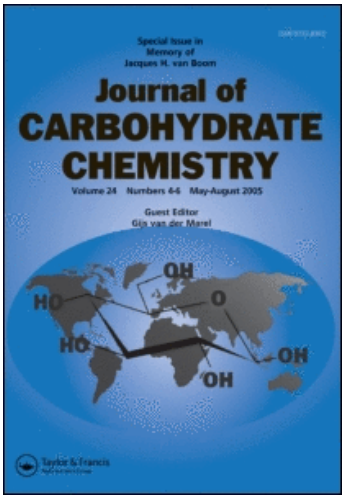

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Synthesis of Methyl 2-*O*-, 3-*O*-, 4-*O*-, 6-*O*-, 2,3-Di-*O*- and 4,6-Di-*O*-β-D-Galactopyranosyl-β-D-glucopyranoside

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SYNTHESIS OF METHYL 2-*O*-, 3-*O*-, 4-*O*-, 6-*O*-, 2,3-DI-*O*- AND 4,6-DI-*O*-β-D-GALACTOPYRANOSYL-β-D-GLUCOPYRANOSIDE

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ABSTRACT

Synthesis of methyl *O*-β-D-galactopyranosyl-(1→2)-β-D-glucopyranoside **1**, methyl *O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranoside **2**, methyl *O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **3**, methyl *O*-β-D-galactopyranosyl-(1→6)-β-D-glucopyranoside **4**, methyl *O*-β-D-galactopyranosyl-(1→4)-[*O*-β-D-galactopyranosyl-(1→6)]-β-D-glucopyranoside **5**, and methyl *O*-β-D-galactopyranosyl-(1→2)-[*O*-β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside **6**, using 2,3,4,6 tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate or 2,3,4,6 tetra-*O*-acetyl-α-D-galactopyranosyl bromide as a glycosyl donor and selectively protected derivatives of methyl *O*-β-D-glucopyranoside as glycosyl acceptors are described.

INTRODUCTION

A series of previous studies suggested that Gal-binding lectins expressed at the tumor cell surface play an important role in tumor cell aggregation and metastasis in both experimental and human cancers.[2-6] On the other hand, carbohydrates at the tumor cell surface may be recognized by lectins expressed on defined organs. For example, Ashwell receptors on liver cells appear to play a role in initiating deposition of Gal- or GalNAc-expressing tumor cells in liver.[7-9] We showed previously that methyl β-D-lactoside and

lacto-*N*-tetraose suppress metastatic potential of B16 melanoma cells.[10] However this phenomenon is probably not due to expression of Gal-binding lectin on B16 cells but rather to recognition of GM3 ganglioside (highly expressed on B16 cells) by Gg3Cer or LacCer expressed on microvascular endothelial cells.[11] To further pursue our studies on the role of carbohydrate-carbohydrate and carbohydrate-lectin interaction in tumor cell metastasis, we needed different structural variants of methyl β-D-lactoside. We now describe the synthesis of terminal β-D-galactose containing di- and trisaccharides, which will be compared in terms of their ability to inhibit B16 metastasis.

RESULTS AND DISCUSSION

For the synthesis of title compounds three acceptors **7, 9,** and **11** were prepared. Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside **7** was prepared from methyl β-D-glucopyranoside[12] essentially by the method of Vasella *et. al.*[13] in three steps: i) Ph_3CCl, Py-DMF ii) BnBr, NaH and iii) CF_3COOH-H_2O in 76.3% yield. To confirm the presence of an unprotected -OH group at C-6, a small portion of **7** was acetylated to give **8**. The ^1H NMR spectrum of **8** showed characteristic deshielded signals for H-6 and H-6'at 4.33 and 4.23 ppm, respectively, due to acetylation at C-6.

Glycosylation of **7** with 2,3,4,6 tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate[14] **12** using $BF_3.Et_2O$ as promoter gave the (1→6)-linked disaccharide **14** in 76% yield. The β-stereochemistry of the newly formed glycosidic linkage was evident from the coupling constant from the anomeric proton of the galactose residue which appeared at 4.59 ppm (J = 8.0 Hz). Compound **14** was deacetylated with NaOMe/MeOH to give **15** which was *O*-debenzylated by hydrogenolysis in the presence of 10% palladium on carbon to afford methyl β-D-galactopyranosyl-(1→6)-β-D-glucopyranoside[15] **4**.

The 4,6-di-*O*-galactosylated trisaccharide **5** was synthesized from methyl 2,3 di-*O*-benzyl-β-D-glucopyranoside[16] **9** which was prepared from methyl β-D-glucopyranoside in three steps: i) $PhC(OMe)_2$, *p*-TsOH ii) BnBr, NaH iii) AcOH-H_2O. The presence of a free OH group at C-4 and C-6 of **9** was confirmed by acetylation which gave **10**.[17] The ^1H NMR spectrum of **10** showed characteristic deshielded signals for H-4, H-6 and H-6' at 5.03, 4.23, and 4.08 ppm, respectively.

Glycosylation of **9** with 2.4 equivalents of **12** in the presence of $BF_3.Et_2O$ gave a complex mixture. In separating the compounds of the mixture by gel-permeation chromatography on Sephadex® LH-20 the desired trisaccharide **23** was obtained in 24.8% yield. The pool of disaccharide containing fractions was composed of β-(1→6) glycosylated disaccharide **16** and its 4-*O*-acetyl derivative **17** along with **20**, which was the 6-*O*-acetyl derivative of β-(1→4) glycosylated disaccharide. The formation of acetates

