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SYNTHETIC MUCIN FRAGMENTS: METHYL 3-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-β-D-GALACTOPYRANOSIDE AND METHYL 3-*O*-(2-ACETAMIDO-2-DEOXY-3-*O*-β-D-GALACTO-PYRANOSYL-β-D-GLUCOPYRANOSYL)-β-D-GALACTOPYRANOSIDE*

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ABSTRACT

Methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**5**) was obtained crystalline by way of its 3-*O*-allyl derivative, which was in turn obtained by ring-opening of a presumed 3,4-*O*-stannylene derivative of methyl β-D-galactopyranoside, followed by benzylation. Condensation of **5** with 2-methyl-(2-acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-β-D-glucopyrano)-[2,1-*d*]-2-oxazoline in 1,2-dichloroethane in the presence of *p*-toluenesulfonic acid afforded the disaccharide derivative methyl 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**6**). Deacetylation of **6** in methanolic sodium methoxide afforded the disaccharide derivative **7**, which was acetalated with α,α-dimethoxytoluene to afford the 4′,6′-*O*-benzylidene acetal (**10**). Catalytic hydrogenolysis of the benzyl groups of **7** afforded the title disaccharide **8**. Glycosylation of **10** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in 1:1 benzene–nitromethane in the presence of mercuric cyanide gave the fully protected trisaccharide derivative **12**. Systematic removal of the protecting groups of **12** then furnished the title trisaccharide **14**. The structures of **5, 8,** and **14** were all confirmed by ^{13}C-n.m.r. spectroscopy. The ^{13}C-n.m.r. chemical shifts for methyl α- and β-D-galactopyranoside, and also those of their 3-*O*-allyl derivatives, are recorded, for the sake of comparison, in conjunction with those of compound **5**.

INTRODUCTION

In inauguration of a program for the synthesis of mucinous-type glycoconjugate fragments, we recently described the synthesis of the disaccharide methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranoside[2]. The parent disaccharide, trivially known[3] as "Lacto-*N*-biose II", forms part of the core struc-

*Synthetic Studies in Carbohydrates, Part XLII, for Part XLI, see ref. 1.
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ture, and also occurs in the side chain, attached to a terminal 2-acetamido-2-deoxy-D-galactose unit that is further *O*-glycosylically linked to protein in such glycoconjugates[4].

In subsequent publications, we demonstrated that the peracetylated glycosyl bromide derived from this disaccharide could be effectively utilized as a glycosyl donor for the synthesis of other oligosaccharides; therein, we described the synthesis of *p*-nitrophenyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside[5], and also that of the benzyl α-glycoside of a related trisaccharide[1], 2-acetamido-3-*O*-[3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl]-2-deoxy-D-galactose, that occurs as a terminal, three-sugar unit in the carbohydrate moiety of mucinous-type glycoproteins.

As a further contribution to this program, we now describe the synthesis of the disaccharide methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (**8**), and of the trisaccharide methyl 3-*O*-(2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**14**).

Augé and Veyrières[6] had already described the synthesis of the parent trisaccharide of **14** by way of condensation of an oxazoline derived from the disaccharide unit β-Gal-(1→3)-GlcNAc with a suitably protected D-galactose derivative. However, as in the case of the disaccharide methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranoside[2], our aim remained primarily the same, namely, to utilize this trisaccharide (as its per-*O*-acetylglycosyl halide) for further oligosaccharide syntheses. Hence, we adopted the present route that utilizes readily accessible intermediates and furnishes the trisaccharide in a form that may conveniently be converted into a per-*O*-acetylglycosyl bromide.

Moreover, it was also coincident with our projected synthetic targets to procure a trisaccharide intermediate that is amenable to subsequent manipulations with a minimal number of synthetic operations. For example, in a projected synthesis of an Le^a type of antigenic determinant, it was desired to obtain a trisaccharide intermediate having a free hydroxyl group on C-4 of the 2-acetamido-2-deoxy-D-glucopyranose residue. This desire dictated the use of a 4,6-*O*-benzylidene acetal as a "temporary" protecting-group in the present synthesis. 4,6-*O*-Benzylidene acetals are known[7] to afford, on reductive ring-opening in acidic media and in the presence of sodium cyanoborohydride, a 6-*O*-benzyl substituent and a free hydroxyl group on C-4.

