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Note

Regioselective synthesis of a glycomimetic trisaccharide of Sialyl Lewis (sLe^x)

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ABSTRACT

Sialyl Lewis (sLe^x) is the smallest naturally occurring carbohydrate ligand that binds to E-Selectin on the activated endothelium. We report here the total synthesis of acetic acid-sLe^x analog (**12**), for testing as a therapeutic agent. Methoxyethyl 4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**3**) was prepared starting from the methoxyethyl-β-D-lactoside (**2**), which was selectively benzoylated to give the methoxyethyl 2,6-di-*O*-benzoyl-4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). Glycosylation of acceptor **4** with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**5**) in the presence of cupric bromide and tetrabutylammonium bromide afforded the corresponding methoxyethyl 2,6-di-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**6**). Selective removal of the 4″,6″-*O*-isopropylidene group from **6** gave the deprotected trisaccharide **7**. The regioselective esterification of *O*-3″ of trisaccharide **8** (obtained from the dibutylstannylene derivative of **7**) with benzyl-2-bromoacetate and tetrabutylammonium bromide afforded the 3″-*O*-carbobenzyloxymethyl trisaccharide derivative **9**, which on saponification and hydrogenolysis with palladium–charcoal afforded the target trisaccharide **12** glycomimetic of Sialyl Lewis (sLe^x) trisaccharide omitting the sialic acid moiety.

Cell-surface glycoconjugates are known to act as cell–cell recognition molecules via the specific binding between carbohydrates on one cell and the protein receptors on the opposing cell.[1] The selectin biomolecules are a family of carbohydrate binding proteins that mediate the tethering and rolling of the leukocytes in the blood vessel endothelium at the sites of inflammation.[2,3] They are also implicated in the hematogenous metastasis of some cancer cells.[4,5] The sialyl Lewis X (sLe^x) tetrasaccharide,[6–8] α-Neu*p*5Ac-(2→3)-β-Gal*p*-(1→4)-α-Fuc*p*-(1→3)-Glc*p*NAc, has been generally recognized as a common ligand for all selectin biomolecules.[9,10]

Numerous analogs and mimetics of the Le^x and sLe^x have been designed and synthesized previously[11–17] to elucidate the mechanism of selectin recognition and to afford novel therapeutic agents for the treatment of allergy, microbial infection, and inflammatory and autoimmune diseases.[18–20] Recently, Asnani and Auzanneau[21,22] used our strategy, which was reported for the synthesis of partially benzoylated 3′,4′-*O*-isopropylidene-β-D-lactosides, to prepare key intermediates for the assembly of sialyl Lewis X (sLe^x) analogs.[23,24] The aim of our work is to synthesize the glycomimetic trisaccharide of sLe^x, lacking the sialic acid moiety, for testing as a therapeutic agent.

In a previous work, we have shown that 3′,4′-*O*-isopropylidene-β-D-lactosides could be selectively benzoylated at positions 2,2′,6,6′ having free hydroxyl groups at *O*-3 and potentially free at *O*-3′, but the structure of the resulting tetrabenzoate **4** was not fully characterized.[23,24]

In the initial stage of the synthesis (Scheme 1), the catalytic O-deacetylation of the hepta-acetate (**1**)[25] afforded methoxyethyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (**2**), which on treatment with 2,2-dimethoxypropane in the presence of camphorsulfonic acid[26] gave the methoxyethyl 4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**3**). Regioselective benzoylation of the 3′,4′-*O*-isopropylidene derivative (**3**) at low temperature produced mainly, methoxyethyl 2,6-di-*O*-benzoyl-4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**) as fine needles in excellent yield.

The structural elucidation of the methoxyethyl-β-D-lactoside acceptor (**4**) was accomplished using a two dimensional ^{1}H–^{1}H COSY experiment beginning with resonances of H-2′ and H-2. The chemical shifts of the other ^{1}H resonances were established by tracing the connectivity of the cross-peaks on the COSY contour map. Additional support for the proposed structure **4** was achieved through a close examination of the ^{13}C NMR spectrum, ^{13}C–^{1}H correlation, and DEPT experiments.

