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SYNTHESIS OF METHYL (OR PROPYL) 2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSIDE 6-(α-D-GLUCOPYRANOSYL PHOSPHATE) AND DERIVATIVES FOR THE STUDY OF THE PHOSPHORIC ESTER LINKAGE IN THE *Micrococcus lysodeikticus* CELL-WALL*

CHRISTOPHER D. WARREN, NASIR-UD-DIN, AND ROGER W. JEANLOZ†

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

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ABSTRACT

Methyl 2-acetamido-3-*O*-allyl-2-deoxy-4-*O*-methyl-α-D-glucopyranoside, methyl 2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside, and methyl 2-acetamido-3,4-di-*O*-allyl-2-deoxy-α-D-glucopyranoside, prepared from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, were coupled with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl phosphate (**13**), to give the phosphoric esters methyl 2-acetamido-3-*O*-allyl-2-deoxy-4-*O*-methyl-α-D-glucopyranoside 6-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl phosphate) (**16**), methyl 2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside 6-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl phosphate) (**23**), and methyl 2-acetamido-3,4-di-*O*-allyl-2-deoxy-α-D-glucopyranoside 6-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl phosphate) (**17**). Compound **13** was prepared from penta-*O*-acetyl-β-D-glucopyranose by the phosphoric acid procedure, or by acetylation of α-D-glucopyranosyl phosphate. Removal of the allyl groups from **16** and **17** gave **23** and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 6-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl phosphate) (**19**), respectively. *O*-Deacetylation of **23** gave methyl 2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside 6-(α-D-glucopyranosyl phosphate) (**26**) and *O*-deacetylation of **19** gave methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 6-(α-D-glucopyranosyl phosphate) (**24**). Propyl 2-acetamido-2-deoxy-α-D-glucopyranoside 6-(α-D-glucopyranosyl phosphate) (**25**) was prepared by coupling **13** with allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside, followed by catalytic hydrogenation of the product to give the propyl glycoside, which was then *O*-deacetylated. Compounds **24**, **25**, and **26** are being employed in structural studies of the *Micrococcus lysodeikticus* cell-wall.

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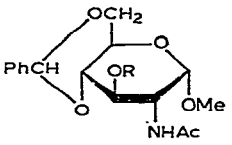
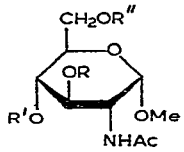
†To whom inquiries should be sent.

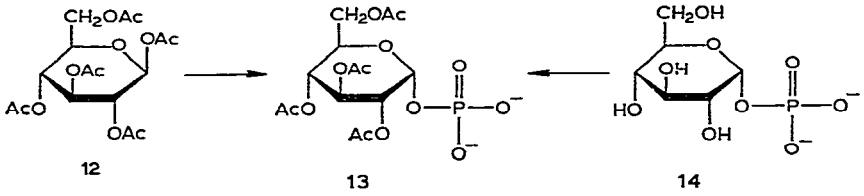


1 R = H
2 R = All

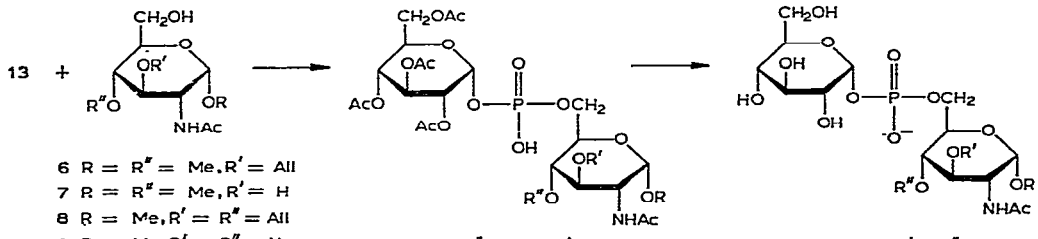
All = $CH_2CH{=}CH_2$



3 R = All, R′ = R″ = H
4 R = All, R′ = H, R″ = Bz
5 R = All, R′ = Me, R″ = Bz
6 R = All, R′ = Me, R″ = H
7 R = R″ = H, R′ = Me
8 R = R′ = All, R″ = H
9 R = R′ = R″ = H
10 R = R′ = H, R″ = Tr
11 R = R′ = All, R″ = Tr



12 13 14



6 R = R″ = Me, R′ = All
7 R = R″ = Me, R′ = H
8 R = Me, R′ = R″ = All
9 R = Me, R′ = R″ = H
15 R′ = All, R′ = R″ = Bzl

Pr = $CH_2CH_2CH_3$

Pre = $CH{=}CH{-}CH_3$

16 R = R″ = Me, R′ = All
17 R = Me, R′ = R″ = All
18 R = Me, R′ = R″ = Pre
19 R = Me, R′ = R″ = H
20 R = R″ = Me, R′ = Pre
21 R = All, R′ = R″ = Bzl
22 R = Pr, R′ = R″ = H
23 R = R″ = Me, R′ = H

24 R = Me, R′ = R″ = H
25 R = Pr, R′ = R″ = H
26 R = R″ = Me, R′ = H

INTRODUCTION

Structural studies of the *Micrococcus lysodeikticus* cell-wall indicate the presence of 2-acetamido-2-deoxy-D-glucose 6-phosphate residues[1,2], and suggest that these residues perform a function similar to that of the *N*-acetylmuramic acid 6-phosphate residues generally present in bacterial cell-walls, including those of *Micrococcus lysodeikticus*, namely, that of linking the external, antigenic polysaccharide to the peptidoglycan chains[3]. In the *Micrococcus lysodeikticus* cell-wall, the antigenic polysaccharide consists of polysaccharide chains of D-glucose and 2-acetamido-2-deoxy-D-mannuronic acid residues and it is likely that the terminal D-glucose residue of this chain is linked to a muramic acid 6-phosphate or 2-acetamido-2-deoxy-D-glucose 6-phosphate residue *via* a phosphoric ester group[4]. Evidence for this type of structure

