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THE SYNTHESIS OF P^1-[2-ACETAMIDO-2-DEOXY-3-*O*-(β-D-GLUCOPYRANOSYLURONIC ACID)-α-D-GLUCOPYRANOSYL] P^2-DOLICHYL DIPHOSPHATE (*N*-ACETYLHYALOBIOSYLURONIC DOLICHYL DIPHOSPHATE)*†

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ABSTRACT

Starting from 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranose (**20**), a crystalline intermediate prepared by a conventional sequence of reactions, the total synthesis of *N*-acetylhyalobiosyluronic dolichyl diphosphate was achieved. One of the key steps involved the transformation of the disaccharide **20** into the methyloxazoline **26**, which was then converted into the stable, crystalline disaccharide phosphate derivative in ~30% yield. The methyloxazoline **26** was directly prepared from the corresponding methyl α-glycoside by acetolysis. Similarly, the allyl α-glycoside was transformed into **26**.

INTRODUCTION

The mode of biosynthesis of hyaluronic acid, which is composed of repeating units of (1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl (*N*-acetylhyalobiouronic acid) residues, is still unclear[2], although recent findings[3,4] suggest the possible involvement of a lipid-linked disaccharide intermediate in the formation of the polysaccharide chains. In order to establish whether the "lipid intermediate" found in mammalian systems[3,4] is *N*-acetylhyalobiosyluronic dolichyl diphosphate, the total chemical synthesis of this compound was performed.

*Dedicated to Professor K. Heyns on the occasion of his 70th birthday.
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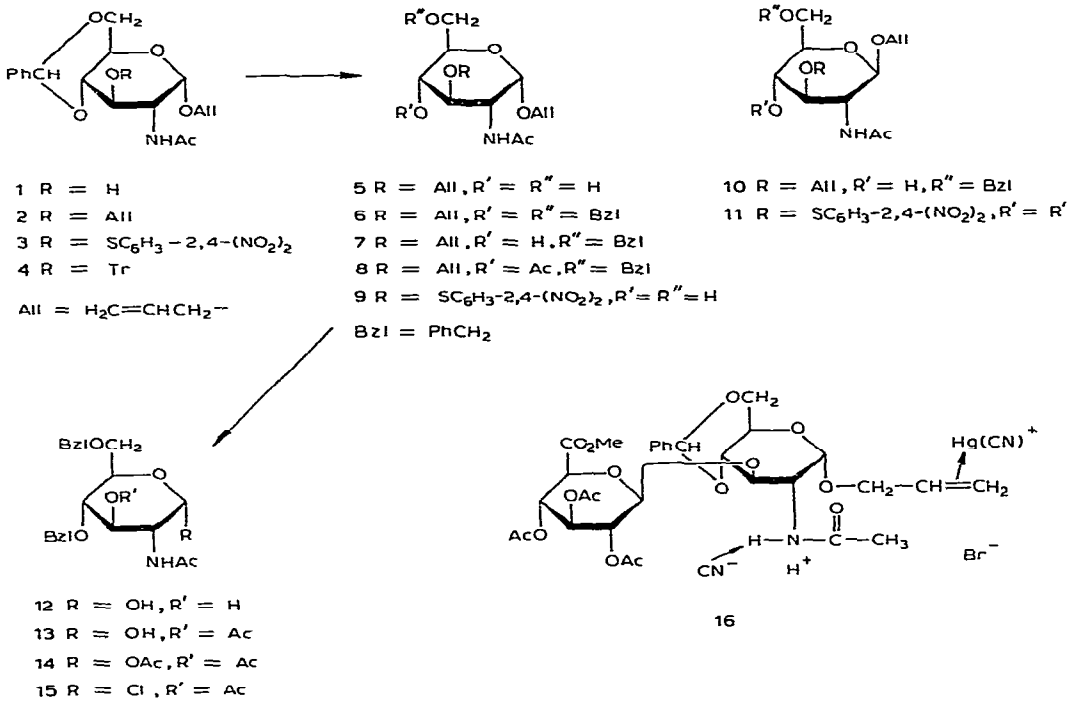
RESULTS AND DISCUSSION

Two routes were contemplated, both starting from allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[5] (**1**). In the first, the synthesis of a suitably protected 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate derivative that could be coupled with a protected D-glucosyluronic acid residue was attempted. As a protecting group for the 3-hydroxyl group that could be sufficiently stable to the conditions of phosphorylation and be removable in neutral medium (to avoid any effect on the phosphate group), the allyl group was investigated. Compound **1** was



converted into the 3-*O*-allyl derivative **2** by treatment of a solution in *N*,*N*-dimethylformamide with allyl bromide in the presence of silver oxide, a method that avoided the formation of unwanted side-products, mainly the *N*-allyl derivative, and gave better yields than the conventional procedures that use either sodium hydride[6], or sodium hydroxide[7]. Allyl 2-acetamido-3-*O*-allyl-2-deoxy-α-D-glucopyranoside (**5**), obtained by mild hydrolysis of **2** with acid, was benzylated to give the 4,6-di-*O*-benzyl derivative **6** and, in a small proportion, a compound that was separated by column chromatography and that had the elementary analysis and the i.r. spectrum expected for a mono-*O*-benzyl derivative. The latter compound did not give a trityl ether after prolonged treatment with chlorotriphenylmethane in anhydrous pyridine, but a trityl ether was formed (t.l.c.) after hydrogenation of the mono-*O*-benzyl derivative (or the

acetylated mono-*O*-benzyl derivative), indicating that the second component formed was, indeed, the 6-*O*-benzyl derivative **7**, which, by acetylation, gave **8**. An additional compound was isolated in small proportion from the mother liquors of **7**. On the basis of its specific rotation, it was assigned structure **10**, the β anomer having possibly been formed by interconversion[8] of the allyl α-glycoside under the benzylation conditions.

Removal of the allyl ether and glycoside groups from **6** by treatment with chlorotris(triphenylphosphine)rhodium[9], followed by mercuric chloride[10], gave the reducing 4,6-di-*O*-benzyl derivative **12**, which crystallized as the α anomer. Brief treatment of **12** with acetyl chloride gave the reducing 3-*O*-acetyl derivative **13** as the crystalline α anomer. Acetylation of **12** with acetic anhydride and pyridine gave the 1,3-diacetate **14**. The 3-*O*-acetyl α-chloride **15**, obtained in pure form by preparative t.l.c. (as an unstable syrup), was formed by prolonged treatment of **12** with acetyl chloride. Chloride **15** was immediately converted into the methyloxazoline **16** by chloride ion catalysis with tetraethylammonium chloride and sodium hydrogencarbonate in acetonitrile by the procedure of Lemieux and Driguez[11]. Under these conditions, **16** was obtained as a mixture with **13**. In view of the low yields achieved, and the difficulty encountered in the purification of both **15** and **16**, this approach was not pursued further.

Protection of OH-3 of **1** with the 2,4-dinitrophenylsulfenyl group was next examined, as successful removal of this group under very mild conditions had been reported for *O*-isopropylidene derivatives of hexoses[12]; however, it had not been used for hexosamine derivatives. Compound **1** was treated with a slight excess of 2,4-dinitrobenzenesulfenyl chloride, and **3** was obtained in crystalline form after column chromatography on Florisil (as the acidity of silica gel might have caused the partial hydrolysis of the acid-labile 2,4-dinitrophenylsulfenyl group). For the removal of the 4,6-*O*-benzylidene group of **3** in the presence of the 2,4-dinitrophenylsulfenyl group, which is also labile in 50% acetic acid, various conditions were tried: treatment of **3** dissolved in an inert solvent with a trace of *p*-toluenesulfonic acid, or with a weakly acidic ion-exchange resin, and short-time treatment with concentrated formic acid. The last method gave the expected product **9**, but it was necessary to purify the reaction mixture on a Florisil column in order to remove (*a*) a trace of unreacted **3**, (*b*) some **1**, and (*c*) a small amount of a product moving in t.l.c. just behind **9**, to which was assigned structure **11** for the same reasons described for **10**. Compound **9** was obtained in pure, crystalline form, but was not used further, because of the formation of the side-products just described and the lengthy purification steps, and because satisfactory elementary analyses for nitrogen and sulfur could not be obtained.

