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SYNTHESIS OF *p*-NITROPHENYL 2-ACETAMIDO-2-DEOXY-4-*O*-β-D-GALACTOPYRANOSYL-β-D-GLUCOPYRANOSIDE, AND *p*-NITROPHENYL 6-*O*-(2-ACETAMIDO-2-DEOXY-3-*O*- AND -4-*O*-β-D-GALACTOPYRANOSYL-β-D-GLUCOPYRANOSYL)-α-D-MANNOPYRANOSIDE*

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ABSTRACT

A facile synthesis of *p*-nitrophenyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside was accomplished by saponification of the product obtained by reaction of 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl chloride and Amberlyst A-26 *p*-nitrophenoxide. The reaction of *p*-nitrophenyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**7**) with the easily accessible 2-methyl-[4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline proceeded readily, to give the protected trisaccharide derivative which, on deacetonation, followed by *O*-deacetylation, produced one of the title trisaccharides, namely, *p*-nitrophenyl 6-*O*-(2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-α-D-mannopyranoside. Synthesis of the other trisaccharide, *p*-nitrophenyl 6-*O*-(2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-α-D-mannopyranoside was accomplished by a similar reaction-sequence when the corresponding 2-methyl-[3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline (**19**) reacted with **7**. Preparation of oxazoline **19** was achieved *via* acetolysis of methyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside. The structures assigned to the final saccharides were supported by ^{1}H- and ^{13}C-n.m.r.-spectral data.

INTRODUCTION

For the past few years, we have engaged in the synthesis of aryl saccharides, because these synthetic derivatives can be further employed for modification of enzyme assay-procedures for certain glycosidases and glycosyltransferases. For example, the synthetic disaccharide *p*-nitrophenyl 2-*O*-α-L-fucopyranosyl-β-D-galacto-

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pyranoside[2] has been successfully employed in a rapid assay procedure for α-(1→2)-L-fucosidase. Similarly, with the aid of *o*-nitrophenyl 2-*O*-α-L-fucopyranosyl-β-D-galactopyranoside[3], we were able to develop a convenient assay-method for α-(1→2)-L-fucosyltransferase in human serum. The availability[4] of the synthetic disaccharides *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**2**), *p*-nitrophenyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**5**), and *p*-nitrophenyl 2-acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**6**) provided a rapid method for linkage-specificity studies of exo-β-D-galactosidase[5]. We now describe a facile synthesis of disaccharide **5**, and also a comparison of its ^{13}C-n.m.r. spectrum with those of the analogs **2** and **6**.

Our interest in the synthesis of **5** and the trisaccharides **11** and **22** was increased because these compounds may be further employed for the identification of the enzymic product obtained by the reaction of D-galactosyltransferases and their corresponding acceptors. We had observed[6] that *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside may be used as an acceptor for the β-(1→4)-D-galactosyltransferase present in human serum, yielding disaccharide **5**. Serum D-galactosyltransferase has been found to be elevated in various types of cancer[6–9], including cancer of the ovary[7,9]. However, discrepant data had been reported[10] with fetuin as the acceptor for this enzyme in the sera of ovarian cancer patients. These conflicting results indicated the need for appropriate acceptors for specific assay-methods for human D-galactosyltransferase. As mentioned in a recent publication[11], we have successfully accomplished the synthesis of *p*-nitrophenyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside, a possible acceptor for β-(1→4)-D-galactosyltransferase. Thus, the availability of trisaccharide **22** as a reference compound should facilitate the assay procedure for this enzyme.

RESULTS AND DISCUSSION

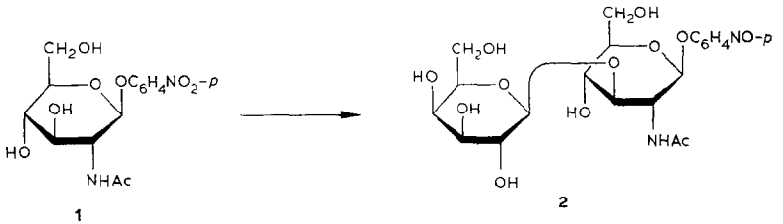
Recently, a number of syntheses of 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (*N*-acetyllactosamine) have been reported from different laboratories[12–14]. It has become apparent that *N*-acetyllactosamine can now be readily obtained from the commercially available 3-*O*-β-D-galactopyranosyl-D-arabinose under the modified reaction-conditions recently reported by Alais and Veyrières[14]. However, we observed that, using this preparative procedure, chromatographic separation of the final product is essential for its absolute purification. The crystalline *N*-acetyllactosamine[14] was converted into 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl chloride (**3**) as described by Kaifu and Osawa[15].

