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Synthesis of Oligosaccharides That Form Parts of the Complex Type of Carbohydrate Moieties of Glycoproteins. Three Glycosides Prepared for Conjugation to Proteins

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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 *p*-nitrophenyl 3-*O*-benzoyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside, *p*-nitrophenyl 3,6-di-*O*-benzyl-α-D-mannopyranoside and *p*-nitrophenyl 3,4-di-*O*-benzyl-α-D-mannopyranoside gave protected tri- and pentasaccharides. The oligosaccharide glycosides *1*, *2* and *3* were obtained after de-protection of these condensation products. These oligosaccharides will, after suitable conversions, be conjugated to proteins for biological studies.

The complex (*N*-acetyl-D-lactosaminic) type of carbohydrate portions which occur in a multitude of glycoproteins have been suggested to be involved in several biologically important phenomena.[1] Syntheses of different oligosaccharides derived from these carbohydrate portions have been reported.[2–14] In order to study certain biological properties of these oligosaccharides they should be prepared as glycosides suitable for covalent attachment to proteins. We now report the synthesis of three such glycosides, one (*1*) with three and two (*2* and *3*) with five sugar residues.

RESULTS AND DISCUSSION

For the synthesis of trisaccharide glycoside *1* a *p*-nitrophenyl-α-D-mannoside blocked at O-3, O-4 and O-6 and an *N*-acetyl-D-lactosamine precursor were needed. The syntheses of such precursors, namely *p*-nitrophenyl 3-*O*-benzoyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside [15] (*4*) and 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 [2,3] (*5*) have been reported earlier. Compounds *4* and *5* were condensed using silver trifluoromethanesulfonate(silver triflate)–2,4,6-trimethylpyridine as promoter. These conditions are known to give high yields of β-glycosides.[16,17] After work-up and chromatography on silica gel the trisaccharide derivative *6* was obtained in 60 % yield. Compound *6* was converted into *7* (66 %) by successive treatment with sodium methoxide in methanol, hydrazine hydrate in boiling ethanol and acetic anhydride–pyridine.[17] Removal of the *O*-benzylidene and *O*-acetyl groups of *7* was accomplished by treatments with aqueous trifluoroacetic acid and sodium methoxide in methanol, respectively. After purification by gel filtration the trisaccharide glycoside *1* was obtained in 76 % yield as an amorphous powder.

For the synthesis of the pentasaccharide glycoside *2* a mannoside blocked at O-3 and O-6 was needed. *p*-Nitrophenyl α-D-mannopyranoside was treated with bis(tributylstannyl)oxide–benzyl bromide–tetrabutylammonium bromide [18,19] to yield, after chromatography, crystalline *p*-nitrophenyl 3,6-di-*O*-benzyl-α-D-mannopyranoside (*8*) (94 %). Glycosyl bromide *5* and *8* were

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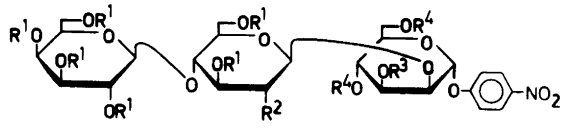


	R^1	R^2	R^3	R^4
6	Ac	NPhth	Bz	*O*-benzylidene
7	Ac	NHAc	Ac	*O*-benzylidene
1	H	NHAc	H	H

condensed as described above and after work-up and chromatography the pentasaccharide derivative *9* was obtained in 41 % yield. Treatment of *9* with hydrazine hydrate followed by acetylation gave *10* (73 %). The nitro group in *10* was reduced by catalytic hydrogenation (Pt catalyst) and the resulting amino group was acylated with trifluoroacetic anhydride to give *11* (71 %). Compound *11* was then de-protected by treatment with sodium methoxide in methanol followed by catalytic hydrogenation (Pd–C catalyst). After gel filtration the pentasaccharide glycoside *2* was obtained in 65 % yield.

