Synthesis of Three Oligosaccharides that form Part of the Complex Type of Carbohydrate Moeity of Glycoproteins containing Intersecting *N*-Acetylglucosamine

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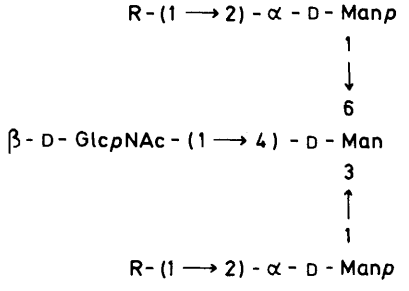
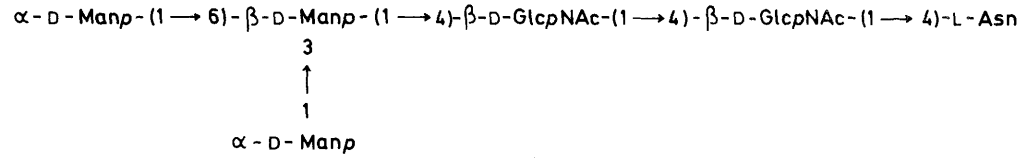
The oligosaccharides (**1**)—(**3**) have been synthesized by conventional methods. 4-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-(α-D-mannopyranosyl)-D-mannose (**1**), 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3,6-bis-*O*-[2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-D-mannose (**2**), and 3,6-bis-*O*-{2-*O*-[2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose (**3**) represent partial structures of the carbohydrate moiety in those glycoproteins of the complex type in which a 2-acetamido-2-deoxy-β-D-glucopyranosyl group is linked to O-4 of the β-D-mannopyranosyl residue of the invariant core (**4**). *p*-Methoxybenzyl groups were used for temporary protection of hydroxy groups.

The glycoproteins of the *N*-glycosidic type contain an invariant oligosaccharide core structure (**4**) and variation is provided by substitution with mono- or oligo-saccharide groups at different positions of this core structure.[1,2] A number of oligosaccharides, representative of different glycoproteins belonging to the complex or *N*-acetyl-lactosamine type, have been synthesized [3–6] and studied in different biological systems.[7,8] In some glycoproteins a 2-acetamido-2-deoxy-β-D-glucopyranosyl group is linked to O-4 of the β-D-mannopyranosyl residue in (**4**), and we now report the synthesis of three oligosaccharides (**1**)—(**3**) containing this unusual feature.



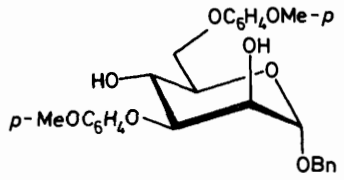
(**1**) R = H
(**2**) R = β-D-GlcpNAc
(**3**) R = β-D-Galp(1→4)-β-D-GlcpNAc



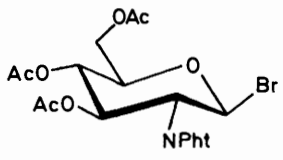
(**4**)

Results and Discussion

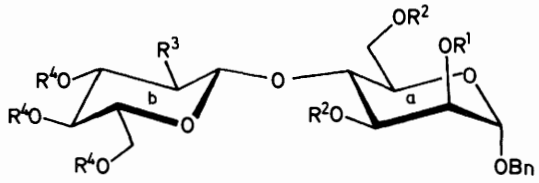
Benzyl 3,6-bis-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (**5**) was glycosylated at the 4-position with one equivalent of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[9] (**6**) using silver trifluoromethanesulphonate–*s*-collidine as promoter. Benzyl 3,6-bis-*O*-(*p*-methoxybenzyl)-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranoside (**7**) was obtained in 66% yield after purification on silica gel. Compound (**7**) was treated with hydrazine hydrate and acetylated to yield benzyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-*O*-acetyl-3,6-bis-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (**8**) (75%) after purification on silica gel. Treatment of (**8**) with sodium methoxide in methanol and benzylation with sodium hydride–benzyl bromide yielded benzyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-2-*O*-benzyl-3,6-bis-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (**9**) (66%). A compound showing higher mobility on t.l.c. was also isolated and identified as the *N*-benzyl derivative of (**9**). It was possible to transform this derivative into compound (**9**) by treatment with potassium t-butoxide in dimethyl sulphoxide[10] in 78% yield. Oxidative cleavage of the *p*-methoxybenzyl ethers[11] with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) yielded crystalline benzyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-2-*O*-benzyl-α-D-mannopyranoside (**10**) (85%). An aliquot of (**10**) was subjected to catalytic hydrogenation, sodium borohydride reduction, and methylation analysis[12] which showed the



