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OLIGOSACCHARIDE OXAZOLINES: PREPARATION, AND APPLICATION TO THE SYNTHESIS OF GLYCOPROTEIN CARBOHYDRATE STRUCTURES*†

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ABSTRACT

O-α-D-Mannopyranosyl-(1→3)-*O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose, isolated from mannosidosis urine, was converted, *via* the per-*O*-acetylglycosyl chloride, into *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-2-methyl-(2-acetamido-3,6-di-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline. This compound was employed to glycosylate allyl or benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside, to give the corresponding tetrasaccharide derivatives, from which the protective allyl or benzyl groups were removed to yield *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucopyranose. This compound was then converted, *via* the per-*O*-acetylglycosyl chloride, into a per-*O*-acetylated tetrasaccharide oxazoline (**20**) that was treated, under anhydrous conditions, with dibenzyl phosphate, to yield a glycosyl (dibenzyl phosphate). Removal of the benzyl groups gave *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate, the synthetic precursor of a tetrasaccharide "lipid intermediate". In an attempt to prepare the tetrasaccharide oxazoline **20** by a shorter route, the propyl per-*O*-acetylglycoside **11** was prepared by catalytic hydrogenation of allyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-

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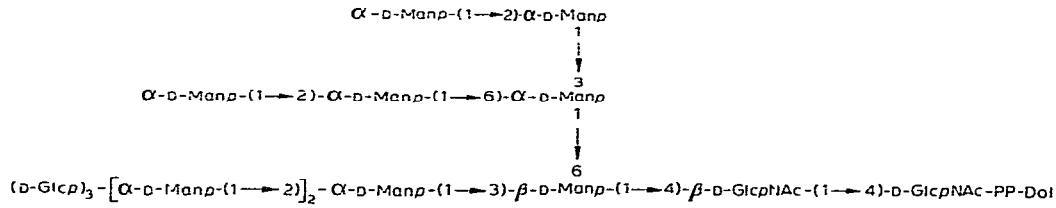
(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside, followed by *O*-acetylation. When **11** was subjected to acetolysis, a complex mixture resulted, containing only a trace of the desired compound.

INTRODUCTION

The oligosaccharide chains of the *N*-glycoproteins are assembled on a "lipid intermediate" prior to transfer to protein. In at least one biosynthetic system[2], the fully formed, "lipid intermediate" has the branched structure **1**, similar to that found in "high mannose" chains[3]. Oligosaccharide "lipid intermediates" having compositions similar to that of **1** have been isolated from a variety of sources, including, in this laboratory, calf pancreas[4].

Many of the enzymic reactions that construct the oligosaccharide moiety in such compounds as **1** have not yet been characterized. In particular, only limited information is available[5] on the mechanism of addition of up to nine residues of D-mannose to P^1-di-*N*-acetylchitobiosyl P^2-dolichyl diphosphate[6], to give an oligosaccharide derivative having the correct linkages and branching pattern, as in **1**.



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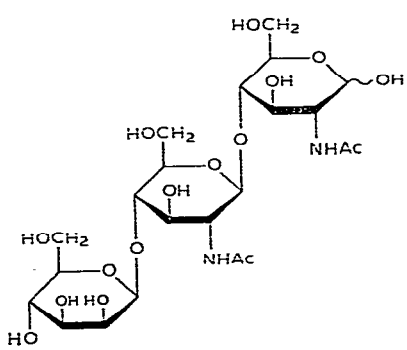
One of the best methods for investigating the biosynthesis of complex glycans is to employ exogenous, glycosyl acceptors of known structure. Thus, in order to study the biosynthesis of such oligosaccharide derivatives as **1**, a series of "lipid intermediates" having different numbers of D-mannosyl residues was needed. Chemical synthesis is the best procedure for obtaining suitable acceptors, because it yields relatively large quantities of pure compounds having known structures. How-



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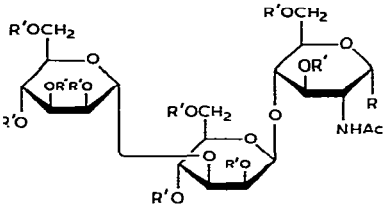
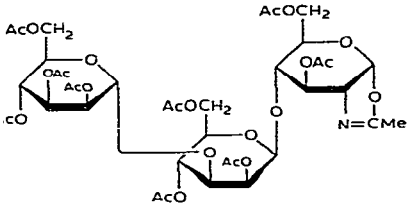


3 R = H/OH , R′ = H
4 R = Cl , R′ = Ac
5 R = R′ = Ac



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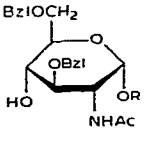
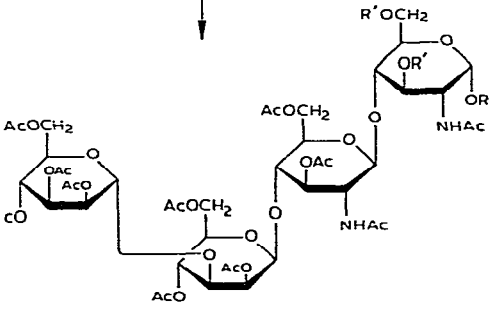
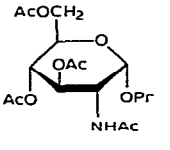


7 R = All
8 R = Bzl
All = $H_2C{=}CHCH_2$
Bzl = $PhCH_2$





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9 R = $CH_2CH{=}CH_2$, R′ = Bzl
10 R = Pr , R′ = H
11 R = Pr , R′ = Ac
12 R = $CH{=}CH{-}CH_3$, R′ = Bzl
13 R = Pr , R′ = Bzl
14 R = H, R′ = Bzl
15 R = R′ = H
16 R = R′ = Bzl
17 R = Cl , R′ = Ac

Pr = $(CH_2)_2CH_3$

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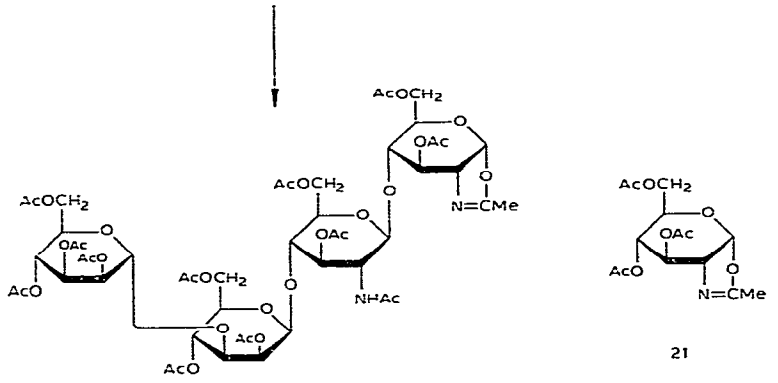