For the synthesis of the pentasaccharide glycoside *3* a mannoside blocked at O-3 and O-4 was needed. 2,6-Di-*O*-acetyl-3,4-di-*O*-benzyl-D-mannosyl bromide [14] was condensed with *p*-nitrophenol using silver triflate-2,4,6-trimethylpyridine as promoter. *p*-Nitrophenyl 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranoside (*12*) was obtained in 37 % yield and this compound was then deacetylated to give *p*-nitrophenyl 3,4-di-*O*-benzyl-α-D-mannopyranoside (*13*) (83 %). Glycosyl bromide *5* and *13* were condensed as described above and the pentasaccharide derivative *14* was obtained in 78 % yield. The 2-deoxy-2-phthalimido groups of *14* were exchanged with 2-acetamido-2-deoxy groups as above to yield crystalline *15* (85 %). Then the *p*-nitrophenyl group *15* was converted into a *p*-*N*-trifluoroacetamidophenyl group as above to give crystalline *16* (51 %) which was finally de-protected to give, after gel filtration, the required pentasaccharide glycoside *3* (65 %).

The structures of *1*, *2* and *3* are evident from their modes of synthesis and from methylation analyses [20] and were also confirmed by ^{1}H and ^{13}C NMR spectroscopy. The spectra of *1*–*3* and their protected derivatives were invariably in agreement with those of similar compounds prepared earlier.

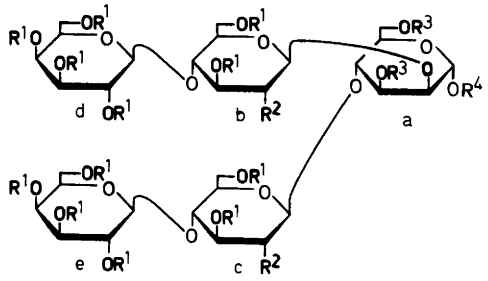
Biological experiments performed with these oligosaccharides will be reported separately.

EXPERIMENTAL

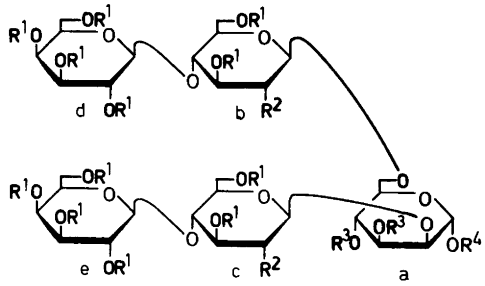
General. Methods and instrumentation for TLC, GLC–MS, NMR and analysis have been described.[3]

Inasmuch as the rational names of compounds *9*–*11* and *14*–*16* are incomprehensible, the reader is referred to the figures for information on the structures of these compounds.

p-*Nitrophenyl 2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl-(1→4)*-O-*3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→2)*-O-*3*-O-*benzoyl-4,6-di*-O-*benzylidene-α*-D-*mannopyranoside (6).* A mixture of mannoside *4* [15] (1.3 g), silver triflate (0.79 g), 2,4,6-trimethylpyridine (0.37 g) and molecular sieves (2 g, 3Å) in anhydrous dichloromethane (10 ml) was cooled to −40 °C under nitrogen. Bromide *5* [2,3] (2.4 g) in anhydrous dichloromethane (10 ml) was added



	R^1	R^2	R^3	R^4
9	Ac	NPhth	Bn	*p*-nitrophenyl
10	Ac	NHAc	Bn	*p*-nitrophenyl
11	Ac	NHAc	Bn	*p*-*N*-trifluoroacetamidophenyl
2	H	NHAc	H	*p*-*N*-trifluoroacetamidophenyl



