Carbohydrate Research, 169 (1987) 201–212
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF *O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→3)-*O*-β-D-GALACTOPYRANOSYL-(1→4)-2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE AND TWO RELATED TRISACCHARIDES CONTAINING THE "LACTO-*N*-BIOSE II" UNIT*

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(Received December 29th, 1986; accepted for publication in revised form, June 15th, 1987)

ABSTRACT

Condensation of 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) with benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside in boiling benzene and in the presence of mercuric cyanide afforded a trisaccharide that was *O*-deacetylated to give benzyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside, the benzyl groups of which were cleaved by catalytic hydrogenolysis to furnish the title trisaccharide. Alternatively, bromide **1** was allowed to react with 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose in 1:1 benzene–nitromethane in the presence of mercuric cyanide, followed by *O*-deacetylation, column chromatography, and reacetylation to give *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose. Acetolysis, followed by catalytic hydrogenation and subsequent *O*-deacetylation furnished the title trisaccharide. A similar condensation of bromide **1** with 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside and 2-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-galactopyranoside produced two trisaccharide derivatives, the acetal groups of which were cleaved in hot, 60% aqueous acetic acid, and the resulting diol intermediates *O*-deacetylated to furnish the desired trisaccharides 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside and 2-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside.

*Dedicated to Dr. R. Stuart Tipson. Synthetic Studies in Carbohydrates, Part LIII. For Part LII, see ref. 1. Presented, in part, at the 13th International Carbohydrate Symposium, Cornell, Ithaca, New York, August 10–15, 1986. This investigation was supported by PHS Grants No. CA-35329 and CA 36021, awarded by the National Cancer Institute, DHHS.

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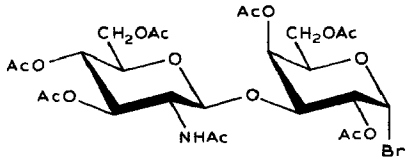

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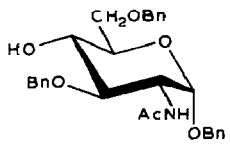

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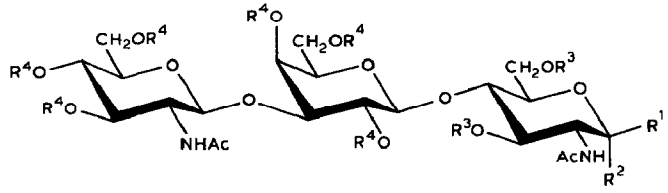


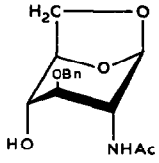
3 $R^1 = H, R^2 = OBn, R^3 = Bn, R^4 = Ac$
4 $R^1 = R^4 = H, R^2 = OBn, R^3 = Bn$
5 $R^1, R^2 = OH, H, R^3 = R^4 = H$

INTRODUCTION

For the past few years, our group has been actively engaged in the synthesis of some oligosaccharides that are primarily intended for use in studies related to glycosidases and glycosyltransferases. More recently, we extended our efforts to encompass the synthesis of a variety of oligosaccharides that are amenable to further manipulation to produce artificial or synthetic antigens for use as immunogens. Our interest in this latter aspect of our work was, to a large extent, enhanced by the fact that a number of carbohydrate structures are increasingly being recognized as tumor-associated antigens. Thus, in furtherance of both our biochemical and immunological studies, we herein describe the synthesis of three trisaccharides, namely, *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose, 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside, and 2-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**5**), (**14**)*, and (**18**)*, respectively.

It is noteworthy that the carbohydrate sequence β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-GlcNAc occurs as part of the polylactosamine backbone of the type II chain of glycoconjugates[2]. In recent studies, it was shown that certain human cancers exhibit an unusually high accumulation of polyfucosylated, repeated *N*-acetyllactosamine chains[3–6]. The fucosyl groups of such polylactosamine chains

*The choice of the nitrophenyl glycoside (*i.e.*, whether 2- or 4-substituted) was dictated only by the availability of the starting intermediates.



