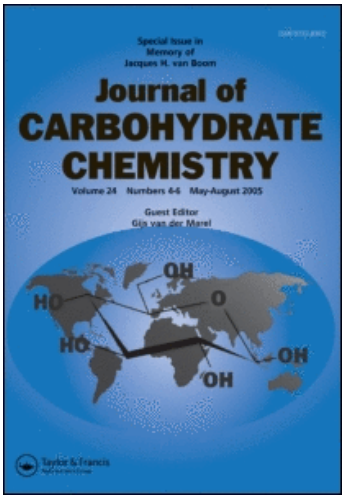

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Synthetic Mucin Fragments: Methyl-3-O-(2-Acetamido-2-Deoxy-4-O- and 6-O-β-D-Galactopyranosyl-β-D-Glucopyranosyl)-β-D-Galactopyranoside and Methyl 3-O-(2-Acetamido-2-Deoxy-4-O- and 3-O-Methyl-β-D-Glucopyranosyl-3-D-Galactopyranoside

Pakesh K. Jain[a]; Saeed A. Abbas[a]; Khushi L. Matta[a]
[a] Department of Gynecologic Oncology, Roswell Park Memorial Institute New York State Hepartment of Health, Buffalo, New York, U.S.A.

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SYNTHETIC MUCIN FRAGMENTS: METHYL-3-O-(2-ACETAMIDO-2-DEOXY-4-O- AND 6-O-β-D-GALACTOPYRANOSYL-β-D-GLUCOPYRANOSYL)-β-D-GALACTOPYRANOSIDE AND METHYL 3-O-(2-ACETAMIDO-2-DEOXY-4-O- AND 3-O-METHYL-β-D-GLUCOPYRANOSYL-β-D-GALACTOPYRANOSIDE[1]

Rakesh K. Jain, Saeed A. Abbas, and Khushi L. Matta*

Department of Gynecologic Oncology
Roswell Park Memorial Institute
New York State Department of Health
Buffalo, New York 14263 (U.S.A.)

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ABSTRACT

Glycosylation of methyl 3-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (2) with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (1), catalyzed by mercuric cyanide, afforded a trisaccharide derivative, which was not separated, but directly O-deacetylated to give methyl 3-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (8). Hydrogenolysis of the benzyl groups of 8 then furnished the title trisaccharide (9). A similar glycosylation of methyl 3-O-(2-acetamido-3-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (obtained by acetylation of 4, followed by hydrolysis of the benzylidene acetal group) with bromide 1 gave a tribenzyl trisaccharide, which, on catalytic hydrogenolysis, furnished the isomeric trisaccharide (12). Methylation of 4 and 2 with methyl iodide-silver oxide in 1:1 dichloromethane-N,N-dimethylformamide gave the 3-O- and 4-O-monomethyl ethers (13) and (15), respectively. Hydrogenolysis of the benzyl groups of 13 and 15 then provided the title monomethylated disaccharides (14) and (16), respectively. The structures of trisaccharides 9 and 12, and disaccharides 14 and 16 were all established by ^{13}C NMR spectroscopy.

INTRODUCTION

For the past several years, we have been actively engaged in the synthesis of some mucin-type oligosaccharide fragments that could effectively be utilized in studies related to glycosyltransferases, serving either as acceptor-substrates or as reference compounds.[2] Moreover, on being linked to appropriate aglycons, some of these oligosaccharide fragments could readily be derivatized to serve as synthetic or artificial antigens.[3]

