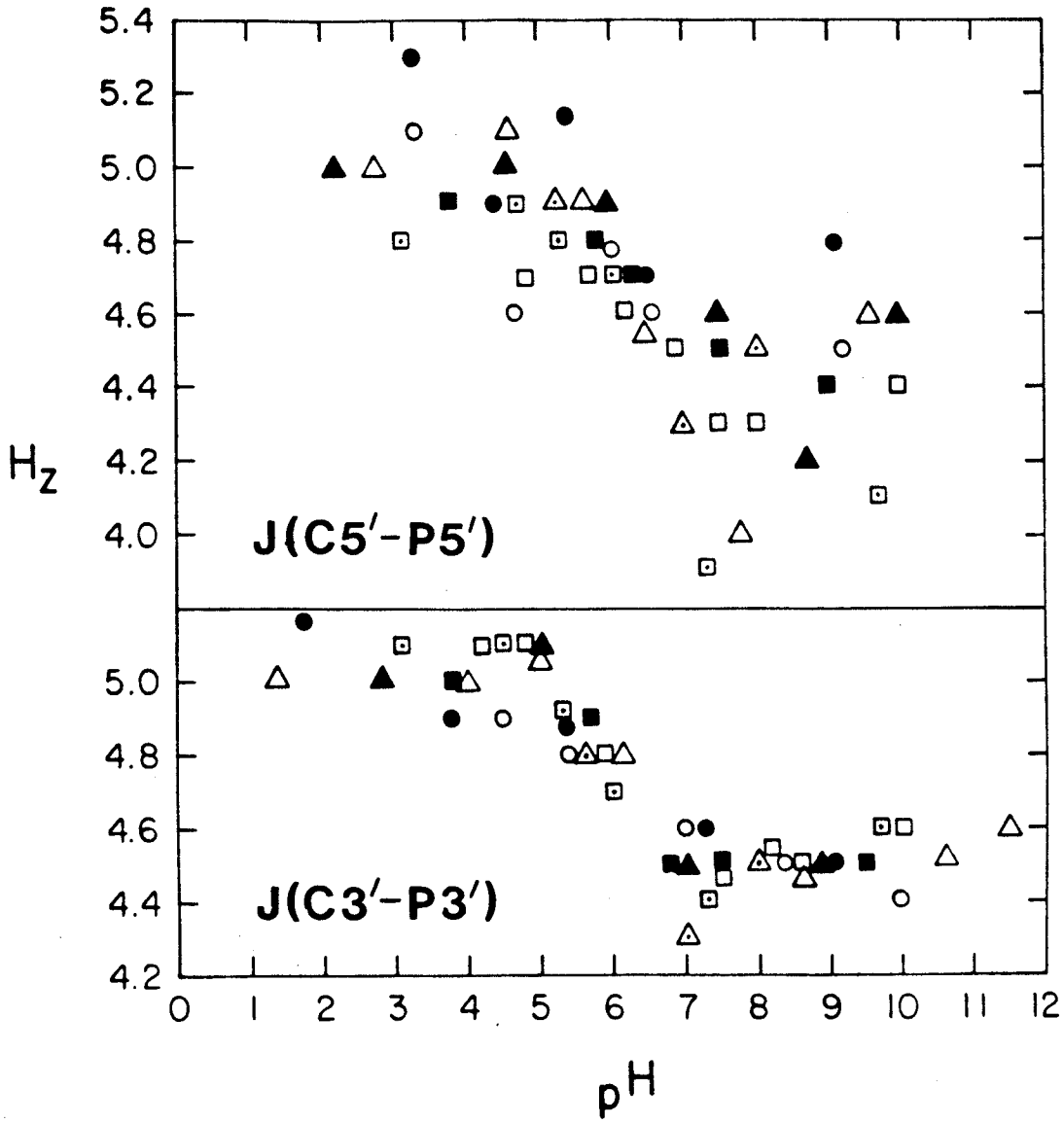
While this and other[47,49] studies observe geminal couplings for mononucleotides and nucleoside diphosphates not exceeding 5.3 Hz, an unusually large (7.0 Hz)[65] ^{2}J(C5'-P5') coupling has been noted in 3',5'-cyclic nucleotides. This may reflect a

FIGURE 7

The pH dependence of the geminal coupling constants J(C5'-P5') (top) and J(C3'-P3') (bottom) (uncorrected for the deuterium isotope effect).

The symbols used are:

ribonucleotides	circles
arabinonucleotides	squares
2'-deoxyribonucleotides	triangles
uracil base	solid symbols
cylosine base	open symbols
3',5'-diphosphates	dotted symbols


5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
Hz
J(C5′-P5′)
5.0
4.8
4.6
4.4
4.2
J(C3′-P3′)
0 1 2 3 4 5 6 7 8 9 10 11 12
pH