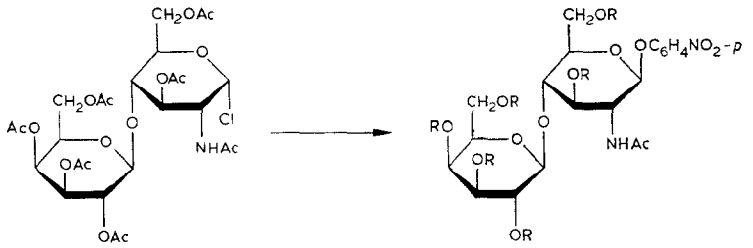
Among the various methods[16,17] described for the preparation of *p*-nitrophenyl glycosides, the use of resin-bound *p*-nitrophenoxide[17] was preferred in the present investigations. Thus, treatment of sugar halide **3** with Amberlyst A-26 *p*-nitrophenoxide[17] in 2-propanol and dichloromethane gave **4**, which was purified by chromatography on a column of silica gel. On saponification[18], compound **4** afforded amorph-

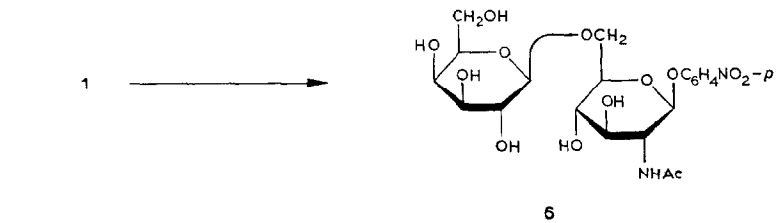
ous **5** in 85% yield. As described later, the structure of disaccharide **5**, along with those of its analogs **2** and **6**, were confirmed by ^{13}C-n.m.r. spectroscopy.

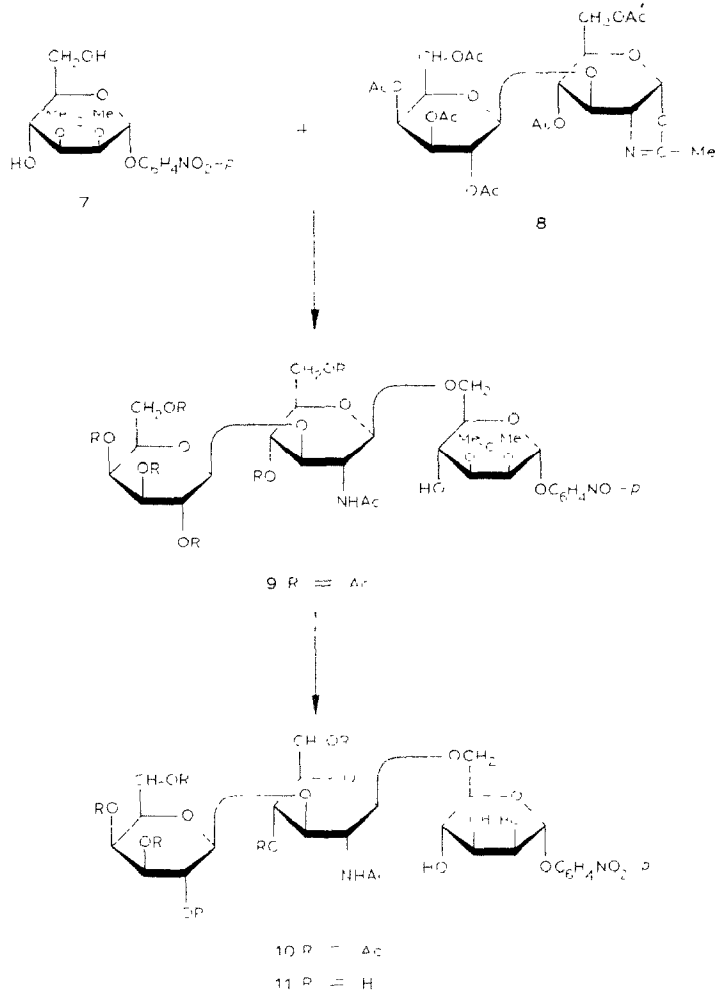
In a recent publication[11] on the synthesis of β-D-GlcNAc-(1→6)-α-D-Man-1→$OC_6H_4NO_2$-*p*, 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline reacted with *p*-nitrophenyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**7**); we have already described the reasons for preferring a 4,6-diol for the synthesis of 6-*O*-substituted glycosides. In the present studies, a similar strategy was applied for the synthesis of the title trisaccharides. Thus, treatment of diol **7** with oxazoline[19] **8** gave the trisaccharide derivative **9** which, without purification, was treated with 65% acetic acid, to give amorphous **10** in an overall yield of 22%. Its ^{13}C-n.m.r. spectrum clearly exhibited the signals for three anomeric carbon atoms at δ 99.01 (C-1), 99.78 (C-1′), and 100.83 (C-1″). *O*-Deacetylation[18] of **10** provided trisaccharide **11**; its ^{1}H- and ^{13}C-n.m.r. spectra confirmed the structure assigned.

Effective use of acetolysis of methyl 2-acetamido-2-deoxy-D-glucopyranoside derivatives has been made in the preparation of certain oxazolines, particularly the 3-O-substituted disaccharide oxazolines[19]. For the preparation of oxazoline **19**, we aimed at its preparation *via* acetolysis. For this purpose, a facile method for preparing