	R^1	R^2	R^3	R^4
14	Ac	NPhth	Bn	*p*-nitrophenyl
15	Ac	NHAc	Bn	*p*-nitrophenyl
16	Ac	NHAc	Bn	*p*-*N*-trifluoroacetamidophenyl
3	H	NHAc	H	*p*-*N*-trifluoroacetamidophenyl

dropwise with stirring. The mixture was allowed to attain room temperature and kept overnight, washed with aqueous hydrogen chloride, water, saturated aqueous sodium hydrogencarbonate and water. After concentration to dryness the product was purified on silica gel with toluene–ethyl acetate (1:1). Compound *6* was obtained as an amorphous power (1.8 g, 60 %), $[\alpha]_D^{21}$ +53° (*c* 1.0, $CHCl_3$); TLC (solvent as above): R_F 0.56; ^{13}C NMR ($CDCl_3$): δ 20.5 (OAc), 54.9 (C'-2), 60.1–78.3 (C-6, C'-6, C''-6, ring C), 95.8, 96.6 (C-1, C'-1), 101.1 (C''-1), 101.8 (*C*HPh), 116.0–160.1 (aromatic C), and 169.4–170.0 (*C*=O).

p-*Nitrophenyl 2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl-(1→4)*-O-*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyransyl-(1→2)*-O-*3*-O-*acetyl-4,6-di*-O-*benzylidene*-α-D-*mannopyranoside (7).* A catalytic amount of sodium was added to a solution of compound *6* (910 mg) in anhydrous methanol (50 ml). The mixture was left at room temperature overnight, neutralised with acetic acid and concentrated to dryness. The product was dissolved in 90 % aqueous ethanol (30 ml), hydrazine hydrate (4 ml) was added and the solution was refluxed for 10 h. After cooling the solution was concentrated to dryness, and the product was treated with acetic anhydride–pyridine (1:1, 30 ml) at 100 °C for 30 min. After concentration the product was purified on silica gel with toluene–ethyl acetate (1:4) to yield trisaccharide derivative *7* as an amorphous powder (520 mg, 66 %), $[\alpha]_D^{21}$ +42° (*c* 1.0, $CHCl_3$); TLC (solvent as above): R_F 0.37; ^{13}C NMR ($CDCl_3$); δ 20.6–24.9 (NHAc, OAc), 53.7 (C'-2), 60.9, 62.4, (C'-6, C''-6), 65.2–78.5 (C-6, ring C), 96.7 (C-1), 100.7 (C'-1), 101.0 (C''-1), 102.0 (*C*HPh), 116.3–160.9 (aromatic C), and 169.2, 170.7 (*C*=O).

p-*Nitrophenyl O*-β-D-*galactopyranosyl-(1→4)*-O-*2-acetamido-2-deoxy*-β-D-*glucopyranosyl-(1→2)*-α-D-*mannopyranoside (1).* Compound *7* (325 mg) was treated with 90 % aqueous trifluoroacetic acid (5 ml) for 5 min at room temperature and concentrated to dryness. The product was dissolved in methanol (50 ml) and a catalytic amount of sodium was added. The solution was left at room temperature overnight, neutralised with acetic acid and concentrated to dryness. The product was purified on a Bio-Gel P2 column (5×80 cm) irrigated with water. After freeze-drying trisaccharide *1* was obtained as an amorphous powder (158 mg, 76 %), $[\alpha]_D^{21}$ +38° (*c* 1.0, H_2O); TLC (ethyl acetate–methanol–acetic acid–water, 12:3:3:2): R_F 0.28; ^1H NMR (D_2O, 85 °C): δ 2.03 (3 H, NHAc), 4.45 (d, 1 H, H''-1, $J_{1,2}$ 6.5 Hz), 4.69 (broad d, 1 H, H'-1, $J_{1,2} \simeq 8$ Hz), 5.70 (d, 1 H, H-1, $J_{1,2}$ 1.5 Hz), 7.22–8.28 (4 H, aromatic H); ^{13}C NMR (D_2O): δ 23.5 (NHAc), 56.0 (C'-2), 61.0, 62.1 (C-6, C'-6, C''-6), 69.6–79.6 (ring C), 96.4 (C-1), 100.6 (C'-1), 104.1 (C''-1), 117.6, 127.1, 143.2, 162.1 (aromatic C), and 175.9 (*C*=O). Methylation analysis [20] of *1* gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 3,4,6-tri-*O*-methyl-D-mannose and 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose.

