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SYNTHESIS OF NUCLEOSIDE 5′-(β-D-GLUCOPYRANOSYL MONOPHOSPHATES) BY THE SUGAR ORTHO ESTER ROUTE

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ABSTRACT

exo-3,4,6-Tri-*O*-acetyl-1,2-*O*-(*tert*-butyl orthoacetyl)-α-D-glucopyranose reacts with nucleoside 5′-monophosphoric acids in *N*,*N*-dimethylformamide to give the nucleoside 5′-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl phosphoric acids). Reactions with 2′-deoxynucleoside 5′-monophosphoric acids gave the corresponding nucleoside 5′-(glucosyl monophosphates) in the 2′-deoxy series.

INTRODUCTION

1,2-*trans*-Glycosyl phosphates may be synthesized by the addition of phosphoric acid derivatives to sugar ortho esters[1]. The original reports of this synthesis used protected phosphoric acids to avoid formation of unwanted diesters. We showed in later work that diester formation could be avoided simply by using an excess of phosphoric acid[2]. Preliminary reports have appeared of the synthesis of a nucleotide sugar analog, a nucleoside 5′-(sugar monophosphate), by this route[3]. We hoped to make the synthesis especially simple by using underivatized nucleoside monophosphoric acids, relying on the preferential addition of the stronger acid to the ortho ester[4], although addition of the hydroxyl groups of ribose or the exocyclic substituents of the heterocyclic rings of the bases was possible in theory. This paper reports synthesis of both the "normal" and the 2′-deoxynucleoside 5′-(sugar monophosphates) by this approach. We note also that, while this series of compounds is related to the nucleoside 5′-(glycosyl diphosphates) ("sugar nucleotides"), there is an even closer relationship to the cytidine 5′-(*N*-acetylneuraminic acid monophosphate) of Roseman[5] and to a similar derivative of 3-deoxy-D-*manno*-octulosonic acid reported by Heath[6].

RESULTS AND DISCUSSION

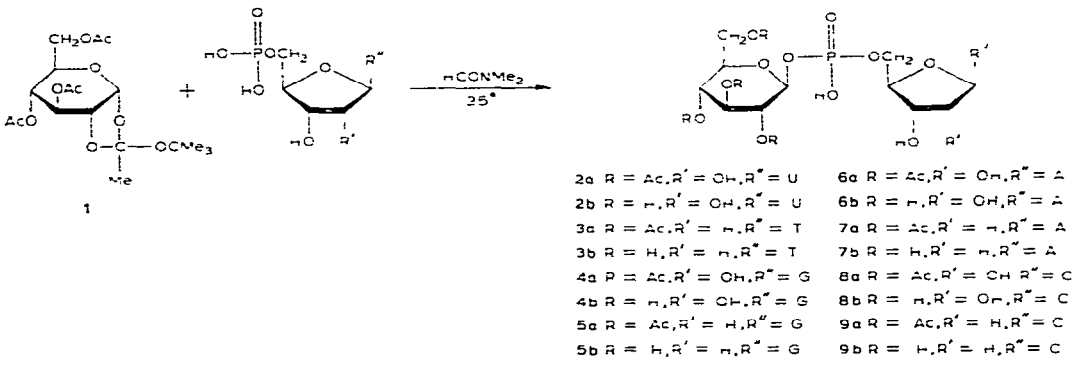
A solution of *exo*-3,4,6-tri-*O*-acetyl-1,2-*O*-(*tert*-butylorthoacetyl)-α-D-glucopyranose (**1**) and anhydrous uridine 5′-monophosphoric acid (UMP) in dry *N*,*N*-dimethylformamide (DMF) showed a single new u.v.-absorbing spot, R_F 0.5 (t.l.c., solvent *B*), after 20 h at 25°. A new phosphate-containing spot at R_p 0.64 also appeared upon electrophoresis, corresponding to the expected uridine 5′-(glucosyl monophos-

TABLE I

^{13}C AND ^{31}P CHEMICAL SHIFTS OF NUCLEOSIDE 5′-(β-D-GLUCOPYRANOSYL MONOPHOSPHATES) (p.p.m.)[a,b]