arose mainly from studies on acid- and alkali-catalyzed hydrolysis. In order to rationalize the results of those studies, it was necessary to investigate the behavior of model compounds. This paper reports the synthesis of three suitable compounds, propyl 2-acetamido-2-deoxy-α-D-glucopyranoside 6-(α-D-glucopyranosyl phosphate) (**25**), methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 6-(α-D-glucopyranosyl phosphate) (**24**), and methyl 2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside 6-(α-D-glucopyranosyl phosphate) (**26**). Compounds **24** and **25** are models for a phosphoric ester linkage between C-1 of a D-glucose residue and C-6 of a 2-acetamido-2-deoxy-D-glucose residue linked in the peptidoglycan chain at C-1 only, whereas **26** is a model for the analogous situation in which the 2-acetamido-2-deoxy-D-glucose residue is linked at both C-1 and C-4. In related work[1], other compounds have been prepared that are models for a phosphoric ester linkage between a D-glucose residue and C-6 of a residue of muramic acid.

RESULTS AND DISCUSSION

The synthesis of the phosphoric diesters **24**, **25**, and **26** was achieved in three stages. In the first, derivatives of methyl or allyl 2-acetamido-2-deoxy-D-glucopyranoside (*a*) having readily removable protecting groups at O-3 and O-4, or (*b*) having an *O*-methyl group at O-4 and an easily removable substituent at O-3 were prepared. In the second stage, efficient methods for preparing 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl phosphate (**13**) were developed. In the third stage, **13** was coupled with the partially protected 2-acetamido-2-deoxy-D-glucopyranoside to give a per-*O*-acetyl phosphoric diester from which the acetyl groups were removed by an alkaline treatment that was mild enough to avoid hydrolysis of the product. At first, allyl groups were selected for protection, as they may be removed under very mild conditions[5,6] unlikely to hydrolyze a phosphoric diester linkage. Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[7] (**1**) was allylated at O-3 to give **2**, from which the benzylidene group was removed by mild, acid hydrolysis to give methyl 2-acetamido-3-*O*-allyl-2-deoxy-α-D-glucopyranoside (**3**) in good yield. Conversion of **3** into methyl 2-acetamido-3-*O*-allyl-2-deoxy-4-*O*-methyl-α-D-glucopyranoside (**6**) was achieved by selective benzoylation at O-6 to give **4**, methylation at O-4 with methyl iodide and silver oxide, and *O*-debenzoylation of the resulting **5**. Compound **6** was employed (*a*) as starting material for the synthesis of the phosphoric diester **16** and (*b*) for conversion, by removal of the allyl substituent, into methyl 2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside[8] (**7**), in order to determine whether or not such a compound as **7**, having free hydroxyl groups at C-3 and C-6, could be used for phosphoric diester synthesis, with the expectation that the reaction would take place almost exclusively at C-6. The allyl group in **6** was removed very readily in the conventional way, by isomerization to the 1-propenyl derivative with tris(triphenylphosphine)rhodium chloride[5]; this result contrasts with the poor yields and side reactions unexpectedly encountered in similar treatment of the phosphoric diesters, especially when two vicinal allyl groups were involved, as with **17** (see later).

For the synthesis of methyl 2-acetamido-3,4-di-*O*-allyl-2-deoxy-α-D-glucopyranoside (**8**), methyl 2-acetamido-2-deoxy-α-D-glucopyranoside[9] (**9**) was converted into the known 6-trityl ether[10] **10**, which was allylated with allyl bromide and sodium hydroxide to give **11**. Finally, *O*-detritylation by mild, acid treatment gave **8**.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl phosphate (**13**) was prepared by two different methods. The first is a modification of the phosphoric acid procedure[11], similar in some respects to that previously developed for the corresponding derivative of D-galactose[12]. Fusion under vacuum of a mixture of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose[13] with crystalline phosphoric acid, followed by cautious neutralization with ammonium hydroxide, gave a syrup that contained **13**. Unlike 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl phosphate[12], compound **13** did not crystallize and had to be purified by preparative layer chromatography (p.l.c.) before it was useful for phosphoric diester synthesis. In the alternative approach, α-D-glucopyranosyl phosphate (**14**) was acetylated with acetic anhydride and, as the base, either tetraethylammonium acetate[14] or pyridine. In the latter case, it is known[14] that a cyclic 1,2-phosphate will be formed in a competing reaction, but because the hydroxyl groups of **14** were acetylated very quickly, it was found possible to stop the reaction before this cyclic by-product was formed to any great extent. P.l.c. gave **13** in good yield.

For the phosphoric diester synthesis, equal amounts of **13**, in the pyridinium form, and one of the derivatives of 2-acetamido-2-deoxy-D-glucose having OH-6 unprotected (**6** or **8**) was thoroughly dried and then treated with a solution of 2,4,6-triisopropylbenzenesulfonyl chloride[15] in anhydrous pyridine. Total exclusion of moisture was critical for the success of the coupling reaction. Earlier, this was achieved by repeated additions and evaporations of dry pyridine[15] or toluene[16], prior to the addition of the coupling reagent. However, in this study, drying the starting compounds over phosphorus pentaoxide in a vacuum desiccator was found to be sufficient. The coupling reaction usually took place during two days at room temperature, after which time the product was isolated by p.l.c., **6** and **8** giving **16** and **17**, respectively. These fully protected phosphoric diesters were solids that showed no crystalline form under a microscope, but did have definite melting points, and were characterized by optical rotation, i.r. spectrum, and elementary analysis.

