Synthesis of a Tri-, a Penta-, and a Hepta-saccharide Containing Terminal *N*-Acetyl-β-D-lactosaminyl Residues, Part of the ' Complex-type ' Carbohydrate Moiety of Glycoproteins

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Silver trifluoromethanesulphonate-promoted condensation of 3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide with benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside, benzyl 3,6-di-*O*-benzyl-α-D-mannopyranoside, and benzyl 3,6-di-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside gave the protected tri-, penta-, and hepta-saccharide derivatives in 66, 72, and 56% yields, respectively. The free oligosaccharides were obtained after exchanging of the 2-deoxy-2-phthalimido-groups for 2-acetamido-2-deoxy-groups and deblocking.

Two main categories of carbohydrate moieties occur in mammalian glycoproteins; those linked *O*-glycosidically to L-serine or L-threonine residues, and those linked *N*-glycosidically to L-asparagine residues in the protein.[1] The latter category comprises two major types; the ' high-mannose ' type (oligomannosidic type) and the ' complex ' type (*N*-acetyl-D-lactosamine type).

We now report the synthesis of *O*-β-D-galactopyranosyl-(1 → 4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-D-mannopyranose (1), 2,4-di-*O*-[β-D-galactopyranosyl-(1 → 4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl]-D-mannopyranose (2), and 3,6-di-*O*-[β-D-galactopyranosyl-(1 → 4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl]-D-mannopyranose (3) which are parts of the ' complex ' type of carbohydrate moiety.

A preliminary account of the synthesis of the penta- and hepta-saccharides has appeared.[2] A synthesis, albeit in low yield, of the trisaccharide (1) has been reported [3] earlier as well as syntheses of the trisaccharide 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannose,[4] the pentasaccharide glycoside methyl 3,6-di-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl]-α-D-mannopyranoside,[5] and of the trisaccharide *O*-β-D-mannopyranosyl-(1 → 4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose.[6,7]

RESULTS AND DISCUSSION

The key intermediate in the synthesis of the three oligosaccharides (1), (2), and (3) is the D-lactosamine derivative 3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (4). Glycosidation with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromides in the presence of silver trifluoromethanesulphonate [8,9] gives a high yield of the β-anomer, and the bromide (4) could be anticipated to behave analogously to yield the oligosaccharides (1), (2), and (3).

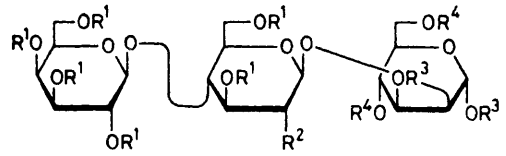
For the synthesis of compound (4) hexa-*O*-acetyl-D-lactal (5) was treated with cerium(IV) ammonium nitrate and sodium azide [10] to give the azidonitrates (6), (7), and (8) in the ratio *ca.* 1 : 4 : 8. The mixture was not further purified but hydrogenated (Pd–C catalyst) when the 1-nitrate groups were removed [11,12] and the 2-azido-groups reduced to 2-amino-groups. The hydrogenated material was treated first with phthalic anhydride in aqueous ethanol and then with acetic anhydride–pyridine. Chromatography on silica gel followed by crystallization yielded the acetates (9) and (10). For preparative purposes, however, a syrupy mixture [yield 47% from a mixture of (6), (7), and (8)] consisting mainly of the acetates (9) and (10), obtained after partial purification on silica gel, was used in the following step. Reaction of this mixture with hydrogen bromide gave the β-bromide (4).

Benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (11) was obtained in 90% yield by treatment of benzyl 2,3*R*;4,6*R*-di-*O*-benzylidene-α-D-mannopyranoside [13,14] with lithium aluminium hydride–aluminium chloride (1.1 mol per mol of glycoside).[14]