In the second route investigated, the first step was the synthesis of the disaccharide moiety, followed by phosphorylation. A β-interglycosidic linkage is obtained selectively either by a Koenigs–Knorr condensation when a participating group, such as an *O*-acetyl group[13], is present at O-2, or by a Bredereck condensation between a trityl ether and a glycosyl bromide derivative[14]. However, such a reaction

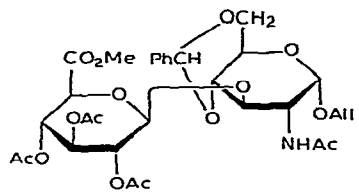
has been described only with 6-trityl ethers[14,15], as the introduction of a trityl group on a secondary hydroxyl group is often difficult and can usually be achieved only under extreme conditions[16]. As a 3-trityl ether of a hexosamine had previously been obtained in our laboratory[17], **4** was synthesized, and condensation thereof with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[18] was attempted under the conditions described by Bredereck *et al.*[14]. Two new products were formed (t.l.c.) which were isolated by column chromatography and which had the characteristics (t.l.c., i.r., elementary analysis) of disaccharide derivatives, but their structures differed from the ones expected, and they were, therefore, not further investigated.

Compound **1** was coupled with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[18] under the conditions of a Koenigs–Knorr reaction described by Flowers and Jeanloz[13]. The disaccharide derivative obtained after column chromatography of the mixture, and crystallization of the residue, was a complex in which the mercury salt was strongly bound to the sugar moiety. Similar observations have been reported for compounds possessing an allylic, or a closely related, group that had been treated with mercuric salts[10]. Structure **16** was tentatively assigned to this derivative on the basis of its elementary analysis, i.r. spectrum, and chemical behavior, and by analogy with the structure recently proposed by Nashed *et al.*[19] for an intermediate formed during the conversion of a 1-propenyl β-glycoside into a methyloxazoline by a mercuric ion-catalyzed cyclization. The mechanism involved is probably similar to that reported earlier[20], but the intermediates in those reactions had not been isolated. This hypothesis is supported by the observation that a disaccharide component, possibly an oxazoline derivative, was found in small proportion together with **17** and was eluted from the silica gel column after this compound.

Removal of the 4,6-*O*-benzylidene group from **17** gave crystalline **18**, which was acetylated to give **19**. All of the derivatives of *N*-acetylhyalobiouronic acid showed a tendency to crystallize with water, which could only be removed under conditions that would also cause some decomposition. The peracetylated methyl ester **19** was obtained as the anhydrous derivative after two treatments by column chromatography.

Isomerization of the allyl to the 1-propenyl group of **19** was achieved with chlorotris(triphenylphosphine)rhodium[9] as described for **6**, and crystalline 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranose (**20**) was obtained by treatment with mercuric chloride and mercuric oxide. Compound **20** was treated with acetyl chloride, and the corresponding disaccharide glycosyl chloride (**21**) was converted into the methyloxazoline by chloride ion catalysis with tetraethylammonium chloride and sodium hydrogencarbonate[11]. A one-step procedure[21], which yields the methyloxazoline by direct treatment of the methyl α-D-glycoside with the acetolysis mixture of Sorkin and Reichstein[22], was applied* to the conversion of methyl 2-acetamido-4,6-di-*O*-acetyl-

*While this work was in progress, Matta and Barlow[24] reported the preparation of two disaccharide oxazolines by treatment of the corresponding methyl α-D-glycosides by the acetolysis procedure[21].



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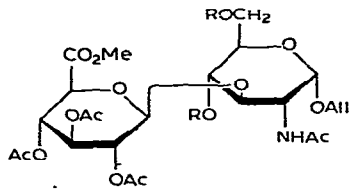
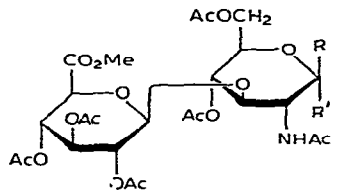
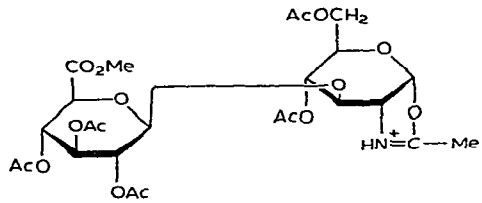
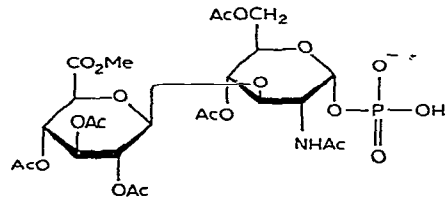


18 R = H
19 R = Ac



20 R = H, R' = OH
21 R = H, R' = Cl
22 R = H, R' = OPO_3H_2
23 R = H, R' = OPO(OPh)$_2$
24 R = OH, R' = H





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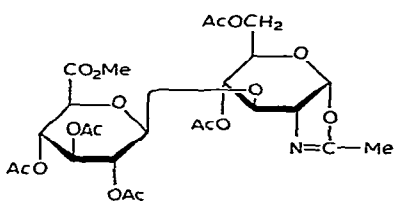
2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranoside[13,23] into the oxazoline **26**. The β-D-(1→3)-interglycosidic linkage is stable to acetolysis[13], and it had been shown[21] that neither methyl nor acetic ester groups were hydrolyzed under the conditions used. The pure oxazoline **26** was obtained in 73% yield, and crystallized directly from the syrupy residue. This procedure eliminated two synthetic steps entailing lengthy purifications, as well as the preparative t.l.c. of **26**. The same acetolysis procedure was used successfully for the direct conversion of the α-allyl glycoside **19** into the oxazoline **26**. In this case, the oxazolinium acetate **27** crystallized from the syrupy residue; the syrup was also found suitable for use in the subsequent reaction without purification.



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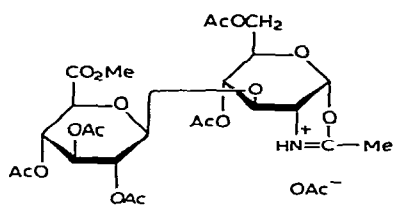

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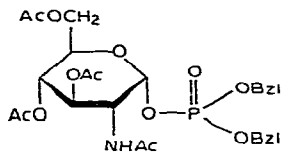
The stable, protected disaccharide phosphate **23** was prepared as the pure α-D anomer by the method of Khorlin *et al.*[25]. It crystallized directly from the reaction mixture, and was obtained in ~30% yield. When dibenzyl, instead of diphenyl, hydrogenphosphate, was condensed with the oxazoline **26** under the conditions recently described by Warren *et al.*[26], no disaccharide dibenzyl phosphate derivative could be obtained. Instead, two products were isolated from the complex reaction-mixture by preparative t.l.c.; one, in 6% yield, was 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranosyl dihydrogenphosphate (**22**), the structure of which was assigned on the basis of elementary analysis, mobility in t.l.c., and i.r. spectrum; it was probably formed by scission of the labile benzyl groups. The second compound isolated in small proportion was the monosaccharide dibenzyl phosphate derivative **28** (t.l.c.).



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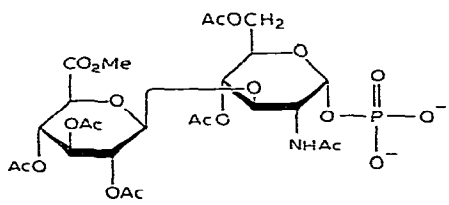

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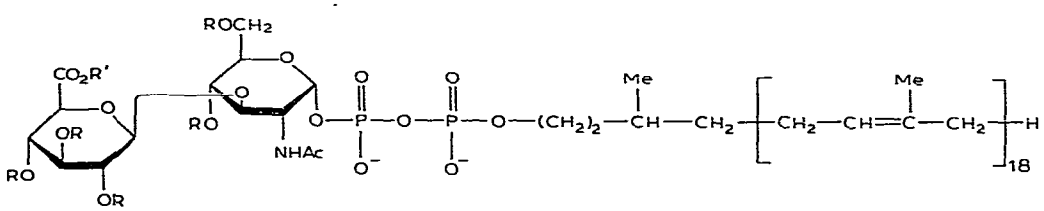

30 R = Ac, R' = Me
31 R = R' = H

The two phenyl groups were removed from pure, crystalline compound **23** by catalytic hydrogenolysis in absolute methanol in the presence of activated platinum oxide[27]. It was found necessary to reactivate the catalyst[28], as the reaction had a tendency to stop when ~50% of **23** had been converted into **29**. The reaction, however, did not proceed to completion, and some unreacted starting-material (<10%) was always present, as shown by t.l.c. Compound **29**, obtained as an amorphous material by lyophilization of its aqueous solution, was converted into the tris(tributylammonium) salt, and this was used immediately for the preparation of **30**.