In the removal of protecting groups, *O*-deallylation was performed conventionally by isomerization of the allyl to a 1-propenyl group, followed by hydrolysis. It was not possible to use potassium *tert*-butoxide[17] for this isomerization, owing to the presence of ester groups, and so tris(triphenylphosphine)rhodium chloride[5] was the reagent of choice. This had worked well in the preparation of **7** from **6** (see earlier) but, with **17**, the reaction was very sluggish, requiring multiple additions of catalyst, and a prolonged reaction-time. When the isomerized material containing **18** was subjected to hydrolysis with mercuric chloride[6], t.l.c. showed the presence, in addition to **19**, of two other compounds lacking unsaturated groups. This result arose through partial hydrogenation of the allyl or 1-propenyl groups to give propyl groups (resistant to hydrolysis), a side reaction previously reported[18]. When

16, which has only one allyl group, was similarly treated, the isomerization to **20** and subsequent hydrolysis, were more facile. Apparentiy, the difficulty in the isomerization of the allyl groups in **17** is associated with both the presence of a phosphoric ester and the presence of vicinal substituents that are involved in the reaction.

In an attempt to overcome this problem, direct coupling of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**9**) with **13** was tried, in the hope that reaction would occur preferentially at O-6. Unfortunately, analysis of the product indicated the presence of three phosphoric diesters, showing that reaction at O-3 and O-4 had also occurred. Therefore, *O*-deacetylation was performed by brief treatment with a dilute solution of sodium methoxide in methanol, after which, separation of **24** was readily achieved by p.l.c.

In another approach to the synthesis of a phosphoric diester having unprotected hydroxyl groups at C-3 and C-4, allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[18] (**15**) was coupled with **13**. The resulting compound was hydrogenated to remove the benzyl groups, and the allyl group was concomitantly converted into a propyl group. The resulting compound (**22**) was not pure (t.l.c.) and *O*-deacetylation was performed without prior purification of **22**. The product (**25**) was shown to be pure by both elementary analysis and chromatographic methods.

As mentioned earlier, the 4-methyl ether **16** was more readily *O*-deallylated than **17**; it gave **23**, which was *O*-deacetylated by the same mild method used for **19** and **22**, to yield the deprotected phosphoric diester **26**. In order to determine whether **23** (and hence **26**) could be obtained by a direct coupling-reaction, not employing an intermediate having a protecting group at O-3, methyl 2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside (**7**) was coupled with **13**, and the product was shown to contain only one phosphoric diester; it was obtained in good yield and corresponded on chromatograms to **23**. Thus, the 3-OH group in **7**, unlike that in **9**, is sterically hindered enough to prevent reaction with the glycosyl phosphate **13** under the conditions employed in this study.

The synthetic phosphoric diesters **24** and **26** were examined by field-desorption mass spectrometry[19]. In each case, the spectrum showed a molecular ion, together with fragmentation ions arising from D-glucose and 2-acetamido-2-deoxy-D-glucose 6-phosphate residues (from **24**) or the 4-methyl ether of the latter (from **26**), thus providing unequivocal proof of the structures assigned to **24** and **26**. These structures were also confirmed by treatment of the compounds with cation-exchange resin[20] (H^+ form) at 65°, the products identified being D-glucose and 2-acetamido-2-deoxy-D-glucose 6-phosphate (from **24**), and 2-acetamido-2-deoxy-4-*O*-methyl-D-glucose 6-phosphate (from **26**).

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP-2 apparatus and correspond to "corrected melting points". Optical rotations were determined for solutions in 1-dm semimicro tubes with a Perkin–Elmer model 141

polarimeter. I.r. spectra were recorded with a Perkin–Elmer model 237 spectrophotometer. The cation-exchange resin used was AG-50W X8 (200–400 mesh) (BioRad Lab., Richmond, CA, 94804, unless stated otherwise) and in all instances the amount of resin used was in at least a two-fold excess over the quantity necessary to effect complete ion-exchange. All proportions of solvents are v/v. Evaporations were conducted *in vacuo*, with the bath temperature kept below 30°. Microanalyses were performed by Dr. W. Manser, CH-8704 Herrliberg (Switzerland).

Chromatographic separations. — T.l.c. was performed on precoated plates of Silica gel G (E. Merck A.-G., Darmstadt, Germany). The plates supplied (20 × 20 cm) were cut to a length of 6 cm and used without pretreatment. Preparative-layer chromatography was performed on precoated plates (2-mm or 0.5-mm thickness) of Silica Gel G (Merck). Unless otherwise stated, the spray reagent was 1:1:18 (v/v) anisaldehyde–sulfuric acid–ethanol[21], and the plates were heated to 125°. Unsaturation was detected with a 1% aqueous solution of potassium permanganate in 2% sodium carbonate. The spray reagent of Dittmer and Lester[22] was used to detect phosphate groups. Solvent systems used for chromatography were: *A*, 60:25:4 chloroform–methanol–water; *B*, 60:35:6 chloroform–methanol–water; and *C*, 10:10:3 chloroform–methanol–water. When plates were eluted more than once with the same solvent, they were dried in a stream of air for at least 30 min between each elution. The R_F values were calculated from measurement of the distance from the origin of the chromatogram to the point of maximum intensity of the spot after development. Column chromatography was performed on silica gel (70–325 mesh; Merck), used without pretreatment. The proportion of weight of substance to weight of silica gel was 1:60 to 1:90.

Methyl 2-acetamido-3-O-allyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**2**). — A suspension of methyl 2-acetamido-4,6-*O*-benzylidene-α-D-glucopyranoside[7] (**1**, 300 mg) in 1:1 benzene–tetrahydrofuran (100 ml) was treated with powdered sodium hydroxide (1.5 g) and allyl bromide (85 μl) for 3 h at boiling temperature under reflux, and for 12 h at room temperature with vigorous stirring. The mixture was filtered, and the insoluble material washed with hot, 1:1 benzene–chloroform (30 ml). The combined filtrate and washings were evaporated, the residue was dissolved in chloroform, and the solution was washed with water, dried (sodium sulfate), and evaporated. The residue was chromatographed on a column of silica gel with 19:1 chloroform–ethanol to give a syrup that crystallized from chloroform–ether as needles (267 mg, 79%), changing into long needles at 242–247°, m.p. 284–285° (dec.), $[\alpha]_D^{20}$ +30° (*c* 0.6, chloroform); ν_{max}^{KBr} 3295 (NH), 1645 (allyl), 1630 (Amide I), and 1550 cm^{-1} (Amide II); t.l.c. (19:1 chloroform–ethanol) R_F 0.37.

