Syntheses of core chain trisaccharides related to human blood group antigenic determinants

R. U. LEMIEUX, S. Z. ABBAS,[1] AND B. Y. CHUNG[2]

Department of Chemistry, University of Alberta, Edmonton, Alta., Canada T6G 2G2

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Trisaccharides related to the core chain of human blood group determinants were synthesized by way of the phthalimido-chloride method; namely, β-D-Gal*p*-(1→3)-β-D-GlcNAc*p*-(1→6)-D-Gal*p* (**3**), β-D-Gal*p*-(1→3)-β-D-GlcNAc*p*-(1→3)-D-Gal*p* (**5**), β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→6)-D-Gal*p* (**4**), and β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→3)-D-Gal*p* (**6**). The syntheses of the 8-methoxycarbonyloctyl β-glycosides of these trisaccharides are also reported.

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Faisant appel à la méthode du chlorure phtalimide, on a synthétisé les trisaccharides correspondant à la chaîne centrale des déterminants du groupe sanguin humain: β-D-Gal*p*-(1→3)-β-D-GlcNAc*p*-(1→6)-D-Galp (**3**), β-D-Gal*p*-(1→3)-β-D-GlcNAc*p*(1→3)-D-Gal*p* (**5**), β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→6)-D-Gal*p* (**4**) et β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→3)-D-Gal*p* (**6**). On rapporte également les synthèses des méthoxycarbonyl-8-octyl β-glycosides de ces trisaccharides.

[Traduit par le journal]

The core structure of the oligosaccharide structures which lead to the human ABH and Lewis antigenic determinants as terminal units can occur as linear or branched chains (1). In the case of the blood group specific glycoproteins, the first disaccharide unit (βDGal(1→3)αDGalNAc) is attached to either a serine or threonine residue of the core protein as an *O*-glycoside. The oligosaccharide is then elaborated to produce the structures which are incorporated into the basic chain shown in Fig. 1. This chain is taken from the composite megalosaccharide structure proposed by Lloyd *et al.* (2) to include the known structural features. It is not meant to indicate a specific structure.

In the case of the ABH active glycosphingolipids which have been isolated from human red cells, the basic core structure, as illustrated in Fig. 1 for a very simple structure, begins with a βDGal(1→4)-βDGlc (lactose) unit attached to the ceramide. The oligosaccharide is then mainly elaborated in this case by way of *N*-acetyllactosamine residues. Kościelak[3] has classified these as both oligo- and poly(glycosyl)ceramides.

The Ii antigens (5) consist of a chemically ill-defined group of human antigenic determinants. The I antigens are well expressed on the red cells of the majority of adults and the i antigens are found on red cells from the umbilical cords of newborn infants and the red cells of a rare group of adults which lack the I antigens (6–8). The change from i to I specificity occurs gradually during the first year of life (6) and appears to be related to the branching of the core oligosaccharide structures (9, 10). Recently the i specificity has been associated with at least two *N*-acetyllactosamine units which are linked 1→3′ (11). The I specificities appear numerous since a wide range of anti-I antibodies of different specificities are recognized (12). These antibodies are termed cold agglutinins because these do not agglutinate red cells at body temperature. These can occur in the plasma as autoantibodies and give rise to haemolytic disorders (13). It has become evident (14) that many of these anti-I antibodies are directed against carbohydrate structures and that at least some of these structures constitute the inner portions of the carbohydrate chains in the blood-group specific glycoproteins and glycolipids.

We set out several years ago[4] to synthesize portions of the structures shown in Fig. 1 as possible candidates for Ii activities (14). In this context and judging from the structures in Fig. 1, thirteen trisaccharide structures are indicated. In this regard, it must be noted that, since most of the anti-I cold agglutinins (antibodies) are monoclonal, it cannot be expected that a trisaccharide will have strong I activity unless the combining site is directed to the central sugar unit and only to portions of the two terminal units, as has now been

[1]University of Alberta Postdoctoral Fellow 1975–1977. Present address: Chembiomed Ltd., W5-56 Chemistry Bldg., University of Alberta, Edmonton, Alta., Canada T6G 2G2.

[2]University of Alberta Postdoctoral Fellow 1975–1977. Present address: Dept. of Chemistry, Korea University, Seoul, Korea.

[3]J. Kościelak. Joint Meeting of International Soc. Hematology/International Soc. Blood Transfusion, Montreal, Aug. 16–22, 1980. Abstracts of Papers, No. 298.

