*Carbohydrate Research*, 139 (1985) 115–121
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

# SYNTHESIS OF A TETRA- AND A NONA-SACCHARIDE WHICH CONTAIN α-L-FUCOPYRANOSYL GROUPS AND ARE PART OF THE COMPLEX TYPE OF CARBOHYDRATE MOIETY OF GLYCOPROTEINS

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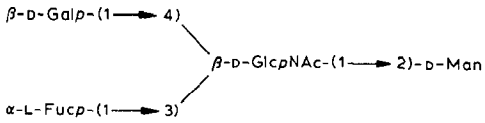
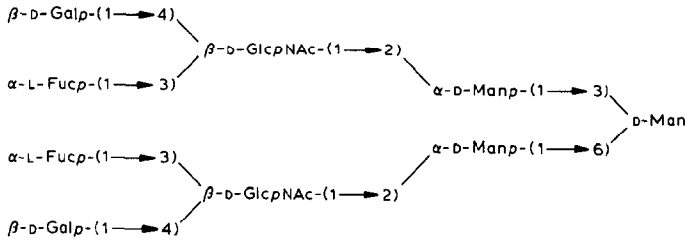
(Received January 31st, 1984; accepted for publication, October 8th, 1984)

ABSTRACT

Methyl trifluoromethanesulfonate-promoted condensation of ethyl 6-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-thio-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside with benzyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside and benzyl 2,4-di-*O*-benzyl-3,6-di-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside gave a tetrasaccharide and a nonasaccharide derivative, respectively. The tetrasaccharide **1** and the nonasaccharide **2** were obtained after removal of the protecting groups and *N*-acetylation.




**1**

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## INTRODUCTION

In the preceding paper[1], the synthesis of oligosaccharides which contain α-L-fucopyranosyl groups and which are part of the complex type of carbohydrate moiety of glycoproteins was reported[1]. In these syntheses, 2-acetamido-2-deoxy-3-*O*-α-L-fucopyranosyl-β-D-glucopyranoside was transferred to D-mannose-containing aglycons *via* its suitably protected ethyl 1-thio-β-glycoside, using methyl triflate as promoter.

We now report on the analogous synthesis of tetrasaccharide **1** and nonasaccharide **2**, using a thioglycoside of trisaccharide **3** as glycosylating agent. The synthesis of trisaccharide **3** has been reported before[2]. Oligosaccharides **1** and **2** are structural elements of glycopeptides found in the urine from patients with fucosidosis[3]. The trisaccharide moiety **3** is part of blood-group antigens[4] and glycolipids isolated from human cancer cells[5,6].