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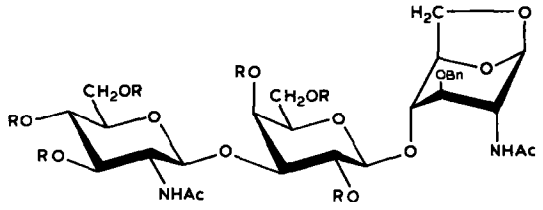
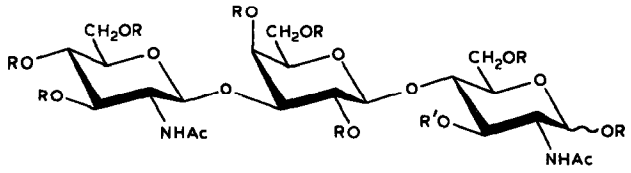


7 R = H
8 R = Ac



9 R = Ac, R′ = Bn
10 R = Ac, R′ = H

were found to reside at O-3 of the 2-acetamido-2-deoxy-D-glucopyranosyl residues, thereby giving rise to the X as well as to the di- or trimeric X determinants. An α-L-(1→3)-fucosyltransferase has been invoked as being responsible for the biosynthesis of such polyfucosylated structures[4]. Furthermore, we have recently observed the presence of an α-L-(1→3)-fucosyltransferase capable of transferring L-fucose from GDP-L-fucose to O-3 of the 2-acetamido-2-deoxy-β-D-glucopyranosyl group of disaccharide β-D-Glc*p*NAc-(1→3)-β-D-Gal-(1→OMe that we have synthesized[7]. An enzyme activity that incorporates L-fucose into 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside has also been reported[8].

Thus, for a better understanding of the biosynthetic pathways of these polyfucosylated polylactosamine structures, it was of interest to synthesize trisaccharide **5** and utilize it as an acceptor-substrate for α-L-(1→3)-fucosyltransferase. It might be reasonable to anticipate that an L-fucosyl group could be incorporated either into O-3 of the terminal 2-acetamido-2-deoxy-β-D-glucopyranosyl group or into O-3 of the reducing 2-acetamido-2-deoxy-D-glucopyranose residue of the trisaccharide, β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-GlcNAc, thus giving rise either to the tetrasaccharide, α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-GlcNAc, or to the alternative, isomeric tetrasaccharide, β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-GlcNAc, respectively. It could also be possible that, with a prolonged incubation period, two such L-fucosyl groups might be incorporated into both positions, with the end product being the pentasaccharide, α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-GlcNAc.

Finally, although trisaccharides **14** and **18** can be expected to be useful in specificity studies of α-L-fucosyltransferases, we chose to synthesize both com-

11

12 $R^1, R^1 = CHC_6H_4OMe\text{-}4, R^2 = Ac$
13 $R^1 = H, R^2 = Ac$
14 $R^1 = R^2 = H$

15

16 $R^1, R^1 = CHC_6H_4OMe\text{-}4, R^2 = Ac$
17 $R^1 = H, R^2 = Ac$
18 $R^1 = R^2 = H$

pounds as their corresponding nitrophenyl glycosides. This choice was dictated by a desire to further employ them as synthetic antigens, after reduction of their nitro groups and subsequent coupling of the resulting amino groups (as their diazonium salts) to a protein.

RESULTS AND DISCUSSIONS

We have previously described the synthesis and use[9–11] (as a glycosyl donor) of the disaccharide bromide 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**1**). As a further illustration of the versatility of **1** for higher oligosaccharide synthesis, we herein describe the syntheses of the three trisaccharides, **5**, **14**, and **18**.

For the synthesis of the trisaccharide *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**5**), two glycosyl acceptors, namely, benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[12] (**2**) and 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose[13] (**6**), were found to be equally useful as intermediates. However, under the reaction conditions employed, condensation of bromide **1** with compound **6** gave a better yield than that with **2**. Nonetheless, the preparation of **2** is by far simpler, and better yielding than that of **6**.

Thus, when **2** was allowed to react with bromide **1** in boiling benzene, in the presence of mercuric cyanide, the fully protected trisaccharide derivative, benzyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**3**), was obtained in ~35% yield after column chromatographic purification. The ^{1}H-n.m.r. spectrum of analytically pure, amorphous **3** showed signals in support of the overall structure expected. *O*-Deacetylation of **3** in methanolic sodium methoxide gave, in 83% yield, benzyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**4**), the ^{13}C-n.m.r. spectrum of which was in agreement with the structure expected (see Table I). Catalytic hydrogenolysis of the benzyl groups of **4** then furnished the desired trisaccharide **5**.