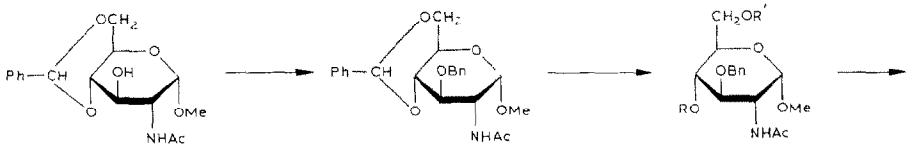




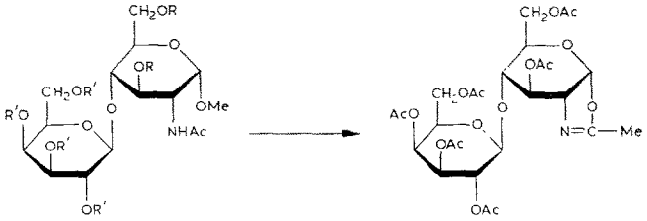




methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**15**) in two steps from methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[19] (**12**) was developed. Thus, benzylation of **12** with benzyl chloride in *N*,*N*-dimethylformamide in the presence of powdered potassium hydroxide produced **13**, which, on selective ring-opening of the benzylidene group in the presence of sodium cyanoborohydride and HCl–ether[20], afforded **15** in 81% yield; its ^{1}H- and ^{13}C-n.m.r. spectra confirmed the structure assigned. Reaction of **15** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in benzene in the presence of mercuric cyanide[21] gave **16**, which, on *O*-deacetylation followed by hydrogenolysis, produced methyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (**18**) in 80% yield. The inter-sugar linkage in **18** was supported by its ^{1}H-n.m.r. spectrum, which showed a doublet at δ 4.85 (*J* 6 Hz, H-1′). Exposure of **18** to an acetolysis mixture of acetic anhydride,



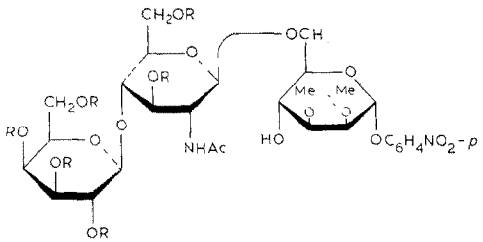
12 13 14 R = R′ = H
15 R = H, R′ = Bn
Bn = $CH_2C_6H_5$



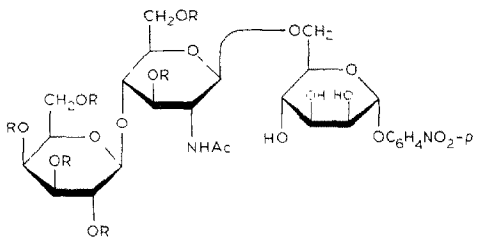
16 R = Bn, R′ = Ac
17 R = Bn, R′ = H
18 R = R′ = H

19

7 + 19 →



20 R = Ac



21 R = Ac
22 R = H

TABLE I

^{13}C-NMR CHEMICAL SHIFTS[a]

Compound	*GlcNAc residue*								*Gal group*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C=O*	CH_3	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*
1[b]	98.28	55.16	73.71	69.93	77.24	60.42	169.21	22.95						
2	97.51	53.83	83.81	68.06	76.58	60.09	169.90	22.87	103.76	70.35	72.79	68.06	75.60	60.44
5	97.87	54.45	71.68	80.31	75.11	59.77	168.85	22.83	103.72	70.43	73.04	68.01	75.44	60.36
6	98.37	55.10	73.77	70.54	74.90	68.97	169.05	22.91	104.17	70.43	73.16	67.86	75.90	60.18

[a] In p.p.m. downfield from Me_4Si (internal) at 25.2 MHz, in Me_2SO-d_6. [b] From ref. 24.

TABLE II

EFFECTS OF β-D-GALACTOSYLATION ON CHEMICAL SHIFT

Compound	D-*Galactopyranosyl group*	*Position on β-D-GlcNAc residue*	*Shielding effect at position of substitution (p.p.m.)*	*Shielding effects at carbon atoms adjacent to position of substitution (p.p.m.)*
2	β-	O-3	C-3 (+10.10)	C-2 (−1.33) C-4 (−1.87)
5	β-	O-4	C-4 (+10.38)	C-3 (−2.03) C-5 (−2.13)
6	β-	O-6	C-6 (+8.55)	C-5 (−2.34)

acetic acid, and sulfuric acid gave a mixture of components which, on chromatography on a column of silica gel, afforded the expected oxazoline **19***, identical with an authentic sample prepared from chloride **3** by the method of Kaifu and Osawa[15]. As already mentioned, *N*-acetyllactosamine is now readily obtainable, and we consider that preparation of oxazoline **19**, starting from *N*-acetyllactosamine, is practical.

Condensation of oxazoline **19** with diol **7** in 1,2-dichloroethane in the presence of *p*-toluenesulfonic acid gave the 6-*O*-substituted derivative **20** in 53% yield. *O*-Deisopropylidenation, followed by saponification, produced the title trisaccharide **22** in 77% yield; its structure was confirmed by ^{1}H- and ^{13}C-n.m.r. spectroscopy.

Recently, Vernon and co-workers[22] reported the ^{13}C-n.m.r. spectra of the 6-aminohexyl glycosides of all of the positional isomers of *O*-β-D-galactopyranosylated 2-acetamido-2-deoxy-β-D-glucopyranose. Availability of the synthetic *p*-nitrophenyl disaccharides **2**, **5**, and **6** prompted us to examine their ^{13}C-n.m.r. spectra in detail, as it is obvious that such studies of these reference compounds will further aid in determining the structures of oligosaccharides in which a β-D-galactopyranosyl group is linked to a 2-acetamido-2-deoxy-D-glucose residue; our results on these ^{13}C-n.m.r. spectra are summarized in Tables I and II. It was observed that the chemical shifts of all of the carbon atoms of the β-D-galactopyranosyl group remain almost constant for disaccharides **2**, **5**, and **6**. It is well established[23] that alkylation of a hydroxyl group causes a 7–10-p.p.m., downfield shift in the resonance of a carbon atom originally bearing a hydroxyl group, and inspection of Tables I and II shows that this holds here. Thus, C-3 of **2**, C-4 of **5**, and C-6 of **6** showed downfield shifts of 10.1, 10.38, and 8.55 p.p.m., respectively, relative to that[24] of the parent glycoside **1**. In the ^{13}C-n.m.r. spectra of disaccharides **2**, **5**, and **6**, the C-1 signal was observed at 98 ±0.5 p.p.m., and this value is appreciably lower than that reported by Vernon and co-workers[22] for the corresponding 6-aminohexyl glycosides, indicating the effect of the *p*-nitrophenyl group on the signal of the anomeric carbon atom.