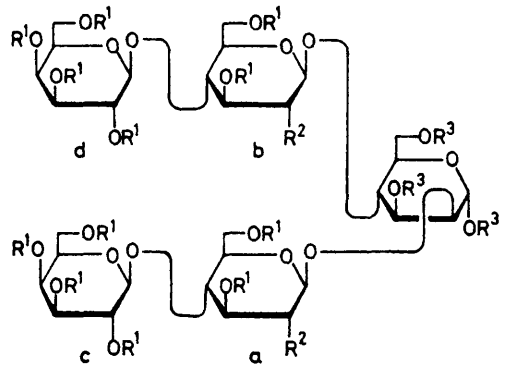
The bromide (4) was condensed with compound (11) using silver trifluoromethanesulphonate–*s*-collidine as promotor.[8,9] After chromatography on silica gel, the trisaccharide derivative (12) was obtained in 66% yield. Compound (12) was treated sequentially with sodium methoxide in methanol, hydrazine hydrate [9] in ethanol, and acetic anhydride–pyridine. After silica gel chromatography compound (13) was obtained in 73% yield. De-*O*-acetylation and de-*O*-benzylation by catalytic hydrogenation afforded, after gel-filtration and freeze-drying, the free trisaccharide (1), $[\alpha]_D$ −22°, as a powder. Methylation analysis [15] of the alditol of compound (1) revealed the presence of 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose, and 1,3,4,5,6-penta-*O*-methyl-D-mannitol.

Benzyl 3,6-di-*O*-benzyl-α-D-mannopyranoside (14) was prepared in 44% yield by treatment of benzyl α-D-mannopyranoside with bis(tributylstannyl) oxide followed by benzyl bromide.[16] A sample of compound (14) was methylated and the *O*-benzyl groups removed by catalytic hydrogenation. On analysis,[15] only 2,4-di-*O*-methyl-D-mannose could be detected.

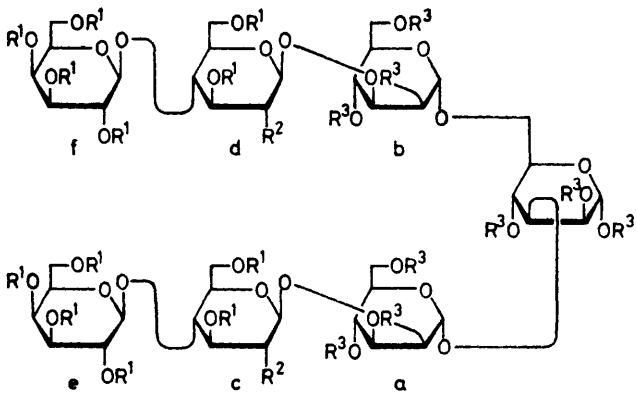
The bromide (4) was condensed with compound (14) as described above. The pentasaccharide derivative (15) was obtained as a syrup in 72% yield after chromato-



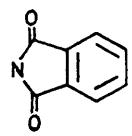
	R^1	R^2	R^3	R^4
(12)	Ac	NPhth	CH_2Ph	4,6-*O*-Benzylidene
(13)	Ac	NHAc	CH_2Ph	4,6-*O*-Benzylidene
(1)	H	NHAc	H	H



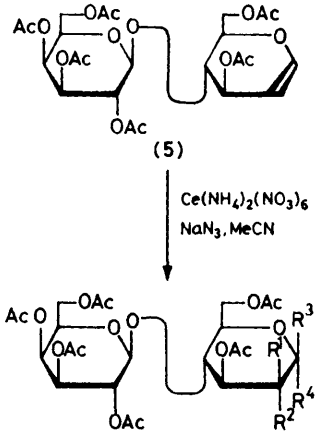
	R^1	R^2	R^3
(15)	Ac	NPhth	CH_2Ph
(16)	Ac	NHAc	CH_2Ph
(2)	H	NHAc	H



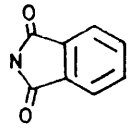
	R^1	R^2	R^3
(18)	Ac	NPhth	CH_2Ph
(19)	Ac	NHAc	CH_2Ph
(3)	H	NHAc	H

NPhth = 

graphy on silica gel. Compound (15) was transformed into the corresponding *N*-acetyl-derivative (16) in 70% yield by the same procedure used for the transformation of compound (12) into (13). Finally, the pentasaccharide derivative (16) was deprotected to give the free pentasaccharide (2), $[\alpha]_D$ −13°, as a powder. Methylation analysis of the alditol of the pentasaccharide (2) revealed the presence of 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose, and 1,3,5,6-tetra-*O*-methyl-D-mannitol.