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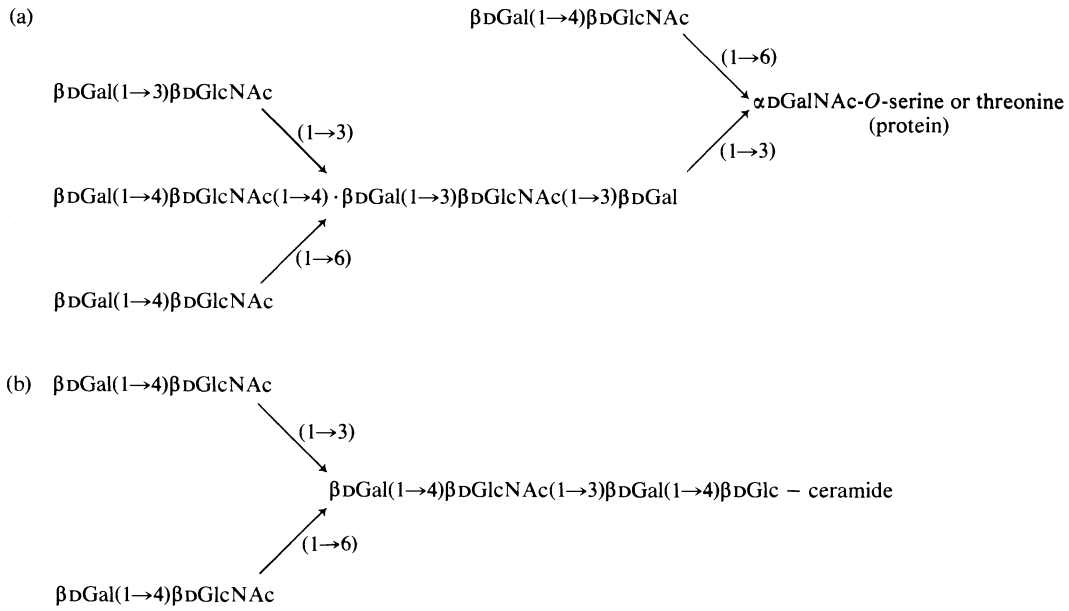


FIG. 1. (a) The composite "megalosaccharide" structure for the branched carbohydrate chains in blood-group specific glycoproteins by Lloyd *et al.* (2). (b) The core structure for the H (Type 2) active glycosphingolipid isolated by Watanabe and co-workers (3) and Kościelak *et al.* (4) have shown that poly(glycosyl)ceramides can have up to 59 glycosyl units and are multibranched.

established for the IMa determinant (15). Consequently, to properly delineate the I activities, it may prove necessary to synthesize tetra-, penta-, and even higher saccharides including branched structures.

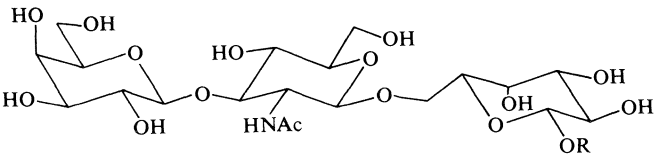
We wish to report herein the synthesis of the four core chain trisaccharides both as the reducing trisaccharides (**3** to **6**) and as the glycosides (**9** to **12**). These were synthesized by way of condensation of hexa-*O*-acetyl-2-deoxy-2-phthalimido-α,β-D-lactosyl chloride (16) (**15**) and the corresponding derivative (17) (**14**) of lacto-*N*-biose I with the alcohols **1**, **2**, **7**, **8**, and **13**.

The condensations of the phthalimido-chlorides with the primary hydroxyl of **1** proceeded without complications and in acceptable yield (about 75%). However, the condensations with the secondary hydroxyl group of **2** were not satisfactory. In the case of the Type 1 reagent **14** to form the blocked derivative of **5**, the yield was only 24%. Indeed, attempts to prepare the Type 2 trisaccharide **6** by condensing **2** with the lactosamine derivative **15** also failed. Although the matter was not investigated in detail, we consider that the diisopropylidene compound **2** is unsatisfactory as a starting material because the cationic intermediate, which is liberated on reaction of the silver-triflate – *sym*-collidine complex with the phthalimido-chloride (**14** or **15**), attacks the *O*-isopropylidene groups at an overall faster rate than it attacks the hydroxyl group. Such an occurrence would not be without precedent (18, 19).

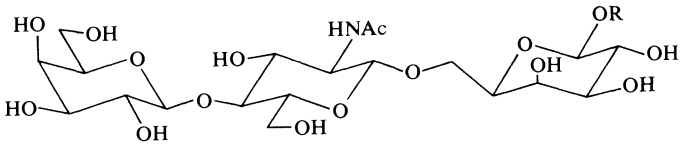
In order to achieve a synthesis of **6**, the condensation of hexa-*O*-acetyl-2-deoxy-2-phthalimido-β-D-lactosyl chloride (**15**) with the galactose derivative **13** was examined. In this case, the reaction proceeded smoothly to provide the desired condensation product in 80% yield. The eventual overall yield of **6**, however, proved to be unsatisfactory.

Although the synthesis of oligosaccharides, such as compounds **3** to **6**, which may correspond to antigenic determinants, can play an important role as inhibitors in studies of antibody–antigen interaction, the synthesis of the oligosaccharide attached to an aglycon which can serve as a linking arm for the preparation of artificial antigens and immunoadsorbents (20) should also provide structures which are useful in such inhibition studies. The trisaccharides **3** to **6** were synthesized as β-glycosides (**9** to **12**) of 8-methoxycarbonyloctanol since this aglycon has proven highly attractive as a linking arm (21). These structures were synthesized starting from the alcohols **7** and **8**.

