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Note

Unsaturated ketonucleotides: synthesis of α and β anomers of 1-(2,3-dideoxy-6-*O*-diethoxyphosphoryl-D-*glycero*-hex-2-enopyranosyl-4-ulose)thymine

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Numerous clinical and experimental nucleoside drugs are known to act in the form of their nucleotides [1–3]. This has prompted investigations of bio-active nucleotides as chemotherapeutic agents in their own right. Unfortunately, the charged nucleotides are usually less active than the corresponding nucleosides [4] because of their poor membrane penetration and their rapid extracellular cleavage [5]. For this reason, uncharged phosphate triester forms of nucleotides obtained from chemotherapeutic nucleoside analogues have been synthesized and investigated as possible membrane-soluble pro-drugs of nucleotides [6–7]. Thus, it has been found that simple 5′-(dialkyl phosphate) derivatives of Ara A and Ara C are resistant to chemical and enzymatic degradation, and exert an effect on DNA synthesis by mammalian cells.

In our laboratory, various unsaturated ketohexopyranosyl nucleosides have been synthesized in the last few years and their significant in vitro and in vivo inhibitory activity against various types of cancer cells has been clearly demonstrated [8,9]. In this paper, we report the first synthesis of unsaturated ketonucleotides in order to compare their activity with the parent nucleosides.

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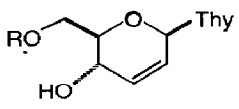
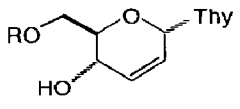
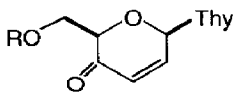


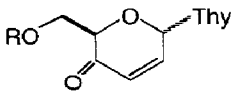
1 R= H
3 R= PO(OEt)$_2$



2 R= H
4 R= PO(OEt)$_2$



5 R= PO(OEt)$_2$
7 R= H



6 R= PO(OEt)$_2$
8 R= H

Numerous attempts at direct phosphorylation of unsaturated ketonucleosides failed and afforded only the cleavage products of the starting material. To avoid this problem, we decided to carry out the oxidation step on the unsaturated parent nucleotide. At low temperature with diethyl phosphorochloridate in pyridine [6], phosphorylation occurs mostly at O-6′ and treatment of **1** or **2** gave **3** and **4** in good yield. Some 4′,6′-bis(diethyl phosphate) was also formed but was easily separated from the 6′-(diethyl phosphate) during the purification process. Many oxidation procedures (e.g., pyridinium chlorochromate or pyridinium dichromate–molecular sieves, Me_2SO–dicyclohexylcarbodiimide, and Me_2SO–acetic anhydride) were tried on **3** and **4**, but all failed. Only the action of Me_2SO–trifluoroacetic anhydride [10,11] at low temperature afforded compounds **5** and **6** in moderate yield (ca. 50%).

In the ^{13}C NMR spectra, phosphorus couplings were observed to the methylene and methyl carbons of the ethyl moiety and to the 5′ and 6′ carbons of the sugar; no longer range couplings were observed. The multiplicity of the signals assigned to the methylene carbons of the ethyl groups was not the same for all compounds. In **3**, **4**, and **5**, the signal appeared as a pair of doublets. This can be attributed to the non-equivalence of the two diastereotopic ethyl groups [6]. By contrast, a single doublet was observed for **6**, showing that the resonances of the two ethyl groups coincided. Moreover, in **4**, **5**, and **6**, the β carbon (C-5′) to the phosphate group possesses a larger coupling constant than the α carbon (C-6′) to the phosphate. This phenomenon has already been noticed for some other compounds [12]. It is well established [13,14] that the rigidity of the unsaturated carbonyl system forces the ring atom into one plane, limiting the hex-2-enopyranos-4-ulose ring to two sofa conformations with H-2, H-3, and H-4 in the plane of the ring. The sofa conformation where H-5′ is perpendicular to the ring is preferred because, in the alternative, the bulky substituents at C-1 and C-5 would both be axial for the β anomer **5**. On the other hand, for **5** and **6**, the H-5′ signal appeared as a doublet of quartets and $J_{5',P}$ was 2.7 and 2.6 Hz, respectively. It has been shown previously [15] that, when HCCOP fragments of phosphate esters adopt a "W" conformation, the $^4J_{H,P}$ coupling exhibits a maximum value of 2.7 Hz. If this relationship is