Compound	*2*	*4*	*5*	*6*	*8*	*5-Me*	*1′*	*2′*	*3′*	*4′*	*5′*	*1″*	*2″*	*3″*	*4″*	*5″*	*6″*	^{31}P
2a, Ac_4UMPGlc	152.2	166.5	103.04	142.23			89.2	74.4	70.3	83.5	65.4	95.7	72.1	72.7	68.5	73.4	62.3	−2.05
2b, UMPGlc	153.2	167.8	103.1	142.0			89.2	74.2	70.3	83.9	65.5	98.4	73.9	75.8	69.9	76.9	61.2	
3a, Ac_4dTMPGlc	152.1	166.8	112.0	138.0		12.28	85.7	39.5	72.7	86.2	65.9	95.7	71.7	72.05	68.5	73.4	62.3	−2.21
4a, Ac_4GMPGlc	154.4	150.5	116.9	160.2	138.6		87.8	74.2	70.9	83.9	65.8	95.7	71.9	72.3	68.4	73.4	62.1	−2.12
5a, Ac_4dGMPGlc	154.4	151.0	117.9	159.4	139.8		84.2	39.2	71.2	86.4	66.2	95.7	71.9	72.7	68.5	73.4	62.2	−2.18
6a, Ac_4AMPGlc	153.6	149.6	118.2	156.1	139.2		87.7	74.7	70.7	84.1	65.7	95.6	71.9	72.9	68.4	73.4	62.2	−2.48
7a, Ac_4dAMPGlc	153.2	149.0	122.6	155.9	140.5		84.4	39.7	71.2	86.5	65.9	95.8	71.9	72.6	68.6	73.5	62.2	−2.24
9a, Ac_4dCMPGlc	157.4	166.7	97.6	142.5			86.3	40.4	71.8	86.8	65.8	95.7	72.2	72.8	68.7	73.5	62.4	−2.14[c]

[a]The acetyl resonances have been omitted, pD 7–8. [b]Assignments are according to ref. 7. [c]Ac_4CMPGlc, **8a**.



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2a R = Ac, R′ = OH, R″ = U
2b R = H, R′ = OH, R″ = U
3a R = Ac, R′ = H, R″ = T
3b R = H, R′ = H, R″ = T
4a R = Ac, R′ = OH, R″ = G
4b R = H, R′ = OH, R″ = G
5a R = Ac, R′ = H, R″ = G
5b R = H, R′ = H, R″ = G

6a R = Ac, R′ = OH, R″ = A
6b R = H, R′ = OH, R″ = A
7a R = Ac, R′ = H, R″ = A
7b R = H, R′ = H, R″ = A
8a R = Ac, R′ = OH, R″ = C
8b R = H, R′ = OH, R″ = C
9a R = Ac, R′ = H, R″ = C
9b R = H, R′ = H, R″ = C

phate). UMP itself appeared at R_p 1.47. The material having R_p 0.64 was isolated by preparative t.l.c. Elemental analysis suggested that it was the tetraacetylated uridine 5′-(glucosyl monophosphate) (**2a**). The ^{13}C-n.m.r. spectrum of **2a** showed eleven resonances, in addition to the acetyl and uracil carbon resonances (Table I). As expected, the C-1″ resonance was split into a doublet by the phosphorus nucleus, $J_{P-C-1''}$ 4.8 Hz. The C-5′ and C-4′ resonances were split into two doublets showing $J_{P-C-5'}$ 4.8 and $J_{P-C-4'}$ 8.4 Hz. The ^{31}P-n.m.r. proton-decoupled spectrum of **2a** showed a single phosphorus resonance at −2.05 p.p.m. The proton-coupled ^{31}P spectrum was split into a sextuplet. The i.r. spectrum gave strong bands at 1765 (C=O) and 1235 cm^{-1} (P=O). Catalytic deacetylation of **2a** with sodium methoxide gave **2b**, homogeneous by electrophoresis and t.l.c.

