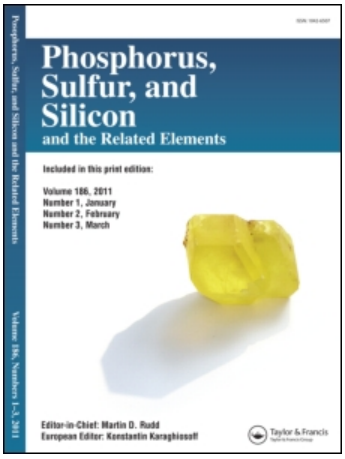

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STUDIES ON THE CONFORMATIONS OF O-URIDINE-5'-YL O-ALKYL N-PHOSPHORYL SERINE METHYL ESTERS BY NUCLEAR MAGNETIC RESONANCE (NMR) AND CIRCULAR DICHROISM (CD)

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STUDIES ON THE CONFORMATIONS OF O-URIDINE-5′-YL O-ALKYL N-PHOSPHORYL SERINE METHYL ESTERS BY NUCLEAR MAGNETIC RESONANCE (NMR) AND CIRCULAR DICHROISM (CD)

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The conformations of O-uridine-5′-yl O-alkyl N-phosphoryl serine methyl esters (1) and (2) have been investigated by nuclear magnetic resonance (NMR) and circular dichroism (CD) techniques. Nuclear Overhauser effect (NOE) measurements and CD studies revealed that the glycosyl bonds in both compounds favor the syn orientation. The stereosensitive ^{31}P-^{13}C coupling constants were also measured and used for the analysis of the trans and gauche conformations. The three-dimensional structural characteristics of compounds (1) and (2) were discussed.

Keywords: Conformation; NMR; CD

INTRODUCTION

Nucleotide peptides exist naturally as intermediates in biochemical pathways in tRNA.[1] For instance, nucleotide-phosphoramidates (containing covalent P-N bond) are intermediates in nucleotide transfers catalyzed by the capping enzyme and DNA ligase.[2] Modified nucleotide peptides are very important antiviral prodrugs[3] and have been identified in prebiotic biosynthesis of proteins.[4] Recently the first synthesis of purine and pyrimidine N-phosphoryl serine methyl ester were achieved.[5] It is found that intramolecular phosphoryl transfer reaction can take place in uridine (U),

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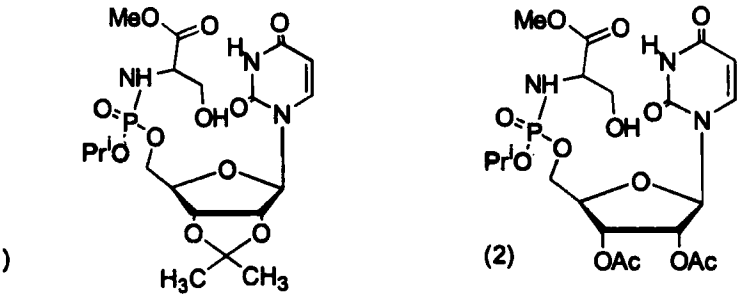
but not in adenosine.[5] It is speculated that the selectivity on nucleoside bases is related to the three-dimensional structure of the molecules. Hence, we report the NMR and CD studies on the conformations of O-uridine-5′-yl O-alkyl N-phosphoryl serine methyl esters (1) and (2) in this report.

RESULTS AND DISCUSSION

The chemical structures of O-[2′, 3′-isopropylidene]uridine-5′-yl O-isopropyl N-phosphoryl serine methyl ester (1), and O-[2′,3′-diacetyl]uridine-5′-yl O-isopropyl N-phosphoryl serine methyl ester (2) are illustrated in Scheme 1.



SCHEME 1 Structures of Compounds (1) and (2)

Conformation of the Glycoside Bond

The uridine nucleoside is generally believed to favor the anti conformation in solution. However, the results from our NMR and CD experiments indicated the contrary. As shown in Figure 1, there are significant NOE cross-peaks between H-1′ and H-2′ of the ribose ring and H-6 of the uracil of both compounds (1) and (2). This is consistent with the chemical shift analyses. Compared to the unsubstituted uridine, the resonance signals from H-2′ and H-3′ are shifted downfield, whereas peaks from H-1′, H-4′ and H-5′ appear more upfield, respectively. The corresponding ^{13}C resonances (except the C-1′ and C-5′ signals) follow similar pattern. These

chemical shift changes are caused in part by the magnetic anisotropy of the 2-keto group of the uracil base over the ribose ring.[6]

TABLE I Selected ^{1}H and ^{13}C Chemical Shifts (δ in ppm) and Coupling Constants (J in Hz) of Uridine, Compounds (1) and (2) in DMSO-d_6