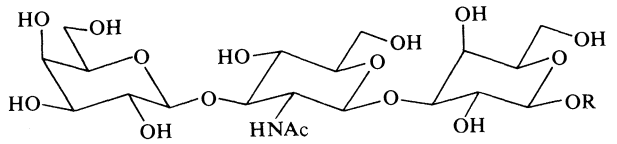
The ^{1}H- and ^{13}C-chemical shifts for the glycosides **9** to **12** are presented in Table 1. Inspection of these data will show that the effects of substitutional changes are in accord with previous experi-



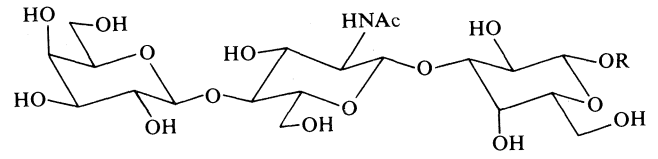
3 R = H(α, β) **9** R = $(CH_2)_8COOCH_3$



4 R = H(α, β) **10** R = $(CH_2)_8COOH_3$



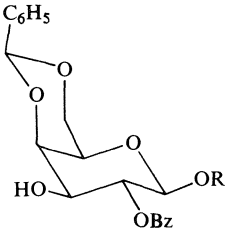
5 R = H(α, β) **11** R = $(CH_2)_8COOCH_3$



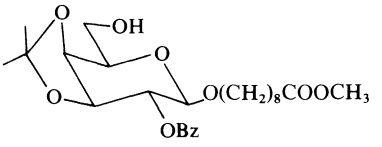
6 R = H(α, β) **12** R = $(CH_2)_8COOCH_3$

ence in these regards (22) and clearly confirm the structural assignments. The ^{13}C chemical shifts are seen to be characteristic of a given disaccharide unit (βDGal(1→3)βDGlcNAc, βDGal(1→4)βDGlcNAc, βDGlcNAc(1→6)βDGal, or βDGlcNAc(1→3)βDGal). The only significant changes which occur are at C1 because of a change in aglycon. These observations support the contention that ^{13}C nmr can play an important role in structural investigation of complex oligosaccharides especially since these appear to be less sensitive to changes in environment, such as the specific interunit chemical shifts which may be observed in the case of ^{1}H-chemical shifts (22).

In the preparations of compounds **9** to **12**, intermediates are encountered wherein it is necessary to remove the phthaloyl group in order to convert the phthalimido group to an acetamido group. Initially, we performed this by alkaline hydrolysis to the amino acid and this stage was followed by peracetylation and then conversion of the carboxyl group of the bridging arm to methyl ester using diazomethane. Since then, a controlled hydrazinolysis of the phthalimido group, to avoid transformation of the methyl ester grouping of the linking arm to acyl hydrazide, was developed by Bundle and Josephson (23), which is the method of choice (24).



7 R = $(CH_2)_8COOCH_3$
13 R = CH_2CCl_3



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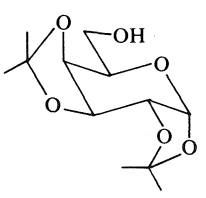

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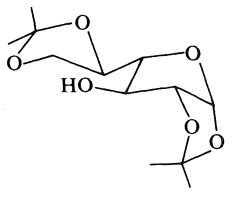

2

Compounds **3** to **6** and **9** to **12** have now been examined for I activity by examining their ability to

inhibit the precipitation of an I active glycoprotein by a variety of anti-I agglutinins (25, 26). As was to be expected, compounds **4** and **10** were excellent inhibitors and, interestingly, provided, under the test conditions used, identical 50% inhibitions. An accompanying paper (27) follows up on this observation by way of the preparation of the diastereoisomeric 6-*C*-methyl derivatives of **4**. The D-isomer proved to be superior to **4** as an inhibitor whereas the L-isomer was virtually ineffective. In this way, it proved possible to establish the conformation of **4** which is bound by the monoclonal anti-I Ma antibody (15).

Experimental

The general experimental procedures in terms of preparation of solvents and reagents, evaporations, chromatographic separations, and analytical methods were the same as previously described (17).

*1,2;5,6-Di-*O*-isopropylidene-α-D-galactofuranose (**2**)*

Prepared as previously described (28) (see also Morgenlie (29) and Paulsen and Behre (30)).

*8-Methoxycarbonyloctyl 2-*O*-benzoyl-4,6-*O*-benzylidine-β-D-galactopyranoside (**7**)*[5]

A solution of chloroacetyl chloride (27.3 mL, 0.34 mol) and pyridine (0.34 mol) in dichloroethane (100 mL) was added dropwise over 2 h to a stirred solution of 8-methoxycarbonyloctyl 4,6-*O*-benzylidene-β-D-galactopyranoside (123 g, 0.28 mol) (31) in dichloromethane and kept near −78°C. The temperature was then allowed to rise to −10°C and the solution was poured into vigorously stirred water (5 L). The product was isolated in the usual way and then benzoylated without further purification. The ^{13}C nmr spectrum of the syrupy product (220 g) indicated that one component represented about 80% of the mixture. This crude product (215 g) was added to dry methanol (2 L) and the mixture was cooled to −30°C. On adding a saturated (−5°C) solution of ammonia in methanol (180 mL), a clear solution was obtained (32). When none of the starting material remained (tlc using ethyl acetate – *n*-hexane (1:1)), the solution was taken to dryness. Column chromatography (silica gel and graded elution using ethyl acetate and *n*-hexane) of a portion of the solid residue provided a fraction (77% yield) which crystallized from ethanol. Recrystallization from ethanol provided the analytical sample, mp 119–120°C, $[\alpha]_D^{24}$ +7.1° (*c* 1, $CHCl_3$). *Anal.* calcd. for $C_{30}H_{38}O_9$: C 66.41, H 7.06; found: C 66.21, H 7.09.

Both the ^1H and ^{13}C nmr spectra were in accord with those expected for the title compound. The position of the signal for H2 at 5.33 ppm (dd, 8 and 10 Hz) confirmed the structural assignment. Although *O*-chloroacetyl groups can be removed by treatment with thiourea (32), ammonolysis proved more convenient in this case.

