Carbohydrate Research, 193 (1989) 125–139
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF SOME OLIGOSACCHARIDES CONTAINING THE *O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→2)-*O*-α-D-MANNOPYRANOSYL UNIT. POTENTIAL SUBSTRATES FOR UDP-GlcNAc:α-D-MANNOPYRANOSYL-(1→6)-*N*-ACETYL-β-D-GLUCOSAMINYL-TRANSFERASE (GnT-V)*

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(Received September 19, 1988; accepted for publication, November 29th, 1988)

ABSTRACT

Four different oligosaccharides containing the 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl sequence as a terminal disaccharide unit were synthesized, namely: 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-β-D-mannopyranoside (**27**), 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**29**), allyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**31**), and allyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (**33**). A common glycosyl donor, namely, 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl bromide was employed for the synthesis of **27**, **29**, **31**, and **33**, the structures of which were all established by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

A variety of *N*-acetyl-β-D-glucosaminyltransferases are known[2–5] to be involved in the biosynthesis of the asparagine (Asn)-linked oligosaccharides as shown in Scheme 1. All of these enzymes use UDP-D-GlcNAc as the glycosyl donor, but they only differ in their specificity for different acceptor-substrates. Of these transferases, UDP-GlcNAc:α-D-mannopyranosyl-(1→6)-*N*-acetyl-β-D-glucosylaminyltransferase (GlcNAc-transferase V, or GnT-V) has attracted considerable interest

*Synthetic Studies in Carbohydrates, Part LXV. For Part LXIV, see ref. 1. Presented, in part, at the Sixth Annual Chemistry Graduate Students' Symposium, SUNY at Buffalo, Buffalo, New York, May 25–26, 1988. This investigation was supported by Grants No. CA-35329, from the National Cancer Institute, U.S. Public Health Service, and CH419, from the American Cancer Society.

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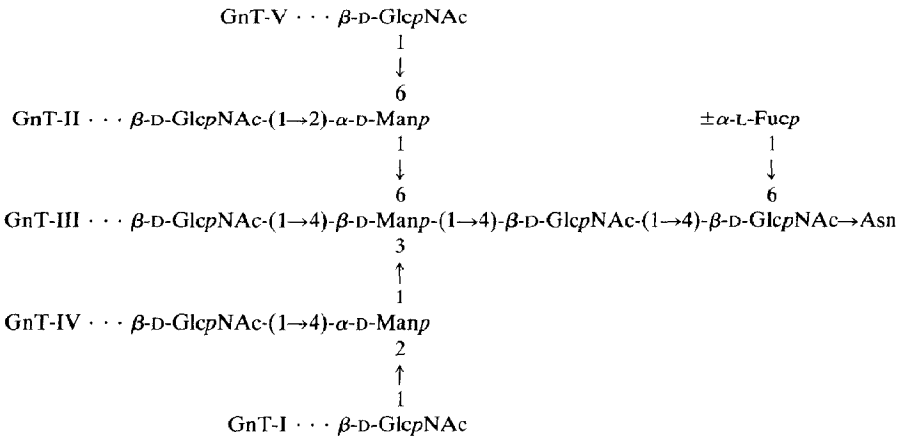
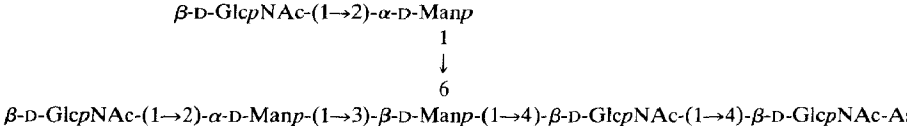


GnT-VI · · · *N*-Acetyl-β-D-glucosaminyltransferase responsible for the formation of β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3) repeating unit.

Scheme 1. Possible 2-acetamido-2-deoxy-β-D-glucopyranosyl groups that can be attached to tri-D-mannopyranosyl cores of asparagine-linked oligosaccharides and the *N*-acetyl-β-D-glucosaminyltransferases responsible for their addition[3].



Scheme 2. A heptasaccharide acceptor for Glc*p*NAc-transferase V.

as a potential tumor marker. This enzyme catalyzes the transfer of a 2-acetamido-2-deoxy-β-D-glucopyranosyl group from UDP-D-GlcNAc to O-6 of the (1→6)-α-D-mannosyl residue that forms part of the trimannosyl core of Asn-linked glycans, thus initiating the synthesis of highly-branched structures that appear to be associated with neoplastic transformations[3,6] and metastatic potential of certain tumor cells[7].

The heptasaccharide shown in Scheme 2 is known to act as a substrate for GnT-V (as well as for other glycosyltransferases), and it has been used to assay this enzyme[8]. However, at the outset of these investigations, we speculated that such a larger molecule as a heptasaccharide might not be a prerequisite for recognition by the enzyme; rather, a structure no larger than a trisaccharide might be sufficient as a substrate. With a similar intention, Hindsgaul and associates independently synthesized[8], during these investigations, the trisaccharide β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Man*p*-O$(CH_2)_8CO_2Me$, and demonstrated[6,9] that it can effectively be utilized as a selective acceptor for GnT-V. On our part, however, we have generally preferred the use of benzyl and nitrophenyl glycosides in studies

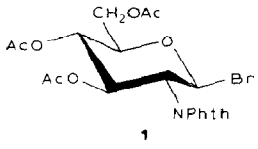
related to *N*-acetyl-β-D-glucosaminyltransferases that are involved in the biosynthesis of mucin-type *O*-linked glycans[10–12]. Because of their chromophore group, such compounds (and also the enzymic products derived therefrom) have the advantage of being readily detectable by chromatographic techniques, *e.g.*, t.l.c. or h.p.l.c., for their isolation and quantitative determination when assaying glycosyltransferases. Moreover, on reduction of their nitro group, nitrophenyl glycosides can readily be manipulated to provide conjugates that proved to be useful in affinity chromatography[13], and as synthetic or artificial antigens[14,15]. This latter property is also shared by allyl glycosides which can be copolymerized with acrylamide to produce polymer-supported substrates[16]. Therefore, it was of interest to synthesize some of the oligosaccharides described herein as their 4-nitrophenyl or allyl glycosides.