(5) Bn = Benzyl



(6) NPht = Phthalimido

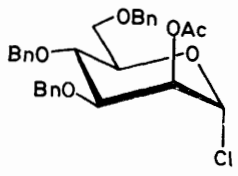


	R^1	R^2	R^3	R^4
(7)	H	CH_2-C$_6$H$_4$-OMe	NPht	Ac
(8)	Ac	CH_2-C$_6$H$_4$-OMe	NHAc	Ac
(9)	Bn	CH_2-C$_6$H$_4$-OMe	NHAc	Bn
(10)	Bn	H	NHAc	Bn

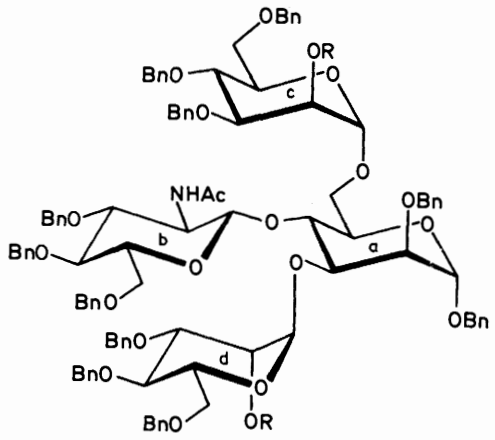
presence of 1,2,3,5,6-penta-*O*-methyl-D-mannitol and 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucose. Other benzyl α-D-mannosides with temporary protecting groups in the 3- and 6-position were also used in attempts to obtain the disaccharide (**10**). Thus, benzyl 3,6-di-*O*-allyl-α-D-mannopyranoside and benzyl 3,6-di-*O*-but-2-enyl-α-D-mannopyranoside were condensed with the bromide (**6**) in yields similar to those obtained in the above synthesis. However, it was difficult to remove these temporary protecting groups, and it was only possible to isolate compound (**10**) in low yield. Benzyl 3,6-di-*O*-benzoyl-α-D-mannoside gave no selectivity in the condensation step.

Condensation of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[13] (**11**) (*ca.* 3 equiv.) with compound (**10**) using silver trifluoromethanesulphonate as promoter gave benzyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3,6-bis-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2-*O*-benzyl-α-D-mannopyranoside (**12**) in 58% yield. After treatment with sodium methoxide in methanol, compound (**12**) was transformed into benzyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-2-*O*-benzyl-3,6-bis-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**13**). A ^{13}C n.m.r. spectrum of (**12**) showed *inter alia* four signals in the anomeric region, δ_C 96.9 ($J_{C\text{-}1^a\text{-}1^a\text{-}H}$ 173 Hz), 98.9 ($J_{C\text{-}1^c\text{-}1^c\text{-}H}$ 170 Hz), 100.8 ($J_{C\text{-}1^d\text{-}1^d\text{-}H}$ 173 Hz), and 101.1 ($J_{C\text{-}1^b\text{-}1^b\text{-}H}$ 159 Hz),* indicating three α-D-mannoside linkages and one β-D-glucosamido linkage. The free tetrasaccharide (**1**) was obtained after catalytic hydrogenation of (**13**). Methylation analysis of the alditol of (**1**) gave 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-mannose, and 1,2,5-tri-*O*-methyl-D-mannitol.

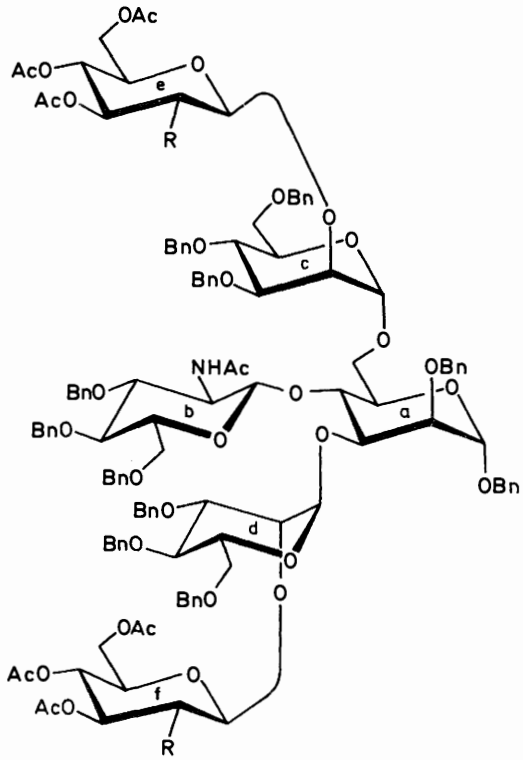
Coupling of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[9] (**6**) with (**13**) promoted by silver trifluoromethanesulphonate gave the hexasaccharide derivative