*8-Methoxycarbonyloctyl 2-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**8**)*

A solution of compound **7** (3.5 g, 6.45 mmol) in 80% aqueous acetic acid (60 mL) was kept at 75°C for 1 h. Solvent removal left a residue which was triturated with hexane (200 mL). The residue (2.7 g) was dissolved in dry *N,N*-dimethylformamide

[5]Prepared by Y. Fouron, University of Alberta postdoctoral fellow, 1975–1977.

TABLE 1. The $^1H^a$ and ^{13}C nmr[b] chemical shifts for glycosides in D_2O (R = $(CH_2)_8COOCH_3$)

Compound	βDGal[c]			βDGlcNAc						βDGal[d]—OR			
	H1[e]	C1	C2	H1[f]	C1	C2	C3	C4	C5	C6	H1[f]	C3	C4	C5	C6
βDGal—OR (22)	—	—	—	—	—	—	—	—	—	—	4.62	72.8	68.6	75.0	60.8
βDGal(1→3)βDGlcNAc—OR (22)	4.69	103.5	70.7	4.81	100.9	54.7	82.6	68.8	75.4	60.9	—	—	—	—	—
βDGal(1→3)βDGlcNAc(1→6)βDGal—OR (**9**)	4.73	103.6	70.7	4.88	101.2	54.6	82.6	68.9	75.4	60.9	4.66	72.8	68.8	73.4	70.7
βDGal(1→3)βDGlcNAc(1→3)βDGal—OR (**11**)	4.73	103.5	70.7	5.05	102.8	55.1	82.5	68.9	75.4	60.9	4.67	82.4	68.4	74.7	60.9
βDGal(1→4)βDGlcNAc—OR (22)	4.74	103.0	71.0	4.79	101.0	55.2	72.6	78.9	74.8	60.3	—	—	—	—	—
βDGal(1→4)βDGlcNAc(1→6)βDGal—OR (**10**)	4.77	102.9	71.0	4.86	101.4	55.2	72.6	78.8	75.0	60.3	4.66	72.8	68.7	73.4	70.6
βDGal(1→4)βDGlcNAc(1→3)βDGal—OR (**12**)	4.77	102.9	71.0	5.02	103.0	55.7	72.6	78.7	75.0	60.3	4.66	82.4	68.3	74.7	60.8

[a] At 400 MHz (305 K) and setting the chemical shift for the methoxyl group of the side chain at 3.98 ppm.
[b] At 22.6 MHz (305 K) and setting the chemical shift for C6 of the terminal βDGal group at 61.0 ppm.
[c] δ (± 0.05 ppm): 72.6 (C3), 68.6 (C4), 75.4 (C5).
[d] δ (± 0.05 ppm): 102.8 (C1), 70.6 (C2).
[e] Doublet, spacing 7.8 Hz.
[f] Doublet, spacing 8.0 Hz.

(30 mL), and dimethoxypropane (1.17 g, 11.2 mmol) and *p*-toluenesulphonic acid (50 mg) were added. The reaction and isolation of the product were the same as previously described (31) for the preparation of 2,2,2-trichloroethyl 3,4-*O*-isopropylidene-β-D-galactopyranoside. The crude product (2.8 g) was dissolved in dry pyridine (30 mL) and triphenylmethyl chloride (2.28 g) was added. The resulting solution was kept at 65°C for 12 h. The product was isolated in the usual manner and chromatographed on silica gel using a mixture of hexane – ethyl acetate (8:1). The first fraction provided a solid which crystallized from hexane (2.0 g), mp 102–103°C, $[\alpha]_D^{25}$ −5.8° (*c* 1.2, $CHCl_3$). The ^1H nmr was consistent with that expected for the 6-*O*-trityl derivative of the title compound **8**. Hydrogenolysis of this product (1.95 g) in ethanol using 5% palladium-on-charcoal at 200 psi of hydrogen pressure gave a solid which was triturated with hexane (100 mL). The residue crystallized from a mixture of hexane–ether (1.18 g, 37% yield from **7**), mp 47–49°C, $[\alpha]_D^{25}$ +17.6° (*c* 0.8, $CHCl_3$). *Anal.* calcd. for $C_{26}H_{38}O_9$: C 63.13, H 7.74; found: C 63.28, H 7.66. The ^{13}C nmr spectrum was in complete accord with the structural assignment.

*2,2,2-Trichloroethyl 2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (**13**)*

2,2,2-Trichloroethyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (mp 198–199°C, $[\alpha]s^{24}$ −60.40° (*c* 1, $CHCl_3$)) was readily prepared from 2,2,2-trichloroethyl-β-D-galactopyranoside (33) following the procedure published by Chittenden and Buchanan (34) for the preparation of the analogous benzyl glycoside. In order to have the benzoyl group migrate from the 3- to the 2-position, a solution of the compound (5.0 g, 9.9 mmol) in dimethylsulfoxide (20 mL) was added to 0.05 *N* aqueous sodium hydroxide (20 mL). After 2 min at room temperature, the solution was poured into ice water and immediately extracted with chloroform (250 mL) to afford a product which was subjected to chromatographic separation on a silica gel column using ethyl acetate – chloroform as solvent. The second fraction to appear in the eluate contained a compound (1.7 g, 35% yield) which crystallized readily, mp 206–208°C, $[\alpha]_D^{23}$ −17.5° (*c* 0.9, $CHCl_3$), which was isomeric (^1H nmr) to the starting material, and which necessarily, from a quartet centered at 5.43 ppm with spacings of 8 and 10 Hz, had the benzoyl group at the 2-position. *Anal.* calcd. for $C_{22}H_{21}O_7Cl_3$: C 52.45, H 4.20, Cl 21.11; found: C 52.40, H 4.29, Cl 21.24.