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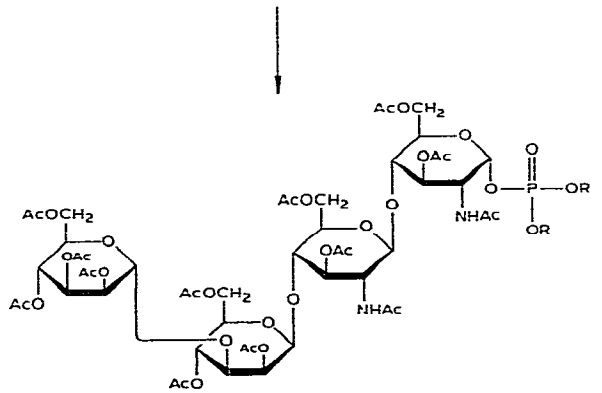


22 R = Bzl
23 R = H

ever, with the exception of the trisaccharide[7,8] **2**, the carbohydrate moieties of oligosaccharide "lipid intermediates" are too complex to be synthesized easily. One way in which to overcome this problem is to utilize the partially degraded oligosaccharides that are excreted by patients with lysozomal storage diseases. Thus, a series of compounds having 2–9 mannosyl residues could be isolated from mannosidosis urine[9]. These compounds have structures derived from "high mannose" oligosaccharides, except that the terminal residue of 2-acetamido-2-deoxy-D-glucose is missing. Replacement of this residue, and conversion of the resulting compound into an α-D-glycosyl phosphate, ready for the formation of a dolichyl diphosphate diester[10,11], would give suitable starting-materials for the synthesis of "lipid intermediates".

In previous work, we have shown that derivatives of 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose (di-*N*-acetylchitobiose) can be conveniently synthesized[12] by the "oxazoline procedure", and that oxazolines are also useful synthetic precursors for glycosyl phosphates[11]. We now

describe the application of these methods to trisaccharide **3**, isolated from mannosidosis urine. Compound **3** was converted into the per-*O*-acetyloxazoline **6**, which was employed for glycosylating derivatives of 2-acetamido-2-deoxy-D-glucose having only O-4 available for reaction. The resulting tetrasaccharides, **9** and **16**, were deprotected, and the products converted into the tetrasaccharide oxazoline **20**, which was transformed by a two-step process into the per-*O*-acetylated tetrasaccharide phosphate **23**.

RESULTS AND DISCUSSION

In previous studies, conversion of 2-acetamido-2-deoxy-D-glucose[13] or chitobiose derivatives[12] into oxazolines has been efficiently achieved by preparation of the corresponding glycosyl chloride, followed by chloride-ion catalysis. For the preparation of α-D-glycopyranosyl chlorides, the sugar was stirred with acetyl chloride at room temperature[14]. When this reaction was attempted with *O*-α-D-mannopyranosyl-(1→3)-*O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**3**), only a part of the material dissolved, and a large proportion of unreacted compound had to be filtered off, before the reaction mixture could be processed, and examined by t.l.c. This revealed the formation of the per-*O*-acetylglycosyl chloride **4**, but also the presence of the 1-*O*-acetyl compound **5** as a major by-product. In an attempt to improve the yield of **4**, saturation of the acetyl chloride with gaseous hydrogen chloride[15] was tried, but the improvement was marginal. A much better result was obtained when a vigorously stirred suspension of **3** in acetyl chloride was treated with a small volume of concentrated hydrochloric acid, after which the reaction tube was sealed. This gave a relatively efficient conversion of **3** into the glycosyl chloride **4**, t.l.c. giving no indication of cleavage of the (1→3)-α-D-mannopyranosyl or (1→4)-β-D-mannopyranosyl linkages during the reaction. Thus, **4** was suitable for conversion into the oxazoline **6**, without prior purification, by use of the standard conditions for chloride-ion catalysis in the presence of a base[13]. The oxazoline **6** contained only a trace of the 1-*O*-acetyl derivative **5** (according to t.l.c.), and was used for synthesis without chromatographic purification. For characterization, a sample of **6** was purified by preparative t.l.c., which afforded an amorphous solid (in 42% yield from **3**) giving the diagnostic, brown color reaction for oxazolines in t.l.c., and showing the characteristic, i.r. absorption band at 1675 cm^{-1} (C=N), and a ^{1}H-n.m.r. signal at δ 6.14, J 7.5 Hz (H-1).

In our previous applications[12] of the "oxazoline procedure" to the synthesis of chitobiose derivatives, an allyl glycoside was employed for the "aglycon", because it introduced a useful functional group, and because allyl derivatives give, in t.l.c., a diagnostic color reaction that is valuable for distinguishing the desired reaction-product from unwanted materials arising from the oxazoline during the coupling reaction. For the same reasons, allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[12] (**7**) was initially used in these studies. The conditions for the reaction of the trisaccharide oxazoline **6** with the alcoholic component **7** were similar to those employed previously. Thus, a mixture of **6** and **7** was dissolved in anhydrous

1,2-dichloroethane, and treated with the calculated amount (carefully weighed) of anhydrous *p*-toluenesulfonic acid. Unlike other, recent studies[8] employing similar conditions, the aglycon **7** was in excess, because of the relative inaccessibility of the starting trisaccharide **3** (precursor of **6**). Formation of the desired tetrasaccharide **9** was readily observed by t.l.c., which showed that the optimum reaction-time was ~7 h. Compound **9** was separated from unchanged **7** by *O*-deacetylation, preparative-layer chromatography (from which **7** was recovered and could be recycled), and *O*-reacetylation. After a final, chromatographic purification, the tetrasaccharide **9** was obtained in a yield (22%, based on **6**) comparable to that resulting from a similar glycosylation with a disaccharide oxazoline[7].

In an attempt to recover some of the starting material **3** from the reaction mixture after *O*-deacetylation, a by-product was recovered from the p.l.c. plates, and acetylated. The main component (according to t.l.c.) of the resulting product was purified by p.l.c., and characterized by its elemental analysis and i.r. and ^{1}H-n.m.r. spectra, and by acid hydrolysis, followed by g.l.c. analysis of the hydrolyzate after per-*O*-(trimethylsilyl)ation. The sum of this evidence indicated that the compound was a per-*O*-acetylated D-mannosyl disaccharide **18**, resulting from scission of the β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose linkage during the coupling reaction.