Furthermore, it seemed reasonable to postulate that, in the saccharide sequence β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Man*p*OR (where R is any aglycon or glycosyl group), HO-2 of the β-D-mannosyl residue may not necessarily be involved as part of the recognition site for GnT-V. As a test of this postulate, we attempted the synthesis of some potential substrates containing the β-D-glucopyranosyl rather than the β-D-mannopyranosyl residue. This would obviate the need for β-D-mannopyranosyl derivatives, the synthesis of which is hitherto wrought with difficulty. We describe herein the synthesis of the trisaccharides **27**, **29**, and **31**, and the tetrasaccharide **33**.

RESULTS AND DISCUSSIONS

A common glycosyl donor, namely, 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl bromide (**7**) was employed for the synthesis of all of the four title oligosaccharides. Bromide **7** was obtained in five, good yielding steps from methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside[17] (**2**). Glycosylation of **2** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**1**), and deacylation–reacetylation in the usual manner[18] afforded, in 88% yield, the peracetate **3**, the ^{1}H-n.m.r. spectrum of which contained signals in support of the overall expected structure (see Experimental section). After catalytic hydrogenation, crude triol **4** was directly acetylated to give methyl 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (**5**). Compound **5** was subjected to acetolysis to afford the peracetate **6**, which was readily converted into **7** by treatment with hydrogen bromide in dichloromethane. In the ^{1}H-n.m.r. spectrum of **6**, a low-field doublet at δ 5.93 (~0.8 H*, J ~1.5 Hz) was indicative of an anomeric mixture that was rich in the α-D anomer. A similar doublet at δ 6.29 (~1 H*, J ~1.5 Hz) in the spectrum of **7** strongly suggested that it existed almost exclusively as the α-D anomer.

*Compared to the acetyl-group methyl protons.



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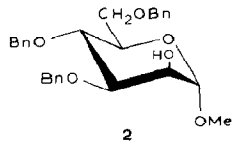

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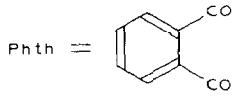
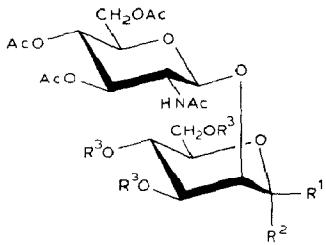
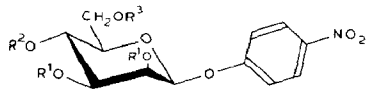
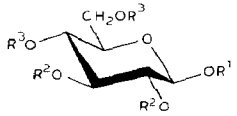




3 $R^1 = H, R^2 = OMe, R^3 = Bn$
4 $R^1 = R^3 = H, R^2 = OMe$
5 $R^1 = H, R^2 = OMe, R^3 = Ac$
6 $R^1, R^2 = H, OAc, R^3 = Ac$
7 $R^1 = H, R^2 = Br, R^3 = Ac$

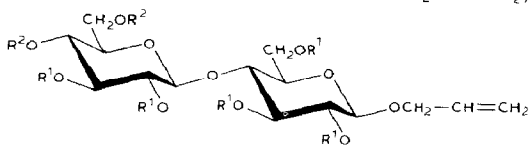
For the synthesis of trisaccharide **27**, 4-nitrophenyl 2,3-*O*-isopropylidene-β-D-mannopyranoside (**11**) was required as a glycosyl acceptor. This was readily obtained in excellent yield from 4-nitrophenyl β-D-mannopyranoside[19] (**8**) by way of the 6-*tert*-butyldiphenylsilyl ethers **9** and **10**. The ^{1}H-n.m.r. spectrum of





8 $R^1 = R^2 = R^3 = H$
9 $R^1 = R^2 = H, R^3 = Bu^tPh_2Si$
10 $R^1, R^1 = CMe_2, R^2 = H, R^3 = Bu^tPh_2Si$
11 $R^1, R^1 = CMe_2, R^2 = R^3 = H$

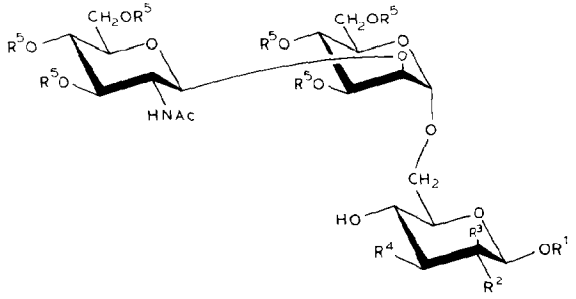
12 $R^1 = C_6H_4NO_2\text{-}4, R^2 = R^3 = H$
13 $R^1 = C_6H_4NO_2\text{-}4, R^2 = H, R^3, R^3 = CHC_6H_4OMe\text{-}4$
14 $R^1 = C_6H_4NO_2\text{-}4, R^2 = Ac, R^3, R^3 = CHC_6H_4OMe\text{-}4$
15 $R^1 = C_6H_4NO_2\text{-}4, R^2 = Ac, R^3 = H$
16 $R^1 = CH_2{-}CH{=}CH_2, R^2 = R^3 = H$
17 $R^1 = CH_2{-}CH{=}CH_2, R^2 = H, R^3, R^3 = PhCH$
18 $R^1 = CH_2{-}CH{=}CH_2, R^2 = Ac, R^3, R^3 = PhCH$
19 $R^1 = CH_2{-}CH{=}CH_2, R^2 = Ac, R^3 = H$



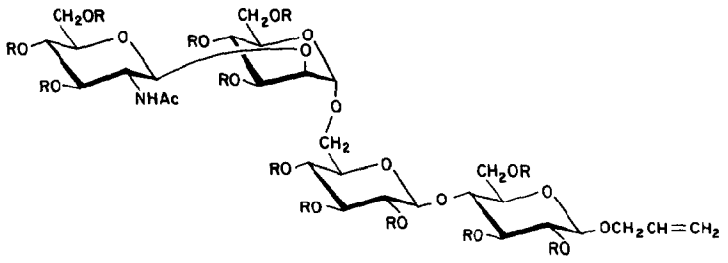
20 $R^1 = R^2 = H$
21 $R^1 = H, R^2, R^2 = PhCH$
22 $R^1 = Ac, R^2, R^2 = PhCH$
23 $R^1 = Ac, R^2 = H$
24 $R^1 = R^2 = Ac$

chromatographically pure **11** was in conformity with the structure expected (see Experimental section). Glycosylation of **11** with **7** in acetonitrile and in the presence of mercuric cyanide and 4A molecular sieves gave, in 67% yield, 4-nitrophenyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2,3-*O*-isopropylidene-β-D-mannopyranoside (**25**). The ^{1}H-n.m.r.spectrum of **25** contained signals in support of its overall structure; two doublets at δ 8.20 and 7.19 (2 H each, J ~10 Hz) accounted for the aromatic-ring protons, whereas a cluster of singlets (δ 2.29–1.89, 21 H) evidenced the presence of seven acetyl-group methyl protons, and the isopropylidene methyl protons occurred as well-separated singlets at δ 1.59 and 1.44. *O*-Deacetylation of **25** in methanolic sodium methoxide, followed by cleavage of the acetal group of **26** with aqueous trifluoroacetic acid in chloroform, furnished **27** in 64% yield.