When a less-pure preparation of **23** was used, *i.e.*, when a trace of oxazoline **26** (or **27**) was present, a new product was formed during the course of the hydro-

genolysis; its mobility in t.l.c. was much higher than that of **23** or **26** (or **27**), and it crystallized out of the reaction mixture during the hydrogenolysis. On the basis of its chemical behavior, i.r. spectrum, and elementary analysis, and of the observation that it was retained on a column of Amberlite IR-45 (acetate), structure **25** was tentatively assigned to it; its formation may be based on a mechanism analogous to that postulated by Maley *et al.*[29], although these authors had not isolated the intermediate, 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucosylammonium phosphate; the reaction between the positively charged ammonium group and the rather strongly acidic phosphate ion was, in the latter case, intramolecular.

The fully protected *N*-acetylhyalobiosyluronic dolichyl diphosphate derivative **30** was obtained by condensing compound **29** with P^1-dolichyl P^2-diphenyl diphosphate, tributylammonium salt[30], prepared from mammalian dolichol, a polyprenol containing a mixture of isoprenologs having 17 to 22 isoprene units, a saturated, α-isoprene unit, and *cis* double bonds. The reaction mixture contained four phosphate-positive components, two of which were carbohydrate derivatives; these reacted very poorly with the phosphate-specific spray[35], and they were localized on the t.l.c. plates by the combined use of the reagents for revealing unsaturation[6] and phosphoric esters[35]. The desired product **30** (tributylammonium salt), obtained as an amorphous, hygroscopic material, was pure according to t.l.c. in five solvent systems when detected with spray reagents specific for carbohydrate[33], unsaturated compounds[6], and phosphoric esters.[35] Proof of the structure of **30** was obtained from its physicochemical behavior and i.r. spectrum, by polarimetry, and by the determination of its phosphate and hexosamine contents after acid hydrolysis. The ratio of phosphate group to 2-acetamido-2-deoxy-D-glucose residue was found to be in agreement with the theoretical value.

In order to avoid scission of the alkali-labile β-D-(1→3)-linkage, as well as a base-catalyzed elimination-reaction in the D-glucopyranosyluronic acid residue[31], the removal of the methyl and acetic ester protecting groups of **30** was accomplished with triethylamine in the cold. Under these conditions, only minor degradation of the molecule was observed (t.l.c.), and *N*-acetylhyalobiosyluronic dolichyl diphosphate (**31**, triethylammonium salt) was obtained, after purification by preparative t.l.c., as a syrup. It was pure according to t.l.c. in five solvent systems when detected with the spray reagents described for **30**. Compound **31** was further characterized by i.r. spectrophotometry and g.l.c. analysis of the carbohydrate components after acid hydrolysis followed by methanolysis. The ratio of D-glucuronic acid to 2-acetamido-2-deoxy-D-glucose residues was found in agreement with structure **31**. For most solvent systems[1], the R_F values of the synthetic compound **31** showed a close resemblance to those reported for the disaccharide–lipid intermediate isolated from a mammalian system[3].

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 n.m.r. spectrum of **23** was recorded at 60 MHz with a Varian T-60 spectrometer for a solution in chloroform-*d* containing 1% of tetramethylsilane as the internal standard. Preparative t.l.c. was performed on precoated PLC plates (20 × 20 cm) of Silica Gel F 254 (Merck). All solvent proportions are v/v. Evaporations were conducted *in vacuo*, with the bath temperature below 30°. The microanalyses were made by Dr. W. Manser, Zurich, Switzerland. Column chromatography was performed on Silica Gel Davison (60–200 mesh, grade 950, Davison Chemical, Baltimore, MD 21226) or, in the case of acid-labile derivatives, on Florisil (activated magnesium silicate, 60–100 mesh, Fisher Scientific, Pittsburgh, PA 15219) without pretreatment. The ratio of diameter of the column to its length was 1:8 to 1:12. The volume of the fractions eluted was 2–3 ml per g of substance to be chromatographed.

Analytical procedures. — G.l.c. of the per(trimethylsilyl) ethers was performed with a Perkin–Elmer model 900 gas chromatograph equipped with a flame-ionization detector, in a column (305 × 0.3 cm) of Gas-Chrom Q (80–100 mesh) coated with 0.1% of OV-17 (Applied Science Laboratories, State College, PA) programmed for a rise of 8°/min from 120 to 300°, with nitrogen as the carrier gas; hexa-*O*-(trimethylsilyl)-*myo*-inositol was used as the internal standard. Per(trimethylsilyl)ation was performed as previously described[32]. T.l.c. was performed on precoated plates of Silica Gel G (layer thickness, 0.25 mm; Merck, Darmstadt, Germany) and Cellulose F (layer thickness, 0.10 mm; Merck). The solvent system used for t.l.c. and preparative t.l.c. (p.t.l.c.) was 19:1 chloroform–ethanol, unless stated otherwise. The zones were detected by spraying the chromatogram with (*A*) 1:1:18 anisaldehyde–conc. sulfuric acid–ethanol[33], (*B*) aniline hydrogenphthalate solution[34], followed by heating on a hot plate for a few min, (*C*) 1% aqueous potassium permanganate in 2% sodium carbonate[6], and (*D*) the reagent of Dittmer and Lester[35]. The content of 2-acetamido-2-deoxy-D-glucose in compound **30** (100 μg) was determined by hydrolysis with 4M hydrochloric acid (125 μl) for 4 h at 100°. After evaporation (nitrogen gas), removal of the residual HCl with water by re-evaporation gave a residue that was acetylated with 1:2 acetic anhydride–pyridine (100 μl) overnight at room temperature. The solvents were removed, and the residue was freed of reagents by three additions and evaporations of toluene. The residue, dissolved in methanol (100 μl), was *O*-deacetylated with triethylamine (100 μl) overnight at 4° and for 2 h at room temperature. Methanol (200 μl) was added, and the solvent was evaporated (nitrogen gas). Methanol was again added, and the solvent was evaporated (3 times). The residue was dried *in vacuo* over phosphorus pentaoxide, and then analyzed by the method of Reissig *et al.*[36]. The proportion of acid-labile phosphate was determined after digestion of the sample (10 μg) with conc. sulfuric acid by the method of Shin[37]. The proportions of glucuronic acid and of 2-acetamido-2-deoxyglucose in **31** (200 μg) were determined by hydrolysis with 0.5M hydrochloric acid, acetylation, methanolysis, acetylation, and *O*-deacetylation with methanolic hydrogen chloride, followed by g.l.c.

Allyl 2-acetamido-3-O-allyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**2**). — To a solution of allyl 4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[7] (**1**, 4.7 g) in dry *N*,*N*-dimethylformamide (75 ml) were added allyl bromide (5 ml) and freshly prepared silver oxide (10 g). The mixture was stirred for 1 h at room temperature and then under reflux for 2 h, when t.l.c. showed that ~75% of the starting material (R_F 0.3) had been converted into **2** (R_F 0.5). The mixture was cooled to room temperature, and allyl bromide (4 ml) and freshly prepared silver oxide (5 g) were added. After being stirred overnight at room temperature, the mixture was filtered through charcoal–Celite, and the filtrate evaporated. Several additions of toluene to and evaporations from the residue removed residual *N*,*N*-dimethylformamide, and **2** crystallized from chloroform–ether as needles (4.85 g), m.p. 230–232°, $[\alpha]_D^{20}$ +80° (*c* 0.704, chloroform); ν_{max}^{KBr} 3285, (NH), 1650–1640 (CH_2–CH=CH_2, Amide I), 1560 (Amide II), 1455 (Ar), 735, and 685 cm^{-1} (Ph).

Anal. Calc. for $C_{21}H_{26}NO_6$: C, 64.93; H, 6.75; N, 3.61; O, 24.71. Found: C, 65.05; H, 7.01; N, 3.59; O, 24.80.

Allyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(2,4-dinitrophenylsulfenyl)-α-D-glucopyranoside (**3**). — Compound **1** (150 mg) and 2,4-dinitrobenzenesulfenyl chloride (160 mg) were dissolved in warm, dry 1,2-dichloroethane (10 ml). Dry pyridine (0.1 ml) was added to the cooled solution, and the mixture was treated as previously described[12]. The crystalline residue (175 mg) was chromatographed on a column of Florisil (60–100 mesh, 8 g), which was eluted with 1:1 benzene–ethyl acetate. The fractions having R_F 0.4 were combined, the solvent was evaporated, and the crystalline residue (115 mg) was recrystallized from ethyl acetate–benzene, to give **3** as clusters of yellow needles (75 mg), dec. 234°, $[\alpha]_D^{20}$ −54° (*c* 0.100, chloroform); ν_{max}^{KBr} 3275 (NH), 1660–1650 (CH_2–CH=CH_2, Amide I), 1600, 1590 (Ar), 1525 (Amide II), 1520, 1350 (Ar NO_2), 1310 (SO), 1100 (Ar), 1060 (CS), 795, 730, and 685 cm^{-1} (Ph).