Two possible routes were envisaged for the conversion of the tetrasaccharide **9** into oxazoline **20**. The simpler would be the catalytic hydrogenation of **9**, resulting in removal of the benzyl substituents and concomitant reduction of the allyl to a propyl group, followed by acetylation, and acetolysis, which has been shown[16] to convert acetylated methyl and allyl α-D-glucopyranosides into oxazolines. Trial experiments with propyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**19**) suggested that this should also pertain to propyl glycosides. The second possibility would be isomerization of the allyl group of **9** to a 1-propenyl group, hydrolysis with dilute acid or mercuric chloride[17] to give the free sugar, treatment with acetyl chloride–hydrochloric acid to give the glycosyl chloride, and chloride-ion catalysis[13].

When **9** was hydrogenated in the presence of palladium-on-charcoal, it was readily converted into the propyl glycoside **10**. However, after acetylation to give **11**, acetolysis led to a complex mixture containing, according to t.l.c., only a trace of the required oxazoline **20** (see later), and so this route was not further pursued. Therefore, the tetrasaccharide **9** was treated with tris(triphenylphosphine)rhodium(II) chloride in the presence of 1,4-diazabicyclo[2.2.2]octane[18], in order to isomerize the allyl to a 1-propenyl group. The extent of the reaction was judged by t.l.c., and by treating a small sample of the reaction mixture with mercuric chloride. This showed that the efficiency of the isomerization reaction was unpredictable; on some occasions, it proceeded quite rapidly to near-completion after a single addition of rhodium catalyst and base, but, at other times, multiple additions of catalyst and base, and long reaction-times were needed. Under these forcing conditions, some hydrogenation of the allyl or 1-propenyl group was inevitable[12,19], and the resulting propyl derivative **13** appeared in t.l.c. as a spot having the same R_F value as the starting compound **9**,

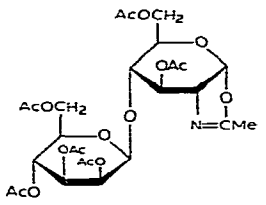
but showing no unsaturation when the plate was sprayed with an alkaline solution of potassium permanganate, and remaining unaffected by treatment of the reaction mixture with mercuric chloride. In contrast, the mercuric chloride treatment converted the 1-propenyl glycoside **12** into the free sugar **14**, which was isolated by p.l.c., and had the expected i.r. and ^{1}H-n.m.r. spectra.

Because of the difficulties encountered in attempts at isomerizing the allyl group of **9**, other catalysts were tried, including rhodium(III) chloride[20] and palladium-on-charcoal, in the presence[21] or absence[22] of *p*-toluenesulfonic acid. None of these were particularly effective, but a moderate yield of **14** could be obtained (without the isolation of the 1-propenyl derivative **12**), by treatment of **9** with palladium-on-charcoal in a mixture of aqueous ethanol and acetic acid[23], followed by p.l.c. However, this reaction had to be carefully monitored by t.l.c., which showed the occurrence of several, competing side-reactions. Removal of the benzyl groups from **14** was efficiently achieved by catalytic hydrogenolysis, to give the triol **15**, having the expected analysis and spectra.

Because of the difficulties encountered with the removal of the allyl group from **9**, further glycosylations with the trisaccharide oxazoline **6** employed benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[7] (**8**) instead of the allyl glycoside **7**. The coupling conditions were the same as those employed for **7**, but, because **16** had, in t.l.c., no characteristics to distinguish it from unwanted materials, its migration position was decided by reference to the corresponding allyl derivative **9**. Isolation of the tetrasaccharide **16** was performed by the same procedure as that employed for **9**, *i.e.*, *O*-deacetylation, separation from unchanged **8** and by-products by p.l.c., and *O*-reacetylation. Compound **16** had the expected analysis and spectra, and the benzyl groups were removed by catalytic hydrogenolysis without any complication, to give the triol **15** having properties indistinguishable from those of this compound as prepared by the other route.

For conversion into the tetrasaccharide oxazoline **20**, compound **15** was treated with hydrochloric acid in acetyl chloride, as for the preparation of **4**, and the resulting glycosyl chloride **17**, without purification, was subjected to chloride-ion catalysis. Examination of the resulting product by t.l.c. showed that, besides the desired oxazoline **20**, the trisaccharide oxazoline **6** and 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[13] (**21**) were formed to a minor extent. The formation of **17** must, therefore, have been accompanied by some scission of the β-D-(1→4) linkage between the two residues of 2-acetamido-2-deoxy-D-glucose. The extent of this side reaction was not decreased by lowering the proportion of hydrochloric acid used in the preparation of the glycosyl chloride **17**.

The desired oxazoline **20** was readily purified by multiple-development p.l.c., and its identity was confirmed by the presence of diagnostic peaks and signals in the i.r. and ^{1}H-n.m.r. spectra, by elemental analysis, and by its lability to mild treatment with acid. The tetrasaccharide oxazoline **20**, the trisaccharide oxazoline **6**, and the disaccharide oxazolines **24** and **25** (respectively derived from 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucopyranose[7] and di-*N*-acetylchitobiose[12]) had similar



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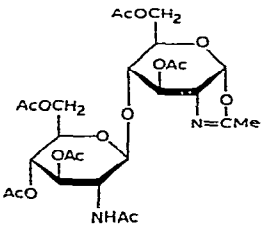

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mobilities in single-elution t.l.c., but could be effectively separated by multiple development. This t.l.c. showed that acetolysis of compound **11** (mentioned previously) had not produced a significant amount of the desired oxazoline **20**.

Treatment of the tetrasaccharide oxazoline **20** with dibenzyl phosphate in 1,2-dichloroethane gave the glycosyl dibenzyl phosphate **22**. This reaction was acutely sensitive to traces of water or methanol, and, unless these were eliminated by the use of carefully dried, starting-compound **20** (as shown by ^{1}H-n.m.r.), solvent, and apparatus, the oxazoline suffered acid-catalyzed hydrolysis rather than phosphorylation. Even though every possible precaution was taken, examination of the reaction mixture by t.l.c. always revealed the presence of some by-products, together with **22**. The dibenzyl glycosyl phosphate **22** was sufficiently stable to be purifiable by preparative-layer chromatography, after which, brief, catalytic hydrogenolysis removed the benzyl groups, to give the per-*O*-acetylglycosyl phosphate **23**.

Compound **23** had the expected i.r. and ^{1}H-n.m.r. spectra, and the optical rotation and n.m.r. spectrum were consistent with **23** having the α-D-anomeric configuration in the glycosyl phosphate residue. This would also be expected from the method of formation: it is known[11,24] that the per-*O*-acetylated oxazolines **21** and **25**, respectively derived from 2-acetamido-2-deoxy-D-glucose and di-*N*-acetylchitobiose, specifically yield α-D-linked phosphates when treated with dibenzyl phosphate, and benzylated oxazolines behave similarly[25]. In t.l.c., the per-*O*-acetyl tetrasaccharide phosphate **23** was readily separated from the per-*O*-acetylglycosyl phosphates derived from 2-acetamido-2-deoxy-D-glucose and di-*N*-acetylchitobiose[10,11].

