2′,3′-Dideoxy-3′-*C*-(phosphonomethyl)adenosine, the Phosphonate Analogue of 2′-Deoxyadenosine 3′-Phosphate

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Dedicated to Professor F. Cramer on the occasion of his 60th birthday

Z. Naturforsch. **38b**, 1665–1668 (1983); received July 6, 1983

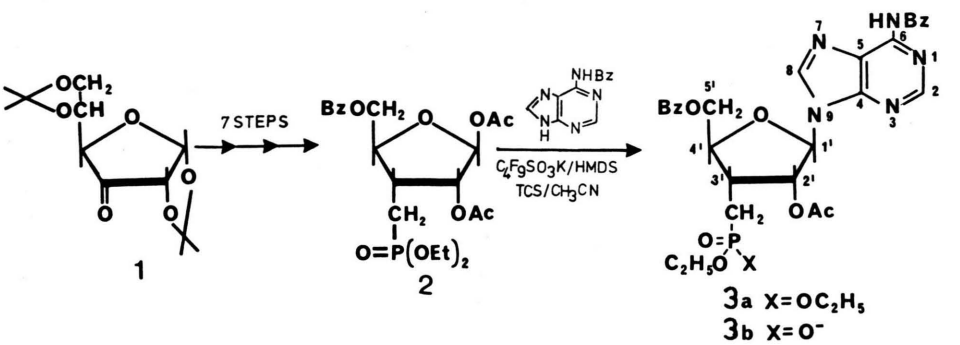
Nucleotide Analogue, Phosphonate, ^{1}H NMR Spectra, ^{13}C NMR Spectra, ^{31}P NMR Spectra, Conformation

The title compound **7** is prepared in a five-step synthesis starting from 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-*O*-(diethoxyphosphonomethyl)-β-D-ribofuranose (**2**). The preferred conformation of **7** is derived from its ^{1}H and ^{13}C NMR data.

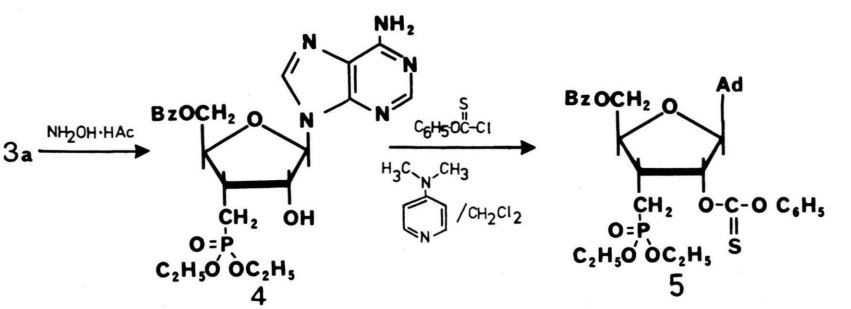
2′-Deoxyribonucleoside 3′-phosphates in which the 3′-oxygen function is replaced by a methylene group are of special interest as precursors in the preparation of modified oligonucleotides which may act as inhibitors of nucleases, particularly the restriction endonucleases. As a representative example of these 2′,3′-dideoxy-3′-*C*-(phosphonomethyl)nucleosides we synthesized 2′,3′-dideoxy-3′-*C*-(phosphonomethyl)adenosine (**7**) [1].

We obtained the crystalline 1,2-di-*O*-acetate **2** in good yield from 1,2:5,6-di-*O*-isopropylidene-α-D-ribo-3-hexofuranosulose (**1**) according to the procedure by Moffatt and coworkers [2].

The subsequent formation of the glycosidic bond



with 6-(benzoylamino)purine was achieved by the one-pot synthesis of Vorbrüggen [3] with potassium perfluorobutanesulfonate, hexamethyldisilazane (HMDS), and chlorotrimethylsilane (TCS) in acetonitrile (*ca.* 60% yield of **3a**). The monoester **3b** was obtained as a byproduct, which presumably arises by reesterification of **3a** with TCS followed by hydrolysis. The 2′-*O*-acetyl group of **3a** was