(11)

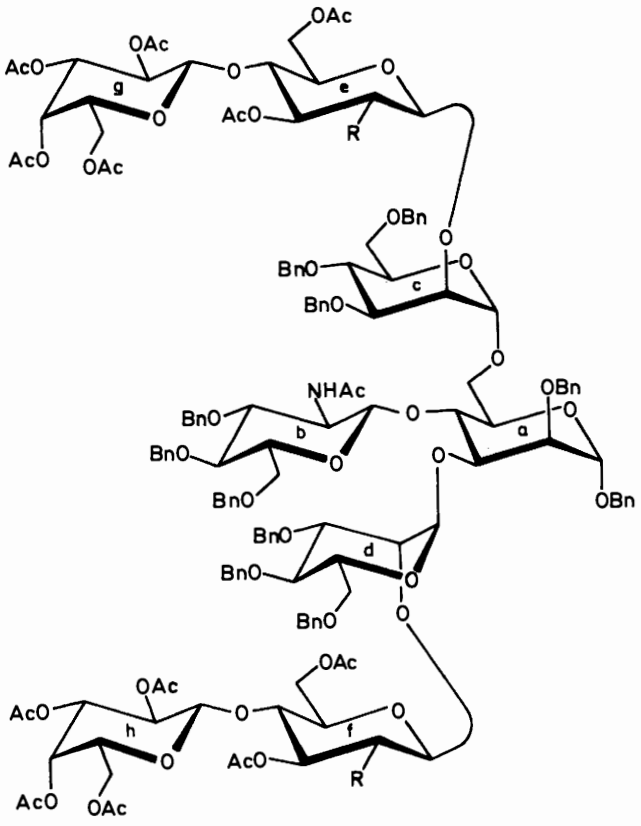


	R
(12)	Ac
(13)	H



	R
(14)	NPht
(15)	NHAc

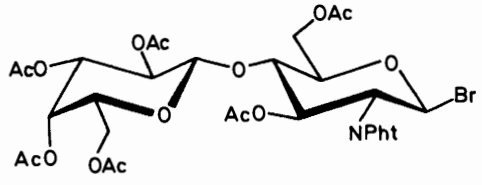
* Superscripts a—h in this paper refer to the respective monosaccharide rings labelled in the structure.



R

(16) NPht

(17) NHAc



(18)

(14) in 51% yield. Hydrazinolysis and acetylation of (14) gave, after purification, compound (15) which was transformed into the free hexasaccharide (2) by transesterfication and catalytic hydrogenation. Methylation analysis of the alditol of (2) gave 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucose, 3,4,6-tri-*O*-methyl-D-mannose, and 1,2,5-tri-*O*-methyl-D-mannitol.

The octasaccharide derivative (16) was obtained in 57% yield by condensation of 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl bromide[3] (18) with compound (13). Subsequent treatment, as for (14), gave the free octasaccharide (3). Methylation analysis of the alditol of (3) gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-3,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucose, 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucose, 3,4,6-tri-*O*-methyl-D-mannose, and 1,2,5-tri-*O*-methyl-D-mannitol. Attempts to make the octasaccharide using silver trifluoromethanesulphonate–*s*-collidine failed, probably because of formation of imidates at the *N*-acetamido group on the β-Glc-*N*-Ac residue, as indicated by ^{13}C n.m.r. spectroscopy.[14]

Experimental

General Methods.—M.p.s were corrected. Concentration of solutions was performed at bath temperatures below 40 °C. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter. ^{13}C N.m.r. spectra (25 MHz) were recorded using a JEOL FX-100 spectrometer. ^1H N.m.r. spectra (100 or 400 MHz) using JEOL FX-100 or JEOL GX-400 spectrometers. Deuteriochloroform was used as solvent for protected saccharides, and deuterium oxide for free saccharides. Chemical shifts are given relative to internal tetramethylsilane (^{13}C, ^1H; $CDCl_3$), relative to external tetramethylsilane (^{13}C; D_2O), and relative to HOD (^1H; D_2O). 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 8% sulphuric acid (charring). For column chromatography Merck silica gel 60 (0.040—0.063 mm) was used. Elemental analysis was performed by Analytische Laboratorien, Elbach, West Germany. Light petroleum refers to the faction boiling in the range 40—60 °C.