Furthermore, it may be pertinent to point out that, by virtue of being β-D-glycosidically linked to methyl groups, both of our synthetic di- and tri-saccharides would apparently bear a close resemblance to the naturally occurring units, and might thus prove more useful in studies related to the biosynthetic pathways of other, more-complex oligosaccharides. For example, our synthetic disaccharide β-GlcNAc-(1→3)-β-Gal-1→OMe is more likely to be a better acceptor than the related β-GlcNAc-(1→3)-α-Gal-1→OMe for β-D-(1→3)-galactosyltransferase[8].

RESULTS AND DISCUSSION

Methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**5**) was prepared in a manner analogous to that described[9] for the synthesis of other, partially *O*-alkylated D-galactopyranose derivatives. Compound **5** was obtained as an analytically pure, crystalline solid, rather than as a syrup*, as previously indicated[10]. The ^{13}C-n.m.r. spectrum of **5** was in complete accord with the structure expected. Thus, in the spectrum of **5** (see Table I), the signal for C-6 was shifted by ~8.4 p.p.m. downfield in comparison to that of the parent methyl β-D-galactopyranoside, clearly indicating that O-6 carried a benzyl substituent. The signals for C-2 (79.62 p.p.m.) and C-4 (75.45 p.p.m.) were also downfield, by ~9.3 and 7.5 p.p.m., respectively, from those of their counterparts in the spectrum of the parent D-galactoside, as would be expected for substitution at O-2 and O-4. The signal for C-3 (73.50 p.p.m.), however, remained close to that (73.17 p.p.m.) of the parent D-galactoside, indicating that O-3 was unsubstituted; the resonance for C-3 of methyl 3,4-di-*O*-benzyl-β-D-galactopyranoside was observed at 82.1 p.p.m., and that for the 3,6-di-*O*-benzyl isomer occurred at 80.3 p.p.m.[11]; compare, also, with that for the 3-*O*-allyl-substituted compound, given in Table I). Additionally, the identity of **5** was unequivocally established by the fact that, on condensation with oxazoline **1**, and subsequent deblocking, it furnished a (1→3)-linked disaccharide (see later).

Condensation of compound **5** with 2-methyl-(2-acetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-β-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**1**) in 1,2-dichloroethane, in the presence of *p*-toluenesulfonic acid, and purification of the crude product by column chromatography on silica gel, afforded the protected disaccharide derivative (**6**). *O*-Deacetylation of **6** in methanolic sodium methoxide gave, in 95.9% yield, amorphous tri-*O*-benzyl disaccharide (**7**), catalytic hydrogenolysis of the benzyl

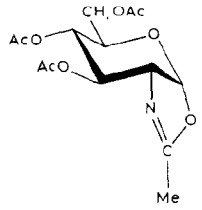
TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS OF SOME METHYL D-GALACTOPYRANOSIDES[a]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*	*CH_2O*	*H_2C=*	*=CH*	*$C_6H_5CH_2$*
2-β	104.22	70.29	73.17	67.96	74.93	60.28	55.61	—	—	—	—
2-α	99.80	68.29	69.49	68.70	70.89	60.52	54.27	—	—	—	—
3-β	104.18	69.20	80.81	64.49	74.80	60.13	55.66	69.20	115.73	135.62	—
3-α[b]	99.84	67.25	77.36	65.42	70.87	60.41	54.21	69.24	115.72	135.81	—
5(β)	104.67	79.62	73.50	75.45	73.45	68.70	56.80	—	—	—	73.96, 74.51, 74.86

[a]In Me_2SO-d_6, with Me_4Si as the internal standard. [b]This compound was prepared from methyl α-D-galactopyranoside, exactly as described for the β anomer; its spectrum is recorded for comparison.

*In ref. 10, it was not explicitly delineated that compound **5** was a syrup, but that was implied, as only the specific rotation was noted. However, the specific rotation given by Flowers[10] was close to that recorded by us; see the Experimental section.