## RESULTS AND DISCUSSION

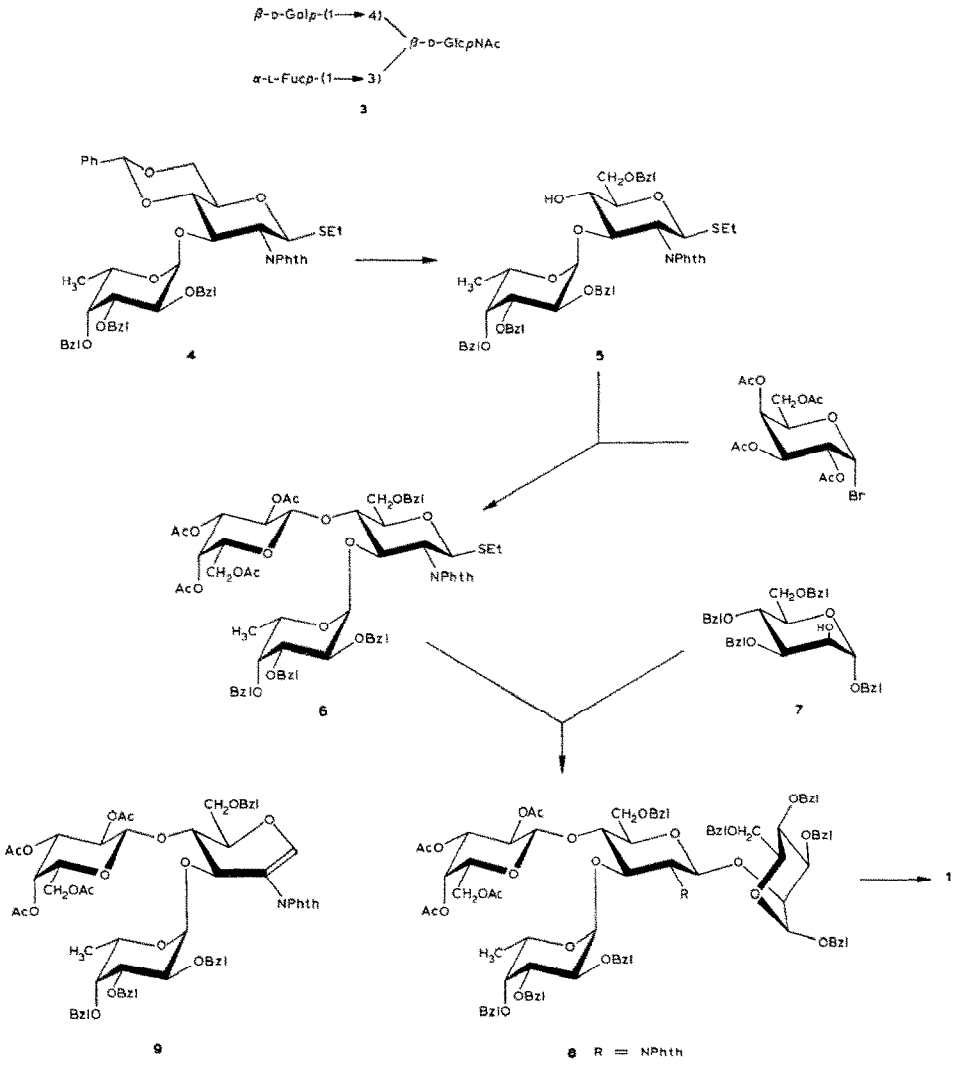
Tetrasaccharide **1** and nonasaccharide **2** contain the common trisaccharide residue **3**. A suitably protected ethyl thioglycoside of **3**, namely **6**, was therefore prepared starting from the ethyl thioglycoside[1] **4**. Reductive opening of the benzylidene ring in **4** with trimethylamine–borane complex–aluminium trichloride in tetrahydrofuran[7] yielded the benzyl ether **5** (66%) with HO-4 of the 2-amino-2-deoxy-D-glucopyranosyl moiety unsubstituted. Silver triflate-promoted glycosylation[8] of **5** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide yielded **6** (53%). As expected, the hydroxyl group in **5** was not very reactive and attempts to achieve glycosylation using other methods were unsatisfactory.

For the synthesis of the protected tetrasaccharide derivative **8**, a solution of equimolar amounts of the thioglycoside **6** and benzyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside[9] **7** in ether was treated with methyl triflate[1] at room temperature in the presence of 4 Å molecular sieves. In addition to the desired tetrasaccharide derivative **8** (67%), the glycal **9** (18%) was also formed.

Treatment of **8** first with hydrazine hydrate in boiling ethanol and then with acetic anhydride–pyridine gave **10** (87% after chromatography). *O*-Deacetylation of **10**, followed by catalytic hydrogenolysis and purification of the product on Biogel P-2, gave **1** (86%).

The synthesis of the nonasaccharide **2** involved the thioglycoside **6** and the trimannoside[1,10] **11**, which were condensed, using methyl triflate as promoter, to give the nonasaccharide derivative **12** (61%). Removal of the protecting groups and chromatography then yielded the nonasaccharide **2** (58%).