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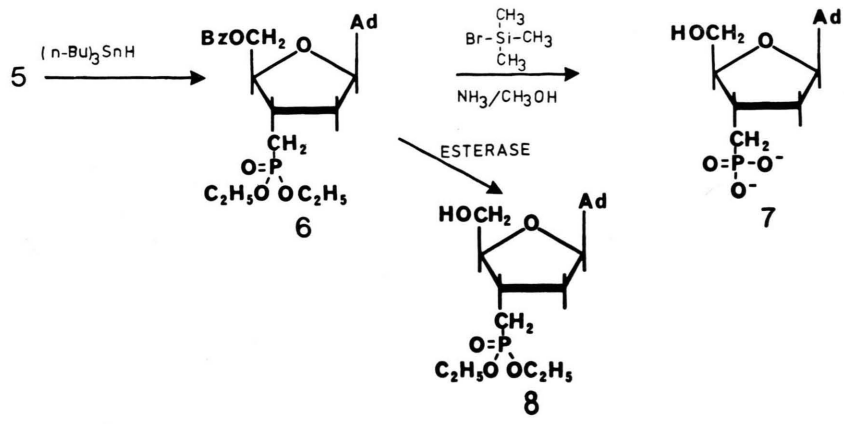


selectively removed by reaction [4] with hydroxylammonium acetate in pyridine at room temperature in *ca.* 60–70% yield.

Under these conditions simultaneous loss of the N^6-benzoyl group occured as this is more labile than the 2′-*O*-acetyl function. A phosphonic monoester was also formed as a byproduct in this step and was separated by column chromatography. **4** was quantitatively converted to 5′-*O*-benzoyl-3′-deoxy-3′-*C*-(diethoxyphosphonomethyl)-2′-*O*-(phenoxythiocarbonyl)adenosine (**5**) by reaction with phenyl chlorothionocarbonate [5] in the presence of 4-(dimethylamino)pyridine [6] in dichloromethane.

Reductive cleavage of **5** with tributyltin hydride in toluene at 60 °C furnished 5′-*O*-benzoyl-2′,3′-dideoxy-3′-*C*-(diethoxyphosphonomethyl)adenosine (**6**) in quantitative yield. After reesterification of **6** with bromotrimethylsilane in dichloromethane and treatment with methanolic ammonia the ammonium salt of **7** was obtained. This was purified on a DEAE-Sephadex column (A–25, HCO_3-form) with a water/triethylammonium hydrogencarbonate gradient (yield 80%). Alkaline hydrolysis of **6** with NH_3 gave the monoester of **7**, while esterase (EC 3.1.1.1) from pig's liver caused selective loss of only the 5′-*O*-benzoyl protecting group to give **8**.

The constitution and configuration of the final product **7** were established unambiguously from its

Table I. NMR data of **7**.

^{1}H NMR (400 MHz)[a]				^{13}C NMR (101 MHz)[b]		
	δ	J [Hz]			δ	J_{PC} [Hz]
2	8.147	1′,2′a	2.0	2	153.4 d	
8	8.448	1′,2′b	7.4	4	149.3 s	
1′	6.335	2′a,2′b	−14.0	5	119.5 s	
2′a	2.871	2′a,3′	7.3	6	156.3 s	
2′b	2.467	2′b,3′	11.0	8	141.0 d	
3′	2.62	3′,4′	9.1	1′	84.9 d	
4′	3.922	3′,PCH(a)	3.5	2′	40.2 t	3.5
5′a	3.859	3′,PCH(b)	9.9	3′	35.4 d	3.2
5′b	3.736	3′,P	10 ± 1	4′	89.2 d	16.6
PCH(a)	1.729	4′,5′a	2.8	5′	62.3 t	
PCH(b)	1.501	4′,5′b	4.5	PCH_2	32.2 t	129.3
		5′a,5′b	−12.9			
		PCH(a), PCH(b)	−14.5			
		PCH(a), P	(−)17.9			
		PCH(b), P	(−)15.9			

$\delta_P = +19.1$[c]

[a] Solvent: D_2O, chemical shifts relative to sodium 3-(trimethylsilyl)propane sulfonate (DSS), Bruker WM-400 spectrometer; [b] solvent: D_2O, chemical shifts relative to internal 1,4-dioxane, $\delta = 67.4$, Bruker WM-400; [c] solvent: D_2O, pD 10.3, chemical shifts relative to ext. H_3PO_4, determined at 40.5 MHz, Varian XL-100 spectrometer.

