*Carbohydrate Research*, 53 (1977) 67–84


# THE SYNTHESIS OF ALLYL 2-ACETAMIDO-3,6-DI-*O*-BENZYL-2-DEOXY-α-D-GLUCOPYRANOSIDE AND OF CHITOBIOSE DERIVATIVES BY THE OXAZOLINE PROCEDURE*

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(Received September 7th, 1976; accepted for publication, September 23rd, 1976)

## ABSTRACT

Controlled, partial benzylation of allyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside gave a mixture of the 3,4-di-, 3,6-di- (**15**), and 3,4,6-tri-*O*-benzyl derivatives, the major product being **15**. Condensation of **15** with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline gave a disaccharide which, after purification, removal of the allyl group, and hydrogenolysis of the benzyl substituents, gave 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranose. This compound was further converted into di-*N*-acetyl-hexa-*O*-acetylchitobiose by acetylation, or into 2-methyl-[4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline, a starting material for the preparation of di-*N*-acetyl-α-chitobiosyl phosphate.

## INTRODUCTION

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate (*N*,*N*′-diacetyl-3,6,3′,4′,6′-penta-*O*-acetyl-α-chitobiosyl phosphate) (**1**) is the starting material for the chemical synthesis of $P^1$-di-*N*-acetyl-α-chitobiosyl $P^2$-dolichyl pyrophosphate[1] (**2**), which is probably a precursor in the biosynthesis of the carbohydrate chains of *N*-glycoproteins[2] [glycoproteins having a 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine carbohydrate–protein linkage]. The extension of this procedure to the synthesis of dolichyl pyrophosphate diesters (**4**) that contain an

*Amino Sugars CVIII. This is publication No. 716 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School and Massachusetts General Hospital. This investigation was supported by research grants from the National Institute of Arthritis, Metabolism, and Digestive Diseases (AM-03564 and AM-05067), National Institutes of Health, U.S. Public Health Service.

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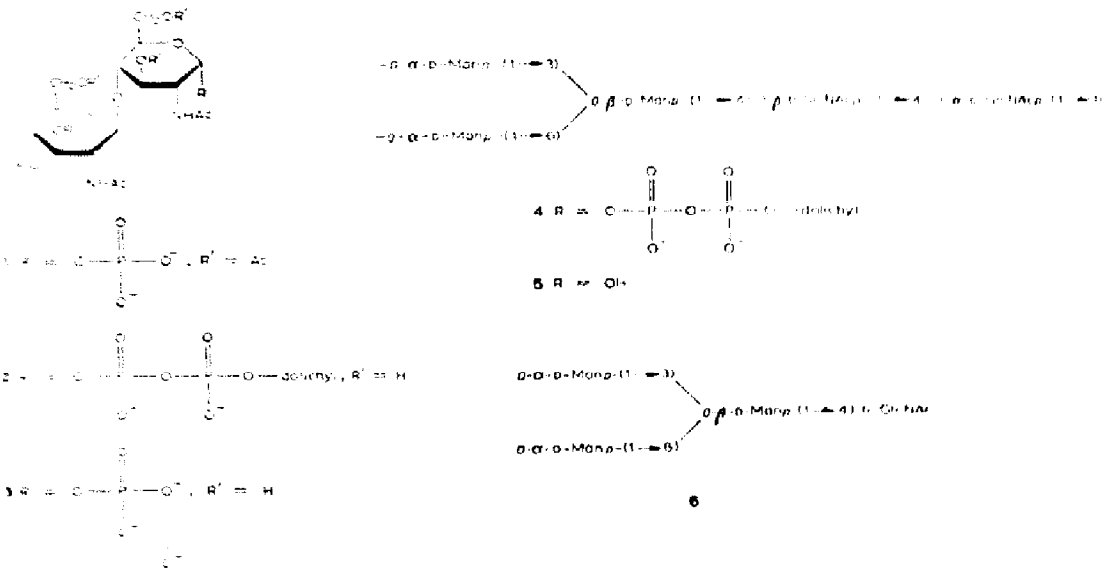
oligosaccharide moiety and are probably also intermediates in glycoprotein biosynthesis[2] requires, as the starting material, compounds containing several D-mannopyranosyl residues linked to a di-*N*-acetylchitobiosyl residue (**5**). The oligosaccharides (**6**) accumulated in the urine of patients[3,4] suffering from mannosidosis are very similar to these oligosaccharides, but lack the terminal 2-acetamido-2-deoxy-D-glucose residue. Linking these natural oligosaccharides (**6**) to a 2-acetamido-2-deoxy-D-glucose residue through a $\beta$-D-(1→4) linkage would give **5**, which, in turn, could be converted into a glycosyl phosphate, the synthetic precursor of the pyrophosphate diesters **4**.



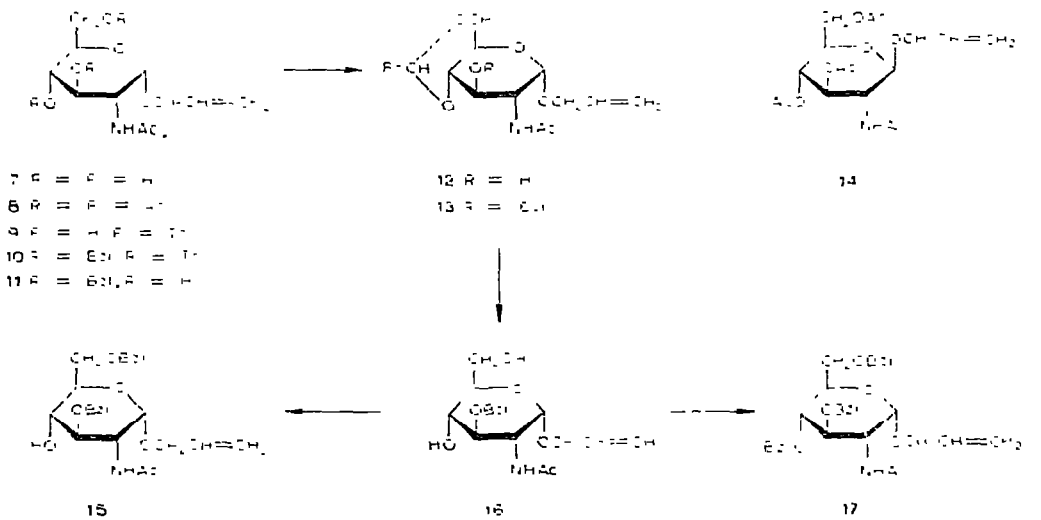
This paper describes the synthesis of allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-$\alpha$-D-glucopyranoside (**15**), which is a suitably protected, synthetic intermediate for the synthesis of **4** from **7**. The usefulness of **15** was demonstrated by the synthesis, in good yields, of derivatives of di-*N*-acetylchitobiose, and of 2-methyl-[2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-$\beta$-D-glucopyranosyl)-3,6-di-*O*-acetyl-1,2-dideoxy-$\alpha$-D-glucopyrano]-[2,1-*d*]-2-oxazoline[5] (**28**), the starting material for preparing di-*N*-acetyl-$\alpha$-chitobiosyl phosphate[1] (**3**).

Chitobiose derivatives have been synthesized previously by Schmitt and Sinaÿ[6] by a different procedure, but the steps involved in the preparation of the synthetic intermediates and in the removal of protecting groups from the final compound were more complex than those described, and would therefore be less readily applicable to the synthesis of oligosaccharide phosphates of biochemical interest.