4-Nitrophenyl 2,3-di-*O*-acetyl-β-D-glucopyranoside (**15**) was chosen as a glycosyl acceptor for the synthesis of the modified substrate **29**. Compound **15** was readily prepared from 4-nitrophenyl β-D-glucopyranoside[20] (**12**) by treatment with



25 $R^1 = C_6H_4NO_2$-4, $R^2 = H$, $R^3, R^4 = O_2CMe_2$, $R^5 = Ac$
26 $R^1 = C_6H_4NO_2$-4, $R^2 = H$, $R^3, R^4 = O_2CMe_2$, $R^5 = H$
27 $R^1 = C_6H_4NO_2$-4, $R^2 = R^5 = H$, $R^3 = R^4 = OH$
28 $R^1 = C_6H_4NO_2$-4, $R^2 = R^4 = OAc$, $R^3 = H$, $R^5 = Ac$
29 $R^1 = C_6H_4NO_2$-4, $R^2 = R^4 = OH$, $R^3 = R^5 = H$
30 $R^1 = CH_2{-}CH{=}CH_2$, $R^2 = R^4 = OAc$, $R^3 = H$, $R^5 = Ac$
31 $R^1 = CH_2{-}CH{=}CH_2$, $R^2 = R^4 = OH$, $R^3 = R^5 = H$



32 R = Ac
33 R = H

4-methoxybenzaldehyde–zinc chloride complex, followed by acetylation and subsequent cleavage of the acetal group. Condensation of diol **15** with **7** in nitromethane, in the presence of mercuric cyanide and 4A molecular sieves, gave in 67% yield the partially protected trisaccharide **28**, which was *O*-deacetylated to furnish in excellent yield the desired **29**.

Allyl 2,3-di-*O*-acetyl-β-D-glucopyranoside (**19**) and allyl *O*-(2,3-di-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**23**) were required as glycosyl acceptors for the synthesis of **31** and **33**, respectively. Thus, allyl β-D-glucopyranoside[21] (**16**) was treated with a benzaldehyde–zinc chloride complex to give **17**, which was conventionally acetylated to afford diacetate **18**, the acetal group of which was cleaved in hot, aqueous acetic acid to furnish **19**. Starting with allyl 4-*O*-β-D-glucopyranosyl-β-D-glucopyranoside[16] (**20**), a similar procedure was employed for the preparation of **23**, which had been previously[16] obtained by treatment of **20** with α,α-dibromotoluene in boiling pyridine, followed by acetylation and debenzylidenation. Glycosylation of diol **19** with **7** in 1:1 benzene–nitromethane, in the presence of mercuric cyanide and 4A molecular sieves, afforded in moderate yield trisaccharide derivative **30**, which was subjected to Zemplén transesterification to provide the desired **31**. A similar glycosylation of **23** with **7**, followed by acetylation (to facilitate chromatographic separation) of the product mixture, gave the fully protected tetrasaccharide derivative **32**, which was likewise *O*-deacetylated to furnish **33**. The ^{13}C-n.m.r. spectra of trisaccharides **27**, **29**, and **31**, and tetrasaccharide **33** were all in good agreement with the structures assigned (see Experimental section).

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at ~25° with a Perkin–Elmer 241 polarimeter. T.l.c. was conducted on aluminum sheets, precoated with 0.2 mm layers of Silica Gel 60F-254 (E. Merck, Darmstadt, Germany); the components were located either by exposure to u.v. light, or by spraying the plates with 5% H_2SO_4 in ethanol and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). Unless otherwise indicated, the following solvents systems (v/v) were used for chromatography: (*A*) 4:1 chloroform–acetone, (*B*) 13:6:1 chloroform–methanol–water, (*C*) 3:2 chloroform–acetone, (*D*) 1:1 ethyl acetate–hexane, (*E*) 5:4:1 chloroform–methanol–water, and (*F*) 9:1 chloroform–methanol. N.m.r. spectra were recorded at ~25°; ^{1}H-n.m.r. spectra with a Varian EM-390, and ^{13}C-n.m.r. spectra either with a Varian XL-100 or a Bruker WP-200 instrument, at 90, 25.2, and 50.3 MHz, respectively; the positions of the peaks (δ) are expressed from the tetramethylsilane signal. Organic solutions were generally dried with anhydrous Na_2SO_4. Pyridine was dried over KOH, and dichloromethane and 1,2-dichloroethane were dried over 4A molecular sieves. Nitromethane and acetonitrile were distilled from P_2O_5 immediately before being

used, and benzene was dried with Na. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, New Jersey 07940.

Methyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6- tri-O-benzyl-α-D-mannopyranoside (**3**). — A mixture of methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside[17] **2** (1.8 g, 3.9 mmol), silver trifluoromethanesulfonate (1.47 g, 5.7 mmol), 2,4,6-trimethylpyridine (0.65 g, 5.4 mmol), and 4A molecular sieves (4 g) in dichloromethane (30 mL), protected from light and moisture, was stirred for 30 min in an atmosphere of N_2. A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**1**, 2.7 g, 5.4 mmol) in dichloromethane (15 mL) was added dropwise, with stirring, during 20 min, and the stirring was continued for a further 40 min. More portions of silver trifluoromethanesulfonate (0.73 g, 2.8 mmol) and 2,4,6-trimethylpyridine (0.33 g, 2.7 mmol) were added, followed by the dropwise addition of a solution of bromine **1** (1.35 g, 2.7 mmol) in dichloromethane (10 mL), and the stirring was continued for an additional 3 h. T.l.c. (90:10:1, v/v, dichloromethane–ethyl acetate–acetone) then revealed the presence of a major product, faster-migrating than **2**; some slower- and some faster-migrating impurities (presumably due to decomposition of **1**) were also revealed by t.l.c. The mixture was diluted with an equal volume of dichloromethane, and the solids were filtered off (a bed of Celite) and washed with dichloromethane. The filtrate and washings were combined, successively washed with ice-cold water, cold 3% aqueous HCl, cold saturated $NaHCO_3$, and water, dried, and evaporated to dryness. The foamy solid so obtained (~5.8 g) was boiled for 3 h under N_2 in a mixture of ethanol (90 mL) and hydrazine hydrate (18 mL). It was then concentrated to dryness to give a residue, which was dissolved in pyridine (100 mL) and acetic anhydride (50 mL) and heated for 30 min at ~90°. The acetic anhydride and pyridine were evaporated under diminished pressure, and the residue was taken up in chloroform, successively washed with water, aqueous $NaHCO_3$, and water, dried, and evaporated. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 0–20% acetone in chloroform. On evaporation, the fractions corresponding to the product gave a solid which crystallized from ethyl acetate to afford **3** (2.7 g; 88% based on **2**), m.p. 189–191°, $[\alpha]_D^{25}$ +14° (*c* 0.7, chloroform); t.l.c. (*A*) R_F 0.28; ^{1}H-n.m.r. ($CDCl_3$): δ 7.28–7.22 (m, 15 H, arom.), 3.30 (s, 3 H, OMe), 1.97 (m, 9 H, 3 OAc), and 1.72 (s, 3 H, NAc).

Anal. Calc. for $C_{42}H_{51}NO_{14}$: C, 63.54; H, 6.48; N, 1.76. Found: C, 63.66; H, 6.21; N, 1.62.

Methyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-O-acetyl-α-D-mannopyranoside (**5**). — A mixture of **3** (3.4 g) and 10% Pd–C (3.4 g) in glacial acetic acid (80 mL) was shaken under H_2 at ~345 kPa for 24 h at room temperature. The suspension was filtered through a bed of Celite, the solid was thoroughly washed with glacial acetic acid, and the filtrate and washings were combined and concentrated under diminished pressure to give **4** as a foamy solid (2.2 g), which was slightly contaminated (t.l.c. solvent *B*) with some faster-migrating impurities.

A solution of crude **4** (2.1 g) in 1:2 acetic anhydride–pyridine (75 mL) was kept overnight at room temperature. It was then concentrated to dryness, and several portions of toluene were added to, and evaporated from the residue which was then dissolved in dichloromethane. Addition of ether–hexane caused the precipitation of **5** (1.89 g, 72.5%), amorphous, $[\alpha]_D^{25}$ −12° (*c* 0.6, chloroform); t.l.c. (*C*) R_F 0.41; ^{1}H-n.m.r. ($CDCl_3$): δ 3.29 (s, 3 H, OMe), 2.00–1.94 (cluster of s, 18 H, 6 OAc), and 1.85 (s, 3 H, NAc).

Anal. Calc. for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.05; N, 2.16. Found: C, 49.67; H, 5.92; N, 2.09.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,4,6-tetra-O-acetyl-D-mannopyranose (**6**). — A solution of **5** (1.65 g) in acetic anhydride (40 mL) containing 1% by volume of conc. H_2SO_4 was stirred for 12 h at room temperature. The mixture was then diluted with dichloromethane (200 mL), successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated, and the residue dissolved in a small volume of ethyl acetate. Addition of ether caused the precipitation of **6** (1.55 g, 90%) as an amorphous solid, $[\alpha]_D^{25}$ −10° (*c* 0.8, chloroform); t.l.c. (*A*) R_F0.27; ^{1}H-n.m.r. ($CDCl_3$): δ 5.93 (d, ~0.8 H, *J* ~1.5 Hz, H-1) and 2.10–1.92 (cluster of s, 24 H, 7 OAc and NAc).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07. Found: C, 49.37; H, 5.71; N, 1.90.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl bromide (**7**). — To a cold (~0°, bath), stirred solution of **6** (0.58 g) in dichloromethane (7 mL) was added a 31% solution of HBr in glacial acetic acid (7 mL), and stirring was continued for 12 h. The mixture was then poured into ice–water and extracted with dichloromethane, successively washed with water, cold saturated $NaHCO_3$, and water, dried, and concentrated to give **7** (0.47 g, 79%), amorphous, $[\alpha]_D^{25}$ +53° (*c* 0.7, chloroform); t.l.c. (*A*) R_F 0.32; ^{1}H-n.m.r. ($CDCl_3$): δ 6.29 (d, ~1 H, *J* ~1.5 Hz, H-1), 2.09–1.99 (cluster of s, 18 H, 6 OAc), and 1.92 (s, 3 H, NAc).

4-Nitrophenyl 6-O-tert-butyldiphenylsilyl-β-D-mannopyranoside (**9**). — To a cold (0°, bath), stirred solution of 4-nitrophenyl β-D-mannopyranoside[19] (**8**; 4.8 g, 15.9 mmol) and imidazole (2.7 g, 40 mmol) in dry *N,N*-dimethylformamide (60 mL) was added *tert*-butylchlorodiphenylsilane (6.3 mL, 24 mmol), and stirring was continued for 2 h at ~0°. The mixture was then poured into ice–water and extracted with chloroform. The chloroform solution was successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated, and the residue applied to a column of silica gel and eluted with 49:1 (v/v) chloroform–methanol. On evaporation, the fractions corresponding to the product afforded **9** (8.3 g, 96.5%), amorphous, $[\alpha]_D^{25}$ −84° (*c* 1.2, chloroform); t.l.c. (*C*) R_F 0.31; ^{1}H-n.m.r. ($CDCl_3$): δ 8.08 and 7.05 (d, 2 H each, *J* ~10 Hz, arom.), 7.73–7.16 (m, 10 H, arom.), and 1.06 (s, 9 H, CMe_3).

Anal. Calc. for $C_{28}H_{33}NO_8Si \cdot 0.5\ H_2O$: C, 61.29; H, 6.25; N, 2.55. Found: C, 61.51; H, 6.09; N, 2.48.