p-*Nitrophenyl 3,6-di*-O-*benzyl*-α-D-*mannopyranoside (8).* *p*-Nitrophenyl α-D-mannopyranoside (3.2 g) and bis(tributylstannyl)oxide (9.0 ml) in toluene (350 ml) was boiled under reflux for 2 h in a Dean-Stark trap. The solution was concentrated to ≃100 ml and benzyl bromide (60 ml) and tetrabutylammonium bromide (1.4 g) were added. The mixture was stirred for 24 h at 80 °C and concentrated to dryness. The product was purified on silica gel with toluene–ethyl acetate (1:1) yielding *8* (4.82 g, 94 %). Crystal-

lisation from ethyl acetate–light petroleum gave prisms, m.p. 112 °C, $[\alpha]_{578}^{22}$ +101° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): 67.0 (C-2), 67.6 (C-4), 69.6 (C-6), 72.1 (C-5), 72.4, 73.5 (CH_2Ph), 79.1 (C-3), 97.9 (C-1), 116.4, 125.7, 127.6, 127.8, 128.0, 128.2, 128.4, 128.6, 137.7, 142.7, 160.8 (aromatic). *Anal.* $C_{26}H_{27}NO_8$: C,H,N.

Pentasaccharide derivative 9. A mixture of mannoside *8* (438 mg), silver triflate (1.18 g), 2,4,6-trimethylpyridine (557 mg) and molecular sieves (4 g, 3Å) in anhydrous dichloromethane (20 ml) was cooled to −40 °C under nitrogen. Bromide *5* [2,3] (3.50 g) in anhydrous dichloromethane (20 ml) was added in two portions after 0 and 1 h reaction time. The mixture was allowed to attain room temperature after the first addition and was then cooled to −25 °C before the final addition. Thereafter the mixture was allowed to attain room temperature (8 h) and worked up as above. The product was purified on silica gel with light petroleum–ethyl acetate (1:2). Compound *9* was obtained as a syrup (712 mg, 41 %), $[\alpha]_{578}^{22}$ +47° (*c* 0.9, $CHCl_3$); TLC (toluene–ethyl acetate, 1:2), R_F 0.55; ^{13}C NMR ($CDCl_3$): 20.4, 20.7 (OAc), 55.2, 55.5 (C-2^b, C-2^c), 61.0–62.4 (4 C, C-6^{b-e}), 66.9–78.6 (C-6^a, ring C, CH_2Ph), 96.4 (C-1^a), 98.0 (2 C, C-1^b, C-1^c), 101.1 (2 C, C-1^d, C-1^e), 116.5, 125.4, 142.6, 161.1 (*p*-nitro-Ph), 123.4–138.6 (aromatic), and 167.8–170.2 (*C*=O).

Pentasaccharide derivative 10. Compound *9* (680 mg) was treated with sodium methoxide in methanol, hydrazine hydrate (2.0 ml) in ethanol (30 ml), and acetic anhydride–pyridine (1:1, 15 ml) as described for compound *7*. The product was purified on silica gel with chloroform–acetone (3:2) yielding *10* as a syrup (457 mg, 73 %), $[\alpha]_{578}^{22}$ +19° (*c* 1.3, $CHCl_3$); TLC (solvent as above): R_F 0.42: ^{13}C NMR ($CDCl_3$): 20.5, 20.8 (OAc), 23.1 (NHAc), 54.3 (2 C, C-2^b, C-2^c), 61.0–62.4 (4 C, C-6^{b-e}), 66.9–78.6 (C-6^a, ring C, CH_2Ph), 96.8 (C-1^a), 99.9 (C-1^b), 100.9, 101.2 (2 C, 1 C, (C-1^b, C-1^d, C-1^e), 116.8, 125.7, 142.8, 161.3 (*p*-nitro-Ph), 127.6–138.6 (aromatic), and 169.1–170.5 (*C*=O).