1 Galβ1→2Glcβ1→OMe

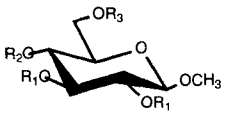
2 Galβ1→3Glcβ1→OMe

3 Galβ1→4Glcβ1→OMe

4 Galβ1→6Glcβ1→OMe

5 Galβ1→4
Glcβ1→OMe
Galβ1→6

6 Galβ1→2
Glcβ1→OMe
Galβ1→3



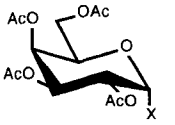
7 $R_1 = R_2 = Bn$, $R_3 = H$

8 $R_1 = R_2 = Bn$, $R_3 = Ac$

9 $R_1 = Bn$, $R_2 = R_3 = H$

10 $R_1 = Bn$, $R_2 = R_3 = Ac$

11 $R_1 = H$, $R_2 = R_3 = Ac$



12 X = O-C(NH)CCl$_3$

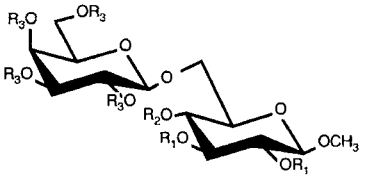
13 X = Br

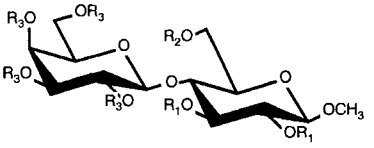
Scheme 1

such as **17** and **20**, due to the acetylation of the glycosyl acceptor through a transesterification side reaction in acid-catalyzed glycosylations involving acetylated glycosyl donors is well documented.[18-22]

The site of the glycosidic linkage in disaccharide **16** was confirmed by acetylation. The ^{1}H NMR spectrum of the acetylated product was identical to that of compound **17** and showed a deshielded signal at 4.79 ppm for H-4 from the glucose residue. This downfield shift of H-4 observed upon acetylation was an indication of the presence of a (1→6) glycosidic linkage in **16**. The coupling constant of the anomeric proton of galactose in both the spectra was 8.0 Hz, which confirmed the β-linkage in both **16** and **17**. Debenzylation of **17** gave **18** which was transformed into its acetate **19** for NMR studies. Compound **19** upon deacetylation gave the 6-*O*-glycosylated disaccharide which was identical to **4**. Compound **20** was likewise debenzylated to give **21**, which on acetylation gave known **22**. Deacetylation of **22** yielded methyl β-D-lactoside **3**.[23,24]

O-Debenzylation of the trisaccharide **23** gave **24**. To confirm the sites of glycosidic linkages in **23**, a small portion of **24** was acetylated. In the ^{1}H NMR spectra of **25** the signals for H-2 and H-3 of glucose residue were deshielded due to acetylation, which signified that glycosidation had occurred at *O*-4 and *O*-6 in **23**. Compound **25** was then





14 $R_1 = R_2 = Bn$, $R_3 = Ac$

15 $R_1 = R_2 = Bn$, $R_3 = H$

16 $R_1 = Bn$, $R_2 = H$, $R_3 = Ac$

17 $R_1 = Bn$, $R_2 = R_3 = Ac$

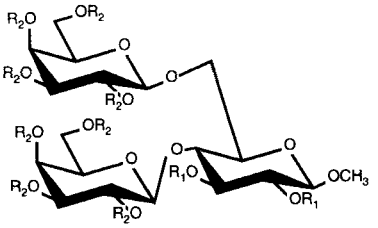
18 $R_1 = H$, $R_2 = Ac$, $R_3 = Ac$

19 $R_1 = R_2 = R_3 = Ac$

20 $R_1 = Bn$, $R_2 = R_3 = Ac$

21 $R_1 = H$, $R_2 = R_3 = Ac$

22 $R_1 = R_2 = R_3 = Ac$



23 $R_1 = Bn$, $R_2 = Ac$

24 $R_1 = H$, $R_2 = Ac$

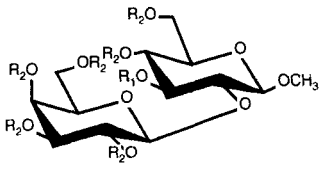
25 $R_1 = R_2 = Ac$

Scheme 2

deacetylated to give **5**, which showed signals for three anomeric protons at 4.51 (J = 7.8 Hz), 4.45 (J = 7.8 Hz) and 4.41 ppm (J = 8.0 Hz).

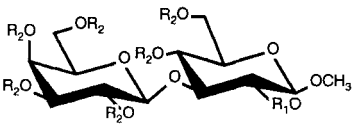
Glycosylation of **9** with 2 equivalents of 2,3,4,6 tetra-*O*-acetyl-α-D-galactopyranosyl bromide **13** in the presence of silver triflate also gave the same profile of glycosylated compounds, but the formation of the side-products **17** and **20** was not significant.

For the synthesis of 2-*O*- and 3-*O*- galactosylated disaccharides and 2,3-di-*O*-glycosylated trisaccharide, methyl 4,6 di-*O*-acetyl-β-D-glucopyranoside[17,25] **11** was used as an acceptor. Compound **11** was prepared from **10** by hydrogenolysis (H_2, 10% Pd-C), which was very sluggish and required prolonged stirring under 50 lb/sq.in. pressure of hydrogen.



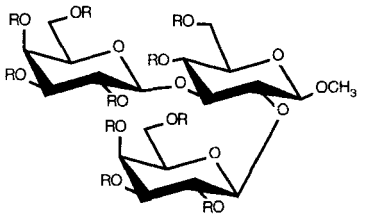
26 $R_1 = H$, $R_2 = Ac$

27 $R_1 = R_2 = Ac$



28 $R_1 = H$, $R_2 = Ac$

29 $R_1 = R_2 = Ac$



30 R = Ac

Scheme 3

Glycosylation of **11** with 1 equivalent of **13** in the presence of silver triflate gave a mixture of three saccharides. Gel-permeation chromatography of this mixture on Sephadex®LH-20, followed by silica gel chromatography gave **26**, **28**, and **30** in 36.6%, 9.5% and 6.0% yield, respectively. Acetylation of compound **26** gave **27**. Comparison of the ^1H NMR spectrum of compound **27** with that of compound **26** showed a characteristic deshielded signal for H-3 of glucose at 5.17 ppm due to acetylation, thereby confirming the formation of a (1→2) glycosidic linkage in **26**. The signals for the anomeric protons of galactose and glucose residues appeared at 4.72 and 4.42 ppm with coupling constants of 8.0 and 7.4 Hz, respectively, thus confirming the formation of a β-glycosidic linkage.