Anal. Calc. for $C_{19}H_{25}NO_6$: C, 62.80; H, 6.93; N, 3.85; O, 26.42. Found: C, 62.67; H, 6.91; N, 3.75; O, 26.37.

Methyl 2-acetamido-3-O-allyl-2-deoxy-α-D-glucopyranoside (**3**). — A suspension of **2** (240 mg) in 60% acetic acid (15 ml) was kept for 1 h at 80°. The resultant solution was cooled, concentrated, and toluene was repeatedly added to it and distilled off. The residue was chromatographed on a column of silica gel with 4:1 chloroform–

ethanol to give a syrup (162 mg, 89%) that crystallized as needles from chloroform (147 mg, 81%), m.p. 178–179°, $[\alpha]_D^{20}$ +117° (*c* 0.82, methanol); ν_{max}^{KBr} 3400–3275 (broad, OH, NH), 1655–1625 (allyl, Amide I), and 1575 cm^{-1} (Amide II); t.l.c. (9:1 chloroform–ethanol) R_F 0.12.

Anal. Calc. for $C_{12}H_{21}NO_6$: C, 52.35; H, 7.69; N, 5.09; O, 34.87. Found: C, 52.32; H, 7.64; N, 5.00; O, 34.81.

Methyl 2-acetamido-3-O-allyl-6-O-benzoyl-2-deoxy-α-D-glucopyranoside (**4**). — A solution of **3** (127 mg) in dry pyridine (15 ml) was cooled to −60° and treated with freshly distilled benzoyl chloride (54 μl) for 6 h at −20°, and for 16 h at −10°. The solution was diluted with dichloromethane (20 ml), and successively washed with ice-cold, saturated solutions of sodium hydrogensulfate, sodium hydrogencarbonate, and ice-cold water, dried (sodium sulfate), and evaporated to give a syrup that was chromatographed on a column of silica gel with 19:1 chloroform–ethanol. The syrupy product crystallized from chloroform–heptane as microneedles (141 mg, 80%), m.p. 216–218°, $[\alpha]_D^{20}$ +100° (*c* 0.49, chloroform); ν_{max}^{KBr} 3275 (NH), 1665 (allyl), 1645 (Amide I), and 1540 cm^{-1} (Amide II); t.l.c. (14:1 chloroform–ethanol) R_F 0.35.

Anal. Calc. for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69; O, 29.52. Found: C, 60.07; H, 6.73; N, 3.81; O, 29.61.

Methyl 2-acetamido-3-O-allyl-6-O-benzoyl-2-deoxy-4-O-methyl-α-D-glucopyranoside (**5**). — A solution of **4** (120 mg) in dry tetrahydrofuran (6 ml) was mixed with iodomethane (3 ml) and silver oxide (200 mg), and the mixture was boiled for 2 h under reflux. After a further addition of silver oxide (100 mg), the mixture was boiled for 2 h under reflux, and then stirred for 6 h at 22°. The mixture was filtered, the residue was washed with warm chloroform, and the combined filtrate and washings were evaporated. The residue was chromatographed on a column of silica gel with 24:1 dichloromethane–ethanol, to give a product that crystallized from ether–pentane as prisms (82 mg, 66%), m.p. 189–192°, $[\alpha]_D^{20}$ +95° (*c* 0.76, chloroform); ν_{max}^{KBr} 3290 (NH), 1670 (allyl), 1645 (Amide I), 1555 (Amide II), 1490, and 1455 cm^{-1} (Ar); t.l.c. (24:1 chloroform–ethanol) R_F 0.44.

Anal. Calc. for $C_{20}H_{27}NO_7$: C, 61.06; H, 6.92; N, 3.56; O, 28.47. Found: C, 60.98; H, 6.96; N, 3.52; O, 28.29.

Methyl 2-acetamido-3-O-allyl-2-deoxy-4-O-methyl-α-D-glucopyranoside (**6**). — A solution of **5** (70 mg) in dry methanol (10 ml) was treated with 0.1M methanolic sodium methoxide (0.2 ml) for 20 h at 4°, and then diluted with methanol (5 ml), and de-ionized with Rexyn 300 (H^+, OH^-) ion-exchange resin (3 ml, Fisher Scientific Co., Fair Lawn, N.J. 07410). The solution was evaporated, and the residue was chromatographed on a column of silica gel with 14:1 chloroform–ethanol to give 46 mg (89%) of material that crystallized from ethanol–ether as clusters of needles, m.p. 198–199°, $[\alpha]_D^{20}$ +25° (*c* 0.16, chloroform); ν_{max}^{KBr} 3290 (NH), 1650 (Amide I), 1640 (allyl), and 1555 cm^{-1} (Amide II); t.l.c. (9:1 chloroform–ethanol) R_F 0.27.

Anal. Calc. for $C_{13}H_{23}NO_6$: C, 53.97; H, 8.01; N, 4.84. Found: C, 53.63; H, 8.08; N, 4.56.

Methyl 2-acetamido-2-deoxy-4-O-methyl-α-D-glucopyranoside (**7**). — A solution

of **6** (20 mg) in 90% ethanol (4 ml) was treated with tris(triphenylphosphine)rhodium chloride (5 mg, Ventron, Danvers, MA 01923) and 1,4-diazabicyclo[2.2.2]octane (1.6 mg). The mixture was boiled for 3 h under reflux. The resulting solution was cooled and evaporated, and the residue was dispersed in water and extracted with ether. The extract was dried (magnesium sulfate) and evaporated. A solution of the residue in methanol (6 ml) was treated with cation-exchange resin (H^+, 100–200 mesh; 2 ml) for 12 h at 37°. The suspension was filtered and the filtrate evaporated. The residue was chromatographed on a column of silica gel with 4:1 chloroform–ethanol to give a material (14 mg, 81%) that crystallized from ethanol–ether as needles, m.p. 228–229°, mixed m.p. 225–226° (lit.[8] m.p. 232–233°).