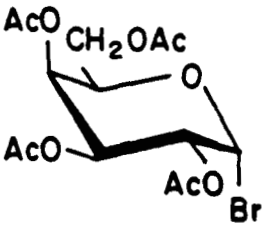
Of particular interest to those syntheses was the procurement of certain compounds that can be employed in the study of galactosyl transferases. This class of enzymes has been enthusiastically studied for some time because they may serve as good markers for a number of malignancies.[4] It thus seemed of interest to characterize the enzyme(s) that catalyze the introduction of the various galactosylic linkages. In this regard, we have recently reported the characterization of an enzyme that catalyzes the incorporation of D-galactose in a β-(1→3)-linkage into 2-acetamido-2-deoxy-D-glucopyranose (D-GlcNAc) in ascites from ovarian cancer patients.[5] The synthetic disaccharide β-D-GlcpNAc-(1→3)-β-D-Galp-1→OR (R = methyl or p-nitrophenyl) was employed as an acceptor-substrate for the characterization of this enzyme. Thus, in an effort to obtain the desired reference compounds, we synthesized three trisaccharides which were expected to form on incorporation of a β-D-galactopyranosyl group into the 2-acetamido-2-deoxy-D-glucopyranosyl residue of the aforementioned disaccharide (R = Me). These are: β-D-Galp-(1→3)-β-D-GlcpNAc-(1→3)-β-D Galp-1→OMe; β-D-Galp-(1→4)-β-D-GlcpNAc-(1→3)-β-D-Galp-1→OMe; and β-D-Galp-(1→6)-β-D-GlcpNAc-(1→3)-β-D-Galp-1→OMe. We have previously described the synthesis of the first one of these trisaccharides,[6] and we herein describe the synthesis of the latter two isomeric trisaccharides. In addition, we also report the synthesis of two monomethylated derivatives of the disaccharide β-D-GlcpNAc-(1→3)-β-D-Galp-1→OMe, which are to be used as modified substrates in the study of this class of enzymes. As we have previously argued, such modified compounds would be expected to act as substrates for a unique and single enzyme, despite the presence of other, related enzymes.[7]

RESULTS AND DISCUSSION

Glycosylation of methyl 3-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside[8] (2) with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (1) in 1:1 benzene-nitromethane and in the presence of powdered $Hg(CN)_2$, followed by O-deacetylation of the product mixture (containing trisaccharide derivative 7) with 0.05 M methanolic sodium methoxide afforded, in 67% overall yield, trisaccharide derivative 8. Catalytic hydrogenation of 8 in glacial acetic acid and in the presence of 10% Pd-C furnished, in excellent yield, methyl 3-O-(2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside 9.

For the synthesis of the isomeric trisaccharide 12, methyl 3-O-(2-acetamido-3-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (6) was required as a starting material. Compound 6 was readily obtained, in high yield, by acetylation of 4 (ref 6) in 1:2 acetic anhydride-pyridine, followed by hydrolysis of the benzylidene acetal group of the intermediate 5. On glycosylation with bromide 1, compound 6 afforded the trisaccharide derivative 10, which was not isolated, but directly O-deacetylated to give, in 72% yield, the tribenzyl trisaccharide 11. Hydrogenolysis of the benzyl groups of 11 then furnished methyl 3-O-(2-acetamido-2-deoxy-6-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside 12. The ^{13}C NMR spectra of both 9 and 12 were in agreement with the structures assigned (see Table 1).

In an earlier attempt to synthesize trisaccharide 12, we chose to utilize the readily accessible methyl 3-O-(2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside[8] (3). However, instead of the desired 12, a product mixture, which appeared to be homogeneous by TLC (solvent B), was isolated after removal of the protecting groups. As it could be gathered from its ^{13}C NMR spectrum, this mixture contained, in addition to the expected 12, a trisaccharide contaminant, presumably, the β-(1→4)-linked isomer. That the contaminant was a closely related trisaccharide was implied by the presence of only three anomeric carbon-atom resonances at δ 104.23-102.20. Also, rather than a single carbon-atom resonance for C-3 of the methyl



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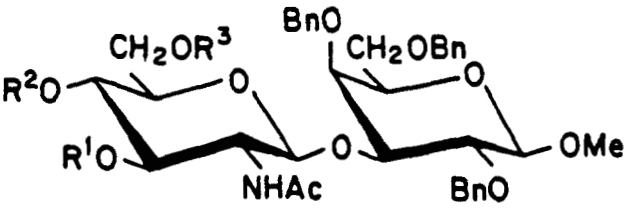
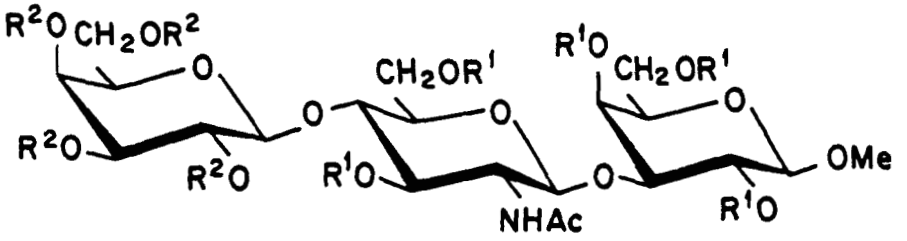
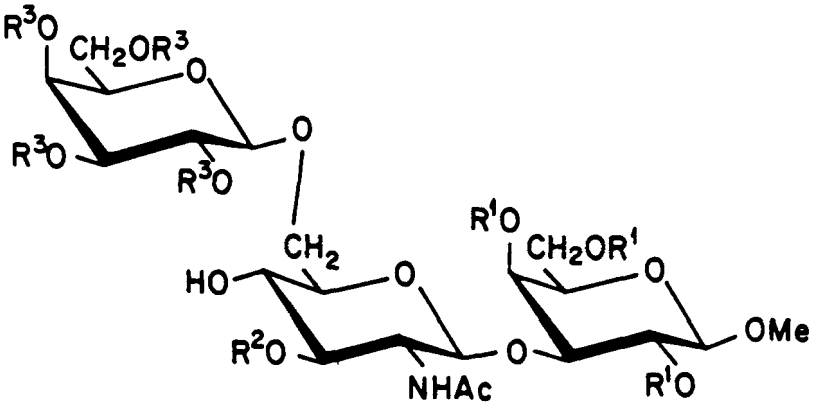