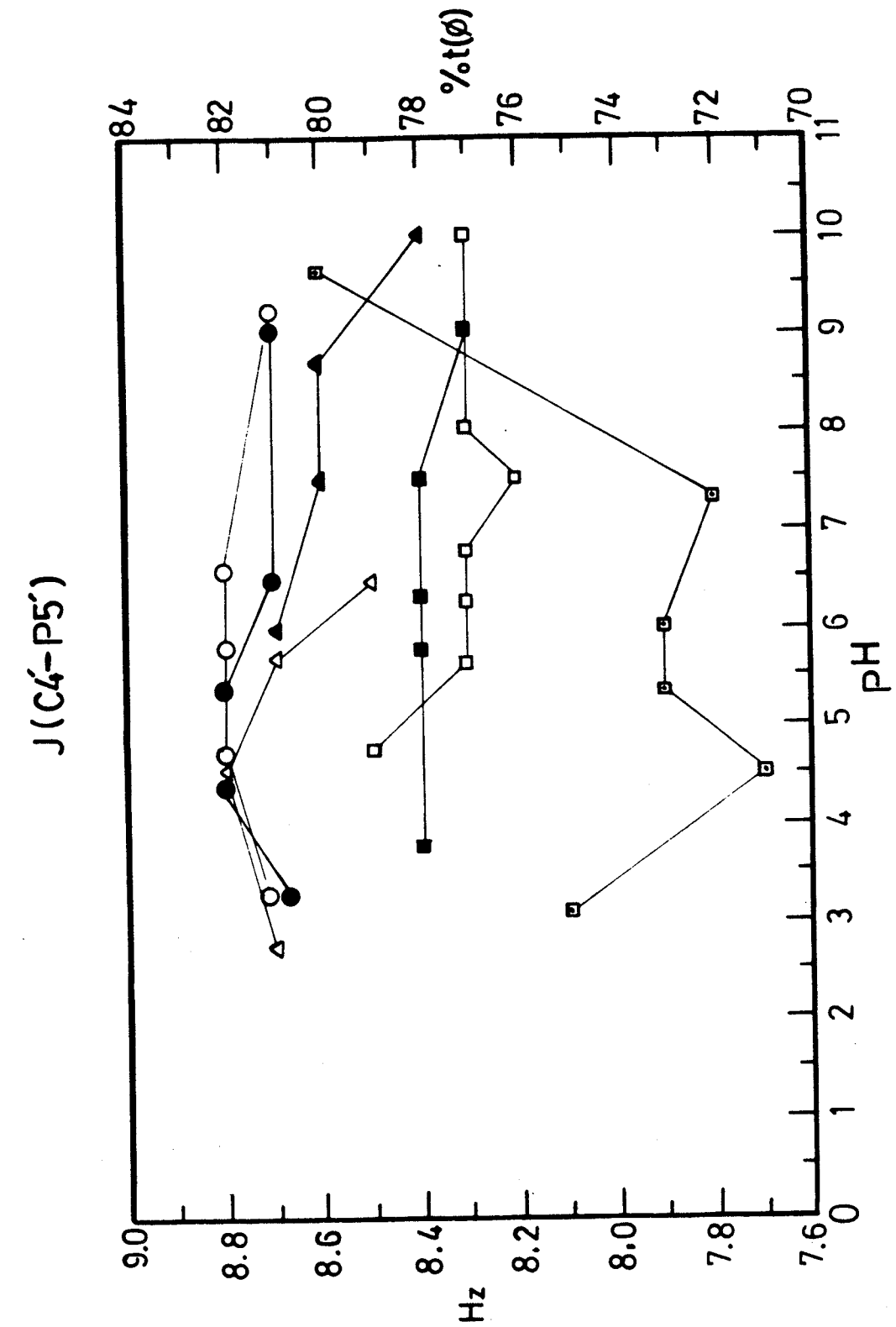
strained system, caused by perhaps, changes in the P-O-C bond bond angle[66] or the P-O[66,67] torsion angle.

B. Conformation about the C5'-O5' Bond (ϕ)

The effect of pH on the vicinal ^{3}J(C4'-P5') carbon-phosphorus coupling constant values for the 5'-nucleotides in the ribose (5'-UMP, 5'-CMP), 2'-deoxyribose (5'-dUMP, 5'-dCMP) and arabinose (5'-aUMP, 5'-aCMP) series is shown in Fig. 8 . (Note that the ordinate axis is labelled in both Hz and % t(ϕ) following Niemczura and Hruska[49]). For these molecules, the couplings fall into the narrow range of 7.7 to 8.8 Hz, over the entire pH range studied. This points to a preference (71-82%) for the trans orientation of the P5'-O5' and C5'-C4'- fragment, with little dependence on the nature of the sugar. These results are in line with published ^{1}H-^{13}C coupling constant data[29]. It should be noted, though, that the arabinose derivatives fall at the lower end of the observed range, reflecting possibly, a slight destabilization of t(ϕ) caused by the 2'-endo hydroxyl. This may be manifest directly or mediated via the ψ bond in light of the ψ-ϕ bond correlation which has been observed for pyrimidine 5'-nucleotides[68]. A complete proton analysis of the 5'-arabino-nucleotides would be of great use in answering this question.

FIGURE 8

The pH dependence of the vicinal coupling constant J(C4'-P5') (uncorrected for the deuterium isotope effect), for a series of 5'-nucleotides and 3',5'-aCDP. The symbols are as in Fig. 7.


J(C4'-P5')
Hz
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
%t(ø)
84
82
80
78
76
74
72
70
pH
0
1
2
3
4
5
6
7
8
9
10
11

