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SYNTHESIS OF A DIMERIC LEWIS X HEXASACCHARIDE DERIVATIVE CORRESPONDING TO A TUMOR-ASSOCIATED GLYCOLIPID

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ABSTRACT

The dimeric Lewis X hexasaccharide *p*-trifluoroacetamidophenylethyl *O*-β-D-galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (**14**), which is a derivative of a tumor-associated glycolipid, was synthesized from thioglycoside intermediates. A protected disaccharide was used as a key-intermediate for synthesis of the *p*-nitrophenylethyl glycoside of suitably protected *O*-β-D-Gal*p*-(1→4)-*O*-β-D-Glc*p*N-(1→3)-*O*-β-D-Gal*p*-(1→4)-β-D-Glc*p*N, which, after selective deblocking, was di-L-fucosylated and deprotected to give **14**.

INTRODUCTION

In 1984, Hakomori *et al.*[1] reported isolation and characterization of glycolipids from adenocarcinoma tissue having the general structure shown. These structures were not present to any appreciable extent in corresponding normal tissue, and were therefore regarded as tumor-associated. Other, related structures have also been reported[2] to occur only in tumor tissue. These tumor-associated carbohydrate structures are potentially useful in cancer diagnosis and treatment. However, only small amounts can be obtained from natural sources, and chemical synthesis is therefore currently the best way to obtain enough material for extensive biological experimentation.

As part of a program aimed at synthesizing tumor-associated carbohydrate structures, we have synthesized the hexasaccharide glycoside[3] **14** , carrying a *p*-trifluoroacetamidophenylethyl linking arm that makes attachment to proteins, lipids, or solid matrixes possible *via* the corresponding isothiocyanate derivative[4]. We now report full experimental details for the preparation of **14**.

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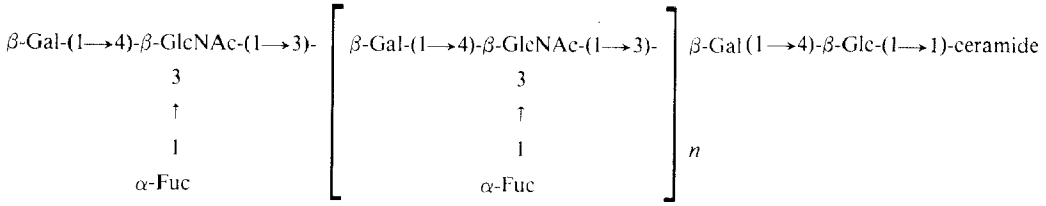


where n = 1 or 2.

RESULTS AND DISCUSSION

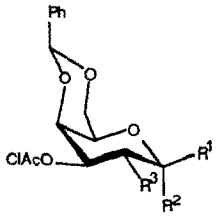
The synthesis was based on thioglycosides as building blocks. Thioglycosides are useful in oligosaccharide synthesis[5] because they are stable under most reaction conditions and can be activated at the anomeric center by treatment with methyl triflate[6], dimethyl(methylthio)sulfonium triflate (DMTST)[7], or halogen[8,9]. All these activation methods were used in the present synthesis.

Our strategy was to synthesize the disaccharide **6**, which has an ethylthio group on C-1, a *p*-methoxybenzyl group on O-3 and a chloroacetyl group on O-3′. The thioethyl group in **6** was converted into a *p*-nitrophenylethyl group, and the chloroacetyl group was removed, to give the acceptor disaccharide **8**, which was coupled with the donor disaccharide **6** to give the linear tetrasaccharide **9**. The *p*-methoxybenzyl groups in **9** were removed, and the resulting tetrasaccharide **10** was di-L-fucosylated, giving the hexasaccharide **11**, which was deblocked to give the target structure. The following steps were performed.

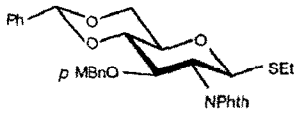
Methyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[10] was selectively chloroacetylated at O-3 by using chloroacetyl chloride (1.1 equiv.) and pyridine (5 equiv.) in dichloromethane, giving compound **1** in 59% yield. Smaller amounts of unreacted starting-material and diacylated product were also observed. Acetylation of **1** with acetyl chloride and pyridine in dichloromethane gave compound **2** in 89% yield. Treatment of **2** with bromine and tetraethylammonium bromide in dichloromethane gave **3** in 84% yield.

Ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[11] was *p*-methoxybenzylated, by using *p*-methoxybenzyl chloride and sodium hydride in *N,N*-dimethylformamide, giving compound **4** in 75% yield. The 4,6-benzylidene acetal ring in **4** was opened by treatment with sodium cyanoborohydride[12,13] and HCl–diethyl ether in tetrahydrofuran, giving the OH-4 compound **5** in 65% yield. No OH-6 compound was observed.

The glycosyl donor, bromide **3**, was coupled with the glycosyl acceptor **5** in the presence of silver triflate and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in dichloromethane, giving the disaccharide **6** in 74% yield. In this glycosidation step ,the amount of DTBMP (1 equiv.) was critical to the outcome of the reaction. Lower proportions resulted in acid-catalysed loss of the *p*-methoxybenzyl group, and