2 $R^1 = R^3 = Bn$, $R^2 = H$
3 $R^1 = Bn$, $R^2 = R^3 = H$
4 $R^1 = H$, R^2, $R^3 = PhCH$
5 $R^1 = Ac$, R^2, $R^3 = PhCH$
6 $R^1 = Ac$, $R^2 = R^3 = H$



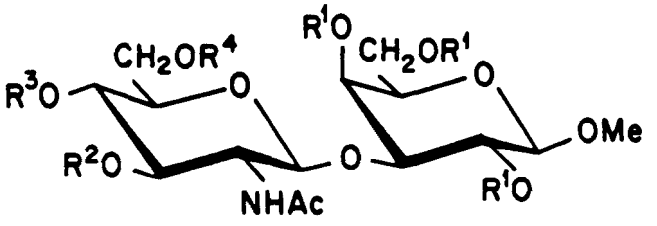
7 $R^1 = Bn$, $R^2 = Ac$
8 $R^1 = Bn$, $R^2 = H$
9 $R^1 = R^2 = H$



10 R^1 = Bn, R^2 = R^3 = Ac
11 R^1 = Bn, R^2 = R^3 = H
12 R^1 = R^2 = R^3 = H

β-galactoside residue of 12, three resonances (δ 82.56, 82.25, and 81.28) were observed, suggesting the presence of two additional interglycosidic-linkages, involving secondary carbon-atoms.

It would thus appear that the presence of a benzyl group at C-3 of the 2-acetamido-2-deoxy-D-glucopyranosyl residue of disaccharide 3 exerted an activation sufficient enough to allow for glycosylation at the normally unreactive hydroxyl group at C-4 of such a compound. By contrast, such activation was practically absent in the presence of an acetyl group at C-3 of disaccharide 6, allowing for an overall good yield of pure 12.



13 R^1 = Bn, R^2 = Me, R^3,R^4 = PhCH
14 R^1 = R^3 = R^4 = H, R^2 = Me
15 R^1 = R^2 = R^4 = Bn, R^3 = Me
16 R^1 = R^2 = R^4 = H, R^3 = Me

Disaccharides 4 and 2 were methylated with methyl iodide-silver oxide, in a 1:1 (v/v) mixture of dichloromethane-N,N-dimethylformamide to afford, in 69 and 74% yields, the 3-O- and 4-O-monomethylated disaccharide derivatives (13) and (15), respectively. The use of such a mixed solvent system in the methylation reaction appeared to be superior to the use of neat N,N-dimethylformamide as solvent; the proportion of faster-migrating (TLC) contaminants (presumably, due to N-alkylation), previously encountered in analogous reactions,[9] was substantially reduced.

Hydrogenolysis of the benzyl groups of 13 and 15 then furnished the desired methyl 3-O-(2-acetamido-2-deoxy-3-O-methyl-β-D-glucopyranosyl)-β-D-galactopyranoside (14) and its 4-O-isomer (16), respectively. The ^{13}C NMR spectra were in full accord with the structures assigned (see Table 1).