*Methyl 2-acetamido-3,4-di-*O*-allyl-6-*O*-trityl-α-*D*-glucopyranoside* (**11**). — A solution of methyl 2-acetamido-6-*O*-trityl-α-D-glucopyranoside[10] (**10**,145 mg) in dry tetrahydrofuran (4 ml) and benzene (10 ml) was treated with powdered sodium hydroxide (1 g) and allyl bromide (54 μl), and the mixture was boiled for 4 h under reflux. To the cooled mixture, sodium hydroxide (0.40 g) was added. The mixture was vigorously stirred for 12 h at room temperature, and then diluted with benzene (10 ml). The solid was filtered off and washed with warm 9:1 chloroform–ethanol (10 ml). The filtrate and washings were combined and evaporated. The residue was dissolved in chloroform, and the solution was washed with water (3 × 6 ml), dried (sodium sulfate), and evaporated. The syrupy residue was chromatographed on a column of silica gel, and elution with 24:1 dichloromethane–ethanol gave a material that crystallized from 2-isopropoxypropane–hexane to give microcrystals (130 mg, 74%), m.p. 167–169°, $[\alpha]_D^{20}$ +77° (*c* 0.39, chloroform); ν_{max}^{KBr} 3290 (NH), 1670–1635 (allyl, Amide I), 1580 (Ar), 1540 (Amide II), and 1455 cm^{-1} (Ar); t.l.c. (19:1 chloroform–ethanol) R_F 0.75.

Anal. Calc. for $C_{34}H_{39}NO_6$: C, 73.23; H, 7.05; N, 2.51; O, 17.21. Found: C, 73.26; H, 6.97; N, 2.40; O, 17.23.

*Methyl 2-acetamido-3,4-di-*O*-allyl-2-deoxy-α-*D*-glucopyranoside* (**8**). — A solution of **11** (110 mg) in acetic acid (4 ml) was heated on a water bath at 80°. The hot solution was diluted with water (2 ml), and heating was continued for 1 h. The solution was diluted with cold water (200 ml) and lyophilized. The residue was chromatographed on a column of silica gel in 14:1 dichloromethane–ethanol to give a syrup that crystallized from 2-isopropoxypropane, affording **8** (58 mg, 80%) as needles, m.p. 186–187°, $[\alpha]_D^{20}$ +105° (*c* 0.98, methanol); ν_{max}^{KBr} 3290 (NH), 1665–1630 (allyl, Amide I), and 1555 cm^{-1} (Amide II); t.l.c. (9:1 chloroform–ethanol) R_F 0.28.

Anal. Calc. for $C_{15}H_{25}NO_6$: C, 57.13; H, 7.99; N, 4.44; O, 30.44. Found: C, 57.06; H, 7.90; N, 4.35; O, 30.55.

*2,3,4,6-Tetra-*O*-acetyl-α-*D*-glucopyranosyl phosphate* (**13**). — (*a*) *From 1,2,3,-4,6-penta-*O*-acetyl-β-*D*-glucopyranose* (**12**). To crystalline phosphoric acid (1.2 g, Tridom Chemical Inc., Hauppauge, N.Y. 11787), dried *in vacuo* over magnesium perchlorate for 48 h at room temperature and then fused by heating at 65° (oil bath), was added **12** (ref. 13) (1.0 g). The mixture was stirred *in vacuo* (oil pump equipped with a carbon dioxide–acetone trap) for 2 h at 65°. A solution of the product in dry

tetrahydrofuran was cooled to −10°, and treated with 15M ammonium hydroxide (~2 ml) to bring the pH to ~6. The precipitated salts were filtered off (Celite). Evaporation of the filtrate gave crude **13** (1.0 g, ammonium$^+$), which was purified by p.l.c. The product, in methanol (2 ml), was applied to ten 20 × 20-cm plates (2 mm thick) which, after drying, were eluted twice with solvent *B*. After detection of **13** with the phosphate-specific spray reagent (it was the major phosphate-containing band), the appropriate zones of silica gel were removed from each plate, combined and ground to a powder, and then stirred overnight with solvent *C*. Filtration (Celite) and evaporation gave a residue that was extracted with 2:1 chloroform–methanol. The resulting solution was filtered and evaporated to give **13** (0.37 g, 27%, ammonium$^+$) showing a single spot in t.l.c. (solvent *B*).

For synthetic purposes, **13** (0.37 g) was converted into the pyridinium$^+$ salt by dissolution in water and slow passage through a column of cation-exchange resin (pyridinium$^+$). The column was washed with 3 vol. of water and the combined eluates were evaporated to dryness. Portions of toluene (2 ml) were added and evaporated off (three times), and then the amorphous **13** (0.37 g, pyridinium$^+$) was dissolved in dry dichloromethane (3.7 ml). Aliquots of this solution were employed for synthesis of phosphoric diesters.

For characterization, **13** (0.37 g, ammonium$^+$) was dissolved in water (5 ml) and converted into the potassium form by stirring with a large excess of cation-exchange resin (K^+) for 24 h at room temperature. The resin was filtered off and washed with water (10 ml), and the combined filtrates were evaporated to a solid. Crystallization from ether–methanol gave the dipotassium salt of **13** (0.21 g, 50%), m.p. 136–137°, $[\alpha]_D^{20}$ +110° (*c* 0.70, 1:1 methanol–water).

Anal. Calc. for $C_{14}H_{19}K_2O_{13}P$: C, 33.33; H, 3.80. Found: C, 33.34; H, 4.09.

(*b*) *From α-D-glucopyranosyl dipotassium phosphate.* Compound **14** (1.0 g, Sigma Chemical Company, St. Louis, MO 63118) was converted into the pyridinium$^+$ form by dissolution in water (10 ml) and passage through a column of cation-exchange resin (pyridinium$^+$), as just described for **13**. After washing of the column, evaporation of the combined eluates, and repeated addition and evaporation of toluene, α-D-glucopyranosyl pyridinium phosphate (**14**) was obtained as a syrup.

