# A NUCLEAR MAGNETIC RESONANCE INVESTIGATION INTO THE EFFECT OF THE SYN CONFORMATION OF THE N-GLYCOSYL BOND ON THE OVERALL CONFORMATION OF SOME PYRIMIDINE NUCLEOSIDES, NUCLEOTIDES, AND DINUCLEOSIDE MONOPHOSPHATES

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The Faculty of Graduate Studies and Research
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of the Requirements for the Degree of
DOCTOR OF PHILOSOPHY

by

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## ABSTRACT

A series of nucleic acid model compounds, $m^6dU$, 3'-$m^6dUMP$, 5'-$m^6dUMP$, 3',5'-$m^6dUDP$, d(Tp$m^6$U), d($m^6$UpT) and d($m^6$Up$m^6$U), were synthesized and compared to a similar series of dT derivatives to investigate the effect of the syn conformation of the N-glycosyl bond on the overall conformation of pyrimidine nucleosides, nucleotides, and nucleotidyl fragments of dinucleoside monophosphates in solution. Proton chemical shifts ($\delta$) and coupling constants were determined at 360 MHz for 3',5'-dTDP and 3',5'-$m^6dUDP$ at two pH values and compared with respect to the conformational information they contained. In addition, the proton chemical shifts and coupling constants (also at 360 MHz) were determined for the dinucleoside monophosphates, d(TpT), d(Tp$m^6$U), d($m^6$UpT), and d($m^6$Up$m^6$U), and compared with the reported chemical shifts and coupling constants for the dT and $m^6dU$ nucleotides available in the literature. The carbon chemical shifts at 22.63 MHz and carbon-phosphorus coupling constants for the entire series of dT and $m^6dU$ nucleosides, nucleotides, nucleoside diphosphates, and dinucleoside monophosphates (including the mixed dinucleoside monophosphates of dT and $m^6dU$) are reported and discussed in light of the conformational information available from the $^1H$ NMR data. Also, the proton-carbon vicinal coupling constants between the anomeric proton, $H_{1'}$, and $C_2$ and $C_6$ of the pyrimidine ring of the nucleosides and nucleotides are reported. An attempt was made to use the observed $^3J$(H1'-C2) and $^3J$(H1'-C6) in the $m^6dU$ series to make a quantitative estimate of the syn and anti conformer populations in dT.

ABSTRACT (Continued)

The effect of pH and temperature changes on the proton and $^{13}C$ NMR was examined. The data provide information about the conformation of the 2'-deoxyfuranose ring and the orientation of nucleotide units about the exocyclic $C_{4'}—C_{5'}$, $C_{5'}—O_{5'}$, and $C_{3'}—O_{3'}$ bonds for the molecules listed above. It was found that the syn base, in general, has shifted the normal 2'-endo-3'-endo equilibrium normally found in anti pyrimidine deoxyribose derivatives. In addition, destabilization of the $g_+$ ($C_{4'}—C_{5'}$) conformer and, to a lesser extent, the t ($C_{5'}—O_{5'}$) conformer which are assumed by the units in helical DNA is apparent. Finally, stabilization of the t ($C_{3'}—O_{3'}$) conformer, particularly at the 3',5'-diphosphate level in basic solution in which the phosphates are doubly ionized is indicated.

This study also provides information about correlations between the sugar pucker and the $C_{4'}—C_{5'}$ and $C_{3'}—O_{3'}$ conformations and the conformational requirements for, and consequences of, UV-induced photodimer formation in the d(TpT) fragment.

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FOR FERNANDO

# CHAPTER I

# INTRODUCTION

Nuclear magnetic resonance spectroscopy (NMR) has been established as a definitive method for the study of the conformation and dynamics of molecules in solution. NMR was first used for organic structure determination and for physical studies of the nature of molecular environments and interactions. As confidence in the technique grew, researchers in the field began to investigate the complicated problems of biochemical processes with the aid of NMR spectroscopy. These applications have grown in number over the past twenty-five years and have made substantial contributions to the understanding of life processes. Recent reviews have summarized the role of magnetic resonance in the study of peptides[1], proteins and enzymes[2,3], lipids and membranes[4], carbohydrates and polysaccharides[5], and of nucleosides, nucleotides, and nucleic acids[6,7].