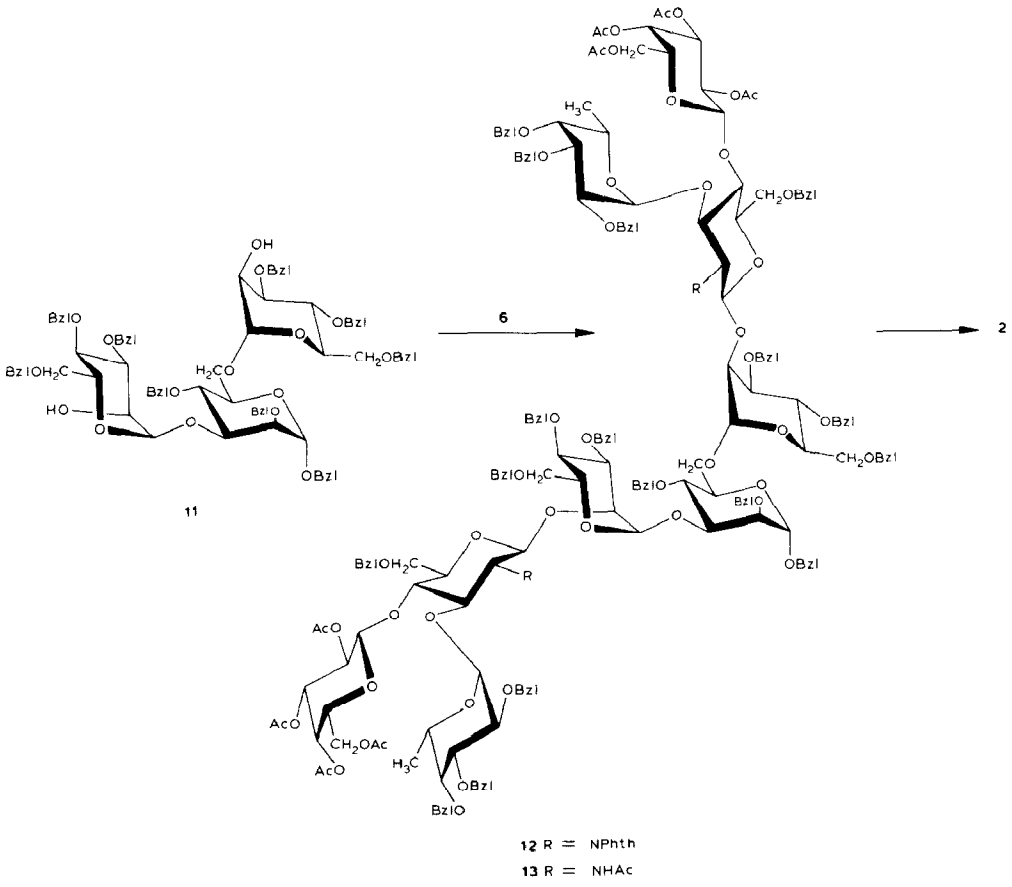
All intermediates gave $^{1}H$- and $^{13}C$-n.m.r. spectra in agreement with the postulated structures. The structures and purity of **1** and **2** were determined by $^{1}H$- (400 MHz and 100 MHz) and $^{13}C$- (25 MHz) n.m.r. spectroscopy and by chromatography.




## EXPERIMENTAL

*General methods.* — These were as described in the preceding paper[1]. Satisfactory elemental analyses were not obtained for syrupy compounds, but their purity was established by chromatography and their structures were determined by n.m.r. spectroscopy.

*Ethyl 6-O-benzyl-2-deoxy-2-phthalimido-1-thio-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside* (**5**). — Aluminium trichloride (1.0 g) was added to a solution of the benzylidene derivative[1] **4** (1.25 g) and trimethylamine–borane complex (0.5 g) in tetrahydrofuran (10 mL) at room temperature. The




solution was stirred at 60° for 2 h, cooled, and partitioned between ice-cold M sulfuric acid and toluene. The organic layer was washed with aqueous sodium hydrogencarbonate and water, and concentrated, and the product was purified by column chromatography (toluene–ethyl acetate, 8:1), to give **5** as a syrup (0.83 g, 66%), $[\alpha]_{578}^{22}$ +29° (*c* 0.83, chloroform), $R_F$ 0.49. $^{13}$C-N.m.r. data ($CDCl_3$): δ 15.1 ($CH_3CH_2$), 16.5 (C-6′), 24.1 ($CH_3CH_2$), 53.8 (C-2), 68.5–84.1 (C-1, ring C, $PhCH_2$), 100.7 (C-1′), 123.0–133.6, 138.1, 138.4, 138.6, 138.8 (aromatic), 169.9, 168.6 (Pht).

*Ethyl 6-O-benzyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-*D*-galactopyranosyl)-1-thio-3-O-(2,3,4-tri-O-benzyl-α-*L*-fucopyranosyl)-β-*D*-glucopyranoside* (**6**). — A solution of silver triflate (9.7 g) and 2,4,6-trimethylpyridine (3.4 mL) in dichloromethane–toluene (50 mL, 3:2) was added dropwise to a stirred mixture of **5** (8.0 g), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (9.73 g), and ground molecular sieves (10 g, 4 Å) in toluene (50 mL) at −20° under dry nitrogen. The mixture was stirred for 30 min, aqueous 10% sodium thiosulfate (50 mL) and toluene (100 mL) were then added, and the mixture was filtered through