*4,6-Di-O-acetyl-3-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl chloride (**14**)*

This reagent was prepared as described in a separate communication (17).

*3,6-Di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl chloride (**15**)*

This reagent was prepared by way of one of the two procedures described in a separate communication (16). The route involving the azidonitration is recommended.

General methods

(a) *Glycosylation reactions*

In general, a solution of the phthalimido-chloride (either **14** or **15**) (3.5 mmol) in nitromethane (10 mL) was added dropwise to a solution of the alcohol (3.5 mmol), silver trifluoromethylsulfonate (3.7 mmol), and *sym*-collidine (3.7 mmol) in nitromethane (20 mL) kept at −15° to −30°C. The resulting mixture was stirred at −15°C for 1 h and then at room temperature for 2 h. Chloroform was then added and the solids removed by filtration prior to washing, first with water, then cold dilute hydrochloric acid, then with dilute aqueous sodium bicarbonate solution, and finally with water. The solid foam which remained after solvent removal was dissolved in ethyl acetate and this solution was applied to a short column of neutral alumina. The product obtained on elution with ethyl acetate was purified as is indicated.

(b) *Hydrolysis of O-isopropylidene groups*

In general, the compound (1 mmol) was dissolved in 95% aqueous trifluoroacetic acid (35) at room temperature. After 15 min the solvents were rapidly removed *in vacuo* near room temperature. The residue was then processed as is indicated.

(c) *Removal of the phthaloyl group and* N*-acetylation*

For the products (about 0.5 mmol) formed on glycosylation of the di-*O*-isopropylidene compounds **1** and **2**, both the acetyl and phthaloyl groups were removed simultaneously by reaction with a large excess of hydrazine hydrate (2 mL) in ethanol (20 mL) at reflux. After solvent removal, the residue was dissolved in 50% aqueous methanol (20 mL), acetic anhydride (5 mL) added, and the solution stirred for 2 h. Solvent removal left a residue which was processed as is indicated.

In the case of the products formed on the glycosylation of the compounds **7** and **8**, the *O*-isopropylidene or the *O*-benzylidene group was first removed by acid hydrolysis. Refluxing 80% acetic acid was used to hydrolyze the benzylidene group. A 10% solution (10 mL) of 99% trifluoracetic acid in dichloromethane was used to remove the *O*-isopropylidene group. The resulting product was taken up in 40% aqueous methanol containing an excess of sodium hydroxide and the solution was heated under reflux for 3 h in order to saponify the methyl ester. The solution was then evaporated to dryness. The residual salt-cake was acetylated overnight with an excess of a mixture (2:1) of pyridine – acetic anhydride. Solvent removal left a residue which was treated with cold 0.1 *N* aqueous hydrochloric acid prior to extraction with dichloromethane. The crude acidic product thus obtained was heated under reflux for 3 h with 40% aqueous methylamine in methanol in order to effect deacetylation and removal of the phthaloyl group. The product was then reacetylated using pyridine – acetic anhydride (2:1) in the usual manner.

The resulting product was then dissolved in dichloromethane for treatment with an excess of diazomethane in ether. The peracetate of the desired compound was then isolated by chromatographic separation on a silica gel column (2.5 × 15 cm) using first ethyl acetate – benzene (1:1) and then this solvent mixture containing 5% methanol. It is to be stressed at this point that this multistep procedure was used only because it was convenient at the time these preparations were made. It is not recommended. The procedure developed by Bundle and Josephson (23) and which was recently used in this laboratory (24) is more convenient and proceeds in better yield.

*6-O-[4,6-Di-O-acetyl-3-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (**16**)*

The crude product from the reaction of **1** with **14** was applied to a silica-gel column and the chromatogram was developed with ethyl acetate – hexane (1:1). The second fraction contained a colorless solid which was recrystallized from ethanol (76% yield), mp 119–121°C, $[\alpha]_D^{25}$ −39.1° (*c* 0.6, $CHCl_3$). The ^1H nmr spectrum required the presence of the phthalimido group, the two isopropylidene groups, and the six acetyl groups. *Anal.* calcd. for $C_{44}H_{55}NO_{23}$: C 54.71, H 5.73, N 1.45; found: C 53.84, H 5.64, N 1.42.

*6-O-[2-Acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (**17**)*

The product from the hydrazinolysis and then *N*-acetylation

of **16** was passed through a column of Dowex 1-X 8 (OH^- form) using methanol as solvent. Solvent removal left a residue which crystallized from methanol – diethyl ether (74% yield). Recrystallization from methanol–acetone gave an analytically pure compound, mp 225–227°C (dec.), $[\alpha]_D^{24}$ −50.3° (*c* 0.5, H_2O). The ^1H and ^{13}C nmr were consistent with the structural assignment.