## RESULTS AND DISCUSSION

For the synthesis of allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-$\alpha$-D-glucopyranoside (**15**), the starting compound was allyl 2-acetamido-2-deoxy-$\alpha$-D-gluco-

pyranoside[7] (**7**), prepared by a modified, Fischer glycosidation. In order to determine whether the product contained an appreciable proportion of the β anomer, as observed for the methyl glycoside[8], **7** was converted[6] into the 3,4,6-tri-*O*-acetyl derivative **8**, which could be separated by t.l.c. from the β-D anomer **14**, a compound also prepared by treatment of 2-methyl-(2-acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**18**) with allyl alcohol. This t.l.c. showed that the **7**, obtained by direct crystallization from the glycosidation mixture, had contained only a small proportion of the β-D anomer. Compound **7** was converted into the 4,6-*O*-benzylidene derivative **12**, which was benzylated to give the 3-*O*-benzyl derivative **13**



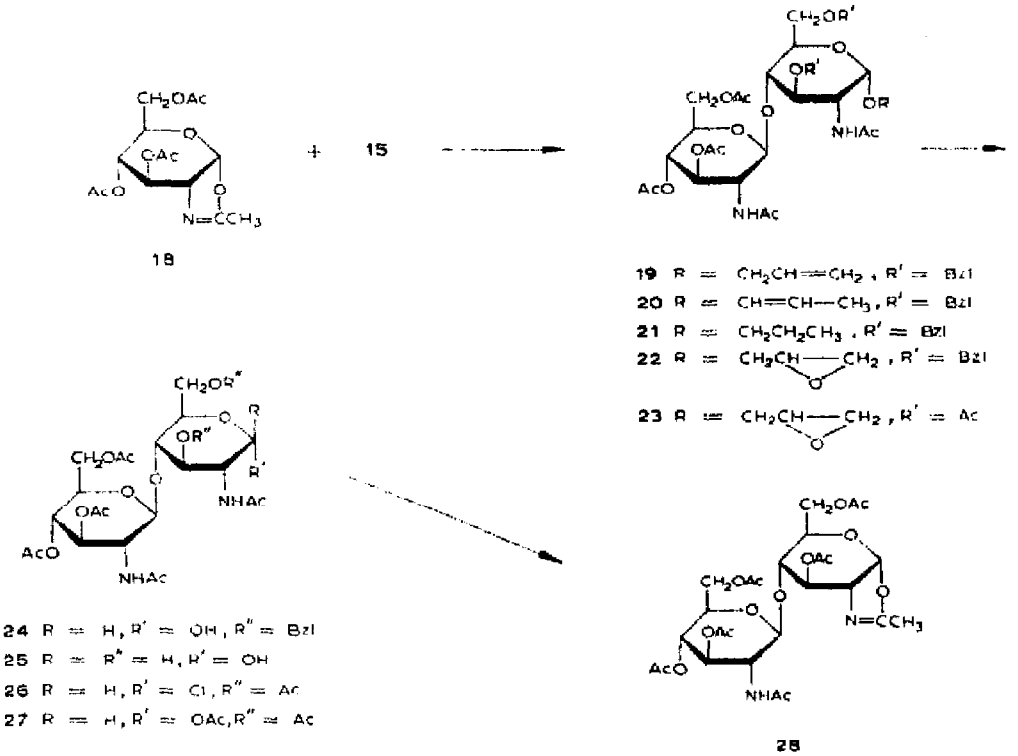
Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**16**) was obtained by mild, acid hydrolysis of **13**. The desired 3,6-di-*O*-benzyl derivative **15** was prepared from **16** by selective, partial benzylation*. This procedure is preferable to the alternative route involving tritylation at O-6, protection of the hydroxyl group at C-4, detritylation, benzylation at O-6, and removal of the O-4 substituent, a route that not only is longer but requires[10] an alkali-stable group for protection at O-4. Neither allyl nor benzyl ethers are suitable for this purpose, as they are already in use as protective groups at other positions, and protection by acetyl groups involves the risk of migration following removal of the trityl group from O-6.

Treatment of **16** with 1 molar equivalent of α-bromotoluene in *N,N*-dimethylformamide, in the presence of a small proportion of sodium hydroxide, gave a mixture of three products (t.l.c.) together with a small proportion of unchanged **16**. The product having the highest chromatographic mobility was shown to be allyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**17**) by comparison

*After this work had been completed, Jaquinet and Sinaÿ[9] reported the use of selective benzylation for preparing the corresponding benzyl glycoside

with the product obtained from **16** or **7** under exhaustive *O*-benzylating conditions. Of the other two products, the one with the higher mobility in t.l.c. was the major product; it was expected to be the 3,6-di-*O*-benzyl derivative **15**, owing to the known, relative ease of benzylation of the primary hydroxyl group, as compared with the sterically hindered, secondary hydroxyl group at C-4. After separation by preparative-layer chromatography, both compounds had the elementary analysis and i.r. spectrum expected for a di-*O*-benzyl derivative, but, surprisingly, neither of them gave a trityl ether when subjected to prolonged treatment with chlorotriphenylmethane in anhydrous pyridine. Therefore, **7** was converted into the 6-*O*-trityl derivative **9**, which was fully benzylated to give **10**. Mild, acid hydrolysis of **10** gave authentic allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**11**), which cochromatographed with the slower-moving of the two partially benzylated compounds, indicating that the faster-moving, major component was, indeed, the 3,6-di-*O*-benzyl derivative **15**. By this convenient, selective benzylation procedure, **15** was obtained in a yield of 65% based on **16**. When the benzylation was performed with a mixture of barium oxide and barium hydroxide, instead of sodium hydroxide, formation of the 3,4-di-*O*-benzyl derivative **11** was minimized, but a large proportion of the starting compound **16** was recovered unchanged.

For the coupling of a second residue of 2-acetamido-2-deoxy-D-glucose to allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**15**) to form a disaccharide having a β-D-(1→4) linkage, the oxazoline method was chosen because of its anomeric specificity[11], and because the conditions employed for the preparation of the oxazoline are mild and unlikely to affect the glycosidic linkages of an oligosaccharide. Glycosidations with 1,2-oxazolines have usually been performed in toluene–nitromethane, with *p*-toluenesulfonic acid as the reaction catalyst[11–13]. When a mixture of **15** and 2-methyl-(2-acetamido-3,4,6-tri-*O*-acetyl-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[14] (**18**) in 1:1 toluene–nitromethane was treated with enough *p*-toluenesulfonic acid to give pH 4, and then heated to 80°, most of the oxazoline precipitated as the *p*-toluenesulfonium salt. After prolonged heating and stirring, some of the salt had redissolved, but the reaction mixture turned black, and t.l.c. revealed the formation of numerous by-products and of only a trace of the disaccharide **19**, which migrated very close to the starting compound **15**. By changing the solvent to 1,2-dichloroethane, the solubility of the *p*-toluenesulfonium salt of **18** was greatly increased, so that the formation of **19** took place smoothly, although some competing side-reactions still occurred. Moreover, the unchanged 3,6-di-*O*-benzyl compound **15** could be readily recovered by chromatography, and recycled, and the yield of **19** after three treatments of **15** with **18** was 43%, based on **15**. Such a yield may be considered satisfactory in view of the poor nucleophilic properties of the hindered OH-4 group in **15**, and compares favorably with the yield obtained by Schmitt and Sinaÿ[6], who did not use the oxazoline procedure. When the synthesis of **19** was attempted with repeated additions of **18**, without intermediate processing, in an attempt to drive the glycosidation nearer to completion[13], the yield of **19** was unchanged after two additions of **18** (and a reaction time of 5 h), and the recovery of the starting compound