Anal. Calc. for $C_{24}H_{25}N_3O_{10}S$: C, 52.65; H, 4.60; N, 7.67; S, 5.86. Found: C, 52.73; H, 4.62; N, 7.22; S, 5.89.

Allyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-trityl-α-D-glucopyranoside (**4**). — A solution of **1** (500 mg) in dry pyridine (15 ml) was treated under exclusion of moisture with chlorotriphenylmethane (750 mg) for 3 days at room temperature, and then under reflux for 3 h, when t.l.c. showed that ~70% of the starting material had been converted into **4** and some slower-moving, decomposition products. The mixture was cooled to room temperature, re-treated with chlorotriphenylmethane (250 mg) under reflux for a further 2 h, cooled, and then poured onto crushed ice. The precipitate was dissolved in chloroform (25 ml), and the solution was washed with cold water (4 × 10 ml), dried (sodium sulfate), and evaporated; repeated additions and evaporations of toluene gave a residue that was dried *in vacuo* over Drierite. It was chromatographed on a column of Florisil (60–100 mesh; 65 g), which was first eluted with chloroform to remove triphenylmethanol; 19:1 chloroform–ethanol then eluted **4**, which crystallized from chloroform–ether–hexane as plates (53 mg) changing at 162–170° into needles, m.p. 180–190°, $[\alpha]_D^{20}$ +91° (*c* 0.456, chloroform); t.l.c. (*A*)

R_F 0.3; ν_{max}^{KBr} 3420–3300 (broad NH), 2940, 2860 (≡C), 1675 (CH_2–CH=CH_2), 1665 (Amide I), 1600 (Ar), 1520 (Amide II), 1490, 1450 (Ar), 1360 (≡C), 1130, 1090, 1050 (Ph), 750, 740, and 700–680 cm^{-1} (Ph).

Anal. Calc. for $C_{37}H_{37}NO_6$: C, 75.11; H, 6.30; N, 2.37. Found: C, 75.49; H, 6.17; N, 2.28.

Allyl 2-acetamido-3-O-allyl-2-deoxy-α-D-glucopyranoside (**5**). — A mixture of **2** (3 g) and 50% acetic acid (40 ml) was stirred on a boiling-water bath for 30 min, when t.l.c. (19:1 chloroform–ethanol) showed complete conversion of **1** into **3**. Evaporation, followed by several additions and evaporations of toluene, and then of methanol, afforded **5** as a crystalline solid that was recrystallized from methanol–acetone–ether to give needles (2.10 g), m.p. 150–151°, $[\alpha]_D^{20}$ +139° (*c* 0.135, methanol); t.l.c. (9:1 chloroform–ethanol) R_F 0.2; ν_{max}^{KBr} 3500–3400 (OH), 3310 (NH), 1650–1640 (CH_2–CH=CH_2, Amide I), and 1545 cm^{-1} (Amide II).

Anal. Calc. for $C_{14}H_{23}NO_6$: C, 55.80; H, 7.69; N, 4.65; O, 31.86. Found: C, 55.73; H, 7.61; N, 4.61; O, 31.90.

Allyl 2-acetamido-3-O-allyl-4,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**6**). — To a solution of **5** (2.3 g) in dry *N*,*N*-dimethylformamide (80 ml) were added barium oxide (5 g), barium hydroxide octahydrate (1.5 g), and α-bromotoluene (2 ml), and the mixture was stirred overnight at room temperature. Chloroform (150 ml) was added, and the mixture was stirred for a few min on a boiling-water bath, cooled to room temperature, filtered, and the filtrate washed with water (3 × 100 ml). The water washes were extracted with chloroform (3 × 50 ml), the chloroform solution and extracts were combined and evaporated, and the residual syrup was dried *in vacuo* over phosphorus pentaoxide. A solution of the residue (2.8 g) in chloroform was applied to a column of silica gel (110 g). Unreacted α-bromotoluene was eluted with chloroform, and **6** with 19:1 chloroform–ethanol. The latter was recrystallized twice from acetone–ether–pentane, to give needles (2.17 g), m.p. 126–127°, $[\alpha]_D^{20}$ +102° (*c* 0.209, acetone); t.l.c. R_F 0.5; ν_{max}^{KBr} 3310 (NH), 1650–1640 (CH_2–CH=CH_2, Amide I), 1545 (Amide II), 1125 (Ar), 725, and 675 cm^{-1} (Ph).

Anal. Calc. for $C_{28}H_{35}NO_6$: C, 69.83: H, 7.33; N, 2.91. Found: C, 69.75; H, 7.26; N, 2.84.

Allyl 2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**7**). — The later fractions from the silica gel column used for the purification of **6** gave a compound having R_F 0.4 (*A*). Evaporation of the solvent, and recrystallization of the crystalline residue (195 mg) from acetone–ether–pentane gave **7** as needles (117 mg), m.p. 100–101°, $[\alpha]_D^{20}$ +110° (*c* 0.486, acetone); ν_{max}^{KBr} 3515 (OH), 3300 (NH), 1665–1650 (CH_2–CH=CH_2, Amide I), 1545 (Amide II), 1455, 1125 (Ar C=C), 725, and 685 cm^{-1} (Ph).

Anal. Calc. for $C_{21}H_{29}NO_6$: C, 64.43; H, 7.47; N, 3.58. Found: C, 64.35; H, 7.42; N, 3.47.

Attempted tritylation of **7**. — Compound **7** (10 mg) in dry pyridine was treated with chlorotriphenylmethane (20 mg), and stirred for 5 days at room temperature;

examination of the reaction mixture (t.l.c.) then showed that no new compound had been formed.

Allyl 2-acetamido-4-O-acetyl-3-O-allyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**8**). — Compound **7** (20 mg) was acetylated with pyridine (5 ml) and acetic anhydride (2 ml) in the usual way. The residue (26 mg) obtained after evaporation was crystallized, and recrystallized, from ether–hexane, to give **8** as clusters of needles (17 mg), m.p. 113–114°, $[\alpha]_D^{20}$ +87° (*c* 0.132, chloroform); t.l.c. R_F 0.5; ν_{max}^{KBr} 3310 (NH), 1735 (OAc), 1660, 1645 (CH_2–CH=CH_2, Amide I), 1645 (Amide II), 1455 (Ar C=C), 1235 (OAc), 725, and 685 cm^{-1} (Ph).

Anal. Calc. for $C_{23}H_{31}NO_7$: C, 63.72; H, 7.21; N, 3.23. Found: C, 63.65; H, 7.21; N, 3.20.

Allyl 2-acetamido-2-deoxy-3-O-(2,4-dinitrophenylsulfenyl)-α-D-glucopyranoside (**9**). — A solution of the sulfenate **3** (40 mg) in 99% formic acid (3 ml) was stirred for 1 h at room temperature, and evaporated, and the yellow, glassy residue (35 mg) was dried in a desiccator overnight, dissolved in 3:2 benzene–ethyl acetate (3 ml), and the solution applied to a column of Florisil (2 g) which was eluted with the same solvent. The fractions having R_F 0.3 were combined, and evaporated, and the crystalline residue was recrystallized from ethyl acetate–ether, to give **9** (29 mg) as clusters of yellow needles, m.p. 190–195° (with dec.), $[\alpha]_D^{20}$ +114° (*c* 0.022, ethyl acetate); t.l.c. R_F 0.5; ν_{max}^{KBr} 3500–3400 (OH), 3295 (NH), 1710 (CO), 1660–1645 (CH_2–CH=CH_2, Amide I), 1600, 1585 (Ar), 1540–1520 (Amide II, Ar NO_2), 1350 (Ar NO_2), 1315 (SO), 1055 (CS), 790, and 730 cm^{-1} (Ph).

Anal. Calc. for $C_{17}H_{21}N_3O_{10}S$: C, 44.44; H, 4.61; N, 9.15; S, 6.98. Found: C, 44.57; H, 4.53; N, 8.21; S, 7.40.