C. Conformation about the C3'-O3' Bond (ϕ')

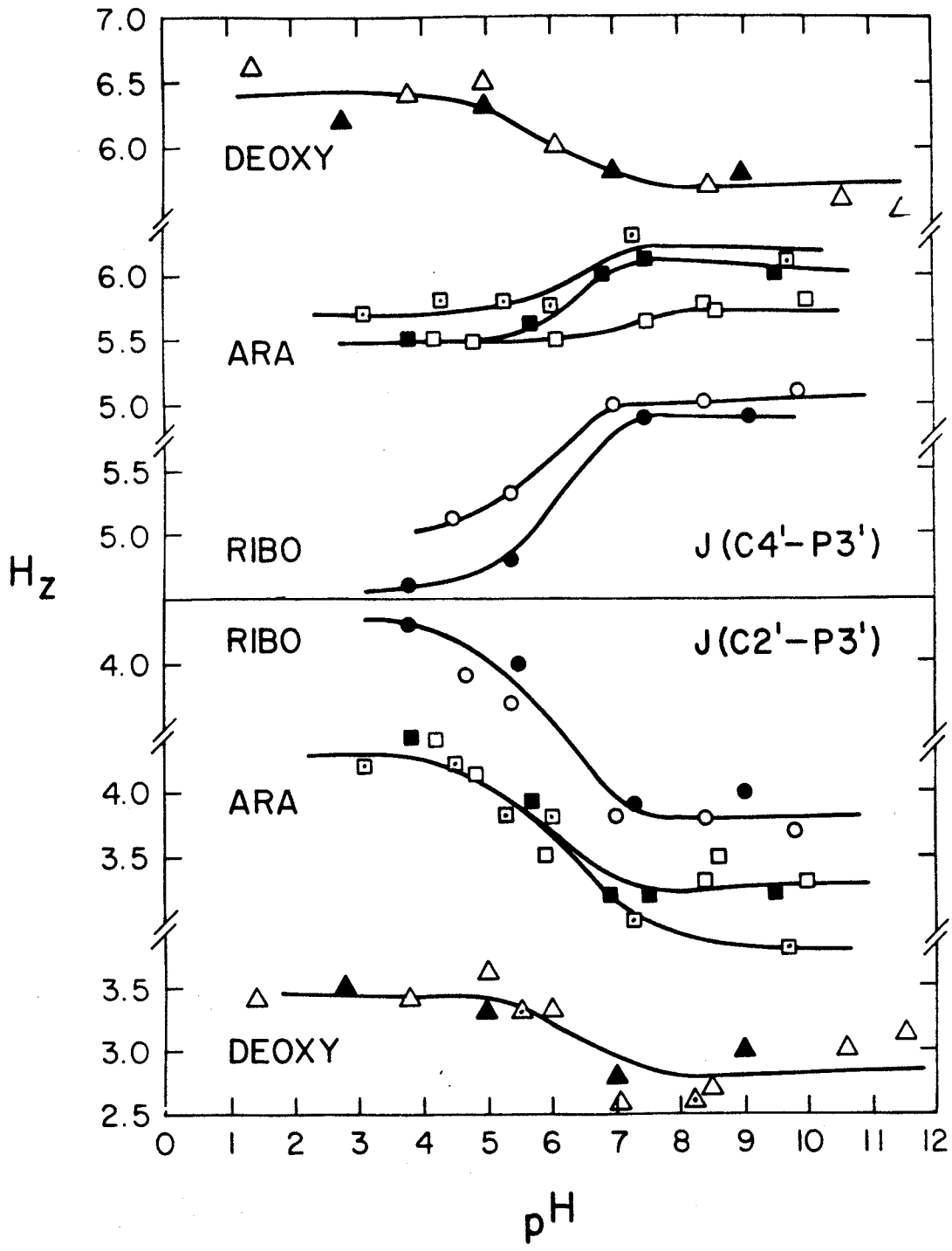
1. Vicinal ^{13}C-^{31}P Coupling Constants

The pH dependence of the vicinal J(C2'-P3') and J(C4'-P3') couplings for the ribose (3'-UMP, 3'-CMP), 2'-deoxyribose (3'-dUMP, 3'-dCMP) and arabinose (3'-aUMP, 3'-aCMP, 3',5'-aCMP) derivatives are shown in Fig. 9. It is important to note that in the figure a discontinuous numbering of the vertical axis was used to separate the titration curves according to the nature of the sugar, to reveal trends which would otherwise be masked by the considerable overlap. Since this magnifies differences between the three series, it should also be noted that the three sets of data are confined to the following, nonoverlapping ranges: J(C2'-P3'): 2.6 - 4.4 Hz; J(C4'-P3'): 4.6 - 6.6 Hz.

At pH < 5.0 the J(C2'-P3') couplings are similar for the ribose and arabinose derivatives (~4.3 Hz) while those for the 2'-deoxyribose derivatives are smaller (~3.4 Hz). Secondary ionization of the phosphate in the pH 5-7 range induces a decrease (~1.5 Hz) for both 3'-UMP and 3'-CMP. The pH-induced decreases for 3'-aUMP and 3'-aCMP are smaller (~1.0 Hz). In the arabinose series, the pH-induced decreases in J(C2'-P3') show a small dependence on the presence of a 5'-phosphate (~1.5 Hz in the 3',5'-aCDP plot). For the 2'-deoxyribose molecules small decreases in J(C2'-P3') (~0.5 Hz) are seen upon secondary ionization of the 3'-phosphate. Overall the nature of the base (U or C) has little effect on J(C2'-P3').

FIGURE 9

The pH dependence of the vicinal coupling constants J(C4'-P3') (top) and J(C2'-P3') (bottom) for a series of 3'-nucleotides and 3'-5'-diphosphates (uncorrected for the deuterium isotope effect). Symbols as in Fig. 7.


7.0
6.5
6.0
DEOXY
6.0
5.5
5.0
ARA
5.5
5.0
RIBO
J (C4'-P3')
RIBO
J (C2'-P3')
4.0
4.0
3.5
ARA
3.5
3.0
DEOXY
2.5
0 1 2 3 4 5 6 7 8 9 10 11 12
pH
Hz

The J(C4'-P3') couplings show a greater dependence on structure. At pH < 5.0 they fall in the range 4.5 - 6.6 Hz with the ribonucleotide values at the lower end, the 2'-deoxyribonucleotides at the upper end, and the arabinonucleotides in mid-range. In contrast with J(C2'-P3'), J(C4'-P3') for the ribose and arabinose molecules experience phsophate ionization-induced <u>increases</u> in the pH 5-7 range, while the 2-deoxyribose compounds still show a small decrease under these conditions. The increase for the ribonucleotides (1.0 - 1.3 Hz) is larger than the increase for the arabinose derivatives (0.3 - 0.5 Hz). For the 2'-deoxyribose molecules, however, ^{3}J(C4'-P3') like ^{3}J(C2'-P3') decreases (0.5 - 1.0 Hz) in going from pH 5 to 7. The above trends are in excellent agreement with those reported for 3'-dTMP[49] and 3'-UMP[47].

