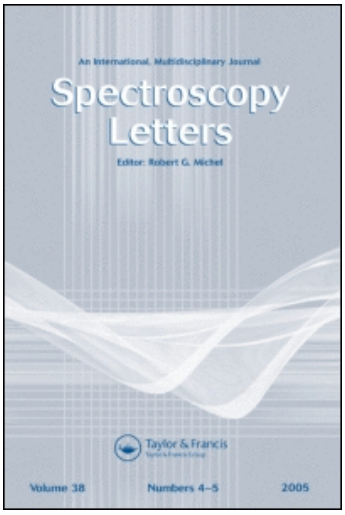


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

^{13}C Nuclear Magnetic Resonance Studies of the Conformations of Serine-3'- and 5'-Thymidine Monophosphate Conjugates

Yuping Feng[a]; Bo Han[a]; Bo Tan[a]; Yufen Zhao[a]
[a] Key Laboratory for Bioorganic Phosphorus Chemistry of Education Ministry, Department of Chemistry, Tsinghua University, Beijing, P.R. China

Online publication date: 16 December 2003

SPECTROSCOPY LETTERS
Vol. 36, Nos. 5 & 6, pp. 419–427, 2003

^{13}C Nuclear Magnetic Resonance Studies of the Conformations of Serine-3′- and 5′-Thymidine Monophosphate Conjugates

Yuping Feng, Bo Han, Bo Tan, and Yufen Zhao*

Key Laboratory for Bioorganic Phosphorus Chemistry of
Education Ministry, Department of Chemistry,
Tsinghua University, Beijing, P.R. China

ABSTRACT

The differences in the backbone conformation between *O*-thymidine-3′-(**1**) and 5′-yl *O*-alkyl N-phosphoryl serine methyl esters (**2**) have been investigated by solution ^{13}C NMR spectroscopy. The stereo-sensitive vicinal ^{31}P–^{13}C coupling constants were measured and used in the conformational analysis for the P–O5′–C5′, P–O3′–C3′, and P–N–C_α bonds. Three-dimensional structural characteristics of dephosphorylation reactions of Compounds are also discussed.

Key Words: ^{13}C NMR; Conformation; Dephosphorylation; Serine-thymidine; Monophosphate conjugates.

*Correspondence: Yufen Zhao, Key Laboratory for Bioorganic Phosphorus Chemistry of Education Ministry, Department of Chemistry, Tsinghua University, Beijing 100084, P.R. China; E-mail: fengyp@mail.tsinghua.edu.cn.

DOI: 10.1081/SL-120026608
0038-7010 (Print); 1532-2289 (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

INTRODUCTION

It is known that many enzymes' activities are regulated through phosphorylation and dephosphorylation mechanisms. In most cases the active sites of amino acid residues such as enzymes consist of hydroxyl on serine or threonine.[1] These reactions also play a fundamental role in the energetics of all living organisms.[2] In addition to normal metabolism, abnormal cancer systems are both modulated and affected by reversible phosphorylation mechanisms.[3] However, the chemistry of the dephosphorylation mechanism is still not very clear in biological systems.

Modified nucleotide peptide conjugates are of significance as potential model compounds for designing antiviral nucleoside prodrugs, which were useful for therapy for cancer and viral diseases.[4,5] Some artificial compounds exhibit remarkable antiviral activities.[6] For example, researchers have shown that nucleotide-5′-phosphoramidates are new efficient anti-HIV inhibitors and have attracted attention as antiviral nucleoside prodrugs.[7,8] Recently, the first syntheses of 3′- and 5′-N-phosphoryl serine methyl ester of pyrimidines (Compound **1**–**4**) were achieved.[9] These studies demonstrated that dephosphorylation would only take place in Compounds **2**, **3** and **4** that have a phosphate at the 5′-position. However, the same reaction did not occur in the 3′-type Compound **1**. To understand the chemistry of the dephosphorylation mechanism, it is of great interest to carry out the investigation on structure–activity relationships and it is clear that the selectivity of the reaction is generally associated with the three-dimensional structure of the molecules. ^{13}C NMR spectroscopy has proved very useful in studying backbone conformations via the carbon–phosphorus coupling constants. We now report on the structural characteristics of dephosphorylation reactions of these compounds by ^{13}C NMR spectroscopy.