The use of NMR spectroscopy for the conformational analysis of nucleic acids and their components in solution has been particularly extensive, as can be seen in the reviews of Davies[6] and Kearns[7]. Early proton NMR studies of nucleoside conformation were reported by C. D. Jardetzky[8-10]. These investigations were hampered by the low resolving power of the available magnetic fields ($B \leq 1.4T$ or 60 MHz for $^1H$), and were confined to discussions of sugar ring pucker based on spin couplings measured from the splittings of isolated resonances. A similar study of thymidine by Lemieux[11] showed that some proton-proton couplings could be determined for the deoxyribose ring at these fields. For the most part, the early work on nucleosides was confined to the interpretation of the dependence of base ring proton

shifts and resolvable proton-proton couplings in terms of the conformational changes induced by perturbations from the medium (i.e., pH, concentration, temperature, solvent composition, etc.)[12-18]. These perturbations were discussed primarily with respect to changes in base stacking and internal hydrogen bonds.

The conformational analysis of nucleosides and nucleotides by $^1H$ NMR was aided by the development of high resolution, high field spectrometers operating at 100 MHz and above for protons[19-25]. Hruska, Smith, and co-workers[19-22] combined high resolution spectra with computer calculated and simulated spectra to extract all the chemical shifts and coupling constants from the tightly coupled six-spin system of the sugar ring of uridine and α- and β-pseudouridine. These studies were the first to demonstrate the application of high resolution NMR spectra to the complete conformational analysis of the ribofuranose ring and the backbone of nucleosides and, later, nucleotides[23-25].

Instrumental advances further expanded the range of commercially available spectrometers. Today, facilities are available to record routinely 360 MHz and 400 MHz proton spectra, while members of the Department of Chemistry at the Carnegie-Mellon Institute in Pittsburgh have built a spectrometer operating at 600 MHz - ten times the field strength used in the original work on nucleosides in the early 1960's.

The early work on nucleosides and nucleotides has already been extended to many dinucleoside phosphates[26-36], a dinucleotide[36], trinucleoside diphosphates[37-39], and to polynucleotides[37,40].

Danyluk and co-workers[34,35,41,42] have shown that synthesis of an oligonucleotide in which one or more residues are selectively deuterated allows the proton analysis of the monomer components in an oligomer. Recently, Altona and co-workers[28,38] have shown that high field spectra (360 MHz), combined with extensive homonuclear and heteronuclear (i.e., $^{31}P$) decoupling experiments and aided by computer simulation of the observed spectrum, yields an unambiguous assignment of resonances up to the trimer level. With the development of the 600 MHz machine, this limit might be extended to the tetramer or pentamer level, but the complete proton analysis of larger oligonucleotides is restricted by the extensive peak overlap accompanying the increased number of resonances.

Aside from the use of the conventional proton chemical shift and coupling constant analysis, there has been an increase in the use of other spectral parameters for structure determination. Applications of the proton spin-lattice relaxation time, $T_1$[43-45], and nuclear Overhauser effect (NOE)[44,45] measurements have attempted to answer questions concerning the dynamics of nucleosides in addition to supplementing the information on their conformation obtained from other methods. These studies have focused primarily on the N-glycosyl bond conformation and on the dynamics of the ribofuranose ring, but a recent application[46] has demonstrated the utility of these methods in determining exocyclic or backbone conformations.