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP2 hot-stage equipped with a microscope, and correspond to "corrected melting points". Optical rotations were determined in 1-dm, semimicro tubes with a Perkin–Elmer No. 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer spectrophotometer, Model 237. ^{1}H-N.m.r. spectra were recorded at 60 MHz with a Varian T-60 spectrometer, with chloroform-*d* as the solvent (containing 1% of tetramethylsilane as the internal standard). The cation-exchange resin used was AG-50W X-8 (200–400 mesh; Bio-Rad Laboratories, Richmond, CA 94804). Evaporations were conducted

in vacuo, with the bath temperature kept below 30°. Dichloromethane, acetonitrile, and 1,2-dichloroethane were dried by distillation from phosphorus pentaoxide, and addition of 3A molecular sieve (No. M-9882, Sigma Chemical Co., St. Louis, MO 63178). Dimethyl sulfoxide was dried by distillation *in vacuo*, and addition of 4A molecular sieve (No. M-0133, Sigma). Other solvents were dried (where stated) by treatment with molecular sieve followed by addition of calcium hydride (in lump form; Fisher Scientific Co., Pittsburgh, PA 15219). The microanalyses were performed by Dr. W. Manser, CH-8704 Herrliberg, Zurich, Switzerland, and by Galbraith Laboratories Inc., Knoxville, TN 37821.

Chromatographic methods. — T.l.c. and preparative t.l.c. (p.t.l.c.) were performed on precoated plates of Silica Gel G, 0.25-mm thick (E. Merck AG, Darmstadt, Germany); for t.l.c., the plates supplied were cut to a length of 6 cm before use, but otherwise were used without pretreatment. All proportions of solvents are v/v. Preparative-layer chromatography (p.c.) was performed on precoated Silica Gel F254 PLC plates, 2-mm thick (Merck), or on precoated plates of Silica Gel F254, 0.5-mm thick (Merck). The spray reagent, unless otherwise stated, was 1:1:18 anisaldehyde–sulfuric acid–ethanol[26], and the plates were heated to 125°. Unsaturation was detected by spraying with a solution of 1% potassium permanganate in 2% aqueous sodium hydrogencarbonate. Phosphate groups were detected with the spray reagent described by Dittmer and Lester[27]. When plates were eluted more than once, they were dried in air between each elution. Column chromatography (l.c.) was performed on Silica gel (0.05–0.2 mm, 70–325 mesh, Merck). Gas–liquid chromatography (g.l.c.) was performed with a Perkin–Elmer Model 900 instrument, equipped with a flame-ionization detector; sugars were analyzed as per(trimethylsilyl) ethers, in a column (300 × 0.3 cm) of Gas Chrom Q coated with 3% of OV 17. The column temperature was programmed to rise from 120° to 300° at 8°/min.

O-(*2,3,4,6-Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1*→*3*)-O-(*2,4,6-tri*-O-*acetyl*-β-D-*mannopyranosyl*-(*1*→*4*)-*2-methyl*-(*2-acetamido-3,6-di*-O-*acetyl-1,2-dideoxy*-α-D-*glucopyrano*)-[*2,1*-d]-*2-oxazoline* (**6**). — A stirred suspension of *O*-α-D-mannopyranosyl-(1→3)-*O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**3**; 140 mg, 0.26 mmol) in acetyl chloride (20 mL) was treated dropwise with conc. hydrochloric acid (0.4 mL). The reaction tube was sealed, and the mixture was stirred for 48 h at room temperature; then, the tube was opened, and the excess of reagents was removed (*a*) by evaporation under a stream of nitrogen gas, and (*b*) *in vacuo*. The residue was dried by repeated addition and evaporation of dry toluene (2 mL), and, finally, for several hours in a vacuum desiccator over potassium hydroxide, giving **4**, t.l.c.: R_F 0.67 (10:1 chloroform–methanol), together with a small proportion of **5**, R_F 0.60.

The glycosyl chloride **4**, without purification, was dissolved in dry acetonitrile (3 mL) and treated with sodium hydrogencarbonate (0.1 g), and tetraethylammonium chloride (0.1 g). The mixture was stirred, with exclusion of moisture, for 3 h at room temperature, diluted with dichloromethane (100 mL), and the solution washed with water (3 mL), dried ($NaHCO_3$), the suspension filtered, and the filtrate evaporated;

the residue was dried by three additions and evaporations of toluene (2 mL), to give oxazoline **6** (contaminated by a small proportion of the 1-*O*-acetyl derivative **5**, but suitable for the glycosylation of **7** and **8** without purification). In t.l.c. (10:1 chloroform–methanol), **6** was not separated from the glycosyl chloride **4**.

For characterization, a solution of a sample of **6** (from 30 mg of **3**) in dichloromethane (1 mL) was chromatographed on two 0.25-mm, t.l.c. plates, which were developed twice with 30:1 chloroform–methanol. The band containing **6** was located by cutting a 0.5-cm strip from the center of each plate and spraying with the anisaldehyde reagent, and **6** was extracted from the silica gel by stirring for 30 min with 2:1 chloroform–methanol (3 × 25 mL). Filtration and evaporation, followed by trituration with hexane, gave **6** (21 mg, 42%), an amorphous material, m.p. 75–77°, $[\alpha]_D^{13}$ −10° (*c* 1.4, 1,2-dichloroethane); ν_{max}^{KBr} 2965, 1745 ($OCOCH_3$), 1675 (C=N), 1435, 1375, 1230 (broad, $OCOCH_3$), 1190, 1085, 1050 (broad), 980, 920, and 900 cm^{-1}; n.m.r.: δ 6.14 (d, $J_{1,2}$ 7.5 Hz, H-1), 5.6 (m, 2 H), 5.22 (d, 2 H, *J* 3 Hz), 5.17 (s, 2 H), 4.96 (s, 2 H), 4.33 (m, 6 H), 3.73 (m, 5 H), 2.33, 2.20, 2.17, 2.12, and 2.07 (s, 30 H, $OCOCH_3$ and CH_3 of oxazoline).

Anal. Calc. for $C_{38}H_{51}NO_{24}$: C, 50.39; H, 5.67; N, 1.55; O, 42.39. Calc. for $C_{38}H_{51}NO_{24} \cdot 0.5\ C_6H_{14}$: C, 51.90; H, 6.16; N, 1.47; O, 40.46. Found: C, 52.55; H, 6.15; N, 1.48; O, 39.91.