4-Nitrophenyl 6-O-tert-butyldiphenylsilyl-2,3-O-isopropylidene-β-D-mannopyranoside (**10**). — To a solution of **9** (8 g) in dry acetone (60 mL) were added 2,2-dimethoxypropane (60 mL) and 4-toluenesulfonic acid monohydrate (1.2 g). The mixture was stirred for 4 h at room temperature, made neutral by the addition of triethylamine, and then evaporated. The residue was purified in a column of silica gel with chloroform as the eluent to give **10** (8.1 g, 94%), a thick syrup, $[\alpha]_D^{25}$ −74.5° (*c* 1.3, chloroform); t.l.c. (*D*) R_F 0.39; ^{1}H-n.m.r. ($CDCl_3$): δ 8.06 and 7.05 (d, 2 H each, *J* ~10 Hz, arom.), 7.70–7.20 (m, 10 H, arom.), 5.51 (d, 1 H, *J* ~2 Hz, H-1), 1.55 and 1.40 (s, 3 H each, CMe_2), and 1.06 (s, 9 H, CMe_3).

Anal. Calc. for $C_{31}H_{37}NO_8Si$: C, 64.22; H, 6.43; N, 2.42. Found: C, 63.93; H, 6.42; N, 2.37.

4-Nitrophenyl 2,3-O-isopropylidene-β-D-mannopyranoside (**11**). — A solution of **10** (1.7 g, 2.9 mmol) in dry oxolan (35 mL) was treated with a M solution of tetrabutylammonium fluoride in oxolan (4.1 mL), and the stirring was continued for 2 h at room temperature. The mixture was concentrated to dryness, and the residue was purified in a column of silica gel with 15% acetone in chloroform as the eluent to afford a solid residue which was dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the precipitation of **11** (0.98 g, 98%), amorphous, $[\alpha]_D^{25}$ −127° (*c* 1.2, chloroform); t.l.c. (*A*) R_F 0.17; ^{1}H-n.m.r. ($CDCl_3$): δ 8.15 and 7.05 (d, 2 H each, *J* ~10 Hz, arom.), 5.57 (d, 1 H, *J* ~2 Hz, H-1), and 1.53 and 1.40 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{15}H_{19}NO_8$: C, 52.78; H, 5.61; N, 4.10. Found: C, 52.65; H, 5.52; N, 4.00.

4-Nitrophenyl 4,6-O-(4-methoxybenzylidene)-β-D-glucopyranoside (**13**). — A mixture of 4-nitrophenyl β-D-glucopyranoside[20] (**12**; 12 g), anhydrous $ZnCl_2$ (12 g), and 4-methoxybenzaldehyde (150 mL) was stirred for 2 days at room temperature, and then poured into ice–water and ether, and stirred for 2 h. The precipitate was filtered off and thoroughly washed with ice–water and ether, and dried. On crystallization from pyridine–ether; it afforded **13** (11.5 g, 69%), m.p. 190–192°, $[\alpha]_D^{25}$ −51° (*c* 0.5, 4:1 chloroform–methanol), t.l.c. (*A*) R_F 0.22; ^{1}H-n.m.r. [CD_3OD plus a little $(CD_2)_2SO$]: δ 8.20 and 7.22 (d, 2 H each, *J* ~10 Hz, $C_6H_4NO_2$-4), 7.40 and 6.85 (d, 2 H each, *J* ~10 Hz, C_6H_4OMe-4), 5.50 (s, 1 H, PhCH), 5.18 (d, 1 H, *J* ~7.5 Hz, H-1), and 3.73 (s, 3 H, OMe).

Anal. Calc. for $C_{20}H_{21}NO_9$: C, 57.28; H, 5.05; N, 3.34. Found: C, 56.99; H, 5.06; N, 3.27.

4-Nitrophenyl 2,3-di-O-acetyl-4,6-O-(4-methoxybenzylidene)-β-D-glucopyranoside (**14**). — A solution of **13** (5 g) in pyridine (60 mL) and acetic anhydride (30 mL) was stirred overnight at room temperature. The crystalline material that separated was filtered off and thoroughly washed with ether to afford **14** (5.8, 97%), m.p. 252–253°, $[\alpha]_D^{25}$ −47° (*c* 0.55, chloroform); t.l.c. (*D*) R_F 0.48; ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 8.21 and 7.21 (d, 2 H each, *J* ~10 Hz, $C_6H_4NO_2$-4), 7.31 and 6.88 (d, 2 H each, *J* ~10 Hz, C_6H_4OMe-4), 5.79 (d, 1 H, *J* ~7.5, H-1), 5.58 (s, 1 H, PhCH), 3.73 (s, 3 H, OMe), and 2.01 (s, 6 H, 2 OAc).

Anal. Calc. for $C_{24}H_{25}NO_{11}$: C, 57.25; H, 5.01; N, 2.78. Found: C, 57.09; H, 4.81; N, 2.69.

4-Nitrophenyl 2,3-di-O-acetyl-β-D-glucopyranoside (**15**). — Acetal **14** (4 g) was stirred in 60% aqueous acetic acid (200 mL) for 1 h at ~70°. The acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several added portions of toluene, to give a solid which crystallized from methanol to afford **15** (2.5 g, 82%), m.p. 167–169°, $[\alpha]_D^{25}$ −46° (*c* 0.6, 4:1 chloroform–methanol); t.l.c. (*A*) R_F 0.17; ^{1}H-n.m.r. [CD_3OD plus a little $(CD_3)_2SO$]: δ 8.17 and 7.15 (d, 2 H each, *J* ~10 Hz, $C_6H_4NO_2$-4), 5.42 (d, 1 H, *J* ~8 Hz, H-1), and 2.06 and 2.01 (s, 3 H each, 2 OAc).

Anal. Calc. for $C_{16}H_{19}NO_{10}$: C, 49.87; H, 4.97; N, 3.64. Found: C, 49.69; H, 4.83; N, 3.44.

Allyl 4,6-O-benzylidene-β-D-glucopyranoside (**17**). — $ZnCl_2$ (3 g) was quickly added, with stirring, to benzaldehyde (11 mL), and stirring was continued for 0.5 h. Allyl β-D-glucopyranoside[21] **16** (3 g) was then added, the mixture was stirred overnight at room temperature, and poured into a stirred 1:1 mixture of ice–water and hexane (~150 mL). The precipitate was filtered off, thoroughly washed with cold water and hexane, dried in the air, and dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the precipitation of **17** (2.8 g, 67%), amorphous, $[\alpha]_D^{25}$ −53° (*c* 1.1, chloroform): t.l.c. (*A*) R_F 0.27; ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.18 (m, 5 H, arom.), 6.10–5.63 (m, 1 H, $CH{=}CH_2$), and 5.42 (s, 1 H, PhCH).