The i.r. spectrum of **2b** showed a strong band at 1240 cm^{-1} (P=O) but no carbonyl absorption. The ^{13}C-n.m.r. spectrum showed eleven resonances, in addition to the uracil carbon resonances (Table I). As in **2a**, C-1″, C-4′, and C-5′ resonances were split into three separate doublets by the phosphorus nucleus, with $J_{P-C-1''}$ 6.0, $J_{P-C-5'}$ 4.8, and $J_{P-C-4'}$ 8.4 Hz. The expected splitting of C-2″ by phosphorus could not be observed because of overlapping resonances. The C-1″–C-5″ resonances showed a downfield shift of 1–3 p.p.m. whereas the C-6″ resonance displayed an upfield shift of 1 p.p.m. in comparison to that of **2a**. This observation was identical to that reported[12] before for glycosyl phosphates. The ^{1}H-n.m.r. spectrum of **2b** at 300 MHz showed the H-1″ signal as a pseudo-triplet at δ 4.82 which collapsed to a doublet, $J_{1'',2''}$ 8 Hz, upon decoupling phosphorus. Compound **2a** was subjected to enzymic analysis. Treatment of 0.5 μmol of **2a** with alkaline phosphate monoesterase was without effect, but phosphate diesterase cleaved **2a** to UMP. Control runs showed, by contrast, that the phosphate diesterase had no effect on UMP but that the phosphate monoesterase catalyzed the hydrolysis of UMP to uridine. D-Glucose and UMP were formed when **2b** was treated with 0.3M hydrochloric acid for 40 h at 25°.

Compounds **3a–9a** were prepared by analogous procedures although, as noted in the experimental section, limited solubility of some of the nucleotides in DMF led to heterogeneous reaction-mixtures. The hydrolytic behavior of **3a–9a** and of **3b–9b** was analogous to that described for **2a** and **2b** except that in the 2′-deoxy series, the

TABLE II

SPECIFIC ROTATIONS[a]

Compound	$[\alpha]_D^{25}$ *(degrees)*	*Compound*	$[\alpha]_D^{25}$ *(degrees)*
Ac_4UMPGlc (2a)	+4.2[b]	5′-dGMP	−7.9[b], pH 2.4 (−31[c])
UMPGlc (2b)	+1.4[b]	Ac_4AMPGlc (6a)	−17.2[b]
UDP-α-D-Glc	+43.3[b], +43.6[d]	5′-AMP	−26[c]
5′-UMP	+3.5[c]	Ac_4dAMPGlc (7a)	−10.2[b]
UDP-β-D-Glc	+7.8[e]	5′-dAMP	−27.1[b], pH 3.2
Ac_4dTMPGlc (3a)	+3.2[b]	Ac_4CMPGlc (8a)	+16.3[b]
5′-dTMP	−4.4[c]	5′-CMP	−27.1[c]
Ac_4GMPGlc (4a)	−9.2[b]	Ac_4dCMPGlc (9a)	−30.7[b]
5′-GMP	−7.8[b], pH 2.4	5′-dCMP	+35[c]
Ac_4dGMPGlc (5a)	+12.7[b]		

[a]The measured rotations are in water, *c* 0.5–1.2, pH 6–8 unless otherwise indicated. [b]Our data. [c]See ref. 8. [d]See ref. 9. [e]See ref. 10.

TABLE III

T.L.C. AND ELECTROPHORETIC MOBILITIES

Compound	R_p[a]	R_FA[b]	R_FB[c]
Ac_4UMPGlc (2a)	0.64	0	0.5
5′-UMP	1.47	0	0
Ac_4dTMPGlc (3a)	0.60	0	0.5
5′-dTMP	1.37	0	0
Ac_4GMPGlc (4a)	0.57	0	0.4
5′-GMP	1.34	0	0
Ac_4dGMPGlc (5a)	0.64	0	0.6
5′-dGMP	1.23	0	0
Ac_4AMPGlc (6a)	0.46	0	0.4
5′-AMP	1.06	0	0
Ac_4dAMPGlc (7a)	0.65	0	0.55
5′-dAMP	1.19	0	0
Ac_4CMPGlc (8a)	0.46	0	0.35
5′-CMP	1.38	0	0
Ac_4dCMPGlc (9a)	0.55	0	0.7
5′-dCMP	1.44	0	0
2b–9b	0.77 (2b), 0.56 (6b)		0
Ac_3-*tert*-butylglucose ortho ester (**1**)		0.5	

[a]Electrophoretic mobility relative to picrate. [b]R_F in solvent *A*. [c]R_F in solvent *B*.

free bases were also formed during hydrolysis by hydrochloric acid. Tables I–III give data for the ^{13}C- and ^{31}P-n.m.r. spectra, the specific rotations, and the chromatographic behavior for these compounds.