1 $R^1 = SCH_3$, $R^2 = H$, $R^3 = OH$
2 $R^1 = SCH_3$, $R^2 = H$, $R^3 = OAc$
3 $R^1 = H$, $R^2 = Br$, $R^3 = OAc$



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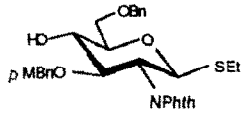
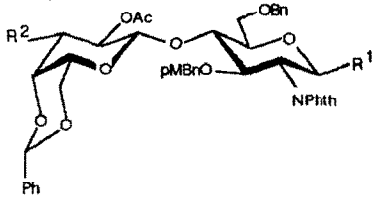
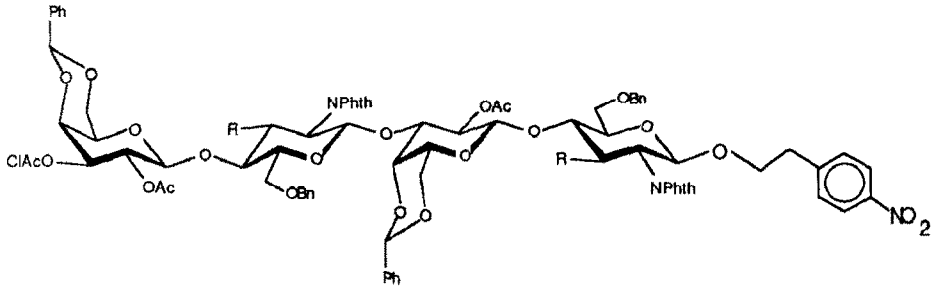


5

*p*MBn = *p*-methoxybenzyl
Phth = phthaloyl



6 $R^1 = SEt$, $R^2 = OCOCH_2Cl$
7 $R^1 = O(CH_2)_2\text{-}C_6H_4\text{-}NO_2$, $R^2 = OCOCH_2Cl$
8 $R^1 = O(CH_2)_2\text{-}C_6H_4\text{-}NO_2$, $R^2 = OH$



9 R = OpMBn
10 R = OH

higher proportions resulted in formation of the corresponding orthoester as a by-product. Similar results have been reported before[14,15].

The thioethyl group in **6** was converted into a *p*-nitrophenylethyl group by treatment with *p*-nitrophenethyl alcohol in dichloromethane, using methyl triflate as

glycosidation promotor and DTBMP as acid acceptor, giving **7** in 70% yield. Treatment of **7** with hydrazine acetate in 1:1 ethyl acetate–methanol removed the chloroacetyl group selectively, giving the OH-3 compound **8** in 83% yield.

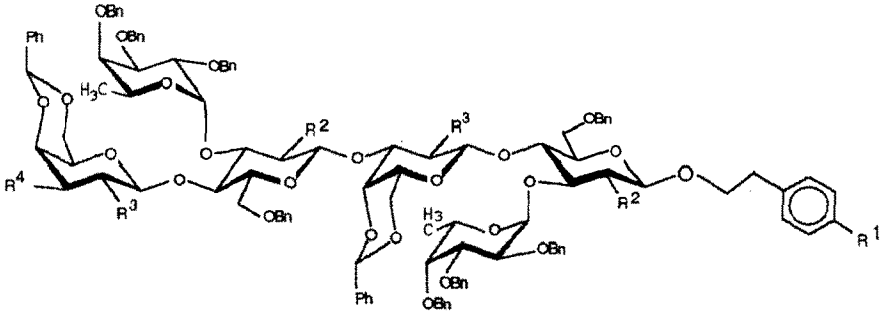
Glycosidation of **8** with **6**, using DMTST as promotor and DTBMP as acid acceptor, gave the tetrasaccharide **9** in 63% yield. The two *p*-methoxybenzyl groups were removed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in water–dichloromethane, to give the diol **10** in 92% yield. Di-L-fucosylation of **10** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[11], using silver triflate–collidine as promotor, gave the hexasaccharide **11** in 70% yield.

The phthalimido groups in **11** were removed by treatment with hydrazine acetate in 2:3 toluene–ethanol giving free amino groups, which were *N*-acetylated with acetic anhydride in 1:1 dichloromethane–methanol giving compound **12** in 86% yield. Reduction of the nitro group in **12** by treatment with aluminum amalgam, followed by treatment of the product with trifluoroacetic anhydride and then with methanolic sodium methoxide gave compound **13** in 61% yield. Finally, **13** was hydrogenolyzed over Pd/C to give the deprotected hexasaccharide **14** in 94% yield.

The structure and purity of **14** were verified by n.m.r. spectroscopy, f.a.b. mass spectrometry, and methylation analysis.

EXPERIMENTAL

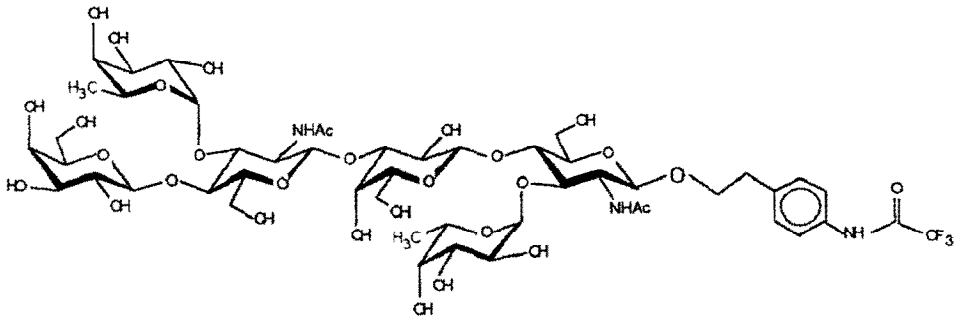
General methods.—Melting points are corrected. Concentrations and evaporations were performed under diminished pressure at $<40°$ (bath). Optical rotations were measured for 0.4–1.0% solutions at room temperature (22–25°) with a Perkin–Elmer 241 polarimeter. T.l.c. was performed on Silica Gel F_{254} (Merck) with detection by u.v., or by charring with sulfuric acid, or both. Column chromatography was performed on silica gel (Matrex Silica Si 60A, 35–70μm, Amicon) using toluene–ethyl acetate mixtures as eluant unless otherwise stated.Organic solutions were dried with magnesium sulfate. Molecular sieves (3 or 4A, Fluka) were desiccated overnight at 300°. Hydrazine acetate was prepared from 1:1 hydrazine hydrate–acetic acid in methanol, and crystallized from diethyl ether. Elemental analyses were not obtained for some syrupy or amorphous compounds. These were purified by column chromatography, and characterized by n.m.r. spectroscopy. N.m.r. spectra were recorded for solutions in $CDCl_3$, using JEOL JNM FX-100, GX-270,GX-400 and Bruker AM 500 MHz instruments, and chemical shifts are given in p.p.m. relative to internal tetramethylsilane, unless otherwise stated. All ^1H assignments were based on 2D experiments. N.m.r. spectra recorded for all new compounds were in agreement with the structures postulated, and only selected data are reported. For some compounds, ^1H shift values and coupling constants (values in parentheses) are given in tabular form. In these tables, the sugar residues are given as GlcNA, GlcNB, GalB etc., where A and B designate increasing distance from the reducing end. For fucose residues, however, A and B designations are arbitrary.