*6-O-[2-Acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-D-galactopyranose [βDGal(1→3)βD-GlcNAc(1→6)DGal] (**3**)*

The product from the hydrolysis of **17** using trifluoroacetic acid was recrystallized from methanol (64% yield), mp 175–178°C (dec.), $[\alpha]_D^{23}$ −0.9° (*c* 0.8, H_2O) (lit. (36) mp 177–184°C, $[\alpha]_D^{20}$ −9.5° to −1.2° (1 h) (*c* 0.84, water)). *Anal.* calcd. for $C_{20}H_{35}NO_{16}\cdot H_2O$: C 42.62, H 6.61, N 2.38; found: C 42.65, H 6.39, N 2.20.

*6-O-[4,6-Di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (**18**)*

The crude product from the reaction of **1** with **15** crystallized on solvent removal (80% yield) and was recrystallized from ethanol, mp 201–202°C, $[\alpha]_D^{29}$ −19.6° (*c* 0.8, $CHCl_3$). The ^1H nmr spectrum confirmed the presence of the phthalimido group, the two isopropylidene groups, and the six acetyl groups. *Anal.* calcd. for $C_{44}H_{55}NO_{23}$: C 54.71, H 5.73, N 1.45; found: C 55.04, H 5.68, N 1.50.

*6-O-[2-Acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-1,2-3,4-di-O-isopropylidene-α-D-galactopyranose (**19**)*

The product from the hydrazinolysis and then *N*-acetylation of **18** was treated as described above in the preparation of **17**. The yield was 87%, mp 212–214°C, $[\alpha]_D^{23}$ −47.2° (*c* 0.9, H_2O). The ^1H and ^{13}C nmr spectra were consistent with the structural assignment. *Anal.* calcd. for $C_{26}H_{43}NO_{16}\cdot H_2O$: C 48.51, H 7.04, N 2.17; found: C 48.49, H 6.69, N 2.06.

*6-O-[2-Acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-D-galactopyranose [βDGal(1→4)βD-GlcNAc(1→6)DGal] (**4**)*

The product from the hydrolysis of **19** using trifluoroacetic acid was recrystallized from ethyl acetate – methanol (73% yield), mp 155–159°C (dec.), $[\alpha]_D^{24}$ +9.6 (*c* 0.5, H_2O) (lit. (36) $[\alpha]_D^{20}$ +4.0° (*c* 1, H_2O)). *Anal.* calcd. for $C_{20}H_{35}NO_{16}\cdot H_2O$: C 42.62, H 6.61, N 2.48; found: C 42.18, H 6.04, N 2.16.

*3-O-[4,6-Di-O-acetyl-3-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-1,2;5,6-di-O-isopropylidene-α-D-galactofuranose (**20**)*

The crude product from the reaction of **2** with **14** was chromatographed on a silica-gel column using ethyl acetate – hexane (1:1). A fraction (24% yield) crystallized, mp 104–107°C, and was recrystallized from ethanol, mp 106–109°C, $[\alpha]_D^{25}$ −15.5° (*c* 0.5, $CHCl_3$). The ^1H nmr spectrum confirmed the presence of the phthalimido group, the two isopropylidene groups, and the six acetyl groups. *Anal.* calcd. for $C_{44}H_{55}NO_{23}\cdot H_2O$: C 53.71, H 5.84, N 1.42; found: C 53.63, H 5.51, N 1.79.

*3-O-[2-Acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-1,2;5,6-di-O-isopropylidene-α-D-galactofuranose (**21**)*

The product from the hydrazinolysis and then *N*-acetylation of **20** was crystallized from methanol – ethyl acetate (50% yield), mp 182–184°C (dec.), $[\alpha]_D^{24}$ −13.4° (*c* 0.4, H_2O). The ^1H and ^{13}C nmr spectra were consistent with the structural assignment.

*3-O-[2-Acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-D-galactopyranose [βDGal(1→3)βD-GlcNAc(1→3)DGal or lacto-N-triose I] (**5**)*

The product from the trifluoroacetic acid hydrolysis of **21** crystallized from methanol (60% yield), mp 139–141°C (dec.), $[\alpha]_D^{23}$ +13.6° (*c* 0.5, H_2O). These physical constants are substantially lower than those reported by Kuhn *et al.* (37), mp 183–185°C, $[\alpha]_D^{23}$ +19.3° (*c* 2, H_2O), and essentially confirmed by Augé and Veyrières (38). Because of the paucity of material (46 mg), this matter was not investigated further except that sugar analysis required the presence of two galactose units per unit of *N*-acetylglucosamine (performed by Professor E. A. Kabat).

*2,2,2-Trichloroethyl 3-O-[3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (**22**)*

The product from the reaction of **13** with **15** crystallized in 80% yield (mp 228–231°C) and was recrystallized from ethanol, mp 252–254°C, $[\alpha]_D^{24}$ +8.6° (*c* 0.8, $CHCl_3$). The ^1H nmr spectrum confirmed the presence of the three aromatic rings and the six acetyl groups. *Anal.* calcd. for $C_{54}H_{56}NO_{24}Cl_3$: C 53.62, H 4.66, N 1.15, Cl 8.79; found: C 53.26, H 4.58, N 1.04, Cl 8.71.

*2,2,2-Trichloroethyl 2-O-acetyl-3-O-[2-acetamido-3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl]-4,6-O-benzylidene-β-D-galactopyranoside (**23**)*

The product of hydrazinolysis of **22** (1.82 g, 1.5 mmol) was acetylated with pyridine – acetic anhydride (2:1). A solution of the peracetate in ethyl acetate was passed through a short column of neutral alumina. Solvent removal left a crystalline solid (mp 144–149°C, 1.35 g, 85% yield) which was recrystallized from ethyl acetate – hexane, mp 150–152°C, $[\alpha]_D^{23}$ −1.9° (*c* 0.8, $CHCl_3$). The ^1H nmr spectrum displayed the presence of the benzylidene group and eight acetyl groups. *Anal.* calcd. for $C_{43}H_{54}NO_{23}Cl_3$: C 48.75, H 5.13, N 1.32; found: C 48.60, H 5.19, N 1.23.