Fucosylation of the disaccharide acceptor **4** with the glycosyl donor methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**5**) in the presence of the glycosyl promoter cupric bromide, tetra-*n*-butylammonium bromide, and activated powdered molecular sieves 4 Å, in methylene chloride, gave the expected trisacccharide

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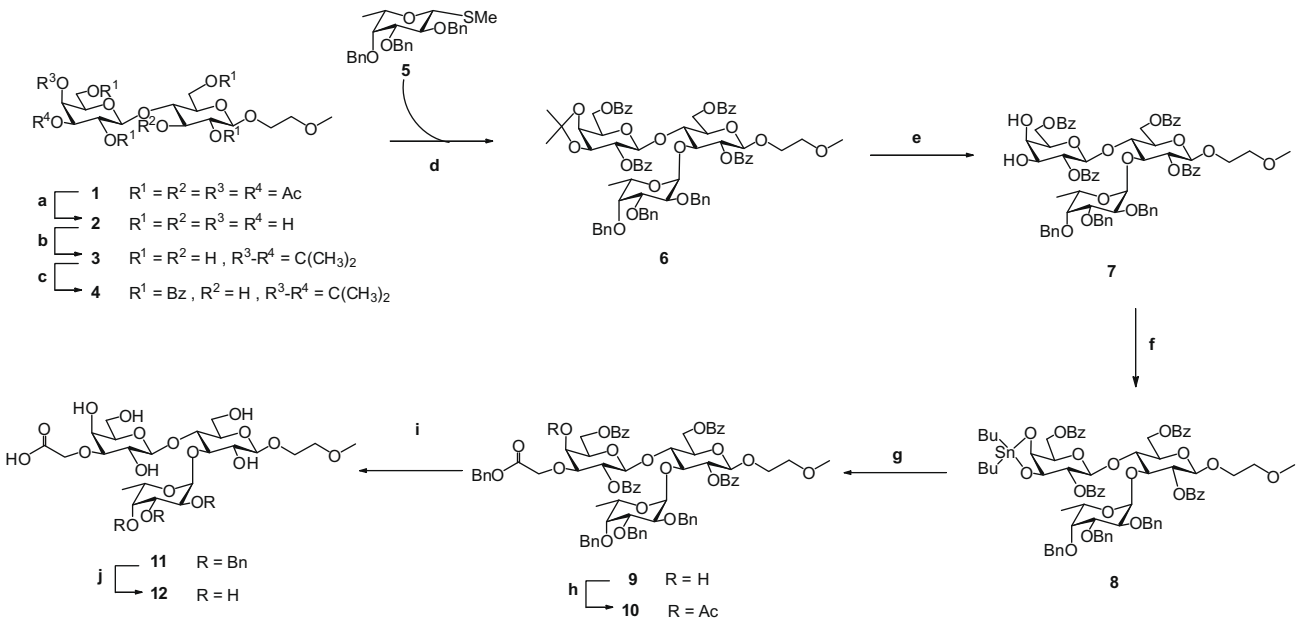

Scheme 1. Reagents and conditions: (a) (1) CH_3OH, Na-metal, 2 h rt; (2) Amberlite IR-120 (H+) resin; (b) (1) camphorsulfonic acid, 2,2-dimethoxypropane, 48 h rt; (2) 10:1 CH_3OH–H_2O, reflux 3 h; (c) BzCl, C_5H_5N, –45 °C, 3–4 h; (d) (1) acceptor **4**, 5:1 CH_2Cl_2–DMF, $CuBr_2$, *n*-Bu_4NBr, MS 4 Å; (2) donor **5**, CH_2Cl_2, 48 h; (e) 80% aq AcOH, 80 °C, 4 h; (f) Bu_2SnO, CH_3OH, reflux 1 h; (g) C_6H_6, $BrCH_2COOBn$, *n*-Bu_4NBr, MS 4 Å, 70 °C, 3 h; (h) Ac_2O, C_5H_5N; (i) = (a); (j) 10% Pd–C, H_2 (g), 1 atm.

methoxyethyl 2,6-di-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**6**) in excellent yield.[27] The ^{1}H NMR spectrum gave the expected three proton doublet signal for the fucosyl moiety at δ 1.02 ($J_{5,6}$ = 6.3 Hz, CH–C*H*$_3$) and one proton doublet signal at δ 5.74 ($J_{1',2'}$ = 3.0 Hz, H-1′), hence confirming the assigned structure. The ^{13}C NMR spectrum showed three anomeric carbons at δ 102.2, 100.9 (C-1 and C-1″), and 98.0 (C-1′) and an additional signal at δ 17.8 corresponding to the *C*H$_3$ group of the fucose moiety.