Comp.	*Nucleus*	δ (1′)	δ (2′)	δ (3′)	δ (4′)	δ (5′)	$J_{1,2'}$	$J_{P\text{-}C4'}$	$J_{P\text{-}N\text{-}Ca}$
U	^{1}H	6.18	4.40	4,40	4.26	4.02	5.16		
	^{13}C	89.10	71.10	74.85	85.90	62.20			
(1)	^{1}H	5.80	4.91	4.77	4.20	4.03	1.00		
	^{13}C	92.02	83.20	79.14	84.76	63.15		7.74	<1.00
(2)	^{1}H	5.94	5.34	5.32	4.25	4.02	6.39		<1.00
	^{13}C	92.02	83.64	86.57	84.82	65.12		8.07	

The above observations are supported by the CD studies. The molar ellipticity of the major CD bond at around 270nm ([Q]max) is known to reflect both the orientation of the glycosyl glycosyl group and the puckering of the furanose ring. Typical [Q]max values for anti and syn orientations are 10000 and 100–1000 deg $cm^{-1}mol^{-1}$, respectively.[7] The CD spectra shown in Figure 2 clearly indicate that the 2′,3′-isopropylidene ring and the 5′-substitution induce the syn orientation for the glycosyl group in both compounds (1) and (2).

Conformation of the Sugar Ring and the Sugar-Phosphate Back Bone

Proton coupling constants ($^{2}J_{1'2'}$) are very sensitive to the conformations of the ribose ring, thus providing an approximate way to monitor the conformation changes. A $^{2}J_{1'2'}$ value of ~0.0 Hz indicates a C'_3 -endo(N) structure for the sugar ring and a gauche-gauche (g^+) conformation for the $C'_4 - C'_5$ bond.[8] However, a $^{2}J_{1'2}$ value of ~9.5Hz means that the conformational orientation about the $C'_4 - C'_5$ bond changes from (g+) into trans-gauche (g-) or gauche- trans (t) and the conformation of the sugar ring is $C_{2'}$-endo(S).[9] Therefore, the experimental data in Table I demonstrated that compound (1) favors C'_3 -endo and (g+) conformation about the $C'_4 - C'_5$ bond, i.e., 5′- oxygen atom lay above the sugar ring (see Figure 3(a)). The $^{2}J_{1'2}$of compound (2) is 6.39Hz, suggesting that it has a

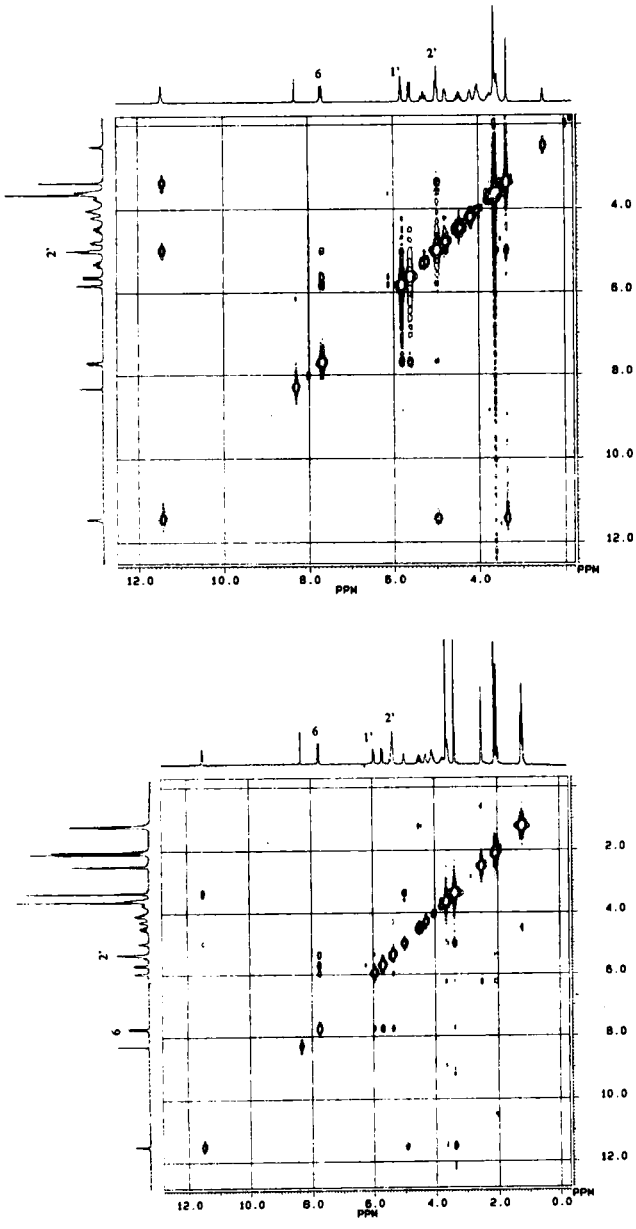


FIGURE 1 Proton NOE spectra of compound (1) in DMSO (a) and compound (2) in DMSO (b). The mixing time was 760ms

ribose average intermediate conformation between C'_3 -endo and $C_{2'}$-endo. The conformation about C'_4-C'_5 bond is not (g+), but (g-) or (t). These results are different from those of compound (1) because the two compounds have different substitution groups on their riboses.