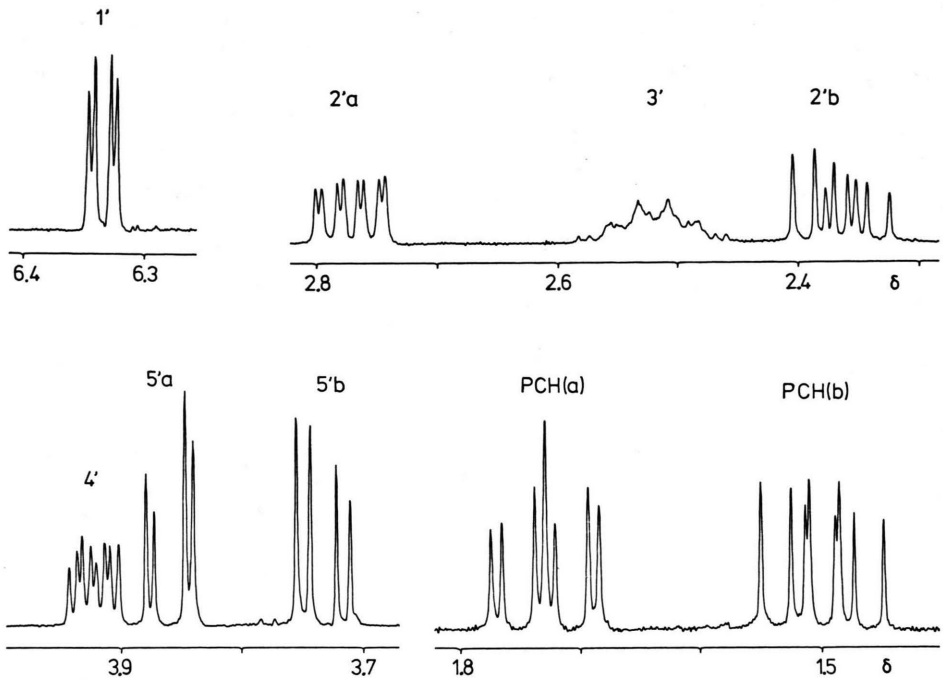
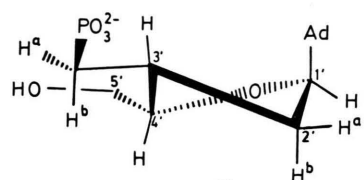


Fig. 1. 400 MHz ^{1}H NMR spectrum of **7** (base protons omitted).

NMR data (Table I). The 400 MHz ^{1}H NMR spectrum (Fig. 1) could be fully analyzed. $^3J_{P,3'}$ could not be determined to better than ± 1 Hz because of the broad ill-resolved 3′-H multiplet. This is caused by 3′-H having six vicinal partners with different coupling constants of which four have rather similar values (between 9 and 11 Hz). The large vicinal coupling between 2′-H and 3′-H on one hand and between 3′-H and 4′-H on the other show that the furanose ring prefers an *N*-type conformation [7] in which these two pairs of protons assume antiperiplanar orientations. The large difference between the two coupling constants of the PCH_2 protons to 3′-H suggests an antiperiplanar arrangement of the P–C bond to either C(3′)–C(4′) or to C(2′)–C(3′). The latter possibility can be excluded on the basis of the large value [8] of $^3J_{P,C(4')}$, although intuitively one might think that it would be favoured as it would allow the formation of an intramolecular hydrogen bond between P–O$^-$ and 5′–OH. NOE difference spectra with irradiations at $\delta_H = 8.45$ and 8.15, respectively, led to measurable enhancements only in the former case. The effects were of approximately the same size at 1′–H, 2′–H_a and 3′–H. Thus the chemical shift of $\delta = 8.45$ is assigned to 8-H and simultaneously it is shown that the torsional angle about the glycosidic bond is not restricted to a narrow range but that both *syn*- and *anti*-conformers are populated. The preferred conformation of **7** in aqueous solution is depicted in the formula.



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In the fast atom bombardment (FAB) mass spectrum of **7** (Kratos MS 50 S, glycerol matrix), the molecular ion and characteristic fragment ions for both the base and the ribose phosphonic acid moieties are observed. The positive ion mass spectrum has peaks at 136 d (base + 2H), 194 d (ribose phosphonic acid —H) and 330 d (M + H), while the negative ion spectrum has peaks at 134 d (base), 193 d (ribose phosphonic acid —2H) and 328 d (M–H).

We are presently working on the synthesis of the cytidine, guanosine and thymidine analogues of **7**.

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