The use of other nuclei in the magnetic resonance studies of nucleic acids has grown in recent years. With the introduction of

pulsed Fourier transform techniques, carbon-13 NMR has been employed in the conformational analysis of these molecules. Although not as extensively used as proton NMR, $^{13}C$ studies have the advantage of a wider chemical shift dispersion of the resonances. In addition, the low natural abundance, combined with proton noise decoupling, yields a spectrum in which each distinct carbon gives rise to only one line, in the case of nucleosides. Early studies by Jones et al.[47-50] on nucleosides and Dorman and Roberts[51] on nucleotides hinted at the utility of $^{13}C$ shifts in conformational work although no effects due to base stacking, as detected from proton work, could be observed. Reports published by Lemeiux and co-workers[52] and Davies[53] have described the utility of proton-carbon couplings for conformational studies. The ribose carbons were found to give rise to broad resonances from unresolved long-range couplings. Only the large one-bond coupling was measurable. The base carbons, however, are better resolved, and the three-bond $^{1}H$-$^{13}C$ coupling from the anomeric proton into the base ring carbons could be used to determine the conformation about the N-glycosyl bond. For nucleotides and larger oligomers, the carbon-phosphorus spin-spin couplings can be measured directly under conditions of proton noise decoupling and serve as another conformational probe[54-56]. Carbon-13 relaxation measurements[57-59] can be used to study the overall motion of nucleic acids in solution and can detect the presence of internal motion in a molecule.

Nuclei, such as phosphorus and nitrogen-15, have not been used as extensively as protons or carbon-13. $^{31}P$ NMR[34,35,60,61] has been utilized primarily as an aid in proton analysis, while the low sensitivity and natural abundance of $^{15}N$ has limited the widespread use of this nucleus[62-64].

The major emphasis of the afore-mentioned studies has been placed on conventional nucleic acid derivatives, i.e., the naturally occurring purine and pyrimidine nucleosidyl units. In these molecules, the orientation about the N-glycosyl bond is believed to be predominantly anti (for the pyrimidine base) or in equilibrium between the syn and anti conformations (as is the case for the purine base). To date, there have been few studies which investigate the effect of the syn conformation on the overall conformation of nucleic acid derivatives.

This thesis presents a systematic comparison between two pyrimidine nucleosides - thymidine and 6-methyldeoxyuridine. The former molecule is believed to exist predominantly in the anti conformation while the latter is expected to exist predominantly in the syn conformation. Proton and carbon-13 high-resolution nuclear magnetic resonance data will be used to determine quantitative conformational properties for a selected series of nucleosides, nucleotides, nucleoside diphosphates, and dinucleoside monophosphates containing the 5-methyluracil and 6-methyluracil bases. The information, in turn, will be compared in order to determine the conformational preferences for model nucleic acid compounds which contain a pyrimidine ring in the syn or anti conformation about the N-glycosyl

bond. The data will also be examined to see what effect phosphorylation or incorporation into a dinucleoside monophosphate has on the N-glycosyl bond.

The results presented should further our knowledge of nucleic acid structure and function.

# CHAPTER II

# EXPERIMENTAL CONSIDERATIONS

The purpose of the conformational analysis of a molecule is to determine the position of an atom or group of atoms relative to the other members of the molecule. In this thesis, an attempt will be made to determine the conformation of a related group of nucleosides, nucleotides, and dinucleoside phosphates in solution. In this chapter, the state of the art of the conformational analysis of nucleic acid derivatives is reviewed. The more common techniques are discussed, divided according to the magnetic nucleus used, and further subdivided according to what portion of the molecule the information probes.