Anal. Calc. for $C_{16}H_{20}O_6$: C, 62.32; H, 6.54. Found: C, 62.53; H, 6.50.

Allyl 2,3-di-O-acetyl-4,6-O-benzylidene-β-D-glucopyranoside (**18**). — Compound **17** (1.3 g) in pyridine (30 mL) and acetic anhydride (15 mL) was kept overnight at room temperature. Acetic anhydride and pyridine were evaporated under reduced pressure, the last traces being removed by coevaporation with several added portions of toluene, and the residue was dissolved in a small volume of dichloromethane. Addition of ether–hexane precipitated **18** (1.46 g, 88%), amorphous, $[\alpha]_D^{25}$ −86° (*c* 0.7, chloroform); t.l.c. (*A*) R_F 0.65; ^{1}H-n.m.r. ($CDCl_3$): δ 7.43–7.20 (m, 5 H, arom.), 6.03–5.58 (m, 1 H, $CH{=}CH_2$), 5.45 (s, 1 H, PhCH), and 2.00 (s, 6 H, 2 OAc).

Anal. Calc. for $C_{20}H_{24}O_8$: C, 61.21; H, 6.16. Found: C, 61.14; H, 6.02.

Allyl 2,3-di-O-acetyl-β-D-glucopyranoside (**19**). — Compound **18** (1.44 g) in 60% aqueous acetic acid (70 mL) was stirred for 6.5 h at ~55°. T.l.c. (*C*) then showed the disappearance of **18** and the presence of a product, slower-migrating than **18**, together with some faster-migrating contaminants. Acetic acid was evaporated under diminished pressure, and several portions of toluene were added to, and evaporated from, the residue which was then dissolved in chloroform and applied to a column of silica gel. On elution with 9:1 (v/v) chloroform–acetone, concentration of the fractions corresponding to the product afforded **19** (1.02 g, 91%), syrup, $[\alpha]_D^{25}$ −51° (*c* 1.8, chloroform); t.l.c. (*C*) R_F 0.36; ^{1}H-n.m.r. ($CDCl_3$): δ 6.03–5.57 (m, 1 H, $CH{=}CH_2$), and 2.05 and 2.03 (s, 6 H, 2 OAc).

Anal. Calc. for $C_{13}H_{20}O_8$: C, 51.31; H, 6.63. Found: C, 51.34; H, 6.61.

Allyl O-(4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-β-D-glucopyranoside (**21**). — This compound was prepared from allyl *O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside[16] (**20**) as described for **16** (to give **17**). After the aforedescribed processing, the solid residue so obtained was dissolved in a small volume of methanol. Addition of ether precipitated **21** (1.8 g, 73%), amorphous, $[\alpha]_D^{25}$ −42° (*c* 0.5, 4:1 chloroform–methanol); t.l.c. (*B*) R_F 0.7; ^{1}H-n.m.r. ($CDCl_3$ plus a little CD_3OD): δ 7.50–7.23 (m, 5 H, arom.), 6.07–5.67 (m, 1 H, $CH{=}CH_2$), and 5.45 (s, 1 H, PhCH).

Anal. Calc. for $C_{22}H_{30}O_{11}$: C, 56.16; H, 6.43. Found: C, 55.87; H, 6.16.

Allyl O-(2,3-di-O-acetyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**22**). — Compound **21** (1.7 g) was acetylated as described for **17** (to give **18**), to give a solid residue which crystallized from dichloromethane–ether–hexane to afford **22** (2.3 g, 94%), m.p. 236–238°, $[\alpha]_D^{25}$ −53° (*c* 1.1, chloroform); t.l.c. (*A*) R_F 0.68 {lit.[16] m.p. 234–236° (from ethanol), $[\alpha]_D^{20}$ −43° (*c* 1, chloroform)}; ^{1}H-n.m.r. spectral data ($CDCl_3$) were comparable to those previously reported[16].

Allyl O-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**23**). — Compound **22** (2.25 g) was deacetalated in hot, 60% aqueous acetic acid as described for **18** (to give **19**), to give a solid residue which was applied to a column of silica gel. Elution with solvent *A*, and concentration of the fractions corresponding to the product afforded, after crystallization from dichloromethane–ether–hexane, **23** (1.7 g, 87%), m.p. 193–195°, $[\alpha]_D^{25}$ −35° (*c* 1.2, chloroform); t.l.c. (*C*) R_F 0.43 {lit.[16], m.p. 194–196° (from toluene), $[\alpha]_D^{23}$ −37° (*c* 2.6, chloroform)}; ^{1}H-n.m.r. spectral data ($CDCl_3$) were similar to those previously reported[16].

4-Nitrophenyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1→6)-2,3-O-isopropylidene-β-D-mannopyranoside (**25**). — A mixture of **11** (0.34 g, 1 mmol), bromide **7** (1.02 g, 1.5 mmol), powdered $Hg(CN)_2$ (0.25 g, 1 mmol), and powdered 4A molecular sieves (1.2 g) in acetonitrile (25 mL) was stirred for 3 h at room temperature. T.l.c. (*C*) then showed the presence of a major product, slower-migrating than **11**; some unchanged **11** was also revealed in t.l.c. The acetonitrile was evaporated under diminished pressure, the residue suspended in chloroform (125 mL), and the solids were filtered off through a bed of Celite and thoroughly washed with chloroform. The filtrate and washings were combined and successively washed with water, aqueous M KI solution, aqueous $NaHCO_3$, and water, dried, and concentrated, and the concentrate applied to a column of silica gel. Elution with a solvent gradient consisting of 0–25% acetone in chloroform gave first unchanged **11** (0.1 g), followed by **25** (0.45 g, 66.5% based on reacted **11**), amorphous, $[\alpha]_D^{25}$ −30° (*c* 1.2, chloroform); t.l.c. (*C*) R_F 0.19; ^{1}H-n.m.r. ($CDCl_3$): δ 8.20 and 7.19 (d, 2 H each, *J* ~10 Hz, arom.), 2.29–1.89 (cluster of s, 21 H, 6 OAc and NAc), and 1.59 and 1.44 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{41}H_{54}N_2O_{24}$: C, 51.35; H, 5.68; N, 2.92. Found: C, 51.10; H, 5.57; N, 2.56.