(*i*). Acetylation was performed by treatment with tetraethylammonium acetate tetrahydrate (10 g, Aldrich Chemical Co., Inc., Milwaukee, WI 53233). Water was removed by repeated additions and evaporations of pyridine (10 ml), and then of toluene (10 ml), and the resulting gum was treated with acetic anhydride (20 ml). The mixture was kept overnight at room temperature, treated with pyridine (10 ml) and water (10 ml), and kept for a further 2 h at room temperature. Evaporation, followed by two additions and evaporations of toluene (10 ml), gave a residue containing inorganic material. This was removed by dissolution of the residue in chloroform and treatment with ether to the point of turbidity. After a few min, the crystalline solid (inorganic) was filtered off, and evaporation of the filtrate gave **13** (0.6 g, 61%, pyridinium$^+$), showing one major spot in t.l.c. (solvent *B*), corresponding to the product obtained by method (*a*).

(*ii*). Alternatively, acetylation was performed by treatment with acetic anhydride (2.5 ml) and pyridine (5 ml). The mixture was kept for 3 h at room temperature, whereupon t.l.c. (solvent *B*) showed a major product (R_F 0.38) that corresponded to 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl phosphate (**13**) as prepared by method (*a*), plus several other compounds having higher R_F values (presumably including an acetylated, cyclic 1,2-phosphate). Water was added (until no more heat was evolved), and after evaporation, followed by two additions and evaporations of toluene (5 ml each), the product was purified by p.l.c. as described in (*a*) to give **13** (0.63 g, 64%, pyridinium$^+$), $[\alpha]_D^{20}$ +114° (*c* 2.2, dichloromethane).

Phosphoric diester synthesis. — A mixture of equal quantities (20–50 mg) of **13** (pyridinium$^+$) and the substituted derivative of methyl (or allyl) 2-acetamido-2-deoxy-α-D-glucopyranoside was kept *in vacuo* over phosphorus pentaoxide for 24–48 h at room temperature, and then treated with the appropriate volume of a solution of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS, Aldrich) in dry pyridine, with rigorous exclusion of moisture. The reaction tube was stoppered tightly, and the reactants were thoroughly mixed with a Vortex mixer to give a clear solution, which was kept for 48 h at room temperature. The mixture was treated with methanol (1 ml) and kept overnight at room temperature, and then the solvents were evaporated (nitrogen) and residual pyridine was removed by two additions and subsequent evaporations of toluene. The residue was dissolved in methanol and examined (*a*) by t.l.c. with solvent *A* (detection with the anisaldehyde, potassium permanganate, and phosphate-specific spray reagents), and (*b*) by withdrawing a very small portion and treating the sample with an excess of 3% sodium methoxide in dry methanol to effect *O*-deacetylation, followed by t.l.c. When these steps showed that a good yield of the required compound had been formed in the condensation, the compound was isolated by p.l.c. of the crude product, solvent *A* or *B* being used for elution of the plates, and the potassium permanganate and phosphate-specific spray reagents for detection (for compounds containing allyl groups); otherwise, a 1-cm strip was cut from the plate and sprayed with the anisaldehyde reagent. The silica gel was removed from the plate, ground to a fine powder, and stirred overnight at room temperature with solvent *C*. After filtration (Celite), the resulting solution was evaporated to dryness, and the residue triturated with 2:1 chloroform–methanol to give a suspension that was filtered through sintered glass and evaporated (nitrogen) to yield the required phosphoric diester as an amorphous solid. For compounds **24–26**, methanol was employed for the second extraction, instead of 2:1 chloroform–methanol. As the amorphous compounds **24–26** were rather unstable, it was generally not possible to remove the solvents completely and obtain acceptable elementary analyses, and homogeneity was demonstrated only by t.l.c.

Methyl 2-acetamido-3-O-allyl-2-deoxy-4-O-methyl-α-D-glucopyranoside 6-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl phosphate) (**16**). — Compounds **13** (30 mg) and **6** (30 mg) were mixed and treated with TPS (36 mg) in pyridine (0.7 ml) by the general method just described. P.l.c. was performed as described for the preparations of **17** and **21** (see later paragraph), but t.l.c. of the product showed that it contained

a contaminant (not a carbohydrate) migrating just ahead of **16** (R_F 0.65, solvent *A*). Therefore, the chromatography was repeated on 2 thin-layer plates (0.25 mm thick), after which, processing, as described in the general methods, gave pure **16** (30 mg, 41% based on **6**), m.p. 141–144°, $[\alpha]_D^{20}$ +70° (*c* 1.27, 1:1 chloroform–methanol); ν_{max}^{KBr} 3400 (OH, v. broad), 2970, 1750, 1665 (Amide I), 1560 (Amide II), 1375, 1230 (broad), 1095, 1050, 950, 875, and 675 cm^{-1}.

Anal. Calc. for $C_{27}H_{42}NO_{18}P$: C, 46.34; H, 6.05; N, 2.00. Found: C, 46.43; H, 6.14; N, 1.89.