Alternatively, 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (**6**) was glycosylated with bromide **1** in 1:1 benzene–nitromethane in the presence of mercuric cyanide. After the usual processing, examination of the crude mixture by t.l.c. (solvent *B*) revealed that the product was contaminated with a marginally slower-migrating impurity. It was, therefore, directly *O*-deacetylated, and the resulting mixture separated by column chromatography to afford intermediate **7**, which was reacetylated in 2:1 pyridine–acetic anhydride to furnish, in ~71% yield (based on **6**), *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (**8**). Acetolysis of **8** with acetic anhydride–acetic acid–sulfuric acid gave, in 73% yield, the amorphous trisacchar-

TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS[a]

Residue	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*NAc*
Benzyl 3,6-dibenzyl-α-D-Glc*p*NAc	**4**	96.10	52.12	77.70	76.76	70.44	66.28	22.35
β-D-Gal*p*-(1→4)		102.65	69.97	82.12	68.23	74.13	60.84	
β-D-Glc*p*NAc-(1→3)		102.06	56.21	74.72	70.35	76.22	59.48	22.98
GlcNAc	**5**	90.16	53.69	69.52	81.42	68.33	60.09	22.45
β-D-Gal*p*-(1→4)		103.48	69.66	81.60	67.02	75.11	60.76	
β-D-Glc*p*NAc-(1→3)		101.92	56.08	74.19	70.31	76.67	60.09	22.91
4-Nitrophenyl β-D-Glc*p*NAc	**14**	97.47	53.77	83.63	68.02	76.53	60.12	22.86
β-D-Gal*p*-(1→3)		103.22	69.29	81.54	67.05	75.20	60.77	
β-D-Glc*p*NAc-(1→3)		101.78	56.26	73.92	70.28	76.53	60.38	22.96
2-Nitrophenyl α-D-Gal*p*NAc	**18**	97.18	48.10	76.13	66.63	72.82	60.07	22.47
β-D-Gal*p*-(1→3)		103.49	69.68	81.87	66.85	74.82	60.76	
β-D-Glc*p*NAc-(1→3)		101.63	56.43	73.99	70.32	76.59	60.07	23.00

[a]For solutions in $(CD_3)_2SO$ with Me_4Si as the internal standard. Carbonyl, aromatic, and $C_6H_5CH_2$ carbon resonances are not shown.

ide derivative **9**, the ^{1}H-n.m.r. spectrum of which exhibited two additional, acetyl group methyl proton signals, thereby confirming the cleavage of the 1,6-anhydro ring. On hydrogenolysis in glacial acetic acid, and in the presence of 10% palladium-on-carbon, **9** provided the trisaccharide derivative **10**, which was then *O*-deacetylated to afford, also, the title trisaccharide **5**.

Glycosylation of 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside[16] (**11**) with bromide **1** in a manner analogous to that described for the reaction of **1** with **6**, but at 50°, followed by column chromatographic purification, gave the fully protected trisaccharide **12** in ~67% yield. A similar reaction of bromide **1** with 2-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-galactopyranoside[17] (**15**) afforded, in 78% yield, amorphous **16**. The ^{1}H-n.m.r. spectra of both **12** and **16** contained signals supporting the overall structures designated (see Experimental section).

Cleavage of the 4-methoxybenzylidene acetal groups of **12** and **16**, in hot, 60% aqueous acetic acid then gave diols **13** and **17**, respectively. The complete removal of the acetal groups from both compounds was evidenced by the absence, in the ^{1}H-n.m.r. spectra, of the two singlets in the vicinity of δ 5.50 and 3.80, attributable to the acetal methine and methoxyl groups, respectively, of **12** and **16**. The absence of four aromatic proton resonances in the ^{1}H-n.m.r. spectra of both **13** and **17** was, also, consistent with the cleavage of the acetal groups.

The title trisaccharides **14** and **18** were obtained by Zemplén transesterification of **13** and **17**, respectively, in 0.5M methanolic sodium methoxide. It might be pertinent to note that a higher concentration of methoxide ion was required to

ensure complete deesterification. A similar resistance to complete transesterification was previously encountered in the case of other, related oligosaccharides, and it was often necessary to resort to more drastic conditions to achieve complete deesterification[10,14,15]. The ^{13}C-n.m.r. spectra of trisaccharides **5**, **14**, and **18** were all in good agreement with the structures assigned (see Table I).