Pentasaccharide derivative 11. Compound *10* (397 mg) was dissolved in ethyl acetate (10 ml), platinum oxide (80 mg) was added and the mixture was treated with hydrogen at atmospheric pressure. When the hydrogen consumption had ceased the mixture was filtered and concentrated to dryness. The product was dissolved in anhydrous pyridine (5.0 ml), cooled to −20 °C under nitrogen and trifluoroacetic anhydride (0.15 ml) was added. The solution was allowed to attain room temperature and kept overnight. The mixture was distributed between dichloromethane and water and the organic phase was washed with water, dilute sulfuric acid, water, saturated aqueous sodium hydrogencarbonate and water. The product was purified on silica gel with chloroform–acetone (3:2) yielding *11* as a syrup (291 mg, 71 %), $[\alpha]_{578}^{22}$ +17° (*c* 1.0, CH_2Cl_2); TLC (solvent as above): R_F 0.46; ^{13}C NMR ($CDCl_3$): 20.6, 20.8 (OAc), 23.1 (2 C, NHAc), 54.3 (2 C, C-1^b, C-1^c), 61.0–62.5 (4 C, C-6^{b-e}), 67.0–78.5 (C-6^a, ring C, CH_2Ph), 96.8 (C-1^a), 99.9 (C-1^b), 101.0, 101.3 (2 C, 1 C, C-1^c, C-1^d, C-1^e), 117.4, 122.5, 130.8, 154.2 (*p*-trifluoroacetamido Ph), 127.6–138.7 (aromatic), and 169.3–170.8 (*C*=O).

p-*Trifluoroacetamidophenyl 2,4-di*-O-[β-D-*galactopyranosyl-(1→4)*-O-*2-acetamido-2-deoxy-β*-D-*glucopyranosyl-α*-D-*mannopyranoside (2)*. Sodium (10 mg) was added to a solution of *11* (260 mg) in anhydrous methanol (20 ml). The mixture was kept at room temperature for 3 h, neutralised with acetic acid and concentrated to dryness. The product was dissolved in 90 % aqueous acetic acid (20 ml) and hydrogenated at 400 kPa over 10 % palladium–charcoal (500 mg). After filtration and concentration the product was purified by gel-filtration on a Sephadex G-15 column (2.5×90 cm) irrigated with water. After freeze-drying *2* was obtained as an amorphous powder (103 mg, 65 %), $[\alpha]_{578}^{22}$ +10° (*c* 0.4, H_2O); TLC (ethyl acetate–methanol–acetic acid–water, 4:3:3:2): R_F 0.79; ^1H NMR (D_2O, 85 °C): 2.00 (s, 2×3 H, NHAc), 4.47 (d, 2 H, $J_{1,2}$ 6.4 Hz, H-1^d, H-1^e), 4.64 (m, 2 H, H-1^b, H-1^c), 5.50 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1^a), and 7.13–7.53 (4 H, aromatic); ^{13}C NMR (D_2O): 23.3, 23.6 (2 C, NHAc), 56.3 (2 C, C-2^b, C-2^c), 61.3–62.2 (5 C, C-6^{a-e}), 72.2–79.7 (ring C), 96.6 (C-1^a), 101.0 (C-1^b), 102.7 (C-1^c), 104.1 (2 C, C-1^d, C-1^e), 111.4, 122.8 ($COCF_3$), 118.7, 125.1, 130.8, 155.0 (aromatic), 157.3, 158.8 ($COCF_3$), and 175.5, 175.9 (*C*=O). Methylation analysis [20] of *2* gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose, and 3,6-di-*O*-methyl-D-mannose.