Benzyl 3,6-*Bis*-O-(p-*methoxybenzyl*)-α-D-*mannopyranoside* (5).—Benzyl α-D-mannopyranoside (15.3 g) was suspended in a mixture of toluene (400 ml) and bis(tributyltin) oxide (37 ml). The mixture was refluxed for 2 h with removal of water. Tetrabutylammonium bromide (20 g) and *p*-methoxybenzyl bromide (22 ml) was added and the solution was stirred at 90 °C overnight. The chilled reaction mixture was washed with water and evaporated. The residue was dissolved in 90% aqueous methanol and washed with light petroleum, and the methanol phase was evaporated to dryness. Purification on silica gel with toluene–ethyl acetate (2:1) as eluant yielded compound (5) as a syrup (7.1 g, 24%); $[\alpha]_D^{21}$ +33° (*c* 1 in $CHCl_3$); R_F 0.44 [toluene–ethyl acetate (1:1)]; δ_H (99.60 MHz; $CDCl_3$) 3.68 (6 H, OMe), 4.85 (1 H, $J_{1,2}$ *ca.* 2 Hz, 1-H), and 6.65—7.30 (ArH); δ_C (25.00 MHz; $CDCl_3$) 55.0 (2 C, OMe), 67.4—79.3 (ring C, C-6, and CH_2Ph), 98.9 (C-1), and 113.7—159.3 (Ar).

Benzyl 3,6-*Bis*-O-(p-*methoxybenzyl*)-4-O-(3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl*)-α-D-*mannopyranoside* (7).—Silver trifluoromethanesulphonate (1.77 g), *s*-collidine (0.83 g), and ground molecular sieves (3Å, 2 g) were added to a solution of compound (5) (3.20 g) in dichloromethane (30 ml) and the mixture was cooled to −40 °C under nitrogen. A solution of bromide (6) (3.12 g) in dichloromethane (10 ml) was added dropwise during 30 min. When no starting material was detected on t.l.c. [toluene–ethyl acetate (1:1)], the mixture was filtered and concentrated. Chromatography on silica gel with the above eluant yielded the disaccharide (7) as a syrup (3.85 g, 66%); $[\alpha]_D^{21}$ +94° (*c* 1 in $CHCl_3$); R_F 0.55 (developer as above); δ_C (25.00 MHz; $CDCl_3$) 20.2—20.5 (3 C, $COCH_3$), 55.0 (2 C, OMe), 55.2 (C-2^b), 61.4—77.6 (ring C, 2 C-6, and CH_2Ph), 97.6 and 98.6 (2 C, C-1^a and C-1^b), 113.5—159.1 (Ar), and 167.7—170.4 (C=O).

Benzyl 4-O-(2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*)-2-O-*acetyl*-3,6-*bis*-O-(p-*methoxybenzyl*)-α-D-*mannopyranoside* (8).—Compound (7) (3.45 g) was dissolved in 90% aqueous ethanol (200 ml), hydrazine hydrate (12 ml) was added, and the solution was refluxed for 6 h. Removal of solvent and co-distillation with ethanol and with toluene left a dry residue which was dissolved in acetic anhydride–pyridine (1:1; 40 ml) and the mixture was kept for 1 h at 100 °C. After concentration the reaction mixture was suspended in chloroform (2 × 10 ml) and filtered. Purification of the filtrate on silica gel with chloroform–acetone (8:1) as eluant gave the disaccharide (8) (2.45 g, 75%), m.p. 179—182 °C (from ethanol); $[\alpha]_D^{21}$ +26° (*c* 1 in $CHCl_3$); R_F 0.49 [light petroleum–acetone (1:1)]; δ_C (25 MHz; $CDCl_3$) 20.6—21.0 ($OCOCH_3$), 23.1

($NCOCH_3$), 54.8 (C-2^b), 55.2 (2 C, OMe), 61.9—75.3 (ring C, 2 C-6, and CH_2Ph), 96.9 (C-1^a), 100.3 (C-1^b), 113.8—159.5 (Ar), and 169.3—170.6 (C=O) (Found: C, 61.1; H, 6.2; N, 1.6. $C_{45}H_{55}NO_{17}$ requires C, 61.28; H, 6.29; N, 1.59%).