a layer of Celite. The organic layer was washed with water and concentrated, and the residue was purified by column chromatography (toluene–ethyl acetate, 3:1) to give **6** (5.87 g, 53%), m.p. 169° (from ether), $[\alpha]_{578}^{22}$ +6° (*c* 0.87, chloroform), $R_F$ 0.37. $^{13}$C-N.m.r. data ($CDCl_3$): δ 14.9 ($CH_3CH_2$), 16.7 (C-6′), 20.4 (2 C), 20.5 (2 C) (Ac), 23.7 ($CH_3CH_2$), 55.5 (C-2), 60.4 (C-6″), 66.6–81.5 (C-1, ring C, Ph$CH_2$), 97.5 (C-1′, $J_{C\text{-}1',H\text{-}1'}$ 167 Hz), 99.6 (C-1″, $J_{C\text{-}1'',H\text{-}1''}$ 164 Hz), 123.5, 125.2–128.9, 131.8, 134.0, 138.0, 138.4, 138.8, 138.9 (aromatic), 167.8 (Pht), 168.5, 169.6 (2 C), 169.8 (OAc).

*Anal.* Calc. for $C_{64}H_{71}NO_{19}S$: C, 64.6; H, 6.0; N, 1.2; S, 2.7. Found: C, 64.5; H, 6.1; N, 1.2; S, 2.7.

*Benzyl 3,4,6-tri*-O-*benzyl-2*-O-[*6*-O-*benzyl-2-deoxy-2-phthalimido-4*-O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-3*-O-*(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-β*-D-*glucopyranosyl*]-*α*-D-*mannopyranoside* (**8**) *and 1,5-anhydro-6*-O-*benzyl-2-deoxy-2-phthalimido-4*-O-*(2,3,4,6-tetra-O*-acetyl-*β*-D-*galactopyranosyl)-3*-O-*(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)*-D-arabino-*hex-1-enitol* (**9**). — Methyl triflate (157 μL) was added to a stirred mixture of benzyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside[9] (**7**, 155 mg), thioglycoside **6** (341 mg), and ground molecular sieves (4 Å, 2 g) in ether (25 mL) at room temperature. Triethylamine (400 μL) was added after 24 h. The mixture was stirred for 10 min, filtered through a layer of Celite, and concentrated, and the residue was purified by column chromatography (toluene–ethyl acetate, 3:1), to yield **8** as a syrup (321 mg, 67%), $[\alpha]_{578}^{22}$ −9° (*c* 0.8, chloroform), $R_F$ 0.55. $^{13}$C-N.m.r. data ($CDCl_3$): δ 16.7 (C-6″), 20.5 (OAc), 56.5 (C-2′), 60.3 (C-6‴), 63.2–79.7 (ring C, Ph$CH_2$), 96.5 (C-1′), 96.7 (C-1), 97.5 (C-1″), 99.5 (C-1‴), 123.3, 127.1–130.0, 131.9, 133.8, 137.4–138.9 (aromatic), 167.2 (Pht), 168.5, 170.0 (C, 3 C, OAc).

Eluted second was **9**, obtained as a syrup (58 mg, 18%), $[\alpha]_{578}^{22}$ −39° (*c* 0.7, dichloromethane), $R_F$ 0.30. $^{13}$C-N.m.r. data ($CDCl_3$): δ 16.5 (C-1′), 20.6 (2 C), 20.7 (2 C) (OAc), 61.3 (C-6″), 66.9, 67.1, 67.5, 68.7, 71.0, 71.1, 71.9, 72.6, 73.1, 73.6, 74.5, 75.2, 75.4, 76.1, 77.7, 79.1 (ring C, Ph$CH_2$), 99.0 (C-1′), 100.6 (C-1″), 107.7, 123.2, 127.4–128.4, 132.2, 133.7, 137.9, 138.3, 138.6, 138.9, 145.9 (C-1,2, aromatic), 167.9 (Pht), 169.2, 170.1, 170.3, 170.4 (OAc).