The success of this synthesis depends critically upon thorough drying of the

solvent, as protonated sugar ortho esters are very rapidly hydrolyzed[11]. We have found that the most effective final drying procedure for the DMF is to stir it with powdered P_4O_{10} briefly and then to filter the dried solvent rapidly into the reaction flask[12]. Some of the P_4O_{10} inevitably dissolves in the solvent and leads to formation of the glycosyl phosphate. Separation of the glycosyl phosphate[12] from the sugar nucleotide is easy (Table III). Also, because we have generally used an excess of the ortho ester in these syntheses rather than an excess of the phosphoric acid as previously described[2,12], we find some phosphate diester formation. Again, this product is readily separated from the sugar nucleotide, because the phosphoric diester has a larger R_F value (~0.7, solvent *B*) than most of the sugar nucleoside monophosphates (Table III)[12].

The analytical results, enzymic analysis, and n.m.r. data leave little doubt as to the structure of this series of compounds, but a comment is in order on the question of the configuration at C-1 of glucose (C-1″).

The 1,2-*trans* (β) configuration is the expected product of the addition to the ortho ester[11]. The ^{13}C-n.m.r. data (Table I) show that the C-1″ resonances for the peracetylated derivatives are all in the range 95.6–95.7 p.p.m. at pH 7. Upon deacetylation, this resonance shifts downfield to 98.5 p.p.m. This value is coincident with the value reported by Barker *et al.*[13] for β-D-glucopyranosyl phosphate; the α anomer shows the C-1″ resonance at 95 p.p.m. UDP-α-D-glucose, however, shows[13] a resonance for C-1″ at 97 p.p.m. We measured the ^{1}H-n.m.r. spectra of **2b** (already discussed) and of **3a** to confirm the β configuration. The H-1″ resonance of **3a** appeared as a pseudo-triplet at δ 5.14, which was identified by decoupling the phosphorus nucleus. For both **2b** and **3a**, the H-1″ resonance showed $J_{1'',2''}$ 8 Hz. This coupling is consistent only with the β configuration[14]. As the C-1″ resonance is identical for all of these compounds, all presumably have the β configuration.

This conclusion is supported by the optical-rotation data (Table II). A β-D-glucosyl residue should contribute little to the net rotation of the molecule, in contrast to the contribution expected from an α-D-glucosyl residue[15]. Thus, we expected that the rotations of all of these derivatives would be close to those of the corresponding nucleoside 5′-phosphates. Table II shows this to be so.

EXPERIMENTAL

General methods. — Proton n.m.r. spectra were recorded with a Varian T-60 instrument at 60 MHz, or a Bruker WM-300 instrument at 300 MHz, proton-decoupled C-13 n.m.r. spectra with a Bruker-90 instrument at 22.63 MHz, and P-31 n.m.r. spectra with a Bruker HFX-10 instrument at 36.4 MHz. Solutions (50–100 mg/mL) in D_2O were used, with 1,4-dioxane as the external standard (C-13) and 85% H_3PO_4 as external standard for ^{31}P-n.m.r. I.r. spectra were recorded with a Perkin–Elmer 237B spectrophotometer. U.v. spectra were recorded with a Cary-15 instrument. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. Evaporations were carried out under an air current. T.l.c. was performed on an aluminum support