The conformations of P-O (5′)-C (5′) bond at minimum energy are shown in Newman projection formula (Fig. 3(b)). Their rotamer population depends on the observed $^3J_{P\text{-}C4}$,and can be calculated according to equation (1):

$$\begin{cases} {}^3J = P_{g+}J_+ + P_tJ_t + P_{g-}J_- \\ 1 = P_{g+} + P_t + P_{g-} \end{cases} \qquad \text{Equation (1)}$$

The observed $^3J_{P\text{-}C4}$ coupling constants have been obtained from ^{13}C NMR spectra (see Table I). Using ${}^3J_t = 10Hz$ and ${}^3J_g = 2Hz$ from Kaplus relation for P-O-C-C molecular fragment,[10] it is estimated that the ratio of P_t is 0.70 for compound (1) and 0.73 for compound (2). In other words, the phosphate atom is trans to C- 4′ and gauche to each of the H- 5′,H-5" atoms for rotamer in the two compounds.

Conformation of Phosphoryl Amino Acid Methyl Ester

C.B. Xue and coworkers demonstrated that the small value (≤1.00Hz) of the geminal coupling constant, $^2J_{P\text{-}N\text{-}C}$a in phosphoryl amino acid methyl ester is related to the anti orientation of the P=O bond with respect to the N-C bond.[11] All the $^2J_{P\text{-}N\text{-}C}$a of compounds (1) and (2) are roughly 1.0 Hz (Table I), indicating that the phosphoryl group is still trans with respect to the N-Ca group in compounds (1) and (2). On the other hand, the ^1H, ^{13}C chemical shifts of δ_{CH3} (~1.34 ppm; ~23.40 ppm) and δ_{CH} (~4.62 ppm; ~63.90ppm) in the isopropyl group of compounds (1) and (2) are similar to that in diisopropyl phosphoryl serine respectively. Thus, it is reasonable to conclude that the isopropyl group is far from the uridine base. Moreover, since the phosphorly center is tetrahedral, there are four groups attached to it. Hence, above analyses suggest that that the N-phosphoryl serine methyl ester should be closed to the uracil base of compounds (1) and (2). There maybe hydrogen bonds between the 2-keto of the uracil and the hydroxyl group of the serine side chain and between the N(3)H of the uracil and the C=O group of the serine. The results from the semiempirical quantum mechanics computation support this assumption in some degree. The model established by full geomethic structure optimiza-

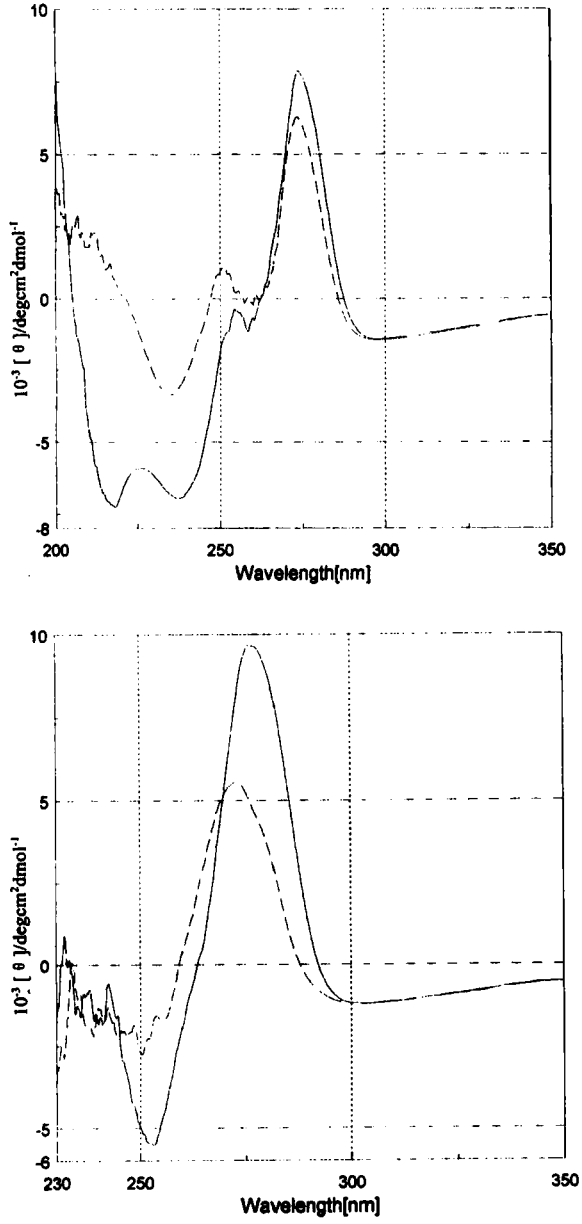


FIGURE 2 CD spectra of U and compound (1) in H_2O (a) and U and compound (2) in DMSO (b)