11 $R^1 = NO_2$, R^2 = NPhth, R^3 = OAc, $R^4 = O\text{-}COCH_2Cl$

12 $R^1 = NO_2$, R^2 = NHAc, $R^3 = R^4$ = OH

13 $R^1 = NHCOCF_3$, R^2 = NHAc, $R^3 = R^4$ = OH



14

Methyl 4,6-O-benzylidene-3-O-(chloroacetyl)-1-thio-β-D-galactopyranoside (**1**).—A solution of methyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[10] (5.34 g, 17.9 mmol) in 1:25 pyridine–dichloromethane (625 mL) was treated with chloroacetyl chloride (1.56 mL, 19.6 mmol) at 0° while being stirred. After 1 h water (10 mL) was added, and the mixture was successively washed with M sulfuric acid and water, dried, and evaporated. Column chromatography of the residue gave **1** (3.95 g, 10.5 mmol, 59%). Crystallization from ethyl acetate–petroleum ether gave material having m.p. 174°, $[\alpha]_{578}$ +87° (*c* 0.5, chloroform); R_F 0.76 (1:3 toluene–ethyl acetate); n.m.r. data: ^{13}C, δ 10.6 (MeS), 40.9 (CH_2Cl), 65.7, 69.1, 69.7, 73.5, 76.5 (C-2,3,4,5,6), 85.1 (C-1), 101.0 (PhCH), 126.2–137.6 (aromatic C), and 167.3 (C=O); ^1H, δ 3.61 (m, H-5), 4.03 (dd, $J_{5,6a}$ 1.7, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.11 (t, $J_{1,2} = J_{2,3} = 9.6$ Hz, H-2), 4.35 (d, H-1), 4.36 (dd, $J_{5,6b}$ 1.1 Hz, H-6b), 4.46 (dd, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, H-4), and 4.96 (dd, H-3).

Anal. Calc. for $C_{16}H_{19}ClO_6S$: C, 51.3; H, 5.1; S, 8.6. Found: C, 51.0; H, 5.0; S, 8.4.

Methyl 2-O-acetyl-4,6-O-benzylidene-3-O-(chloroacetyl)-1-thio-β-D-galacto-

pyranoside (**2**).—Acetyl chloride (2.1 mL, 29 mmol) was added to a stirred solution of **1** (5.48 g, 14.6 mmol) and pyridine (10 mL) in dichloromethane (50 mL) at 0°. The mixture was stirred for 2 h at this temperature. Then, water (10 mL) was added, and the mixture was successively washed with M sulfuric acid and water, dried, and evaporated.Column chromatography (3:1 petroleum ether–ethyl acetate) of the residue gave **2** (5.41 g, 13.0 mmol; 89%). Crystallization from ethyl acetate–isooctane gave material having m.p. 115–116°, $[\alpha]_{578}$ +60° (*c* 0.5, chloroform); R_F 0.54 (1:1 toluene–ethyl acetate); n.m.r. data: ^{13}C, δ 10.2 (MeS), 20.8 (Me acetyl), 40.6 (CH_2Cl), 65.7, 69.0, 69.4, 73.3, 74.5 (C-2,3,4,5,6), 81.9 (C-1), 101.1 (Ph*C*H), 126.3–137.5 (aromatic C), 167.0 (C=O chloroacetyl), and 169.4 (C=O acetyl); ^1H, δ 3.62 (m, H-5), 4.04 (dd, $J_{5,6a}$ 1.7, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.37 (dd, $J_{5,6b}$ 1.7 Hz, H-6b), 4.40 (d,$J_{1,2}$ 9.9 Hz, H-1), 4.46 (dd, $J_{3,4}$ 3.6, $J_{4,5}$ 0.9 Hz, H-4), 5.05 (dd, $J_{2,3}$ 9.9 Hz, H-3), and 5.51 (dd, H-2).

Anal. Calc. for $C_{18}H_{21}ClO_7S$: C,51.9; H,5.1; S,7.7 Found: C, 51.8; H, 5.1; S, 7.7.