Benzyl 4-O-(2-*Acetamido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-2-O-*benzyl*-3,6-*bis*-O-(p-*methoxybenzyl*)-α-D-*mannopyranoside* (**9**).—A catalytic amount of sodium was added to a solution of compound (**8**) (1.73 g) in methanol (30 ml). After being kept overnight at room temperature the methoxide was neutralised with acetic acid and the mixture was evaporated. The residue was suspended in tetrahydrofuran (40 ml) and the mixture was added to light petroleum-washed sodium hydride (*ca.* 0.4 g) in a sealed flask. After being stirred for 30 min, benzyl bromide (1.8 ml) was added and the mixture was boiled under reflux for 1 h. Excess of reagent was destroyed with methanol and the mixture was diluted with toluene and washed with water. Chromatography on silica gel with toluene–ethyl acetate (3:1) as eluant gave compound (**9**) (1.28 g, 61%), m.p. 136—139 °C (from ethyl acetate–light petroleum); $[\alpha]_D^{21}$ +37° (*c* 1 in $CHCl_3$); R_F 0.32 [toluene–ethyl acetate (3:1)]; δ_C (25 MHz; $CDCl_3$) 23.4 ($COCH_3$), 55.1 (2 C, OMe), 56.8 (C-2^b), 68.7—82.1 (ring C, 2 C-6, and CH_2Ph), 97.8 (C-1^a), 100.4 (C-1^b), 113.6—159.3 (Ar), and 170.0 (C=O) (Found: C, 72.7; H, 6.6; N, 1.2. $C_{65}H_{71}NO_{13}$ requires C, 72.67; H, 6.66; N, 1.30%).

Benzyl 4-O-(2-*Acetamido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-2-O-*benzyl*-α-D-*mannopyranoside* (**10**).—DDQ (230 mg) was added to a stirred solution of compound (**9**) (495 mg) in moist dichloromethane (20 ml). After 1 h at room temperature the mixture was diluted with dichloromethane and washed successively with aqueous sodium hydrogen carbonate and water. The product was purified on silica gel with light petroleum–acetone (2:1) as eluant to yield the disaccharide derivative (**10**) (0.33 g, 85%) as a syrup. Crystallisation from ethanol gave needles, m.p. 170—172 °C; $[\alpha]_D^{21}$ +73° (*c* 1 in $CHCl_3$); R_F 0.31 (developer as above); δ_C (25.00 MHz; $CDCl_3$) 23.2 ($COCH_3$), 55.8 (C-2^b), 60.8 (C-6^a), 68.5—82.3 (ring C, C-6^b, and CH_2Ph), 98.1 (C-1^a), 102.0 (C-1^b), 127.1—138.8 (Ar), and 171.0 (C=O) (Found: C, 70.55; H, 6.7; N, 1.6. $C_{49}H_{55}NO_{11}$ requires C, 70.57; H, 6.65; N, 1.68%).

Benzyl 4-O-(2-*Acetamido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-3,6-*bis*-O-(2-O-*acetyl*-3,4,6-*tri*-O-*benzyl*-α-D-*mannopyranosyl*)-2-O-*benzyl*-α-D-*mannopyranoside* (**12**).—A solution of disaccharide derivative (**9**) (0.66 g) and the chloride (**11**) (1.3 g) in toluene (20 ml) with ground molecular sieves (4Å, *ca.* 1 g) was cooled to 0 °C under nitrogen. To the stirred solution was added dropwise a solution of silver trifluoromethanesulphonate (0.86 g) in toluene (10 ml) during 30 min. Pyridine (0.5 ml) was added after another 15 min and the reaction mixture was filtered and concentrated. Purification by chromatography on silica gel with toluene–ethyl acetate (3:1) as eluant yielded the tetrasaccharide derivative (**12**) as a syrup (0.82 g, 58%), $[\alpha]_D^{21}$ +53° (*c* 1 in $CHCl_3$); R_F 0.39 [toluene–ethyl acetate (2:1)]; δ_C (25.00 MHz; $CDCl_3$) 21.0 and 21.2 (2 $OCOCH_3$), 23.2 ($NCOCH_3$), 56.2 (C-2^b), 66.0—81.8 (ring C, C-6s, and CH_2Ph), 96.8 (C-1^a, $J_{C\text{-}1\text{-}1\text{-}H}$ 173 Hz), 97.2 (C-1^c, $J_{C\text{-}1\text{-}1\text{-}H}$ 170 Hz), 99.0 (C-1^d, $J_{C\text{-}1\text{-}1\text{-}H}$ 173 Hz), 100.5 (C-1^b, $J_{C\text{-}1\text{-}1\text{-}H}$ 159 Hz), 127.6—138.8 (Ar), and 170.0 and 170.2 (3 C=O).