It should be pointed out that neither the nature of the sugar (ribose, 2'-deoxyribose, or arabinose), nor the base (cytosine or uracil) have a significant effect on either of the vicinal C-P couplings in alkali solution. At pH > 7 the majority of the ^{3}J(C2'-P3') and ^{3}J(C4'-P3') values fall into the narrow ranges of 2.7 - 3.3 Hz, and 5.7 - 6.2 Hz respectively.

The largest pyrimidine base influence is found for the ^{3}J(C4'-P3') coupling of ribonucleotides in acid solution. The difference between the cytosine and uracil derivatives here is 0.6 Hz.

2. The SUM (^3J(C2'-P3') + ^3J(C4'-P3'))

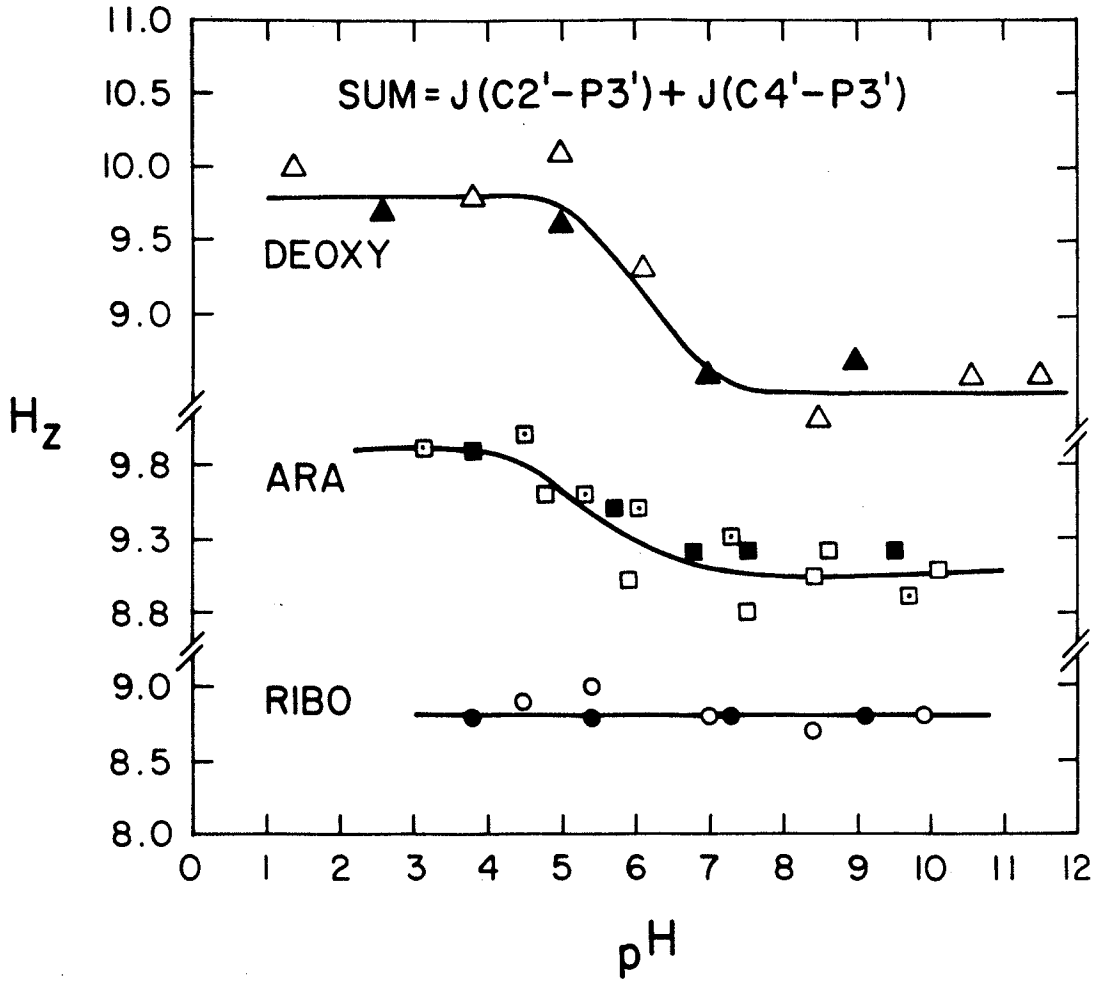
A useful quantity to consider is the SUM of the two C-P vicinal couplings (SUM = ^3J(C2'-P3') + ^3J(C4'-P3')). The dependence of the SUM values on pH are shown in Fig. 11. Again note that a discontinuous numbering system has been used on the ordinate axis (see section ϕ').

The observed trends are quite sensitive to the type of sugar, but overall, the nature of the pyrimidine base (U or C) has little influence on the couplings (<0.5 Hz). In light of this observation the following discussion will concentrate on the effects that the different sugar moieties have on the value of SUM. The ribose derivatives show virtually no variation of the SUM (8.7 - 9.0 Hz) over the pH range (3.8 - 9.8) studied. An examination of the magnitudes of individual couplings involved, reveals that the increase in ^3J(C4'-P3') upon ionization matches the decrease in ^3J(C2'-P3') with the result being the observed pH independent SUM. This is in excellent agreement with the data of Alderfer & Ts'o[47], for 3'-UMP (SUM = 8.8 - 9.0 Hz for the pH range 4.2 - 8.4).