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at 25–27° with a Perkin–Elmer 241 polarimeter. T.l.c. was conducted on aluminum sheets, precoated with 0.2-mm layers of Silica Gel $60F_{254}$ (E. Merck, Darmstadt, Germany). The components were located either by exposure to u.v. light or by spraying the plates with 5% H_2SO_4 in ethanol and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). The following solvent systems (v/v) were used for chromatography: (*A*) 2:1 chloroform–acetone; (*B*) 3:1 chloroform–acetone; (*C*) 5:1 chloroform–acetone; (*D*) 19:1 chloroform–acetone; and (*E*), 3:2:2 ethyl acetate–2-propanol–water. N.m.r. spectra were recorded at ~25° and, unless otherwise indicated, ^{1}H-n.m.r. spectra with a Varian EM-390 instrument and ^{13}C-n.m.r. spectra with a Varian XL-100 instrument at 25.2 MHz in the F.t. mode; the positions of the peaks (δ) are indicated from the Me_4Si signal. Organic solutions were generally dried with anhydrous Na_2SO_4. Nitromethane was distilled from P_2O_5 immediately before being used, and benzene was dried with Na. Elemental analyses were performed by Robertson Laboratory, Madison, New Jersey, U.S.A., or by Galbraith Laboratories, Inc., Knoxville, Tennessee, U.S.A.

Benzyl O-*(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**3**). — A stirred solution of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[12] (**2**; 1 g, 2 mmol) in dry benzene (80 mL) was boiled until ~20 mL of the solvent had distilled off. After cooling, a solution of 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide[9] (**1**; 2.5 g, 3.6 mmol) and $Hg(CN)_2$ (0.9 g, 3.6 mmol) was added, and the mixture was boiled, with stirring, for 16 h. T.l.c. (solvent *B*) then revealed the presence of a major product, slower-migrating than **2**; a small proportion of **2** and a slower-migrating contaminant (presumably resulting from the decomposition of **1**) were also revealed in t.l.c. The mixture was diluted with an equal volume of benzene, the solution successively washed with water, M KI solution, aqueous $NaHCO_3$, and water, dried, and concentrated. The crude product mixture was purified in a column of silica gel using solvent *C* as the eluent. The first fractions that emerged from the column contained **2** (0.3 g), and the next fraction to be eluted (0.1 g) contained mainly (t.l.c., solvent *B*) the major product, together with a slightly faster-migrating contaminant. On evaporation of the solvent, the fractions corresponding to the product gave a solid residue, which

was dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the precipitation of **3** (0.8 g, 35%); amorphous, $[\alpha]_D^{25}$ +68° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.20 (m, 15 H, arom.), and 2.20–1.65 (cluster of s, 24 H, 6 OAc and 2 NAc).

Anal. Calc. for $C_{55}H_{68}N_2O_{22}$: C, 59.55; H, 6.19; N, 2.53. Found: C, 59.33; H, 5.92; N, 2.43.

*Benzyl O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-O-β-*D*-galacto-pyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-α-*D*-glucopyranoside* (**4**). — Compound **3** (0.55 g) in 0.2M methanolic sodium methoxide (25 mL) was stirred for 16 h at room temperature. T.l.c. (4:1 chloroform–methanol) then showed the disappearance of **3** and the presence of a single product, slower migrating than **3**. The base was neutralized by the dropwise addition of glacial acetic acid, the solvent evaporated under diminished pressure, and the residue dissolved in a fresh portion of methanol. The solution was de-ionized with Amberlite IR-120 (H^+) cation-exchange resin, and the resin filtered off and thoroughly washed with methanol. The methanol was evaporated and the residue redissolved in ethanol. Addition of ether caused the precipitation of **4** (0.35 g, 83%); amorphous, $[\alpha]_D^{25}$ +76° (*c* 0.4, 3:2 chloroform–methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.20 (m, 15 H, arom. and 1.90 (s, 6 H, 2 NAc); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{43}H_{56}N_2O_{16}\cdot H_2O$: C, 59.01; H, 6.46; N, 3.20. Found: C, 59.11; H, 6.62; N, 3.06.