tion (AM1 method) revealed two pairs of intrmolecular hydrogen bonds. The calculated distances between the (U) C (2)=O to HO-β (Ser) and (U) N (3)H to O=C (Ser) are listed in Table II. The hydroxyl group on the serine side chain is activated by this intramolecular hydrogen bonding. This means that the nucleophility of the oxygen atom in the hydroxyl group is increased, thus its ability to attack the P atom is enhanced. However, there are no such hydrogen bonds in adenosine. Therefore, the phosphoryl transfer reaction only happened to the O-pyrimidine-5′-yl O-alkyl N-phosphoryl serine methyl esters but not to the adenine.

TABLE II Some Distances Calculated by the AM1 Method

Compound	*(U) C(2)=O to HO-β (Ser)*	*(U)N(3)H to O=C(Ser)*
(1)	2.126 (Å)	2.227 (Å)
(2)	2.131 (Å)	2.238 (Å)

CONCLUSION

NMR and CD studies demonstrated that compounds (1) and (2) exist predominantly in syn conformation for their glycoside bond. The subtly differences in the ribose ring conformations for the two compound is due to their different substitution groups. In both compounds, the N-phosphoryl amino acid methyl ester locates near the base of uridine. There are probably two pairs of hydrogen bonds between the pyrimidine base and the serine methyl ester. These unique structural characteristics for O-uridine O-alkyl N-phosphoryl amino acid methyl esters fulfil the specific structural requirements of the phosphoryl transfer reactions.

EXPERMENTAL PROCEDURES

The compounds were preparated according to the published procedures.[5] All the NMR experiments were carried out on Bruker Ac-200p and AM-500 FT NMR spectrometers. The ^{1}H and ^{13}C NMR chemical shifts are referred to TMS and $CDCl_3$, respectively. The ^{31}P NMR spectra were obtained using 85% phosphoric acid as an external reference. The CD

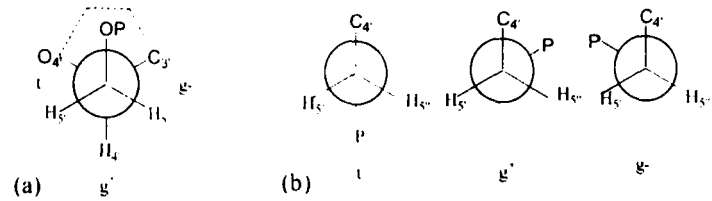


FIGURE 3 Newman Projection Viewed along $C'_4 - C'_5$ (a) and P-O (5')-C (5') (b). Three rotamers: g+, t and g refer to the conformational domain containing the 60°, 180° (-180°), and 300 (-60°), respectively

spectra were collected on Jasco J-715 spectropolarimeter. All the measurements were performed at room temperature.

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References

1. R. Hall; The Modified Nucleoside on Nucleic Acids, Columbia University press, New york and London 317–345 (1971).
2. S. Suman B. Schwer; *Molecular Microbiology*, **17**(3), 405–410 (1995).
3. E.J. Mcintee, R.P. Remmel et al; *J. Med. Chem.*, **40**, 3323–3331 (1997).
4. H. Fu, L.Z. Li, et al. *J. Am. Chem. Soc.* **121**, 291–295 (1999).
5. Y.F. Zhao et al; *Synth. Commun.* In press.
6. Martin P. Schweizer, E.B. Banta, et al; *J. Am. Chem. Soc.* **95**, 3370–3378 (1973).
7. Takehiko Wada, Narutoshi Minamimoto, et al; *Chem. Lett.* 1025–1026 (1998).
8. F.E. Hruska, A.A. Smith et al; *J. Amer. Chem. Soc.* **93**, 4334–4336 (1971).
9. G. Govil, R.V. Hosur; "Conformations of Biological Molecules" Berlin Heridelberg New York (1982), Chapter 3.
10. Y.P. Feng, X. Chen, Y.F. Zhao; *Phosphorus, Sulfur, and Silicon*, **118**, 219–225 (1996).
11. C.B. Xue, Y.W. Yin et al; *J. Chem. Soc. Peakin Trans*, **2**, 431–434 (1990).