**15** was less; after three additions, and a reaction time of 7 h, both the yield of **19** and the recovery of **15** were markedly decreased. When the reaction mixture was stirred overnight at 80°, the yield of **19** became almost zero. In other attempts to improve the yield of **19**, different solvents were tried, but only dichloromethane gave a yield approaching that obtained with 1,2-dichloroethane. It was also found that anhydrous *p*-toluenesulfonic acid is necessary, as the hydrate gave very low yields of **19**. Lemieux and Driguez[14] reported that use of trifluoromethanesulfonic acid increases the yield in this type of glycosidation, although at the risk of decreasing the anomeric specificity. In the presence of this reagent, however, the formation of **19** took place much more slowly, and the eventual extent of the conversion of **15** into **19** was only about half that obtained previously.

After a preliminary chromatographic purification, which did not separate **19** from **15**, the crude **19** was *O*-deacetylated, and the product separated from **15** by preparative-layer chromatography. Reacetylation gave pure, crystalline allyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**19**), characterized by elementary analysis, i.r. and $^1$H-n.m.r. spectra, optical rotation, and conversion into *N*,*N*'-diacetylhexa-*O*-acetylchitobiose.

For the preparation of derivatives of chitobiose, **19** was converted into the 1-propenyl glycoside **20** by treatment with tris(triphenylphosphine)rhodium chloride, and the 1-propenyl group was removed with mercuric chloride[18] to give the reducing disaccharide **24**. In some experiments, a small proportion of the isomerized product was resistant to the treatment with mercuric chloride. After preparative-layer chromatography, and crystallization, a compound was isolated that had the same i.r. spectrum, and almost the same elementary analysis, as the allyl glycoside **19**. However, it could be distinguished from **19** by mixed m.p., $^1$H-n.m.r. spectrum, and the absence of unsaturation. As the $^1$H-n.m.r. spectrum indicated the presence of a propyl group, the propyl glycoside **21** was prepared by partial hydrogenation of **19**, to give a crystalline compound identical with the product just described. The formation of **21** from **19** or **20** in the presence of tris(triphenylphosphine)rhodium chloride may be explained by the excellent catalytic properties of this reagent[19] for hydrogenation, and its ability to transfer hydrogen from an alcoholic solvent to an unsaturated derivative[20]: the occurrence of this type of reaction during the isomerization of allyl groups by this catalyst has not been reported previously. The benzyl groups of **24** were removed by catalytic hydrogenolysis, to give 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranose (**25**).

It is evident that **25** would have been obtained by fewer synthetic stages had the benzyl glycoside[9] been employed instead of the allyl glycoside **15**. However, the object of this work was not primarily to develop a new synthesis of *N,N'*-diacetylhexa-*O*-acetylchitobiose, but to obtain a derivative, such as **24**, having OH-1 available for the preparation of a glycosyl phosphate without removal of the benzyl groups at O-3 and O-6. In addition, the allyl group of the intermediate compounds shows a diagnostic color reaction with the anisaldehyde spray[21], and it can be detected by the potassium permanganate spray on preparative-layer chromatograms. Finally, allyl glycosides are useful starting-compounds for preparing 1,2-epoxypropyl glycosides (for use as enzyme inhibitors[22]) and 3-(2-aminoethylthio)propyl glycosides that can be linked to solid supports for affinity chromatography[7].

Thus, **19** was treated with chloroperoxybenzoic acid[23] to give the epoxypropyl derivative **22**. Removal of the benzyl groups by catalytic hydrogenolysis, followed by purification by preparative-layer chromatography, and *O*-acetylation with acetic anhydride–pyridine, afforded 2,3-epoxypropyl 2-acetamido-4-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**23**), which, on thin-layer chromatograms, gives a vivid, diagnostic-color reaction with the anisaldehyde spray[21] that is rather similar to that obtained with allyl derivatives.

*O*-Acetylation of **25** with acetic anhydride and pyridine gave *N,N'*-diacetylhexa-*O*-acetylchitobiose, identical by thin-layer chromatography and mixed melting point with the compound prepared from chitin[24]. Alternatively, **25** was treated with acetyl chloride[25] to give the glucopyranosyl chloride **26**, which was efficiently converted, without purification, into 2-methyl-[2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-1,2-dideoxy-α-D-gluco-

pyrano]-[2,1-*d*]-2-oxazoline (**28**) by an adaptation of the method of Lemieux and Driguez[14]. For comparison, **28** was prepared from *N,N'*-diacetylhexa-*O*-acetylchitobiose[24] by *O*-deacetylation, and treatment of the product with acetyl chloride, to give the peracetylated glycosyl chloride **26**. It was found unnecessary to employ hydrogen chloride[5] in this reaction. The oxazoline **28** could be obtained from **26** by treatment with silver nitrate plus 2,4,6-trimethylpyridine[5], but the resulting product had to be purified chromatographically before it was useful for comparison, or for synthetic purposes[1]. A purer product was obtained by conversion of **26** into 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose by the action of mercuric acetate in glacial acetic acid[25], followed by treatment with ferric chloride, *i.e.*, an adaptation of the method of Bach and Fletcher as reported by Matta *et al.*[26]. However, the best method of preparing **28** was treatment of **26** with tetraethylammonium chloride plus sodium hydrogencarbonate in acetonitrile, the procedure recently reported by Lemieux and Driguez[14] for the preparation of 2-methyl-(2-acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**18**). Compound **28** prepared according to this procedure was suitable for synthetic purposes[1] without further purification, but it was less pure, in t.l.c., than the sample of **28** synthesized from **15**, because peracetylated chitobiose prepared from chitin is contaminated with 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5,6-di-*O*-acetyl-2,3-dideoxy-*aldehydo*-D-*erythro*-hex-2-enose[27], and traces of this or related compounds are always present in **26** and **28** prepared from chitobiose, unless they are purified chromatographically.

We have shown[1] that **28** can be converted into 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate (**1**), which is the synthetic precursor of $P^1$-di-*N*-acetylchitobiosyl $P^2$-dolichyl pyrophosphate (**2**). Therefore, the present work makes procedures available for (*a*) synthesizing compounds containing a di-*N*-acetylchitobiose moiety, and (*b*) converting these compounds into α-linked D-glycopyranosyl phosphates, and thence, into the dolichyl pyrophosphate diesters required for related biosynthetic studies[28].

## 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. N.m.r. spectra were recorded at 60 MHz with a Varian T-60 spectrometer for solutions in chloroform-*d* containing 1% of tetramethylsilane as the internal standard, unless otherwise stated. Evaporations were conducted *in vacuo*, with the bath temperature kept below 30°. Microanalyses were performed by Dr. W. Manser, CH-8704 Herliberg, Switzerland, or by Galbraith Laboratories Inc., Knoxville, Tennessee 37921, U.S.A.