	R^1	R^2	R^3	R^4
(4)	H	NPhth	Br	H
(6)	N_3	H	H	ONO_2
(7)	H	N_3	H	ONO_2
(8)	H	N_3	ONO_2	H
(9)	H	NPhth	H	OAc
(10)	H	NPhth	OAc	H

NPhth = 

For the synthesis of the heptasaccharide (3) a mannotrisaccharide derivative protected in all positions except *O*-2 of the non-reducing terminal α-D-mannopyranosyl groups was needed. The synthesis of benzyl 3,6-di-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside (17) has been reported earlier.[4]

The bromide (4) was condensed with the protected trisaccharide (17) as above and the protected heptasaccharide derivative (18) was obtained in 56% yield after chromatography. The *N*-acetyl-derivative (19) and the free heptasaccharide (3), $[\alpha]_D$ +14°, were obtained from (18) in 68 and 80% yields, respectively, as above. Methylation analysis of the alditol of the heptasaccharide (3) afforded 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose, 3,4,6-

tri-*O*-methyl-D-mannose, and 1,2,4,5-tetra-*O*-methyl-D-mannitol.

None of the free oligosaccharides (1), (2), and (3), and only some intermediates, were obtained crystalline. However, all compounds were chromatographically homogeneous and gave n.m.r. spectra which were consistent with the proposed structures. The ^{1}H n.m.r. spectra (see Experimental section) of the oligosaccharides (1), (2), and (3) show good agreement with those from related natural oligosaccharides.[17]

The biological properties of these oligosaccharides, *e.g.* interaction with carbohydrate-binding proteins, will be reported separately.

EXPERIMENTAL

Melting points are corrected. Concentration was performed at bath temperatures below 40 °C. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter. N.m.r. spectra were recorded in the pulsed Fourier-transform mode using JEOL FX-100 or Bruker WP-200 instruments at 25 °C. Chemical shifts are given in δ relative to internal $SiMe_4$ (^{1}H and ^{13}C, chloroform solutions), relative to external $SiMe_4$ (^{13}C, deuterium oxide solutions) and relative to internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulphonate (^{1}H, deuterium oxide solutions). For t.l.c. Merck silica gel 60 F-254 plates were used. Compounds were located by quenching of u.v. fluorescence, or by spraying with sulphuric acid. For column chromatography Merck silica gel 60 (0.040—0.063 mm) was used. The loadings on columns were usually 1 : 50—100. Procedures and equipment for methylation and sugar analysis were the same as described earlier,[15,18] except that hydrolyses were performed with 4M aqueous hydrochloric acid for 2 h at 100 °C. Elemental analysis was performed by Analytische Laboratorien, Elbach, W. Germany.

1,3,6-*Tri*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-2-*deoxy*-2-*phthalimido*-α- *and* -β-D-*glucopyranose*, (9) *and* (10).—Compound (5) [19] (20.0 g) in acetonitrile (170 cm^3) was added to a mixture of sodium azide (3.25 g) and cerium(IV) ammonium nitrate (45.0 g). The mixture was stirred for 16 h at −15 °C under nitrogen. Diethyl ether (400 cm^3) and water (250 cm^3) were added and the mixture was shaken. The organic phase was washed with water and concentrated to dryness. The residue was suspended in ethyl acetate (100 cm^3) at 40 °C, filtered, and light petroleum added. A *crystalline mass* (15.4 g, 65%) was obtained. The ^{1}H n.m.r. (99.60 MHz, $CDCl_3$) showed, *inter alia*, signals in the ratio *ca.* 8 : 1 : 4 at δ 5.58 (d, J 8.5 Hz), 6.15 (d, J 3.5 Hz), and 6.28 (d, J 4.2 Hz), attributed to the anomeric protons of compounds (8), (6), and (7), respectively. T.l.c. (toluene–ethyl acetate, 1 : 1) showed spots at R_F 0.60 [(6) and (8)] and 0.50 (7) and, in addition, a trace (estimated at <5%) of a compound with R_F 0.13. This compound, tentatively identified as the 1-*N*-acetamido-product,[10] was not detected in the ^{1}H n.m.r. spectrum discussed above.