EXPERIMENTAL

General Methods. Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured at 25°C with a Perkin-Elmer 241 polarimeter. TLC 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 uv 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). Unless otherwise indicated, the solvent systems used for chromatography were (v/v): (A) 2% acetone in chloroform, (B) 5:4:1 chloroform-methanol-water, and (C) 13:6:1 chloroform-methanol-water. NMR spectra were recorded at ~25°C; ^1H NMR spectra at 90 MHz with a Varian EM-390, and ^{13}C NMR spectra at either 25.2 or 100.6 MHz with a Varian XL-100 or a Bruker AM-400 instrument, respectively. The positions of the peaks (δ) are relative to the Me_4Si signal (δ= 0.0). Organic solutions were generally dried with anhydrous Na_2SO_4. Ag_2O was prepared by the method of Helferich and Klein.[10] Elemental analyses were performed by Robertson Laboratory, 29 Samson Avenue, Madison, New Jersey, U.S.A.

TABLE 1

Proposed ^{13}C NMR Chemical Shifts[a]

Residue or Group	Compound	C-1	C-2	C-3	C-4	C-5	C-6	CH_3CO	OCH_3
β-D-GalpOMe	b	103.79	69.29	82.22	67.02	74.69	60.22		55.56
β-D-GlcpNAc-(1→3)		101.88	56.38	74.22	70.28	76.57	60.77	22.98	
β-D-GalpOMe	9	104.00	69.27	82.39	67.12	74.75	60.30		55.70
β-D-GlcpNAc-(1→3)		102.00	55.46	73.73	81.21	75.48	60.41	23.02	
β-D-Galp-(1→4)		103.92	70.51	71.97	68.09	74.75	60.30		
β-D-GalpOMe	12	103.81	69.39	81.95	67.32	74.62	60.26		55.69
β-D-GlcpNAc-(1→3)		101.92	56.33	74.28	70.69	74.98	68.00	22.98	
β-D-Galp-(1→6)		103.81	70.69	73.12	68.00	75.73	60.36		
β-D-GalpOMe	14	104.03	69.10	82.17	67.12	74.76	60.28		55.70
3-O-Me-β-D-GlcpNAc-(1→3)		101.99	54.50	83.06	69.29	76.52	60.64	22.99	58.60
β-D-GalpOMe	16	103.94	69.35	82.47	67.06	74.76	60.23		55.71
4-O-Me-β-D-GlcpNAc-(1→4)		101.91	56.75	74.15	79.66	75.40	60.44	22.99	59.56

[a] For solutions in DMSO-d_6, with Me_4Si as the internal standard. Carbonyl carbon resonances are not shown. [b] Methyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside. The chemical shifts of the last-mentioned compound are included for comparison.

Methyl 3-O-(2-Acetamido-3-O-acetyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (5). Methyl 3-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside[6] (4; 2.9 g, 3.8 mmol) was kept overnight at room temperature in a mixture of 1:2 (v/v) acetic anhydride-pyridine (60 mL). It was then processed in the usual manner to give a solid residue, which was dissolved in a small volume of ethyl acetate. Addition of ether-hexane caused the precipitation of 5 (3 g, 98%), amorphous, $[\alpha]_D$ -57.8° (c 0.7, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.50-7.20 (m, 20 H, arom), 5.47 (s, 1 H, PhCH), 3.50 (s, 3 H, OMe), 2.00 (s, 3 H, OAc), and 1.57 (s, 3 H, NAc).

Anal. Calc for $C_{45}H_{51}NO_{12}$: C, 67.73; H, 6.46; N, 1.76. Found: C, 67.48; H, 6.27; N, 1.78.

Methyl 3-O-(2-Acetamido-3-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (6). Compound 5 (2.5 g) in 80% aqueous acetic acid (100 mL) was stirred for 1 h at ~80°C. The acetic acid was evaporated under diminished pressure, the last traces being removed by co-evaporation with several added portions of toluene. The residue was purified in a column of silica gel by using ethyl acetate as the eluant, to afford amorphous 6 (2.1 g, 94%); $[\alpha]_D$ -34.8° (c 1.37, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.50-7.20 (m, 15 H, arom), 3.50 (s, 3 H, OMe), 1.97 (s, 3 H, OAc), and 1.55 (s, 3 H, NAc).

Anal. Calc for $C_{38}H_{47}NO_{12}$: C, 64.29; H, 6.69; N, 1.97. Found: C, 64.18; H, 6.72; N, 1.88.