*Chromatographic separations.* — T.l.c. was performed on precoated plates of Silica gel G (E. Merck A.G., Darmstadt, Germany). The plates supplied (20 × 20 cm) were cut to a length of 6 cm, and used without pretreatment. Preparative-layer chromatography was performed on precoated PLC plates, Silica gel G (Merck). The spray reagent, unless otherwise stated, was 1:1:18 (v/v) anisaldehyde–sulfuric acid–ethanol[21], and the plates were heated to 125°. Unsaturation was detected with a 1% aqueous solution of potassium permanganate in 2% sodium carbonate. All proportions of solvents are v/v. The $R_F$ value was calculated from measurement of the distance from the origin of the chromatogram to the point of maximum intensity of the spot after development.

*Allyl 2-acetamido-2-deoxy-α-D-glucopyranoside* (**7**). — The following procedure gave a product that crystallized much more readily, and in higher yield, than that obtained by the method of Lee and Lee[7]. A mixture of 2-acetamido-2-deoxy-D-glucose (20 g), allyl alcohol (225 ml, dried over molecular sieve), and boron trifluoride etherate (2.5 ml) was boiled for 2 h under reflux, with stirring. A 2% solution of hydrogen chloride in allyl alcohol (5 ml) was added, and the mixture was boiled for a further 1 h under reflux, cooled, treated with ether to the point of turbidity, and kept overnight at 4°. The resulting, crystalline mass was filtered off, and washed with ether, giving a product (12.0 g) having $[\alpha]_D^{20}$ +148° (*c* 1, ethanol). This product gave a single major spot in t.l.c. in 60:25:4 chloroform–methanol–water ($R_F$ 0.75), and was suitable for synthetic purposes without purification. A second crop of crystals (1.23 g) had $[\alpha]_D^{20}$ +142° (*c* 1, ethanol). Recrystallization of the first crop from ethyl acetate gave plates, m.p. 159–160° (softening at 154°); n.m.r. data ($D_2O$ with 4,4-dimethyl-4-silapentane-1-sulfonate as the internal standard): $\delta$ 2.03 (3 H, NHCOC$H_3$), 3.63, 3.85 and 4.18 (m, 6 H, 4 pyranose-ring H and C$H_2$OH), 4.98 (d, *J* 3 Hz), 5.23 (d, *J* 3.5 Hz), and 5.45 (d, *J* 6 Hz, 4 H, anomeric H and C$H_2$=C*H*–C$H_2$ group).

*Anal.* Calc. for $C_{11}H_{19}NO_6$: C, 50.56; H, 7.34; N, 5.36; O, 36.75. Found: C, 50.47; H, 7.34; N, 5.36; O, 36.70.

Lee and Lee[7] reported m.p. 172–174°, $[\alpha]_D^{25}$ +148.8° (*c* 1.62, water), but no figures for elementary analysis.

*Allyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside* (**8**). — Compound **7** was acetylated with 1:2 acetic anhydride–pyridine (1 ml) for 24 h at room temperature. The mixture was treated with water (0.5 ml), and then evaporated. After two additions and evaporations of toluene, **8** was obtained, giving a single spot in t.l.c. As it was required only for chromatographic purposes, it was not recrystallized.

*Allyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside* (**14**). — A mixture of 2-methyl-(2-acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[14] (**18**) (0.5 g), allyl alcohol (4 ml), and anhydrous *p*-toluenesulfonic acid (0.7 mg in 100 µl of toluene; prepared by fusing *p*-toluenesulfonic acid hydrate at 110° *in vacuo* over phosphorus pentaoxide, and dissolving the residue in dry toluene) was kept for 1 h at 80°, when t.l.c. (10:1 chloroform–methanol) showed a single, major spot ($R_F$ 0.58) having the purple color diagnostic of an allyl derivative and indicating the complete reaction of the oxazoline. The mixture was cooled, and

the acid was neutralized with pyridine (1 ml). Evaporation, followed by two additions and evaporations of toluene, afforded a residue that crystallized from ethanol–ether–hexane to give **14** (0.25 g); this was recrystallized from ethyl acetate or ethanol, m.p. 162–166°, $[\alpha]_D^{20}$ −22° (*c* 1.0, methanol); lit.[22] m.p. 160°, $[\alpha]_D^{23}$ −15.1° (*c* 2.06, chloroform)

The α anomer **8** was well separated from the β anomer **14** by t.l.c. in 10:1 chloroform–methanol. $R_f$ 0.70 (α) and 0.58 (β), showing that **8** was contaminated by less than 3% of **14**.

*Allyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside* (**12**). — A mixture of **7** (0.5 g) and benzaldehyde (3 ml) was stirred and cooled to −10°, and then treated with 99% formic acid (2.5 ml, ICN Pharmaceuticals, Life Sciences Group, 26201 Miles Road, Cleveland, Ohio 44128). The mixture was stirred at room temperature under anhydrous conditions for 1 h, when t.l.c. (5:1 chloroform–methanol) showed that 60% of **7** had been converted into **12**. (After a longer period of reaction, the proportion of **12** had not appreciably increased, and t.l.c. showed the formation of by-products). The mixture was poured into a rapidly stirred, saturated solution of potassium carbonate (7.5 ml) while being cooled to −10°. The suspension of **12** and potassium salts was treated with hexane (20 ml) and water (10 ml), and stirred for 30 min at room temperature. The solid product was filtered off and washed with water and hexane, to give crude **12** (~0.5 g) showing a single, major spot ($R_F$ 0.50) in t.l.c. (5:1 chloroform–methanol), but containing residual salt. Recrystallization from hot ethanol–water gave crystals (0.25 – 0.37 g), m.p. 234–237° (subliming into very long needles), $[\alpha]_D^{20}$ +99° (*c* 1, *N*,*N*-dimethylformamide); $\nu_{max}^{KBr}$ 3410, 3300, 3070, 2940, 2860, 1640, 1550, 1455, 1420, 1375, 1325, 1275, 1225, 1175, 1160, 1135, 1115, 1070, 1025, 1000, 975, 960, 930, 770, 750, and 690 $cm^{-1}$.

*Anal.* Calc. for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.65; N, 4.01; O, 27.48. Found: C, 61.79; H, 6.67; N, 4.01; O, 27.50.

*Allyl 2-acetamido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside* (**13**). — A mixture of **12** (0.42 g), α-bromotoluene (0.45 ml), and *N*,*N*-dimethylformamide (9 ml) was treated with barium oxide (1.5 g) plus barium hydroxide octahydrate (0.4 g), and stirred at room temperature under anhydrous conditions. The reaction was monitored by t.l.c. (10:1 chloroform–methanol), and, when the conversion of **12** ($R_F$ 0.40) into **13** ($R_F$ 0.84) was apparently complete, the mixture was diluted with chloroform (50 ml) and filtered through Celite. After evaporation, and removal of residual *N*,*N*-dimethylformamide by additions and evaporations of toluene, the product crystallized from ethanol, to give **13** (0.4 g) as needles, m.p. 224–228°, $[\alpha]_D^{20}$ +88° (*c* 1.1, chloroform); $\nu_{max}^{KBr}$ 3280, 3100, 2940, 2860, 1650, 1560, 1500, 1450, 1375, 1360, 1225, 1180, 1125, 1090, 1060, 1020 (broad), 975, 960, 925, 730, and 685 $cm^{-1}$.