For further characterization, **6** was compared, by t.l.c. migration (20:1 chloroform–methanol, 2 elutions), with *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-2-methyl-(2-acetamido-3,6-di-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**24**); the migration was clearly different. For determination of the D-mannose and 2-acetamido-2-deoxy-D-glucose contents of **6**, samples of **6** (1 mg) and **3** (1 mg) were subjected to methanolysis by treatment with M hydrogen chloride in methanol (0.2 mL) for 2 h at 85°. After evaporation, the released sugars were acetylated with 1:1 acetic anhydride–pyridine (0.2 mL) overnight at room temperature, the products *O*-deacetylated by treatment with 0.5M hydrogen chloride in methanol for 1 h at 65°, and the products per-*O*-(trimethylsilyl)ated. Analysis by g.l.c. showed that the ratio of areas of the peaks for D-mannopyranosides and 2-acetamido-2-deoxy-D-glucopyranosides was 2.19:1 for the starting compound **3**, and 1.92:1 for **6** (~2:1 for both compounds, within experimental error; calc.: 2.0:1.0). When the *O*-deacetylation step was performed with 4M ammonia in methanol for 1 h at 65°, the ratio was 1.84:1 for both **3** and **6**.

O-(*2,3,4,6-Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1→3*)-O-(*2,4,6-tri*-O-*acetyl*-β-D-*mannopyranosyl*)-(*1→4*)-*2-acetamido-1,3,6-tri*-O-*acetyl*-*2-deoxy*-D-*glucopyranose* (**5**). — A mixture of compound **3** (30 mg, 0.55 mmol) with 1:2 acetic anhydride–pyridine (2 mL) was stirred to give a clear solution; this was kept overnight at room temperature, treated with water (0.5 mL), and evaporated (N_2 gas). After two additions and evaporations of toluene (0.25 mL), the residue was taken up in 1,2-dichloroethane, and the resulting suspension filtered (sintered glass). Evaporation gave **5** (58 mg, 100%), amorphous, m.p. 106–109°, $[\alpha]_D^{20}$ 0.0° (*c* 2.3, 5:1 chloroform–methanol); t.l.c. (10:1 chloroform–methanol): R_F 0.60 (α anomer; t.l.c. showed a

mixture of the anomers); *cf.*, R_F 0.67 for compound **6**; ν_{max}^{KBr} 3370 (NH) 2970, 1745 ($OCOCH_3$), 1675 (amide I), 1535 (amide II), 1430, 1375, 1230 (v. broad $OCOCH_3$), 1140, 1050 (v. broad), 980, and 940 cm^{-1}; n.m.r.: δ 6.1 (d, $J_{1,2}$ 8 Hz, H-1β), 2.17, and 2.09, 2.02, 1.97, and 1.92 (m, 33 H, 10 $OCOCH_3$ and $NHCOCH_3$).

Anal. Calc. for $C_{40}H_{55}NO_{26} \cdot H_2O$: C, 48.83; H, 5.84; N, 1.42. Found: C, 48.49; H, 5.71; N, 1.27.

Allyl O-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1*→*3*)-O-(*2,4,6-tri*-O-*acetyl*-β-D-*mannopyranosyl*)-(*1*→*4*)-O-(*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1*→*4*)-*2-acetamido-3,6-di*-O-*benzyl-2-deoxy*-α-D-*glucopyranoside* (**9**). — A mixture of **6** (derived from 0.26 mmol of **3**, without purification) and **7** (100 mg, 0.23 mmol) was dissolved in anhydrous 1,2-dichloroethane (4 mL), and the stirred solution was treated with a solution of *p*-toluenesulfonic acid in toluene (prepared by fusing *p*-toluenesulfonic acid hydrate at 110° *in vacuo* over phosphorus pentaoxide, and dissolving the residue in dry toluene, to give a concentration of ~1 mg/14 μL) to give a pH of ~4 (pH paper). The reaction tube was sealed, and the contents were kept for 7 h at 80°, when t.l.c. (20:1, chloroform–methanol, 2 elutions) showed a partial conversion of **7** into a new compound **9**, having a lower mobility and giving the characteristic, purple color-reaction of an allyl derivative with the anisaldehyde spray. (Thus, **9** was readily distinguished from other compounds, arising from the oxazoline **6**, that did not give this color reaction). The reaction mixture was cooled, treated with 1.5% methanolic sodium methoxide until pH paper showed the presence of an excess of base, and then kept for 2 h at room temperature, when t.l.c. (60:35:6 chloroform–methanol–water) showed the formation of a major product, R_F ~0.5, and a large amount of unwanted material staying near the origin. After treatment with a cation-exchange resin (pyridinium$^+$) to remove the excess of base, the resin was filtered off and washed with 10:10:3 chloroform–methanol–water (2 mL). The filtrate and washings were combined, and evaporated to dryness, and the residue was dissolved in methanol (1 mL). The resulting solution was applied to two p.c. plates, which were eluted with 60:35:6 chloroform–methanol–water. The band corresponding to the desired product was located by spraying with the potassium permanganate reagent, and viewing under u.v. light (for recovery of unchanged **7**, see later). The silica gel was removed from the plate and stirred overnight with 10:10:3 chloroform–methanol–water. The suspension was filtered (Celite), the filtrate evaporated to dryness, and the residue dried by 3 additions and evaporations of toluene (1 mL), before treatment with 1:2 acetic anhydride–pyridine (4 mL). The acetylation mixture was kept overnight at room temperature, treated with water (1 mL), and evaporated. The residue was dried by 2 additions and evaporations of toluene (1 mL), and examination by t.l.c. (10:1 chloroform–methanol) showed the presence of **9**, together with minor contaminants. Final purification was achieved by a second p.c., on two 0.5 mm-thick, preparative plates, with 10:1 chloroform–methanol. Compound **9** was located by spraying with the potassium permanganate reagent and by u.v. illumination, was extracted from the silica gel by stirring overnight with 2:1 chloroform–methanol, and was isolated by filtration (Celite), and

evaporation. The residue was taken up in 1,2-dichloroethane (2 mL), the suspension was filtered (sintered glass), and the filtrate evaporated, to yield **9** (30 mg, 22% based on **6**), amorphous, m.p. 95–97°, $[\alpha]_D^{20}$ +5° (*c* 2.1, 5:1 chloroform–methanol); t.l.c. (10:1 chloroform–methanol): R_F 0.8 (the same as **7**); ν_{max}^{KBr} 3480 (NH), 2950 (broad), 1750 ($OCOCH_3$), 1675 (amide I), 1535 (amide II), 1460, 1375, 1230 (broad), 1140, 1050 (broad), 945, 730, and 690 cm^{-1} (Ar); ^{1}H-n.m.r.: δ 7.44, 7.25, (s, 10 H, 2 $C_6H_5CH_2$), 2.22 (s, 27 H, 9 $OCOCH_3$), 2.01, 1.98, 1.91, 1.73, and 1.70 (s, 6 H, 2 $NHCOCH_3$).