Compound **28** was likewise acetylated to give **29**. The ^1H NMR spactrum of **29** showed a signal for H-1 of galactose at 4.55 ppm (J = 7.8 Hz) and a deshielded signal for H-2 of glucose, which confirmed a β-(1→3) glycosidic linkage in **29**.

Compounds **27**, **28**, and **30**, were deacetylated to give **1**, **2**, and **6**, respectively. Compounds **1** and **2**, each showed two signals for anomeric protons at 4.72 (J = 7.83 Hz), 4.49 (J = 7.89 Hz) and 4.65 (J = 7.6 Hz) and 4.42 ppm (J = 7.5 Hz) respectively. Compound **6** showed three signals for anomeric protons at 4.80 (J = 8.0 Hz), 4.73 (J =

8.0 Hz), and 4.51 ppm (J = 8.0 Hz). From the coupling constants of each anomeric proton it was evident that all newly formed glycosidic linkages are β- in stereochemistry.

In conclusion, non-ambiguous synthesis of **1-6** is achieved.

EXPERIMENTAL

General procedures. Optical rotations were determined with a Perkin-Elmer Model 241 MC polarimeter at 25 °C, unless mentioned otherwise. ^{1}H NMR spectra were recorded on a Bruker WM-500 spectrometer. FABMS and high-resolution MS were performed on a JEOL JMS-HX 110/da-5000 mass spectrometer/data system. Chemical shifts (δ) are referenced to internal Me_4Si (0.0 ppm) for $CDCl_3$ and to acetone (2.225 ppm) for D_2O. TLC and HP TLC were conducted on Silica Gel 60 F-254 plates (Merck, Darmstadt, Germany) and visualized by spraying with 0.5% orcinol in 10% aq. H_2SO_4, followed by heating. Flash chromatography[26] was performed on Silica Gel (230-400 mesh, EM Science, Gibbstown, NJ) and gel-permeation chromatography was performed on Sephadex® LH-20 and G-10 (Pharmacia). Purity of the compounds was judged on the basis of their ^{1}H NMR spectra and high-resolution MS. 3-Nitrobenzyl alcohol (NBA) was used as a matrix and sodium acetate was added to the matrix to obtain $(M + Na)^+$ ions.

Methyl 2,3,4-tri-*O*-Benzyl-β-D-glucopyranoside (7). This compound was prepared from methyl β-D-glucopyranoside (purchased from Aldrich, Milwaukee) by the method of Vasalla *et.al.*[13] with slight modifications. To a solution of methyl β-D-glucopyranoside (1.0 g, 4.9 mmol) in a mixture of pyridine and DMF (1:1, 20 mL), triphenylmethyl chloride (2.7 g, 9.83 mmol) was added and the reaction mixture stirred at 100 °C for 24 h. Evaporation of the solvent *in vacuo* afforded a yellow syrup which was diluted with $CHCl_3$ and successively washed with water and aqueous $NaHCO_3$. Evaporation of the solvents after drying over $MgSO_4$ gave methyl 6-*O*-triphenylmethyl-β-D-glucopyranoside. This was dissolved in DMF (20 mL) and sodium hydride (944 mg, 50% dispersion in mineral oil) was added. After stirring for 10 min, benzyl bromide (2.89 mL, 24.2 mmol) was added and the reaction mixture was stirred at 20 °C for 24 h. Evaporation of the solvent gave a residue which was dissolved in ethyl acetate and washed with water. Drying of the organic extract over $MgSO_4$ followed by filtration and evaporation of solvents gave methyl 2,3,4-*O*-tri-*O*-benzyl-6-*O*-triphenylmethyl-β-D-glucopyranoside (2.9 g, 83.5%), after chromatography (SiO_2; 9:1, hexane-ethyl acetate). A solution of this compound (2.5 g, 3.5 mmol) in dicholoromethane (200 mL) was treated with a mixture of CF_3COOH and H_2O (8:2, 100 mL) for 30 min at 25 °C. The reaction mixture was cooled to 0 °C and quenched with triethylamine. Evaporation of the solvent gave a residue which was dissolved in ethyl acetate and successively washed with water

and aqueous $NaHCO_3$. Evaporation of the solvent and purification by chromatography on silica gel (2:1, hexane-ethyl acetate) gave the title compound (1.5 g, 91.4%).

Methyl 6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (8). To a solution of **7** (250 mg, 0.53 mmol) in CH_2Cl_2 (25 mL), a mixture of acetic anhydride and pyridine (1:2, 5 mL) was added at 0 °C and the reaction mixture was stirred for 6 h. Evaporation of the solvents gave a residue which was chromatographed on silica gel (4:1, hexane-ethyl acetate) to give **8** in quantitative yield: $[\alpha]_D$ +9.9° (*c* 4.48, chloroform); ^1H NMR δ 7.4-7.2 (m, 15H, Ph), 4.93, 4.90, 4.84, 4.78, 4.70, and 4.55 (3 *AB*-System, 6 H, $PhCH_2$), 4.33 (dd, 1 H, $J_{5,6}$ = 2.0, $J_{6,6'}$ = 8.0 Hz, H-6), 4.30 (dd, 1 H, $J_{1,2}$ = 8.0 Hz, H-1), 4.23 (dd, 1 H, H-6'), 3.66 (dd, $J_{2,3}$ = 8.7, $J_{3,4}$ = 8.6 Hz, H-3), 3.55 (s, 3 H, OCH_3), 3.53 (dd, 1 H, H-4), 3.5 (m, 1 H, H-5), 3.42 (dd, 1 H, H-2), 2.02 (s, 3 H, OAc).

