Carbohydrate Research, 110 (1982) 55–67
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

SYNTHESIS OF THREE DISACCHARIDES FOR THE PREPARATION OF IMMUNOGENS BEARING IMMUNODETERMINANTS KNOWN TO OCCUR ON GLYCOPROTEINS

GÖRAN EKBORG*, YOSHIAKI SONE†, AND CORNELIS P. J. GLAUDEMANS
National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205 (U.S.A.)

(Received May 12th, 1982; accepted for publication, June 3rd, 1982)

ABSTRACT

p-Nitrophenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside was condensed with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide, the product deprotected, and the disaccharide glycoside converted into *p*-trifluoroacetamidophenyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside. *p*-Nitrophenyl 3-*O*-benzoyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside was condensed with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide, and the product was deprotected, to yield *p*-nitrophenyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside. *p*-Nitrophenyl 2-acetamido-3,4-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside was condensed with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide, and, after reduction, trifluoroacetylation, and deprotection, *p*-trifluoroacetamidophenyl 2-acetamido-2-deoxy-6-*O*-α-L-fucopyranosyl-β-D-glucopyranoside was obtained.

INTRODUCTION

In the last few years, it has been found that some tumor cells express altered surface antigens not present on cells from the normal corresponding tissue[1]. Thus, it has become of great interest to have antibodies that are specific for these tumor-associated antigens, so as to be able to analyze and detect cell transformation and, in some cases, possibly even selectively effect tumor destruction[2].

Glycoproteins and glycolipids are frequently found associated with cell membranes, and it seems likely that oligosaccharidic determinants of these biopolymers actively partake in defining the immunological behavior of cells. Thus, monoclonal immunoglobulins having anticarbohydrate specificity are playing an increasingly important role in this area of cell biology. Although a great number of monoclonal immunoglobulins derived from plasmacytomas are known, many of them have

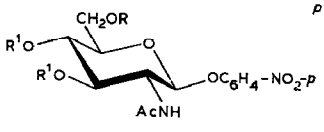
*Visiting Associate from Sweden.
†Visiting Fellow from Japan.

specificities for determinants not encountered on glycoproteins or glycolipids[3]. Since the monumental discovery of Köhler and Milstein[4] that continuously secreting, hybridoma cultures can be obtained by somatic cell-hybridization, it has become possible to obtain monoclonal immunoglobulins of predetermined specificity. For this reason, it is important to have available immunogens of sharply defined chemical structure having determinants which leave no doubt as to the specificity of the monoclonal immunoglobulin elicited against it. In the literature are reports of the preparation of monoclonal immunoglobulins by using large carbohydrate segments of glycoproteins as the immunogen[5], and, consequently, the fine specificity of the resulting, monoclonal antibody is not well defined. In our laboratory, we have begun the preparation of some *disaccharide* glycosides capable of being linked to protein carrier by standard methods[6]. We here report the synthesis of three such disaccharides, known to occur on glycoproteins, in a form suitable for linkage to carrier proteins.

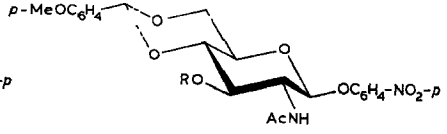
RESULTS AND DISCUSSION

For the synthesis of the first disaccharide, *p*-trifluoroacetamidophenyl 2-acetamido-2-deoxy-4-*O*-*β*-D-galactopyranosyl-*β*-D-glucopyranoside (**12**), we chose as the "aglycon" *p*-nitrophenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (**7**). The use of the corresponding 3,6-di-*O*-benzoyl derivative would have been more convenient, as it is readily obtainable in good yield by benzoylation of **1** at −45°, and would also have had the advantage of the possible removal of all protecting groups in one step to afford the final product. However, in our hands, it failed to give a glycosidic product in the condensation reaction with the per-*O*-benzoyl-*α*-D-galactopyranosyl bromide, instead giving rise to an orthoester that could not be rearranged to the corresponding disaccharide, but decomposed to unchanged aglycon and hydrolysis products that were slow-moving in t.l.c.

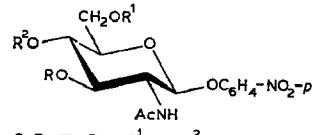
Treatment of **4** (ref. 7) with benzyl bromide, barium carbonate, and barium



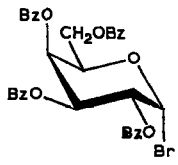
1 R = R¹ = H
2 R = Tr, R¹ = Bz
3 R = H, R¹ = Bz
Bz = PhCO
Tr = Ph_3C