Methyl 3-O-(2-Acetamido-3,6-di-O-benzyl-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (8). A stirred mixture of methyl 3-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside[6] (2; 2.5 g, 2.9 mmol) and powdered $Hg(CN)_2$ (2.3 g, 9 mmol) in 1:1 benzene-nitromethane (350 mL) was boiled until ~100 mL of the solvent had distilled off. The temperature was then adjusted to ~50°C, and a solution of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (1; 3.7 g, 9.0 mmol) in 1:1 benzene-nitromethane (20 mL) was added and the stirring was continued for 26 h at 50°C, more portions of $Hg(CN)_2$ (0.3 g) and bromide 1 (1 g, in 10 mL of 1:1 benzene-nitromethane) were added after 16 h. The mixture was then cooled, diluted with benzene,

successively washed with saturated aqueous $NaHCO_3$, 10% aqueous KI solution and water, dried, and evaporated to a syrup. Examination by TLC (9:1 chloroform–acetone) showed the disappearance of 2 and the presence of a major product, faster-migrating than 2; several slower-migrating, minor contaminants were also revealed in TLC. The crude product mixture (~7 g, containing 7) was thoroughly dried *in vacuo*, dissolved in 0.05 M sodium methoxide in methanol (200 mL), and stirred for 3 h at room temperature. The base was neutralized by the dropwise addition of glacial acetic acid. The solution was evaporated to dryness, and the residue applied to a column of silica gel. On elution with 7% methanol in chloroform, evaporation of the fractions corresponding to product gave amorphous 8 (2 g, 67%), $[\alpha]_D$ +1.6° (c 1.2, chloroform); ^1H NMR ($CDCl_3$): δ 7.00–7.50 (m, 25 H, arom), 3.47 (s, 3 H, OMe), and 1.47 (s, 3 H, NAc).

Anal. Calc for $C_{56}H_{66}NO_{16}$: C, 66.64; H, 6.61; N, 1.39. Found: C, 66.68; H, 6.79; N, 1.58.

Methyl 3-O-(2-Acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-gluco-pyranosyl)-β-D-galactopyranoside (9). A mixture of 8 (1.4 g) and 10% Pd–C (1.4 g) in glacial acetic acid (35 mL) was shaken under H_2 at ~345 kPa for 2 days at room temperature. The suspension was filtered (a bed of Celite) and the solids were thoroughly washed with water. The filtrate and washings were combined and concentrated. The residue so obtained was purified in a column of silica gel by using solvent B as the eluant to afford 9 (0.72 g, 93%), amorphous, $[\alpha]_D$ +4.5° (c 1.3, water); ^{13}C NMR, see Table 1.

Anal. Calc for $C_{21}H_{37}NO_{16}$: C, 45.07; H, 6.68. N, 2.50. Found: C, 44.98; H, 6.52; N, 2.42.

Methyl 3-O-(2-Acetamido-2-deoxy-6-O-β-D-galactopyranosyl-β-D-gluco-pyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (11). Diol 6 (1.05 g, 1.5 mmol) was treated with bromide 1 (0.95 g, 2.3 mmol) in the presence of $Hg(CN)_2$ (0.6 g, 2.4 mmol) in a manner analogous to that described for 2 (to give 7). After the usual processing, the crude product (containing 10) was O-deacetylated in 0.05 M methanolic sodium methoxide (50 mL), and the mixture was purified on a column of silica gel with 4:1 (v/v) chloroform–methanol as the eluant to give 11 (0.9 g, 72%); amorphous; $[\alpha]_D$ −8.65° (c 0.47, methanol).

Anal. Calc for $C_{42}H_{55}NO_{16}.H_2O$: C, 59.48; H, 6.79; N, 1.65. Found: C, 59.25; H, 6.49; N, 1.68.

Methyl 3-O-(2-Acetamido-2-deoxy-6-O-β-D-galactopyranosyl-β-D-glucopyranosyl-β-D-galactopyranoside (12). Compound 11 (0.8 g) was hydrogenolyzed exactly as described for 8 (to give 9) to afford, after column-chromatographic purification with solvent B as the eluant, amorphous 12 (0.43 g, 80%); $[\alpha]_D$ +10.76° (c 1.2, water); ^{13}C NMR, see Table 1.

Anal. Calc for $C_{21}H_{37}NO_{16}$: C, 45.07; H, 6.68; N, 2.50. Found: C, 44.80; H, 6.73; N, 2.39.