Allyl 2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**10**). — From the mother liquors of **7** was isolated a compound that migrated just ahead of **7** in t.l.c., and the behavior of which was very similar to that of **7**. It was separated from **7** by p.t.l.c. The band having R_F 0.45 (*A*) was removed, the silica gel extracted with chloroform, the suspension filtered, and the filtrate evaporated. The residue (14 mg) was crystallized and recrystallized from acetone–ether–hexane, to give **10** (9 mg) as platelets, m.p. 94–97°, $[\alpha]_D^{20}$ +54° (*c* 0.05, acetone); i.r. spectrum similar to that of **7**.

Anal. Calc. for $C_{21}H_{29}NO_6 \cdot H_2O$: C, 61.60; H, 7.63; N, 3.42. Found: C, 61.86; H, 7.25; N, 3.18.

Allyl 2-acetamido-2-deoxy-3-O-(2,4-dinitrophenylsulfenyl)-β-D-glucopyranoside (**11**). — The later fractions eluted from the Florisil column in the purification of **9** showed two spots in t.l.c. [1:1 benzene–ethyl acetate (*A*)], a minor one having R_F 0.4, corresponding to **9**, and a major one having R_F 0.35. These fractions were combined (for further purification), and evaporated, the residue was dissolved in ethyl acetate, and the solution was applied to a p.t.l.c. plate that was eluted with 1:1 benzene–ethyl acetate. The band having R_F 0.35 was extracted with ethyl acetate, the solution filtered through Celite, and the filtrate evaporated. The residue (4 mg) was crystallized and recrystallized from ethyl acetate–benzene, to give **11** as clusters of yellow

needles, m.p. 197–200° (with dec.), $[\alpha]_D^{20}$ +44° (*c* 0.016, ethyl acetate); ν_{max}^{KBr} 3500–3400 (broad, OH), 3290 (NH), 1730 (shoulder, OAc), 1715 (CO), 1665–1645 (CH_2–CH=CH_2, Amide I), 1595, 1580 (Ar), 1535–1520 (Amide II, Ar NO_2), 1345 (Ar NO_2), 1310 (SO), 1055 (CS), 795, and 730 cm^{-1} (Ph).

Anal. Calc. for $C_{17}H_{21}N_3O_{10}S \cdot 0.5C_6H_6 \cdot 0.5CH_3CO_2C_2H_5$: C, 48.71; H, 5.20; N, 7.75. Found: C, 48.86; H, 5.69; N, 7.24.

2-Acetamido-4,6-di-O-benzyl-2-deoxy-α-D-glucopyranose (**12**). — A solution of **2** (1 g) and 1,4-diazabicyclo[2.2.2]octanetriethylenediamine (1 g) in 9:1 methanol–water (50 ml) was heated to boiling, chlorotris(triphenylphosphine)rhodium (0.5 g) was added, and the mixture was boiled for 4 h under reflux, and then stirred overnight at room temperature; t.l.c. then showed total conversion of the allyl into the 1-propenyl group. The mixture was filtered, and the filtrate evaporated; the residue was dissolved in 9:1 acetone–water (40 ml), and the solution treated with a mixture of mercuric chloride (2 g) and mercuric oxide (1 g) for 1 h at room temperature with stirring. The mixture was filtered, the filtrate evaporated, and the residue dried *in vacuo* overnight. It was then suspended in chloroform, and chromatographed on a column of silica gel (250 g). Compound **12** (824 mg) was eluted with chloroform, and recrystallized twice from absolute ethanol (720 mg, 87%), m.p. 211–212° (with dec.), $[\alpha]_D^{20}$ +99 (3 min) → +55° (24 h; *c* 1.122, ethanol); t.l.c. in 9:1 chloroform–ethanol (*A*, *B*): R_F 0.3; ν_{max}^{KBr} 3400 (OH, shoulder), 3300 (NH), 1655 (Amide I), 1560 (Amide II), 1495, 1455, 1135, 1115 (Ar), 855, 730, and 685 cm^{-1} (Ph).

Anal. Calc. for $C_{22}H_{27}NO_6$: C, 65.82; H, 6.78; N, 3.49. Found: C, 65.62; H, 6.89; N, 3.32.

2-Acetamido-3-O-acetyl-4,6-di-O-benzyl-2-deoxy-α-D-glucopyranose (**13**). — Compound **11** (10 mg) was treated with cold acetyl chloride (0.5 ml) for 1 h at 4°; t.l.c. then showed that the starting material had been converted into a faster-moving compound giving a positive reaction with aniline hydrogenphthalate[34]. The solution was evaporated *in vacuo*, and the residue freed of acetyl chloride by repeated evaporation with toluene. The crystalline residue was recrystallized from ether to give **13** (7 mg) as needles, m.p. 188–191°, $[\alpha]_D^{20}$ +55 (3 min) → +30° (24 h; *c* 0.547, chloroform); t.l.c. (*A*, *B*): R_F 0.3; ν_{max}^{KBr} 3410 (shoulder, OH), 3310 (NH), 1735 (OAc), 1650 (Amide I), 1545 (Amide II), 1500, 1455 (Ar), 745–730, and 685 cm^{-1} (Ph).

Anal. Calc. for $C_{24}H_{29}NO_7$: C, 65.00; H, 6.59; N, 3.16. Found: C, 64.95; H, 6.60; N, 3.12.

2-Acetamido-1,3-di-O-acetyl-4,6-di-O-benzyl-2-deoxy-α-D-glucopyranose (**14**). — Compound **12** (50 mg) was acetylated with acetic anhydride (2 ml) and pyridine (3 ml) in the usual way, to give **14** as a colorless syrup which crystallized after approximately 1 year, from a trace of abs. ethanol at room temperature, as needles, m.p. 126–127°, $[\alpha]_D^{20}$ +44° (*c* 0.364, chloroform); t.l.c.: R_F 0.4; ν_{max}^{KBr} 3440–3350 (broad, NH), 1750–1720 (OAc), 1650 (Amide I), 1540 (Amide II), 1495, 1455 (Ar), 745 (Ph), 710 (C–Cl), and 685 cm^{-1} (Ph).

Anal. Calc. for $C_{26}H_{31}NO_8 \cdot 0.33\ CHCl_3$: C, 60.20; H 6.01; N, 2.67. Found: C, 60.22; H, 6.10; N, 2.80.

Attempted preparation of 2-methyl-[2-acetamido-3-O-acetyl-4,6-di-O-benzyl-2-deoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline. — Compound **12** (30 mg) was treated with acetyl chloride (1.5 ml) overnight at room temperature. The solution was diluted with dichloromethane (40 ml), and the mixture poured onto crushed ice. The organic layer was successively washed with cold, saturated, aqueous sodium hydrogencarbonate (3 × 30 ml) and ice–water, dried (sodium sulfate), and evaporated, and the residue was dried *in vacuo* over soda-lime. The colorless syrup (39 mg) failed to crystallize; examination by t.l.c. showed that it was a mixture of the expected 2-acetamido-3-*O*-acetyl-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl chloride (**15**) (R_F 0.5) with a slower-moving contaminant (R_F 0.3) in the ratio of ~5:1. Compound **15** was purified by preparative t.l.c., to give a syrup (30 mg); ν_{max}^{KBr} no OH absorption, 670 cm^{-1} (C–Cl), and the rest of the spectrum similar to that of **12**.

Compound **15** (40 mg) was immediately dissolved in dry acetonitrile (1 ml) that contained tetraethylammonium chloride (16 mg) and anhydrous sodium hydrogencarbonate (16 mg). The mixture was treated according to Lemieux and Driguez[11], to give a colorless syrup (45 mg) that showed two spots in t.l.c. in 1:1 ether–hexane. The major component had R_F 0.3, and, with the anisaldehyde reagent, gave a red color characteristic of an oxazoline, $[\alpha]_D^{20}$ +68° (*c* 0.010, chloroform); ν_{max}^{KBr} 1670 (C=N). The minor component in t.l.c. corresponded to **13**, and crystallized from the mixture. The oxazoline could not be obtained in pure form.

Allyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranoside (**17**) *and its complex* (**16**) *with mercuric cyanide and mercuric bromide.* — A mixture of methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[18] (2 g), **1** (1.6 g), and mercuric cyanide (1.4 g) in 10:3 anhydrous nitromethane–benzene (130 ml) was stirred for 24 h at 40°. Further quantities of the glycosyl bromide (1.5 g) and mercuric cyanide (1 g) were added, and the mixture was treated as described by Flowers and Jeanloz[13]. Examination of the residue by t.l.c. showed a single spot reacting with anisaldehyde and potassium permanganate, and having R_F 0.5. This residue (4.3 g) was suspended in 1:1 ether–ethyl acetate and chromatographed on a column of silica gel (250 g) which was eluted with 1:1 ether–ethyl acetate. The fractions having R_F 0.5 in t.l.c. were combined and evaporated, and an aliquot of the crystalline residue was recrystallized from acetone–ether–hexane, to give **16** as prisms, m.p. 157–160°, changing to needles, m.p. 183–185°, $[\alpha]_D^{20}$ +32° (*c* 0.471, acetone); ν_{max}^{KBr} 3615, 3490, 3385 (OH), 3300 (NH), 2150–2100 (CN^-, C≡N), 1750 (ester C=O), 1675–1650 (CH_2–CH=CH_2, Amide I), 1540 (Amide II), 1455 (Ar), 740, 690 (PH), and 635 cm^{-1} (C–Br).