Changes in the magnitude of the two couplings in the arabinose series, however, are not equal, with the ionization-induced increase in ^3J(C4'-P3') being smaller than the decrease in ^3J(C2'-P3'). This results in a pH dependent SUM whose value decreases (0.5 - 1.0 Hz) in the pH 5 to 7 range. The SUM values here range from 9.8 - 9.0 Hz over the pH range 3.1 - 9.6.

FIGURE 10

The pH dependence of the SUM = [J(C4'-P3') + J(C2'-P3')] for a series of 3'-nucleotides and 3',5'-diphosphates (uncorrected for the deuterium isotope effect). Symbols as in Fig. 7.


SUM = J(C2'-P3') + J(C4'-P3')
DEOXY
ARA
RIBO
Hz
pH
11.0
10.5
10.0
9.5
9.0
9.8
9.3
8.8
9.0
8.5
8.0
0
1
2
3
4
5
6
7
8
9
10
11
12

The 2'-deoxyribose derivatives show the largest change in their SUM, with values ranging from 9.8 - 8.5 Hz in the pH range 1.4 to 11.5. These results are in excellent agreement with those of Niemczura and Hruska[49] for 3'-dTMP. In contrast with the ribose and arabinose derivatives the 2'-deoxyribose molecules demonstrate a decrease for both ^{3}J(C4'-P3') and ^{3}J(C2'-P3') upon phosphate ionization. This parallel dependence of the individual couplings is reflected in the strong dependence of the SUM on the ionization state of the 3'-phosphate.

3. Electronegativity Effects

One factor complicating interpretation of the vicinal $^{13}C-^{31}P$ coupling constants in terms of the C3'-O3' conformational preferences is the electronegativity effect of the 2'-hydroxyl on J(C2'-P3'). In the ribonucleotides and arabinonucleotides, C2' and C4' are identically bonded: two carbons, one oxygen and one hydrogen atom. Thus the J(C2'-P3') and J(C4'-P3') couplings for the two series of molecules can be compared in conformational terms with minimal concern for electronegativity corrections (assuming the C2' configuration is not important). However, replacement of the 2'-hydroxyl by a hydrogen in the 2'-deoxynucleotides should lead to an increase in J(C2'-P3'). No theoretical estimates of the magnitude of these effects have been reported

The data in Figure 10 suggest that the electronegativity

effects can not be primarily responsible for the changes in the vicinal ^{13}C-^{31}P couplings of the 2'-deoxyribonucleotides relative to the ribo- and arabinonucleotides. For example, at pH < 5.0, the J(C2'-P3') couplings in the 2'-deoxyribose molecules are smaller by about 1.0 Hz rather than larger as would be expected if the electronegativity effect dominated. Furthermore, the J(C4'-P3') couplings in the 2'-deoxyribose molecules are larger than in the ribose and arabinose molecules by 1.0 - 1.5 Hz. These opposing trends in J(C2'-P3') and J(C4'-P3') are easily interpreted in terms of conformational changes about the C3'-O3' bond, but are not consistent with a dominant role for any inductive effect of the 2'-hydroxyl.

Additional evidence that inductive effects are of secondary importance is provided by the work on conformationally locked 3',5'-cyclic nucleotides by Lapper et.al.[65], who noted that the C2'-P3' couplings on the one hand, and the C4'-P3' couplings on the other, were identical to within 0.5 Hz for a series of ribose and 2'-deoxyribose derivatives.

4. Effects of Secondary Phosphate Ionization

The changes in J(C2'-P3') and J(C4'-P3') induced by phosphate ionization will reflect (a) conformational effects and (b) electronic (through-bond) effects. The conformational changes will include changes in the relative importance of the t(ϕ') and g^-(ϕ')

domains of the C3'-O3' bond (Fig. 5) and also in the positions of the minima of the potential energy profile for rotation about C3'-O3'. The electronic effects of phosphate ionization should be similar for the C2'-C3'-O3'-P3' and C4'-C3'-O3'-P3' fragments, and thus J(C2'-P3') and J(C4'-P3') should show parallel titration behaviour if electronic rather than conformational factors were dominant. However, for the ribose and arabinose molecules, phosphate ionization leads to an <u>increase</u> in J(C4'-P3') and a <u>decrease</u> in J(C2'-P3') (Fig. 10). These inverse trends can be understood in conformational (but not electronic) terms. By contrast, both sets of vicinal couplings for the 2'-deoxyribose molecules (3'-dUMP and 3'-dCMP) decrease upon phosphate ionization. It would seem, therefore, that electronic factors primarily define the overall trends in the ionization induced changes for the 2'-deoxyribose molecules. One can not discount, however, a combination of conformational changes (i.e., in $t(\phi')$, $g^{-}(\phi')$ weighting plus changes in positions of energy minima). These subtle effects of the nature and orientation of the 2'-substituents suggest caution when comparing, in conformational terms, the data for the three series of nucleotides.

5. Analysis of the C3'-O3'-Bond

Before proceeding with the conformational analysis of the C3'-O3' bond, a brief recap of the theoretical arguments involved in its analysis, would be beneficial at this point.