p-*Nitrophenyl 2,6-di*-O-*acetyl-3,4-di*-O-*benzyl-α*-D-*mannopyranoside (12)*. A mixture of *p*-nitrophenyl (3.23 g), molecular sieves (7 g, 3Å), silver triflate (5.97 g), and 2,4,6-trimethylpyridine (1.31 g) in anhydrous dichloromethane (30 ml) was cooled to −40 °C under nitrogen. A solution of 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-D-mannosyl bromide [14] (7.85 g) in dichloromethane (10 ml) was added with stirring over 45 min. The mixture was allowed to attain room temperature, pyridine (1.5 ml) was added and the mixture was worked up as above. The product was purified on silica gel with toluene–ethyl acetate (6:1). Compound *12* was obtained as a syrup (3.22 g, 37 %), $[\alpha]_{578}^{22}$ +81° (*c* 2.3, $CHCl_3$); TLC (toluene–ethyl

acetate, 4:1): R_F 0.57; ^{13}C NMR ($CDCl_3$): δ 20.6, 20.8 (OAc), 62.7 (C-6), 67.9 (CH_2Ph), 71.1 (C-2),72.0 (CH_2Ph), 73.5 (C-5), 75.2 (C-4), 77.6 (C-3), 96.0 (C-1), 116.4, 125.6, 142.8, 160.3 (*p*-nitro-Ph), 128.0–137.8 (aromatic), and 169.9, 170.3 (*C*=O).

p-*Nitrophenyl 3,4-di-O-benzyl-α-*D-*mannopyranoside (13).* Compound *12* was deacetylated as described for *2* and the product was purified on silica gel with toluene–ethyl acetate (3:1). Compound *13* was obtained as an amorphous powder (2.12 g, 83 %), $[\alpha]_{578}^{22}$ +111° (*c* 1.0, $CHCl_3$); TLC (toluene–ethyl acetate, 1:2): R_F 0.45; ^{13}C NMR ($CDCl_3$): δ 61.3 (C-6), 68.1 (CH_2Ph), 72.5, 73.2, 73.3 (3 C, C-2, C-5, CH_2Ph), 75.3 (C-4), 79.4 (C-3), 97.6 (C-1, $^1J_{C,H}$ 173 Hz),[21] 116.3, 125.8, 142.6, 160.7 (*p*-nitro-Ph), and 127.8–138.1 (aromatic).

Pentasaccharide derivative 14. Compound *14* was prepared from *13* (423 mg) analogously to *6* by using bromide *5* [2,3] (2.43 g), molecular sieves (2 g, 3Å) and silver triflate (1.15 g)–2,4,6-trimethylpyridine (0.54 g). The product was purified on silica gel with light petroleum–chloroform–acetone (2:2:1). Compound *14* was obtained as an amorphous powder (1.30 g, 78 %), $[\alpha]_{578}^{22}$ +58° (*c* 1.0, $CHCl_3$); TLC (toluene–ethyl acetate, 1:2): R_F 0.60; ^{13}C NMR ($CDCl_3$): δ 20.5 (OAc), 55.0 (2 C, C-2^b, C-2^c), 60.8–62.3 (4 C, C-6^{b-e}), 66.9–78.5 (C-6^a, ring C, CH_2Ph), 95.9 (C-1^a), 97.3, 98.3 (2 C, C-1^b, C-1^c), 101.0, 101.1 (C-1^d, C-1^e), 116.6, 125.4, 142.6, 161.1 (*p*-nitro-Ph), 123.4–138.0 (aromatic), and 167.4–170.1 (*C*=O).