MATERIALS AND METHODS

Materials

All the compounds were prepared according to published procedures.[9]

NMR Spectroscopy

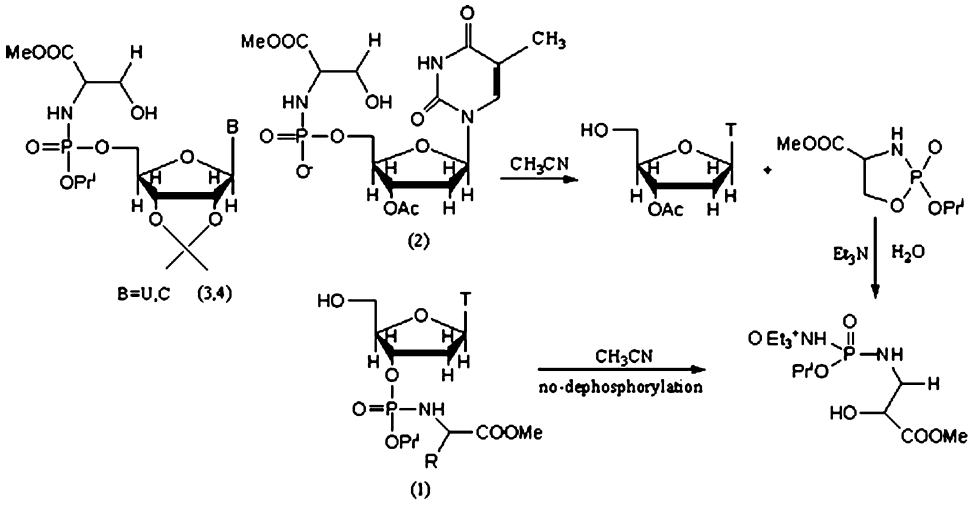
NMR experiments were carried out on either a Brüker Ac-200p or an AM-500 FT NMR spectrometer. ^{13}C NMR chemical shifts were referred to $CDCl_3$ and the ^{31}P NMR chemical shifts were obtained using 85% phosphoric

acid as an external standard. All the measurements were performed at room temperature.

RESULTS AND DISCUSSION

The chemical structures of *O*-thymidine-3′-yl *O*-isopropyl N-phosphoryl serine methyl ester (Compound **1**), *O*-[3′-acetyl] thymidine-5′-yl O-isopropyl N-phosphoryl serine methyl ester (Compound **2**) are illustrated in Scheme 1. It was found that when treated with one equivalent of Et_3N, Ser-3′-thymidine monophosphate was stable at 22°C for one week in anhydrous CH_3CN. However, Ser-5′-thymidine monophosphate and the corresponding Ser-5′-uridine monophosphate, Ser-5′-cytosine monophosphate, were completely dephosphorylated after 48 h in the reaction media (Scheme1). Kinetic data, k, which were obtained by ^{31}P NMR were 8.6×10^{-4}, 3.3×10^{-4}, 2.8×10^{-4} (s^{-1}), respectively. The results indicated that distinct chemical properties could be attributed to the different molecular conformations.

The pyrimidine nucleoside is generally believed to favor the anti-conformation in solution. However, results from CD experiments in acetonitrile clearly indicated that the 2′, 3′-O-cyclic furanose ring, and a substitution at the 5′ position, induced the *syn* orientation for the glycosyl group in Compound **2**.[10] This is also consistent with the ^1H NMR analyses.



Scheme 1. Structures and dephosphorylation reactions of compounds.

Table 1. ^{1}H NMR (δ in ppm) of furanose for compounds **1** and **2** in DMSO-d_6.

Comp.	δ(1′)	δ(2′)	δ(3′)	δ(4′)	δ(5′)
Thymidine	6.14 6.15	2.05	4.22	3.74	3.55
(1)	6.18 6.19	2.21	4.86	4.03	3.61
(2)	5.85 5.87	2.28	4.98	3.99	3.93

Compared to the unsubstituted thymidine, the ^{1}H resonance signals from H-2′ and H-3′ of Compound **2** are shifted downfield (see Table 1). These chemical shift changes are caused in part by the magnetic anisotropy of the 2-keto group of the thymine base over the ribose ring.

Effect of Substitution Positions on ^{13}C Resonance of Furanose

Compared to the spectrum of the unsubstituted thymidine, the ^{13}C resonance signals from C-2′ and C-4′ of Compound 1 were shifted about 2 ppm up-field, and the signal from C-3′, where the phosphoryl group was attached, was shifted down-field by about 6 ppm. However ^{13}C signals from C-4′ of Compound **2** appeared more up-field than that of thymidine and the chemical shift of C-5′ where the phosphoryl group was attached was increased from 61.32 ppm to 63.12 ppm (Table 2). These differences in chemical shift are due to the different positions of the phosphorus (3′ vs. 5′) in the compounds.