coated with silica gel 60 with fluorescent indicator, with either solvent *A* (3:1 ether–light petroleum) or solvent *B* [60:35:6 (v/v) chloroform–methanol–water]. Spots were detected by using a sulfuric acid–ethanol spray and heating. Preparative t.l.c. was performed on precoated p.l.c. plates, Silica Gel GF (Analtech, 20 × 20 cm, 1000-μm thick). Paper chromatograms were developed in 4:1:1 1-butanol–ethanol–water, and processed by the silver nitrate dip-procedure[16]. Paper electrophoresis was conducted on Schleicher and Schuell No. 589 orange ribbon paper-strips in 0.15M NH_4HCO_3 (pH 7.9). The relative mobilities, R_p, are given by cm(phosphate)/cm(picrate). Phosphate spots were revealed by the spray reagent of Bandurski and Axelrod[17]. The orthoester derivative of D-glucose was prepared as previously described[18]. Anhydrous ethers were prepared by distillation and storage over molecular sieve 4 Å. *N*,*N*-Dimethylformamide was purified by distillation from calcium hydride, storage over molecular sieve 4 Å, and final drying by brief treatment with P_4O_{10}. Nucleotides were dried *in vacuo* over P_4O_{10} for 7–10 days at 25°. Microanalyses were performed by Galbraith Analytical Laboratories, Knoxville, Tenn. Phosphate monoesterase (*E. coli*) and phosphate diesterase (snake venom) were obtained from Boehringer. Filtrations were performed with a sintered-glass filter, with Celite as filter aid. Compounds **2a**–**9a** were deacetylated in 0.2M methanolic sodium methoxide. U.v. spectra were measured in water at pH 6–8. The absorbances and specific rotations are corrected for the presence of $CaSO_4$, as determined from the ash content.

*Uridine 5′-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl phosphoric acid)(Ac$_4$-UMPGlc)* (**2a**). — To a solution of 3,4,6-tri-*O*-acetyl-1,2-*O*-(*tert*-butyl orthoacetyl)-α-D-glucopyranose (**1**, 0.5 g, 1.24 mmol) in dry DMF (1.5 mL) at ~50°, was added anhydrous uridine 5′-phosphoric acid (0.09 g, 0.28 mmol). The mixture was stirred at room temperature. The mixture became homogeneous after 0.5 h. The solution was stirred for 20 h at 25°. T.l.c. (solvent *A*) showed diminution of the orthoester spot (R_F 0.5) after this time and concurrent appearance of material having zero mobility. T.l.c. (solvent *B*) showed the appearance of a new spot at R_F 0.5. Electrophoresis at pH 7.9 showed two phosphorus-containing spots, one corresponding to UMP (R_p 1.47) and the other to compound **2a** (R_p 0.64). The latter was isolated by preparative t.l.c. on silica gel plates with solvent *B* in a yield of ~38 mg (21%); λ_{max} 262 nm (ε 10,400) (reported for UMP, 10,000). The material contained some $CaSO_4$ derived from the t.l.c. plates.

Anal. Calc. for $C_{23}H_{30}N_2O_{18}P \cdot 7/8CaSO_4$: C, 35.75; H, 3.89; N, 3.63; P, 4.02. Found: C, 35.88; H, 4.35; N, 3.77; P, 4.11.

*Uridine 5′-(β-*D*-glucopyranosyl phosphoric acid) (UMPGlc)* (**2b**). — To a solution of **2a** (0.08 g) in water (0.5 mL), sodium methoxide in methanol (0.5 mL, 0.2M) was added with stirring and the mixture was kept for 48 h at room temperature. T.l.c. (solvent *B*) showed a slow-moving spot (R_F 0), and no starting material (R_F 0.5). The excess of methanol and water was removed to give **2b** as an amorphous powder, yield 57 mg (97%); λ_{max} 262 nm (ε 10,150).

Anal. Calc. for $C_{15}H_{21}N_2O_{14}PNa \cdot 4\ H_2O$: C, 31.1; H, 5.0. Found: C, 30.8; H, 5.02.

The syntheses of **3a–9a** followed the same general procedure as that described for **2a**. The principal differences concerned the solubilities of the 5'-nucleotides in DMF. This behavior is noted in the following descriptions. Compounds **3a–9a** were converted into **3b–9b** as described for the conversion of **2a** into **2b**. Single products were formed, as shown by t.l.c. analysis (solvent *B*).

*Thymidine 5'-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl phosphoric acid)* (*Ac_4dTMPGlc*) (**3a**). — Thymidine 5'-phosphoric acid dissolved readily in DMF and **3a** was isolated in a yield of 0.106 g (39%): ν_{max} 1240 (P=O) and 1765 cm^{-1} (C=O); λ_{max} 267 nm (ε 9200) (reported for thymine, 9650).