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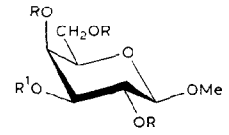
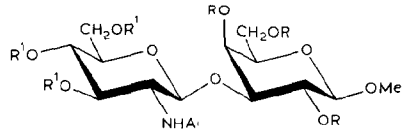
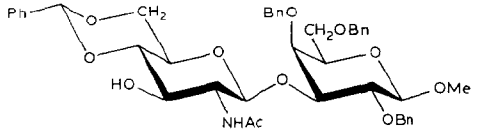


2 R = R' = H
3 R = H, R' = $-CH_2-CH=CH_2$
4 R = Bn, R' = $-CH_2-CH=CH_2$
5 R = Bn, R' = H

Bn = $PhCH_2$



6 R = Bn R' = Ac
7 R = Bn, R' = H
8 R = R' = H
9 R = R' = Ac



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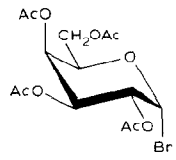

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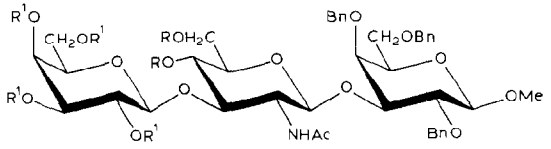
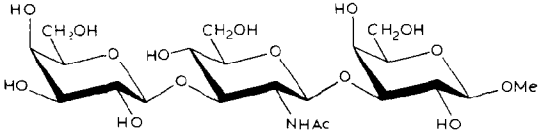


12 R,R = PhCH, R' = Ac
13 R,R = PhCH, R' = H



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groups of which furnished, in 75% yield, the crystalline, title disaccharide **8**, having a ^{13}C-n.m.r. spectrum in accord with the structure assigned (see Table II). Compound **8** was also converted into its analytically pure peracetate (**9**).

Acetalation of triol **7** with α,α-dimethoxytoluene (benzaldehyde dimethyl

TABLE II

PROPOSED ^{13}C-N.M.R CHEMICAL SHIFTS FOR DI- (**8**) AND TRI-SACCHARIDE (**14**)[a]

Residue	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*	*CH_3CO*	*C=Ó*
Me α-Gal	[b]	99.40	67.23	78.97	67.67	70.76	60.74	54.19	—	—
β-GlcNAc		101.89	56.27	74.51	70.24	76.49	60.36	—	22.90	170
Me β-Gal	**8**	103.79	69.29	82.22	67.02	74.69	60.22	55.56	—	—
β-GlcNAc		101.88	56.38	74.22	70.28	76.57	60.77	—	22.98	169.94
Me β-Gal	**14**	103.87	69.28	82.19	67.12	74.73	60.28	55.70	—	—
β-GlcNAc		101.50	54.81	84.53	68.43	76.09	60.46	—	23.07	170.37
β-Gal		103.64	70.47	72.64	68.10	75.60	60.46	—	—	—

[a]In Me_2SO-d_6, with Me_4Si as the internal standard. [b]Methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranoside[2].

acetal) in *N,N*-dimethylformamide, in the presence of *p*-toluenesulfonic acid, gave, in fair yield, the benzylidenated derivative (**10**).

Glycosylation of **10** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**11**) in 1:1 (v/v) benzene–nitromethane, and processing in the usual manner, afforded, in 88% yield, after recrystallization from ethyl acetate–ether–hexane, the trisaccharide derivative (**12**). *O*-Deacetylation of **12** in methanolic sodium methoxide gave the partially protected derivative (**13**), which was subjected to catalytic hydrogenolysis in glacial acetic acid in the presence of 10% palladium-on-carbon, to furnish the title trisaccharide **14**. The structure of **14** was clearly evidenced by the appropriate signals in its ^{13}C-n.m.r. spectrum (see Table II).

Comments on the ^{13}C-n.m.r. assignments. — The assignment of ^{13}C signals for disaccharide **8** were made by comparison of its spectrum with those of methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranoside (see Table II) and methyl β-D-galactopyranoside (**2**; see Table I).

A glance at Table II reveals that the resonances for the carbon atoms of the 2-acetamido-2-deoxy-β-D-glucopyranosyl group in both **8** and its methyl α anomer remained close to each other, apparently because there were no subtle changes at this part of the molecule.

Whereas, in the ^{13}C-n.m.r. spectrum of the methyl α anomer, the resonance for C-1 was observed at δ 99.40, that for C-1 of compound **8** occurred at δ 103.79, in agreement with the presence of a β-linked aglycon. That O-3 of compound **8** was the site of glycosylation was clearly revealed by the noticeable (~9 p.p.m.) downfield shift of the C-3 signal, by comparison to its counterpart in the spectrum of **2**. Slight upfield shifts, because of substitution at O-3, were also observed for the resonances of C-2 and C-4 of **8**.