A mixture of compounds (6), (7), and (8) (10.0 g) was dissolved in ethyl acetate (250 cm^3) and hydrogenated at 400 kPa over 10% palladium–charcoal (1.0 g). After filtering and concentrating, the reduction mixture was treated for 2 h at room temperature with phthalic anhydride (10.0 g) in 90% aqueous ethanol (100 cm^3) and kept at pH *ca.* 9 by addition of sodium carbonate. The reaction mixture was concentrated to dryness and the residue was dissolved in pyridine (50 cm^3). Acetic anhydride (50 cm^3) was added, and the mixture was kept at room temperature overnight, followed by heating on a boiling water-bath for 1 h. After cooling the solution was poured into water and extracted with methylene chloride. The organic phase was washed with saturated sodium hydrogencarbonate and water. Concentration followed by purification on silica gel with light petroleum–ethyl acetate (1 : 3) gave a syrupy mixture (5.4 g, 47%) of the *acetates* (9) and (10) together with a trace (^{1}H n.m.r.) of the corresponding α-D-*manno*-isomer. This mixture was used in the subsequent step. Crystallization from methanol gave the α-*acetate* (9), m.p. 229—230 °C, $[\alpha]_D^{21}$ +66° (*c* 1.0, $CHCl_3$); δ_H (99.60 Mz, $CDCl_3$), 1.94—2.18 (21 H, OAc), 3.80—5.40 (12 H, m, remaining H), 6.20 (1 H, d, $J_{1,2}$ 4.0 Hz, H-1), 6.38 (1 H, dd, $J_{2,3} \approx J_{3,4} \approx 8$ Hz, H-3), and 7.76 (4 H, m, phthaloyl); δ_C (25.05 MHz, $CDCl_3$), 20.4—21.0 (7 C, OAc), 52.8 (C-2), 60.9, 61.6 (C-6, C′-6), 66.8—78.5 (7 C, ring C), 90.4 (C-1), 100.9 (C′-1), 123.6, 131.1, 134.4 (phthaloyl), and 167.2—170.1 (C=O) (Found: C, 53.2; H, 5.0; N, 1.75. $C_{34}H_{39}NO_{19}$ requires C, 53.35; H, 5.10; N, 1.83%). Likewise, crystallization from methanol gave the β-*acetate* (10), m.p. 263—265 °C, $[\alpha]_D^{21}$ +32° (*c* 1.0, $CHCl_3$); δ_H (99.60 MHz, $CDCl_3$), 1.94—2.18 (21 H, OAc), 3.80—5.38 (12 H, m, remaining H), 5.84 (1 H, dd, $J_{2,3} \approx J_{3,4} \approx 8$ Hz, H-3), 6.48 (1 H, d, $J_{1,2}$ 8.5 Hz, H-1), and 7.76 (4 H, m, phthaloyl); δ_C (25.05 MHz, $CDCl_3$), 20.5—20.8 (7 C, OAc), 53.9 (C-2), 60.8, 62.0 (C-6, C′-6), 66.7—78.4 (7 C, ring C), 89.7 (C-1), 100.9 (C′-1), 123.7, 131.4, 134.7 (phthaloyl), and 167.4—170.2 (C=O) (Found: C, 53.2; H, 5.1; N, 1.75. $C_{34}H_{39}NO_{19}$ requires C, 53.35; H, 5.10; N, 1.83%).

3,6-*Di*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl* *Bromide* (4).—A mixture (4.0 g) of the acetates (9) and (10) was stirred in dichloromethane saturated with hydrogen bromide (100 cm^3) for 4 h at room temperature. The solution was concentrated to dryness, dissolved in ethyl acetate, and decolourizing carbon was added. The mixture was boiled for 10 min and filtered. Crystallization from ethyl acetate–light petroleum gave the *bromide* (4) (3.1 g, 75%), m.p. 109—110 °C, $[\alpha]_D^{21}$ +24° (*c* 1.0, $CHCl_3$) (sugar analysis [18] gave D-galactose and D-glucosamine only); δ_H (99.60 MHz, $CDCl_3$), 1.92—2.18 (18 H, OAc), 3.80—5.40 (12 H, m, remaining H), 5.70 (1 H, dd, $J_{2,3} \approx J_{3,4} \approx 8$ Hz, H-3), 6.39 (1 H, d, $J_{1,2}$ 10.0 Hz, H-1) and 7.80 (4 H, m, phthaloyl); δ_C (25.05 MHz, $CDCl_3$) 20.4—21.1 (6 C, OAc), 58.6 (C-2), 60.2, 60.8 (C-6, C′-6), 62.2—76.3 (7 C, ring C), 77.6 (C-1), 101.0 (C′-1), 123.7, 131.2, 134.5 (phthaloyl), and 167.2—170.1 (C=O) (Found: C, 48.95; H, 4.65; Br, 10.0; N, 1.75. $C_{32}H_{36}BrNO_{17}$ requires C, 48.88; H, 4.58; Br, 10.16; N, 1.78%).