*O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-β-*D*-glucopyranose* (**8**). — A stirred solution of 2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose[13] (**6**; 0.54 g, 1.84 mmol) in 1:1 benzene–nitromethane (140 mL) was boiled until ~70 mL of the solvent had distilled off. After cooling to room temperature, disaccharide bromide **1** (1.75 g, 2.50 mmol), and $Hg(CN)_2$ (0.63 g, 2.50 mmol) were added, and stirring was continued overnight at room temperature. T.l.c. (solvent *B*) revealed the presence of a product, slightly slower-migrating than **6**; a small proportion of **6** was also revealed in t.l.c. More portions of bromide **1** (1.0 g, 1.43 mmol) and $Hg(CN)_2$ (0.36 g, 1.43 mmol) were added and stirring was continued overnight at room temperature. The mixture was then diluted with benzene and processed as described for **2** (to give **3**), to afford a solid residue (~1.6 g), which contained (t.l.c., solvent *B*) the product, as well as a marginally slower-migrating contaminant. This crude product was taken up in methanol (50 mL) containing M sodium methoxide in methanol (1 mL), and stirred for 4 h at room temperature. The base was neutralized with a few drops of glacial acetic acid, the solution evaporated to dryness, the residue dissolved in methanol, and the solution de-ionized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off and washed with methanol, and the filtrate and washings were combined and concentrated to a small volume, and the concentrate was applied to a column of silica gel. On elution with 13:6:1 (v/v) chloroform–methanol–water, evaporation of the fractions corresponding to the product af-

forded **7** (0.86 g) as a white amorphous solid, which was thoroughly dried, taken up in a mixture of acetic anhydride (20 mL) and pyridine (40 mL), and stirred overnight at room temperature. Acetic anhydride and pyridine were evaporated under diminished pressure, the last traces being removed by coevaporation with several portions of toluene, and the residue was dissolved in chloroform and applied to a short column of silica gel. Elution with solvent *A* and evaporation of the fractions corresponding to the acetylated product gave **8** (1.2 g, 71%, based on **6**); amorphous, $[\alpha]_D^{26}$ −37° (*c* 0.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30 (s, 5 H, arom.) and 2.20–1.66 (cluster of s, 24 H, 6 OAc and 2 NAc).

Anal. Calc. for $C_{41}H_{54}N_2O_{21}$: C, 54.05; H, 5.99; N, 3.08. Found: C, 53.93; H, 6.01; N, 2.88.

O-*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-1,6-di-*O*-acetyl-3-*O*-benzyl-*D*-glucopyranose* (**9**). — Compound **8** (0.5 g) was dissolved in a precooled mixture of 70:30:1 (v/v) acetic anhydride–acetic acid–conc. H_2SO_4 (25 mL), and the mixture stirred for 15 min at ~0° (bath). It was then allowed to gradually warm up to room temperature, and the stirring was continued for an additional 3 h. After neutralization with saturated aqueous sodium acetate, the mixture was diluted with dichloromethane (100 mL), successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated. The concentrate was applied to a column of silica gel and eluted with solvent *D* to afford **9** (0.4 g, 73%); amorphous, $[\alpha]_D^{26}$ +63° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.23 (s, 5 H, arom.) and 2.06–1.56 (cluster of s, 30 H, 8 OAc and 2 NAc).

Anal. Calc. for $C_{45}H_{60}N_2O_{24}$: C, 53.56; H, 5.93; N, 2.77. Found: C, 53.12; H, 6.19; N, 2.59.

O-*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-1,6-di-*O*-acetyl-2-deoxy-*D*-glucopyranose* (**10**). — A mixture of **9** (0.5 g) and 10% Pd–C (0.5 g) in glacial acetic acid (30 mL) was shaken under H_2 at ~345 kPa for 16 h at room temperature. The suspension was filtered through a bed of Celite, the solid thoroughly washed with glacial acetic acid, and the filtrate and washings combined and evaporated. The residue so obtained was subjected to column chromatography on silica gel with solvent *D* as the eluent to give **10** (0.4 g, 89%); amorphous, $[\alpha]_D^{27}$ +69° (*c* 0.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 2.20–1.80 (cluster of s, 30 H, 8 OAc and 2 NAc).

Anal. Calc. for $C_{38}H_{54}N_2O_{24}$: C, 49.46; H, 5.86; N, 3.04. Found: C, 49.29; H, 6.22; N, 2.91.