Methyl 2,3-di-*O*-Benzyl-β-D-glucopyranoside (9). This compound was prepared as described in ref.19.

Methyl 4,6-di-*O*-Acetyl-2,3-di-*O*-benzyl-β-D-glucopyranoside (10). This compound was prepared as described in ref.20.

Methyl 4,6-di-*O*-Acetyl-β-D-glucopyranoside (11). A solution of **10** (4.8 g, 10.4 mmol) in methanol (15 mL) was hydrogenated in the presence of 10% palladium on carbon (200 mg) under 50 lb/sq. in. pressure of hydrogen. The catalyst was filtered off and washed with methanol. The filtrate and washings were combined and evaporated to a syrup which was chromatographed on silica gel (5:1, toluene-methanol) to afford **11**: high resolution FABMS: calcd for $C_{11}H_{18}NaO_8$ $[M + Na]^+$, 301.0900; found, 301.0893.

Methyl 2,3,4-tri-*O*-Benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-β-D-glucopyranoside (14). To a solution of **7** (0.99 g, 2.14 mmol) in dichloromethane (15 mL) at -10 °C, **12** (1.29 g, 2.47 mmol) and $BF_3.Et_2O$ (0.25 mL, 2.07 mmol) were added and the reaction mixture was allowed to stir for 4 h. After the completion of reaction, the reaction mixture was cooled to 0 °C and neutralized by the addition of triethylamine. Evaporation of the solvents *in vacuo* and gel-permeation chromatography of the residue on Sephadex® LH-20 (4:1, chloroform-methanol) gave the title disaccharide **14** (1.3 g, 76%) as a crystalline product: $[\alpha]_D$ +3.9° (*c* 0.95, chloroform); ^1H NMR δ 7.4-7.1 (m, 15 H, Ph), 5.37 (d, 1 H, J = 3.5 Hz, H-4b), 5.24 (dd, 1 H, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.4 Hz, H-2b), 4.98 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, H-3b), 4.93-4.52 (3 *AB*-System, 6 H, $PhCH_2$), 4.59 (d, 1 H, H-1b), 4.28 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1a), 3.64 (dd, 1 H, $J_{2,3}$ = 9.0, $J_{3,4}$ = 7.4 Hz, H-3a), 3.57 (s, 3 H, OCH_3), 3.50 (dd, $J_{4,5}$ = 9.5 Hz, H-4a), 3.39 (dd, 1 H, H-2a), 3.36 (t, 1 H, H-5a), 2.14, 2.0, and 1.97 (3s, 9 H, OAc); high resolution FABMS: calcd for $C_{42}H_{50}NaO_{15}$ $[M + Na]^+$, 817.3046; found, 817.3032.

Methyl 2,3,4-tri-*O*-Benzyl-6-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (15). To a solution of **14** (3.5 g, 4.4 mmol) in methanol (30 mL), a catalytic amount of NaOMe was added. The reaction mixture after stirring for 24 h at 20 °C was neutralized with Amberlyst® A-15 and filtered. Concentration of the filtrate and chromatography of the residue on silica gel (9:1 chloroform-methanol) gave **15** (2.4 g, 86.4%): $[\alpha]_D$ +3.6° (*c* 1.07, MeOH); ^{1}H NMR δ 7.4-7.2 (m, 15 H, Ph), 4.31 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1b), 4.26 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1a), 3.55 (s, 3 H, OCH_3); high resolution FABMS: calcd for $C_{34}H_{41}O_{11}$ [M - H]$^-$, 625.2648; found, 625.2638.

Methyl 6-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (4). A) To a solution of **15** (2.4 g, 3.8 mmol.) in MeOH (100 mL), 10% palladium on carbon was added and the suspension was shaken under 40 lb/sq. in. the pressure of hydrogen for 24 h. Removal of the catalyst by filtration and evaporation of the the solvent gave a gummy mass which was purified by passing through a Sephadex® G-10 column to afford **4** (1.2 g, 80%).

B) A solution of **19** in MeOH was treated with NaOMe as described for **15** to give **4** in quantitative yield: $[\alpha]_D$ -5.1° (*c* 1.22, water); ^{1}H NMR δ 4.45 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1b), 4.38 (d, 1 H, $J_{1,2}$ = 7.8, H-1a), 4.22 (d, 1 H, H-6a), 3.92 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-4b), 3.84 (d, 1 H, H-6'a), 3.57 (s, 3 H, OCH_3), 3.49 (t, 1 H, H-2b), 3.27 (t, 1 H, H-2a); high resolution FABMS: calcd for $C_{13}H_{23}O_{11}$ [M - H]$^-$, 355.1241; found, 355.1241.

Methyl 2,3-di-*O*-Benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (16), Methyl 4-*O*-Acetyl-2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (17), Methyl 6-*O*-Acetyl-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (20) and Methyl 2,3-di-*O*-Benzyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (23). To a stirred mixture of **9** (848 mg, 2.2 mmol), **12** (2.6 g, 5.37 mmol), and powdered molecular sieves AW-300 in CH_2Cl_2 (50 mL) at 0 °C, $BF_3.Et_2O$ (0.4 mL, 3.2 mmol) was added. The reaction mixture was stirred for 2 h at room temperature, neutralized by adding a few drops of triethylamine, and filtered through Celite.® Evaporation of the filtrate gave a complex mixture which was separated by gel-permeation chromatography on Sephadex® LH-20 (1:1 $CHCl_3$-MeOH) to afford trisaccharide **23** (580 mg, 24.8%): $[\alpha]_D$ -0.7° (*c* 5.15, chloroform); ^{1}H NMR δ 7.2-7.4 (m, 10 H, Ph), 5.39 (d, 1 H, $J_{3,4}$ = 3.2 Hz, H-4c), 5.29 (dd, 1 H, $J_{3,4}$ = 3.4 Hz, H-4b), 5.20 (dd, 1 H, $J_{2,3}$ = 10.2 Hz, H-2c), 5.15 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, H-2b), 5.01 (dd, 1 H, H-3c), 4.97 (dd, 1 H, H-3b), 4.86, 4.85, 4.65 and 4.63 (2*AB*-System, 4 H, $PhCH_2$), 4.66 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1c), 4.64 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1b), 4.26 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1a), 3.54 (s, 3 H, OCH_3), 3.62 (dd, 1 H, $J_{2,3}$ = 8.7 Hz, H-3a), 3.36