Benzyl 3-O-*Benzyl*-4,6-O-*benzylidene*-α-D-*mannopyranoside* (11).—Lithium aluminium hydride (1.0 g) was added to a solution of benzyl 2,3*R*;4,6*R*-di-*O*-benzylidene-α-D-mannopyranoside [13,14] (2.55 g) in ether–dichloromethane (1 : 1) (60 cm^3). Aluminium chloride (3.0 g) in ether (30 cm^3) was added dropwise to the refluxing mixture. After 2 h reflux the reagents were decomposed by addition of ethyl acetate and water. After work-up, the product was purified on silica gel with chloroform–acetone (19 : 1) to yield the *mannoside* (11) as a syrup (2.30 g, 90%), $[\alpha]_D^{21}$ +54° (*c* 1.0, $CHCl_3$), R_F 0.48 (solvent as above) (lit.,[14] m.p. 88—89 °C, $[\alpha]_D^{21}$ +55°); δ_H (99.60 MHz, $CDCl_3$) 3.77—4.80 (10 H, remaining H), 4.95 (1 H, d, $J_{1,2}$ small, H-1),

5.59 (1 H, s, C*H*Ph), 7.20—7.40 (15 H, m, aromatic); δ_C (25.05 MHz, $CDCl_3$) 63.7 (C-5), 68.6, 69.7 (C-6, CH_2Ph), 73.0 (C-2), 76.0 (C-3), 78.8 (C-4), 99.6 (C-1), 101.3 (*C*HPh), and 126.0—138.0 (aromatic).

Benzyl 3,6-*Di*-O-*benzyl*-α-D-*mannopyranoside* (14).—A solution of benzyl α-D-mannopyranoside [20] (2.7 g) and bis-(tributylstannyl) oxide (7.6 cm³) in toluene (300 cm³) was refluxed for 4 h with continuous removal of water. After evaporation of the toluene, benzyl bromide (50 cm³) was added and the mixture was kept under nitrogen for 3 days at *ca.* 90 °C. After work-up the product was purified on silica gel with chloroform–acetone (5 : 1) to yield the *mannoside* (14) as a syrup (2.0 g, 44%), $[\alpha]_D^{21}$ +42° (*c* 0.6, $CHCl_3$), R_F 0.42 (solvent as above); δ_H (99.60 MHz, $CDCl_3$) 3.60—4.80 (12 H, m, ring H and CH_2Ph), 4.95 (1 H, d, $J_{1,2}$ small, H-1), and 7.1—7.4 (15 H, m, aromatic); δ_C (25.05 MHz, $CDCl_3$) 67.5, 67.9 (*C*H_2Ph), 69.0 (C-4), 70.1, 71.0 (*C*H_2Ph, C-6), 71.9 (C-2), 73.5 (C-5), 79.6 (C-3), 98.7 (C-1), and 127.6—137.9 (aromatic).

Trisaccharide Derivative (12).—Silver trifluoromethanesulphonate (670 mg) and *s*-collidine (315 mg) were added to a solution of mannoside (11) (766 mg) in dichloromethane (10 cm³) and the mixture was cooled to −40 °C under nitrogen. Bromide (4) (2.0 g) in dichloromethane (10 cm³) was added dropwise with stirring. The mixture was allowed to attain room temperature overnight. After filtration the mixture was extracted with dilute aqueous hydrogen chloride, water, saturated sodium hydrogencarbonate, and water. The product was purified on silica gel with toluene–ethyl acetate (1 : 1) to yield the *trisaccharide* (12) as a pure syrup (1.31 g, 66%). Crystallization from methanol yielded crystals, m.p. 167—168 °C, $[\alpha]_D^{21}$ +19° (*c* 1.0, $CHCl_3$); δ_H (99.60 MHz, $CDCl_3$) 1.88—2.20 (18 H, OAc), 3.50—5.36 (24 H, m, remaining H), 5.40 (1 H, s, C*H*Ph), 5.78 (1 H, dd, $J_{2',3'} \approx J_{3',4'} \approx 8$ Hz, H′-3), 7.10—7.40 (15 H, m, aromatic), and 7.72 (4 H, m, phthaloyl); δ_C (25.05 MHz, $CDCl_3$) 20.6—21.4 (6 C, OAc), 54.8 (C′-2), 60.8—78.6 (*C*H_2-Ph, ring C, C-6, C′-6, C″-6), 96.4 (C′-1), 97.2 (C-1), 101.0 (C″-1), 101.4 (*C*HPh), 123.2—138.3 (aromatic), and 168.9—170.1 (C=O) (Found: C, 61.8; H, 5.55; N, 1.15. $C_{59}H_{63}NO_{23}$ requires C, 61.42; H, 5.47; N, 1.21%).