Benzyl 4-O-(2-*Acetamido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-2-O-*benzyl*-3,6-*bis*-O-(3,4,6-*tri*-O-*benzyl*-α-D-*mannopyranosyl*)-α-D-*mannopyranoside* (**13**).—Compound (**12**) (0.74 g) was dissolved in CH_2Cl_2 (1 ml) and the solution was treated with a catalytic amount of sodium methoxide in methanol (20 ml) at room temperature and kept overnight. After neutralisation with acetic acid and evaporation the residue was purified on silica gel with toluene–ethyl acetate (1:2) eluant. Tetrasaccharide derivative (**13**) was isolated in 89% (0.74 g) yield, $[\alpha]_D^{21}$ +52° (*c* 1 in $CHCl_3$); R_F 0.51 [toluene–ethyl acetate (1:2)]; δ_C (25.00 MHz; $CDCl_3$) 23.2 ($NCOCH_3$), 56.6 (C-2^b), 65.8—81.4 (ring C, C-6s, and CH_2Ph), 96.9 (C-1^a), 98.9 C-1^c), 100.8 and 101.1 (C-1^b and C-1^d), 127.7—138.6 (Ar), and 170.5 (C=O).

4-O-(2-*Acetamido*-2-*deoxy*-β-D-*glucopyranosyl*)-3,6-*di*-O-(α-D-*mannopyranosyl*)-D-*mannose* (**1**).—Compound (**13**) (170 mg) was dissolved in 90% aqueous acetic acid (20 ml) and hydrogenated at 400 kPa over 10% palladium–charcoal (200 mg) overnight. After filtration and concentration the product was desalted on a BioGel P-2 column irrigated with water. Freeze-drying gave compound (**1**) (71 mg, 96%) as an amorphous powder, $[\alpha]_D^{21}$ +55° (*c* 1 in H_2O); R_F 0.54 [ethanol–water–isobutyl alcohol–pyridine–acetic acid (100:30:10:10:3)]; δ_H (399.78 MHz; D_2O) 2.03 (3 H, s, Ac), 4.53 (0.3 H, d, $J_{1,2}$ 8.3 Hz, 1^b-H when 1^a-H is β), 4.56 (0.7 H, d, $J_{1,2}$ 8.3 Hz, 1^b-H when 1^a-H is α), 4.84 (0.3 H, d, $J_{1,2}$ 1 Hz, 1^a-H_β), 4.92 (0.7 H, d, $J_{1,2}$ 2 Hz, 1^c-H when 1^a-H is α), 4.93 (0.3 H, d, $J_{1,2}$ 1.7 Hz, 1^c-H when 1^a-H is β), 5.09 (0.7 H, d, $J_{1,2}$ 2.5 Hz, 1^a-H_α) 5.19 (0.7 H, d, $J_{1,2}$ 1.7 Hz, 1^d-H when 1^a-H is α), and 5.21 (0.3 H, d, $J_{1,2}$ 1.4 Hz, 1^d-H when 1^a-H is β); δ_C (25.00 MHz; D_2O) 23.3 ($COCH_3$), 57.3 (C-2^b), 62.2—78.4 (C-6s and ring C), 94.7 (0.3 C, $J_{C\text{-}1\ 1\text{-}H}$ 163 Hz, C-$1^a\beta$), 94.9 (0.7 C, $J_{C\text{-}1\text{-}1\text{-}H}$ 171 Hz, C-$1^a\alpha$), 101.4, 102.2, and 102.7 (C-1^b, C-1^c, and C-1^d), and 175.7 (C=O).

Benzyl 4-O-(2-*Acetamido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-2-O-*benzyl*-3,6-*bis*-O-[3,4,6-*tri*-O-*benzyl*-2-O-(3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl*)-α-D-*mannopyranosyl*]-α-D-*mannopyranoside* (**14**).—Compound (**13**) (130 mg) and the bromide (**6**) (0.15 g) were dissolved in dichloromethane (10 ml). Ground molecular sieves (4Å, *ca.* 0.5 g) were added and the mixture was chilled to −18 °C under nitrogen. A solution of silver trifluoromethanesulphonate (80 mg) in toluene (5 ml) was added dropwise to the mixture during 30 min. After filtration and evaporation, purification of the residue on a silica gel column with toluene–ethyl acetate (1:1) as eluant gave the hexasaccharide (**14**) (101 mg, 52%) as a syrup, $[\alpha]_D^{21}$ +21° (*c* 1 in $CHCl_3$); R_F 0.48 (developer as above); δ_C (25.00 MHz; $CDCl_3$) 20.5 and 20.7 ($OCOCH_3$), 23.0 ($NCOCH_3$), 54.5 and 56.6 (3 C, C-2^b, C-2^e, and C-2^f), 61.4—79.0 (ring C, C-6s, and CH_2Ph), 96.5 (C-1^a), 97.0 and 97.2 (2 C, C-1^e and C-1^f), 97.5 (C-1^c), 99.3 (C-1^d), 99.9 (C-1^b), 123.2—138.9 (Ar), and 169.5—170.7 (C=O).