Methyl 2-acetamido-2-deoxy-4-O-methyl-α-D-glucopyranoside 6-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl phosphate) (**23**). — (*a*) *From* **16**. A solution of **16** (25 mg) and 1,4-diazabicyclo[2.2.2]octane (5 mg) in 9:1 ethanol–water (2 ml) was stirred at 77° and treated with tris(triphenylphosphine)rhodium chloride (10 mg). The mixture was stirred for 2 h at 77°, at which time t.l.c. (solvent *A*) showed nearly complete conversion of **16** into the 1-propenyl derivative **20**. After the addition of more rhodium derivative (10 mg), the mixture was stirred a further 1 h at 77°, when t.l.c. showed no residual allyl group (anisaldehyde). The solvents were evaporated off, and the residue, consisting of unpurified **20**, was dissolved in 5:1 acetone–water (2 ml), and the resulting solution treated with mercuric chloride (25 mg). After 15 min at room temperature, t.l.c. (solvent *A*) showed the formation of **23** (R_F 0.40), and of a by-product (presumably a propyl derivative) that resisted hydrolysis, had the same R_F value as **16** and **20**, but gave a negative test for unsaturation (potassium permanganate reagent). After evaporation of the solvents, the residue was dissolved in 2:1 chloroform–methanol and **23** was purified by p.l.c. on one plate (0.5 mm thick, 20 × 20 cm) with solvent *B* and was detected with the anisaldehyde reagent. Processing by the general method gave **23** as the mercuric salt (11 mg, 40.5%), m.p. 151–155°, $[\alpha]_D^{20}$ +97° (*c* 1.1, 1:1 chloroform–methanol); ν_{max}^{KBr} 3380 (OH,NH), 2955, 1750, 1655 (Amide I), 1545 (Amide II), 1375, 1230 (broad), 1125, 1040, and 960 cm^{-1}; t.l.c. (solvent *B*) R_F 0.50.

Anal. Calc. for $C_{24}H_{38}NO_{18}P \cdot 5Hg^{2+}$: C, 37.95; H, 5.04; N, 1.84. Found: C, 38.07; H, 4.96; N, 2.20.

(*b*) *From* **13** *and* **7**. Compound **13** (25 mg) and **7** (25 mg) were mixed and treated with TPS (30 mg) in dry pyridine (0.62 ml) by the general method. P.l.c. on one plate (2 mm thick, 20 × 20 cm), with solvent *B* for elution and the anisaldehyde reagent for detection, gave **23** (27 mg, 41% based on **7**), pure according to t.l.c. (solvents *A* and *B*) and cochromatographing with the product obtained by method *a*.

Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 6-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl phosphate) (**19**). — A solution of **17** (50 mg) and 1,4-diazabicyclo-[2.2.2]octane (10 mg) in 9:1 ethanol–water (2 ml) was stirred at 78° and treated with tris(triphenylphosphine)rhodium chloride (10 mg). The mixture was stirred for 2 h at 78°, whereupon t.l.c. (solvent *A*) showed partial conversion of **17** into the 1-propenyl derivative **18**, having the same R_F value as **17** but lacking the color reaction with the anisaldehyde reagent that is characteristic of an allyl group[18]. In order to complete the conversion, it was necessary to perform four additional treatments with

tris(triphenylphosphine)rhodium chloride (10 mg), at 2-h intervals, while stirring at 78°. After evaporation of solvents, partial purification of **18** was achieved by p.l.c. on two plates, (0.5 mm thick, 20 × 20 cm), with solvent *B* for elution and the potassium permanganate spray for detection. Processing as described under "phosphoric diester synthesis" gave **18** (35 mg, 70%), t.l.c. (solvent *A*) R_F 0.62, showing the presence of some triphenylphosphine oxide as a pale-blue spot having a lower R_F value.

The 1-propenyl groups of **18** (35 mg) were hydrolyzed by dissolution in 5:1 acetone–water (3 ml), and treatment with mercuric chloride (50 mg). After 15 min at room temperature, t.l.c. (solvent *A*) showed formation of **19** (R_F 0.25) and of two by-products (R_F 0.62 and 0.50) that gave a negative test for unsaturation (potassium permanganate), and which presumably contained one or two propyl ether groups. Compound **19** was purified by p.l.c. on one plate (0.5 mm thick, 20 × 20 cm) with solvent *B* for elution and the anisaldehyde spray for detection. Processing, as described under "phosphoric diester synthesis", gave **19** as the mercuric salt (7 mg, 14%), m.p. 165–167°, $[\alpha]_D^{20}$ +69° (*c* 0.7, 1:1 chloroform–methanol); ν_{max}^{KBr} 3390 (OH, NH), 2950, 1750, 1660 (Amide I), 1545 (Amide II), 1375, 1230, 1145, 1110, 1045, and 955 cm^{-1}.

Anal. Calc. for $C_{23}H_{36}NO_{18}P \cdot 0.5Hg^{2+}$: C, 37.04; H, 4.86; N, 1.88. Found: C, 37.48; H, 5.15; N, 2.26.

Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 6-(α-D-glucopyranosyl phosphate) (**24**). — (*a*) *From* **19**. Compound **19** (1 mg) was treated with 1.5% sodium methoxide in dry methanol, and the mixture kept for 30 min at room temperature, after which time t.l.c. showed disappearance of **19** and formation of **24** (R_F 0.35, solvent *C*). The excess of base was removed by addition of cation-exchange resin (pyridinium$^+$), and the resin was removed by filtration and washed with 1:1 methanol–water. Evaporation gave amorphous **24**, which migrated more slowly in t.l.c. than the corresponding propyl glycoside **25** (R_F 0.52, solvent *C*). The sample of **24** prepared by this route was employed for chromatographic purposes only (see *b*).

(*b*) *From* **13** *and* **9**. A mixture of **13** (50 mg) and **9** (ref. 9) (50 mg) was treated with TPS (60 mg) as described in the general method for phosphoric diester synthesis. The product was isolated by p.l.c. on two plates, (2 mm thick, 20 × 20 cm) with solvent *B* and detection with the anisaldehyde reagent, to give an amorphous solid (35 mg), but t.l.c. (solvent *B*) showed that this was not homogenous. Therefore, *O*-deacetylation was performed (as described in *a*), after which, t.l.c. (solvent *C*) showed the formation of **24** (R_F 0.35, identified by comparison with the product from *a*) plus two other compounds, having a higher and a lower R_F value, respectively (presumably phosphoric diesters linked to O-3 and O-4 of the 2-acetamido-2-deoxy-D-glucose residue). Purification of **24** was achieved by p.l.c. on two plates (0.25 mm thick, 20 × 20 cm), with solvent *C* (two elutions) and detection with the anisaldehyde reagent. Processing by the general method previously described gave **24** (16 mg, 12% based on **9**), as an amorphous solid having no definite m.p. and containing residual solvent, $[\alpha]_D^{20}$ +87° (*c* 0.95, 1:1 methanol–water), $[\alpha]_D^{20}$ +121° (after correction

for solvent content); ν_{max}^{KBr} 3380 (OH, NH), 2940, 1650 (Amide I), 1550 (Amide II), 1375, 1230, 1145, 1090, 1035, 940, and 875 cm^{-1}.