It is also well established[25] that the carbon atoms of the hydroxymethyl group

*In different experiments, the yield varied from 40 to 70%.

TABLE III

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

Atoms	*Compound*	
	11	**22**
C-1	98.63	98.60
C-2	71.12	71.12
C-3	69.31	69.31
C-4	67.34	67.36
C-5	73.31	73.28
C-6	70.38	70.37
C-1′	101.75	101.93
C-2′	55.21	55.80
C-3′	83.34	73.28
C-4′	69.58	79.52
C-5′	76.06	75.50
C-6′	61.65	60.98
C=O	175.11	175.01
CH_3	23.12	23.07
C-1″	104.27	103.71
C-2″	71.48	71.73
C-3″	73.31	73.28
C-4″	69.31	69.31
C-5″	76.13	76.10
C-6″	61.80	61.75

[a]In p.p.m. downfield from Me_4Si (external) at 25.2 MHz, in D_2O.

of aldohexopyranoses exhibit their signals in the region of 60–63 p.p.m. from Me_4Si (external) with D_2O as the solvent, and this region is generally free from signals from other types of carbon atoms that occur in carbohydrate structures. Thus, it could be anticipated that structures **11** and **22** would give signals for two carbon atoms in this region, the substitution having caused a deshielding of 7–10 p.p.m. Inspection of Table III shows that this occurs. Thus, the absence of a signal for C-6 at 60–63 p.p.m., and the presence of the C-6 signal at 70.38 p.p.m. in the spectrum of **11** and 70.37 p.p.m. in the spectrum of **22**, confirm the position of the new glycosidic linkage in trisaccharides **11** and **22**, respectively. The complete absence of a C-6 signal in the region of 60–63 p.p.m. in the spectrum of **11** and **22** also confirmed that glycosylation had occurred only at the 6-hydroxyl group of acetal **7** in both. The C-1′ resonance at δ 101.75 in the ^{13}C-n.m.r. spectrum of **11**, and at δ 101.93 in the spectrum of **22**, further supported the β-D configuration for both trisaccharides.

The ^{13}C-n.m.r. spectra of partially benzylated derivatives of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside are summarized in Table IV. The pronounced, downfield shift of 8.81 p.p.m. exhibited by C-6 on benzylation, and the upfield shift

TABLE IV

^{13}C-N.M.R. CHEMICAL SHIFTS[a] (25.2 MHz)

Compound	*GlcNAc residue*									*Gal group*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C=O*	*CH_3*	*OMe*	*C-1′*	*C-2′*	*C-3′*	*C-4′*	*C-5′*	*C-6′*
Methyl 2-acetamido-2-deoxy-α-D-gluco-pyranoside[b]	98.6	54.25	71.9	70.40	72.20	61.40			55.60						
14	98.04	52.18	79.86	70.13	73.42	60.51	169.00	22.47	54.11						
15	98.05	52.15	79.85	70.25	71.40	69.32	168.93	22.45	54.18						
17	97.81	51.97	76.32	77.77	70.14	67.43	168.94	22.41	54.21	103.16	71.03	73.34	68.05	74.96	59.70
18	98.51	54.03	70.49	79.51	71.13	60.78	175.00	22.73	56.01	103.62	71.76	73.32	69.34	76.12	61.80

[a]Solvent Me_2SO-d_6, except for D_2O for **18**. The reference standard (Me_4Si) was internal for solutions in Me_2SO-d_6, and external for solutions in D_2O.
[b]From ref. 26.

(2.02 p.p.m.) of C-5, confirmed the position of substitution in **15**. The introduction of the β-D-galactopyranosyl group at O-4 of **15** caused a deshielding of the inter-sugar C-4 by 7.52 p.p.m. In the ^{13}C-n.m.r. spectrum of disaccharide **18**, the site of substitution was readily identified by the occurrence of a 9.1-p.p.m. downfield-shift of the C-4 signal, in comparison to that of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside[26]. The C-1 resonance at δ 103.62 in the ^{13}C-n.m.r. spectrum of **18** supported the β-D configuration. In the benzylated methyl glycosides **14**, **15**, and **17**, the α-methoxyl carbon atom resonates at ~54 p.p.m., whereas, in fully deblocked disaccharide **18**, it resonates at ~56 p.p.m.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at room temperature. Ascending t.l.c. was conducted on plates coated with a 0.25-mm layer of silica gel 60 PF-254 (E. Merck, Darmstadt, Germany); the components were located by exposure to u.v. light, or by spraying the plate with 5% sulfuric acid in ethanol and heating. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A. N.m.r. spectra were recorded with Varian EM-390 and XL-100 instruments; ^{1}H-n.m.r. spectra (100 MHz) and ^{13}C-n.m.r. spectra (25.2 MHz) were determined by the Fourier-transform (F.t.) mode; the positions of the peaks are expressed in δ from the signal for tetramethylsilane.