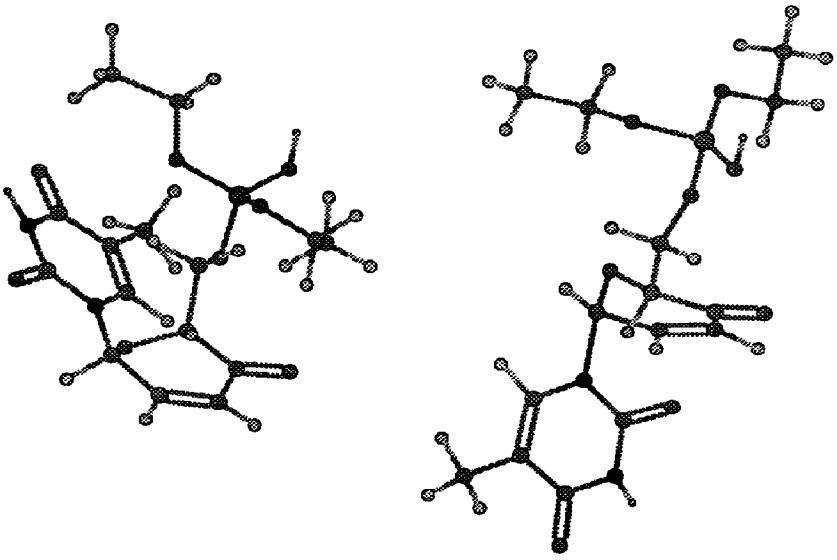


Fig. 1. Lowest energy conformations for **5** ($E = -72.34$ kcal/mol) and **6** ($E = -74.01$ kcal/mol).

absent, $^4J_{H,P}$ is nearly zero. So, in the α and β compounds **6** and **5**, the atoms H-5′, C-5′, C-6′, O-6′, and P-6′ are in the same plane and in a "W" relationship even if the two ethoxy groups are not equivalent as in **5**.

In order to substantiate these conclusions, **5** and **6** were examined computationally by means of MACROMODEL 4.0 program [16]. Following preliminary energy minimization analysis, the resulting structures were subjected to a multiconformer run that encompassed all three rings. In each case, over 1000 conformers were generated and minimized to ensure proper identification of the global energy minimum. Final optimization was accomplished in MM2* (Fig. 1).

Experiments on the cytotoxicity of **5** and **6**, compared with the parent nucleoside analogues **7** and **8** [17], are now in progress. The results will be published elsewhere.

1. Experimental

General methods.—Flash column chromatography was performed on Silica Gel 60 (240–400 mesh, Merck). $[\alpha]_D^{20}$ values were determined on solutions in MeOH (*c* 0.1). NMR spectra were recorded at room temperature on a Bruker AMX 500 spectrometer with internal $(CH_3)_4Si$ for ^1H and ^{13}C spectra, and on a Bruker MSL 300 with Me_3PO_4 as external reference for ^{31}P spectra. The positions in carbohydrate moieties are designated by primes. All reactions were carried out under scrupulously dry conditions and under an N_2 atmosphere.

*1-(2,3-Dideoxy-6-O-diethoxyphosphoryl-β-*D*-erythro-hex-2-enopyranosyl)thymine* (**3**).—A solution of **1** [17] (508 mg, 2 mmol) in pyridine (20 mL) was cooled to 0°C and diethyl phosphorochloridate (0.63 mL, 2 mmol) was added dropwise with

stirring. The mixture was then allowed to rise slowly to room temperature and stirring was continued for 12 h. After concentration at 40°C under vacuum, the residue was dissolved in CH_2Cl_2 (80 mL), and the solution was washed twice with water (10 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography (95:5 CH_2Cl_2–CH_3OH) afforded **3** as an oil (625 mg, 80%); $[\alpha]_D^{20}$ +2°; ^{1}H NMR ($CDCl_3$): δ 6.45 (d, 1 H, $J_{2',3'}$ 10.2 Hz, H-3'), 6.37 (s, 1 H, H-1'), 5.70 (d, 1 H, H-2'), 4.32–4.07 (m, 8 H, H-4',5',6'a,6'b, CH_2), 3.61 (d, 1 H, J 8.1 Hz, OH), 1.31 (m, 6 H, CH_3); ^{13}C NMR: δ 137.69 (s, C-3'), 122.30 (s, C-2'), 110.25 (s, C-4'), 76.78 (s, C-1'), 72.16 (d, $J_{5',P}$ 5.6 Hz, C-5'), 64.49 (dd, J 6, 18.9 Hz, CH_2OP), 61.84 (s, C-6'), 16.05 (d, J 4.8 Hz, CH_3CH_2OP); ^{31}P NMR: δ −5.55. Anal. Calcd for $C_{15}H_{23}N_2O_8P \cdot H_2O$: C, 44.12; H, 6.17; N, 6.86; P, 7.59. Found: C, 44.33; H, 6.29; N, 6.87; P, 7.45.