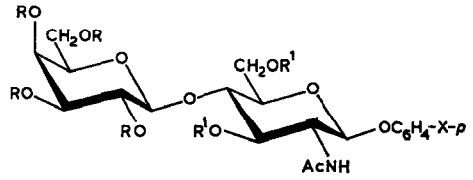
4 R = H
5 R = Bn
Bn = $PhCH_2$



6 R = Bn, R¹ = R² = H
7 R = R¹ = Bn, R² = H
8 R = R² = Bn, R¹ = H



+ 7

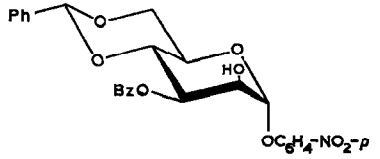


9 R = Bz, R^1 = Bn, X = NO_2

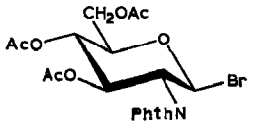
10 R = Bz, R^1 = Bn, X = $NHCOCF_3$

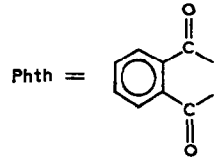
11 R = Bz, R^1 = H, X = $NHCOCF_3$

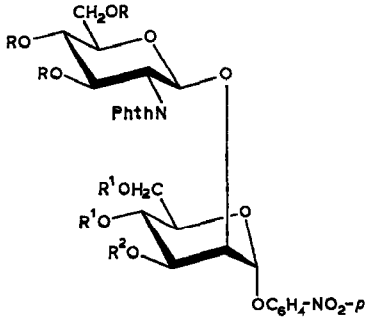
12 R = R^1 = H, X = $NHCOCF_3$



+







13 R = Ac, R^1 = PhC<, R^2 = Bz

14 R = Ac, R^1 = H, R^2 = Bz

hydroxide octahydrate in *N,N*-dimethylformamide yielded the 3-*O*-benzyl derivative **5** in almost quantitative yield. The crude **5** was homogeneous in t.l.c., and was therefore not purified for use in the next step. The anisylidene group of compound **5** was readily removed by brief hydrolysis with hot, 85% aqueous acetic acid, to give crystalline **6** in 68% yield. Compound **6** was then partially benzylated with benzyl bromide, barium carbonate, and barium hydroxide octahydrate in *N,N*-dimethylformamide, using essentially the same conditions and work-up as described by Jacquinet and Sinaÿ[8]. Chromatography of the products on a column of silica gel yielded a small amount of the 3,4,6-tri-*O*-benzyl derivative, and a fraction consisting of the desired 3,6-di-*O*-benzyl derivative (**7**) and a minor component (**8**). Compounds **7** and **8** were separated by fractional recrystallization, to give **7** in 41% yield and 14% of **8**. Compound **7** was identified as the 3,6-di-*O*-benzyl derivative by the shift, in the ^{13}C-n.m.r. spectrum, of the C-6 signal from 66.68 for precursor **6** to 71.66 p.p.m. for compound **7**. Compound **8** was shown to be the 3,4-di-*O*-benzyl derivative, as evidenced by the large, downfield shift of the signal assigned to C-4 (77.54 for **8** *vs.* 69.84 for **7** and 69.89 p.p.m. for **6**). Compound **7** was then treated with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide by using the silver triflate–2,4,6-collidine complex[9] in toluene–nitromethane solution at an initial temperature of −20°. It proved necessary, however, to extend the reaction time overnight at a slowly rising temperature to achieve the maximum yield of the desired, fully protected disaccharide derivative **9**. After work-up and chromatography on a column of silica gel, compound **9** was obtained in 57% yield.

For deprotection, the *p*-nitrophenyl group in **9** was first converted into a *p*-trifluoroacetamidophenyl group. Compound **9** was hydrogenated over Adams's catalyst in ethyl acetate solution, and the product was treated *in situ* with trifluoroacetic anhydride–pyridine at 60°, to give **10** in 88% yield after purification. Hydrogenation of **10** in glacial acetic acid in the presence of 10% palladium-on-charcoal then yielded **11**, which was debenzoylated to **12** by use of a catalytic amount of sodium methoxide in methanol. When needed for linking to a carrier protein, the remaining trifluoroacetyl group can be readily removed[10] by treatment of **11** (which is stable) with methanolic ammonia.

The ^{1}H-n.m.r. spectrum of **12** showed, *inter alia*, a one-proton doublet at δ 5.21 with a coupling constant of 8 Hz, assigned to the 2-acetamido-2-deoxy-β-D-glucosyl residue, and a one-proton doublet for the anomeric H of the β-D-galactosyl group, at δ 4.55, with a coupling constant of 7.5 Hz. At δ 2.05, a three-proton singlet was observed for the *N*-acetyl group. In the ^{13}C-n.m.r. spectrum, signals were observed, at δ 102.95 and 102.55, for C-1 of the β-D-galactosyl group and the 2-acetamido-2-deoxy-β-D-glucosyl residue, respectively. The signal assigned to C-4 of the residue was observed at δ 81.30, shifted downfield from δ 70.16 for "unsubstituted" **1**, in agreement with substitution at C-4. These data, and the observed specific rotation of $[\alpha]_{578}^{23}$ −10.8°, are in agreement with the structure postulated for **12**.

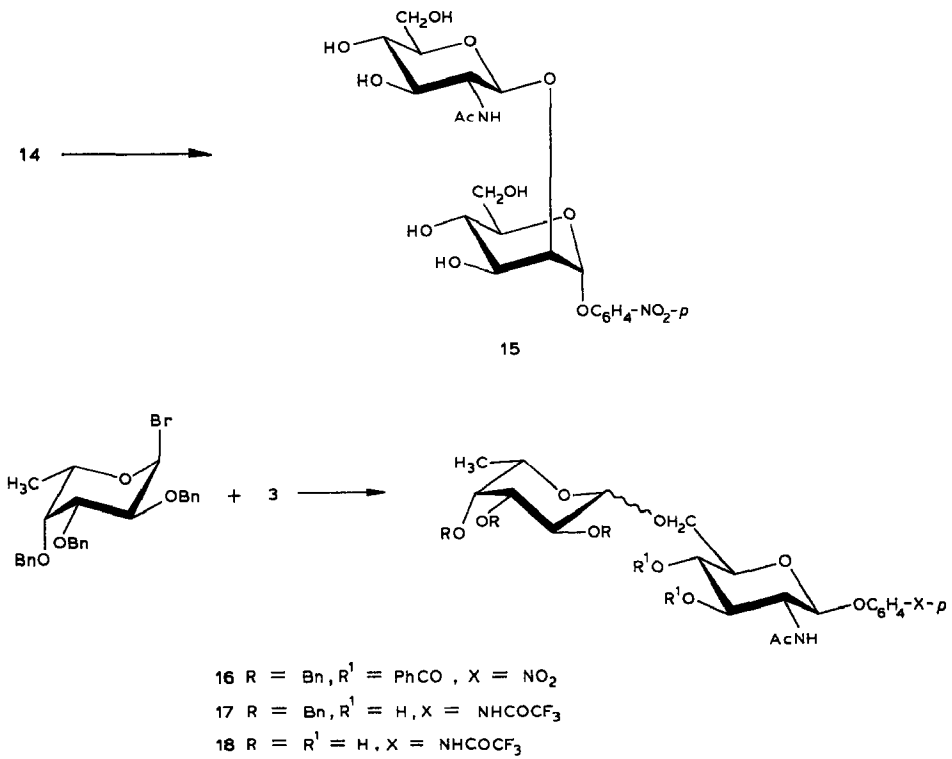
For the second disaccharide, *p*-nitrophenyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside (**15**), the Helferich modification[11] of the

Koenigs–Knorr reaction was employed. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[12] was treated with *p*-nitrophenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside[13] in dry acetonitrile in the presence of mercuric cyanide as a promoter. After two days at +4°, the mixture was worked up and, after chromatography on a column of silica gel, the fully protected disaccharide derivative **13** was obtained in 31% yield. Use of silver triflate as a promoter[9,12] resulted in an unrecognizable product. If the reaction was carried out at higher temperatures (*e.g.*, room temperature), extensive formation of an elimination product lowered the yield of the desired derivative **13**. This elimination product was not fully identified, but its n.m.r. spectra (^{1}H and ^{13}C) suggested that it could be **13** that had lost a mole of acetic acid.