p-*Nitrophenyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-glucopyranoside* (**2**). — Disaccharide **2** was prepared from *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**) as described by Matta and Barlow[4]; ^{1}H-n.m.r. (Me_2SO-d_6): δ 1.83 (s, 3 H, NAc), 5.42 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 7.24 and 8.24 (2 m, 2 × 2 H, aromatic), and 7.92 (d, 1 H, $J_{NH,2}$ 8 Hz, NH).

p-*Nitrophenyl 2-acetamido-3,6-di-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside* (**4**). — A solution of chloride **3** (500 mg) in 2-propanol (10 mL) and dichloromethane (2 mL) was stirred at room temperature in the presence of Amberlyst A-26 *p*-nitrophenoxide (1.0 g). After 24 h, more resin (1.0 g) was added, and stirring was continued for 2 days more. The suspension was filtered, and the filtrate evaporated to dryness. The residue was purified by chromatography on a column of silica gel, eluting first with chloroform, and then with 19:1 (v/v) chloroform–acetone (to remove unreacted chloride), and finally with 9:1 (v/v) chloroform–acetone, giving **4** in a yield of 81% (on the basis of chloride recovered); m.p. 155–156° (from acetone–hexane), $[\alpha]_D$ −36.7° (*c* 1.3, chloroform); t.l.c. (3:1 chloroform–acetone): R_f 0.5; ^{1}H-n.m.r. data ($CDCl_3$): δ 2.0–2.2 (cluster of singlets, 21 H, 6 Ac + 1 NAc), 6.2 (d, 1 H, $J_{1,2}$ 9 Hz, H-1), and 7.1 and 8.2 (2 m, 2 × 2 H, aromatic).

p-*Nitrophenyl 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranoside* (**5**). — A suspension of compound **4** (200 mg) in dry methanol (10 mL) was

stirred overnight in the presence of a catalytic amount of the macroreticular[18] Amberlyst A-26 (OH^-). The disaccharide that was precipitated was redissolved by the addition of a few drops of water. The resin was filtered off, and the filtrate was evaporated to give amorphous **5** (115 mg, 85%); $[\alpha]_D$ −4.2° (*c* 0.5, Me_2SO); t.l.c. (13:6:1 chloroform–methanol–water): R_F 0.76; ^{1}H-n.m.r. data (Me_2SO-d_6): δ 1.82 (s, 3 H, NAc), 5.24 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 7.2 and 8.2 (2 m, 2 × 2 H, aromatic), and 7.92 (d, 1 H, $J_{NH,2}$ 8 Hz, NH).

Anal. Calc. for $C_{20}H_{28}N_2O_{13} \cdot H_2O$: C, 45.97; H, 5.78; N, 5.36. Found: C, 45.77; H, 5.86; N, 5.31.

p-*Nitrophenyl 2-acetamido-2-deoxy-6-O-β-D-galactopyranosyl-β-D-glucopyranoside* (**6**). — Disaccharide **6** was prepared from **1** as described by Matta and Barlow[4]; ^{1}H-n.m.r. data (Me_2SO-d_6): δ 1.83 (s, 3 H, NAc), 4.18 (d, 1 H, $J_{1',2'}$ 7 Hz, H-1'), 5.10 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 7.3 and 8.3 (2 m, 2 × 2 H, aromatic), and 7.88 (d, 1 H, $J_{NH,2}$ 8 Hz, NH).

p-*Nitrophenyl 6-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,3-O-isopropylidene-α-D-mannopyranoside* (**9**). — A solution of oxazoline **8** (902 mg, 1.5 mmol) and **7** (341 mg, 1 mmol) in a 0.01M solution of *p*-toluenesulfonic acid in 1,2-dichloroethane (10 mL) was stirred at 65°, and the reaction was monitored by t.l.c. After 12 h, the acid was neutralized with a few drops of pyridine, and the solution was evaporated to a dark-brown residue which was dissolved in chloroform (100 mL), and the solution washed twice with water (2 × 25 mL), dried, and evaporated. The solid residue was used as such for the next reaction.

p-*Nitrophenyl 6-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-mannopyranoside* (**10**). — A mixture of **9** (1.0 g) with 65% acetic acid (50 mL) was stirred for 1.5 h at 65°, cooled, and evaporated. Several additions and evaporations of toluene gave a solid mass which was purified by chromatography on a column of silica gel, with elution with 9:1 (v/v) chloroform–ethanol, to give amorphous **10** (200 mg) in an overall yield of 22%; $[\alpha]_D$ +41.2° (*c* 1.3, dichloromethane); ^{1}H-n.m.r. data (Me_2SO-d_6): δ 1.83, 1.87, 1.97, 2.0, and 2.07 (cluster of singlets, 21 H, 6 Ac + 1 NAc), 5.43 (d, 1 H, $J_{1,2}$ ~1 Hz, H-1), 7.23 and 8.23 (2 m, 2 × 2 H, aromatic), and 7.73 (d, 1 H, $J_{NH,2'}$ 9 Hz, NH); ^{13}C-n.m.r. (Me_2SO-d_6): δ 20.21, 20.28 ($OCOCH_3$), 22.77 ($NHCOCH_3$), 53.72 (C-2'), 60.93 (C-6'), 61.82 (C-6''), 77.72 (C-3'), 99.01 (C-1), 99.78 (C-1'), 100.83 (C-1''), and 168.76–169.74 (C=O).