Anal. Calc. for $C_{24}H_{32}N_2O_{17}P \cdot 3/7CaSO_4$: C, 40.62; H, 4.51; N, 3.94; P, 4.37. Found: C, 40.6; H, 4.81; N, 4.09; P, 4.15.

This material was desalted on a Biogel P-2 column to give material having ε 10,200 at 267 nm.

Anal. Calc. for $C_{24}H_{32}O_{17}N_2P \cdot 3\ H_2O$: C, 40.85; H, 5.39; N, 3.97; P, 4.40. Found: C, 40.75; H, 5.19; N, 4.05; P, 4.50.

*Guanosine 5'-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl phosphoric acid)* (*Ac_4GMPGlc*) (**4a**). — Guanosine 5'-phosphoric acid dissolved in DMF after stirring for 3 h at room temperature, and **4a** was isolated in a yield of 27 mg (20%): ν_{max} 1240 (P=O) and 1760 cm^{-1} (C=O); λ_{max} 252 nm (ε 13,100) (reported for GMP, 13,700).

Anal. Calc. for $C_{24}H_{31}N_5O_{17}P \cdot 2/7CaSO_4$: C, 39.4; H, 4.24; P, 4.24. Found: C, 39.2; H, 4.93; P, 3.49.

*2'-Deoxyguanosine 5'-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl phosphoric acid)* (*Ac_4dGMPGlc*) (**5a**). — 2'-Deoxyguanosine 5'-phosphoric acid reacted with the orthoester as a heterogeneous mixture in DMF to give 18 mg (9%) of **5a**; ν_{max} 1240 (P=O) and 1760 cm^{-1} (C=O); λ_{max} 252 (ε 13,200) (reported for GMP 13,700).

Anal. Calc. for $C_{24}H_{31}N_5O_{16}P \cdot 4/7CaSO_4$: C, 38.19; H, 4.11; P, 4.11. Found: C, 37.76; H, 4.66; P, 3.36.

*Adenosine 5'-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl phosphoric acid)* (*Ac_4AMPGlc*) (**6a**). — The reaction mixture with adenosine 5'-phosphoric acid remained heterogeneous, and **6a** was isolated in a yield of 12 mg (7%): ν_{max} 1250 (P=O) and 1760 cm^{-1} (C=O); λ_{max} 259 nm (ε 14,500) (reported for AMP 15,400).

Anal. Calc. for $C_{24}H_{31}N_5O_{16}P \cdot 13.6\ CaSO_4$: C, 11.41; H, 1.23; N, 2.77; P, 1.23. Found: C, 11.42; H, 2.15; N, 2.48; P, 1.06.

*2'-Deoxyadenosine 5'-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl phosphoric acid)* (**7a**) (*Ac_4dAMPGlc*). — The mixture became homogeneous after 3 h; yield of **7a** 38 mg (18.5%); ν_{max} 1245 (P=O) and 1770 cm^{-1} (C=O); λ_{max} 260 nm (ε 14,900) (reported for AMP 15,400).

Anal. Calc. for $C_{24}H_{31}N_5O_{15}P \cdot 5/14CaSO_4$: C, 40.62; H, 4.37; P, 4.37. Found: C, 40.63; H, 4.94; P, 4.02.

*Cytidine 5'-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl phosphoric acid)* (*Ac_4-CMPGlc*) (**8a**). — The mixture remained heterogeneous; yield of **8a** 12 mg (5.7%);

ν_{max} 1240 (P=O) and 1750 cm^{-1} (C=O); λ_{max} 271 nm (ε 8700) (reported for CMP 9000).

Anal. Calc. for $C_{23}H_{31}O_{17}N_3P \cdot 2\ H_2O$: C, 40.12; H, 5.1. Found: C, 40.34; H, 5.45.

*2'-Deoxycytidine 5'-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl phosphoric acid)* (*Ac_4dCMPGlc*) (**9a**). — The mixture remained heterogeneous; yield of **9a** 35 mg (14.5%): ν_{max} 1240 (P=O) and 1760 cm^{-1} (C=O); λ_{max} 271 nm (ε 8700) (reported for CMP 9000).

Anal. Calc. for $C_{23}H_{31}N_3O_{16}P \cdot 3/14CaSO_4$: C, 41.5; H, 4.66. Found: C, 41.4; H, 5.15.

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