*Anal.* Calc. for $C_{25}H_{29}NO_6$: C, 68.29; H, 6.66; N, 3.19; O, 21.84. Found: C, 68.29; H, 6.70; N, 3.22; O, 21.83.

*Allyl 2-acetamido-3-O-benzyl-2-deoxy-α-D-glucopyranoside* (**16**). — A mixture of **13** (0.1 g) and 60% acetic acid (2 ml) was stirred for 1 h at 70°, or until t.l.c.

(10:1 chloroform–methanol) showed complete conversion of **13** ($R_F$ 0.84) into **16** ($R_F$ 0.25). Evaporation, followed by two additions and evaporations of toluene, gave a solid which was recrystallized from ethanol, to give **16** (55 mg), m.p. 164.5–166.5°, $[\alpha]_D^{20}$ +122° (*c* 0.4, ethanol): $\nu_{max}^{KBr}$ 3300, 2940, 2860, 1640, 1550, 1500, 1450, 1375, 1345, 1230, 1170, 1125, 1100, 1050 (broad), 1025, 1000, 930 (broad), 860, 725, and 680 $cm^{-1}$.

*Anal.* Calc. for $C_{18}H_{25}NO_6$: C, 61.53; H, 7.19; N, 3.99; O, 27.32. Found: C, 61.58; H, 7.24; N, 4.09; O, 27.32.

*Allyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**17**), *allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**11**), *and allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**15**) *from* **16**. — *A*. A mixture of **16** (0.5 g, not recrystallized), powdered sodium hydroxide (0.25 g), α-bromotoluene (0.26 g), and *N*,*N*-dimethylformamide (13 ml) was stirred at room temperature. The reaction was monitored by t.l.c., which showed the rapid formation from **16** ($R_F$ 0.25) of **11** ($R_F$ 0.46) and **15** ($R_F$ 0.67), and the slower formation of **17** ($R_F$ 0.85). When the proportion of **15** was at the maximum (at 15 min) and a small proportion of **16** remained unchanged, the reaction was stopped by the addition of toluene (200 ml). The precipitate was filtered off (Celite) and washed with toluene, and the filtrates were combined and evaporated; addition and evaporation of toluene removed the residual solvent. A solution of the residue in 2:1 chloroform–methanol (2 ml) was applied to three preparative-layer plates (20 × 20 cm), which were then developed with 20:1 chloroform–methanol. The plates were dried in air, and redeveloped in the same solvent system, and the bands containing compounds **11**, **15**, and **16** were detected with the potassium permanganate spray. The silica gel containing each band was removed from the plate, and stirred overnight with 5:1 chloroform–methanol. Filtration of the solutions through Celite, and evaporation, gave unchanged **16** (83 mg), **11** (161 mg), and **15** (339 mg, 65% based on the amount of **16** that was not recovered).

*B*. A mixture of **16** (90 mg), barium oxide (0.50 g), barium hydroxide octahydrate (0.13 g), α-bromotoluene (90 mg), and *N*,*N*-dimethylformamide (3 ml) was kept for 1 h at 100°, after which t.l.c. (10:1 chloroform–methanol) showed that the reaction had stopped. The mixture was processed and chromatographed as in *A*, to give unchanged **16** (31 mg) and **15** (37 mg), the yield of **11** (t.l.c.) being negligible.

*C*. A mixture of **16** (100 mg), powdered sodium hydroxide (100 mg), α-bromotoluene (100 mg), and *N*,*N*-dimethylformamide (10 ml) was stirred for 5 h at room temperature, when t.l.c. (10:1 chloroform–methanol) showed that **17** was the major product, together with some **11** and **15**. The mixture was processed and chromatographed as in *A*, to yield **17** (92 mg), **15** (33 mg), and **11** (21 mg).

Compound **17** was recrystallized from toluene–hexane: m.p. 128–130° (change of form at 123–124°), $[\alpha]_D^{22}$ +92° (*c* 1.3, chloroform).

*Anal.* Calc. for $C_{32}H_{37}NO_6$: C, 72.26; H, 7.03; N, 2.63; O, 18.05. Found: C, 72.13; H, 7.02; N, 2.58; O, 18.03.

Compound **11** was recrystallized from ethyl acetate; m.p. 165.5–166.5°, $[\alpha]_D^{22}$ +119° (*c* 0.7, chloroform).

*Anal.* Calc. for $C_{25}H_{31}NO_6 \cdot 0.5H_2O$: C, 66.59; H, 7.17; N, 3.11; O, 23.08. Found: C, 66.71; H, 7.03; N, 3.13; O, 23.16.

Compound **15** was recrystallized from ether as needles, m.p. 97–98° (soft. at 89°), $[\alpha]_D^{20}$ +90° (*c* 1.3, chloroform); $\nu_{max}^{KBr}$ 3400, 3300, 3060, 3040, 2930, 2865, 1650, 1530 (broad), 1495, 1450, 1375, 1205, 1120, 1035, 925, 730, and 680 $cm^{-1}$; n.m.r. data: $\delta$ 1.9 (3 H, $NHCOCH_3$), 3.77 (s, 4 H, 2 pyranose-ring protons, $CH_2OH$), 4.10 (d, 1 H), 4.60 (s, 1 H, deuteratable, OH-4), 4.76 (s, 4 H, $CH_2$ of Bzl, split into two singlets at 4.70 and 4.73 by deuteration), 4.93 (d, *J* 4 Hz), 5.05 (d, *J* 3 Hz), 5.25 and 5.38 (4 H, anomeric H and $CH_2$=$CH$–$CH_2$–), and 7.37 (10 H, aromatic ring).

*Anal.* Calc. for $C_{25}H_{31}NO_6$: C, 67.98; H, 7.09; N, 3.17; O, 21.74. Found: C, 67.97; H, 7.15; N, 3.19; O, 21.83.

*Attempted tritylation of* **11** *and* **15**. — Compound **11** or **15** (5 mg) was treated with chlorotriphenylmethane (10 mg) and dry pyridine (0.5 ml) for 2 weeks, and examined by t.l.c. (10:1 chloroform–methanol), which showed that no new compound had been formed from either **11** or **15**.

*Allyl 2-acetamido-2-deoxy-6-O-trityl-α-*D*-glucopyranoside* (**9**). — A mixture of **7** (50 mg), chlorotriphenylmethane (100 mg), and dry pyridine (2.5 ml) was kept for 3 days at room temperature, when t.l.c. (10:1 chloroform–methanol) showed that most of the **7** ($R_F$ 0.06) had been converted into the 6-*O*-trityl derivative **9** ($R_F$ 0.39). Evaporation, followed by two additions and evaporations of toluene, gave a crude product which was purified by preparative-layer chromatography on one plate (20 × 20 cm) in 10:1 chloroform–methanol, compounds being detected with the potassium permanganate spray. The silica gel was removed from the plate, and stirred with 10:1 chloroform–methanol overnight; evaporation of the resulting solution gave a product which was recrystallized from ether–hexane to give **9** (70 mg) as prisms, m.p. 86–90°, $[\alpha]_D^{22}$ +44° (*c* 2.1, chloroform).

*Anal.* Calc. $C_{30}H_{33}NO_6$: C, 71.55; H, 6.62; N, 2.78; O, 19.06. Found: C, 71.64; H, 6.67; N, 2.60; O, 19.05.

*Allyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-trityl-α-*D*-glucopyranoside* (**10**). — Benzylation of **9** (40 mg) was performed with powdered sodium hydroxide (40 mg) and α-bromotoluene (65 mg) in *N*,*N*-dimethylformamide (6.5 ml) for 2 h at room temperature, when t.l.c. (10:1 chloroform–methanol) showed complete conversion of **9** ($R_F$ 0.39) into **10** ($R_F$ 0.90). The mixture was diluted with toluene (50 ml), filtered through Celite, and the filtrate evaporated. After three additions and evaporations of toluene, the residue was taken up in ether, the resulting suspension filtered, and the filtrate evaporated, to give an oily product which was washed three times with hexane. The residue was crystallized from ether–hexane, to give **10** (26 mg) as prisms, m.p. 166–168° (softening at 153°), $[\alpha]_D^{21}$ +70° (*c* 0.85, chloroform).

*Anal.* Calc. for $C_{44}H_{45}NO_6$: C, 77.25; H, 6.65; N, 2.05; O, 14.14. Found: C, 77.19; H, 6.65; N, 1.98; O, 14.16.

*Allyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-α-*D*-glucopyranoside* (**11**). —

Hydrolysis of **10** (21 mg) was performed with 60% acetic acid (0.5 ml) to which was added sufficient 2:1 chloroform–methanol to give a single phase. The mixture was kept for 1.5 h at 70°, when t.l.c. (10:1 chloroform–methanol) showed that **10** ($R_F$ 0.9) had been converted into a product having $R_F$ 0.46. The solvents were evaporated, and the residue was purified by chromatography on a thin-layer plate (20 × 20 cm) in 10:1 chloroform–methanol, the compounds being detected with the potassium permanganate spray. The silica gel was stirred overnight with 5:1 chloroform–methanol; filtration of the resulting suspension and evaporation of the filtrate gave a solid which was recrystallized from ethyl acetate, to give **11** (13 mg), m.p. 163–165°, $[\alpha]_D^{22}$ +119° (*c* 1.3, chloroform); after admixture with the product prepared by partial benzylation of **16**, the m.p. was 165–166°, and the two products cochromatographed in t.l.c.

*Allyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside* (**19**). — To a solution of 2-methyl-(2-acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[14] (**18**, 100 mg) in 1,2-dichloroethane (1 ml) was added **15** (100 mg, dried *in vacuo* over phosphorus pentaoxide for 15 h). The mixture was stirred until all of the **15** had dissolved, and was then treated with a solution of anhydrous *p*-toluenesulfonic acid (2.8 mg) in toluene (0.4 ml; see preparation of **14**) to give a pH of 4. The mixture was stirred for 3 h at 80°, when t.l.c. (10:1 chloroform–methanol) showed that ~30% of **15** ($R_F$ 0.67) had been converted into a product having $R_F$ 0.60 and showing the intense, purple color diagnostic of an allyl derivative. This t.l.c. also revealed the formation of a major by-product ($R_F$ 0.52) of the oxazoline, and of several minor by-products. Therefore, the reaction mixture was cooled, neutralized with pyridine (0.2 ml), and chromatographed on a preparative-layer plate (20 × 20 cm) in 10:1 chloroform–methanol. After being dried in air, the plate was redeveloped with the same solvent mixture, and compounds containing the allyl group were identified with the potassium permanganate spray. This chromatography did not separate **15** from **19**. The mixture of compounds was extracted from the silica gel by stirring it overnight with 5:1 chloroform–methanol. Filtration (Celite) and evaporation, followed by three additions and evaporations of toluene, gave a residue that was treated with an excess of 3% sodium methoxide in methanol. After 2 h at room temperature, t.l.c. (10:1 chloroform–methanol) showed that **19** had been *O*-deacetylated, to give a compound having $R_F$ 0.20, well separated from **15** ($R_F$ 0.67). The mixture was chromatographed on a preparative-layer plate (20 × 20 cm) in 5:1 chloroform–methanol, and detection of the products was performed with the potassium permanganate spray. The upper band (compound **15**) was extracted by stirring the silica gel overnight with 5:1 chloroform–methanol to give, after filtration (Celite) and evaporation, **15** (69 mg). Extraction of the lower band with 60:25:4 chloroform–methanol–water, filtration, and evaporation, followed by three additions and evaporations of toluene, gave a solid residue that was reacetylated with acetic anhydride (0.5 ml) and pyridine (1 ml). The resulting mixture was kept overnight at room temperature, treated dropwise with water until no more heat was evolved, and then evaporated; residual solvent was removed by two additions and evaporations of toluene (2 ml). The residue was taken

up in 5:1 chloroform–methanol, the suspension was filtered (sintered glass), the filtrate was evaporated, and the residue (which, in t.l.c. with 10:1 chloroform–methanol, showed a single spot having $R_F$ 0.60) was triturated with 1:1 hexane–ether and centrifuged (three times) to give **19** (35 mg), m.p. 219–225°. Because most of the unreacted starting-material **15** had been recovered (69 mg), the effective yield of **19**, based on **15**, was 65%. When recovered **15** was re-treated twice with oxazoline **18** (69 mg in the second treatment, and 46 mg in the third), the actual yield of **19** was 75 mg (43%). A sample of **19** was recrystallized from aqueous ethanol to give prisms, m.p. 236–238°, $[\alpha]_D^{20}$ +43° (*c* 0.6, 5:1 chloroform–methanol); $\nu_{max}^{KBr}$ 3300, 2930, 2860, 1740, 1650, 1545, 1450, 1375, 1250, 1230, 1120, 1045, 725, and 680 $cm^{-1}$; n.m.r. data: $\delta$ 1.88 (6 H, $NHCOCH_3$), 1.97, 2.0, and 2.03 (3 s, 9 H, $OCOCH_3$), 3.62 (m) and 4.04 (q, 12 H, pyranose ring H), 4.50 (s, 1 H), 4.63 (s, 4 H, $CH_2$ of Bzl), 4.86 (s, 1 H), 4.95 (d, 2 H, *J* 2.5 Hz), 5.06 (s, 1 H), 7.33 (s, 5 H), and 7.52 (s, 5 H, aromatic ring H).

*Anal.* Calc for $C_{36}H_{50}N_2O_{14}$: C, 60.75; H, 6.55; N, 3.63; O, 29.05. Found: C, 60.65; H, 6.58, N, 3.58, O, 29.08

*1-Propenyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside* (**20**). — A solution of **19** (46 mg) and diazabicyclo[2.2.2]octane (20 mg) in 9:1 ethanol–water (1 ml) was stirred at 85° and treated with $(Ph_3P)_3RhCl$ (5 mg). The mixture was stirred for 1 h at 85°, when t.l.c. (10:1 chloroform–methanol) showed that **19** had been converted into **20**. Although the $R_F$ values of **19** and **20** are similar, the colors of the spots with the anisaldehyde spray are quite different, **20** giving a faint, greenish-brown spot ($R_F$ 0.65), and **19**, the intense, purple color of an allyl derivative ($R_F$ 0.60). In some experiments in which the conversion of **19** into **20** was incomplete, it was necessary to re-treat with equal amounts of base and rhodium complex. For the characterization of **20**, the solvents were evaporated ($N_2$), and the residue was dissolved in chloroform (10 ml). The solution was successively washed with saturated potassium chloride solution, 0.1M hydrochloric acid, and potassium chloride solution, dried (magnesium sulfate), and evaporated, to give a crude product that was triturated with 1:1 hexane–ether. The resulting solid was isolated by centrifugation, to give **20** (41 mg). Although this product gave a single spot in t.l.c., it was not pure, as hydrolysis with mercuric chloride did not proceed to completion (see next paragraph). It was therefore recrystallized from methanol, to give small needles (20 mg), m.p. 260.5–263.5° (softening at 254–256°), $[\alpha]_D^{22}$ +64° (*c* 0.6, chloroform); i.r. spectrum indistinguishable from that of **19**.