*Benzyl 2*-O-[*2-acetamido-6*-O-*benzyl-2-deoxy-4*-O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-3*-O-*(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-β*-D-*glucopyranosyl*]-*3,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**10**). — A solution of **8** (210 mg) and hydrazine hydrate (7 mL) in aqueous 90% ethanol (70 mL) was boiled under reflux overnight, then cooled, and concentrated to dryness. The residue was acetylated with acetic anhydride–pyridine (30 mL, 1:2) at room temperature overnight. The solution was concentrated, and the product was purified by column chromatography (toluene–chloroform–acetone, 2:3:1) to yield **10** as a syrup (168 mg, 87%), $[\alpha]_{578}^{22}$ −26° (*c* 0.6, dichloromethane), $R_F$ 0.54. $^{13}$C-N.m.r. data ($CDCl_3$): δ 16.8 (C-6″), 20.6 (OAc), 23.1 (NAc), 58.9 (C-2′), 60.3 (C-6‴), 66.1–80.0 (ring C, Ph$CH_2$), 97.3, 97.7 (C, 2 C, C-1,1′,1″), 99.5 (C-1‴), 127.0–128.0, 137.4–139.0 (aromatic), 168.7, 169.9, 170.0 (OAc), 171.2 (NAc).

*2-O-(2-Acetamido-2-deoxy-3-O-α-L-fucopyranosyl-4-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-D-mannose* (**1**). — Sodium methoxide in methanol (0.2 mL, 0.3M) was added to a solution of **10** (150 mg) in dichloromethane–methanol (15 mL, 2:3) at room temperature. Acetic acid (0.1 mL) was added after 2 h, when the reaction was complete. The solution was concentrated and a solution of the residue in aqueous 90% acetic acid (50 mL) was hydrogenolysed at 400 kPa over 10% Pd/C (200 mg) overnight. The mixture was filtered and concentrated, and the residue was eluted from a column (2.5 × 80 cm) of Biogel P-2 with water. After freeze-drying, **1** was obtained as an amorphous powder (55 mg, 86%), $[\alpha]_{578}^{22}$ −78° (*c* 0.2, water), $R_F$ 0.33 (ethyl acetate–methanol–acetic acid–water, 4:3:3:2). N.m.r. data: $^1$H (100 MHz, $D_2O$, 85°), δ 1.15 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6‴), 1.99 (s, 3 H, HNAc), 4.43 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1″), 4.62 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1′), 4.66 (q, 1 H, $J_{5,6}$ 6.5 Hz, H-5‴), 4.88 (d, 0.15 H, $J_{1,2}$ 0.5 Hz, H-1β), 5.10 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1‴), 5.15 (d, 0.85 H, $J_{1,2}$ 1.5 Hz, H-1α); $^{13}$C ($D_2O$), δ 17.0 (C-6‴), 24.2 (NAc), 57.6 (0.85 C, C-2′α), 57.8 (0.15 C, C-2′β), 61.7, 63.1, 63.4 (C-6,6′,6″), 68.3, 69.4, 69.6, 70.1 (2 C), 73.0, 73.7, 74.4 (2 C), 75.3, 76.4, 76.6, 77.2, 79.4 (ring C), 93.1 (0.85 C, C-1α), 95.4 (0.15 C, C-1β), 100.0 (C-1‴), 101.8 (0.85 C, C-1′α), 103.0 (0.15 C, C-1″β), 103.6 (C-1″), 176.0 (NAc).

*Benzyl 2,4-di-O-benzyl-3,6-di-O-{3,4,6-tri-O-benzyl-2-O-[6-O-benzyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranosyl}-α-D-mannopyranoside* (**12**). — Compound **12** was prepared from the mannotrioside[1,10] **11** (75 mg) as for **8** by using the thioglycoside **6** (203 mg), methyl trifluoromethanesulfonate (94 μL), and molecular sieves (2 g, 4 Å). The product was purified by column chromatography (toluene–ethyl acetate, 5:2) to yield **12** as a syrup (107 mg, 61%), $[\alpha]_{578}^{22}$ −5° (*c* 0.6, dichloromethane), $R_F$ 0.57. $^{13}$C-N.m.r. data ($CDCl_3$): δ 16.8 (2 C-6, Fuc), 20.5 (OAc), 56.1 (2 C-2, PhtGlc), 60.3 (2 C-6, Gal), 66.3–92.4 (ring C, Ph*C*$H_2$), 96.2 (2 C-1, PhtGlc), 96.6 (C-1, Man), 97.2 [C-1, Man-(1→6)], 97.4 (2 C-1, Fuc), 99.0 [C-1, Man-(1→3)], 99.5 (2 C-1, Gal), 123.2, 126.6–128.5, 131.9, 133.7, 137.1–138.9 (aromatic), 167.2 (Pht), 168.4, 168.6, 169.7 (3 C), 169.8 (2 C), and 170.0 (OAc).