Benzyl 4-O-(2-*Acetamido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-3,6-*bis*-O-[2-O-(2-*acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*)-3,4,6-*tri*-O-*benzyl*-α-D-*mannopyranosyl*]-2-O-*benzyl*-α-D-*mannopyranoside* (**15**).—Compound (**14**) (86 mg) dissolved in 90% aqueous ethanol (18 ml) and the solution was refluxed with hydrazine hydrate (2 ml) overnight. After acetylation and work-up [as for (**8**)] and purification on silica gel with toluene–ethyl acetate (1:3) as eluant, the hexasaccharide (**15**) (36 mg, 44%) was isolated as a syrup, $[\alpha]_D^{21}$ +15° (*c* 1 in $CHCl_3$); R_F 0.35 [toluene–ethyl acetate 1:3)]; δ_C (25.00 MHz; $CDCl_3$) 20.6—21.0 ($OCOCH_3$), 23.1 ($NCOCH_3$), 53.7, 54.8, and 56.6 (3 C, C-2^b, C-2^e, and C-2^f), 62.0—82.2 (ring C, C-6s, and CH_2Ph), 98.5 (C-1^a), 98.8 (C-1^c), 99.3 and 99.7 (2 C, C-1^e and C-1^f), 100.4 (C-1^d), 100.9 (C-1^b), 127.4—139.1 (Ar), and 169.3—171.1 (C=O).

4-O-(2-*Acetamido*-2-*deoxy*-β-D-*glucopyranosyl*)-3,6-*bis*-O-[2-O-(2-*acetamido*-2-*deoxy*-β-D-*glucopyranosyl*)-α-D-*mannopyranosyl*]-D-*mannose* (**2**).—Compound (**15**) (35 mg) was deacetylated in methanol (5 ml) containing a catalytic amount of sodium methoxide. Catalytic hydrogenation and desalting

on a BioGel P-2 column gave, after freeze-drying, the hexasaccharide (**2**) in 88% yield (14 mg), $[\alpha]_D^{21}$ +26° (*c* 1 in H_2O); R_F 0.51 (ethanol–water–isobutyl alcohol–pyridine–acetic acid (100:30:10:10:3)]; δ_H (399.78 MHz; D_2O) 2.06 (9 H, s, NAc), 4.48—4.60 (3 H, doublets, $1^{b,e,f}$-H), 4.85 (0.3 H, d, $J_{1,2}$ < 2 Hz, 1^a-H_β), 4.95 (0.7 H, d, $J_{1,2}$ < 2 Hz, 1^c-H when 1^a-H is α), 4.98 (0.3 H, d, $J_{1,2}$ < 2 Hz, 1^c-H when 1^a-H is β), 5.06 (1 H, br s, 1^d-H), and 5.11 (0.7 H, d, $J_{1,2}$ < 2 Hz, 1^a-H_α); δ_C (25.00 Hz; D_2O) 23.3 and 23.6 (3 C, $COCH_3$), 56.6 and 57.4 (3 C, C-$2^{b,e,f}$), 61.6—63.0 (5 C, C-$6^{b,c,d,e,f}$), 67.1—78.0 (C-6^a and ring C), 94.8 (0.3 C, C-$1^a\beta$), 95.4 (0.7 C, C-$1^a\alpha$), 99.1 (1 C, C-1^c), 100.9, 101.2, and 101.9 (4 C, C-$1^{b,d,e,f}$), and 175.5 and 177.6 (3 C, C=O).

Benzyl 4-O-(2-*Acetamido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-2-O-*benzyl*-3,6-*bis*-O-{3,4,6-*tri*-O-*benzyl*-3,6-*di*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-α-D-*mannopyranosyl*}-α-D-*mannopyranoside* (**16**).—In a typical experiment, a solution of the tetrasaccharide (**13**) (52 mg) and the bromide (**18**) (0.1 g) in toluene–dichloromethane (10:1; 2 ml) was kept under nitrogen at −40 °C with ground molecular sieves (4Å, *ca.* 0.5 g). To the stirred mixture was added a solution of silver trifluoromethanesulphonate (30 mg) in toluene (2 ml) dropwise during 20 min. When no starting material was left the mixture was diluted with dichloromethane (20 ml) and filtered. After two successive column separations with 'iso-octane'–acetone* (1:1) and chloroform–acetone (5:1) as eluant the octasaccharide derivative (**16**) (54 mg, 57%) was obtained, $[\alpha]_D^{21}$ +12° (*c* 1 in $CHCl_3$); R_F 0.41 ['iso-octane'–acetone (1:1)]; δ_C 20.5—20.7 ($COCH_3$), 54.8 and 55.1 (3 C, C-$2^{b,e,f}$), 60.8—74.3 (ring C, C-6s, and CH_2Ph), 96.4 (C-1^a), 96.9 and 97.1 (3 C, C-$1^{c,e,f}$), 99.7 and 100.2—101.4 (4 C, C-$1^{b,d,g,h}$), 123.4—138.3 (Ar), and 169.0—170.3 (C=O).