Conformation of the Sugar-Phosphorous Backbone

Conformational properties of Compounds **1** and **2** can be determined by the ^{3}J(CCOP) values related to the associated dihedral angles. The vicinal phosphorus–carbon coupling constants for these compounds have been

Table 2. ^{13}C NMR chemical shifts (δ in ppm) and coupling constants (J in Hz) of furanose for compounds **1** and **2** in DMSO-d_6.

Comp.	δ(1')	δ(2′)	δ(3′)	δ(4′)	δ(5′)	$J_{P-C2'}$	$J_{P-C3'}$	$J_{P-C4'}$	$J_{P-C5'}$
Thymidine	83.78	39.6	70.39	87.23	61.32				
(1)	83.64	37.65	76.25	85.61	61.12	3.35	4.05	4.77	
(2)	83.83	37.96	74.3	81.97	63.12		2.60	4.76	7.04

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

obtained directly from the ^{13}C NMR spectra and are presented in Table 2. The conformational analysis is completed using a three state model (P_1, P_2 and P_3) consisting of staggered conformations. As shown in Figure 1, P_1, P_2 and P_3 are the relative populations of the three possible conformers (g^+, t and g^- for $\Phi = 60°$, 180° and 300°, respectively). Their conformational population can be estimated from relative equations.[11] The conformational properties of the P–O3′–C3′ bond in Compound **1** were determined from the $^3J_{P-C2'}$, $^3J_{P-C4'}$ values. The weighted average conformations about the P–O3′–C3′ bond, with respect to the C3′–C4′ bond consisting of a three-state model, are illustrated in Figure 1(A) according to the Newman projection formula. Their relative population can easily be obtained by using Eqs. 1–3,[11] where J_g and J_t are the coupling constants for ^3J(CCOP) in the gauche and trans orientations. Typical values of J_g and J_t are 2.1 and 10.1 Hz. Thus, the estimated ratios of population for P_1, P_2, and P_3 are 0.59, 0.30 and 0.11, respectively. This means that the phosphoramidate group for

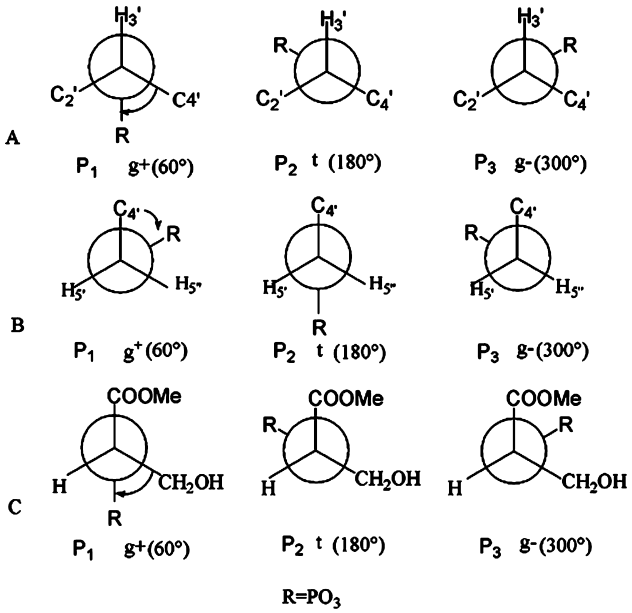


Figure 1. Newman projection diagrams for staggered conformation population consisting of three-state model (P_1, P_2, P_3). A: conformations population around C3′–C4′ bond in **1**; B: conformations population around C5′–C4′ bond in **2**; C: conformations population around C5′–N bond of N-phosphoryl serine methyl ester in **1** and **2**.

Compound **1** is located under the furanose ring and the P–O (3′) bond is gauche to the C (3′)–C (4′) and C (3′)–C (2′) bonds.

$$^{3}J_{P-C2'} = P_1J_g + P_2J_g + P_3J_t \quad (1)$$

$$^{3}J_{P-C4'} = P_1J_g + P_2J_t + P_3J_g \quad (2)$$

$$1 = P_1 + P_2 + P_3 \quad (3)$$

The conformations of P–O (5′)–C (5′) bond in Compound **2** were also subjected to similar analysis to determine the distribution of conformer populations (Figure 1(B)). The conformation populations depended on the observed $3J_{P-C4'}$ value, and can be estimated based on the following equations:

$$^{3}J_{P-C4'} = P_1J_t + P_2J_g + P_3J_g \quad (4)$$

$$1 = P_1 + P_2 + P_3 \quad (5)$$

Using $^{3}J_t = 10.1$ and $^{3}J_g = 2.1$ Hz, the populations of the conformers for P_2 and $P_1 + P_3$ are found to be *ca.* 0.33 and 0.67 for Compound **2**.[12] This indicated that the g^+ and g^- conformations for the P–O (5′)–C (5′)–C (4′) group are adopted. In other words, the phosphate atom is gauche to C-4′ atom.