On comparison with that of **8**, the ^{13}C-n.m.r. spectrum of **14** contained an additional, anomeric carbon-atom resonance at δ 103.64, which was indicative of the presence of another β-galactosyl group. The fact that substitution occurred at O-3

of the 2-acetamido-2-deoxy-β-D-glucopyranosyl unit was evidenced by a signal at low field (84.53 p.p.m.), attributable to C-3 of this residue, which suffered a downfield shift of ~10.3 p.p.m. compared to the same resonance in the spectrum of **8**. A further indication that O-3 of the β-GlcNAc residue of **14** was substituted was that the resonances for C-2 and C-4 in this residue were shifted upfield by ~1.6 and 1.9 p.p.m., respectively, compared to those of their counterparts in the spectrum of **8**.

EXPERIMENTAL

General methods. — These were the same as those already described[1], except that the silica gel used for column chromatography was Baker Analyzed (60–200 mesh), and Solvent *A* was 1:4 (v/v) ethyl acetate–hexane. *N,N*-Dimethylformamide was distilled under diminished pressure, and stored over molecular sieves. Nitromethane was distilled from phosphorus pentaoxide immediately before being used, and benzene was dried with sodium. *p*-Toluenesulfonic acid, employed as a catalyst in glycosylations, was an ~0.02M solution in 1,2-dichloroethane. Organic solutions were generally dried with anhydrous sodium sulfate. Sodium hydride used in alkylation reactions was a 60% dispersion in mineral oil.

Methyl 3-O-allyl-β-D-galactopyranoside (**3**). — A mixture of methyl β-D-galactopyranoside (**2**; 13.6 g, 70 mmol) and dibutyltin oxide (17.4 g, 70 mmol) in benzene (500 mL) was heated for 20 h at reflux temperature, with azeotropic distillation of water. The solution was then concentrated to ~200 mL, tetrabutylammonium iodide (25.9 g, 70 mmol) and allyl bromide (35 mL, 41 mmol) were added, and the mixture was stirred for 7 h at ~60°. It was then evaporated to dryness, to give a dark-red residue which crystallized on addition of ether–hexane. Recrystallization from ethyl acetate afforded compound **3** (12.9 g, 78.6%) as a white solid; m.p. 108–109°, $[\alpha]_D$ +5.7° (*c* 1.0, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{10}H_{18}O_6$: C, 51.27; H, 7.74. Found: C, 51.38; H, 7.74.

Methyl 3-O-allyl-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**4**). — To a cold (0°, bath), stirred solution of **3** (10.4 g, 44.4 mmol) in *N,N*-dimethylformamide (120 mL) was added sodium hydride (10.4 g, 261 mmol), portionwise, during 0.5 h, and stirring was continued for 1.5 h at room temperature. Benzyl chloride (89.5 mL, 777 mmol) was then cautiously added, and stirring continued for 22 h at room temperature. After careful addition of methanol, to decompose the excess of sodium hydride, the mixture was evaporated, the residue dissolved in chloroform, and the solution washed with water, dried, and evaporated, to give a brown residue which was purified in a column of silica gel by elution first with hexane, and then with solvent *A*, to afford **4** (17.9 g, 80%) as a yellowish syrup that contained (t.l.c., Solvent *A*) a marginally faster-migrating contaminant. It was used without purification in the next step.

Methyl 2,4,6-tri-O-benzyl-β-D-galactopyranoside (**5**). — A mixture of the 3-*O*-allyl derivative **4** (15.9 g, 31.6 mmol) and 10% palladium-on-carbon (1.68 g)

with ethanol (100 mL), glacial acetic acid (50 mL), and water (50 mL) was stirred for 48 h at ~75°. The solid material was then filtered off (Celite bed), the filtrate concentrated to a small volume, and the concentrate applied to a column of silica gel. On elution with chloroform, evaporation of the fractions corresponding to the product gave a faint-yellow syrup, which crystallized upon addition of ether–hexane. Recrystallization from the same solvent system furnished compound **5** (11.3 g, 77.2%); m.p. 59–60°, $[\alpha]_D$ +0.7° (*c* 2.9, chloroform); lit.[10] $[\alpha]_D$ +1.0° (*c* 1.9, chloroform); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.67; H, 7.23.

Methyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**6**). — A mixture of compound **5** (9.9 g, 21.3 mmol), oxazoline **1** (7 g, 21.3 mmol), and *p*-toluenesulfonic acid (0.27 g) in 1,2-dichloroethane (70 mL), protected from moisture, was heated for 2 days at ~70° in an atmosphere of nitrogen, an additional amount of **1** (3.9 g) in 1,2-dichloroethane (35 mL) and of *p*-toluenesulfonic acid (0.13 g) in 1,2-dichloroethane (35 mL) being added after 17 h. The mixture was cooled, the acid neutralized by the addition of a few drops of pyridine, and the solution evaporated to dryness. Examination of the crude product by t.l.c. with 9:1 (v/v) chloroform–acetone revealed the presence of a major product, slower-migrating than **5**, and marginally faster-migrating than **1**; some unchanged **5**, and also some slower-migrating contaminants (presumably, decomposition products of **1**) were also revealed by t.l.c. The crude product was applied to a column of silica gel, and eluted with 19:1 (v/v) chloroform–acetone. Evaporation of the first fractions gave unchanged **5** (3 g). On evaporation, the fractions corresponding to the major product gave a solid residue, which was dissolved in a small volume of ethyl acetate. Addition of ether caused the precipitation of **6** (9 g, 53.4%), as a white powder; m.p. 133–136°, $[\alpha]_D$ −26.1° (*c* 1.1, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.53 (s, 3 H, NAc), 1.98 and 2.02 (s, 9 H, 3 OAc), 3.53 (s, 3 H, OMe), and 7.20–7.40 (m, 15 H, aromatic).

Anal. Calc. for $C_{42}H_{51}NO_{14}$: C, 63.54; H, 6.48; N, 1.76. Found: C, 63.53; H, 6.68; N, 1.64.

Methyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**7**). — Compound **6** (8.5 g) was stirred in 0.1M sodium methoxide in methanol (80 mL) for 2.5 h at room temperature. The base was then 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 evaporated, and then several portions of ethanol were added to, and evaporated from, the residue, to afford **7** (7 g, 95.9%); amorphous; $[\alpha]_D$ −23.7° (*c* 1.6, chloroform).

Anal. Calc. for $C_{36}H_{35}NO_{11} \cdot H_2O$: C, 63.05; H, 6.91; N, 2.04. Found: C, 62.73; H, 6.71; N, 1.95.

Methyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (**8**). — A solution of compound **7** (0.5 g) in glacial acetic acid (30 mL) was sha-

ken under hydrogen at 345 kPa for 20 h at room temperature in the presence of 10% palladium-on-carbon (0.3 g). The suspension was filtered through a bed of Celite, the solid was thoroughly washed with methanol, and the filtrate and washings were combined, and evaporated under diminished pressure, to give a solid which crystallized from absolute alcohol, to furnish the disaccharide **8** (0.22 g, 75%); m.p. 256–257°, $[\alpha]_D$ −3.3° (*c* 1.0, water); for ^{13}C-n.m.r. data, see Table II.

Anal. Calc. for $C_{15}H_{27}NO_{11} \cdot H_2O$: C, 43.36; H, 7.04; N, 3.37. Found: C, 43.20; H, 7.00; N, 3.17.

Methyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-acetyl-β-D-galactopyranoside (**9**). — Disaccharide **8** (0.8 g) was acetylated overnight in 1:2 acetic anhydride–pyridine (15 mL). After customary processing, the product was purified in a column of silica gel by using 4:1 (v/v) chloroform–acetone as the eluant, to give **9** (0.11 g, 84%); m.p. 178–180°, $[\alpha]_D$ +21.2° (*c* 1.3, chloroform).

Anal. Calc. for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.05; N, 2.16. Found: C, 49.71; H, 6.09; N, 1.88.

Methyl 3-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**10**). — To a stirred solution of **7** (6.3 g) in *N,N*-dimethylformamide (100 mL) were added *p*-toluenesulfonic acid (0.14 g) and α,α-dimethoxytoluene (7.3 g), and the stirring was continued for 2 days at room temperature. The acid was then neutralized with a little triethylamine, and the solution evaporated to dryness, to give a solid residue which was recrystallized from ethyl acetate to afford compound **10** (3.6 g, 50.4%); m.p. 176°, $[\alpha]_D$ −26.3° (*c* 1.7, chloroform). An additional amount of **10** (1.9 g, 26%) was obtained after purification of the mother liquor in a column of silica gel by using 19:1 chloroform–methanol as the eluant.