(dd, 1 H, H-2a), 2.14, 2.17, 2.06., 2.05, 2.04, 1.97, 1.95, 1.94 (8s, 24 H, OAc); high resolution FABMS: calcd for $C_{49}H_{62}NaO_{24}$ [M + Na]$^+$, 1057.3530; found, 1057.3511.

The pool from disaccharide fractions contained three disaccharides, which were separated by silica gel chromatography (1:1 hexane-ethyl acetate) to obtain **17** (74 mg, 3.7%): $[\alpha]_D$ -5.1° (*c* 1.58, chloroform); ^{1}H NMR δ 7.2-7.4 (m, 10 H, Ph), 5.38 (d, 1 H, $J_{3,4}$ = 3.4 Hz, H-4b), 5.19 (dd, 1 H, H-2b), 4.99 (dd, 1 H, J= 10.3 Hz, H-3b), 4.88, 4.80, 4.68 and 4.62 (2*AB*-System, 4 H, $PhCH_2$), 4.79 (dd, 1 H, $J_{3,4}$ = 9 Hz, H-4a), 4.57 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H-1b), 4.29 (d, 1 H, $J_{1,2}$ = 8 Hz, H-1a), 3.57 (m, 4 H, H-3a, OCH_3), 3.43 (t, 1 H, H-2a), 2.13, 2.04, 1.97, 1.91 (4s, 15 H, OAc); high resolution FABMS: calcd for $C_{37}H_{46}NaO_{16}$ [M + Na]$^+$, 769.2684; found, 769.2737.

Further elution with the same solvent gave **20** (380 mg, 18.7 %): $[\alpha]_D$ +11.8° (*c* 1.3, chloroform); ^{1}H NMR δ 7.2-7.4 (m, 10 H, Ph), 5.29 (d, 1 H, $J_{3,4}$ = 3.3 Hz, H-4b), 5.17 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, H-2b), 4.93 (dd, 1 H, J= 10.3 Hz, H-3b), 4.89, 4.85, and 4.65 (2*AB*-System, 4H, $PhCH_2$), 4.68 (d, 1 H, $J_{1,2}$ = 8 Hz, H-1b), 4.45 (d, 1 H, H-6a), 4.30 (d, 1-H, $J_{1,2}$ = 7.7 Hz, H-1a), 4.12 (H-6'a), 3.96 (dd, 1 H, $J_{3,4}$ = 11 Hz, H-4a), 3.40 (s, 3 H, OCH_3), 3.40 (t, 1 H, $J_{2,3}$ = 8.2 Hz, H-2a), 2.09, 2.06, 2.03, 1.95, 1.94 (5s, 15 H, OAc); high resolution FABMS: calcd for $C_{37}H_{46}NaO_{16}$ [M + Na]$^+$, 769.2684; found, 769.2717.

Futher elution gave **16** (190 mg, 10.1%): $[\alpha]_D$ -4.3° (*c* 2.6, chloroform); ^{1}H NMR δ 7.2-7.4 (m, 10 H, Ph), 5.37 (d, 1 H, $J_{3,4}$ = 3.3 Hz, H-4b), 5.21 (dd, 1 H, $J_{2,3}$ = 10.4 Hz, H-2b), 4.99 (dd, 1 H, H-3b), 4.93, 4.92 and 4.7 (2 *AB*-System, 4 H, $PhCH_2$), 4.61 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1b), 4.29 (d, 1 H, $J_{1,2}$ = 7.4 Hz, H-1a), 3.54 (s, 3 H, OCH_3), 2.13, 2.03, 2.02, 1.90 (4s, 12 H, OAc); high resolution FABMS: calcd for $C_{35}H_{44}NaO_{15}$ [M + Na]$^+$, 727.2578; found, 727.2572.

Methyl 4-*O*-Acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) -β-D-glucopyranoside (18). To a solution of **17** (71 mg, 0.095 mmol) in ethyl acetate, Pd-C (10 mg) was added and the mixture was stirred under an atmosphere of H_2 for 16 h. Filtration of the reaction mixture through a bed of Celite® and evaporation of the solvents gave a syrup. Chromatography of this syrup on silica gel (9:1 $CHCl_3$-MeOH) gave **18** (50 mg, 93%): $[\alpha]_D$ -12.6° (*c* 0.15, chloroform); ^{1}H NMR δ 5.38 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4b), 5.20 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, H-2b), 4.99 (dd, 1 H, H-3b), 4.74 (t, 1 H, H-4a), 4.55 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1b), 4.18 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1a), 3.66 (t, 1 H, H-3a), 3.56 (s, 3 H, OCH_3), 3.41 (t, 1 H, H-2a), 2.13, 2.11, 2.04, 1.97 (4s, 15 H, OAc); high resolution FABMS: calcd for $C_{23}H_{34}NaO_{16}$ [M + Na]$^+$, 589.1744; found, 589.1707.