*2-O-Acetyl 4,6-O-benzylidene-3-O-(chloroacetyl)-α-*D*-galactopyranosyl bromide* (**3**.)—A solution of bromine (0.53 mL, 10 mmol) in dry dichloromethane (5 mL) was added to a stirred solution of **2** (3.95 g, 9.48 mmol) in dry dichloromethane (40 mL) at 0°. After 20 min., tetraethylammonium bromide (1.0 g) was added, and the mixture was stirred for 3 h at room temperature. 1-Hexene (3 mL) was added, and the mixture was stirred until it was completely decolorized (15 min). The mixture was successively washed with cold aqueous sodium hydrogencarbonate and water, dried, and evaporated.The residue crystallized from diethyl ether–hexane to yield **3** (3.58 g, 7.96 mmol, 84%) having m.p. 154–156°, $[\alpha]_{578}$ +293° (*c* 0.6, chloroform); R_F 0.71 (3:1 toluene–ethyl acetate); n.m.r. data:^{13}C,δ 20.6 (Me acetyl), 40.5 (CH_2Cl), 66.8, 67.4, 68.2, 70.7, 72.6 (C-2,3,4,5,6), 89.9 (C-1), 100.7 (Ph*C*H), 126.1–137.1 (aromatic C), 166.8 (C=O chloroacetyl), and 169.6 (C=O acetyl); ^1H,δ 4.13 (m, H-5), 4.59 (dd, $J_{3,4}$ 3.4 Hz, H-4), 5.28 (dd, $J_{1,2}$ 3.8, $J_{2,3}$ 10.5 Hz, H-2), 5.47 (dd, H-3), and 6.82 (d, H-1).

Anal. Calc. for $C_{17}H_{18}BrClO_7$: C, 45.4; H, 4.0. Found: C, 45.0; H, 4.1.

*Ethyl 4,6-O-benzylidene-2-deoxy-3-O-(p-methoxybenzyl)-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**4**). - A solution of ethyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[11] (18.0 g, 40.8 mmol) and *p*-methoxybenzyl chloride (11.0 mL, 81.3 mmol) in dry *N,N*-dimethylformamide (180 mL) was added dropwise to 60% sodium hydride (3.26 g, 80 mmol) at 0° under nitrogen. The mixture was stirred overnight at room temperature. Acetic anhydride (40 mL) was added, and, after 3 h, the mixture was partitioned between toluene and aqueous sodium hydrogencarbonate. The organic layer was washed with water, dried, and evaporated. The product was purified by column chromatography (5:1 petroleum ether–ethyl acetate) and crystallized from dichloromethane–isooctane, to yield **4** (17.1 g, 30.4 mmol; 75%) having m.p. 78–80°, $[\alpha]_{578}$ +59° (*c* 0.5, chloroform);R_F 0.67 (3:1 toluene– ethyl acetate); n.m.r. data: ^{13}C, δ 14.9 (Me ethyl), 24.1 (CH_2S), 54.7 (MeO), 54.9 (C-2), 68.7–81.7 (C-3,4,5,6), 83.0 (C-1), 101.3 (Ph*C*H), 113.4 (aro-

matic C *p*-methoxybenzyl) 123.1–137.3 (aromatic C), 158.8 (aromatic C *p*-methoxybenzyl), 167.4, and 167.7 (C=O phthalimido); ^{1}H, δ 3.71 (m,H-5), 3.83 (dd, $J_{3,4}$ 10.0 Hz, H-4), 4.26 (dd, $J_{1,2}$ 10.6, $J_{2,3}$ 10.0 Hz, H-2) 4.44 (dd, H-3), and 5.34 (d, H-1).

*Ethyl 6-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**5**).—Diethyl ether saturated with hydrogen chloride was added, at room temperature, to a stirred mixture of compound **4** (15.8 g, 28.1 mmol), sodium cyanoborohydride (10.6 g, 169 mmol) and molecular sieves 3A in tetrahydrofuran (300 mL) until the mixture was acidic (as determined with indicator paper). The mixture was stirred for 20 min at room temperature and then triethylamine (15 mL) was added. The mixture was filtered through Celite, washed with water, dried, and evaporated. The syrup resulting was purified twice by column chromatography (3:1 petroleum ether–ethyl acetate), to give pure **5** (10.3 g, 18.3 mmol; 65%), $[\alpha]_{578}$ +39° (*c* 0.8, chloroform); R_F 0.38 (3:1 toluene–ethyl acetate); n.m.r. data: ^{13}C, δ 14.9 (Me ethyl), 24.0 (CH_2S), 54.5 (MeO), 54.9 (C-2), 70.9–79.3 (C-3,4,5,6), 81.2 (C-1), 113.5 (aromatic C *p*-methoxybenzyl), 123.1–137.7 (aromatic C), 158.9 (aromatic C *p*-methoxybenzyl), 167.5, and 168.0 (C=O phthalimido); ^{1}H, δ 2.96 (d, $J_{OH,4}$ 1.8 Hz, OH), 3.67 (m, $J_{4,5}$ 8.4, $J_{5,6a}$ 4.9, $J_{5,6b}$ 4.2 Hz, H-5), 3.76 (dd, $J_{6a,6b}$ 9.8 Hz, H-6a), 3.81 (m, $J_{3,4}$ 8.2 Hz, H-4), 3.84 (dd, H-6b), 4.19 (dd, $J_{1,2}$ 10.4, $J_{2,3}$ 10.3 Hz, H-2), 4.24 (dd, H-3), and 5.27 (d, H-1).