Deblocking of **13** to give **15** was performed in the following way: compound **13** was first treated with hot, 80% aqueous acetic acid to give the derivative **14**. Treatment of **14** with hydrazine hydrate under reflux removed all acyl groups, to give an intermediate which, for purification purposes, was peracetylated with acetic anhydride–pyridine in the usual way. After chromatography on a column of silica gel, a syrup was obtained that was identified by ^{1}H-n.m.r. spectroscopy as peracetylated **15**. *O*-Deacetylation with sodium methoxide in methanol then yielded (after chromatographic purification) the desired disaccharide derivative **15** in 50% overall yield from **13**. The ^{1}H-n.m.r. spectrum of **15** showed a one-proton doublet at δ 5.66 having a coupling constant of 2 Hz, characteristic for H-1 of an α-D-mannosyl residue, and a one-proton doublet at δ 4.58 with a coupling constant of 7.5 Hz, typical for H-1 of a 2-acetamido-2-deoxy-β-D-glucopyranoside. A three-proton singlet was observed at δ 1.81 for the *N*-acetyl group. In the ^{13}C-n.m.r. spectrum, two signals were observed, at 102.20 and 97.82 p.p.m., respectively assigned to the anomeric carbon atoms of the α-D-mannosyl residue and the 2-acetamido-2-deoxy-β-D-glucosyl group. The signal for C-2 of the α-D-mannosyl residue in **15** had shifted to 75.89 p.p.m., downfield from 71.50 p.p.m. for C-2 in "unsubstituted" *p*-nitrophenyl α-D-mannoside. These data, together with a specific rotation of $[\alpha]_{578}^{23}$ +72.7°, confirmed the structure postulated for **15**.

The synthesis of the third disaccharide, *p*-trifluoroacetamidophenyl 2-acetamido-2-deoxy-6-*O*-α-L-fucopyranosyl-β-D-glucopyranoside (**17**), was effected by use of the halide ion-assisted, glycosylation procedure[14–19]. The "aglycon" **3** was prepared from **1** by tritylation and subsequent benzoylation *in situ* in pyridine, to give the crystalline, fully protected derivative **2** in 77% yield. Compound **2** was then detritylated by treatment with hot, 90% aqueous acetic acid to give **3** in a yield of 88%.

In the glycosylation step, compound **3** was treated with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (freshly prepared from the anomeric 1-*p*-nitrobenzoates[20–22] in the presence of tetraethylammonium bromide and molecular sieve 4A in dichloromethane[17] solution, under the conditions described by Lemieux and Driguez[18]). After two days, the mixture was worked up, and the products were separated on a column of silica gel, to give 73% of a mixture of **16α** (α-L-fucoside) and **16β** (β-L-fucoside) in the ratio of ~4:1, as indicated by comparison of the signals assigned to



the anomeric carbon atoms of the L-fucosyl groups. The fact that a small proportion of the β-linked **16β** was obtained along with the desired **16α** is probably due to the higher reactivity of the primary hydroxyl group in **3**, which facilitates *direct* displacement of the α-L-fucosyl bromide, giving a β-linked product.

Compounds **16α** and **16β** could not be resolved at this stage, but resolution could be achieved after deprotection. First, the *p*-nitrophenyl group was transformed into a *p*-trifluoroacetamidophenyl group as already described for **10**. *O*-Debenzoylation and hydrogenolysis then gave, after the usual work-up, and chromatography on a column of silica gel, pure **18** (shown to be anomerically pure by ^{13}C-n.m.r. spectroscopy) in 30% yield from **16** (α and β). In the ^{1}H-n.m.r. spectrum of **18**, signals were observed at δ 5.08, a one-proton doublet with a coupling constant of 8 Hz, for H-1 of the 2-acetamido-2-deoxy-β-D-glucosyl residue; at δ 4.84, a one-proton doublet with a coupling constant of 3 Hz, for H-1 of the α-L-fucosyl group; a three-proton singlet for the *N*-acetyl group; and a three-proton doublet with coupling constant 6 Hz for H-6 of the α-L-fucosyl group. The ^{13}C-n.m.r. spectrum showed signals at 102.55 and 101.91 p.p.m., respectively assigned to the anomeric carbon atoms of the 2-acetamido-2-deoxy-β-D-glucosyl residue and the α-L-fucosyl group. The signal assigned to C-6 of the residue had shifted to 69.16 p.p.m. (possibly to 69.74 p.p.m., see the Experimental section), downfield from 60.62 p.p.m. for unsubstituted *p*-

nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**). The foregoing spectral data, and the specific rotation of $[\alpha]_{578}^{23}$ −60.2°, are in full agreement with the structure assigned to **18**.

EXPERIMENTAL

General methods. — Melting points are corrected, and optical rotations were determined with a Perkin–Elmer 241MC polarimeter. Preparative chromatography was performed with Merck Silica Gel 60; sample to column ratios (w/w) usually were in the 0.5–2.0% range. Solvents were of analytical grade. T.l.c. was performed on glass plates precoated with Merck Silica gel 60 F_{254} (0.25 mm), and components were detected by spraying with 5% ammonium hydrogensulfate in 75% methanol followed by charring at 120°. ^{1}H-N.m.r. spectra were recorded either at 220 MHz with a Varian HR-220 spectrometer operating in the continuous-wave mode, or at 99.55 MHz with a Jeol FX-100 instrument in the Fourier-transform mode. ^{13}C-N.m.r. spectra were recorded either at 25.05 MHz with a Jeol FX-100 spectrometer, or at 75.47 MHz with a Bruker WM-300 spectrometer, both operating in the Fourier-transform mode. All spectra were in agreement with the structures postulated, and only pertinent n.m.r. data are reported. Chemical shifts are given relative to internal tetramethylsilane, in p.p.m., except for solutions in deuterium oxide, when sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate was used.