Methyl 6-*O*-Acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) -β-D-glucopyranoside (21). A solution of **20** (345 mg, 0.46 mmol) in EtOAc was

hydrogenated as described for **18** to give **21** (220 mg, 84%): $[\alpha]_D$ +13.7° (*c* 0.5, chloroform); ^{1}H NMR δ 5.39 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4b), 5.23 (dd, 1 H, $J_{2,3}$ = 10.4 Hz, H-2b), 5.01 (dd, 1 H, H-3b), 4.55 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1b), 4.32 (dd, 1 H, J = 2.0 Hz, 13.7 Hz, H-6a), 4.24 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1a), 3.64 (t, 1 H, H-3a), 3.56 (s, 3 H, OCH_3), 3.41 (dd, 1 H, H-2a), 2.16, 2.10, 2.09, 2.07, 1.97 (5s, 15 H, OAc); high resolution FABMS: calcd for $C_{23}H_{34}NaO_{16}$ [M + Na]$^+$, 589.1744; found, 589.1749.

Methyl 4-*O*-(2,3,4,6-tetra-*O*-Acetyl-β-D-galactopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (24). A solution of **23** (467mg, 0.45 mmol) in EtOAc was hydrogenated as described for **18** to give **24** (372 mg, 96.6%): $[\alpha]_D$ +8.1° (*c* 1.1, chloroform); ^{1}H NMR δ 5.39 (d, 2 H, J = 3.2 Hz, H-4c, H-4c), 5.22 (dd, 1 H, $J_{2,3}$ = 10.4 Hz, H-2c), 5.19 (dd, 1 H, $J_{2,3}$ = 9.0 Hz, H-2b), 5.04 (dd, 1 H, H-3c), 5.0 (dd, 1 H, H-2b), 4.61 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1c), 4.55 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1b), 4.20 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1a), 3.63 (dd, 1 H, $J_{2,3}$ = 8.8 Hz, H-3a), 3.55 (s, 3 H, OCH_3), 3.40 (dd, 1 H, H-2a), 2.17, 2.15, 2.09, 2.08, 2.06., 2.05, 1.98 (6s, 24 H, OAc); high resolution FABMS: calcd for $C_{35}H_{50}NaO_{24}$ [M + Na]$^+$, 877.2590; found, 877.2607.

Methyl 2,3-di-*O*-Acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (25). Acetylation of compound **24** as described for **8** gave **25** in quantitative yield: $[\alpha]_D$ -7.9° (*c* 0.57, chloroform); ^{1}H NMR δ 5.4 (d, 1 H, $J_{3,4}$ = 2.8 Hz, H-4c), 5.34 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4b), 5.21 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, H-2c), 5.16 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, H-3a), 5.08 (dd, 1 H, H-2b), 5.02 (m, 2 H, H-3b, H-3c), 4.86 (dd, 1 H, H-2a), 4.62 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1c), 4.50 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H-1b), 4.35 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1a), 3.47 (s, 3 H, OCH_3), 2.15, 2.14, 2.09, 2.06., 2.05, 2.04, 2.03, 2.02, 1.98, 1.80 (9s, 30 H, OAc); high resolution FABMS: calcd for $C_{39}H_{54}NaO_{26}$ [M+ Na]$^+$, 961.2801; found, 961.2650.

Methyl 4,6-di-*O*-β-D-Galactopyranosyl-β-D-glucopyranoside (5). Compound **25** was deacetylated as described for **15** to yield **5**: $[\alpha]_D$ + 2.5° (*c* 0.59, MeOH); ^{1}H NMR δ 4.51 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1c), 4.45 (d, 1 H, $J_{1,2}$ = 7.8, H-1b), 4.41 (d, 1 H, $J_{1,2}$ = 8.0, H-1c), 4.31 (dd, 1 H, $J_{6,6'}$ = 11.5 Hz, H-6a), 3.93 (m, 3 H, H-4b, H-4c, H-6'a), 3.59 (s, 3 H, OCH_3), 3.32 (t, 1 H, H-2a); high resolution FABMS: calcd for $C_{19}H_{33}O_{16}$ [M - H]$^-$, 517.1769; found, 517.1769.

Methyl 4,6-di-*O*-Acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (26), Methyl 4,6-di-*O*-Acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (28) and Methyl 4,6-di-*O*-Acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (30). To a

mixture of **11** (3.0 g, 1.1 mmol), activated molecular sieves 4A° and silver triflate (333 mg, 1.29 mmol) in CH_2Cl_2 (15 mL) at -10 °C, a solution of **13** (4.86 g, 1.18 mmol) in CH_2Cl_2 (20 mL) was added dropwise. The reaction mixture was stirred at room temperature for 20 h, and filtered through a bed of Celite.® The filtrate was successively washed with water and 10% aqueous $NaHCO_3$. The organic extract was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Gel-permeation chromatography of the residue on Sephadex® LH-20 (1:1, MeOH-$CHCl_3$), followed by chromatography on silica gel (1:1, toluene-ethyl acetate) gave compound **26** (2.47 g, 36.6%), compound **28** (0.64 g, 9.5%) and compound **30** (0.54 g, 6%). Compound **26**: $[\alpha]_D$ -5.3° (*c* 1.6, chloroform); ^{1}H NMR δ 5.4 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-4b), 5.18 (dd, 1 H, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.3 Hz, H-2b), 5.05 (dd, 1 H, $J_{3,4}$ = Hz, H-3b), 4.9 (t, 1 H, J = 9.5 Hz, H-4), 4.79 (d, 1 H, H-1b), 4.34 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1a), 3.17 (ddd, 1 H, $J_{2,3}$ = 8.3, $J_{3,4}$ = 9.5 and $J_{3,OH}$ = 3.3 Hz, H-3a), 3.55 (s, 3 H, OCH_3), 3.56 (m, 1 H, H-5a), 3.41 (dd, 1 H, H-2a), 2.97 (d, 1 H, OH), 2.14, 2.08, 2.06, 2.05, 2.03 and 1.97 (6s, 18 H, OAC); high resolution FABMS: calcd for $C_{25}H_{36}NaO_{17}$ $[M + Na]^+$, 631.1850; found, 631.1840.