## A. NOMENCLATURE

### 1. CHEMICAL NOMENCLATURE

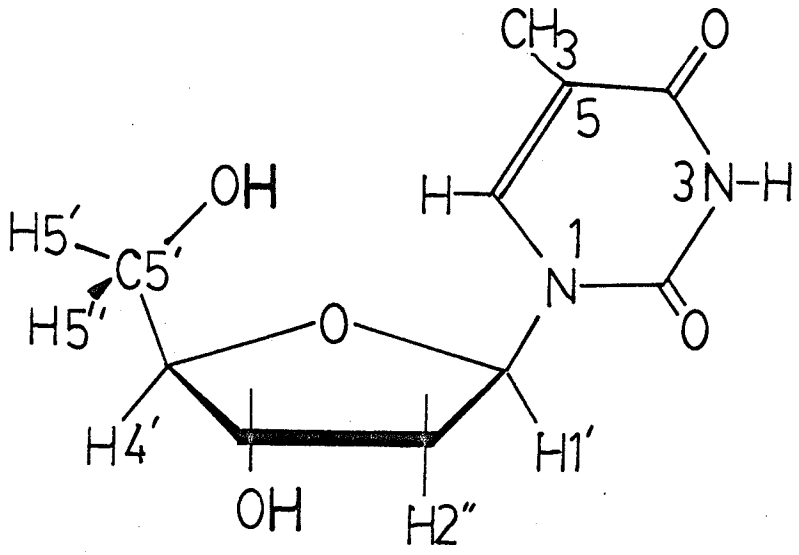
The nomenclature and symbols used in this chapter and throughout the rest of this work follow from the IUPAC-IUB recommendations[65]. For example, thymidine is hereafter referred to as 2'-deoxythymidine and symbolized as dT while the 6-methyl analogue will be called 6-methyl-2'-deoxyuridine and symbolized as $m^6dU$. Figure 2.1 illustrates the structure of these two nucleosides along with the accepted numbering scheme. Nucleotides will be designated as 3'-dTMP, 5'-dTMP and 3',5'-dTDP for the 3'- and 5'-monophosphates and 3',5'-diphosphate derivatives of 2'-deoxythymidine, respectively. Similarly, the nucleotides of 6-methyl-2'-deoxyuridine will be symbolized as 3'-$m^6$dUMP, 5'-$m^6$dUMP and 3',5'-$m^6$dUDP.

The designation of dinucleoside phosphates follows along similar lines. For example, the molecule 2'-deoxythymidyl-(3'-5')-2'-deoxythymidine will be written as d(TpT) while 2'-deoxythymidyl-(3'-5')-6-methyl-2'-deoxyuridine will be abbreviated as d(Tpm$^6$U). All dinucleoside phosphates studied are 3',5'-linked.

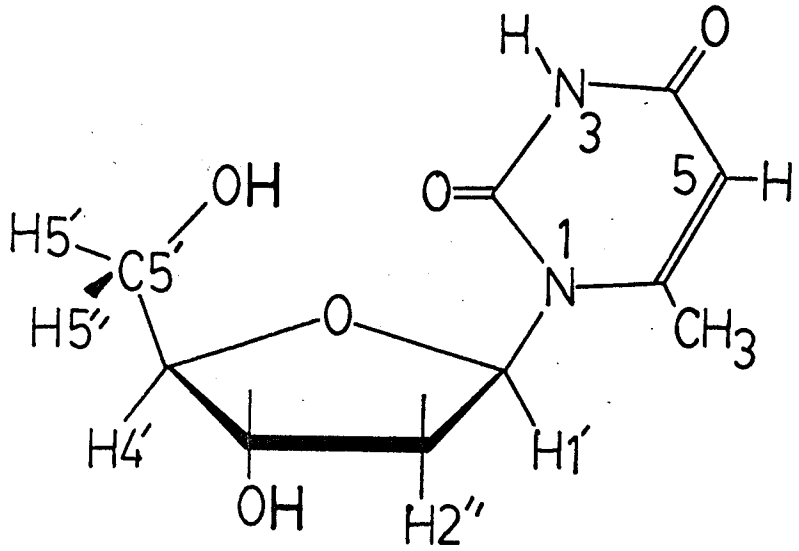
### 2. CONFORMATIONAL NOMENCLATURE

The conformational nomenclature follows that recommended at the 5th Jerusalem Symposium[66] and used in Davies' review[6]. Figure 2.2 shows a dinucleoside phosphate fragment where all the major bonds have been designated by their standard labels. The nucleotide

FIGURE 2.1 contains the structural formulas for a) dT and b) $m^6dU$ with the numbering scheme used for the pyrimidine and deoxyribose rings.