p-*Nitrophenyl 2-acetamido-3,4-di*-O-*benzoyl-2-deoxy-6*-O-*trityl-β*-D-*glucopyranoside* (**2**). — *p*-Nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**; 0.34 g, 1 mmol) was dissolved in anhydrous pyridine (5 mL), chlorotriphenylmethane (0.31 g, 1.1 mmol) was added, and the mixture was kept overnight at room temperature, when t.l.c. showed no remaining starting-material. Benzoyl chloride (0.26 mL, 2.2 mmol) was added, the solution was kept for 2 h, water was added, and, after 15 min, the solution was diluted with dichloromethane, washed with water, and dried (Na_2SO_4). Filtration, and evaporation of the solvents, afforded a solid (0.99 g) which, when recrystallized from ethanol, gave **2** (0.61 g, 77%), m.p. 195–198°, $[\alpha]_{578}^{23}$ −14.3° (*c* 2.9, $CHCl_3$); ^{1}H-n.m.r. data (220 MHz, $CDCl_3$): δ 7.2–8.2 (m, 29 H), 5.94 (d, 1 H, $J_{NH,2}$ 9 Hz, NH), 5.75 (dd, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 5.61 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 4.43 (m, 1 H, H-5), and 1.91 (s, 3 H, Ac). The ^{1}H-n.m.r. spectrum showed the presence of one mol of ethanol.

Anal. Calc. for $C_{42}H_{40}N_2O_{10} \cdot C_2H_5OH$: C, 70.20; H, 5.52; N, 3.33. Found: C, 70.10; H, 5.46; N, 3.34.

p-*Nitrophenyl 2-acetamido-3,4-di*-O-*benzoyl-2-deoxy-β*-D-*glucopyranoside* (**3**). — A solution of compound **2** (3.96 g, 5 mmol) in a mixture of glacial acetic acid (180 mL) and water (20 mL) was heated for 15 min on a steam bath; when t.l.c. (19:1 chloroform–methanol) showed the reaction to be complete. The mixture was cooled, and evaporated to a residue which, on recrystallization from 2-propanol, afforded **3** (2.0 g, 73%). The mother liquors were concentrated, and purified by chromatography on a column of silica gel (19:1 chloroform–methanol), to yield an additional 0.42 g

(15%). An analytical sample had m.p. 204–205°, $[\alpha]_{578}^{23}$ −20.4° (*c* 3.85, $CHCl_3$); ^{1}H-n.m.r. data (220 MHz, $CDCl_3$): δ 7–8.2 (m, 14 H), 6.09 (d, 1 H, $J_{NH,2}$ 8.5 Hz, NH), 6.00 (dd, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 5.70 (d, 1 H, $J_{1,2}$ 9 Hz, H-1), 5.50 (dd, 1 H, $J_{2,3} = J_{3,4} = 10$ Hz, H-3), and 1.89 (s, 3 H, *N*-acetyl CH_3); ^{13}C-n.m.r. data (25.05 MHz, $CDCl_3$): δ 171.95 (*N*-acetyl C=O), 166.68 and 166.09 (*O*-benzoyl C=O), 162.00, 142.93, 133.80, 129.88, 128.89, 128.65, 125.90, and 116.72 (aromatic), 98.29 (C-1), 75.18 (C-5), 72.72 (C-3), 69.74 (C-4), 61.14 (C-6), 54.53 (C-2), and 22.82 (*N*-acetyl CH_3).

Anal. Calc. for $C_{28}H_{26}N_2O_{10}$: C, 61.10; H, 4.76; N, 5.09. Found: C, 60.80; H, 5.02; N, 5.00.

p-*Nitrophenyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-β-*D*-glucopyranoside* (**5**). — To a solution of compound **4** (4.2 g) in anhydrous *N,N*-dimethylformamide (75 mL) were added barium carbonate (8.5 g), barium hydroxide octahydrate (2.5 g), and benzyl bromide (1.7 mL), and the mixture was vigorously stirred for 1.5 h at room temperature. Chloroform (250 mL) was added and the suspension was filtered. Concentration of the filtrate yielding a residue which was triturated with toluene, and dried. The solid (6.12 g, 100%) was sufficiently pure for use in the next step; ^{1}H-n.m.r. data (220 MHz, dimethyl sulfoxide-d_6): δ 8.23 and 6.98 (AB, 2 H, each, $J_{H,H}$ 7.5 Hz, aromatic H), 7.73–7.27 (m, 9 H, aromatic H), 5.68 (s, 1 H, benzylidene H), 5.48 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.77 and 4.63 (AB, 1 H each, $J_{H,H}$ 10 Hz, benzyl CH_2), 3.39 (s, 3 H, OCH_3), and 1.82 (s, 3 H, $COCH_3$).

p-*Nitrophenyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**6**). — A solution of compound **5** (5.50 g) in hot, glacial acetic acid (250 mL) and water (50 mL) was kept on a steambath for 10 min, cooled, and concentrated to a residue which was recrystallized from ethanol, to give **6** (2.92g 68%); an analytical sample had m.p. 213–215°, $[\alpha]_{578}^{23}$ +15° (*c* 1.9, 1:1 $CHCl_3$–MeOH); ^{1}H-n.m.r. data (220 MHz, 3:1 $CDCl_3$–CD_3OD): δ 7.95 and 6.93 (d, 2 H each, $J_{H,H}$ 9.5 Hz, *p*-nitrophenyl H), 7.22–7.04 (m, 5 H, aromatic H), 5.11 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.79 and 4.59 (d, 1 H each, AB, $J_{H,H}$ 11 Hz, benzyl CH_2), 3.93 (dd, 1 H, $J_{3,4} = J_{4,5} = 9$ Hz, H-4), 3.61 (dd, 1 H, $J_{5,6}$ 2, $J_{6,6}$ 12 Hz, H-6), and 1.84 (s, 3 H, *N*-acetyl CH_3); ^{13}C-n.m.r. data (25.05 MHz, 3:1 $CDCl_3$–CD_3OD): δ 171.70 (*N*-acetyl C=O), 161.76, 142.07, 138.07, 127.60, 127.16, 127.91, 115.90 (aromatic C), 97.96 (C-1), 81.49 (C-3), 76.52 (C-5), 74.03 (benzyl CH_2), 68.89 (C-4), 60.68 (C-6), 54.34 (C-2), and 21.64 (*N*-acetyl CH_3).