Compound **28**: $[\alpha]_D$ -4.6° (*c* 1.9, chloroform); ^{1}H NMR δ 5.36 (d, 1 H, $J_{3,4}$ = 3.6 Hz, H-4b), 5.13 (dd, 1 H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.3 Hz, H-2b), 5.03 (dd, 1 H, $J_{3,4}$ = 3.6 Hz, H-3b), 4.91 (t, 1 H, J = 9.5 Hz, H-4a), 4.79 (d, 1 H, H-1b), 4.20 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1a), 3.78 (t, 1 H, J = 9.5 Hz, H-3a), 3.62 (m, 1 H, H-5a), 3.56 (s, 3 H, OCH_3), 3.48 (dd, 1 H, H-2a), 2.15, 2.08, 2.06, 2.04 and 1.97 (5s, 18 H, OAC); high resolution FABMS: calcd for $C_{25}H_{36}NaO_{17}$ $[M + Na]^+$, 631.1850; found, 631.1854.

Compound **30**: $[\alpha]_D$ -14.7° (*c* 0.86, chloroform); ^{1}H NMR δ 5.40 (d, 1 H, $J_{3,4}$ = 3.4 Hz, H-4b), 5.36 (d, 1 H, $J_{3,4}$ = 3.3 Hz, H-4c), 5.22 (dd, 1 H, $J_{1,2}$ = 8.1, $J_{2,3}$ = 10.5 Hz, H-2b), 5.14 (dd, 1 H, $J_{2,3}$ = 10.5, $J_{3,4}$ = 3.4 Hz, H-3b), 5.03 (dd, 1 H, $J_{1,2}$ = 7.8, $J_{2,3}$ = 10.5 Hz, H-2c), 5.17 (dd, 1 H, $J_{2,3}$ = 10.5, $J_{3,4}$ = 3.3 Hz, H-3c), 4.89 (t, 1 H, H-4a), 4.88 (d, 1 H, $J_{1,2}$ = 8.1 Hz, H-1b), 4.82 (dd, 1 H, $J_{1,2}$ = 7.8 Hz, H-1c), 4.46 (d, 1 H, $J_{1,2}$ = 7.2 Hz, H-1a), 4.23-4.08 (m, 6 H, H-6a, H-6'a, H-6b, H-6'b, H-6c, H-6'c), 3.92 (t, 2 H, H-5b, H-5c), 3.67 (dd, 1 H, $J_{3,4}$ = 7.8 Hz, $J_{2,3}$ = 7.2 Hz, H-3a), 3.64-3.58 (m, 1 H, H-5a), 3.55 (t, 1 H, H-2a), 3.53 (s, 3 H, OCH_3), 2.18, 2.12, 2.10, 2.07, 2.06, 2.05, 2.04, 2.03, 1.98, 1.95 (10 s, 30 H, OAc);high resolution FABMS: calcd for $C_{39}H_{54}NaO_{26}$ $[M + Na]^+$, 961.2801; found, 961.2810.

Methyl 3,4,6-tri-*O*-Acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-β-D-glucopyranoside (27). Acetylation of **26** (2.47 g, 4.06 mmol) in CH_2Cl_2 (50 mL) as described for **8** gave **27** (2.59 g, 99.5%): $[\alpha]_D$ +6.7° (*c* 1.24, chloroform); ^{1}H NMR δ 5.36 (d, 1 H, $J_{3,4}$ = 3.4 Hz, H-4b), 5.18 (dd, 1 H, $J_{2,3}$ = 9.0, $J_{3,4}$ = 9.7 Hz, H-3a), 5.18 (dd, 1 H, $J_{1,2}$ = 8.0, $J_{2,3}$ = 6.8 Hz, H-2b), 4.98 (t, 1 H, H-4a), 4.95 (dd, 1 H, H-3b), 4.74 (d, 1 H, $J_{1,2}$ = 8 Hz, H-1b), 4.42 (d, 1 H, $J_{1,2}$ = 7.4 Hz, H-1a), 4.26 (dd, 1 H, $J_{5,6}$ = 4.7, $J_{6,6'}$ = 12.3 Hz, H-6a), 4.18 (dd, 1 H, H-6'a), 4.13 (m, 2

H, H-6b, H-6'b) 3.89 (t, 1 H, H-5b), 3.67 (m, 2 H, H-2a, H-5a), 3.55 (s, 3 H, OCH_3), 2.14, 2.07, 2.05, 2.04, 2.00 and 1.96 (6s, 21 H, OAC); high resolution FABMS: calcd for $C_{26}H_{35}NaO_{17}$ [MH-MeOH]$^+$, 619.1874; found, 619.1875.

Methyl 2,4,6-tri-*O*-Acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-β-D-glucopyranoside (29). A solution of **28** (0.64 g, 1.06 mmol) was acetylated as described for **8** to afford **29** in quantitative yield. $[\alpha]_D$ -27.6° (*c* 1.67, chloroform); ^{1}H NMR δ 5.34 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4b), 5.07 (dd, 1 H, $J_{1,2}$ = 7.8, $J_{2,3}$ = 10.5 Hz, H-2b), 5.01-4.92 (m, 3 H, H-2a, H-4a, H-3b), 4.55 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1b), 4.31 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1a), 4.23-4.16 (m, 3 H, H-6a, H-6'a, H-6'b), 4.04 (dd, 1 H, J = 7.3 Hz, H-6b), 3.90 (t, $J_{2,3}$ = 8.3 Hz, H-3a), 3.88 (t, 1 H, H-5b), 3.67 (m, 1 H, H-5a), 3.46 (s, 3 H, OCH_3), 2.14, 2.11, 2.08, 2.06, 2.04, 2.02 and 1.96 (7s, 21 H, OAC); high resolution FABMS: calcd for $C_{26}H_{35}NaO_{17}$ [MH-MeOH]$^+$, 619.1874; found, 619.1875.