*Ethyl 4-*O*-[2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(chloroacetyl)-β-*D*-galactopyranosyl]-6-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**6**).—A dry solution of silver triflate (2.33 g, 9.07 mmol) in toluene (10 mL) was added to a stirred mixture of **3** (4.23 g,9.41 mmol), **5** (3.40 g, 6.03 mmol), DTBMP (1.25 g, 6.03 mmol), and molecular sieves 4A in dry dichloromethane (150 mL) at −50° under nitrogen. After 30 min at this temperature, sodium thiosulfate (10%, 50 mL) was added, and the mixture was allowed to attain room temperature. The mixture was filtered through Celite, and the organic layer was washed with water, dried, and evaporated. Column chromatography of the residue gave **6** (4.18 g, 4.48 mmol; 74%) $[\alpha]_{578}$ +44° (*c* 0.5 chloroform);R_F 0.36 (3:1 toluene–ethyl acetate); n.m.r. data:^{13}C, δ 14.9 (Me ethyl), 20.8 (Me acetyl), 23.8 (CH_2S), 40.6 (CH_2Cl), 54.7 (MeO), 54.9 (C-2), 65.9–79.2 (C-3,4,5,6,2′,3′,4′,5′,6′), 81.0 (C-1), 100.2 (C-1′), 101.1 (PhCH), 113.1 (aromatic C *p*-methoxybenzyl),123.1–138.1 (aromatic C), 158.5 (aromatic C *p*-methoxybenzyl), 167.1 (C=O chloroacetyl), 167.4, 167.8 (C=O phthalimido), and 169.0 (C=O acetyl); ^{1}H, δ 3.23 (m, H-5′), 3.55 (m, H-5), 3.83 (m, H-6a,6b), 3.95 (dd, $J_{5',6a'}$ 1.7, $J_{6a',6b'}$ 12.4 Hz, H-6a′), 4.12 (dd, $J_{3,4}$ 8.4, $J_{4,5}$ 9.9 Hz, H-4), 4.20 (dd, $J_{1,2} = J_{2,3}$ = 10.3 Hz, H-2), 4.28 (dd, H-3), 4.30 (dd, $J_{3',4'}$ 3.8 Hz, H-4′), 4.33 (dd, $J_{5',6b'}$ 1.5 Hz, H-6b′), 4.64 (d, $J_{1',2'}$ 8.0 Hz, H-1′), 4.82 (dd, $J_{2',3'}$ 10.3 Hz, H-3′), 5.22 (d, H-1), and 5.35 (dd, H-2′).

Anal. Calc. for $C_{48}H_{50}ClNO_{14}S$: C, 61.8; H, 5.4; N, 1.5; S, 3.4. Found: C, 61.1; H, 5.4; N, 1.4; S, 4.0.

p-*Nitrophenylethyl* O-*[2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(chloroacetyl)-β-*D*-*

galactopyranosyl]-(1→4)-6-O-benzyl-2-deoxy-3-O-(p-methoxybenzyl)-2-phthalimido-β-D-glucopyranoside (**7**).—Methyl triflate (1.09 mL, 9.93 mmol) was added at room temperature to a stirred mixture of **6** (1.54 g, 1.65 mmol), *p*-nitrophenethyl alcohol (0.69 g, 4.1 mmol), DTBMP (1.0g, 5.0 mmol), and molecular sieves 4A in dry dichloromethane. The mixture was kept overnight and triethylamine (3 mL) was then added. After 1 h, it was filtered through Celite and evaporated. Column chromatography of the residue gave **7** (1.20g, 1.16 mmol; 70%). Crystallization from ethyl acetate–methanol gave material having m.p. 197–198°, $[\alpha]_{578}$ +14° (*c* 0.6, chloroform); R_F 0.22 (3:1 toluene–ethyl acetate); n.m.r. data: ^{13}C, δ 20.8 (Me acetyl), 35.3 (CH_2 *p*NO_2Ph), 40.6 (CH_2Cl), 54.6 (MeO), 55.5 (C-2), 65.9–78.3 (C-3,4,5,6,2′,3′,4′,5′,6′), 98.2 (C-1), 100.2 (C-1′), 101.0 (PhCH), 113.1 (aromatic C *p*-methoxybenzyl), 122.9–138.0 (aromatic C), 146.7 (aromatic C *p*NO_2Ph), 158.4 (aromatic C *p*-methoxybenzyl), 167.0 (C=O chloroacetyl), 167.4 (C=O phthalimido), and 168.9 (C=O acetyl); ^{1}H, δ 3.21 (m, H-5′), 3.49 (m, H-5), 3.79 (dd, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 10.7 Hz, H-6a), 3.83 (dd, $J_{5,6b}$ 3.1 Hz, H-6b), 3.94 (dd, $J_{5',6a'}$ 1.8, $J_{6a',6b'}$ 12.4 Hz, H-6a′), 4.03 (dd, $J_{1,2}$ 8.4, $J_{2,3}$ 10.5Hz, H-2), 4.07 (dd, $J_{3,4}$ 8.2, $J_{4,5}$ 9.6 Hz, H-4), 4.12 (dd, H-3), 4.28 (dd, $J_{3',4'}$ 3.7, $J_{4',5'}$ 0.8 Hz,H-4′), 4.33 (dd, $J_{5',6b'}$ 1.6 Hz, H-6b′), 4.53 (d, $J_{1',2'}$ 8.0 Hz, H-1′), 4.78 (dd, $J_{2',3'}$ 10.3 Hz, H-3′), 5.01 (d, H-1), and 5.32 (dd, H-2′).