Alderfer and Ts'o[47] have discussed the interpretation of the J(C2'-P3') and J(C4'-P3') couplings in terms of the t(ϕ') and g^-(ϕ') populations (Fig. 5). (For steric reasons, g^+(ϕ') is neglected). However x-ray data[18,34] suggest that a continuous range of C3'-O3' torsion angles, spanning adjacent segments of the t(ϕ') and g^-(ϕ') domains, are accessible to a 3'-nucleotide. This would result from a low energy barrier at the H3', P3' eclipsed orientation (ϕ' = -120°; Fig.5). In addition, some theoretical calculations[45,46] reveal a broad energy minimum in the range of ϕ' values defined by the crystal data. If the barrier is small, or absent in solution, then interpretation of the couplings in terms of "conformer" populations is not valid[42-44]. For lack of a satisfactory model, therefore, the present data shall be interpreted in a qualitative manner. That is, only the following assumptions will be made: if J(C4'-P3') > J(C2'-P3'), the C4', P3' trans situation (t(ϕ') domain) is preferred over the C2', P3' trans situation (g^-(ϕ' domain)); and if J(C4'-P3') < J(C2'-P3'), the C2', P3' trans situation (g^-(ϕ')) is preferred. To facilitate the comparison, the ratio J(C4'-P3')/J(C2'-P3'), denoted by R, will be used.

At pH < 5.0 (monoanionic phosphate) the near equality of the vicinal ^{13}C-^{31}P couplings for the ribose derivatives (R = 1.1; average over all entries) indicate a balance of the t(ϕ') and g^-(ϕ') domains; for the arabinose derivatives a slight preference

for t(ϕ') is indicated (R = 1.3; overall average). Secondary phosphate ionization leads to a stabilization of t(ϕ') in the ribose and arabinose series (R = 1.7 - 2.2 at pH > 7.0) (The most pronounced stabilization appears for 3',5'-aCDP, presumably a consequence of phosphate-phosphate interaction). For the 2'-deoxyribose molecules a preference for t(ϕ') is apparent at pH < 5.0 (R ~ 1.9); secondary phosphate ionization has little effect (R ~ 2.1 at pH > 7.0). Thus, at pH > 7.0, the R values are similar for the three series of molecules (1.7 - 2.2), suggesting similar conformational behaviour. Also the ϕ' conformation is little affected by the nature of the pyrimidine bases examined. A syn pyrimidine base was found[17] to stabilize the t(ϕ') domain in a 3',5'-diphosphate (R > 10 in the syn 6-methyluracil derivative). A purine base-adenine in 3'-AMP - leads to a small destabilization of t(ϕ')[16].

6. Influence of the Sugar Pucker

In general, furanose rings execute an interconversion in solution between the N(3'-endo) and S(2'-endo) puckers, the populations of which are estimated from proton coupling constants[27,28]. It is thought that sugar-pucker-dependent close-contact interactions, involving the oxygen atoms of the 3'-phosphate and the atoms on the sugar ring should influence the potential energies of the t(ϕ') and g^-(ϕ') domains[54,68] and hence their relative weightings in solution.

In this section some of these interactions are considered qualitatively to see whether the conformational trends suggested by Fig. 9 can be understood in terms of a ϕ', sugar-pucker interdependence. Useful for this discussion are the estimates (Table 17)of the 3'-endo populations for the 3'-nucleotides obtained from the pH study of their proton spectra. In general agreement with earlier (but less complete) data[31,36,70], the 2'-deoxyribose-3'-nucleotides show a preference (60-70%) for 2'-endo, whereas the 3'-ribonucleotides show a near balance (or slight preference for 3'-endo). Their pucker is not strongly dependent on the ionization state of the 3'-phosphate. The 3'-arabinonucleotides at pH < 5 resemble the 2'-deoxyribose molecules (ca. 60% 2'-endo) but show a near balance in 2'-endo and 3'-endo at pH > 7.

a. Close Contacts with C5' Substituents

Sasisekharan and coworkers[53,69] have shown that, in the 3'-endo pucker of a ribose or 2'-deoxyribose 3'-nucleotide, contacts are made between the O atoms of the 3'-phosphate and the C5' substituents, which should destabilize large angles in the $g^-(\phi')$ range. Molecular models indicate that similar contacts in the 3'-endo pucker should destabilize $g^-(\phi')$ in a 3'-arabinonucleotide. These contacts with C5' substituents may, however, be discounted as the dominant factor determining the trends in the ϕ' orientational preferences suggested by the J(C,P) data in Fig. 9), since,

TABLE 17

CALCULATED POPULATIONS (%) OF THE N(3'-ENDO) PUCKER FOR THE 3'-NUCLEOTIDES[a]

Compound	3'-endo(%) pH < 5	pH > 7
3'-UMP	56	56
3'-CMP	54	57
3'-dUMP	37	38
3'-dCMP	36	39
3'-aUMP	41	51
3'-aCMP	38	48

a) Assuming %N ≈ 10 $J_{3',4'}$; %S = 100% - N, as in Ref. 36.

if they were dominant, we would expect the smallest $g^-(\phi')$ weighting for those molecules with the largest 3'-endo weighting, namely the 3'-ribonucleotides. However, at pH < 5 the converse is noted, since the smallest $g^-(\phi')$ weighting is shown by the 2'-deoxyribonucleotides that have the smallest 3'-endo population. Furthermore, at pH < 5 the arabinonucleotides have a 2'-endo, 3'-endo distribution like that of the deoxyribonucleotides but a $t(\phi')$, $g^-(\phi')$ distribution like that of the ribonucleotides.

b. Contacts with the C2'-Hydroxyl Group

Pattabiraman et al.[53] have pointed out that, in a 3'-ribonucleotide with a 2'-endo conformation, close contacts between the 3'-phosphate and the 2'-hydroxyl group destabilize small angles in the $t(\phi')$ domain. This destabilization, occurring during the time the ring spends in 2'-endo, would seem to account for the reduced $t(\phi')$ weighting in the 3'-ribonucleotides, relative to the 3'-deoxyribonucleotides, that is observed at pH < 5. However, this reasoning cannot be extended to the data at pH > 7, since at this higher pH, the ϕ' distributions are similar in the two series even though the riboses retain their significant contribution (ca. 45%) from 2'-endo.