4-Nitrophenyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-α-D-mannopyranosyl-(1→6)-2,3-O-isopropylidene-β-D-mannopyranoside (**26**). — Compound **25** (0.42 g) in 20mM methanolic sodium methoxide (65 mL) was stirred overnight at room temperature. T.l.c. (*B*) then showed the disappearance of **26** and the presence of a slower-migrating product; some slower-migrating contaminants that were undetectable under u.v. light were also revealed by t.l.c. The base was neutralized with a few drops of glacial acetic acid, and the solution was de-ionized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off through a bed of Celite and thoroughly washed with methanol, and the filtrate and washings were combined and concentrated to a small volume. The concentrate was applied to a column of silica gel and eluted with 27:12:1 (v/v) chloroform–methanol–water. On evaporation, the fractions corresponding to the product gave a solid which was dissolved in a little methanol. Addition of ether caused the precipitation of **26** (0.21 g, 67%), amorphous, $[\alpha]_D^{25}$ −62° (*c* 0.8, methanol); t.l.c. (*B*) R_F 0.27; ^{1}H-n.m.r. (CD_3OD): δ 8.23 and 7.18 (d, 2 H each, *J* ~10 Hz, arom.), 5.68 (d, 1 H, *J* ~1.5 Hz, H-1), 1.97 (s, 3 H, NAc), and 1.55 and 1.42 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{29}H_{42}N_2O_{18}$: C, 49.29; H, 5.99; N, 3.97. Found: C, 48.99; H, 5.71; N, 3.85.

4-Nitrophenyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-α-D-mannopyranosyl-(1→6)-β-D-mannopyranoside (**27**). — A mixture of **26** (0.2 g), trifluoroacetic acid (0.8 mL), water (0.2 mL), and chloroform (5 mL) was stirred for 4 h at room temperature. It was then evaporated, and several portions of toluene were added to, and evaporated from the residue. T.l.c. (*E*) revealed the presence of a major product, slower-migrating than **26**, together with some faster-migrating contaminants. The crude product was applied to a column of silica gel and eluted first with 7:3 (v/v) chloroform–methanol, and then with solvent *B*. Concentration of the fractions corresponding to the major product gave a residue which was dissolved in a small volume of methanol. Addition of ether precipitated **27** (0.12 g, 64%), amorphous, $[\alpha]_D^{25}$ −51° (*c* 0.9, methanol); t.l.c. (*E*) R_F 0.45; ^{13}C-n.m.r. [$(CD_3)_2SO$]: δ 101.40 (C-1″), 97.87 (C-1), 97.58 (C-1′), 78.97 (C-2′), 66.58 (C-4′), 66.43 (C-6), 61.33 and 61.14 (C-6′,C-6″), 55.87 (C-2″), and 23.12 (NAc).

Anal. Calc. for $C_{26}H_{38}N_2O_{18}$: C, 46.85; H, 5.75; N, 4.20. Found: C, 46.61; H, 5.88; N, 3.94.

4-Nitrophenyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1→6)-2,3-di-O-acetyl-β-D-glucopyranoside (**28**). — A mixture of **7** (0.45 g, 0.72 mmol), **15** (0.27 g, 0.72 mmol), $Hg(CN)_2$ (0.36 g, 1.44 mmol), and 4A molecular sieves (0.23 g) in dry nitromethane (15 mL) was stirred for 21 h at ~40°. It was then cooled, and the solids were filtered off and thoroughly washed with chloroform. The filtrate and washings were combined and processed as described for **11** (to give **25**) to give a

residue which showed in t.l.c. (*C*) the presence of a major product, slower-migrating than **15**; some unchanged **15**, as well as some faster- and slower-migrating contaminants were also revealed in t.l.c. The crude product mixture was applied to a column of silica gel and eluted with a solvent gradient consisting of 0–25% acetone in chloroform. On evaporation, the first fractions that emerged from the column gave unchanged **15** (0.1 g). Continued elution of the column and concentration of the fractions corresponding to the product gave **28** (0.3 g, 67% based on reacted **15**), amorphous, $[\alpha]_D^{25}$ −7° (*c* 1.8, chloroform); t.l.c. (*C*) R_F 0.27; ^{1}H-n.m.r. ($CDCl_3$): δ 8.15 and 7.02 (d, 2 H each, *J* ~10 Hz, arom.), 2.09–1.82 (cluster of s, 24 H, 8 OAc), and 1.77 (s, 3 H, NAc).

Anal. Calc. for $C_{42}H_{54}N_2O_{26}$: C, 50.29; H, 5.44; N, 2.78. Found: C, 50.12; H, 5.35; N, 2.68.

4-Nitrophenyl O-*(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→2)*-O-*α*-D-*mannopyranosyl-(1→6)-β*-D-*glucopyranoside* (**29**). — Compound **28** (0.15 g) was suspended in 20mM sodium methoxide in methanol (20 mL) and stirred. The suspended **28** gradually dissolved, and the stirring was continued overnight at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the resin was filtered off and washed with methanol, and the filtrate and washings were combined and concentrated. The residue was dissolved in a little water and lyophilized to afford **29** (90 mg, 90%), amorphous, $[\alpha]_D^{25}$ −62° (*c* 0.8, water); t.l.c. (*E*) R_F 0.42; ^{13}C-n.m.r. [$(CD_3)_2SO$]: δ 101.50 (C-1″), 100.31 (C-1), 97.57 (C-1′), 79.08 (C-2′), 66.03 (C-6), 61.40, 61.16 (C-6′,6″), and 55.93 (C-2″).

Anal. Calc. for $C_{26}H_{38}N_2O_{18}\cdot 0.5\ H_2O$: C, 46.22; H, 5.81; N, 4.15. Found: C, 46.01; H, 5.65; N, 3.89.