Anal. Calc. for $C_{31}H_{39}NO_{15}\cdot Hg(CN)_2\cdot HBr\cdot 12H_2O$: C, 32.60; H, 5.31; N, 3.46. Found: C, 32.26; H, 5.17; N, 3.83.

The crystalline residue (3.1 g) obtained after column chromatography was dissolved in chloroform, and the solution was successively washed with an aqueous, saturated solution of potassium iodide (2 × 50 ml) and water (2 × 50 ml), dried (sodium sulfate), and evaporated, to give a residue (3 g) that was chromatographed on a column of silica gel. After the mercuric salts, compound **17** was eluted with 1:1

ether–ethyl acetate, and recrystallized twice from acetone–ether–hexane, to give 2.29 g, m.p. 157–160°, $[\alpha]_D^{20}$ +35° (*c* 0.158, acetone); ν_{max}^{KBr} 3500–3400 (OH), 3310 (NH), 1750 (ester C=O), 1680–1650 (CH_2–CH=CH_2, Amide I), 1540 (Amide II), 1460 (Ar), 750, and 690 cm^{-1} (Ph); no absorption at 2150–2100, or below 690 cm^{-1}.

Anal. Calc. for $C_{31}H_{39}NO_{15}\cdot 0.5H_2O$: C, 55.19; H, 5.98; N, 2.08. Found: C, 55.18; H, 5.85; N, 2.15.

After **17**, a second disaccharide derivative was eluted from the column. It migrated just behind **17** in t.l.c. (R_F 0.45), gave a characteristic red color with reagent *A*, and showed unsaturation; ν_{max}^{film} 1670 cm^{-1} (C=N). It was obtained in pure form as a syrup, but was not further investigated.

*Allyl 2-acetamido-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-α-*D*-glucopyranoside* (**18**). — Treatment of compound **17** (1.7 g) with 50% acetic acid (15 ml) in the usual way gave a colorless, glassy residue (1.5 g) that was chromatographed on a column of silica gel (80 g) in 19:1 chloroform–ethanol. The fractions showing R_F 0.25 in the same solvent-system were combined and evaporated, and the residue was crystallized from ethanol–ether to give **18** (1.08 g), m.p. 120–121°, $[\alpha]_D^{20}$ +35° (*c* 1.91, ethanol); ν_{max}^{KBr} 3500 (OH), 3315–3300 (NH), 1750 (ester C=O), 1675–1645 (CH_2–CH=CH_2, Amide I), and 1550 cm^{-1} (Amide II).

Anal. Calc. for $C_{24}H_{35}NO_{15}\cdot H_2O$: C, 48.40; H, 6.26; N, 2.35. Found: C, 48.77; H, 6.05; N, 2.38.

*Allyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-α-*D*-glucopyranoside* (**19**). — Acetylation of compound **18** (1.05 g) with acetic anhydride (5 ml) and pyridine (20 ml) in the usual way gave a partly crystalline residue (1.37 g) that was purified by chromatography on a column of silica gel (80 g). Elution with 19:1 chloroform–ethanol gave a major fraction, R_F 0.5. Evaporation of the eluate, and recrystallization of the partly crystalline residue from acetone–ether–hexane gave **19** monohydrate (645 mg) as plates, m.p. 87–90°, $[\alpha]_D^{20}$ +45° (*c* 0.429, acetone); ν_{max}^{KBr} 3600–3500 (OH), 3360 (NH), 1750 (ester C=O), 1680–1665 (CH_2–CH=CH_2, Amide I), and 1535 cm^{-1} (Amide II).

Anal. Calc. for $C_{28}H_{39}NO_{17}\cdot H_2O$: C, 49.48; H, 6.08; N, 2.06; O, 42.37. Found: C, 49.56; H, 5.82; N, 2.00; O, 42.36.

Compound **19** was obtained in anhydrous form in some preparations, and also after subjecting the monohydrate to a further column chromatography on silica gel; it crystallized from acetone–ether–hexane as plates, m.p. 164–165°, change of form to needles, m.p. 173°, $[\alpha]_D^{20}$ +48° (*c* 3.042, acetone); t.l.c. (*A*, *C*): R_F 0.5; ν_{max}^{KBr} 3300 (NH), 1770, 1750–1730 (ester C=O), 1665 (shoulder, CH_2–CH=CH_2), 1650 (Amide I), and 1555 cm^{-1} (Amide II).

Anal. Calc. for $C_{28}H_{39}NO_{17}$: C, 50.83; H, 5.94; N, 2.12; O, 41.11. Found: C, 50.92; H, 5.97; N, 2.18; O, 41.32.

*2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-α-*D*-glucopyranose* (**20**). — A solution of **19** (1.29 g) and 1,4-diazabicyclo[2.2.2]octanetriethylenediamine (0.6 g) in 9:1 methanol–water (50 ml) was heated to boiling and treated with chlorotris(triphenylphosphine)rhodium (0.5 g).

The mixture was stirred for 4 h under reflux, and then kept overnight at room temperature. T.l.c. showed ~80% conversion of **19** into the 1-propenyl glycoside. The mixture was filtered, and the filtrate evaporated. The residue was dissolved in 9:11 acetone–water (40 ml), and the solution stirred with a mixture of mercuric chloride (2 g) and mercuric oxide (1 g) for 1.5 h at room temperature. The mixture was filtered, the filtrate evaporated, and the residue dissolved in chloroform. The solution was successively washed with an aqueous, saturated solution of potassium iodide (3 × 75 ml) and water (2 × 75 ml), dried (sodium sulfate), and evaporated, and the residue (1.15 g) was dissolved in chloroform (5 ml). The solution was applied to a column of silica gel (90 g). The noncarbohydrate components were eluted with chloroform, and the column was then eluted with 9:1 chloroform–ethanol. The fractions showing R_F 0.3 (*A*, *B*) in the latter solvent-system were combined, and evaporated, the residue was dissolved in a small volume of chloroform, the solution passed through charcoal–Celite, and evaporated, and the residue crystallized and recrystallized from chloroform–ether, to give **20** (508 mg, 42%) as clusters of prisms, m.p. 209–211° (with dec.), $[\alpha]_D^{20}$ +40 (3 min) → +19° (24 h; *c* 0.211, chloroform); ν_{max}^{KBr} 3420 (OH), 3315 (NH), 1765–1735 (ester C=O), 1650 (Amide I), and 1550 cm^{-1} (Amide II).

Anal. Calc. for $C_{25}H_{35}NO_{17}$: C, 48.31; H, 5.68; N, 2.25. Found: C, 48.21; H, 5.63; N, 2.20.

*2-Methyl-[4,6-di-*O*-acetyl-1,2-dideoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-α-*D*-glucopyrano]-[2,1-*d*]-2-oxazoline* (**26**) *and -oxazolinium acetate* (**27**). — *From* **20** via **21**. To compound **20** (70 mg) was slowly added cold acetyl chloride (2.5 ml), and the solution was stirred for 1 h at 4° and then overnight at room temperature. Dichloromethane (30 ml) was added, and the solution was poured onto crushed ice. The organic layer was successively washed with iced water (2 × 50 ml), cold saturated aqueous sodium hydrogencarbonate (3 × 50 ml), and iced water (50 ml), dried (mixture of sodium and magnesium sulfate), and evaporated; the residual yellow syrup (60 mg) was treated by several additions and evaporations of toluene, and then dried *in vacuo* over soda-lime. Examination by t.l.c. showed almost total conversion of **20** into a major component (**21**), R_F 0.35, with only a trace of a faster-moving contaminant. A slower-moving spot, R_F ~0.1, corresponded to a trace of unreacted **20**. 2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranosyl chloride (**21**) failed to crystallize, and was immediately purified by filtration through charcoal–Celite and immediately used for the the preparation of **26**; ν_{max}^{KBr} no OH absorption, 685 cm^{-1} (C–Cl). Compound **21** (50 mg, dried *in vacuo*) was dissolved in anhydrous acetonitrile (1.5 ml) which contained tetraethylammonium chloride (35 mg) and anhydrous sodium hydrogencarbonate (35 mg). The mixture was kept for 20 min at 55°, cooled, and evaporated, and dichloromethane (10 ml) was added. The mixture was processed according to Lemieux and Driguez[11], to give a syrup (42 mg) that crystallized on addition of dichloromethane, to give crystalline **26** (34 mg) which was recrystallized from ethyl acetate–ether–pentane, m.p. 141–146° (with dec.); t.l.c. showed a major

spot, R_F 0.35, giving a characteristic reddish, fast-developing spot with anisaldehyde. Compound **26** was obtained pure by p.t.l.c. in 1:1 ether–ethyl acetate.