Anal. Calc. for $C_{54}H_{53}ClN_2O_{17}$: C, 62.5; H, 5.2; N,2.7. Found: C,62.5; H, 5.2; N, 2.9.

p-*Nitrophenylethyl O-(2-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-6-O-benzyl-2-deoxy-3-O-(p-methoxybenzyl)-2-phthalimido-β-D-glucopyranoside* (**8**).—Hydrazine acetate (0.46 g, 5.0 mmol) was addded to a stirred solution of **7** (1.74 g, 1.68 mmol) in 1:1 ethyl acetate–methanol (20 mL) at room temperature. The mixture was stirred for 3 h and then evaporated. The residue was partitioned between dichloromethane and water. The dichloromethane layer was dried, and concentrated. Column chromatography gave **8** (1.30 g, 1.35 mmol; 81%), $[\alpha]_{578}$ −10° (*c* 0.6, chloroform); R_F 0.33 (1:1 toluene–ethyl acetate); n.m.r. data: ^{13}C, δ 21.1 (Me acetyl), 35.4 (CH_2*p*NO_2Ph), 54.7 (MeO), 55.5 (C-2), 66.3–78.6 (C-3,4,5,6,2′,3′,4′,5′,6′), 98.3 (C-1), 100.3 (C-1′), 101.5 (PhCH), 113.1 (aromatic C *p*-methoxybenzyl), 122.6–138.2 (aromatic C), 146.8 (aromatic C *p*NO_2Ph), 158.5 (aromatic C *p*-methoxybenzyl), 167.4, 167.7 (C=O phthalimido), and 170.2 (C=O acetyl); ^{1}H, δ 3.22 (m, H-5′), 3.46 (dd, $J_{2',3'}$ 10.1, $J_{3',4'}$ 3.7 Hz, H-3′), 3.52 (m, H-5), 3.82 (dd, $J_{5,6a}$ 1.3, $J_{6a,6b}$ 10.9 Hz, H-6a), 3.90 (dd, $J_{5,6b}$3.3 Hz, H-6b), 3.95 (dd, $J_{5',6a'}$ 1.6, $J_{6a',6b'}$ 12.3 Hz, H-6a′), 4.04 (dd, $J_{1,2}$ 8.4, $J_{2,3}$ 10.6 Hz, H-2), 4.05 (dd, $J_{3,4}$ 8.3, $J_{4,5}$ 9.8 Hz, H-4), 4.09 (dd, H-4′), 4.14 (dd, H-3), 4.33 (dd,$J_{5',6'}$ 1.1 Hz, H-6b′), 4.52 (d, $J_{1',2'}$ 8.0 Hz, H-1′), 5.03 (d, H-1), and 5.04 (dd, H-2′).

p-*Nitrophenylethyl O-[2-O-acetyl-4,6-O-benzylidene-3-O-(chloroacetyl)-β-D-galactopyranosyl]-(1→4)-O-[6-O-benzyl-2-deoxy-3-O-(p-methoxybenzyl)-2-phthalimido-β-D-glucopyranosyl]-(1→3)-O-(2-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-6-O-benzyl-2-deoxy-3-O-(p-methoxybenzyl)-2-phthalimido-β-D-glucopyranoside* (**9**). — A solution of DMTST (1.07 g, 4.14 mmol) in dry dichloromethane (5 mL) was added to a stirred mixture of **6** (0.95 g, 0.99 mmol), **8**

(1.29 g, 1.34 mmol), DTBMP (1.2 g, 5.9 mmol) and molecular sieves 4A in dichloromethane at 0° under nitrogen. The mixture was stirred for 3 h at room temperature, and then triethylamine (3 mL) was added. After 30 min at room temperature, the mixture was filtered through Celite, and the filtrate evaporated. Column chromatography (1:1 chloroform–ethyl acetate) followed by crystallization from dichloromethane–isooctane gave **9** (1.07 g, 0.58 mmol, 59%) having m.p. 202–205°, $[\alpha]_{578}$ −16°(*c* 0.7, chloroform); R_F 0.43 (1:1, toluene–ethyl acetate); n.m.r. data: ^{13}C, δ 20.3, 20.9 (2 Me acetyl), 35.3 (CH_2 *p*NO_2Ph), 40.6 (CH_2Cl), 54.6, 54.7 (2 MeO), 55.4, 55.5 (C-2,2'), 66.0–78.7 (C-3,4,5,6,2',3',4',5',6',3'',4'',5'',6'',2''',3''',4''',5''',6'''), 98.1 (C-1), 99.1 (C-1''), 100.4, 100.5 (C-1',1'''), 100.7, 101.1 (2 PhCH), 112.9, 113.1 (2 aromatic C *p*-methoxybenzyl),122.5–138.1 (aromatic C), 146.7 (aromatic C *p*-NO_2Ph), 158.3, 158.4 (2 aromatic C *p*-methoxybenzyl), 167.1 (C=O chloroacetyl), 167.3–167.6 (C=O phthalimido), 168.4, and 169.1 (2 C=O acetyl); ^1H-n.m.r. data are shown in the table.

	H-1	*H-2*	*H-3*	*H-4*	*H-5*
GlcNA	4.93 (8.4)	ND	4.01	3.90	3.35
GlcNB	5.24 (8.1)	4.15 (10.8)	4.26 (8.2)	4.02	3.64
GalA	4.27 (8.0)	4.99 (10.0)	3.46 (3.9)	4.18 (0.6)	3.08
GalB	4.65 (8.0)	5.38 (10.3)	4.88 (3.7)	4.32	3.29

Anal. Calc. for $C_{98}H_{96}ClN_3O_{30}$: C, 64.2; H, 5.3; N, 2.3. Found: C, 63.7; H, 5.3; N, 2.2.