Anal. Calc. for $C_{63}H_{81}N_2O_{30}$: C, 56.20; H, 6.06; N, 2.08; O, 35.65. Found: C, 56.12; H, 6.16; N, 1.96; O, 35.45.

To recover unchanged **7**, the band migrating near the solvent front of the first p.c. (displaying intense u.v. absorption) was removed from the plate and stirred overnight with 2:1 chloroform–methanol. The suspension was filtered (Celite), and the filtrate evaporated, to give **7** (75 mg, 75%).

Benzyl O-(*2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl*)-(*1→3*)-O-(*2,4,6-tri-O-acetyl-β-D-mannopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl*)-(*1→4*)-*2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside* (**16**). — The coupling reaction between **6** (derived from 0.26 mmol of **3**) and **8** (100 mg, 0.20 mmol) was performed as described for the procedure employing **7**, but **16** was identified by t.l.c. (10:1 or 20:1 chloroform–methanol, 2 elutions), by comparison with the allyl derivative **9**; otherwise it could not be distinguished from unwanted materials. *O*-Deacetylation and p.c. were performed as described for **9**, but location of **16** on the chromatogram was achievable only by u.v. illumination. Reacetylation, and a second p.c., as described for **9**, gave **16** (28 mg, 19% based on **6**), amorphous, m.p. 79–83°, $[\alpha]_D^{20}$ +13° (*c* 0.6, 1,2-dichloroethane); ν_{max}^{KBr} 3400 (OH, NH), 2940, 1745 ($OCOCH_3$), 1665 (amide I), 1545 (amide II), 1460, 1375, 1230 ($OCOCH_3$), 1140, 1070, 730, and 690 cm^{-1} (Ar).

Anal. Calc. for $C_{67}H_{83}N_2O_{30} \cdot 2\ H_2O$: C, 56.18; H, 6.12. Found: C, 56.33; H, 6.18.

Recovery of unchanged **8** was performed as described for **7**, to give 74 mg (74%).

In order to determine the identity of a major by-product (indicated by t.l.c.) arising from **6** during the glycosylation of **7** or **8**, a further band, having a low R_F value, was located on the p.c. plates (from the first p.c.—see the preceding). This was achieved by cutting a 1-cm strip from the center of each plate, and spraying it with the anisaldehyde reagent. The material was extracted from the silica gel by stirring overnight with 10:10:3 chloroform–methanol–water, filtration (Celite), and evaporation. Examination of the residue by t.l.c. (10:14:5 chloroform–methanol–water) showed that the main component had an R_F value slightly higher than that of **3**, and that only a trace of **3** was present. After treatment with 1:2 acetic anhydride–pyridine (0.2 mL) overnight at room temperature, followed by addition of water (0.1 mL), evaporation, and two additions and evaporations of toluene (0.1 mL), the major acetylated compound (**18**) [according to t.l.c. (10:1 chloroform–methanol)] was purified by preparative t.l.c. on one plate, with 10:1 chloroform–methanol (2 elutions).

The band corresponding to **18** was located by cutting a 0.5-cm strip from the plate, and spraying it with the anisaldehyde reagent. Extraction of tetra-*O*-acetyl-*O*-(2,3,4,6-tetra-*O*-acetyl-D-mannopyranosyl)-D-mannose (**18**) was performed as described for **9** or **16**, to give a residue that was triturated with hexane, to yield a solid (10 mg, from 98 mg of **6**), m.p. 56–58°, $[\alpha]_D^{20}$ +17° (*c* 2.1, 5:1 chloroform–methanol); ν_{max}^{KBr} 1750 ($OCOCH_3$), 1375, 1230, 1140, 1085, 1050, and 985 cm^{-1}; ^{1}H-n.m.r.: δ 5.3 (m, 5 H), 5.07 (m, 2 H), 4.22 (m, 7 H), 2.27, 2.18, 2.14, 2.12, and 2.10 (s, 24 H, 8 $OCOCH_3$).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.55; H, 5.64. Found: C, 49.90; H, 5.96.

G.l.c. analysis, performed as described for **6**, showed the absence of 2-acetamido-2-deoxy-D-glucose.

Propyl O-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1→3*)-O-(*2,4,6-tri*-O-*acetyl*-β-D-*mannopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1→4*)-*2-acetamido-2-deoxy*-α-D-*glucopyranoside* (**10**). — A solution of **9** (12 mg, 9 μmol) in methanol (2 mL) was mixed with 10% palladium-on-charcoal (10 mg, Fluka-Tridom Chem. Co., Hauppauge, NY 11787), and hydrogenated at 1.5 atm for 5 h, when t.l.c. (10:1 chloroform–methanol) showed the complete conversion of **9** (R_F 0.8) into **10** (R_F 0.4). The catalyst was filtered off and washed with methanol, and the filtrate and washings were combined and evaporated, to give an amorphous material (6 mg, 56%), m.p. 138–140°, $[\alpha]_D^{20}$ +3° (*c* 0.8, 5:1 chloroform–methanol); ν_{max}^{KBr} 3470 (OH, NH), 2975, 2945, 1750 ($OCOCH_3$), 1665 (amide I), 1545 (amide II), 1375, 1230, 1140, and 1050 cm^{-1} (broad).

Anal. Calc. for $C_{49}H_{71}N_2O_{30} \cdot 2\ H_2O$: C, 48.87; H, 6.28; N, 2.33. Found: C, 48.91; H, 6.31; N, 2.06.

For g.l.c. analysis, 0.5 mg of **10** was treated with M hydrochloric acid (0.2 mL), and the mixture was kept in a sealed tube for 4 h at 100°. The hydrolyzate was evaporated (N_2 gas), and two additions and evaporations of water (0.1 mL) were made; the residue was then kept *in vacuo* over potassium hydroxide pellets overnight. The released sugars were converted into methyl glycopyranosides by methanolysis with M hydrogen chloride in methanol for 2 h at 85°, and these were processed, and converted into per(trimethylsilyl) ethers, as described for **6**. Calc.: ratio of D-mannose to 2-acetamido-2-deoxy-D-glucose: 1.0:1.0. Found: 1.0:1.2.