Anal. Calc. for $C_{43}H_{49}NO_{11}$: C, 68.32; H, 6.53; N, 1.85. Found: C, 68.08; H, 6.33; N, 1.83.

Methyl 3-O-[2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**12**). — A stirred solution of compound **10** (2.4 g, 3 mmol) in 1:1 benzene–nitromethane (140 mL) was boiled until ~100 mL of the solvent had distilled off. The temperature was then adjusted to ~50°, mercuric cyanide (1.15 g) and a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**11**; 1.85 g, 4.51 mmol) in 1:1 benzene–nitromethane (20 mL) were added, stirring was continued for 3 h at 50°, more portions of mercuric cyanide (0.38 g) and bromide **11** (0.62 g) in 1:1 benzene–nitromethane (10 mL) were added, and stirring was continued for 18 h at room temperature. After processing in the usual manner, the residue so obtained was dissolved in a small volume of ethyl acetate. Addition of ether–hexane caused the precipitation of **12** (2.84 g, 88%) as a white solid; m.p. 151–153°, $[\alpha]_D$ −20.1° (*c* 1.1, chloroform).

Anal. Calc. for $C_{57}H_{67}NO_{20}$: C, 63.03; H, 6.22; N, 1.29. Found: C, 62.89; H, 6.05; N, 1.14.

Methyl 3-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**13**). — Compound **12** (1 g) was added to 0.1M methanolic sodium methoxide (25 mL), and the suspension stirred at room temperature. The suspended **12** quickly dissolved, and, in ~0.5 h, crystallization ensued. The mixture was kept for 4 h at room temperature, refrigerated overnight, the base neutralized by the addition of a few drops of glacial acetic acid, and the crystalline material filtered off, and thoroughly washed with cold ethanol, to afford compound **13** (0.61 g, 72.3%); m.p. 205–208°, $[\alpha]_D$ −25.9° (*c* 1.6, chloroform). An additional amount of **13** (0.2 g) was obtained by passing a solution of the mother liquor in methanol through a short column of silica gel.

Anal. Calc. for $C_{49}H_{59}NO_{16}$: C, 64.11; H, 6.48; N, 1.53. Found: C, 64.04; H, 6.72; N, 1.57.

Methyl 3-O-(2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**14**). — Compound **13** (0.5 g) was hydrogenolyzed exactly as described for **7** (to give **8**) to give, after recrystallization from aqueous alcohol, compound **14** (0.27 g, 90%); m.p. 256–258°, $[\alpha]_D$ −2.5° (*c* 0.7, water); for ^{13}C-n.m.r. data, see Table II.

Anal. Calc. for $C_{21}H_{37}NO_{16} \cdot H_2O$: C, 43.67; H, 6.81; N, 2.42. Found: C, 43.56; H, 7.06; N, 2.11.

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REFERENCES

1 S. A. ABBAS AND K. L. MATTA, *Carbohydr. Res.*,130 (1984) 137–141.
2 S. A. ABBAS AND K. L. MATTA, *Carbohydr. Res.*, 123 (1983) 53–61.
3 R. KUHN AND H. H. BAER, *Chem. Ber.*, 89 (1956) 504–511.
4 E. A. KABAT, *Methods Enzymol.*, 70 (1980) 3–49.
5 S. A. ABBAS AND K. L. MATTA, *Carbohydr. Res.*, 124 (1983) 115–121.
6 C. AUGÉ AND A. VEYRIÈRES, *J. Chem. Soc., Perkin Trans. 1*, (1977) 1343–1345.
7 P. J. GAREGG, H. HULTBERG, AND S. WALLIN, *Carbohydr. Res.*, 108 (1982) 97–101.
8 B. T. SHEARES AND D. M. CARLSON, *J. Biol. Chem.*, 258 (1983) 9893–9898.
9 S. DAVID, A. THIEFFRY, AND A. VEYRIÈRES, *J. Chem. Soc., Perkin Trans. 1*, (1981) 1796–1801.
10 H. M. FLOWERS, *Carbohydr. Res.*, 39 (1975) 245–251.
11 T. OGAWA, T. NUKADA, AND M. MATSUI, *Carbohydr. Res.*, 101 (1982) 263–270.