In contrast with the situation in ribonucleotides, close contacts between the 3'-phosphate and the 2'-hydroxyl group are not possible in a 2'-endo arabinonucleotide. In fact, in the 2'-endo pucker the close contacts affecting the ϕ' orientation are identical

for an arabinose and a 2'-deoxyribose molecule in both the t(ϕ') and g^-(ϕ') domains, since this pucker provides maximum separation between the 3'-phosphate and the endo-2'-hydroxyl. Thus, in solution the t(ϕ'), g^-(ϕ') distribution of an arabinonucleotide and a 2'-deoxyribonucleotide should not differ during the times spent in 2'-endo. However, molecular models reveal close contacts in a 3'-endo arabinonucleotide that should destabilize t(ϕ'). This destabilization, occurring during the time spent in 3'-endo, would seem to account for the decrease in the t(ϕ') weighting in the arabinonucleotides relative to the deoxyribonucleotides at pH < 5. But again, the reasoning cannot be extended to the pH > 7 data, at which point their ϕ' distributions are similar, even though the arabinonucleotides retain a significant (and , in fact, enhanced) 3'-endo contribution at the higher pH. Thus, it is difficult to provide a simple rationale of the trends in Fig. 9 , which is based on a dominant, steric role for the 2'-hydroxyl group and which applies consistantly over the entire pH range.

c. Other Influences of the C2'-Hydroxyl Group

In view of the present ^1H and ^{13}C data though, the following comments can be made concerning the conformational consequences of phosphate ionization: a) Observation of ^3J(C4'-P3') shows a significant increase in t(ϕ') for the ribose sugars, a smaller increase for the arabinose sugars, and a decrease for the 2'-

deoxyribose sugars, (assuming the coupling constants can be interpreted purely in conformation terms, (see section IV. C. 3.)
b) The sugar conformations remain unchanged for the ribose and 2'-deoxyribose sugars, but the arabinose derivatives move toward C3'-endo, from ~40% to ~50% (average for the U and C bases). In both the ribose and arabinose sugars therefore, unexpected stabilization of the t(ϕ') conformation is observed. Note, as explained above, that space filling models reveal that arabinose sugars in 3'-endo ϕ'(t), and a ribose sugar in the 2'-endo-ϕ'(t) conformation experience similar 2'-hydroxyl-phosphate group close contact interactions.
(c) There is little if any conformational change observed for either the sugar pucker or the ϕ' orientation upon phosphate ionization in the 2'-deoxyribose molecules. Therefore, it can be speculated that the above trends taken in concert, point to an electrostatic attraction between the doubly charged phosphate group and the 2'-hydroxyl proton.

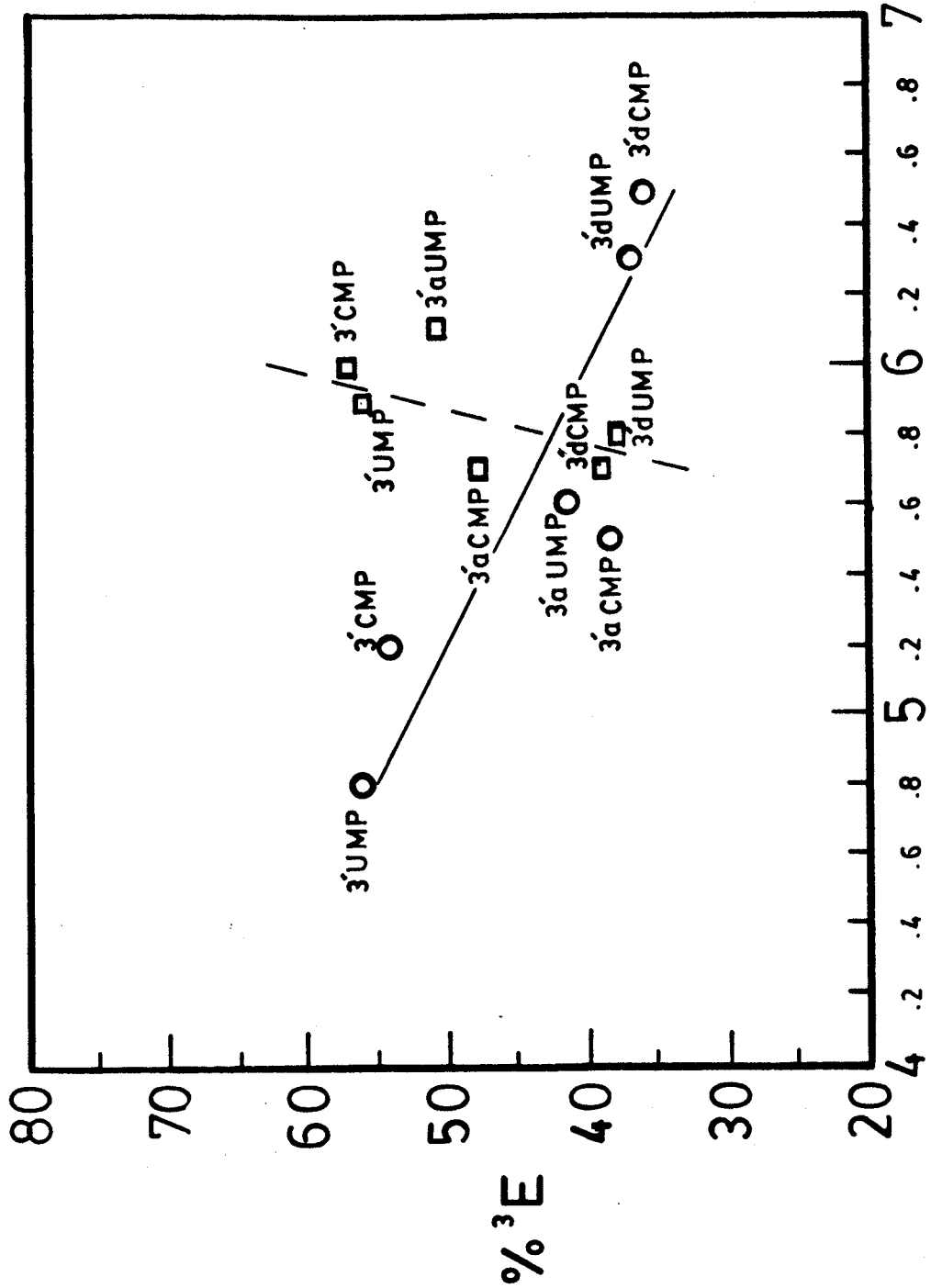
Evidence for this speculation can be drawn from a plot of % 3'-endo vs. ^{3}J(C4'-P3')(Fig. 11), for each 3'-nucleotide at the pH values of 5 and 8. [Note: that the larger values of ^{3}J(C4'-P3') simply reflect a larger contribution from ϕ'(t).] As mentioned previously crystal data and some NMR reports indicate a correlation of C3'-endo with ϕ'(t) and C2'-endo with ϕ'(g^{-}). In addition, the C2-endo-ϕ'(t) conformer is only disallowed for the ribose sugars. Therefore, as argued above, the ribose sugars only receive significant contributions to ϕ'(t) when they are C3'-endo