Conformation of Phosphoryl Amino Acid Methyl Ester

Karplus relations Eqs. 6–8 were used in analysing the conformations of N-phosphoryl serine methyl ester (NMR data in Table 3). The results (Figure 1(C))

$$^{3}J_{P-N-C\alpha-C\beta} = P_1J_g + P_2J_g + P_3J_t \quad (6)$$

$$^{3}J_{P-N-C\alpha-\beta C=O} = P_1J_t + P_2J_g + P_3J_g \quad (7)$$

$$1 = P_1 + P_2 + P_3 \quad (8)$$

showed that the ratio of significant population of P_2 is 0.69 for the N-phosphoryl serine methyl ester in **1**, and 0.61 for the N-phosphoryl serine methyl ester in **2**. This suggests that the N–P=O groups are trans to the hydroxyl group on serine side chain in both compounds.

In addition, the P–N–C_α–C_β dihedral angle can be obtained with Eq. 9 derived from the Karplus relation for the P–O–C–C molecular fragment[13]

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Table 3. The ^{13}C chemical shifts (δ in ppm) and coupling constants (J in Hz) of N-phosphoryl serine methyl esters in compounds in DMSO-d_6.

Comp.	Ipro–CH_3	ipro–CH	α–CH	β–CH_2	β–C═O	$COOCH_3$
(5)	23.20 (4.00)	71.00 (6.00)	56.20	63.80 (5.90)	171.70 (5.90)	51.70
(1)	23.38 (5.20)	70.40 (5.19)	56.61	63.07 (7.62)	172.39 (3.35)	51.69
	23.52 (3.20)					
(2)	23.49 (3.90)	70.38 (5.2)	56.54	63.12 (7.04)	172.30 (4.20)	51.67
	23.36 (3.00)					
(3)	23.40 (4.00)	70.32 (4.06)	56.53	63.16 (5.86)	172.35 (4.20)	51.71
	23.51 (4.43)					

where θ is the dihedral angle between the plane P–O–C_α and C_α–C_β. The corresponding P–N–C_α–C_β dihedral angles were found to be

$$^3J_{P-C} = 9.5\cos^2\theta - 0.6\cos\theta \quad (9)$$

$180^\circ \pm 43^\circ$ or $180^\circ \pm 49^\circ$ in Compound **2** and about $180^\circ \pm 22^\circ$ or $180^\circ \pm 30^\circ$ in Compound **1**. This is because the trans conformation is dominant. Based on the comparison of the estimated data, a possible explanation for this is that average distance between the hydroxyl group of the serine side chain and the phosphorous atom in Compound **2** may be shorter than that in Compound **1**. There are some important differences in the geometric position of the phosphoryl serine methyl ester in the two compounds. In Compound **1**, the phosphoramidate group is located under the sugar ring, whereas, it situated above the ribose ring in Compound **2**.

Our experiments indicated that the respective ^1H and ^{13}C chemical shifts are *ca.* 1.34 and 23.40 ppm for δCH_3, and *ca.* 4.62 and 71.00 ppm for δCH in the isopropyl group of Compounds **1** and **2**. These values are similar to those found for the corresponding diisopropyl phosphoryl serine methyl ester (Compound **5** see Table 3) which has no nucleoside base. Therefore, it is reasonable to say that their isopropyl group is away from the nucleoside base. Moreover, since the phosphoryl center is tetrahedral, there are four groups attached to it. Hence, the N-phosphoryl serine methyl ester should be closer to the thymidine base in Compound **2**. It is also worthy to mention that the ^{13}C signal of β–CH_2 of **2** has a 1.3 ppm downfiled shift because the syn orientation of the glycosyl group is favored in this compound. This suggests that there maybe some hydrogen bonds formed between (Ser)OH and O═C(2). Results from the semi-empirical quantum mechanics computations support this assumption to some degree. Indeed, the calculated molecular model for Compound **3** by a full geometric

