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Preliminary communication

A convenient synthesis of uridine 5′-(2-acetamido-2-deoxy-α-D-manno-pyranosyluronic acid pyrophosphate)*

TATSUMI YAMAZAKI, CHRISTOPHER D. WARREN, ANNETTE HERSCOVICS, and ROGER W. JEANLOZ†

Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, MA 02114 (U. S. A.)

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Teichuronic acid, the antigenic polysaccharide in the cell wall of *Micrococcus lysodeikticus*, consists[1] of repeating units of D-glucose and 2-acetamido-2-deoxy-D-mannuronic acid (ManNAcA). Recent work of Rohr *et al.*[2] suggested that polyprenol-linked polysaccharides containing 2-acetamido-2-deoxy-D-glucose, ManNAcA-GlcNAc, and $(ManNAcA)_2$-GlcNAc residues are involved in different steps of teichuronic acid biosynthesis. The "nucleotide sugar" UDP-ManNAcA (8) is considered to be the donor of sugar to lipid in the formation of such lipid-linked saccharides, and was needed by us for biosynthetic studies. It is difficult to obtain 8 from natural sources[3], and, although a chemical synthesis of 8 has been reported[3], we found it to be impractical, because of the low yield, and the time and effort involved in obtaining a pure product. We now report a rapid and efficient route for the synthesis of 8.

2-Methyl-(2-acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-β-D-mannopyrano)-[2,1-*d*]-2-oxazoline (1), readily prepared from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranosyl chloride by treatment with base[4], was condensed with dibenzyl phosphate in benzene for 15 h at room temperature[5]. Catalytic hydrogenolysis of the resulting dibenzyl derivative in dry methanol in the presence of 10% Pd–C gave 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranosyl phosphate (2) in 61% yield (from 1), after purification by t.l.c.§ (solvent *A*, R_F 0.2), $[\alpha]_D^{20}$ +25° (*c* 1.0, methanol); 2 was further characterized by quantitative *O*-deacetylation, to give 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate[5] (3), $[\alpha]_D^{20}$ +23° (*c* 1.0, water); ^{1}H-n.m.r. (D_2O): δ 5.24 (bd, 1 H, $J_{1,P}$ 8 Hz, H-1), 4.33 (bd, 1 H, $J_{2,3}$ 4.5 Hz, H-2), 4.10 (q, 1 H, $J_{2,3}$ 4.5, $J_{3,4}$ 10 Hz, H-3), 3.85 (s, 2 H, H-6a, -6b), and

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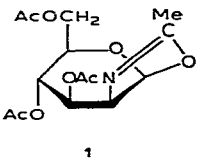
† To whom correspondence should be addressed.

§ Preparative t.l.c. was performed with precoated plates of Silica Gel F-254 (Merck) in the solvent systems (*A*) 60:25:4 and (*B*) 10:10:3 (v/v) chloroform–methanol–water. All compounds were eluted from the silica gel with solvent *B*.



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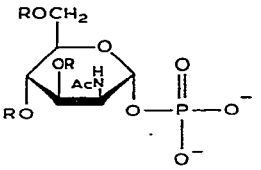
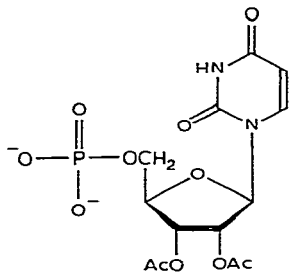


2 R = Ac
3 R = H



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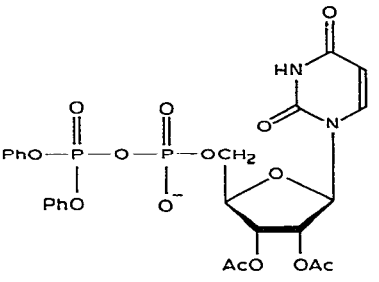

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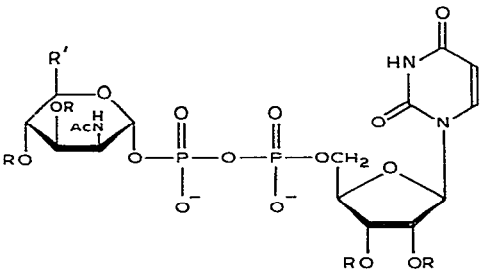

6 R = Ac, R′ = CH_2OAc
7 R = H, R′ = CH_2OH
8 R = H, R′ = CO_2^-

2.04 (s, 3 H, NAc); see Fig. 1. Compound **2** was suitable for condensation without further purification.

2′,3′-Di-*O*-acetyluridine 5′-monophosphate (**4**) was obtained in 72% yield from uridine 5′-(disodium monophosphate) by acetylation with acetic anhydride in pyridine–*N*,*N*-dimethylformamide. The efficient condensation of **4** with **2** was performed by application of the mixed-anhydride method[6,7] and of preparative t.l.c. for isolation of the product from the reaction mixture. The conditions were as follows: treatment of the tributylammonium salt of **4** with diphenyl phosphorochloridate (1.1 equiv.) in dry 1,4-dioxane for 2 h at room temperature under nitrogen, and subsequent evaporation of the reaction mixture, gave syrupy 2′,3′-di-*O*-acetyluridine 5′-(diphenyl pyrophosphate) (**5**), which was immediately treated with a slight excess of the tributylammonium salt of **2** in dry pyridine for 8–10 h at room temperature. Evaporation of the reaction mixture, and purification by