Removal of the isopropylidene group was carried out by warming the fucosylated trisaccharide (**6**) in 80% aqueous acetic acid to give the methoxyethyl 2,6-di-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**7**) in good yield. The ^{1}H and ^{13}C NMR spectra confirmed the disappearance of the isopropylidene signals and permitted the complete elucidation of the proposed structure.

The fucosylated trisaccharide **7** possessing a free diol at positions 3″,4″ was heated at reflux with an equimolar amount of di-*n*-butyltin oxide in absolute CH_3OH according to the conditions of Nashed and Anderson,[28] followed by evaporation of the CH_3OH to give a syrupy solution of 3″,4″-*O*-dibutylstannylene derivative **8**, which was used immediately as such for the following regioselective etherification.

Subsequently, the regioselective etherification of fucosylated trisaccharide **8** was carried out with excess bromoacetic acid benzyl ester and tetrabutylammonium bromide[29] in dry benzene at 70 °C for 3 h, to produce exclusively the 3″-*O*-benzyl acetate trisaccharide derivative (**9**). The ^{1}H NMR spectrum of compound **9** showed a new signal at δ 5.08 (2d, J_{gem} = 12.0 Hz, OC*H*$_2$–COOBn), and loss of one of the OH signals. ^{13}C NMR spectrum showed additional three inverted peaks at δ 67.9 (OCH$_2$–COOBn), 68.8 (OCH$_2$–COOCH$_2$Ar), and the carbonyl group at δ 172.1.

As expected, the regioselective alkylation occurred essentially at the equatorial oxygen.[28] This was evidenced by the acetylation of trisaccharide **9** with acetic anhydride in pyridine at room temperature to give the mono-acetylated trisaccharide derivative **10**. The ^{1}H NMR spectrum of the mono-acetylated trisaccharide **10** was similar to that of **9** except for a broad doublet (J = 2.9 Hz) observed which was shifted to a lower field resonance of δ 5.74 ppm for H-4″, a singlet at δ 1.80 for the methyl protons of the acetyl group, and the absence of the OH signal.

The Zemplén O-debenzoylation in methanolic sodium methoxide converted the tetrabenzoate (**9**) quantitatively into methoxyethyl 3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(3-*O*-carbobenzyloxymethyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**11**), which was then de-ionized with Amberlite IR-120 (H^+) resin. After filtration and concentration, the residual methyl benzoate was removed by the addition and decantation of several portions of *n*-hexane. The crude syrup was used for the hydrogenolysis step without further purification.

The hydrogenolysis of the benzyl protecting group was carried out with 10% Pd–charcoal in CH_3OH to afford the free trisaccharide glucomimetic of sLe^x (**12**) lacking the sialic acid moiety. The ^{1}H NMR spectrum of trisaccharide **12** confirmed the loss of all the low-field Ph–*H* signals, and showed the three diagnostic doublets for the anomeric protons at δ 5.26 ($J_{1',2'}$ = 3.8 Hz) assigned to the α-L-fucose moiety, δ 4.31 (J = 8.4 Hz) and δ 4.27 (J = 7.7 Hz) for the β-D-galactose and β-D-glucose moieties.

Further versatility results from the use of methoxyethyl group as an aglycon because it can be derivatized to the bromide,[25] which is useful as a donor to attach a lipid tail.