*1-(2,3-Dideoxy-6-O-diethoxyphosphoryl-α-*D-erythro-*hex-2-enopyranosyl)thymine* (**4**).—Compound **2** [17] (508 mg, 2 mmol) was treated with diethyl phosphorochloridate as previously described for **1**. After purification, **4** was obtained as an oil (585 mg, 75%); $[\alpha]_D^{20}$ +14°; ^{1}H NMR: δ 6.46 (s, 1 H, H-1'), 6.31 (d, 1 H, $J_{2',3'}$ 10.2 Hz, H-3'), 5.63 (d, 1 H, H-2'), 4.41–4.01 (m, 8 H, H-4',5',6'a,6'b, CH_2), 3.77 (d, 1 H, J 10.8 Hz, OH), 1.34 (dd, J 7.0 and 12.3 Hz, CH_3); ^{13}C NMR: δ 137.19 (s, C-3'), 125.05 (s, C-2'), 111.64 (s, C-4'), 78.69 (d, J 4.82 Hz, C-5'), 66.05 (d, J 4.84 Hz, C-6'), 64.49 (dd, J 2.81 and 10.44 Hz, CH_2OP), 16.05 (d, J 4.80 Hz, CH_3CH_2OP); ^{31}P NMR: δ −5.55. Anal. Calcd for $C_{15}H_{23}N_2O_8P \cdot H_2O$: C, 44.12; H, 6.17; N, 6.86; P, 7.59. Found: C, 44.48; H, 6.11; N, 6.67; P, 7.30.

*1-(2,3-Dideoxy-6-O-diethoxyphosphoryl-β-*D-glycero-*hex-2-enopyranosyl-4-ulose)-thymine* (**5**).—To a solution of dry Me_2SO (2.7 mmol, 0.19 mL) in CH_2Cl_2 (1.5 mL) cooled to −70°C was slowly added $(CF_3CO_2)O$ (1.8 mmol, 0.205 mL) in CH_2Cl_2 (0.75 mL) with stirring. After 10 min, a solution of **3** (390 mg, 1 mmol) in CH_2Cl_2 (1.5 mL) was added within ca. 10 min. The mixture was stirred at −70°C for 30 min, allowed to warm up to −30°C, and stirred again for another 1 h. *N,N*-Diisopropylethylamine (ca. 0.36 mL) was added dropwise until pH 7 and the mixture was then allowed to rise slowly to room temperature before being diluted with CH_2Cl_2 (10 mL), washed twice with water (5 mL), dried, and concentrated. The resulting oil was purified on a flash chromatography column (95:5 CH_2Cl_2–CH_3OH) to afford **5** as an oil (195 mg, 50%); $[\alpha]_D^{20}$ −12°; ^{1}H NMR ($CDCl_3$): δ 6.96 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{3',5'}$ 2.1 Hz, H-3'), 6.83 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1') 6.48 (dd, 1 H, H-2'), 4.58–4.38 (m, 2 H, H-6'a,6'b), 4.35 (dq, $J_{5',P}$ 2.7, $J_{5',6'a}$ 5.5, $J_{5',6'b}$ 11.5 Hz, H-5'), 4.13 (m, 4 H, CH_2OP), 1.33 (dd, 6 H, J 6.2 and 12.5 Hz, CH_3CH_2OP); ^{13}C NMR ($CDCl_3$): δ 164.35 (s, C-4'), 144.49 (s, C-3'), 130.56 (s, C-2'), 77.92 (d, J 8.03, C-5'), 76.81 (s, C-1'), 67.08 (dd, J 4.83 and 6.42 Hz, CH_2OP), 64.24 (d, J 3.21, C-6'), 16.05 (d, J 4.82, CH_3CH_2OP); ^{31}P NMR ($CDCl_3$): δ −7.12. Anal. Calcd for $C_{15}H_{21}N_2O_8P$: C, 46.40; H, 5.45; N, 7.20; P, 7.98. Found: C, 46.09; H, 5.52; N, 6.87; P, 7.35.

*1-(2,3-Dideoxy-6-O-diethoxyphosphoryl-α-*D-glycero-*hex-2-enopyranosyl-4-ulose)-thymine* (**6**).—Compound **4** (450 mg, 1 mmol) was oxidized with Me_2SO–$(CF_3CO_2)O$ as previously described for **5**. After purification, **6** was obtained as an oil (195 mg, 50%); $[\alpha]_D^{20}$ −7°; ^{1}H NMR: δ 6.93 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{3',5'}$ 2.1 Hz,

H-3′), 6.80 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1′), 6.45 (dd, 1 H, H-2′), 4.57–4.43 (m, 2 H, H-6′a,6′b), 4.36 (dq, 1 H, $J_{5',P}$ 2.6, $J_{5',6'}$ 5.3 and 11.3 Hz, H-5′), 4.10 (m, 4 H, CH_2OP), 1.30 (m, 6 H, CH_3CH_2OP); ^{13}C NMR: δ 163.92 (s, C-4′), 144.52 (s, C-3′), 130.44 (s, C-2′), 77.70 (d, J 8.03 Hz, C-5′), 77.49 (s, C-1′), 67.01 (d, J 4.83 Hz, CH_2OP), 64.29 (d, J 5.7 Hz, C-6′), 16.02 (d, J 7.22 Hz, CH_3CH_2OP); ^{31}P NMR: δ −7.35. Anal. Calcd for $C_{15}H_{21}N_2O_8P$: C, 46.40; H, 5.45; N, 7.21; P, 7.98. Found: C, 45.92; H, 5.57; N, 6.93; P, 7.95.

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