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Note

Synthesis of an *O*-acetylated α-D-glucosyl ester of a nucleoside 5′-diphosphate by the orthoester route

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Glycosyl esters of nucleoside 5′-diphosphates are of central importance in the metabolism of many carbohydrates[1]. However, all previous methods for the chemical synthesis of these molecules involved formation of the pyrophosphate bond of the nucleotide[2]. We now report a synthesis based on the condensation of a nucleoside 5′-diphosphate with a glycosyl orthoester, an approach analogous to our syntheses[3] of nucleotide 5′-(glycosyl monophosphate)s. The advantage of this approach lies in the fact that the glycosyl phosphate, frequently difficult to obtain, does not have to be synthesized. All of the common nucleoside 5′-diphosphates are commercially available, and sugar orthoesters are readily synthesized[4].

Addition of phosphoric acids to α-D-glucosyl orthoesters usually leads to the 1,2-*trans* (β-D-glucosyl) anomer[5], but we encountered the unanticipated formation of the 1,2-*cis* (α-D-glucosyl) anomer.

RESULTS AND DISCUSSION

Anhydrous adenosine 5′-diphosphoric acid (ADP; **2**) was added to a solution of the α-D-glucosyl orthoester **1** in 8:1 hexamethylphosphoric triamide–oxolane that had been dried[5] with P_2O_5, and the suspension was stirred for 8 days at 25°. T.l.c. then showed three new, phosphate-containing spots having the R_F values given in Table I, column 2. These corresponded to the bis(tetra-*O*-acetyl-D-glucosyl) phosphoric diester[5], the tetra-*O*-acetyl-D-glucosyl ester of adenosine 5′-monophosphate[3] ($AMPGlcAc_4$), and a tetra-*O*-acetyl-D-glucosyl ester (**3**) of adenosine 5′-diphosphate. AMP** and unreacted ADP (R_F 0 for both) were also present. Electrophoresis of this mixture gave the results shown in Table I, column 3. Compound **3** was purified, and isolated, by a combination of preparative t.l.c. and paper electrophoresis.

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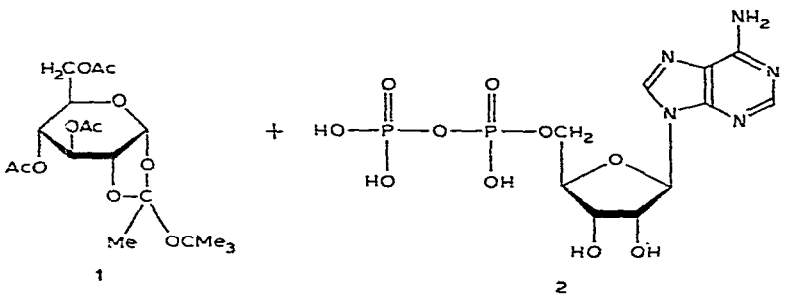
**ADP is known[6] to disproportionate into AMP and ATP.



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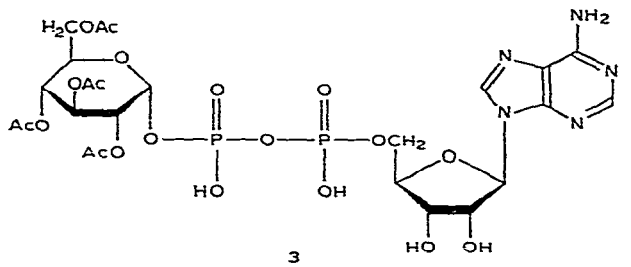

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TABLE I

SOME PROPERTIES OF THE PRODUCTS FROM THE REACTION OF **1** WITH **2**

Compound	R_F[a]	R_p[b]
AMPGlcAc$_4$	0.4	0.46
3	0.22	0.88
(GlcAc$_4$)$_2$ phosphoric diester	0.7	0.38
AMP	0.0	1.16
(**2**	0.0	1.38)

[a]T.l.c. on silica gel with solvent *B*, 60:35:6 (v/v) chloroform–methanol–water. [b]Paper electrophoresis in 0.15M NH_4HCO_3 buffer, pH 7.9; mobilities relative to picrate ion at R_F 1.0.