O-(*2,3,4,6-Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1→3*)-O-(*2,4,6-tri*-O-*acetyl*-β-D-*mannopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1→4*)-*2-acetamido-3,6-di*-O-*benzyl-2-deoxy*-D-*glucopyranose* (**14**). — (*a*). A solution of **9** (90 mg, 67 μmol) in 9:1 ethanol–water (4 mL) was treated with diazabicyclo[2.2.2.]octane (30 mg) and triphenylphosphinerhodium(II) chloride (10 mg), and the mixture was stirred at 80°. After 1 h, t.l.c. (20:1 chloroform–methanol, 2 elutions) showed partial conversion of **9** into the propenyl derivative **12**, having a slightly higher mobility. An additional 10 mg of catalyst was added, and the mixture was kept for a further 1 h at 80°, and then examined by t.l.c. In one experiment, this showed an almost complete conversion into **12**, and the reaction was stopped (see later); in another, three more additions of triphenylphosphinerhodium(II) chloride

(10 mg) were made at 1-h intervals, and t.l.c. showed that the reaction was still incomplete, and formation of **12** had ceased. After evaporation of the solvent (with N_2 gas), the residue was dissolved in 5:1 acetone–water (5 mL), and the solution was treated with mercury(II) chloride (150 mg). The mixture was stirred for 15 min, when t.l.c. (10:1 chloroform–methanol) showed that **12** (R_F 0.8) had been converted into **14** (R_F 0.54, α-D anomer; the β-D anomer had a slightly lower mobility). After dilution with chloroform (20 mL), the suspension was filtered (Celite), and the filtrate was evaporated, to give a residue that was dissolved in methanol (1 mL) and chromatographed on four t.l.c. plates with 10:1 chloroform–methanol. The band containing **14** (both anomers) was located by spraying with the potassium permanganate reagent, and by cutting a 0.5-cm strip from each plate and spraying with the anisaldehyde reagent. Compound **14** was extracted from the silica gel by stirring overnight with 2:1 chloroform–methanol, filtering (Celite), and evaporating, to give a residue that was taken up in 1,2-dichloroethane (5 mL). The resulting suspension was filtered (sintered glass), and the filtrate evaporated, to give **14**, the yield ranging from 35 mg (38%) to 67 mg (73%); amorphous, m.p. 115–118°, $[\alpha]_D^{20}$ −7→−8° (2 h; *c* 1.8, 5:1 chloroform–methanol); ν_{max}^{KBr} 3380 (NH, OH), 2950, 1745 ($OCOCH_3$), 1660 (amide I), 1535 (amide II), 1455, 1375, 1230, 1135, 1050 (broad), 980, 730, and 685 cm^{-1}; ^{1}H-n.m.r.: δ 7.36, 7.23, (s, 10 H, Ar), 7.2 ($CHCl_3$), 2.90 (s, 2 H), 2.22, 2.09, 2.03, 1.97 (s, 27 H, $OCOCH_3$), and 1.76 (s, 6 H, $NHCOCH_3$).

Anal. Calc. for $C_{60}H_{77}N_2O_{30} \cdot 0.5\ CHCl_3$: C, 53.19; H, 5.71; N, 2.05; O, 35.14. Found: C, 52.84; H, 5.85; N, 2.16; O, 34.58.

In an experiment in which t.l.c. had shown a particularly low conversion of **9** into **12**, the material resistant to the action of mercury(II) chloride (upper band in preparative t.l.c.) was extracted from the silica gel with 2:1 chloroform–methanol, to give 30 mg of a product (mainly **13**) showing the absence of unsaturation (potassium permanganate reagent).

(*b*) A solution of **9** (50 mg, 37 μmol) in 2:1:1 ethanol–glacial acetic acid–water (1.25 mL) was treated with 10% palladium-on-charcoal (13 mg; Fluka-Tridom). The mixture was stirred at 70°, and t.l.c. (10:1 chloroform–methanol) showed the gradual conversion of **9** (R_F 0.8) into **14** (R_F 0.54, α-D anomer) plus minor by-products. After 6–7 h, the proportion of by-products, especially a compound having R_F 0.25, began to increase, and the reaction was stopped after 8 h, even though a considerable amount of **9** remained unchanged. The catalyst was filtered off, and the filtrate was concentrated to ~1 mL (N_2 gas) and the concentrate applied to two 0.5-mm, p.c. plates, which were developed twice with 20:1 chloroform–methanol. The bands containing **14** and **9** were detected by spraying with the potassium permanganate reagent, and viewing under u.v. light. Extraction of compounds **14** and **9** from the silica gel was performed by stirring overnight with 2:1 chloroform–methanol, filtering (Celite), and evaporating, to give **14** [20 mg, 39%; identical in all respects with the material obtained by method (*a*)] and unchanged **9** (6 mg, 12%).

O-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-mannopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyrano-

syl)-(*1→4*)-*2-acetamido-2-deoxy*-D-*glucopyranose* (**15**). — (*a*) *From* **14**. A solution of **14** (12 mg, 9 μmol) in methanol (2 mL) was mixed with 10% palladium-on-charcoal (10 mg, Fluka-Tridom) and hydrogenated at 1.5 atm for 2–3 h, when t.l.c. (10:1 chloroform–methanol) showed the conversion of **14** (R_F 0.54) into **15** (R_F 0.2; α anomer). The catalyst was filtered off (sintered glass), and washed with methanol, and the filtrate and washings were combined, and evaporated, to yield **15** (10.5 mg, 100%), amorphous, m.p. 184–184.5°, $[\alpha]_D^{20}$ −11° (*c* 1.6, 5:1 chloroform–methanol, no change in 4 h); t.l.c.: R_F 0.6 (5:1 chloroform–methanol, α-D anomer; β-D anomer has slightly lower R_F value); ν_{max}^{KBr} 3450 (OH, NH), 2950, 1745 ($OCOCH_3$), 1665 (amide I), 1550 (amide II), 1430, 1375, 1230 (broad), and 1050 (broad) cm^{-1}.

Anal. Calc. for $C_{46}H_{65}N_2O_{30} \cdot H_2O$: C, 48.29; H, 5.90; N, 2.45. Found: C, 48.38; H, 6.08; N, 2.55.

(*b*). *From* **16**. A solution of **16** (28 mg, 0.20 μmol) in methanol (4 mL) was mixed with 10% palladium-on-charcoal (25 mg, Fluka-Tridom), and hydrogenated as described in (*a*), to give **15** (25 mg, 100%), identical with the product from **14** according to m.p. and t.l.c.