Anal. Calc. for $C_{38}H_{50}N_2O_{24} \cdot H_2O$: C, 48.72; H, 5.60; N, 2.99. Found: C, 48.87; H, 5.37; N, 2.87.

p-*Nitrophenyl 6-O-(2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-α-D-mannopyranoside* (**11**). — *O*-Deacetylation of compound **10** (150 mg) as described for **5** gave amorphous **11** (80 mg, 73%); $[\alpha]_D$ +37.1° (*c* 0.5, water); t.l.c. [11:9:2 (v/v) chloroform–methanol–water]: R_F 0.65; ^{1}H-n.m.r. data (D_2O): δ 2.46 (s, 3 H, NAc), 6.17 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), and 7.70 and 8.70 (2 m, 2 × 2 H, aromatic).

Anal. Calc. for $C_{20}H_{38}N_2O_{18} \cdot 2\ H_2O$: C, 44.44; H, 6.03; N, 3.99. Found: C, 44.59; H, 5.98; N, 3.79.

Methyl 2-acetamido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**13**). — To a mixture of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**12**; 10 g), *N*,*N*-dimethylformamide (250 mL), and powdered potassium hydroxide (10 g) was added benzyl chloride (15 mL) dropwise, with vigorous stirring, during 15 min at 70–75°. The reaction was continued for 2 h, and the mixture was cooled, and poured into ice–water (4 L) with stirring. The solid thus produced was collected by filtration, washed several times with cold water, and recrystallized from chloroform–hexane, to give pure **13** in 53% yield (6.8 g); m.p. 228–230°, $[\alpha]_D$ +53.7° (*c* 0.9, *N*,*N*-dimethylformamide); ν_{max}^{KBr} 3280 (NH), 1645 (Amide I), 1550 (Amide II), 1500, 740, and 695 cm^{-1} (Ph).

Anal. Calc. for $C_{23}H_{25}NO_6$: C, 66.81; H, 6.58; N, 3.38. Found: C, 67.05; H, 6.81; N, 3.25.

Methyl 2-acetamido-3-O-benzyl-2-deoxy-α-D-glucopyranoside (**14**). — A mixture of **13** (6.0 g) and 80% acetic acid (300 mL) was stirred for 1 h at 100°, cooled, and evaporated. Several additions and evaporations of water, and then of toluene, gave a solid mass that was recrystallized from acetone–hexane, to afford **14** (3.5 g, 74%): m.p. 181–182°, $[\alpha]_D$ +103.4° (*c* 0.8, methanol); ν_{max}^{KBr} 3300 (OH), 1640 (Amide I), 1545 (Amide II), 1500, 740, and 700 cm^{-1} (Ph); ^{1}H-n.m.r. data (Me_2SO-d_6), δ 1.83 (s, 3 H, NAc), 3.3 (s, 3 H, OMe), 5.23 (d, 1 H, *J* 6 Hz, D_2O-exchangeable, OH-4), 7.3 (m, 5 H, aromatic), and 8.0 (d, 1 H, $J_{NH,2}$ 9 Hz, NH).

Anal. Calc. for $C_{16}H_{23}NO_6$: C, 59.06; H, 7.12; N, 4.30. Found: C, 58.82; H, 7.23; N, 4.10.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**15**). — A solution of **13** (0.858 g, 2 mmol) and sodium cyanoborohydride (1.131 g, 18 mmol) in dry oxolane (30 mL) containing 3A molecular sieves (5 g) was cooled to 0°, and hydrogen chloride in diethyl ether was added until the solution was acidic (pH paper). After 3 h at 0°, when t.l.c. indicated complete reaction, the mixture was poured into ice–water, and the product was extracted with dichloromethane. The extract was successively washed with saturated, aqueous sodium hydrogencarbonate and water, dried (magnesium sulfate), and evaporated *in vacuo*, affording a solid that was purified by chromatography on a column of silica gel, with elution with 5:1 (v/v) chloroform–acetone, to give **15** (700 mg, 81.2%); m.p. 144–145° (from acetone–ether–hexane), $[\alpha]_D$ +85.9° (*c* 1.1, chloroform); t.l.c. in 5:1 chloroform–acetone, R_f 0.53; ^{1}H-n.m.r. data (Me_2SO-d_6): δ 1.83 (s, 3 H, NAc), 3.3 (s, 3 H, OMe), 5.35 (d, 1 H, *J* 6 Hz, D_2O-exchangeable, OH-4), 7.3 (m, 10 H, aromatic), and 7.95 (d, 1 H, $J_{NH,2}$ 9 Hz, NH).

Anal. Calc. for $C_{23}H_{29}NO_6$: C, 66.49; H, 7.04; N, 3.37. Found: C, 66.27, H, 7.24; N, 3.43.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**16**). — A solution of the alcohol **15** (2.493 g, 6 mmol) and mercuric cyanide (1.52 g, 6 mmol) in dry benzene (60 mL) was boiled

under a nitrogen atmosphere until 30 mL of the solvent had distilled. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (2.47 g, 6 mmol) in dry benzene (30 mL) was rapidly added, and the mixture was refluxed for 30 h, a further addition of the bromide (1.24 g, 3 mmol) in dry benzene (30 mL) being made after 6 h. The mixture was cooled to room temperature, diluted with benzene, successively washed with 10% aqueous potassium iodide solution and water, dried ($MgSO_4$), and evaporated to dryness, to give a solid residue which was used as such for the next reaction.

*Methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside* (**17**). — A molar solution of sodium methoxide in methanol (4 mL) was added to a solution of compound **16** (4 g) in dry methanol (40 mL), and the mixture was kept overnight at room temperature, made neutral with acetic acid, and evaporated; this was followed by a few additions and evaporations of dry toluene. The solid mass was purified by chromatography on a column of silica gel, with elution with 9:1 (v/v) chloroform–methanol, to afford amorphous **17** in 61% yield (from **15**): $[\alpha]_D$ +70.6° (*c* 1.3, Me_2SO); ^{1}H-n.m.r. data (Me_2SO-d_6): δ 1.83 (s, 3 H, NAc), 3.3 (s, 3 H, OMe), 7.3 (m, 10 H, aromatic), and 8.0 (d, 1 H, $J_{NH,2}$ 9 Hz, NH).

*Methyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside* (**18**). — A solution of **17** (2 g) in glacial acetic acid (100 mL) was hydrogenolyzed with hydrogen in the presence of 10% Pd–C for 2 days. The suspension was filtered, and the filtrate evaporated to dryness. The residue was purified by chromatography on a column of silica gel, with elution with 65:35:8 (v/v) chloroform–methanol–water, to give **18** in 80% yield (1.1 g); m.p. 264–265° (methanol), $[\alpha]_D$ +98.4° (*c* 0.9, Me_2SO); ^{1}H-n.m.r. (D_2O): δ 2.42 (s, 3 H, NAc), 3.76 (s, 3 H, OMe), 4.85 (d, 1 H, $J_{1',2'}$ 6 Hz, H-1'), and 5.63 (1 H, H-1); for ^{13}C-n.m.r. data, see Table IV.

Anal. Calc. for $C_{15}H_{27}NO_{11}$: C, 45.33; H, 6.85; N, 3.53. Found: C, 45.05; H, 7.05; N, 3.68.

*2-Methyl-[3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano]-[2,1-d]-2-oxazoline* (**19**). — A solution of compound **18** (200 mg) in a mixture of acetic anhydride (2.15 mL), acetic acid (1.4 mL), and sulfuric acid (25 μL) was stirred for 2 days at room temperature. It was then diluted with cold dichloromethane (100 mL), washed successively with ice-cold, saturated sodium hydrogencarbonate solution and ice-cold water (2 × 10 mL), dried (anhydrous sodium sulfate), and evaporated to give a solid material, t.l.c. of which in 10:10:1 (v/v) chloroform–ether–methanol showed two minor impurities. The product was purified by chromatography on a column of silica gel, with elution with 10:10:1 (v/v) chloroform–ether–methanol, to afford amorphous **19** (170 mg, 55%); $[\alpha]_D$ +35.9° (*c* 1, chloroform) {lit.[15] $[\alpha]_D$ +17° (*c* 1.1, chloroform)}; ν_{max}^{KBr} 1750 (Ac) and 1675 cm^{-1} (C=N); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.97–2.17 (cluster of singlets, 21 H, 6 Ac + 1 Me of oxazoline) and 5.87 (d, 1 H, *J* 7.5 Hz, H-1).

p-*Nitrophenyl 6-*O*-[2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,3-*O*-isopropylidene-α-D-mannopyranoside* (**20**). — A solution of oxazoline **19** (1.44 g, 2.4 mmol) and **7** (682 mg, 2 mmol) in 0.01M *p*-toluenesulfonic acid in dichloromethane (25 mL) was stirred at

80°, and the reaction was monitored by t.l.c. Even after 4 days, t.l.c. still showed some starting material. More *p*-toluenesulfonic acid (20 mg) was added, and stirring was continued for a further 3 days. The mixture was cooled, made neutral with a few drops of pyridine, and evaporated; the residue was applied to a column of silica gel. Elution with 5:1 (v/v) chloroform–acetone gave pure compound **20** (1.02 g, 53%), amorphous; $[\alpha]_D$ +18.7° (*c* 1.2, chloroform); t.l.c. (3:2 chloroform–acetone): R_f 0.48; ^{1}H-n.m.r. (Me_2SO-d_6): δ 1.3 and 1.4 (s each, 2 × 3 H, isopropylidene methyls), 1.72 (s, 3 H, NAc), 1.87, 1.92, 1.99, 2.03 and 2.07 (cluster of singlets, 18 H, 6 Ac), 5.83 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 7.25 and 8.2 (2 m, 2 × 2 H, aromatic), and 7.67 (d, 1 H, $J_{NH,2}$ 9 Hz, NH).

Anal. Calc. for $C_{41}H_{54}N_2O_{24}$: C, 51.35; H, 5.68; N, 2.92. Found: C, 51.15; H, 5.77; N, 2.92.

p-*Nitrophenyl* 6-O-[*2-acetamido-3,6-di-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-*D-*galactopyranosyl)-β-*D-*glucopyranosyl*]-α-D-*mannopyranoside* (**21**). The isopropylidene group of compound **20** (750 mg) was removed as described for the preparation of **10**, to give a solid residue which was purified by chromatography on a column of silica gel, with elution with 9:1 (v/v) chloroform–methanol, to afford **21** in 63% yield (453 mg); m.p. 132–133° (from acetone–ether), $[\alpha]_D$ +38.9° (*c* 1.1, CH_2Cl_2); t.l.c. (9:1 chloroform–methanol): R_f 0.48; ^{1}H-n.m.r. data (Me_2SO-d_6): δ 1.77 (s, 3 H, NAc), 1.90, 1.95, 2.0, 2.05 and 2.1 (cluster of singlets, 18 H, 6 Ac), 4.5 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 7.5 Hz, H-2), 5.45 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 7.23 and 8.23 (2 m, 2 × 2 H, aromatic), and 7.67 (d, 1 H, $J_{NH,2}$ 9 Hz, NH); ^{13}C-n.m.r. data (Me_2SO-d_6): δ 20.2, 20.32, 20.51 ($OCOCH_3$), 22.6 ($NHCOCH_3$), 53.09 (C-2′), 60.6 (C-6′), 62.15 (C-6″), 76.29 (C-4′), 98.95 (C-1), 99.76 (C-1′), 100.48 (C-1″), 168.75, 169.14, 169.53, and 169.93 (C=O).