Allyl O-*(2-acetamido-3,4,6-tri-*O-*acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→2)-*O-*(3,4,6-tri-*O-*acetyl-α-*D*-mannopyranosyl)-(1→6)-2,3-di-*O-*acetyl-β-*D*-glucopyranoside* (**30**). — A stirred mixture of **19** (0.25 g, 0.82 mmol), powdered $Hg(CN)_2$ (0.21 g, 0.82 mmol), and powdered 4A molecular sieves (0.8 g) in 1:1 benzene–nitromethane (90 mL) was boiled until ~15 mL of the solvent had distilled off. After cooling to room temperature, disaccharide bromide **7** (0.86 g, 1.23 mmol) in 1:1 benzene–nitromethane (18 mL) was added, and the stirring was continued for 9 h at ~45°. T.l.c. (*C*) showed the presence of a major product, slower-migrating than both **7** and **19**; a faster-migrating contaminant was also revealed in t.l.c. The mixture was filtered through a bed of Celite, the solids were thoroughly washed with benzene, and the filtrate and washings were combined and diluted with benzene to a total volume of ~300 mL. The solution was successively washed with water, M KI solution, aqueous $NaHCO_3$, and water, dried, and concentrated to give a solid which was dissolved in a little dichloromethane. Addition of ether–hexane precipitated **30** (0.35 g, 46%), amorphous, $[\alpha]_D^{25}$ −10° (*c* 0.9, chloroform); t.l.c. (*F*) R_F 0.53; ^{1}H-n.m.r. ($CDCl_3$): δ 6.04–5.60 (m, 1 H, $CH{=}CH_2$), 2.10–2.04 (cluster of s, 24 H, 8 OAc), and 1.92 (s, 3 H, NAc).

Anal. Calc. for $C_{39}H_{55}NO_{24}$: C, 50.81; H, 6.01; N, 1.52. Found: C, 50.76; H, 5.94; N, 1.54.

Allyl O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→2)-*O-*α-*D-*mannopyranosyl-(1→6)-β-*D-*glucopyranoside* (**31**). — Compound **30** (0.3 g) in 20mM methanolic sodium methoxide solution (40 mL) was stirred overnight at room temperature. It was then processed as described for **25** (to give **26**), and the crude product was applied to a column of silica gel. Elution with solvent *B* and evaporation of the fraction corresponding to the product gave a solid which was dissolved in water and lyophilized to afford amorphous **31** (0.15 g, 79%), $[\alpha]_D^{25}$ −12° (*c* 0.9, methanol); t.l.c. (*E*) R_F 0.45; ^{13}C-n.m.r. [$(CD_3)_2SO$]: δ 102.29 (C-1), 101.41 (C-1″), 97.34 (C-1′), 78.97 (C-2′), 66.23 (C-6), 61.41, 61.10 (C-6′,6″), and 55.83 (C-2″).

Anal. Calc. for $C_{23}H_{39}NO_{16}\cdot H_2O$: C, 45.77; H, 6.85; N, 2.36. Found: C, 45.68; H, 6.47; N, 2.25.

Allyl O-*(2-acetamido-3,4,6-tri-*O-*acetyl-2-deoxy-β-*D-*glucopyranosyl)-(1→2)-*O-*(3,4,6-tri-*O-*acetyl-β-*D-*mannopyranosyl)-(1→6)-*O-*(2,3,4-tri-*O-*acetyl-β-*D-*glucopyranosyl)-(1→4)-2,3,6-tri-*O-*acetyl-β-*D-*glucopyranoside* (**32**). — A stirred mixture of **23** (0.44 g, 0.74 mmol), powdered $Hg(CN)_2$ (0.2 g, 0.78 mmol), and powdered 4A molecular sieves (0.7 g) in 1:1 benzene–nitromethane (70 mL) was boiled until ~15 mL of the solvent had distilled off. After cooling to room temperature, bromide **7** (0.68 g, 0.97 mmol) in 1:1 benzene–nitromethane (14 mL) was added and the stirring was continued for 5 h at ~40°. It was then processed as described for **19** (to give **30**) to give a residue which showed in t.l.c. (*C*) the disappearance of **7** and the presence of a prominent spot having a mobility similar to that of **23**. This crude product (~1 g) was dried *in vacuo*, mixed with 1:2 acetic anhydride–pyridine (20 mL) and kept overnight at room temperature. The acetic anhydride and pyridine were evaporated under reduced pressure, the last tracers being removed by coevaporation with several added portions of toluene. The crude product was applied to a column of silica gel and eluted with a solvent gradient consisting of 0–20% acetone in chloroform. Evaporation of the earlier fractions that emerged from the column gave a compound (0.22 g) which had a chromatographic mobility (t.l.c., *C*) identical with that of an authentic sample of **24**. Continued elution of the column and evaporation of the fractions corresponding to the product afforded **32** (0.51 g, 55% based on **23**), amorphous, $[\alpha]_D^{25}$ −7° (*c* 1.1, chloroform); t.l.c. (*C*) R_F 0.54; ^{1}H-n.m.r. ($CDCl_3$): δ 5.97–5.60 (m, 1 H, CH=CH_2), 2.10–2.00 (cluster of s, 36 H, 12 OAc), and 1.90 (s, 3 H, NAc).

Anal. Calc. for $C_{53}H_{73}NO_{33}$: C, 50.84; H, 5.88; N, 1.12. Found: C, 51.11; H, 5.55; N, 1.33.

Allyl O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→2)-*O-*α-*D-*mannopyranosyl-(1→6)-*O-*β-*D-*glucopyranosyl-(1→4)-β-*D-*glucopyranoside* (**33**). — Compound **32** (0.4 g) in methanol (60 mL) was treated with M sodium methoxide (1.2 mL), and the mixture was stirred overnight at room temperature. After processing as described for **25** (to give **26**), the residue so obtained was dissolved in a little methanol and precipitated by the addition of ether to afford **33** (0.19 g, 78%), amorphous, $[\alpha]_D^{25}$ −6° (*c* 1.1, methanol); t.l.c. (*E*) R_F 0.29; ^{13}C-n.m.r. [$(CD_3)_2SO$]: δ 103.18 (C-1′), 101.89 (C-1), 101.53 (C-1″), 97.65 (C-1‴), 80.49 (C-4), 78.99 (C-2″), and 66.33 (C-6′).

Anal. Calc. for $C_{29}H_{49}NO_{21}$: C, 46.58; H, 6.61; N, 1.87. Found: C, 46.30; H, 6.83; N, 1.90.

ACKNOWLEDGMENTS

The authors thank Dr. Rakesh K. Jain for valuable discussions. They are also grateful to Mr. Conrad F. Piskorz for his valuable technical assistance and to Mr. J. Potienko for recording most of the ^{13}C-n.m.r. spectra. One of us (S.H.K.) thanks Roswell Park Memorial Institute for a predoctoral research fellowship.

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