Trisaccharide Derivative (13).—A catalytic amount of sodium was added to a solution of compound (12) (960 mg) in methanol (20 cm³); the mixture was left at room temperature overnight and then concentrated to dryness at 25 °C. The product was dissolved in ethanol (20 cm³), hydrazine hydrate (4 cm³) was added, and the solution was refluxed for 6 h. After cooling the solution was concentrated to dryness. The product was then acetylated with acetic anhydride–pyridine (1 : 1) (4 cm³) at room temperature overnight. After concentration the product was purified on silica gel with toluene–ethyl acetate (1 : 4) to yield the *trisaccharide* (13) as a syrup (651 mg, 73%), $[\alpha]_D^{21}$ +2° (*c* 1.0, $CHCl_3$), R_F 0.47 (solvent as above); δ_H (99.60 MHz, $CDCl_3$) 1.84—2.16 (21 H, OAc, NHAc), 3.64—5.36 (26 H, m, remaining H), 5.60 (1 H, s, C*H*Ph), and 7.10—7.40 (15 H, m, aromatic); δ_C (25.05 MHz, $CDCl_3$), 20.4—23.1 (7 C, NHAc, OAc), 54.2 (C′-2), 60.9, 62.5, 64.4 (C-5, C′-6, C″-6), 69.4—78.7 (*C*H_2Ph, C-6, ring C), 98.4 (C-1), 99.5 (C′-1), 100.8 (C″-1), 101.3 (*C*HPh), 125.9—138.4 (aromatic), and 169.0—170.3 (C=O).

O-β-D-*Galactopyranosyl*-(1 ⟶ 4)-O-2-*acetamido*-2-*deoxy*-β-*glucopyranosyl*-(1 ⟶ 2)-D-*mannopyranose* (1).—A catalytic amount of sodium was added to a solution of compound (13) (627 mg) in methanol (15 cm³); the mixture was left at room temperature overnight, neutralized with acetic acid, and concentrated to dryness. The product was dissolved in 90% aqueous acetic acid (50 cm³) and hydrogenated at 400 kPa over 10% palladium–charcoal (400 mg) overnight. After filtration and concentration the product was de-salted by gel filtration on a Sephadex G-25 column (2.5 × 80 cm) irrigated with water. After freeze-drying the *trisaccharide* (1) was obtained as an amorphous powder (203 mg, 63%), $[\alpha]_D^{21}$ −22° (*c* 1.0, H_2O) (lit.,[3] $[\alpha]_D^{20}$ −13°); δ_H (200 MHz, D_2O), 2.05 (3 H, s, NHAc), 4.47 (1 H, d, $J_{1,2}$ 7.5 Hz, H″-1), 4.60 (1 H, d, $J_{1,2}$ *ca.* 8 Hz, H′-1), 4.94 (0.2 H, d, $J_{1,2}$ *ca.* 1 Hz, H$^{\beta}$-1), and 5.20 (0.8 H, d, $J_{1,2}$ *ca.* 1 Hz, H-$^{\alpha}$1); δ_C (25.05 MHz, D_2O), 23.6, 23.8 (NHAc, α- and β-form), 56.1 (C′-2), 61.2, 62.2, 62.8 (C-6, C′-6, C″-6), 68.7—79.8 (ring C), 92.3 (0.85 C, C$^{\alpha}$-1), 95.1 (0.15 C, C$^{\beta}$-1), 100.7 (0.85 C, C′-1 when C-1 is in α-form), 102.5 (0.15 C, C′-1 when C-1 is in β-form) 104.1 (C″-1), and 175.8 (C=O) (Found: C 44.25; H, 6.3; N, 2.7; $C_{20}H_{35}NO_{14}$ requires, C, 44.06; H, 6.43; N, 2.57%).