p-*Nitrophenylethyl* O-*[2-O-acetyl-4,6-O-benzylidene-3-O-(chloroacetyl)-β-*D-*galactopyranosyl]-(1→4)*-O-*(6-O-benzyl-2-deoxy-2-phthalimido-β-*D-*glucopyranosyl)-(1→3)*-O-*(2-O-acetyl-4,6-O-benzylidene-β-*D-*galactopyranosyl)-(1→4)-6-O-benzyl-2-deoxy-2-phthalimido-β-*D-*glucopyranoside* (**10**). - DDQ (252 mg, 1.11 mmol) was added to a stirred solution of **9** (680 mg, 371 μmol) in dichloromethane (15 mL) saturated with water. After 2 h at room temperature, the reaction was complete, and the organic layer was successively washed with aqueous sodium hydrogencarbonate and water, dried, and concentrated. Column chromatography gave **10** (546 mg, 343 μmol; 92%) having $[\alpha]_{578}$ −40° (*c* 0.6, chloroform); R_F 0.42 (1:1, toluene–ethyl acetate); n.m.r. data: ^{13}C, δ 20.4, 20.8 (2 Me acetyl), 35.4 (CH_2 *p*NO_2Ph), 40.5 (CH_2Cl), 55.7, 55.8 (C-2,2''), 66.5–81.8 (C-3,4,5,6,2',3',4',5',6',3'',4'',5'',6'',2''',3''',4''',5''',6'''), 98.1 (C-1), 99.0 (C-1''), 100.5, 101.0 (C-1',1'''), 101.2 (2 PhCH), 122.8–138.2 (aromatic C), 146.7 (aromatic C *p*NO_2Ph), 167.1 (C=O chloroacetyl), 167.7–167.9 (C=O phthalimido), 168.5, and 168.9 (2 C=O acetyl).

p-*Nitrophenylethyl* O-*[2-O-acetyl-4,6-O-benzylidene-3-O-(chloroacetyl)-β-*D-*galactopyranosyl]-(1→4)*-O-*{2,3,4-tri-O-benzyl-α-*L-*fucopyranosyl-(1→3)}*-O-*(6-O-benzyl-2-deoxy-2-phthalimido-β-*D-*glucopyranosyl)-(1→3)*-O-*(2-O-acetyl-4,6-O-benzylidene-β-*D-*galactopyranosyl)-(1→4)-O-{2,3,4-tri-O-benzyl-α-*L-*fucopyranosyl-(1→3)}-[6-O-benzyl-2-deoxy-2-phthalimido-β-*D-*glucopyranoside* (**11**). — A solution of silver triflate (1.32 g, 5.14 mmol) and 2,4,6-trimethylpyridine (680 μL,

5.1 mmol) in 3:2 dichloromethane–toluene (5 mL) was added to a stirred mixture of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[11] (2.17 g, 4.36 mmol), **10** (693 mg, 435 μmol) and molecular sieves 4A in dry dichloromethane (10 mL) at −25° under nitrogen. Stirring was continued for 10 min, and then aqueous sodium thiosulfate (10%, 5 mL) was added, and the mixture was allowed to attain room temperature. Dichloromethane (10 mL) was added, and the mixture was filtered through Celite. The organic layer was washed with water, dried, and evaporated. Column chromatography of the residue gave **11** (644 mg, 266 μmol; 61%) having $[\alpha]_{578}$ −91° (*c* 0.5, chloroform); R_F 0.73 (1:2, toluene–ethyl acetate); n.m.r. data: ^{13}C, δ 15.4, 16.1 (2 C-6, Fuc), 20.3, 20.8 (2 Me acetyl), 35.3 (CH_2 *p*NO_2Ph), 40.6 (CH_2Cl), 56.0, 56.4 (C-2 GlcNA, C-2 GlcNB), 66.2–78.9 (ring C), 97.6 (C-1, FucA), 97.7 (C-1, FucB), 98.1 (C-1 GlcNA), 98.9 (C-1 GlcNB), 99.58 (C-1 GalB), 99.61 (C-1 GalA), 99.8 (2 Ph*C*H), 122.9–139.6 (aromatic C), 146.6 (aromatic C *p*NO_2Ph), 167.1 (C=O chloroacetyl), and 168.0–168.7 (C=O acetyl, C=O phthalimido); ^1H-n.m.r. data are shown in the table.

	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
GlcNA	4.88 (8.5)	4.25 (10.9)	4.38 (8.8)	ND (10.6)	3.28	3.82
GlcNB	5.17 (8.4)	4.46 (10.8)	4.70 (8.7)	4.03 (10.5)	3.60	3.98
GalA	4.36 (8.3)	4.88 (9.8)	3.49 (4.1)	4.15	2.98	3.89
GalB	4.70 (8.3)	5.37 (10.3)	4.82 (4.0)	4.28	3.11	3.98
FucA	4.41 (3.8)	3.45 (10.6)	3.76 (2.7)	2.97	ND (6.5)	0.72
FucB	4.65 (3.9)	3.56 (10.5)	3.82 (2.7)	3.10	ND (6.5)	1.12

Anal. Calc. for $C_{136}H_{136}ClN_3O_{36}$: C, 67.4; H, 5.7; N, 1.7. Found: C, 67.3; H, 5.7; N, 2.0.

p-*Nitrophenylethyl* O-*(4,6*-O-*benzylidene*-β-D-*galactopyranosyl)*-*(1→4)*-*O*-{*2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl*-*(1→3)*}-O-*(2-acetamido-6*-O-*benzyl-2-deoxy*-β-D-*glucopyranosyl)*-*(1→3)*-O-*(4,6*-O-*benzylidene*-β-D-*galactopyranosyl)*-*(1→4)*-*O*-{*2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl*-*(1→3)*}-*2-acetamido-6*-O-*benzyl-2-deoxy*-β-D-*glucopyranoside* (**12**). - Hydrazine acetate (460 mg, 5.0 mmol) was added to a mixture of **11** (553 mg, 228 μmol) in 2:3 toluene–ethanol (10 mL). The mixture was refluxed overnight, and then cooled and evaporated. The residue was partitioned between 1:1 dichloromethane–ethanol and water. The organic layer, which contained the product, was concentrated; n.m.r. data: ^{13}C, δ 103.1, 103.9 (C-1, C-1″). The residue was dissolved in 1:1 dichloromethane–methanol (10 mL), and treated with acetic anhydride (0.5 mL) at room temperature. After 2 h, the mixture was concentrated and co-evaporated with ethanol. Column chromatography (6:20:3, toluene–ethyl acetate–methanol) gave **12** (410 mg, 196 μmol, 86%) having R_F 0.52 (6:20:3, toluene–ethyl acetate–methanol); n.m.r. data: ^{13}C, δ 97.9, 98.2 (2 C-1 Fuc), 99.2,99.8, 99.9, 100.7 (C-1,1′,1″,1‴), and 101.6 (2 Ph*C*H).