Anal. Calc. for $C_{21}H_{24}N_2O_8$: C, 58.30; H, 5.59; N, 6.48. Found: C, 57.90; H, 5.41; N, 6.27.

p-*Nitrophenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**7**). — To a solution of compound **6** (2.59 g) in *N,N*-dimethylformamide (25 mL) were added barium carbonate (4.8 g) and barium hydroxide octahydrate (1.2 g). To the vigorously stirred mixture was then added benzyl bromide (0.9 mL). After 2 h, t.l.c. (9:1 chloroform–methanol) revealed the presence of a minor component corresponding in rate of movement to the 3,4,6-tri-*O*-benzyl derivative, and a major component corresponding to the desired 3,6-dibenzyl ether **7**, the latter moving close

to another minor spot, shown by ^{13}C-n.m.r. spectroscopy to be *p*-nitrophenyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**8**). Chloroform (125 mL) was added, the mixture was filtered, and the filtrate was concentrated, to give a residue from which the remaining *N*,*N*-dimethylformamide was removed by repeated co-distillation with toluene; unreacted benzyl bromide was then extracted by trituration with hexane. The resulting material was purified by chromatography on a column of silica gel with 9:1 chloroform–methanol as the eluant, to yield 0.25 g of the 3,4,6-tribenzyl ether, 1.71 g of the mixed dibenzyl ethers, and 0.66 g of the starting material. Fractional recrystallization from ethanol, and then ethanol–water, gave 0.44 g of **8**, m.p. 215–217°, and 1.27 g (41%) of the desired **7**, m.p. 191–193°, $[\alpha]_{578}^{23}$ −11.3° (*c* 1.65, 9:1 $CHCl_3$–MeOH); ^{1}H-n.m.r. data for **7** (220 MHz, 3:1 $CDCl_3$–CD_3OD): δ 8.09 and 7.07 (both d, 2 H, each, $J_{H,H}$ 10 Hz, AB, *p*-nitrophenyl H), 7.50–7.15 (m, 10 H, aromatic H), 5.25 (d, 1 H, $J_{1,2}$ 8 Hz, H-1) and 1.89 (s, 3 H, *N*-acetyl CH_3); ^{13}C-n.m.r. (25.05 MHz, 3:1 $CDCl_3$–CD_3OD): δ 171.56 (*N*-acetyl C=O), 162.10, 142.51, 135.37, 137.88, 128.47, 128.08, 127.89, 129.64, 125.69, 116.63 (aromatic carbon atoms), 98.11 (C-1), 81.20 (C-3), 75.69 (C-5), 74.42 and 73.64 (benzyl CH_2), 71.16 (C-6), 69.84 (C-4), 55.12 (C-2), and 23.10 (*N*-acetyl CH_3).

Anal. Calc. for $C_{28}H_{30}N_8O_2 \cdot H_2O$: C, 62.20; H, 5.97; N, 5.18. Found: C, 62.60; H, 5.72; N, 5.36.

Compound **8** had $[\alpha]_{578}^{23}$ −3.8° (*c* 1.1, 1:1 $CHCl_3$–MeOH); ^{1}H-n.m.r. data (220 MHz, 7:3 $CDCl_3$–CD_3OD): δ 7.95 and 7.11 (both d, 2 H, each, $J_{H,H}$ 7.5 Hz, AB, *p*-nitrophenyl H), 7.3–7.15 (m, 10 H, aromatic H), 5.27 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 3.97 (dd, 1 H, $J_{4,5}$ 10, $J_{3,4}$ 8 Hz, H-4), and 1.89 (s, 3 H, *N*-acetyl CH_3); ^{13}C-n.m.r. (25.05 MHz, Me_2SO-d_6): δ 98.06 (C-1), 82.03 (C-3), 77.54 (C-4), 75.98 (C-5), 74.18 and 74.03 (benzyl-CH_2), 59.90 (C-6), 54.44 (C-2), and 22.86 (*N*-acetyl CH_3).

p-*Nitrophenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside* (**9**). — To a solution of compound **7** (1.53 g) in 1:1 toluene–nitromethane (20 mL) were added silver trifluoromethanesulfonate (0.55 g) and 2,4,6-trimethylpyridine (200 μL), and the mixture was stirred and cooled to −20°. A solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide in the same solvent mixture (10 mL) was added dropwise during 15 min. Stirring was continued overnight while the reaction mixture slowly attained room temperature. During this time, anhydrous conditions were ensured by keeping the reactants under dry argon; t.l.c. in 2:1 toluene–ethyl acetate then showed only one major product. Pyridine was added to neutrality, the suspension was filtered through Celite, and the filtrate was diluted with chloroform (200 mL), washed twice with water, dried (Na_2SO_4), and concentrated in a rotary evaporator. The residue was purified by chromatography on a column of silica gel, with 4:1 toluene–ethyl acetate as the eluant. Appropriate fractions were pooled, and evaporated, to yield amorphous **9** (0.97 g, 57%); $[\alpha]_{578}^{23}$ +16.4° (*c* 3.1, $CHCl_3$); ^{1}H-n.m.r. data (220 MHz, $CDCl_3$): δ 8.1–6.8 (m, 34 H, aromatic H), 6.00 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ <1 Hz, Gal H-4), 5.77 (dd, 1 H, $J_{2,3}$ 10, $J_{1,2}$ 8 Hz, Gal H-2), 5.61 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, Gal H-3), 5.30 (d, 1 H, $J_{1,2}$ 6.5 Hz, GlcNAc H-1), 4.89 (d, 1 H, $J_{1,2}$ 8 Hz, Gal H-1), 4.61 (dd,

1 H, $J_{5,6}$ 7, $J_{6,6}$ 11 Hz, Gal H-6), 4.14 (dd, 1 H, $J_{5,6}$ 7, $J_{6,6}$ 11 Hz, GlcNAc H-6), and 2.09 (s, 3 H, *N*-acetyl CH_3); ^{13}C-n.m.r. data (25.05 MHz, $CDCl_3$; carbohydrate signals only): D-galactosyl group: δ 99.86 (C-1), 71.55 (C-5), 71.01 (C-3), 70.18 (C-2), 67.99 (C-4), and 61.65 (C-6); 2-acetamido-2-deoxy-D-glucosyl residue: δ 97.18 (C-1), 76.27 (C-3), 74.57 (C-5), 72.81 (C-4), 68.72 (C-6), and 51.13 (C-2).