*Benzyl 3,6-di-O-{2-O-[2-acetamido-6-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-3,4,6-tri-O-benzyl-α-D-mannopyranosyl}-2,4-di-O-benzyl-α-D-mannopyranoside* (**13**). — Compound **12** (92 mg) was treated with hydrazine hydrate (3 mL) in aqueous 90% ethanol (30 mL), followed by acetic anhydride–pyridine (15 mL, 1:2) as described for **8**. The product was purified by column chromatography (toluene–chloroform–acetone, 1:2:1) to yield **13** as a syrup (63 mg, 72%), $[\alpha]_{578}^{22}$ −25° (*c* 0.3, dichloromethane), $R_F$ 0.35. $^{13}$C-N.m.r. data ($CDCl_3$): δ 16.8 (2 C-6, Fuc), 20.6, 20.8 (OAc), 23.3 (2 C, NAc), 58.4, 59.2 (C-2, GlcNAc), 60.3 (2 C-6, Gal), 66.1–80.0 (ring C, Ph*C*$H_2$), 96.0 (C-1, Man), 97.4, 97.6 (4 C-1, GlcNAc, Fuc), 98.0 [C-1, Man-(1→6)], 99.3 [C-1, Man-(1→3)], 99.6 (2 C-1, Gal), 127.0–128.6, 137.0–139.0 (aromatic), 168.6, 168.7, 169.8 (2 C), 169.9 (2 C), 170.0 (2 C) (OAc), 170.9, and 171.4 (NAc).

*3,6-Di*-O-[*2*-O-*(2-acetamido-2-deoxy-3*-O-α-L-*fucopyranosyl-4*-O-β-D-*galactopyranosyl-β*-D-*glucopyranosyl)*-α-D-*mannopyranosyl*]-D-*mannose* (**2**). — Compound **13** (50 mg) was *O*-deacetylated and hydrogenolysed, and the product was purified as described for **1**, to give **2** as an amorphous powder (18 mg, 80%), $[\alpha]_{578}^{22}$ −34° (*c* 0.6, water), $R_F$ 0.36 (2-butanol–ethanol–acetic acid–pyridine–water, 10:100:3:10:30). N.m.r. data: $^1$H (400 MHz, $D_2O$, 85°), 1.17 (d, 6 H, $J_{5,6}$ 6.4 Hz, H-6, Fuc), 2.04, 2.05 (3 s, 0.35 H, 0.65 H, 1 H, HNAc), 4.43 (d, 2 H, $J_{1,2}$ 7.8 Hz, H-1, Gal), 4.62, 4.63, 4.64 (3 d, 1 H, 0.65 H, 0.35 H, $J_{1,2}$ 7.8 Hz, H-1, GlcNAc), 4.71 (q, 2 H, $J_{5,6}$ 6.4 Hz, H-5, Fuc), 4.88 [d, 1 H, $J_{1,2}$ 1.2 Hz, H-1, Man-(1→6)], 4.90 (d, 0.35 H, $J_{1,2}$ 1.4 Hz, H-1β), 5.10 (d, 2 H, $J_{1,2}$ 3.7 Hz, H-1, Fuc), 5.13 [d, 1 H, $J_{1,2}$ 1.2 Hz, 1.5 Hz, H-1, Man-(1→3)], 5.14 (d, 0.65 H, $J_{1,2}$ 1.7 Hz, H-1α); $^{13}$C ($D_2O$), 16.5 (2 C-6, Fuc), 23.6 (2 C, NAc), 56.9 (2 C-2, GlcNAc), 60.9, 62.7 (2 C-6, 4 C-6, Man, GlcNAc, Gal), 66.7–79.3 (ring C), 94.9, 95.4 (0.35 C-1α, 0.65 C-1β), 98.0 [C-1, Man-(1→6)], 99.7, 100.4 [2 C-1, 3 C-1, Fuc, GlcNAc, Man-(1→3)], 103.0 (2 C-1, Gal), and 175.6 (2 NAc).

ACKNOWLEDGMENTS

I thank Dr. J. Lönngren, Professor P. J. Garegg, and Professor B. Lindberg for their interest, and the Swedish Natural Science Research Council for financial support.

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