Pentasaccharide Derivative (15).—Mannoside (14) (140 mg) and bromide (4) (800 mg) were condensed using silver trifluoromethanesulphonate (285 mg) and collidine (135 mg) as promotor, as described for the corresponding preparation of trisaccharide (12). The product was purified on silica gel with toluene–ethyl acetate (1 : 2) to yield the *pentasaccharide* (15) as a syrup (419 mg, 72%), $[\alpha]_D^{21}$ +12° (*c* 0.8, $CHCl_3$), R_F 0.56 (solvent as above); δ_C (25.05 MHz, $CDCl_3$) 20.6, 20.9, (12 C, OAc), 54.7, 55.4 (C^a-2, C^b-2), 60.7, 61.5, 62.3 (4 C, C^{a-d}-6), 66.5—78.4 (*C*H_2Ph, C-6, ring C), 96.6 (C^a-1), 97.1 (C-1), 98.2 (C^b-1), 101.0 (2 C, C^c-1, C^d-1), 123.2—138.8 (aromatic), and 167.7—170.1 (C=O) (Found: C, 58.6; H, 5.3; N, 1.45. $C_{91}H_{100}N_2O_{40}$ requires C, 58.73; H, 5.38; N, 1.51%).

Pentasaccharide Derivative (16).—Compound (15) (397 mg) was treated sequentially with sodium methoxide, hydrazine hydrate, and acetic anhydride–pyridine as described above for the corresponding preparation of compound (13). After purification on silica gel with chloroform–acetone (1 : 1) the *pentasaccharide* (16) was obtained as a syrup (250 mg, 70%), $[\alpha]_D^{21}$ −1° (*c* 1.0, $CHCl_3$), R_F 0.62 (solvent as above); δ_C (25.05 MHz, $CDCl_3$) 20.6—24.9 (14 C, NHAc, OAc), 54.2 (2 C, C^a-2, C^b-2), 60.8, 62.1 (4 C, C^{a-d}-6), 66.7—77.2 (*C*H_2Ph, C-6, ring C), 96.9 (C-1), 99.5 (C^a-1), 101.0 (3 C, C^b-1, C^c-1, C^d-1), 127.2—138.4 (aromatic), and 168.8—170.5 (C=O).

2,4-*Di*-O-[β-D-*galactopyranosyl*-(1 ⟶ 4)-O-2-*acetamido*-2-*deoxy*-β-D-*glucopyranosyl*]-D-*mannopyranose* (2).—Compound (16) (240 mg) was treated with sodium methoxide followed by catalytic hydrogenation as described above for the corresponding preparation of compound (1). After purification of the product by gel-filtration and freeze-drying the *pentasaccharide* (2) was obtained as an amorphous powder (79 mg, 61%), $[\alpha]_D^{21}$ −13° (*c* 1.0, H_2O); δ_H (200 MHz, D_2O) 2.05, 2.07 (6 H, s, NHAc), 4.48 (2 H, d, $J_{1,2}$ 7.5 Hz, H^c-1, H^d-1), 4.55 (1 H, d, $J_{1,2}$ *ca.* 8 Hz, H^b-1), 4.59 (1 H, d, $J_{1,2}$ *ca.* 8 Hz, H^a-1), 4.92 (0.15 H, d, $J_{1,2}$ *ca.* 1 Hz, H$^{\beta}$-1), and 5.19 (0.85 H, d, $J_{1,2}$ *ca.* 1 Hz, H$^{\alpha}$-1) [δ(200 MHz, D_2O, 85 °C) see ref. 2]; δ_C (25.05 MHz, D_2O) 23.3, 23.6 (2 C, NHAc), 56.1 56.4 (C^a-2, C^b-2), 61.3—62.3 (5 C, C-6′s), 68.3—79.8 (ring C), 92.1 (0.85 C, C$^{\alpha}$-1), 95.1 (0.15 C, C$^{\beta}$-1), 100.8 (C^a-1), 102.7 (C^b-1), 104.1 (2 C, C^c-1, C^d-1), and 175.6, 176.0 (C=O) (Found: C, 44.75; H, 6.45; N, 2.95. $C_{34}H_{58}N_2O_{26}$ requires C, 44.86; H, 6.38; N, 3.08%).