CH3
O
5
H
3N-H
OH
H5′
C5′
H5″
1
N
O
O
H4′
H1′
OH
H2″

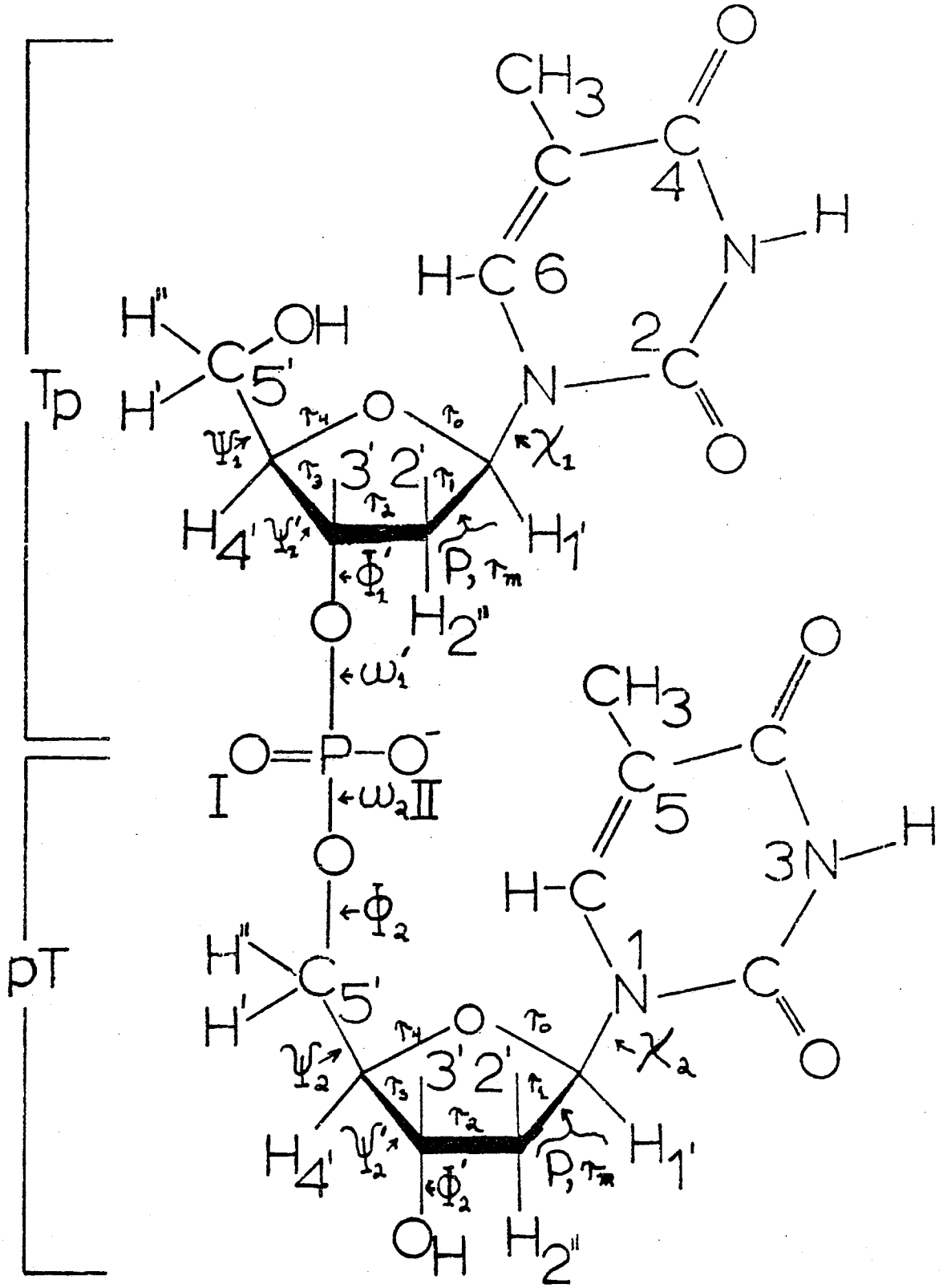
a


H
N
O
3
5
H
OH
H5′
C5′
O
1
H5″
N
CH3
O
H4′
H1′
OH
H2″

b

FIGURE 2.2 shows the structural formula for d(TpT). Superimposed on the figure are the labels for the bonds discussed in this thesis.