Methyl 2-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (1). A solution of compound **27** (1.11 g, 1.71 mmol) in dry methanol (I5 mL) was deacetylated as described for **15**. Evaporation of the solvent gave a syrup (0.57 g, 99%). This was passed through a Sephadex® G-10 column using water as eluant. Lyophilization of the sugar containing fractions gave **1** as a white amorphous powder: $[\alpha]_D$ -13.0° (*c* 1.33, water); ^{1}H NMR δ 4.72 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1b), 4.49 (d, 1 H, $J_{1,2}$ = 7.8, H-1a), 3.92 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-4b), 3.91 (d, 1 H, H-6a), 3.56 (s, 3 H, OCH_3); high resolution FABMS: calcd for $C_{13}H_{23}O_{11}$ [M - H]$^-$, 355.1241; found, 355.1258.

Methyl 3-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (2). Compound **29** (0.69 g, 1.13 mmol) was deacetylated as described for **15** to afford compound **2** (0.35 g, 99.8%): $[\alpha]_D$ +5.7° (*c* 1.25, water); ^{1}H NMR δ 4.65 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1b), 4.42 (d, 1 H, $J_{1,2}$ = 7.5, H-1a), 3.91 (d, 1 H, H-6a), 3.91 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-4b), 3.57 (s, 3 H, OCH_3); high resolution FABMS: calcd for $C_{13}H_{23}O_{11}$ [M - H]$^-$, 355.1241; found, 355.1258.

Methyl 2,3-di-*O*-β-D-Galactopyranosyl-β-D-glucopyranoside (6). Compound **30** was deacetylated as described for **15** to afford **6** in quantitative yield: $[\alpha]_D$ +2.4° (*c* 4.78, water); ^{1}H NMR δ 4.80 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1b), 4.73 (d, 1 H, $J_{1,2}$ = 8.0, H-1c), 4.51 (d, 1 H, $J_{1,2}$ = 8.0, H-1a), 3.93 (m, 3 H, H-4b, H-4c, H-6a), 3.59 (s, 3 H, OCH_3); high resolution FABMS: calcd for $C_{19}H_{33}O_{16}$ [M - H]$^-$, 517.1769; found, 517.1754.

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REFERENCES AND NOTES

1. Corresponding author's present address: Alpha-Beta Technology, One Innovation Drive, Worcester, Massachusetts 01605.

2. R. Lotan, D. Lotan and A. Raz, *Cancer Res.*, **45**, 4349 (1985).

3. L. Meromsky, R. Lotan and A. Raz, *Cancer Res.*, **46**, 5270 (1986).

4. H. J. Gabius, R. Engelhardt, F. Cramer, R. Batge and G. a. Nagel, *Cancer Res.*, **45**, 253 (1985).

5. H. J. Gabius, R. Engelhardt and F. Cramer, *Anticancer Res.*, **6**, 573 (1986).

6. A. Raz, G. Pazerini and P. Carmi, *Cancer Res.*, **49**, 3489 (1989).

7. G. Ashwell and A. G. Morell, *Adv. in Enzymology,* **41**, 99 (1974).

8. J. Beth, K. Oette and G. Uhelenbruk, *Lectins*, Vol. 5; D. van Driesche and T. C Bon-Hansen, Eds.; Walter de Gruyter: Berlin, 1986, p 229.

9. Beuth, H. L. Ko, V. Schirrmacher, G. Uhlenbruck and G. Pulverer, *Clinc. Expl. Metastasis,* **6**, 115 (1988).

10. H. Oguchi, T. Toyokuni, B. Dean, H. Ito, E. Otsugi, V. L. Jones, K. K. Sadozai and S. Hakomori, *Cancer Commun.*, **2**, 311 (1990).

11. N. Kojima, M. Shiota, Y. Sadahira, K. Handa and S. Hakomori, *J. Biol. Chem.*, **267**, 17264 (1992).

12. E. Pacsu, *Methods in Carbohydrate Chem.*, Vol. II; R. L.Whistler and M. L. Wolfrom, Eds.; Academic Press: New York, 1963, p 356.

13. B. Bernet and A. Vasella, *Helv. Chim. Acta.*, **62**, 1990 (1979).

14. R. R. Schmidt and M. Stumpp, *Liebigs Ann. Chem.*, 1249 (1983).

15. W. Majak, M. H. Benn and Y. Y. Huang, *J. Nat. Prod.*, **51**, 985 (1988).

16. P. Kovac and C. P. J. Glaudmans, *J. Carbohydr. Chem.*, **2**, 313 (1983).

17. A. P. Tulloch and A. Hill, *Can. J. Chem.*, **46**, 2485 (1968).

18. P. Kovac and C. P. J. Glaudemans, *Carbohydr. Res.*,**140**, 289 (1985).

19. P. Kovac, *Carbohydr. Res.*,**135**, 237 (1986).

20. V. Pozsgay, J. R. Brisson and H. J. Jennings, *Can. J. Chem.*, **65**, 2764 (1978).

21. V. Pozsgay, J. R. Brisson and H. J. Jennings, *Carbohydr. Res.*, **205**, 133 (1990).

22. N. E. Nifant'ev, G. M. Lipkind, A. S. Sashkov and N. K. Kochetkov, *Carbohydr.Res.*, **223**, 109 (1992).

23. H. P.Wessel, T. Iversen and D. R. Bundle, *Carbohydr. Res.*, **130**, 5 (1984).

24. M. L. Hayes, A. S. Serianni, and R. Barker, *Carbohydr. Res.*, **100**, 87 (1982).

25. V. S. Rao and A. S. Perlin, *Can. J. Chem.*, **61**, 2688 (1983).

26. W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).