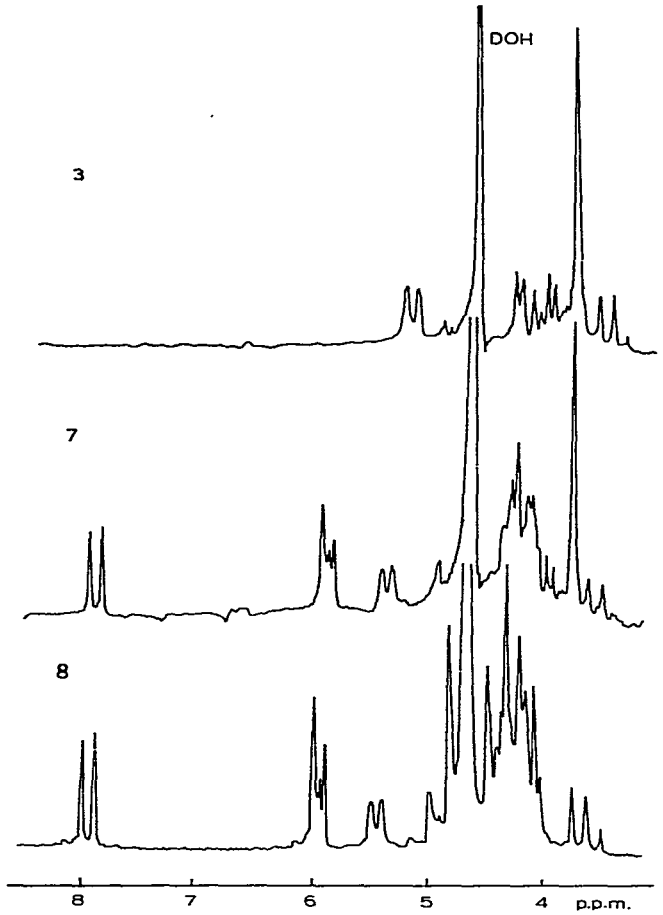

Fig. 1. The 80-MHz, n.m.r. spectra of compounds 3, 7, and 8.

preparative t.l.c. (solvent *B*, R_F 0.8) gave pure, syrupy 2′,3′-di-*O*-acetyluridine 5′-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranosyl pyrophosphate) (6) in 86% yield (based on 4), $[\alpha]_D^{20}$ +19° (*c* 1.0, methanol); ^{1}H-n.m.r. (D_2O): δ 7.95 (d, 1 H, $J_{5,6}$ 8 Hz, H-6 of uracil residue), 6.15—5.80 (m, 3 H, H-5 of uracil residue, H-1 of 2-acetamido-2-deoxy-D-mannosyl and D-ribosyl residues), and 2.20—1.90 (m, 18 H, OAc and NAc). *O*-Deacetylation of 6 in aqueous sodium hydroxide solution afforded uridine 5′-(2-acetamido-2-deoxy-α-D-mannopyranosyl pyrophosphate) (7) as a hygroscopic solid (94% yield), isolated as its lithium salt after preparative t.l.c. (solvent *B*, R_F 0.4), $[\alpha]_D^{20}$ +16° (*c* 0.2, water); ν_{max}^{KBr} 3440 (OH), 1690 (NHCO), and 1130 cm^{-1} (P—O—P); ^{1}H-n.m.r. (D_2O): δ 7.88 (d, 1 H, $J_{5,6}$ 8 Hz, H-6 of uracil residue), 5.90 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1 of D-ribosyl residue), 5.88 (d, 1 H, $J_{5,6}$ 8 Hz, H-5 of uracil residue), 5.37 (bd, 1 H, $J_{1,P}$ 8 Hz, H-1 of 2-acetamido-2-deoxy-D-mannosyl group), 5.0—3.8 (m, 9 H), 3.83 (s, 2 H, H-6a, -6b of

2-acetamido-2-deoxy-D-mannosyl group), and 2.00 (s, 3 H, NAc); see Fig. 1. In related work[4], we have shown that other "nucleotide sugars" can be prepared in similar high yield by this method.

Selective oxidation at C-6 of the 2-acetamido-2-deoxy-D-mannosyl group of 7 was achieved without preparing a special catalyst[3]. By bubbling oxygen into a suspension of 7 in aqueous sodium hydrogencarbonate solution in the presence of an excess of 10% Pt–C (Fluka) for 7 h at 75°, compound 8 was obtained as a hygroscopic lithium salt in 20% yield after purification by preparative, paper chromatography[3], preparative t.l.c. (solvent *B*), and, finally, chromatography on a column of Bio-Rad P-6 in 0.5M pyridine acetate, pH 7.0; $[\alpha]_D^{20}$ +19° (*c* 0.2, methanol); ν_{max}^{KBr} 3450 (OH), 1680 (NHCO), 1630 (CO_2^-), and 1130 cm^{-1} (P–O–P); ^{1}H-n.m.r. (D_2O): δ 7.90 (d, 1 H, $J_{5,6}$ 8 Hz, H-6 of uracil residue), 5.95 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1 of D-ribosyl residue), 5.92 (d, 1 H, $J_{5,6}$ 8 Hz, H-5 of uracil residue), 5.42 (bd, 1 H, $J_{1,P}$ 8 Hz, H-1 of 2-acetamido-2-deoxy-D-mannosyl group), 5.0–3.5 (m, 9 H), and 2.05 (s, 3 H, NAc). The n.m.r.-spectroscopic data for 7 and 8 have not been previously reported. Interestingly, the n.m.r. spectra of 7 and 8 (see Fig. 1) show clear, characteristic, proton signals for each compound. The success of the oxidation step was readily confirmed by disappearance of the strong peak at δ 3.83 in the spectrum, corresponding to the C-6 protons of the 2-acetamido-2-deoxy-D-mannosyl group. In none of the spectra could a clear coupling between H-1 and H-2 of the 2-acetamido-2-deoxy-D-mannosyl group be seen, although a value of $J_{1,2}$ 1.5 Hz has been reported[5].

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