Anal. Calc. for $C_{15}H_{28}NO_{14}P \cdot CHCl_3 \cdot 4H_2O$: C, 30.53; H, 5.57; N, 2.09. Found: C, 30.32; H, 5.45; N, 2.24.

*Allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside 6-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl phosphate)* (**21**). — Compound **13** (50 mg) and **15** (ref. 18, 50 mg) were treated with TPS (60 mg) in pyridine (1.25 ml), and **21** was isolated as described in the general method for phosphoric diester synthesis, by p.l.c. on two plates (0.5-mm thick, 20 × 20 cm); yield 57 mg (55% based on **15**), m.p. 163–166°, $[\alpha]_D^{20}$ +88° (*c* 2.0, 2:1 chloroform–methanol), $[\alpha]_D^{20}$ +94° (after correction for residual solvent); ν_{max}^{KBr} 3400 (NH), 2950, (broad) 1750, 1650 (Amide I), 1550 (Amide II), 1500 (Ar), 1455, 1375, 1230 (broad), 1125, 1075, 1045, 950, 740, and 685 cm^{-1}; t.l.c. (solvent *A*) R_F 0.71.

Anal. Calc. for $C_{39}H_{50}NO_{18}P \cdot 0.5CHCl_3$: C, 52.06; H, 5.58; N, 1.54. Found: C, 51.98; H, 5.72; N, 1.71.

*Propyl 2-acetamido-2-deoxy-α-*D*-glucopyranoside 6-(α-*D*-glucopyranosyl phosphate)* (**25**). — Compound **21** (51 mg) was dissolved in methanol (4 ml) and hydrogenated at 1.5 atm over 10% palladium-on-charcoal (25 mg, Tridom). After 3 h, t.l.c. showed formation of a major product **22** (R_F 0.48, solvent *B*) together with a minor product having a slightly lower R_F value. The catalyst was filtered off and washed with methanol, and a small portion of the filtrate was treated with fresh catalyst and hydrogenated again for 3 h. As t.l.c. showed no change, a small portion of the original filtrate was *O*-deacetylated by treatment with an excess of sodium methoxide for 30 min at room temperature. T.l.c. now showed formation of pure **25** (R_F 0.52, solvent *C*), suggesting that the contaminant in **22** had resulted from partial *O*-deacetylation during hydrogenation. Therefore, the methanolic solution containing **22** was evaporated, and **22**, without purification, was *O*-deacetylated by treatment with 1.5% sodium methoxide in methanol for 30 min at room temperature. The mixture was treated with cation-exchange resin (pyridinium$^+$), the resin was filtered off (sintered glass) and washed with methanol, and the combined filtrates were evaporated to give **25** (27 mg, 95.5%) as an amorphous solid having no definite m.p., $[\alpha]_D^{20}$ +108° (*c* 1.2, 1:1 water–methanol); ν_{max}^{KBr} 3365 (OH, NH), 2940, 1650 (Amide I), 1550 (Amide II), 1490, 1375, 1225, 1090, 1030, 935, 875, 745, and 670 cm^{-1}; t.l.c. (solvent *C*) R_F 0.52.

Anal. Calc. for $C_{17}H_{32}NO_{14}P$: C, 40.40; H, 6.38; N, 2.77. Found: C, 40.42; H, 6.40; N, 2.71.

*Methyl 2-acetamido-3,4-di-*O*-allyl-2-deoxy-α-*D*-glucopyranoside 6-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl phosphate)* (**17**). — A mixture of **13** (50 mg) and **8** (50 mg) was treated with TPS (60 mg) in pyridine (1.25 ml), and the product **17** (58 mg, 50% based on **8**), isolated as described for **21**, had m.p. 117–120°, $[\alpha]_D^{20}$ +74° (*c* 1.5, 2:1 chloroform–methanol); ν_{max}^{KBr} 3425 (NH), 2960, 2875, 1750, 1650 (Amide I), 1550 (broad, Amide II), 1375, 1230 (broad), 1085, 1050, 1020, 950, 875, and 675 cm^{-1}; t.l.c. (solvent *A*) R_F 0.62.

Anal. Calc. for $C_{29}H_{44}NO_{18}P$: C, 47.96; H, 6.11; N, 1.93. Found: C, 48.06; H, 6.11; N, 1.74.

Methyl 2-acetamido-4-O-methyl-2-deoxy-α-D-glucopyranoside 6-(α-D-glucopyranosyl phosphate) (**26**). — Compound **23** (20 mg, obtained by method *b*) was *O*-deacetylated by treatment with 1.5% sodium methoxide in methanol, as described for the preparation (*a*) of **24**. Compound **26** (pyridinium$^+$ form) was obtained pure according to t.l.c. (solvent *C*), as an amorphous solid (17 mg, 77%) having no definite melting point and containing residual solvent; $[\alpha]_D^{20}$ +80° (*c* 1.7, 1:1 methanol–water), $[\alpha]_D^{20}$ +102° (after correction for residual solvent); ν_{max}^{KBr} 3350 (OH, NH), 2940, 1650 (Amide I), 1550 (Amide II), 1495, 1380, 1230, 1120, 1080, 1040, 945, 875, 750, and 675 cm^{-1}.

Anal. Calc. for $C_{16}H_{30}NO_{14}P \cdot C_5H_5N \cdot CHCl_3 \cdot 2H_2O$: C, 36.40; H, 5.55; N, 3.86. Found: C, 36.71; H, 6.32; N, 3.84.

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