*Anal.* Calc. for $C_{36}H_{50}N_2O_{14}$: C, 60.75; H, 6.55; N, 3.63; O, 29.05. Found. C, 60.59; H, 6.54; N, 3.62; O, 29.08.

*2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranose* (**24**). — For the preparation of **24**, it was not necessary to isolate the 1-propenyl derivative **20**. Compound **19** (46 mg) was treated with the rhodium complex as described in the previous paragraph. After evaporation of the solvents, the residue (consisting of **20**, bicyclic base, Rh derivatives,

and $Ph_3PO$) was dissolved in 1:5 water–acetone (6 ml), and the solution was stirred with HgO (100 mg) and $HgCl_2$ (100 mg). After 10 min, t.l.c. (10:1 chloroform–methanol) showed that most of the starting material ($R_F$ 0.60) had been hydrolyzed to give **24** ($R_F$ 0.44), together with a small proportion (~20%) of the $\beta$ anomer, which showed a slightly lower $R_F$ value. A small proportion (~10%) of the starting material was resistant to hydrolysis (see next paragraph). After evaporation of most of the acetone, chloroform (20 ml) was added, and the mixture shaken. The organic layer was separated, and the aqueous layer was extracted twice with chloroform (10 ml). The chloroform solutions were combined, filtered (Celite), washed twice with saturated potassium iodide solution, and dried (magnesium sulfate). The solution was concentrated to ~1 ml, and applied to a preparative-layer plate (20 × 20 cm) which was eluted with 5:1 chloroform–methanol. Compound **24** was detected with the potassium permanganate spray in the lower of two bands, and was extracted from the silica gel with 2:1 chloroform–methanol. After filtration (Celite) of the extract, the filtrate was evaporated, and the residue taken up in 5:1 chloroform–methanol. The suspension was filtered (sintered glass), and the filtrate evaporated, to give a mixture (36 mg) of **24** and its $\beta$ anomer (t.l.c). Recrystallization from aqueous methanol gave pure **24** (20 mg), m.p. 225–226°, $[\alpha]_D^{20}$ +4° (*c* 1, 5:1 chloroform–methanol); $\nu_{max}^{KBr}$ 3440, 3280, 2930, 1740, 1720, 1650, 1540, 1450, 1375, 1250 (a double peak), 1125, 1060 (broad), 970, 725, and 685 $cm^{-1}$.

*Anal.* Calc. for $C_{36}H_{46}N_2O_{14}\cdot 0.5H_2O$: C, 58.45; H, 6.42; N, 3.79. Found: C, 58.34; H, 6.38; N, 3.71.

In order to characterize, as **21**, the compound that had the same $R_F$ value as **20** and was resistant to treatment with mercuric chloride, the upper band of the preparative chromatogram (see previous paragraph) was extracted with 5:1 chloroform–methanol; evaporation of the extract gave a solid, which was recrystallized twice from aqueous methanol to give needles, m.p. 252–254°, $[\alpha]_D^{22}$ +52° (*c* 1.6, chloroform); i.r. spectrum identical with that of **19**; n.m.r. data: $\delta$ 0.95 (t, 3 H, *J* 7 Hz, $CH_3$ adjacent to $CH_2$), and 1.15 (m, 2 H, $CH_2$ adjacent to $CH_2$ and $CH_3$), otherwise identical with the n.m.r. spectrum of **19**; t.l.c.: negative test for unsaturation ($KMnO_4$ spray); in admixture with **19**, the m.p. was depressed to 242–243°.

*Propyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside* (**21**) *from* **19**. — Compound **19** (2 mg) in methanol (0.5 ml) was hydrogenated at 1 atm over 10% Pd-on-charcoal (Fluka, A.G., CH-9470 Buchs, Switzerland) for 2.5 min. Examination by t.l.c. then showed the absence of an allyl ether, and the presence of a trace of debenzylated compound, the main product having the same $R_F$ as **20**. The catalyst was filtered off, and the filtrate evaporated, to give a residue which was crystallized from aqueous methanol; m.p. 251–253°; admixture with the by-product arising during the isomerization of the allyl group of **19** gave m.p. 252–254°.

*2,3-Epoxypropyl 2-acetamido-4-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside* (**23**). — To a solution

of **19** (45 mg) in 1,2-dichloroethane (2 ml) was added 3-chloroperoxybenzoic acid (65 mg), and the mixture was kept overnight at room temperature, when t.l.c. (10:1 chloroform–methanol) showed the conversion of **19** ($R_F$ 0.60) into a new compound having $R_F$ 0.55. The mixture was diluted with chloroform, and the resulting solution was successively washed with saturated $Na_2SO_3$, $NaHCO_3$, $Na_2S_2O_3$, $Na_2CO_3$, and KCl[23], and dried ($MgSO_4$). Evaporation gave a syrup, which was triturated with methanol–ether–hexane to give a solid (**22**) (27 mg), m.p. 194–200°. This epoxypropyl derivative (27 mg) was dissolved in methanol (2 ml), and hydrogenated at 2 atm in the presence of 10% Pd-on-charcoal (Fluka, 25 mg) for 5 h, when t.l.c. (10:1 chloroform–methanol) revealed the formation of a single, major product ($R_F$ 0.2) and traces of several other products. The catalyst was filtered off, and the filtrate was concentrated to 0.5 ml and applied to a thin-layer plate (20 × 20 cm) that was developed with 5:1 chloroform–methanol. The position of the band containing the product was located by cutting strips from the center and sides of the plate, and spraying with the anisaldehyde reagent. The product was extracted from this band of silica gel by treatment overnight with 60:25:4 chloroform–methanol–water. After filtration of the extract, and evaporation of the filtrate, followed by two additions and evaporations of toluene, a residue was obtained that was treated with 1:2 acetic anhydride–pyridine (0.5 ml). The mixture was kept overnight at room temperature, and water (0.5 ml) was then added. Evaporation, followed by two additions and evaporations of toluene, gave a residue that was taken up in chloroform. The suspension was filtered through glass wool, and the filtrate evaporated, to give **23** (11 mg), m.p. 226–232°, $[\alpha]_D^{22}$ +27° (*c* 0.75, chloroform); $\nu_{max}^{KBr}$ 3320, 2940, 1745, 1660, 1535, 1375, 1235, 1125, 1045, and 900 $cm^{-1}$, the i.r. spectrum being almost identical with that of **27**. In t.l.c. (10:1 chloroform–methanol), **23** migrated just ahead of **27**, and gave with the anisaldehyde reagent a vivid, purple color even more intense than that given by allyl ethers. It was not detected by the potassium permanganate reagent. The corresponding $\beta$ anomer shows[22] m.p. 260°, $[\alpha]_D^{23}$ −53.2° (*c* 2.15, chloroform).