The results of elemental analysis of **3** were consistent with the structure assigned. The presence of the diphosphate bridge was indicated by ^{31}P-n.m.r. spectroscopy, which showed a pair of doublets, at 11.1 and 13.4 p.p.m., with $J_{P_1-P_2}$ 20.2 Hz (lit.[7] UDP-α-Glc, 20.6 Hz). We next examined the ^{1}H-n.m.r. spectrum, to confirm the expected β-D-glucosyl configuration, but, to our surprise, the 300-MHz, ^{1}H-n.m.r. spectrum gave clear evidence for the α configuration: on phosphorus decoupling, a quartet at 5.59 p.p.m. collapsed to a doublet, $J_{1'',2''}$ 3.4 Hz (see Fig. 1). Assignment of the α configuration was supported by the specific rotation: $[\alpha]_D^{25}$ +21.3° (*c* 0.3, water), a value close to that measured for ADP-α-Glc itself: $[\alpha]_D^{25}$ +22.2° (*c* 1.1, water). The value expected for the β anomer of **3** is[3] in the vicinity of −20°.

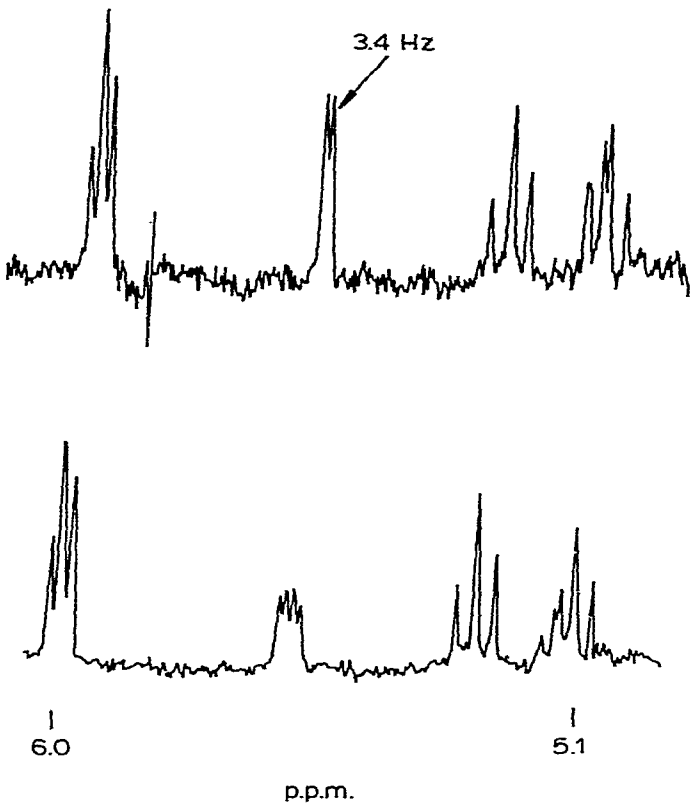

Fig. 1. 300-MHz, ^{1}H-n.m.r. spectra of **3**. (Lower, phosphorus coupled; upper, phosphorus decoupled.)

Anomerization of glycosyl phosphates is well known to occur under the conditions of the MacDonald synthesis[8], and has been observed during an orthoester synthesis of a glycosyl phosphate[9]. We assume that in the present experiment, as well, the β anomer was formed as the kinetic product and gave rise to the α anomer during the relatively long time required for the synthesis.

EXPERIMENTAL

General. — The methods used were the same as those described previously[3].

*Adenosine 5'-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl diphosphate*) (**3**). — To a solution of 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-*tert*-butoxyethylidene)-α-D-glucopyranose (**1**; 0.5 g, 1.24 mmol) in dry (P_2O_5) hexamethylphosphoric triamide (4 mL) at ~50° was added anhydrous adenosine 5'-diphosphoric acid (**2**; 0.1 g, 0.23 mmol), followed by[5] oxolane (0.5 mL). The mixture was stirred for 8 days at 25°, and remained heterogeneous. Compound **3** was isolated as follows. The mixture of **3** and $AMPGlcAc_4$ was separated from **2**, AMP, and the bis(tetra-*O*-acetyl-β-D-glucosyl) phosphate by preparative, thin-layer chromatography on silica gel in solvent *B* (see Table I).

Finally, 3 was separated from $AMPGlcAc_4$ by paper electrophoresis in ammonium hydrogencarbonate buffer, pH 7.9, as the diammonium salt in a yield of 0.003 g; $\lambda_{max}^{pH\ 7.8}$ 259 nm, ε_{mM} 14.50.

Anal. Calc. for $C_{24}H_{39}N_7O_{19}P_2$: C, 36.4; H, 4.9. Found: C, 36.1; H, 5.2.

Compound 3 was converted into 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose plus 2 on treatment with 0.3M HCl for 10 min at 80°. Treatment with 0.2M sodium hydroxide in methanol–water for 50 min at 25° gave D-glucosyl phosphate and AMP, as did incubation with nucleotide pyrophosphatase (venom, Sigma).

ACKNOWLEDGEMENTS

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