O-(*2,3,4,6-Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1→3*)-O-(*2,4,6-tri*-O-*acetyl*-β-D-*mannopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1→4*)-*2-methyl*-(*2-acetamido-3,6-di*-O-*acetyl-1,2-dideoxy*-α-D-*glucopyrano*)-[*2,1*-d]-*2-oxazoline* (**20**). — A stirred suspension of **15** (16 mg, 14 μmol) in acetyl chloride (1.5 mL) was treated with conc. hydrochloric acid (30 μL). The reaction tube was sealed, and the mixture stirred for 24 h at room temperature. The tube was opened, and the excess of reagents was removed by evaporation under a stream of nitrogen gas. After repeated addition and evaporation of dry toluene (0.25 mL), drying of **17** was completed over potassium hydroxide pellets in a vacuum desiccator (2 h). The glycosyl chloride **17**, without purification, was dissolved in dry acetonitrile (1 mL), and treated with sodium hydrogencarbonate (16 mg) and tetraethylammonium chloride (16 mg). The mixture was stirred, with exclusion of moisture, for 3 h at room temperature, when t.l.c. (20:1 chloroform–methanol, 3 elutions) showed the formation of **20** (main spot), together with minor proportions of the trisaccharide oxazoline **6** and the monosaccharide oxazoline **21**, both having higher mobility. The reaction mixture was applied, without processing, to two thin-layer plates, which were developed twice in 15:1 chloroform–methanol. The band containing **20** was located by cutting a 0.5-cm strip from the center of each plate and spraying it with the anisaldehyde reagent, and the shape of the band was observed by viewing the plate under u.v. light. To extract the product from the silica gel, it was stirred for 30 min with 2:1 chloroform–methanol, and the suspension was filtered (Celite). This extraction was repeated twice, and the filtrates were combined, and evaporated, to yield **20** (9 mg, 53%); amorphous, m.p. 117–119°, $[\alpha]_D^{20}$ −12° (*c* 1.05, dichloromethane); ν_{max}^{KBr} 1745 ($OCOCH_3$), 1675 (C=N, and amide I), 1540 (amide II), 1430, 1375, 1230 ($OCOCH_3$), 930, 1050, and 980 cm^{-1}; ^{1}H-n.m.r.: δ 5.81 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.54 (d, *J* 3 Hz), 5.12 (d, *J* 3 Hz), 3.83 (d, *J* 4 Hz), 3.73, 3.6 (d, *J* 4 Hz), 3.44, 2.13, 2.07, 2.0, 1.94, and 1.87 (s, 39 H, 11 $OCOCH_3$, $NHCOCH_3$, CH_3 of oxazoline).

Anal. Calc. for $C_{50}H_{67}N_2O_{31} \cdot CH_3OH$: C, 50.05; H, 5.85; N, 2.28. Found: C, 50.33; H, 6.29; N, 1.95.

O-(*2,3,4,6-Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1*→*3*)-O-(*2,4,6-tri*-O-*acetyl*-β-D-*mannopyranosyl*)-(*1*→*4*)-O-(*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1*→*4*)-*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-α-D-*glucopyranosyl phosphate* (**23**). — Compound **20** (12 mg, 10 μmol) was exhaustively dried, by repeated addition and evaporation of dry toluene (0.5 mL), followed by storage *in vacuo* over phosphorus pentaoxide for 15 h. Dibenzyl phosphate (25 mg) was dissolved in dry 1,2-dichloroethane (0.5 mL), and 150 μL of this solution (equivalent to 7.5 mg, 27 μmol of reagent) was quickly added to the reaction tube, with stirring. The tube was flushed with dry nitrogen, and sealed, and the contents were kept overnight at room temperature, when t.l.c. (10:1 chloroform–methanol) showed the formation of compound **22** (R_F 0.66) having almost the same mobility as **20**, but giving a positive reaction with the phosphate spray. Some hydrolysis product (R_F 0.35) was also formed. The reaction mixture was directly applied to two thin-layer plates, which were eluted with either 10:1 chloroform–methanol, or 2:1 acetone–toluene. The position of the band containing **22** was located by spraying with the phosphate-specific reagent, by cutting a 0.5-cm strip from the center of each plate and spraying with the anisaldehyde reagent, and by viewing the plates under u.v. light. The unstable product **22** was extracted from the silica gel by repeatedly stirring with 2:1 chloroform–methanol, allowing the gel to settle, and decanting the clear, supernatant liquor. The extracts were combined, and evaporated to dryness, and the residue was quickly dissolved in methanol (2 mL), mixed with 10% palladium-on-charcoal (10 mg, Tridom-Fluka), and hydrogenated at 1.5 atm for 1 h. Examination by t.l.c. (10:1 chloroform–methanol) showed that **22** (R_F 0.66) had been converted into a compound remaining at the origin (phosphate spray). T.l.c. (60:35:6 chloroform–methanol–water) showed a single product (R_F 0.33) according to the phosphate spray, but several non-phosphate-containing materials were also present (anisaldehyde reagent). The reaction mixture was made neutral with pyridine (1 drop), and the catalyst was filtered off and washed with methanol. The combined filtrates were concentrated, and the solution was applied to two thin-layer plates, which were developed with 60:35:6 chloroform–methanol–water. The band containing **23** was located by spraying with the phosphate-specific reagent, and **23** was extracted from the silica gel by stirring overnight with 10:10:3 chloroform–methanol–water. After filtration (Celite) and evaporation, the residue was dissolved in 5:1 chloroform–methanol. The resulting solution was filtered (sintered glass), and evaporated (N_2 gas), to yield **23** (3.5 mg, 27%), amorphous, m.p. 157–159°, $[\alpha]_D^{20}$ 0.0° (*c* 0.7, 5:1 chloroform–methanol): ν_{max}^{KBr} 1750 ($OCOCH_3$), 1675 (amide I), 1545 (amide II), 1375, 1230, and 1000 (broad) cm^{-1}.

Anal. Calc. for $C_{50}H_{70}N_2O_{35}P \cdot 2\ H_2O$: C, 45.29; H, 5.62. Found: C, 44.93; H, 5.70.

For further characterization, **23** (1 mg) was treated with M hydrochloric acid (0.2 mL) and the mixture was kept in a sealed tube for 4 h at 100°. After evaporation

(N_2 gas), water (0.2 mL) was twice added and evaporated, and then toluene (0.2 mL) was thrice added and evaporated. Drying of the residue was completed over potassium hydroxide pellets *in vacuo* (24 h), after which the sugars were acetylated, subjected to methanolysis, and converted into per(trimethylsilyl) ethers of methyl D-mannopyranosides and methyl 2-acetamido-2-deoxy-D-glucopyranosides, and these were analyzed by g.l.c. as described for **6**. Calc.: ratio of methyl D-mannosides to methyl 2-acetamido-2-deoxy-D-glucosides: 1.0:1.0. Found: 0.91:1.0.

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