*From methyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-α-*D*-glucopyranoside*[13,23]. — A cold solution of this glycoside (230 mg) in a mixture of acetic anhydride (3.45 ml), glacial acetic acid (2.30 ml), and conc. sulfuric acid (40 μl) was allowed to warm to room temperature, and was kept for 48 h at this temperature. Cold chloroform (20 ml) was added to the solution at 0–5°, and the organic layer was successively washed with cold, saturated sodium hydrogencarbonate solution (3 × 50 ml) and iced water (2 × 50 ml), dried (sodium sulfate), and evaporated, and the residual syrup treated by several additions and evaporations of toluene, and then dried *in vacuo* over phosphorus pentaoxide to give **26** as a colorless syrup which crystallized, and was recrystallized, from ethyl acetate–ether–pentane; needles (155 mg, 73%), m.p. 97–100°, changed to larger needles, m.p. 146–153°, $[\alpha]_D^{20}$ +27° (*c* 0.136, ethyl acetate); t.l.c. in 9:1 chloroform–ethanol (*A*): R_F 0.55; R_F of the starting methyl α-glycoside[13,23]: 0.5; R_F of 2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranose[13]: 0.5; ν_{max}^{KBr} 3485, 3370 (ring CONR), 2980 (CH_3–C=), 1775–1730 (ester C=O), 1670 (C=N), and 1535 cm^{-1} (Amide II); no absorption at ~3300 cm^{-1} (NH).

Anal. Calc. for $C_{25}H_{33}NO_{16}$: C, 49.75; H, 5.51; N, 2.32; O, 42.41. Found: C, 49.57; H, 5.72; N, 2.25; O, 42.21.

From **19**. — A cold solution of **19** (1 g) in a mixture of acetic anhydride (15 ml), glacial acetic acid (10 ml), and conc. sulfuric acid (175 μl) was treated as just described for the methyl α-glycoside, to give a colorless syrup which, on examination by t.l.c., showed two spots, the slower-moving one (R_F 0.35) corresponding to the oxazoline derivative **26**, and the faster-moving spot (R_F 0.45), to the oxazolinium acetate **27**. The syrup crystallized, and was recrystallized, from ethyl acetate–ether, to give **27** as prisms (51 mg, 59%), m.p. 96–100°, $[\alpha]_D^{20}$ +43° (*c* 0.087, ethyl acetate); t.l.c. (*A*): R_F 0.45 (**27**) and 0.35 (**26**); ν_{max}^{KBr} 3485, 3375 (ring CONR), 2890 (CH_3–C=), 1750 (ester C=O), 1670 (C=N) and 1530 cm^{-1} (Amide II); no absorption at ~3300 cm^{-1} (NH).

Anal. Calc. for $C_{27}H_{36}NO_{18}$: C, 48.94; H, 5.48; N, 2.11. Found: C, 48.90; H, 5.54; N, 2.19.

*2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-α-*D*-glucopyranosyl dihydrogenphosphate* (**22**). — A mixture of compound **26** (42 mg) and dibenzyl hydrogenphosphate (75 mg) was dissolved in 1,2-dichloroethane (2 ml), and the solution treated according to Warren *et al.*[26], to give a mixture of two major compounds and several minor contaminants. The two phosphate-containing components were separated by p.t.l.c. in 5:1 chloroform–methanol; the compound showing R_F 0.4 was purified by trituration with ether–hexane, to give **22** as a partly crystalline material (5 mg, 6%), m.p. 134–140° (with dec.), $[\alpha]_D^{20}$ +23° (*c* 0.010, chloroform); ν_{max}^{KBr} 3625–3300 (broad, OH, NH), 1750

(ester C=O), 1660 (Amide I), 1570 (Amide II), and 1265–1230 cm^{-1} (P=O and C=O), no absorption at ~1450, 1125, or below 800 cm^{-1} (Ph).

Anal. Calc. for $C_{25}H_{36}NO_{20}P \cdot H_2O \cdot 0.33CHCl_3$: C, 40.07; H, 5.09; N, 1.85. Found: C, 39.91; H, 4.76; N, 2.30.

The second compound (R_F 0.6) crystallized from ether–hexane as prisms consisting of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl dibenzyl phosphate (**28**), m.p. 130–142° (with dec., softening at 97–100°), $[\alpha]_D^{20}$ +29° (*c* 0.160, chloroform); ν_{max}^{KBr} 3600–3475 (broad, OH), 3300 (NH), 1770–1750 (OAc), 1650 (Amide I), 1550 (Amide II), 1440 (Ar), 1265, 1230 (P=O and C=O), 1130 (Ar), 715, and 680 cm^{-1} (Ph).

Anal. Calc. for $C_{28}H_{34}NO_{12}P \cdot 1.5H_2O$: C, 53.00; H, 5.88; N, 2.21. Found: C, 52.84; H, 5.92, N, 2.37.

*2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranosyl diphenyl phosphate* (**23**). — Compound **26** (or **27**) (100 mg) was treated with diphenyl hydrogenphosphate (50 mg) in anhydrous 1:1 toluene–nitromethane (3 ml) for 24 h at room temperature. T.l.c. in 9:1 chloroform–ethanol showed the presence of some unreacted starting-material. After a further addition of diphenyl hydrogenphosphate (15 mg), the mixture was kept for 24 h at room temperature, and evaporated, and the syrupy residue (169 mg) was dissolved in ethyl acetate (~1 ml). Some material that had crystallized out of the mixture was filtered off, and recrystallized twice from ethyl acetate, to give **23** as prisms (36 mg), m.p. 166–167° (with dec.), $[\alpha]_D^{20}$ +21° (*c* 0.505, cloroform); t.l.c. in ethyl acetate (*A*, *D*): R_F 0.5; in 9:1 chloroform–ethanol: R_F 0.5; ν_{max}^{KBr} 3490 (OH), 3285 (NH), 1750 (ester C=O), 1645 (shoulder, Amide I), 1600, 1590 (Ar), 1550 (shoulder, Amide II), 1490, 1445 (Ar), 1250–1200 (P=O, O–P–O–Ph, and C=O), 780, 755, and 680 cm^{-1} (Ph); n.m.r. data: δ 1.97–2.13 (18 H, 5 OAc and NHAc), 3.70 and 4.08 (s, 2 H), 4.74 (d, *J* 6 Hz), 5.13 (d, $J_{1,2}$ 8 Hz, axial H-1 of interglycosidic linkage), 6.03 (q, $J_{1,2}$ 3.5 Hz, $J_{1,P}$ 7 Hz), and 7.22 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{37}H_{44}NO_{20}P \cdot H_2O$: C, 50.98; H, 5.32; N, 1.61; P, 3.55. Found: C, 50.98; H, 5.30; N, 1.96; P, 3.41.

The mother liquors were chromatographed on two p.t.l.c. plates in 9:1 chloroform–ethanol, and two zones of phosphate and carbohydrate-containing material (*A*, *D*) were detected. The bands were removed, and the silica gel extracted by stirring overnight with 10:10:3 chloroform–methanol–water. The mixture was filtered, the filtrate evaporated, and the residue dried. This was then extracted with 2:1 chloroform–methanol, the mixture filtered, and the filtrate evaporated to give a white residue. The slower-moving component (R_F 0.5, ethyl acetate) crystallized from ethyl acetate, and gave additional **23** (7 mg; total yield 43 mg, 30%). The faster-moving component (R_F 0.6) was not investigated further; by analogy with the formation of **28**, it was possibly 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl diphenyl phosphate.