# d(TpT)


Tp
pT
CH3
C
C
4
N
H
H-C 6
2
C
O
H''
OH
H'
C 5'
N
O
χ1
Ψ1
τ4
τ0
τ3
3' 2'
τ1
τ2
H4'
Ψ2'
H1'
Φ1'
P, τm
H2''
ω1'
O=P-O⁻
I
ω2 II
Φ2
CH3
C
C
5
3N
H
H-C
1
N
C
O
χ2
Ψ2
τ4
τ0
τ3
3' 2'
τ1
τ2
H4'
Ψ2'
H1'
Φ2'
P, τm
OH
H2''

fragment can be conveniently dissected into three segments for conformational discussions. These are the N-glycosyl bond conformations, the sugar conformation or ring pucker of the deoxyribose ring, and the backbone conformation.

In the discussions that follow, the pyrimidine ring is assumed to be planar, as shown by X-ray studies[67,68]. The N-glycosyl bond conformation is then defined by a rotational angle about $C_{1'}$ of the deoxyribose ring and $N_1$ of the pyrimidine ring. (The definition of these and other conformational angles will be presented in the next section.)

There are two methods used to describe the conformation of the deoxyribose ring currently in use. One uses the twenty envelope (E) and twist (T) conformations which were developed into a cycle of pseudorotation by Hall and co-workers[201]. The other uses the pseudorotational concept developed by Altona and Sundaralingam[67] in which each point on the pseudorotational cycle is defined by two parameters: P and $\tau_m$. P is the phase angle of pseudorotation, and $\tau_m$ is the degree of pucker. The two methods are similar, but the method of Hall et al. does not include a parameter describing the amplitude of pucker (i.e., $\tau_m$). In this thesis, the pseudorotational analysis of Altona and Sundaralingam will be used, supplemented by the envelope and twist notation to aid in visualizing the various conformations.

Finally, the backbone conformational angles are discussed according to the classical staggered rotamers for the backbone bonds

$\omega$, $\phi$, $\psi$, $\psi'$, $\phi'$, $\omega'$. The nomenclature used by NMR spectroscopists to differentiate these rotamers ($g_+$, t, $g_-$, etc.) will be employed[6].

The torsion angle (or dihedral angle) about the bond B—C in a fragment composed of the atoms A—B—C—D is defined as the angle of rotation of the far bond C—D relative to the near bond A—B from the eclipsed position of the bonds A—B and C—D. The eclipsed position of the bonds A—B and C—D defines the $0^{\circ}$ torsion angle. The angle of rotation is defined as positive for a clockwise rotation of the C—D bond from the A—B bond when viewed along B—C.

## B. PROTON NMR APPROACH TO NUCLEIC ACID CONFORMATION

### 1. N-GLYCOSYL BOND CONFORMATION

The N-glycosyl bond of a nucleoside is represented by the Newman projection in Figure 2.3. The conformation is defined by the rotational angle of the pyrimidine ring relative to the ribofuranose ring about the bond between $C_{1'}$ and $N_1$ and is symbolized by $\chi$. The zero point is defined as the eclipsed orientation of the $C_{1'}—O_{4'}$ and $N_1—C_2$ bonds with the positive direction defined as an anticlockwise rotation of the pyrimidine ring.

Pyrimidine nucleosides and nucleotides are found to exist in two broad conformational ranges, syn ($\chi = 90^{\circ} \pm 90^{\circ}$) and anti ($\chi = 270^{\circ} \pm 90^{\circ}$). The large majority of pyrimidine derivatives studied, both in the solid state[67,68] and in solution[69,73], prefer the anti conformation or exist as an equilibrium mixture (in solution) of the two conformers[74,75]. A bulky substituent at $C_6$ of the pyrimidine ring favors the syn position[72,73,76].

In general, there are three methods for determining the N-glycosyl bond conformation using proton NMR. They are chemical shift, coupling constant, and relaxation studies.

#### a. CHEMICAL SHIFTS

The first use of chemical shifts for the study of the N-glycosyl bonds monitored the effect of phosphorylation at the 5'-ribose position on the proton chemical shifts of the pyrimidine ring[12,16]. The 5'-mononucleotides were compared with the nucleosides and the

FIGURE 2.3 is a Newman projection depicting the rotation about the N-glycosyl bond. The direction of rotations shown in the figure is positive.