Heptasaccharide Derivative (18).—The protected *manno*-trisaccharide [4] (17) (416 mg) and bromide (4) (800 mg) were

condensed using silver trifluoromethanesulphonate (285 mg) and *s*-collidine (135 mg) as promotor as described above for the corresponding preparation of trisaccharide (12). The product was purified on silica gel with toluene–ethyl acetate (1 : 1) to yield the *heptasaccharide* (18) as a syrup (485 mg, 56%), $[\alpha]_D^{21}$ +10° (*c* 1.0, $CHCl_3$), R_F 0.43 (solvent as above); δ_C (25.05 MHz, $CDCl_3$, 50 °C) 20.4—21.3 (12 C, OAc), 54.9 (2 C, C^c-2, C^d-2), 61.1—78.6 (CH_2Ph, C-6's, ring C), 96.2 (C-1), 96.8 (2 C, C^c-1, C^d-1), 97.3 (C^b-1), 99.1 (C^a-1), 101.1, 101.2 (C^e-1, C^f-1), 123.2—138.8 (aromatic), and 167.6—170.0 (C=O) (Found: C, 63.8; H, 5.6; N, 0.95. $C_{145}H_{156}N_2O_{50}$ requires C, 63.89; H, 5.73; N, 1.03%).

Heptasaccharide Derivative (19).—Compound (18) (465 mg) was treated with sodium methoxide, hydrazine hydrate, and acetic anhydride–pyridine as described above for the corresponding preparation of compound (13). After purification on silica gel with toluene–ethyl acetate (1 : 4) the *heptasaccharide* (19) was obtained as a syrup (296 mg, 68%), $[\alpha]_D^{21}$ +11° (*c* 1.0, $CHCl_3$), R_F 0.42 (solvent as above); δ_C (25.05 MHz, $CDCl_3$) 20.4—23.2 (14 C, NHAc, OAc), 54.0, 54.6 (C^c-2, C^d-2), 60.9—78.4 (CH_2Ph, C-6's, ring C), 97.8 (C-1), 99.1 (2 C, C^c-1, C^d-1), 99.6 (C^b-1), 100.3 (C^a-1), 101.3 (2 C, C^e-1, C^f-1), 125.9—138.9 (aromatic), and 168.9—170.0 (C=O).

3,6-*Di*-O-[β-D-*galactopyranosyl*-(1 → 4)-O-2-*acetamido*-2-*deoxy*-β-D-*glucopyranosyl*-(1 → 2)-O-α-D-*mannopyranosyl*]-D-*mannopyranose* (3).—Compound (19) (274 mg) was treated with sodium methoxide followed by catalytic hydrogenation under the same conditions used for the corresponding preparation of compound (1). After purification of the product by gel-filtration and freeze-drying, the *heptasaccharide* (3) was obtained as an amorphous powder (107 mg, 80%), $[\alpha]_D^{21}$ +14° (*c* 1.0, H_2O); δ(200 MHz, D_2O) 2.06 (6 H, s, NHAc), 4.48 (2 H, d, $J_{1,2}$ 7.5 Hz, H^e-1, H^f-1), 4.59 (2 H, br d, H^c-1, H^d-1), 4.92 (1.25 H, d, $J_{1,2}$ *ca.* 1 Hz, H^β-1, H^b-1), and 5.15 (1.75 H, d, $J_{1,2}$ *ca.* 1 Hz, H^α-1, H^a-1) [δ(200 MHz, D_2O, 85 °C) see ref. 2], δ_C (25.05 MHz, D_2O) 23.5 (2 C, NHAc), 56.1 (2 C, C^c-2, C^d-2), 61.2—81.7 (C-6 s and ring C), 95.0 (0.20 C, C^β-1), 95.4 (0.80 C, C^α-1), 98.0 (C^b-1), 100.6 (3 C, C^a-1, C^c-1, C^d-1), 104.1 (2 C, C^e-1, C^f-1), and 175.9 (C=O) (Found: C, 44.5; H, 6.85; N, 2.3; $C_{46}H_{78}N_2O_{36}$ requires C, 44.75; H, 6.32; N, 2.27%).

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