Benzyl 3,6-*Bis*-O-{2-O-[2-*acetamido*-3,6-*di*-O-*acetyl*-2-*deoxy*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-3,4,6-*tri*-O-*benzyl*-α-D-*mannopyranosyl*}-4-O-(2-*acetamido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-2-*O*-*benzyl*-α-D-*mannopyranoside* (**17**).—Compound (**16**) (119 mg) was dissolved in 90% aqueous ethanol (20 ml) containing hydrazine hydrate (2 ml). After being refluxed overnight the mixture was concentrated to dryness and acetylated (acetic anhydride–pyridine) at room temperature for 20 h. After the same work-up as for compound (**8**), purification on silica gel with toluene–ethyl acetate (1:3) as eluant gave the octasaccharide (**17**) (57 mg, 51%), $[\alpha]_D^{21}$ +16° (*c* 1 in $CHCl_3$); R_F 0.28 (developer as above); δ_C (25.00 MHz; $CDCl_3$) 20.5 and 20.6 ($OCOCH_3$), 23.2 ($NCOCH_3$), 53.6, 54.4, and 56.5 (3 C, C-$2^{b,e,f}$), 60.6—80.7 (ring C, C-6s, and CH_2Ph), 97.9 (C-1^a), 98.4 (C-1^c), 99.1 and 99.4 (C-$1^{e,f}$), 100.2 (C-1^d), 100.8 (C-1^b), 101.3 and 101.6 (C-$1^{g,h}$), 127.2—139.0 (Ar), and 169.1—170.9 (C=O).

3,6-*Bis*-O-{2-O-[2-*acetamido*-2-*deoxy*-4-O-(β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-α-D-*mannopyranosyl*}-4-O-(2-*acetamido*-2-*deoxy*-β-D-*glucopyranosyl*)-D-*mannose* (**3**).—Compound (**17**) (39 mg) was deacetylated, hydrogenated, and desalted as in the preparation of compound (**2**). The product (**3**), was isolated in 95% yield (18 mg), $[\alpha]_D^{21}$ +18° (*c* 1 in H_2O); R_F 0.28 [ethanol–water–isobutyl alcohol–pyridine–acetic acid (100:30:10:10:3)]; δ_H (399.78 MHz; D_2O) 2.06 (9 H, s, NAc), 4.49 and 4.50 (2 H, 2 d, *J* 7.5 Hz, $1^{g,h}$-H), 4.53 (1 H, d, *J* 8 Hz, 1^b-H), 4.60 (2 H, m, *J* 8.5 Hz, $1^{e,f}$-H), 4.88 (0.3 H, d, *J* < 1.5 Hz, 1^a-H_β), 4.99 (0.7 H, d, *J* < 1.5 Hz, 1^c-H when 1^a-H is α), 5.02 (0.3 H, d, *J* < 1.5 Hz, 1^c-H when 1^a-H is β), 5.08 (1 H, d, *J* < 1.5 Hz, 1^d-H), and 5.14 (0.7 H, d, *J* 1.5 Hz, 1^a-H_α); δ_C (25.00 MHz; D_2O) 23.3 and 23.6 (3 C, $COCH_3$), 56.1 and 57.4 (3 C, C-$2^{b,e,f}$), 61.2, 62.2, and 63.0 (7 C, C-6^{b-h}), 67.1—80.1 (C-6^a and ring C), 94.9 (0.3 C, C-$1^a\beta$), 95.4 (0.7 C, C-$1^a\alpha$), 99.0 (C-1^c), 100.7 (C-1^d), 101.0 (2 C, C-$1^{e,f}$), 101.9 (C-1^b), 104.0 and 104.2 (2 C, C-$1^{g,h}$), and 175.9 (3 C, C=O).

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* 'Iso-octane' = 2,2,4-trimethylpentane.

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