*2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-β-D-glucopyranose* (**24**). — From the mother liquors of **23** was

isolated, by p.t.l.c., a phosphate-negative compound having R_F 0.35 in 9:1 chloroform–ethanol (*A*, *B*), which crystallized from chloroform–ether as clusters of prisms (**24**, 19 mg), m.p. 167–169° (with dec.), $[\alpha]_D^{20}$ +12 (3 min) → +18° (24 h; *c* 0.155, chloroform); ν_{max}^{KBr} 3420 (OH), 3315 (NH), 1765–1735 (ester C=O), 1650 (Amide I), 1570 and 1530 cm^{-1} (Amide II).

Anal. Calc. for $C_{25}H_{35}NO_{17}$: C, 48.31; H, 5.68; N, 2.25. Found: C, 48.37; H, 5.00; N, 2.32.

*2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-α-*D*-glucopyranosyl phosphate, bis(tributylammonium) salt* (**29**). — A solution of dried, recrystallized **23** (60 mg) in absolute methanol (2.5 ml) was hydrogenolyzed under anhydrous conditions in the presence of activated[27] platinum oxide (~6 mg) for 1.5 h at room temperature and atmospheric pressure. The catalyst was reactivated according to Posternak and Rosselet[28], and the hydrogenolysis was continued under the same conditions for a further 1.5 h, when t.l.c. in 9:1 chloroform–ethanol (*A*, *D*) showed that most of the starting compound **23** had been converted into a single compound having R_F 0.2; all of the catalyst had been reduced (color change from brown to black). The suspension was filtered, and the residue washed with methanol (5 ml). The filtrate and washings were combined, and evaporated under a stream of nitrogen. Water (50 ml) was added to the syrupy residue, the mixture filtered, and the clear filtrate lyophilized, to give **22** (43 mg) as an amorphous, white material, $[\alpha]_D^{20}$ +25° (*c* 0.089, chloroform); i.r. spectrum almost identical with that of **22** just described. Amorphous **22** (40 mg) was converted into the bis(tributylammonium) salt **29** by dissolution in methanol (4 ml) and treatment with tributylamine (40 mg). The solution was processed according to Warren and Jeanloz[5], to give **29** as a syrup (46 mg) which was used immediately for the preparation of **30**; t.l.c. in 9:1 chloroform–ethanol (*A*, *D*): R_F 0.3; ν_{max}^{KBr} 3600–3490 (broad, OH), 3385 $(NHR_3)^+$, 3300 (shoulder, NH), 1780–1755 (ester C=O), 1660 (Amide I), 1545 (Amide II), and 1265–1220 cm^{-1} (P–O and C=O).

*{2-Methyl-[4,6-di-*O*-acetyl-1,2-dideoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-α-*D*-glucopyrano]-[2,1-*d*]-2-oxazolinium [2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-α-*D*-glucopyranosyl]} hydrogenphosphate* (**25**). — When a trace of oxazoline **26**, or oxazolinium acetate **27**, was present in the reaction mixture described for the hydrogenolysis of **23**, a third phosphate-positive, faster-moving spot was detected by t.l.c., and this new compound, formed in small amount, crystallized out of the methanolic solution at the end of the hydrogenolysis. It was filtered off, and recrystallized from absolute methanol, to give **25** (~3–4%) as needles, m.p. 112–115°, $[\alpha]_D^{20}$ +30° (*c* 0.068, chloroform); t.l.c. in 9:1 chloroform–ethanol (*A*, *D*): R_F 0.55; ν_{max}^{KBr} 3600–3380 (broad, OH), 3300 (shoulder, NH), 2980 (CH_3–C=), 1770–1750 (ester C=O), 1680–1660 (C=N and Amide I), 1535 (Amide II), and 1270–1220 cm^{-1} (P=O and C=O).

Anal. Calc. for $C_{50}H_{69}N_2O_{36}P \cdot H_2O$: C, 45.39; H, 5.41; N, 2.12. Found: C, 45.15; H, 5.45; N, 2.14.

In order to eliminate this side-product from the previously described reaction-

mixture, the solvent was evaporated, the residue dissolved in water, and the solution passed through a column of Amberlite IR-45 (OAc^-) ion-exchange resin (20–50 mesh), on which **25** was retained.

P^1-[*2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranosyl*] P^2-*dolichyl diphosphate* (**30**). — A mixture of **29** (42 mg) and P^1-dolichyl P^2-diphenyl phosphate[30], tributylammonium salt, prepared from pig-liver dolichol (mixture of isoprenologs having 17 to 22 isoprene units), was treated according to Warren and Jeanloz[30], except that unreacted **29** was not removed by extraction with water. The solvent was evaporated from the reaction mixture, the residue was suspended in 5:1 chloroform–methanol (3 ml), and this suspension was applied to two p.t.l.c. plates, which were developed in 65:25:4 chloroform–methanol–water. The band containing the expected product was located by spraying a narrow zone near the center with reagents *C* and *D*. Four phosphate-positive bands were present; the fastest-migrating band gave an intense reaction with the phosphate-specific spray and contained diphenyl phosphate. The second band, R_F 0.42, corresponded to unreacted **29**. The third band, R_F 0.29, gave a very weak reaction with the phosphate-specific reagent, and contained the desired product. The fourth (narrow) band was at the origin. The silica gel and band having R_F 0.29 was removed from the plates, and extracted with 10:10:3 chloroform–methanol–water by stirring overnight. The suspension was filtered through a sintered-glass funnel containing a layer of Celite, the residue was washed with the same solvent, and the filtrate was evaporated. The residue was extracted with 5:1 chloroform–methanol, the extract filtered, the filtrate evaporated, and the residue (11 mg) purified by trituration with ether, to give the tributylammonium salt of **30** as an amorphous, hygroscopic solid (7 mg), $[\alpha]_D^{20}$ +1.0° (*c* 0.030, 2:1 chloroform–methanol); t.l.c. (*A*, *C*, *D*) in 65:25:4 chloroform–methanol–water: R_F 0.29; in 10:10:3 chloroform–methanol–water: R_F 0.66; in 7:3 1-propanol–water: R_F 0.72; and in 65:35:4:4 chloroform–methanol–conc. ammonium hydroxide–water: R_F 0.25; ν_{max}^{KBr} 3560–3320 (OH, NH), 1760 (ester C=O), 1730, 1715 (C=O), 1660 (C=C and Amide I), 1635, 1620 (Amide II), 1610 $(NHR_3)^+$, 1375 (CCH_3), 1215 (P=O and C=O), 990 (P–O), 965 (C–O–P=O), and 875 cm^{-1} (–CH=C).

Anal. Ratio of 2-acetamido-2-deoxy-D-glucosyl residue to phosphate group, calc.: 1:2. Found: 1:2.0 (average of 3 determinations).

P^1-[*2-Acetamido-2-deoxy-3-O-(β-D-glucopyranosyluronic acid)-α-D-glucopyranosyl*] P^2-*dolichyl diphosphate* (**31**). — A solution of **30** (250 μg) in 2:1 chloroform–methanol (500 μl) was cooled to 4° and treated with triethylamine (500 μl) overnight at 4° and then for 2 h at room temperature; t.l.c. in 65:25:4 chloroform–methanol–water then showed that **30** (R_F 0.29) had been converted into a new, slower-migrating product (R_F 0.16). The solvent was evaporated under a stream of N_2, methanol (3 × 1 ml) was added to, and evaporated from, the residue, and this was dried *in vacuo* over phosphorus pentaoxide, giving **31** (190 μg). Compound **31** showed a single major spot in t.l.c. in the solvent systems (*A*, *C*, *D*) described for **30**, and contained a trace of dolichyl phosphate, as well as an unidentified product

migrating ahead of the main spot, possibly P^1-2-acetamido-2-deoxy-α-D-glucopyranosyl P^2-dolichyl diphosphate[30]. Compound **31** (triethylammonium salt) was obtained pure by p.t.l.c. in 65:25:4 chloroform–methanol–water, the zone containing the desired product (R_F 0.16) being extracted as described for **30**. Compound **31** was obtained as a yellowish, hygroscopic syrup (115 μg), $[\alpha]_D^{20}$ −25° (*c* 0.020, 2:1 chloroform–methanol); t.l.c. on silica gel G (*A*, *C*, *D*) in 65:25:4 chloroform–methanol–water: R_F 0.16; in 10:10:3 chloroform–methanol–water: R_F 0.60; in 7:3 1-propanol–water: R_F 0.64; and in 65:35:4:4 chloroform–methanol–conc. ammonium hydroxide–water: R_F 0.15; on Cellulose F (*C*, *D*) in 5:1 isobutyric acid–M ammonium hydroxide: R_F 0.85.

Anal. Ratio of D-glucosyluronic residue to 2-acetamido-2-deoxy-D-glucopyranosyl residue, calc.: 1:1. Found: 1:0.8.

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