Anal. Calc. for $C_{38}H_{50}N_2O_{24}$: C, 49.67; H, 5.49; N, 3.05. Found: C, 49.50; H, 5.62; N, 3.04.

p-*Nitrophenyl* 6-O-(*2-acetamido-2-deoxy-4-O-β-*D-*galactopyranosyl-β-*D-*glucopyranosyl)-α-*D-*mannopyranoside* (**22**). — *O*-Deacetylation of compound **21** (250 mg), as described for **5**, gave amorphous **22** (0.14 g, 77%); $[\alpha]_D$ +41.6° (*c* 1, water); t.l.c. (11:9:2 chloroform–methanol–water): R_f 0.65; ^{1}H-n.m.r. (D_2O): δ 2.48 (s, 3 H, NAc), 6.17 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), and 7.70 and 8.70 (2 m, 2 × 2 H, aromatic).

Anal. Calc. for $C_{26}H_{38}N_2O_{18} \cdot H_2O$: C, 45.61; H, 5.98; N, 4.09. Found: C, 45.60; H, 5.83; N, 4.07.

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REFERENCES

1 S. A. ABBAS, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 113 (1982) 63–70.
2 R. A. DICIOCCIO, C. F. PISKORZ, G. SALAMIDA, J. J. BARLOW, AND K. L. MATTA, *Anal. Biochem.*, 111 (1981) 176–183.
3 R. A. DICIOCCIO, J. J. BARLOW, AND K. L. MATTA, *Clin. Chim. Acta*, 108 (1980) 41–48.
4 K. L. MATTA AND J. J. BARLOW, *Carbohydr. Res.*, 43 (1975) 299–304.
5 R. A. DICIOCCIO, P. J. KLOCK, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 81 (1980) 315–322.
6 K. L. MATTA, C. F. PISKORZ, R. A. DICIOCCIO, AND J. J. BARLOW, *Proc. Am. Assoc. Cancer Res.*, 21 (1980) 326.
7 S. K. CHATTERJEE, M. BHATTACHARYA, AND J. J. BARLOW, *Cancer Lett.*, 8 (1980) 247–253.
8 D. K. PODOLSKY AND M. M. WEISER, *Biochem. Biophys. Res. Commun.*, 65 (1975) 545–550.
9 M. M. WEISER, W. D. KLOHS, D. K. PODOLSKY, AND J. R. WILSON, in M. I. HOROWITZ (Ed.), *The Glycoconjugates*, Vol. 4, Academic Press, New York, 1982, pp. 301–334.
10 I. D. CAPEL, H. M. DORRELL, D. C. WILLIAMS, I. W. F. HANHAM, AND H. N. LEVITT, *Oncology*, 39 (1982) 193–196.
11 S. S. RANA, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 96 (1981) 79–85.
12 J.-C. JACQUINET AND P. SINAŸ, *Carbohydr. Res.*, 46 (1976) 138–142.
13 R. T. LEE AND Y. C. LEE, *Carbohydr. Res.*, 77 (1979) 270–274.
14 J. ALAIS AND A. VEYRIÈRES, *Carbohydr. Res.*, 93 (1981) 164–165.
15 R. KAIFU AND T. OSAWA, *Carbohydr. Res.*, 52 (1976) 179–185.
16 R. H. SHAH AND O. P. BAHL, *Carbohydr. Res.*, 74 (1979) 105–116.
17 T. IVERSON AND R. JOHANSSON, *Synthesis*, (1979) 823–824.
18 L. A. REED, III, P. A. RISBOOD, AND L. GOODMAN, *J. Chem. Soc., Chem. Commun.*, (1981) 760–761.
19 K. L. MATTA AND J. J. BARLOW, *Carbohydr. Res.*, 53 (1977) 47–56.
20 P. J. GAREGG AND H. HULTBERG, *Carbohydr. Res.*, 93 (1981) c10–c11.
21 A. VEYRIÈRES, *J. Chem. Soc., Perkin Trans. 1*, (1981) 1626–1629.
22 J. VERNON, Y. C. LEE, AND M. KOREEDA, *Tetrahedron Lett.*, (1981) 1067–1077.
23 D. E. DORMAN AND J. D. ROBERTS, *J. Am. Chem. Soc.*, 93 (1971) 4463–4472.
24 S. S. RANA, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 101 (1982) 245–253.
25 H. J. JENNINGS AND I. C. P. SMITH, *J. Am. Chem. Soc.*, 95 (1973) 606–608.
26 A. S. SHASHKOV, A. YU. EVSTIGNEEV, AND V. A. DEREVITSKAYA, *Carbohydr. Res.*, 72 (1979) 215–217.