Attempted Preparation of 12 from methyl 3-O-(2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (3). Compound 3 (ref 8; 1.8 g, 2.37 mmol) was allowed to react with bromide 1 (1.5 g, 3.6 mmol) in the presence of $Hg(CN)_2$ (0.95 g, 3.8 mmol) in a manner similar to that described for 2 (to give 7). It was then processed, and the crude product mixture O-deacetylated in 0.05 M methanolic sodium methoxide, and purified on a column of silica gel with 9:1 (v/v) chloroform-methanol as the eluant to give an amorphous solid (1.2 g), $[\alpha]_D$ +4.7° (c 1.6, chloroform).

Anal. Calc for $C_{49}H_{61}NO_{16}.H_2O$: C, 62.73; H, 6.78; N, 1.49. Found: C, 62.81; H, 6.58; N, 1.67.

This material (0.8 g) was hydrogenolyzed as just described to give, in ~93% yield, an amorphous solid, having $[\alpha]_D$ +8.8° (c 1.3, water); ^{13}C NMR (DMSO-d_6): δ 104.23 and 102.20 (C-1, C-1", and C-1'), 82.25 (C-3 of 12), 82.56 and 81.28 (presumably, C-3 and C-4' of contaminant).

Methyl 3-O-(2-Acetamido-4,6-O-benzylidene-2-deoxy-3-O-methyl-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (13). To a stirred mixture of methyl 3-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (4; 1 g) and freshly prepared Ag_2O (2 g) in 1:1 (v/v) dichloromethane-N,N-dimethylformamide (60 mL) was added methyl iodide (2 mL), and the mixture stirred in the dark for 4 h at room temperature. The suspension was filtered through a bed of Celite and the solids thoroughly washed with dichloromethane. The filtrate and washings were combined and the solvent evaporated under diminished pressure to give a residue which was taken up in chloroform. The suspended solid was filtered off and

thoroughly washed with chloroform. The filtrate and washings were combined, successively washed with water, dilute $Na_2S_2O_3$, and water; dried, and concentrated to a small volume. The concentrate was applied to a column of silica gel. Elution with solvent A and evaporation of the solvent fraction corresponding to product afforded amorphous 13 (0.7 g, 69%); $[\alpha]_D$ -25.5° (c 0.9, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.20-7.50 (m, 20 H, arom), 5.47 (s, 1 H, PhCH), 3.47 (s, 3 H, OMe), and 1.57 (s, 3 H, NAc).

Anal. Calc for $C_{44}H_{51}NO_{11}$: C, 68.63; H, 6.69; N, 1.82. Found: C, 68.52; H, 6.85; N, 1.73.

Methyl 3-O-(2-Acetamido-2-deoxy-3-O-methyl-β-D-glucopyranosyl)-β-D-galactopyranoside (14). Compound 13 (0.7 g) was hydrogenolyzed as described for 8 (to give 9) to afford, after silica gel column chromatography with solvent C as the eluant, amorphous 14 (0.25 g, 67%); $[\alpha]_D$ -2.7° (c 0.9, water); ^{13}C NMR, see Table 1.

Anal. Calc for $C_{16}H_{29}NO_{11}\cdot H_2O$: C, 44.74; H, 7.29; N, 3.26. Found: C, 44.80; H, 6.96; N, 3.14.

Methyl 3-O-(2-Acetamido-3,6-di-O-benzyl-2-deoxy-4-O-methyl-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (15). Compound 2 (0.8 g) was O-methylated in a manner analogous to that described for 4 (to give 13) and the product mixture purified in a column of silica gel with solvent A, to afford 15 (0.6 g, 74%), amorphous, $[\alpha]_D$ +4.1° (c 0.7, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.20-7.50 (m, 25 H, arom), 3.50 (s, 3 H, OMe), and 1.47 (s, 3 H, NAc).

Anal. Calc for $C_{51}H_{59}NO_{11}$: C, 71.05; H, 6.91; N, 1.63. Found: C, 70.94; H, 7.18; N, 1.51.

Methyl 3-O-(2-Acetamido-2-deoxy-4-O-methyl-β-D-glucopyranosyl)-β-D-galactopyranoside (16). Compound 15 (0.8 g) was hydrogenolyzed exactly as described for 8 (to give 9), and the crude product mixture purified by column chromatography with solvent C as the eluant to afford amorphous 16 (0.25 g, 65%); $[\alpha]_D$ +5.5° (c 0.7, water); ^{13}C NMR, see Table 1.

Anal. Calc for $C_{16}H_{29}NO_{11}\cdot H_2O$: C, 44.74; H, 7.29; N, 3.26. Found: C, 44.97; H, 6.90; N, 2.99.

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