p-*Trifluoroacetamidophenyl 2-acetamido-3,6-di*-O-*benzyl-2-deoxy-4*-O-(*2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl*)-*β*-D-*glucopyranoside* (**10**). — A suspension of Adams's catalyst (150 mg) in ethyl acetate (20 mL) containing **9** (0.83 g) was stirred under hydrogen at atmospheric pressure until consumption of the gas had ceased. Trifluoroacetic anhydride (0.8 mL) and pyridine (2 mL) were added, and the tightly stoppered flask was heated for 30 min at 60°, cooled, the mixture filtered, and the filtrate concentrated. A solution of the residue in chloroform (150 mL) was washed thrice with water, dried (Na_2SO_4) and concentrated *in vacuo* to a residue which was purified by chromatography on a column of silica gel using 2:1 toluene–ethyl acetate as the eluant, to give homogeneous **10** (0.76 g, 88%); $[\alpha]_{578}^{23}$ +21.5° (*c* 3.4, $CHCl_3$); ^{1}H-n.m.r. data (220 MHz, $CDCl_3$): δ 8.1–7.0 (m, 32 H, aromatic H), 6.68 (d, 2 H, $J_{H,H}$ 8.5 Hz, high-field portion of AB spectrum of *p*-trifluoroacetamidophenyl group), 5.93 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ <1 Hz, Gal H-4), 5.73 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, Gal H-2), 5.52 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, Gal H-3), 5.14 (d, 1 H, $J_{1,2}$ 6 Hz, GlcNAc, H-1), 4.86 (d, 1 H, $J_{1,2}$ 8 Hz, Gal H-1), and 1.97 (s, 3 H, *N*-acetyl CH_3).

p-*Trifluoroacetamidophenyl 2-acetamido-2-deoxy-4*-O(*2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl*)-*β*-D-*glucopyranoside* (**11**). — Catalyst (10% Pd-on-charcoal, 0.40 g) was suspended in glacial acetic acid (30 mL) containing **10** (0.43 g), and the suspension was stirred under hydrogen at atmospheric pressure until consumption of the gas had ceased. The catalyst was removed by filtration, and the filtrate was concentrated to a residue which was purified by chromatography on a column of silica gel using 9:1 chloroform–methanol as the eluant. Appropriate fractions were pooled and evaporated *in vacuo*, to yield **11** as a chromatographically homogeneous oil (0.22 g, 66%), $[\alpha]_{578}^{23}$ +67.5° (*c* 3.4, $CHCl_3$); ^{1}H-n.m.r. data (220 MHz, $CDCl_3$): δ 7.95–6.70 (m, 24 H, aromatic H), 5.56 (dd, 1 H, $J_{3,4}$ 3, $J_{4,5}$ 1 Hz, Gal H-4), 5.70 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, Gal H-2), 5.56 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3 Hz, Gal H-3), 5.06 (d, 1 H, $J_{1,2}$ 8 Hz, GlcNAc H-1), 4.91 (d, 1 H, $J_{1,2}$ 8 Hz, Gal H-1), and 1.93 (s, 3 H, *N*-acetyl CH_3).

p-*Trifluoroacetamidophenyl 2-acetamido-2-deoxy-4*-O-*β*-D-*galactopyranosyl-β*-D-*glucopyranoside* (**12**). — Compound **11** (0.15 g) was dissolved in methanol (10 mL) and treated with 0.2M sodium methoxide in methanol (0.75 mL). After 2.5 h, the solution was de-ionized, and concentrated to dryness. A solution of the residue in water was washed with hexane, and evaporated, to yield a crude product which was purified by chromatography on a column of silica gel, using 3:2:1 ethyl acetate–2-propanol–water as the eluant, to give chromatographically homogeneous **12** (71 mg, 75%); recrystallization from 2-propanol–water gave **12**, m.p. 248–251° (dec.), $[\alpha]_{578}^{23}$ −10.8° (*c* 0.86, 1:1 water–pyridine); ^{1}H-n.m.r. data (99.55 MHz): δ 7.51 and 7.14 (both d, 2 H, each, AB $J_{H,H}$ 9 Hz, *p*-trifluoroacetamidophenyl group), 5.21

(d, 1 H, $J_{1,2}$ 8 Hz, GlcNAc H-1), 4.55 (d, 1 H, $J_{1,2}$ 7.5 Hz, Gal H-1), and 2.05 (s, 3 H, *N*-acetyl CH_3); ^{13}C-n.m.r. data (75.47 MHz, D_2O), D-galactosyl group: δ 105.95 (C-1), 78.07 (C-5, or GlcNAc C-5), 75.58 (C-3, or GlcNAc C-3), 74.03 (C-2), 71.60 (C-4), and 64.03 (C-6); 2-acetamido-2-deoxy-D-glucosyl residue: δ 102.55 (C-1), 81.30 (C-4), 78.39 (C-5, or Gal C-5), 75.19 (C-3, or Gal C-3), 62.99 (C-6), and 58.05 (C-2).