*Anal.* Calc. for $C_{29}H_{42}N_2O_{17}$: C, 50.40; H, 6.10; N, 4.10. Found: C, 50.57; H, 6.30; N, 3.61.

*2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose* (**25**). — A solution of **24** (30 mg, not recrystallized) in methanol (2 ml) was hydrogenated overnight at 2 atm in the presence of 10% Pd-on-charcoal (Fluka, 25 mg). Examination of the solution by t.l.c. (5:1 chloroform–methanol) showed that the **24** ($R_F$ 0.70) had been converted into two products having $R_F$ 0.44 (presumably having one residual benzyl group) and $R_F$ 0.35. The catalyst was filtered off and replaced with a fresh batch, and, after a further period of hydrogenation (8 h), t.l.c. showed one major product ($R_F$ 0.35) that was incompletely resolved into a double spot characteristic of an anomeric mixture. The catalyst was filtered off, and washed with methanol, and the combined filtrates were evaporated to a solid that was recrystallized from methanol, to give **25** (18 mg) as prisms, decomposing and partially melting above 210°, and becoming completely liquid at 242–246°, $[\alpha]_D^{20}$ +14° (*c* 0.9, 5:1 chloroform–methanol): $\nu_{max}^{KBr}$ 3480, 3300, 3090, 2940,

2900, 1745, 1655, 1545, 1430, 1375, 1300, 1230 (broad), 1125, 1045, 965, 905, 775, and 685 $cm^{-1}$.

*Anal.* Calc. for $C_{22}H_{34}N_2O_{14}$: C, 47.97; H, 6.24; N, 5.09; O, 40.66. Found: C, 47.87; H, 6.26; N, 5.06; O, 40.19.

*2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-α-D-glucopyranose* (N,N'-*diacetylhexa-O-acetylchitobiose*) (**27**). — A mixture of **25** (10 mg) with 1:2 acetic anhydride–pyridine (0.5 ml) was kept overnight at room temperature. After the addition of water (0.2 ml), evaporation, followed by two additions and evaporations of toluene (0.2 ml), gave a product which showed a double spot in t.l.c. (10:1 chloroform–methanol), $R_F$ 0.43. The major spot cochromatographed with **27** prepared by the acetolysis of chitin[24], the minor spot probably being the β anomer. Recrystallization from methanol–ether gave very small needles (5 mg) showing a single spot in t.l.c., m.p. 305–306°; in admixture with a sample from chitin (m.p. 306–307°), the m.p. was 306–307°.

*2-Methyl-[2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline* (**28**). — *A. From* **25**. A mixture of **25** (40 mg) and acetyl chloride (3 ml) was stirred for 48 h at room temperature. The reagent was evaporated, and after five additions and evaporations of toluene (2 ml), the product was dissolved in dry acetonitrile (3 ml) and stirred at room temperature with tetraethylammonium chloride (16 mg) and $NaHCO_3$ (16 mg). After 1 h, t.l.c. (10:1 chloroform–methanol) revealed the formation of a major product ($R_F$ 0.48), the spot having the intense, brown color that is diagnostic of an oxazoline (anisaldehyde spray), together with minor products having $R_F$ 0.47 and 0.66 (see later). The solution was diluted with dichloromethane (30 ml), washed twice with water (5 ml) and once with saturated, aqueous KCl, and then dried ($MgSO_4$). After evaporation, the residue was dissolved in methanol (0.5 ml), and the solution diluted with ether (10 ml). The resulting precipitate ($R_F$ 0.47), which was a contaminant, was filtered off and washed with 30:1 ether–methanol. The combined filtrates were evaporated to yield **28** as an amorphous solid (36 mg), which cochromatographed with the product obtained from **27** (see next paragraph) in t.l.c. in 10:1 chloroform–methanol, and had an identical i.r. spectrum. However, the t.l.c. and the optical rotation, $[\alpha]_D^{20}$ +3° (*c* 1, dichloromethane), showed that the compound was contaminated by a trace of the corresponding oxazoline **18** ($R_F$ 0.66) derived from 2-acetamido-2-deoxy-D-glucose.

*B. From* **27**. Compound **27**, obtained by the acetolysis of chitin[24], was recrystallized from methanol, but still contained 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5,6-di-*O*-acetyl-2,3-dideoxy-*aldehydo*-D-*erythro*-hex-2-enose[27], as shown by t.l.c. (10:1 chloroform–methanol) in which it gave a dark-green spot having the same mobility as **27** ($R_F$ 0.43). The contaminated **27** (200 mg) was *O*-deacetylated by treatment with 3% sodium methoxide in methanol. The mixture was kept for 2 h at room temperature, and then treated with AG 50W-X8 cation-exchange resin (pyridinium$^+$, Bio-Rad Laboratories, Richmond, Calif. 94804) for removal of sodium ions. The resin was filtered off and washed with

methanol, the combined filtrates were evaporated ($N_2$), and the residue was dried *in vacuo* over phosphorus pentaoxide overnight. Acetyl chloride (3 ml) was added, and the mixture was stirred for 24 h at room temperature and processed, and the product converted into the oxazoline, as described in Method A, with tetraethylammonium chloride (80 mg) and $NaHCO_3$ (80 mg) in acetonitrile (2 ml). Partial purification, as described in Method A, gave the oxazoline **28**, having the same major component, by t.l.c., as the product obtained from compound **25**, but with other contaminants having lower $R_F$ values and giving, in t.l.c., the green color typical of unsaturated carbohydrates. Compound **28**, as prepared by this route, was also accompanied by a small proportion of the monosaccharide oxazoline ($R_F$ 0.66), presumably arising because of attack by acetyl chloride on the $\beta$-(1→4) linkage of peracetylated chitobiose, but it was suitable for synthetic purposes[1] without further purification.

The oxazoline **28** (83 mg) was chromatographed on a preparative-layer plate (20 × 20 cm) in 10:1 chloroform–methanol. For detection, a strip was cut from the center of the plate and sprayed (anisaldehyde). The purified product was extracted from the band of silica gel with 2:1 chloroform–methanol, and, after stirring overnight, filtration, and evaporation, **28** was obtained as an amorphous solid (55 mg), $[\alpha]_D^{20}$ −3° (*c* 1.1, dichloromethane): $\nu_{max}^{KBr}$ 3280, 3090, 2950, 1745, 1670 (double peak), 1555, 1430, 1375, 1320, 1230 (broad), 1170, 1130, 1035, and 945 $cm^{-1}$; n.m.r. data: $\delta$ 1.27 (1 H, $CH_3$ of oxazoline), 1.95 ($NHCOCH_3$), 2.01, 2.08 and 2.13 (incompletely resolved group of 18 H, s, $CH_3$ of AcO), 3.55 and 4.21 (two unresolved m, 8 H), and 5.15 (m, 2 H); Khorlin *et al.*[5] obtained a crystalline product, m.p. 189–190°, $[\alpha]_D^{20}$ −8 ± 2° (*c* 1.0, chloroform); $\nu_{max}^{KBr}$ 1671, 1658, and 1563 $cm^{-1}$.

*Anal.* Calc. for $C_{20}H_{36}N_2O_{15}$: C, 50.65; H, 5.90; N, 4.54; O, 38.82. Found: C, 50.52; H, 5.97; N, 4.35; O, 38.64.

ACKNOWLEDGMENT

The authors thank Mr. Keyes Linsley for recording the n.m.r. spectra.

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