O-*(2-Acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-*O*-β-*D*-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*D*-glucopyranose* (**5**). — *From* **10**. A solution of **10** (0.37 g) in methanol (50 mL) was treated with M sodium methoxide (5 mL), and the mixture stirred overnight at room temperature. The mixture was then diluted with methanol containing a little water to remove some turbidity, and de-ionized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off (a

ethyl acetate. Addition of ether–hexane caused the precipitation of **13** (0.63 g, 94%); amorphous, $[\alpha]_D^{26}$ +17° (*c* 0.6, methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 8.13 and 7.03 (2 × 2 H, *J* 10 Hz, arom.), and 2.20–1.70 (cluster of s, 24 h, 6 OAc and 2 NAc).

Anal. Calc. for $C_{40}H_{53}N_3O_{24}$: C, 50.04; H, 5.58; N, 4.38. Found: C, 49.91; H, 5.73; N, 4.35.

4-Nitrophenyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**14**). — Compound **13** (0.45 g) was suspended in 0.5M methanolic sodium methoxide (25 mL). The suspension gradually dissolved, and in ~1 h crystallization ensued. The mixture was stirred overnight at room temperature, the base neutralized by the addition of a few drops of glacial acetic acid, and the solid material filtered off and thoroughly washed with cold ethanol to furnish **14** (0.3 g, 90%); amorphous, $[\alpha]_D^{25}$ +5.5° (*c* 0.8, dimethylsulfoxide); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{28}H_{41}N_3O_{18}$: C, 47.52; H, 5.85; N, 5.94. Found: C, 47.56; H, 5.97; N, 5.64.

2-Nitrophenyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-O-(p-methoxybenzylidene)-α-D-galactopyranoside (**16**). — 2-Nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-galactopyranoside[17] (**15**; 0.42 g, 0.9 mmol) was allowed to react with bromide **1** (1.07 g, 1.5 mmol) in 1:1 benzene–nitromethane in the presence of $Hg(CN)_2$ (0.38 g, 1.5 mmol), in a manner analogous to that described for **11** (to give **12**). Column-chromatographic purification of the product mixture on silica gel using solvent *C* as the eluent gave a solid residue which was dissolved in ethyl acetate. Addition of ether–hexane caused the precipitation of **16** (0.79 g, 78%); amorphous, $[\alpha]_D^{25}$ +72° (*c* 1.0, chloroform); ^{1}H-n.m.r. (100 MHz; $CDCl_3$): δ 8.00–6.85 (8 H, arom.), 5.54 (s, 1 H, $MeOC_6H_4CH$), 3.84 (s, 3 H, OMe), and 2.24–1.78 (cluster of s, 24 H, 6 OAc and 2 NAc).

Anal. Calc. for $C_{48}H_{59}N_3O_{25}$: C, 53.48; H, 5.52; N, 3.90. Found: C, 53.65; H, 5.61; N, 3.76.

2-Nitrophenyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**17**). — The acetal group of **16** (0.72 g) was cleaved in hot, 60% aqueous acetic acid as described for **12** (to give **13**), to give a solid residue, which was dissolved in a small volume of methanol. Addition of ether caused the precipitation of **17** (0.55 g, 86%); amorphous, $[\alpha]_D^{27}$ +53° (*c* 1.0, chloroform); ^{1}H-n.m.r. (100 MHz; $CDCl_3$): δ 8.00–7.10 (4 H, arom.), and 2.20–1.70 (cluster of s, 24 H, 6 OAc and 2 NAc).

Anal. Calc. for $C_{40}H_{53}N_3O_{24}\cdot H_2O$: C, 49.13; H, 5.66; N, 4.29. Found: C, 48.81; H, 5.56; N, 4.10.

2-Nitrophenyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**18**). — Compound **17** (0.31 g) was *O*-deacetylated in 0.5M methanolic sodium methoxide as described for **13** (to give **14**) to furnish amorphous trisaccharide **18** (0.15 g, 68%); $[\alpha]_D^{27}$ −133° (*c* 0.8, dimethyl sulfoxide); ^{13}C-n.m.r. see Table I.

Anal. Calc. for $C_{28}H_{41}N_3O_{18}\cdot H_2O$: C, 46.34; H, 5.98; N, 5.79. Found: C, 46.56; H, 6.30; N, 5.59.

ACKNOWLEDGMENTS

The authors are grateful to Mr. Conrad F. Piskorz for his valuable technical assistance, to Mrs. Onda D. Simmons for recording most of the n.m.r. spectra, and to Ms. Carol Wilczak for kindly typing the manuscript.

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