Anal. Calc. for $C_{22}H_{29}F_3N_2O_{12} \cdot H_2O$: C, 44.90; H, 5.31; N, 4.76. Found: C, 44.50; H, 5.45; N, 4.28.

p-*Nitrophenyl 3-O-benzoyl-4,6-O-benzylidene-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranoside* (**13**). — 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (0.70 g, 1.4 mmol) and *p*-nitrophenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside (0.22 g, 0.4 mmol) were dissolved in dry acetonitrile (5 mL), and the mixture was cooled in ice. Mercuric cyanide (0.42 g) was added, and the mixture was stirred for 2 days at 4°; t.l.c. (2:1 toluene–ethyl acetate) then showed the presence of a major, new spot at R_F ~0.5. The mixture was diluted with dichloromethane (75 mL), washed successively with cold, aqueous $NaHCO_3$ solution (3 × 60 mL) and water (60 mL), dried (Na_2SO_4), and concentrated; the residue was purified by chromatography on a column of silica gel, using 2:1 toluene–ethyl acetate as the eluant, to yield **13** (0.13 g, 31%). When crystallized from ethanol, the material had m.p. 238–241°, $[\alpha]_{578}^{23}$ +80.2° (*c* 3.2, $CHCl_3$); ^{1}H-n.m.r. data (220 MHz, $CDCl_3$): δ 8.29–6.84 (m, 18 H, aromatic H), 5.80 (dd, 1 H, $J_{3,4}$ 10, $J_{4,5}$ 11 Hz, GlcNPhth H-4), 5.57 (dd, 1 H, $J_{2,3}$ 3, $J_{3,4}$ 9 Hz, GlcNPhth H-1), 5.27 (s, 1 H, benzylidene proton), 5.07 (dd, 1 H, $J_{2,3} = J_{3,4} = 10$ Hz, GlcNPhth H-3), and 4.45 (dd, 1 H, $J_{3,4}$ 9, $J_{4,5}$ 11 Hz, Man H-4); ^{13}C-n.m.r. data (25.05 MHz, $CDCl_3$): mannosyl residue, δ 96.93 (C-1), 75.25 (C-5), 74.71 (C-2), 68.72 (C-4), 68.23 (C-3), 64.72 (C-6); GlcNPhth group, δ 95.81 (C-1), 71.83, 70.23; 68.86, 61.70 (C-3,4,5,6), 54.53 (C-2).

Anal. Calc. for $C_{46}H_{42}N_2O_{18}$: C, 60.70; H, 4.65; N, 3.08. Found: C, 60.20; H, 4.55; N, 2.99.

p-*Nitrophenyl 2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside* (**15**). — A solution of **13** (0.22 g) in 80% acetic acid was heated for 15 min at 100°, cooled, and concentrated to a residue from which remaining acetic acid was removed by codistillation with toluene. The material was purified by chromatography on a column of silica gel, using 15:1 chloroform–methanol as the eluant, to give the intermediate **14** (0.19 g); ^{1}H-n.m.r. spectroscopy showed complete removal of the benzylidene group. This product was dissolved in a mixture of 85% hydrazine hydrate (1 mL) and 95% ethanol (10 mL), and the solution was boiled under reflux for 1 h, when t.l.c. with ethyl acetate–2-propanol–water showed only one component. The mixture was cooled, and evaporated to a residue which, for purification, was acetylated with acetic anhydride (5 mL) and pyridine (5 mL); after the usual work-up, the product was purified by chromatography on a column of silica gel, using 10:1 chloroform–methanol as the eluant. The acetate (0.20 g) was obtained as a chromatographically homogeneous oil, identified by ^{1}H-n.m.r. spectroscopy; it was *O*-deacetylated with

sodium methoxide in methanol, the solution made neutral with acetic acid, and concentrated, and the residue purified by chromatography on a column of silica gel using 3:2:1 ethyl acetate–2-propanol–water as the eluant. Chromatographically homogeneous **15** was obtained as a syrup, 70 mg (50% from **13**); $[\alpha]_{578}^{23}$ +72.7° (*c* 1.7, H_2O); ^{1}H-n.m.r. data (99.95 MHz, D_2O): δ 7.91 and 6.96 (2 d, 2 H, each, $J_{H,H}$ 9.5 Hz, AB, *p*-nitrophenyl H), 5.66 (d, 1 H, $J_{1,2}$ 2 Hz, Man H-1), 4.58 (d, 1 H, $J_{1,2}$ 7.5 Hz, GlcNAc H-1), and 1.81 (s, 3 H, *N*-acetyl CH_3); ^{13}C-n.m.r. data (25.05 MHz, D_2O), D-mannosyl residue: δ 102.20 (C-1), 76.57 (C-5), 75.89 (C-2), 72.47 (C-3), 69.40 (C-4), and 63.75 (C-6); 2-acetamido-2-deoxy-D-glucosyl group: δ 97.82 (C-1), 78.47 (C-5), 75.27 (C-3), 71.94 (C-4), 63.12 (C-6), and 58.00 (C-2).

p-*Nitrophenyl 2-acetamido-3,4-di*-O-*benzoyl-2-deoxy-6*-O-[*2,3,4-tri*-O-*benzyl-α-(and β)*-L-*fucopyranosyl*]-*β*-D-*glucopyranoside* (**16α** *and* **16β**). — Compound **3** (1.1 g, 2 mmol) in dichloromethane (7 mL), containing tetraethylammonium bromide (0.54 g, 4 mmol) and suspended molecular sieve 4A (2.4 g), was stirred. A solution of freshly prepared 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (from 2.3 g of the anomeric 1-*p*-nitrobenzoates[22]) in dichloromethane (7 mL) was added, and the mixture was stirred for 2 days at room temperature, when t.l.c. (1:1 hexane–ethyl acetate) showed the formation of a major product having R_F ~0.27. Solids were removed by filtration, and the filtrate was washed with water (twice), the aqueous layer being back-extracted with dichloromethane. The combined solution was dried (Na_2SO_4), and concentrated, and the residue was purified by chromatography on a column of silica gel, using the aforementioned solvent as the eluant, to yield a chromatographically homogeneous oil (1.47 g, 73%), shown by ^{13}C-n.m.r. spectroscopy to consist of a mixture of **16α** and **16β** in the ratio of ~4:1; ^{1}H-n.m.r. data (220 MHz, $CDCl_3$): δ 8.09–6.84 (m, 29 H, aromatic H), 5.89 (dd, 1 H, $J_{3,4} = J_{4,5} = 9$ Hz, GlcNAc H-4), 5.52 (d, 1 H, $J_{1,2}$ 8 Hz, GlcNAc H-1), 4.00 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10 Hz, Fuc H-2), 3.86 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 2.5 Hz, Fuc H-3), 3.59 (d or dd, 1 H, $J_{3,4}$ 2.5, $J_{4,5}$ <1 Hz, Fuc H-4), 1.84 (s, 3 H, *N*-acetyl CH_3), and 0.93 (d, 3 H, $J_{5,6}$ 6.5 Hz, Fuc H-6); ^{13}C-n.m.r. data (25.05 MHz, $CDCl_3$), fucosyl group: δ 98.74 (C-1α), 103.56 (C-1β), 79.14 (C-4), 77.59 (C-3), 76.66 (C-2), 66.56 (C-5), and 16.47 (C-6); 2-acetamido-2-deoxy-D-glucosyl residue: δ 98.35 (C-1), 75.79 (C-5), 72.47 (C-3), 69.79 (C-4), 67.01 (C-6), and 54.98 (C-2).