FIGURE 11

Variation of %N(3'-endo) with J(C4'-P3') for a series of 3'-nucleotides. The entries are as labelled in the Figure.

● data for pH < 5

■ data for pH > 7


80
70
60
50
40
30
20
% 3E
4
.2
.4
.6
.8
5
.2
.4
.6
.8
6
.2
.4
.6
.8
7
J (C4'-P3') Hz
3'UMP
3'CMP
3'UMP
3'CMP
3áUMP
3áCMP
3áUMP
3áCMP
3áCMP
3áUMP
3dCMP
3dUMP
3dUMP
3dCMP

whereas the 2'-deoxyribose sugars may populate $\phi'(t)$ while in both sugar conformations. Therefore, even though the ribose sugars receive a larger contribution from 3'-endo the 2'-deoxyribose derivatives show the higher population of $\phi'(t)$.

The case for pH > 7 is quite different (Fig. 11). Here the sugars behave as predicted, i.e. a larger contribution from 3'-endo is reflected in a higher tendancy toward $\phi'(t)$. Again as speculated previously this might reflect a stabilization of the $\phi'(t)$ conformer during the time the ribose sugars spend in 2'-endo. As well, the $\phi'(t)$ conformer must also be stabilized during 3'-endo conformations of the arabinose sugars.

If the above situation is actually the case, a C2'-O-H$\cdots\bar{O}$-P3' hydrogen bond would not be unexpected in the crystal structure of a dianionic 3'-ribo or arabinonucleotide. Partial support for this line of reasoning can be taken from the crystal structure of disodium uridine-3'-phosphate tetrahydrate[71] (Na_2 3'-UMP·$4H_2O$), the only dianionic 3'-nucleotide reported to date. Although the ϕ' conformation here is -106° (~g^-), and there are no intramolecular hydrogen bonds, the shortest intermolecular hydrogen bond is between the 2'O-H (proton donor) and an adjacent 3'-phosphate oxygen (proton acceptor). This demonstrates the strong potential for hydrogen bonding between these two substituents given favorable conformational constraints. Additional dianionic 3'-nucleotide crystal structures for both ribose and arabinose sugars, plus a

comparison of the exchange rate of the 2'-OH proton at pH 5 and 8 should provide considerably more insight into this situation.

V. CONCLUSION

CONCLUSION

This work has dealt mainly with the pH dependence of the vicinal ^{3}J(C2'-P3') and ^{3}J(C4'-P3') coupling constants, for pyrimidine 3'-nucleotides in the ribose (3'-UMP, 3'-CMP), 2'-deoxyribose (3'-dUMP, 3'-dCMP), and arabinose (3'-aUMP, 3'aCMP) series. These couplings have then been interpreted, in a qualitative manner, in terms of the conformational preferences about the C3'-O3' (ϕ') bond. The (ϕ') conformation has been shown to be influenced by sugar-pucker dependent interactions with the 2'-hydroxyl group. There is good agreement between the present data and crystal [72] studies, as well as energy calculations[53], which suggest that a shift toward a 2'-endo pucker should effect a decrease in the t(ϕ') weighting for 3'-ribonucleotides in acid solution. Similar reasoning has also led to the proposal that a shift toward a 3'-endo conformation should effect a decrease in the t(ϕ') conformation for 3'-arabinonucleotides in acid solution. Thus, it has been possible to provide in terms of pucker-dependent 3'-phosphate-2'-hydroxyl contacts, a qualitative description of the gross trends in the ^{3}J(C-P3') data at pH < 5, however, only a speculative interpretation has been offered for the data at pH > 7.

The conformation about the C5'-O5' (ϕ) bond has also been investigated. The 2'-hydroxyl appears to have little influence here, with t(ϕ) conformations falling in the range 70-80%. These conformations appear to be independent of the nature of the

sugar (ribose, 2'-deoxyribose, or arabinose), the type of base (uracil or cytosine) or the pH of the solution.

This work has also provided a detailed description of the synthesis of a series of pyrimidine 3'-, and 5'-nucleotides with 2'-deoxyribose or arabinose sugars. The synthesis of 3',5'-aCDP has also been described.

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