p-*Trifluoroacetamidophenylethyl* O-*(4,6*-O-*benzylidene*-β-D-*galactopyranosyl)*-*(1→4)*-*O*-{*2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl*-*(1→3)*}-O-*(2-acetamido-6*-O-*benzyl-2-deoxy*-β-D-*glucopyranosyl)*-*(1→3)*-O-*(4,6*-O-*benzylidene*-β-D-*galactopyra-*

nosyl)-(1→4)-O-{*2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl-(1→3)*}-*2-acetamido-6*-O-*benzyl-2-deoxy*-β-D-*glucopyranoside* (**13**). — A solution of **12** (410 mg, 196 μmol) in 1:1 tetrahydrofuran–water (20 mL) was treated with aluminum amalgam for 3 h at room temperature. The mixture was filtered through Celite and evaporated. The residue was dissolved in dichloromethane, and treated with pyridine (160 μL) and trifluoracetic anhydride (165 μL, 0.52 mmol) at room temperature. After 4 h, the mixture was diluted with dichloromethane, and washed with aqueous sodium hydrogencarbonate. The organic layer was concentrated, and treated with sodium methoxide in methanol for 10 min, made neutral with acetic acid, and concentrated. Column chromatography (6:20:1 toluene–ethyl acetate–methanol) yielded **13** (260 mg, 121 μmol; 61%) having R_F 0.58 (6:20:3 toluene–ethyl acetate–methanol); n.m.r. data: ^{13}C, δ 97.9, 98.4 (2 C-1 Fuc), 99.2, 99.8, 100.0, 100.7 (C-1,1′,1″,1‴), and 101.4, 101.6 (2 Ph*C*H).

p-*Trifluoroacetamidophenylethyl* O-β-D-*galactopyranosyl-(1→4)*-O-[α-L-*fucopyranosyl-(1→3)*-*O*-(2-acetamido-2-deoxy-β-D-*glucopyranosyl)-(1→3)*-O-(β-D-*galactopyranosyl-(1→4)*-O-[α-L-*fucopyranosyl-(1→3)-2-acetamido-2-deoxy*-β-D-*glucopyranoside* (**14**). — A solution of **13** (220 mg, 102 μmol) in 12:3:2 ethyl acetate–ethanol–water was hydrogenolyzed over Pd/C (10%, 200 mg) at 400 kPa for 12 h, filtered, and the filtrate evaporated. The residue was purified on a Biogel P-2 column, using water as eluant, giving compound **14** (120 mg, 96 μmol; 94%) having $[\alpha]_{578}$ −67° (*c* 0.7, water); R_F 0.72 (4:3:3:2 ethyl acetate–acetic acid–methanol–water); n.m.r. data (D_2O; Me_2CO, $\delta_H = 2.225$; 1,4-dioxane, $\delta_C = 67.4$): ^{13}C, δ 16.1, 16.2 (2 C-6 Fuc), 22.9, 23.1 (2 Me *N*-acetyl), 35.3 (*C*H_2 ethyl), 56.5 (C-2), 56.8 (C-2″), 60.5, 60.6, 62.34, 62.26 (C-6,6′,6″,6‴), 67.5 (C-5 FucA, C-5 FucB), 68.4 (C-2 FucB), 68.6 (C-2 FucA), 69.0 (C-4′), 69.2 (C-4‴), 70.0 (C-3 FucA, C-3 FucB), 71.1 (*C*H_2 ethyl), 71.3 (C-2‴), 71.9 (C-2′), 72.7 (C-4 FucB), 72.8 (C-4 FucA), 73.3 (C-3‴), 73.9, 75.3, 75.6, 75.7 (C-3,5,5′,3″,5″,5‴), 75.9 (C-4‴), 76.1 (C-4), 82.5 (C-3′), 99.4 (C-1 FucA), 99.5 (C-1 FucB), 101.6 (C-1), 102.6 (C-1′,1‴), 103.3 (C-1″), 123.1, 130.4, 133.6, 138.7 (aromatic C), and 174.7, 175.4 (2 C=O N-acetyl); ^1H n.m.r. data are shown in the table.

	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
GlcNA	4.47 (8.4)	3.80	ND	3.55	ND	ND
GlcNB	4.70 (8.3)	3.96 (10.3)	3.87 (8.6)	3.57	ND	ND
GalA	4.42 (7.8)	3.49 (10.0)	3.69 (3.2)	4.09 (1.3)	ND	ND
GalB	4.46 (7.8)	3.49 (9.9)	3.65 (3.4)	3.89 (1.2)	ND	ND
FucA	5.13 (4.0)	3.69 (10.3)	3.90 (3.3)	3.79 (1.5)	4.83 (6.6)	1.18
FucB	5.01 (3.9)	3.64 (10.3)	3.85 (3.3)	3.75 (1.5)	4.78 (6.6)	1.13

Methylation followed by hydrolysis with strong acid gave a mixuture of the expected partially methylated sugars, all identified as their alditol acetates by g.l.c.–m.s. Positive-ion f.a.b.-m.s. showed an M + H ion at *m/z* 1256.

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