p-*Trifluoroacetamidophenyl 2-acetamido-2-deoxy-6*-O-α-L-*fucopyranosyl-β*-D-*glucopyranoside* (**18**). — A suspension of Adams's catalyst (0.12 g) in a solution of **16α** and **16β** in ethyl acetate (50 mL) was stirred under hydrogen at atmospheric pressure. When uptake had ceased, pyridine (4 mL) and trifluoroacetic anhydride (1.6 mL) were added, and the tightly stoppered flask was kept for 30 min at 60°. The flask was cooled, the solids were removed by filtration, and the filtrate was concentrated to an oil which was dissolved in toluene, washed thrice with water and dried (Na_2SO_4). This solution was concentrated to a residue which was purified by chromatography on a column of silica gel with 1:1 toluene–ethyl acetate, to yield chromatographically homogeneous (but unresolved) **17α** and **17β** (0.38 g). This material was *O*-debenzoylated as described for **12**, and the product was repurified by chromatography on a

column of silica gel with 5:1 chloroform–methanol, to afford 0.34 g of the debenzoylated anomers. Reductive debenzylation of this material was achieved by hydrogenation of its solution in ethanol (40 mL), using 10% palladium-on-charcoal (0.24 g) as the catalyst. After the usual work-up, the crude product was purified by chromatography on a column of silica gel with 3:2:1 ethyl acetate–2-propanol–water as the eluant. Separation was effected by carefully monitoring the eluate, and pure, crystalline **18** (as shown by ^{13}C-n.m.r. spectroscopy) was obtained (92 mg, 30%), m.p. 227–229° (dec.), $[\alpha]_{578}^{23}$ −60.2° (*c* 1.7, H_2O); ^{1}H-n.m.r. data (99.59 MHz, D_2O): δ 7.19 and 6.87 (both 2 H each, AB, $J_{H,H}$ 9 Hz, *p*-trifluoroacetamidophenyl group), 5.08 (d, 1 H, $J_{1,2}$ 8 Hz, GlcNAc H-1), 4.84 (d, 1 H, $J_{1,2}$ 3 Hz, Fuc H-1), 1.79 (s, 3 H, *N*-acetyl CH_3), and 0.98 (d, 3 H, $J_{5,6}$ 6 Hz, Fuc H-6); ^{13}C-n.m.r. data (25.05 MHz, D_2O), fucosyl group: δ 101.91 (C-1), 74.37 (C-4), 72.13 (C-3), 70.77 (C-2), 69.74 (C-5, or GlcNAc C-6), and 17.84 (C-6); 2-acetamido-2-deoxy-D-glucosyl residue: δ 102.55 (C-1), 77.69 (C-5), 76.13 (C-3), 72.43 (C-4), 69.16 (C-6, or Fuc C-5), and 58.15 (C-2).

Anal. Calc for $C_{22}H_{29}F_3N_2O_{11} \cdot H_2O$: C, 44.80; H, 5.63; N, 4.62. Found: C, 45.10; H, 5.39; N, 4.72.

ACKNOWLEDGMENT

We thank the Section on Microanalytical Services for performing the elemental analyses.

REFERENCES

1 S.-I. Hakomori, *Annu. Rev. Biochem.*, 50 (1981) 733–764.
2 W. W. Young and S.-I. Hakomori, *Science*, 211 (1981) 487–489.
3 C. P. J. Glaudemans and L. M. Schwartz (Ed.), *Compendium of Immunology*, Vol. IV, Van Nostrand, New York, in press.
4 G. Köhler and C. Milstein, *Nature*, 256 (1975) 495–496.
5 H. Kaprowski, M. Herlyn, and Z. Steplewski, *Science*, 212 (1981) 53–55.
6 (a) D. H. Buss and I. J. Goldstein, *J. Chem. Soc., C*, (1968) 1457–1461; (b) C. R. McBroom, C. H. Samanen, and I. J. Goldstein, *Methods Enzymol.*, 28 B (1972) 213–219.
7 K. Yamamoto, *Bull. Chem. Soc. Jpn.*, 46 (1973) 658–659.
8 J.-C. Jacquinet and P. Sinaÿ, *Carbohydr. Res.*, 46 (1976) 138–142.
9 P. J. Garegg and T. Norberg, *Acta Chem. Scand., Ser. B*, 33 (1979) 116–118, and references cited therein.
10 G. Ekborg, P. J. Garegg, and B. Gotthammar, *Acta Chem. Scand., Ser. B*, 29 (1975) 765–771.
11 B. Helferich and J. Zirner, *Chem. Ber.*, 95 (1962) 2604–2611.
12 R. U. Lemieux, T. Takeda, and B. Y. Chung, *ACS Symp. Ser.*, 39 (1973) 90–115.
13 T. J. Williams, L. D. Homer, J. A. Shafer, I. J. Goldstein, P. J. Garegg, H. Hultberg, T. Iversen, and R. Johansson, *Arch. Biochem. Biophys.*, 209 (1981) 555–564.
14 C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.*, 28 (1963) 3004–3006.
15 R. U. Lemieux, K. B. Hendriks, R. V. Stick, and K. James, *J. Am. Chem. Soc.*, 97 (1975) 4056–5062.
16 R. U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, 97 (1975) 4063–4069.
17 C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, 87 (1965) 2456–2461.
18 R. U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, 97 (1975) 4069–4075.
19 R. U. Lemieux, D. R. Bundle, and D. A. Baker, *J. Am. Chem. Soc.*, 97 (1975) 4076–4083.
20 W. W. Zorbach and T. A. Payne, *J. Am. Chem. Soc.*, 80 (1958) 5564–5567.
21 C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.*, 36 (1971) 3598–3603.
22 M. Dejter-Juszynski and H. M. Flowers, *Carbohydr. Res.*, 18 (1971) 219–226.