*2,2,2-Trichloroethyl 2,4,6-tri-O-acetyl-3-O-[2-acetamido-3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl]-β-D-galactopyranoside (**24**)*

Compound **23** (1.00 g, 0.94 mmol) was kept at 100°C in 60% aqueous acetic acid for 40 min. The product was acetylated in pyridine – acetic anhydride (2:1) in the usual manner to provide a solid which crystallized from ethyl acetate – hexane (0.50 g, 50% yield), mp 138–141°C, $[\alpha]_D^{23}$ +2.2° (*c* 1, $CHCl_3$). *Anal.* calcd. for $C_{40}H_{54}NO_{25}Cl_3$: C 45.52, H 5.15, N 1.32, Cl 10.07; found: C 45.19, H 5.09, N 1.24, Cl 10.0.

*2,2,2-Trichloroethyl 3-O-[2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**25**)*

Compound **24** (190 mg, 0.18 mmol) was dissolved in a triethylamine–water–methanol (1:1:8) mixture (12 mL) and the solution left at room temperature for 16 h. The solution was concentrated to dryness and the residue was passed through a short column of Dowex (2-X8, OH^- form) using water. The aqueous fractions were combined and freeze-dried to give a powder (90 mg, 75% yield), $[\alpha]_D^{23}$ −4.88° (*c* 0.84, H_2O). The ^1H and ^{13}C nmr spectra were consistent with the structural assignment. *Anal.* calcd. for $C_{21}H_{36}NO_{16}Cl_3\cdot H_2O$: C 36.93, H 5.60, N 2.05; found: C 36.48, H 5.07, N 1.85.

*3-O-[2-Acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-D-galactopyranose [βDGal(1→4)βD-GlcNAc(1→3)DGal] (**6**)*

Compound **25** (85 mg) in glacial acetic acid (2 mL) was treated

with a zinc–copper couple (33, 39) (20 mg) at room temperature for 4 h. The product was purified on a silica gel column using chloroform–methanol–water (65:35:8) (40 mg, 57% yield), $[\alpha]_D^{23}$ +17.5° (*c* 0.5, H_2O) (lit. (36) $[\alpha]_D^{20}$ +19.5 (*c* 0.62, H_2O)). *Anal.* calcd. for $C_{20}H_{35}NO_{16}\cdot H_2O$: C 42.62, H 6.61, N 2.48; found: C 42.27, H 6.29, N 2.20.

*8-Methoxycarbonyloctyl 6-O-[4,6-di-O-acetyl-3-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-2-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranoside (**26**)*

The product obtained on the condensation of compounds **8** and **14** was obtained in 71% yield (mp 87–91°C) after passage through a short column of neutral alumina. Since the ^1H nmr spectrum was in good accord with expectation, no attempt was made to achieve higher purity. *Anal.* calcd. for $C_{58}H_{73}NO_{26}$: C 58.04, H 6.13, N 1.16; found: C 58.24, H 6.04, N 1.33.

*8-Methoxycarbonyloctyl 2,3,4-tri-O-acetyl-6-O-[2-acetamido-4,6-di-O-acetyl-3-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl]-β-D-galactopyranoside (**27**)*

Compound **26** (1.35 g, 1.1 mmol) was subjected to the multi-step process for the conversion of all the protecting groups to acetyl groups. The major fraction (amorphous, 372 mg, 30% yield) $[\alpha]_D^{23}$ −13.7° (*c* 0.7, $CHCl_3$), appeared chromatographically pure and possessed an ^1H nmr spectrum consistent with that expected for the title compound. *Anal.* calcd. for $C_{48}H_{71}NO_{27}$: C 52.69, H 6.54, N 1.28; found: C 53.53, H 6.80, N 1.12.

*8-Methoxycarbonyloctyl 6-O-[2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside [βDGal(1→3)βDGlcNAc(1→6)βDGalO(CH$_2$)$_8$COOCH$_3$] (**9**)*

Compound **27** (325 mg, 0.29 mmol) was dissolved in 0.01 *N* sodium methoxide in methanol (15 mL) and the solution was kept overnight at room temperature. After deionization with Amberlite 120H$^+$, the solvent was removed and the residue was dissolved in water, the solution clarified and freeze dried to a colorless powder (196 mg, 97% yield), mp 185–188°C, $[\alpha]_D^{23}$ −20.9° (*c* 1, H_2O). The ^1H and ^{13}C nmr (see Table 1) required a high purity and were consistent with the structural assignment. *Anal.* calcd. for $C_{30}H_{53}NO_{18}\cdot H_2O$: C 49.17, H 7.39, N 1.90; found: C 49.20, H 7.30, N 1.87.

*8-Methoxycarbonyloctyl 6-O-[3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-2-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranoside (**28**)*

The product obtained on the condensation of compounds **8** and **15** was obtained in 64% yield as an amorphous powder, $[\alpha]_D^{23}$ +21.3° (*c* 1.2, $CHCl_3$). The ^1H nmr was consistent with the structural assignment. *Anal.* calcd. for $C_{58}H_{73}NO_{26}$: C 58.04, H 6.13, N 1.16; found: C 57.93, H 6.06, N 1.17.