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

structural optimization (AM1 method) revealed a pair of intramolecular hydrogen bonds. The distance between the C (2)═O (U) to HO–β (Ser) is less then 0.26 nm and the oxygen of the serine is pushed toward the phosphorus atom with a distance of 0.23 nm. It follows that a similar conclusion was drawn for Compound **2** and **4**, because their bases are same as Compound **3**. By this conformation, the hydroxyl group on the serine side chain is activated by these intramolecular hydrogen bonds. This indicates that the nucleophility of the oxygen atom in the hydroxyl group is increased, thus its ability to attack the phosphorus atom is enhanced and it rehybridizes the phosphorus atom from a tetrahedral to a penta-coordinate state, which will be transferred to other species. On the other hand, there are no such hydrogen bonds in Compound **1**. Therefore, a dephosphorylation reaction only occurs to the 5′-phosphoamidate but not to the 3′-phosphoamidate of pyrimidine.

CONCLUSION

This study reveals that differences in substitution positions resulted in differences in backbone conformations of 3′- and 5′-N-phosphoryl serine methyl ester of thymidine that are likely to be related to previously observed differences in dephosphorylation. In Compound **2**, the N-phosphoryl amino acid methyl ester is located above the sugar ring, and there are probably hydrogen bonds existing between the pyrimidine base and the serine side chain. Consequently, the unique structural characteristics of *O*-pyrimidine *O*-alkyl N-phosphoryl amino acid methyl esters **2**–**4** fulfill the specific structural requirements for the dephosphorylation reactions.

ACKNOWLEDGMENT

The authors would like to extend thanks for financial support from the Chinese National Natural Science Foundation (No. 20132020), the Ministry of Science and Technology, the Chinese Ministry of Education and Tsinghua University.

REFERENCES

1. Rosen, O.M.; Krebs, E.G. *Protein Phosphorylation*; Cold Laboratory, Cold Spring Harbor: New York, 1981; 849–854.

2. Stock, J.B.; Ninfa, A.J.; Stock, A.M. Microbiol. Rev. **1989**, *53*, 450–490.
3. Mathews, C.K.; Van Holde, K.E.; Ahern, K.G. *Biochemistry*, 3rd Ed.; An Imprint of Abbison Wesley Longman Inc.: San Francisco, 2000.
4. Jonnes, A.S.; McGuigan, C.; Walker, R.T.; Balzarini, J.; Clercq, E.d. Synthesis, properties and biological activity of some nucleoside cyclic phosphoramidates. J. Chem. Soc., Perkin Trans. **1984**, *1*, 1471–1474.
5. McIntee, E.J.; Remmel, R.P. Probing the mechanism of action and decomposion of amino acid phosphomonoester amides of antiviral nucleoside prodruges. J. Med. Chem. **1997**, *40*, 3323–3331.
6. Fries, K.M.; Joswig, C.; Borch, R.F. Synthesis, and biological valuation of 5-fluoro-2′-deoxyuridine phosphoramide analogs. J. Med. Chem. **1995**, *38*, 2672–2680.
7. Siddiqui, A.Q.; Ballatore, C.; McGuigan, C.; Balzarini, J. The presence of substituents on the aryl moiety of the aryl phosphoramide derivative of d4T enhances anti-HIV efficacy in cell culture: structure–activity relationship. J. Med. Chem. **1999**, *42*, 393–399.
8. Valette, G.; Pompon, A.; Girardet, J.L. Decomposition pathways and in vitro HIV inhibitory effects of IsoddA pronucleotides. J. Med. Chem. **1996**, *39*, 1981–1990.
9. Zhao, Y.F.; Han, B.; Zhao, G.; Fu, H. Synthsis of novel N^{α}, N^{ε}-lysine liked dinucleotides. Synth. Commun. **2000**, *30*, 3141–3151.
10. Wada, T.; Minamimoto, N. Chem. Lett. **1998**, 1025–1026.
11. Dvies, D.B. Conformations of nucleosides and nucleotides. Prog. Nucl. Magn. Reson. Spectrosc. **1978**, *12*, 135–225.
12. Feng, Y.P.; Chen, X.; Zhao, Y.F. $^{31}P-^{13}C$, $^{31}P-^{1}H$ Spin–spin coupling and structural assignment and conformational analysis of epimeric thymidine-3′yl benzoin phosphates. Phosphorus Sulfur Silicon **1996**, *118*, 219–225.
13. Schweizer, M.P.; Banta, E.B. J. Am. Chem. Soc. **1973**, *95*, 3370–3378.

Received October 24, 2002
Accepted August 20, 2003