Pentasaccharide derivative 15. Compound *14* (365 mg) was treated with sodium methoxide in methanol, hydrazine hydrate (1.6 ml) in ethanol (30 ml), and acetic anhydride–pyridine (1:1, 20 ml) as described for compound *7*. The product was purified on silica gel with toluene–ethyl acetate (1:4) yielding *15* (280 mg, 85 %). Crystallisation from ethanol gave small crystals, m.p. 233–235 °C, $[\alpha]_{578}^{22}$ +14° (*c* 0.7, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 20.5, 20.7 (OAc), 23.4, 24.7 (2 C, NHAc), 54.2, 56.5 (2 C, C-2^b, C-2^c), 61.0–62.7 (4 C, C-6^{b-e}), 67.0–78.6 (C-6^a, ring C, CH_2Ph), 95.5 (C-1^a), 97.8 (C-1^c), 101.0, 101.6, 101.8 (C-1^b, C-1^d, C-1^e), 116.4, 125.8, 142.8, 161.0 (*p*-nitro-Ph), 127.7–139.0 (aromatic), and 169.0–171.6 (*C*=O). *Anal.* $C_{78}H_{98}N_3O_{40}$: C,H,N.

Pentasaccharide derivative 16. Compound *15* (800 mg) was subjected to catalytic hydrogenation over platinum oxide catalyst (200 mg) and treatment with trifluoroacetic anhydride (0.2 ml) in pyridine (5.0 ml) as described for *11*. The product was purified on silica gel with chloroform–acetone (2:1) yielding *16* (420 mg, 51 %). Crystallisation from ethanol–water gave small crystals, m.p. 145–148 °C, $[\alpha]_{578}^{22}$ +0.5° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 20.6 (OAc), 23.3 (2 C, NHAc), 54.0 (2 C, C-2^b, C-2^c), 60.8–62.8 (4 C, C-6^{b-e}), 66.8–78.5 (C-6^a, ring C, CH_2Ph), 95.0 (C-1^a), 98.3, 101.0 (1 C, 3 C, C-1^{b-e}), 116.5, 122.7, 130.3, 153.6 (*p*-trifluoroacetamido Ph), 127.7–138.7 (aromatic), and 169.1–170.8 (*C*=O). Found: C 53.12l H 5.39; N 2.29. Calc. for $C_{80}H_{98}N_3O_{39}F_3$: C 53.90; H 5.50; N 2.36.

p-*Trifluoroacetamidophenyl 2,6-di-*O-[*β-*D-*galactopyranosyl-(1→4)-*O-*2-acetamido-2-deoxy-β-*D-*glucopyranosyl]α-*D-*mannopyranoside (3).* Compound *16* (385 mg) was *O*-deacetylated and debenzylated as described for *2*. The product was desalted on a Sephadex G-15 column (2.5×90 cm) irrigated with water. After freeze-drying, *3* was obtained as an amorphous powder (154 mg, 65 %), $[\alpha]_{578}^{22}$ +2° (*c* 0.8, H_2O); TLC (ethyl acetate–methanol–acetic acid–water, 4:3:3:2): R_F 0.76; ^1H NMR (D_2O, 85 °C): δ 1.92, 2.04 (2 s, 2×3 H, NHAc), 4.40, 4.45 (2 d, 2 H, $J_{1,2}$ 7.2 Hz, H-1^d, H-1^e), 4.50 (d, 1 H, $J_{1,2}$ ~7 Hz, H-1^b), 4.68 (d, 1 H, $J_{1,2}$ ~7 Hz, H-1^c), 5.50 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^a), and 7.11–7.53 (4 H, aromatic); ^{13}C NMR (D_2O): δ 23.4, 23.7 (2 C, NHAc), 56.1 (2 C, C-2^b, C-2^c), 61.3–62.2 (4 C, C-6^{b-e}), 68.7–80.0 (C-6^a, ring C), 97.0 (C-1^a), 100.9 (C-1^c), 102.1 (C-1^b), 104.2 (2 C, C-1^d, C-1^e), 111.4, 122.9 ($COCF_3$), 157.2, 158.7 ($COCF_3$), 118.8, 125.1, 130.9, 155.3 (aromatic), and 175.2, 175.7 (*C*=O). Methylation analysis [20] of *3* gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose and 3,4-di-*O*-methyl-D-mannose.

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