*8-Methoxycarbonyloctyl 2,3,4-tri-O-acetyl-6-O-[2-acetamido-3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl]-β-D-galactopyranoside (**29**)*

Compound **28** (1.02 g, 0.84 mmol) was subjected to the multi-step procedure for conversion of all the protecting groups to acetyl groups. The major fraction (318 mg, 44% yield), $[\alpha]_D^{23}$ −18.3° (*c* 0.5, $CHCl_3$), appeared chromatographically pure and possessed an ^1H nmr spectrum consistent with that expected for the title compound. *Anal.* calcd. for $C_{48}H_{71}NO_{27}$: C 52.69, H 6.54, N 1.28; found: C 52.42, H 6.47, N 1.20.

*8-Methoxycarbonyloctyl 6-O-[2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside [βDGal(1→4)βDGlcNAc(1→6)βDGalO(CH$_2$)$_8$COOCH$_3$] (**10**)*

De-*O*-acetylation of **29** (194 mg, 0.17 mmol) as was described for the preparation of **9**, provided a powder (118 mg, 94% yield), mp 202–205°C, $[\alpha]_D^{23}$ −15.7° (*c* 0.8, H_2O). The ^1H and ^{13}C nmr spectra (see Table 1) required high purity and were consistent with structural assignment. *Anal.* calcd. for $C_{30}H_{53}NO_{18}\cdot H_2O$: C 49.17, H 7.39, N 1.90; found: C 48.81, H 7.28, N 1.88.

*8-Methoxycarbonyloctyl 3-O-[4,6-di-O-acetyl-3-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (**30**)*

The product obtained on the condensation of compounds **7** and **14** had to be chromatographed on a silica gel column to afford the title compound as an amorphous solid in 34% yield, $[\alpha]_D^{23}$ +1.6° (*c* 0.7, $CHCl_3$). The ^1H nmr spectrum was consistent with the structural assignment. *Anal.* calcd. for $C_{62}H_{73}NO_{26}$: C 59.65, H 5.89, N 1.12; found: C 59.38, H 5.85, N 1.12.

*8-Methoxycarbonyloctyl 2,4,6-tri-O-acetyl-3-O-[2-acetamido-4,6-di-O-acetyl-3-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl]-β-D-galactopyranoside (**31**)*

Compound **30** (700 mg, 0.56 mmol) was subjected to the multi-step process for the conversion of all the protecting groups to acetyl groups. The major fraction of the chromatogram provided an amorphous solid (135 mg, 22% yield), $[\alpha]_D^{23}$ +11.1° (*c* 0.9, $CHCl_3$). The ^1H nmr spectrum was consistent with the structural assignment and inferred chemical homogeneity. *Anal.* calcd. for $C_{48}H_{71}NO_{27}$: C 52.69, H 6.54, N 1.28; found: C 52.46, H 6.50, N 1.27.

*Methoxycarbonyloctyl 3-O-[2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside [βDGal(1→3)βDGlcNAc(1→3)βDGalO(CH$_2$)$_8$COOCH$_3$] (**11**)*

De-*O*-acetylation of **31** (125 mg, 0.12 mmol) as was described for the preparation of **9** afforded an amorphous powder (61 mg, 72% yield), $[\alpha]_D^{23}$ −4.7° (*c* 0.9, H_2O). The ^1H and ^{13}C nmr spectra (see Table 1) required high purity and were consistent with the structural assignment.

*8-Methoxycarbonyloctyl 3-O-[3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (**32**)*

The product obtained on the condensation of compounds **7** and **15** had to be chromatographed on a silica gel column to afford the title compound in 44% yield as a colorless solid, $[\alpha]_D^{23}$ +31.5° (*c* 0.7, $CHCl_3$). The ^1H nmr spectrum was consistent with the structural assignment. *Anal.* calcd. for $C_{62}H_{73}NO_{26}$: C 59.65, H 5.89, N 1.12; found: C 59.90, H 6.01, N 1.23.

*8-Methoxycarbonyloctyl 2,4,6-tri-O-acetyl-3-O-[2-acetamido-3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl]-β-D-galactopyranoside (**33**)*

Compound **32** (960 mg, 0.77 mmol) was subjected to the multi-step process for the conversion of all the protecting groups to acetyl groups. The major fraction of the chromatogram provided an amorphous solid (256 mg, 30% yield), $[\alpha]_D^{23}$ +4.6° (*c* 0.75, $CHCl_3$). The ^1H nmr spectrum was consistent with the structural assignment and inferred chemical homogeneity.

8-Methoxycarbonyloctyl 3-O-[2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside [βDGal(1→4)βDGlcNAc(1→3)βDGalO-$(CH_2)_8COOCH_3$] (12)

De-*O*-acetylation of **33** (220 mg, 0.21 mmol) as was described for the preparation of **9** afforded an amorphous powder (141 mg, 97% yield), $[\alpha]_D^{23}$ −3.8° (*c* 1, H_2O). The ^1H and ^{13}C nmr spectra (see Table 1) required high purity and were consistent with the structural assignment. *Anal.* calcd. for $C_{30}H_{53}NO_{18}\cdot H_2O$: C 49.19, H 7.39, N 1.90; found: C 48.80, H 7.23, N 1.91.

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