1. Experimental

1.1. General methods

Optical rotations were obtained at 22 °C with a Perkin–Elmer Model 241 Polarimeter 10 cm, 1 mL microcell. ^{1}H NMR spectra were recorded at Glycomed, Inc. Alameda, California, USA, with a Varian Gemini 300 MHz spectrometer, and at Faculty of Science, Alexandria University, Egypt, at 500 MHz (JEOL EX 500 spectrometer) at ambient temperature. ^{13}C NMR spectra were recorded with a Varian Gemini 300 MHz instrument operating at 75.50 MHz or 125.77 MHz, and the chemical shifts were referenced to $CDCl_3$ (δ 77.7 ppm). ^{1}H NMR and ^{13}C NMR chemical shifts (δ) are reported

in parts per million (ppm). Coupling constants (*J*) are reported in Hertz (Hz). Assignment of proton resonance was verified by the two dimensional ^{1}H–^{1}H COSY experiments to trace connectivity. Also, the assignment of carbon resonance was supported by ^{13}C–^{1}H correlation and DEPT experiments. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and doublet of quartet (dq). Thin Layer Chromatography (TLC) was performed on aluminum plates precoated with silica gel (Merck, GF_{254}). Spots were detected under ultraviolet (UV) light and/or charred with a solution of 10% H_2SO_4 in ethanol. For column chromatography, Merck Silica Gel 60 (particle size 70–230 mesh) was used.

1.1.1. High resolution electrospray ionization mass spectrometry (HRESIMS)

High resolution conventional electrospray ionization-mass spectrometry for all the synthesized compounds was acquired in the positive ion mode using an Applied Biosystems API-QSTAR XL quadrupole orthogonal time of-flight (QqTOF)-MS/MS hybrid tandem mass spectrometer (Applied Biosystems International-MDS Sciex, Foster City, California, USA). This instrument is capable of analyzing a mass range of *m/z* 5–40,000, with a resolution of 10,000 in the positive ion mode. ESI was performed with the Turbo Ionspray source operated at 5.5 kV and a temperature varying form 60 to 100 °C. The ESI-MS were recorded with a cone voltage setting (Declustering Potential 1) varying from 150 to 200 volts. The ion spray voltage, Curtain Gas, ion source gas 1, ion source gas 2, focusing potential and Declustering Potential 2 parameters were generally kept constant.

1.2. Methoxyethyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (2)

Methoxyethyl hepta-*O*-acetyl-β-D-lactoside (**1**)[25] (10 g, 14.4 mmol) was dissolved in anhydrous CH_3OH (50 mL) and sodium metal (0.5 g) was added. The mixture was stirred at room temperature for 2 h and then de-ionized with Amberlite IR-120 (H^+) resin. The resin was filtered off and rinsed with CH_3OH and the combined filtrate and washings were concentrated and evaporated to dryness. Crystallization, and re-crystallization, of the residue from CH_3OH furnished **2** (5.46 g, 95%) as needles. $[\alpha]_D$ −5.8 (*c* 0.5, CH_3OH); ^{1}H NMR (500 MHz, D_2O): δ 4.33 (d, 1H, $J_{1',2'}$ = 8.4 Hz, βH-1′), 4.27 (d, 2H, $J_{1,2}$ = 8.4 Hz, βH-1), 3.89–3.15 (m, 12H, C*H* and CH_2 of sugar), 3.50–3.47 (m, 4H, OCH_2CH_2O), 3.22 (s, 3H, OCH_3); HRESIMS: *m/z* calcd for $[C_{15}H_{28}O_{12}+Na]^+$, 423.1479; found, 423.1541.

1.3. Methoxyethyl 2,6-di-*O*-benzoyl 4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (4)

Dry camphorsulfonic acid[26] (143 mg, 0.6 mmol) was added to a solution of **2** (5 g, 12.5 mmol) in 2,2-dimethoxypropane (300 mL). The mixture was stirred for 48 h at room temperature. Following addition of triethylamine (0.85 mL, 6.2 mmol), the mixture was stirred for 15 min and concentrated to dryness and coevaporated with toluene to remove all traces of triethylamine. A solution of the crude product in 10:1 CH_3OH–H_2O (300 mL) was heated at reflux for 3 h. The solvents were evaporated and the residue was purified by flash chromatography (20:1, CH_3Cl–CH_3OH) to afford *methoxyethyl 4-O-(3,4-O-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside* (**3**) (4.9 g, 89%).

The pure 3′,4′-*O*-isopropylidene-β-D-lactoside derivative (**3**) (10 g, 22.7 mmol) was dissolved in absolute pyridine (200 mL), the solution was cooled to −45 °C and benzoyl chloride (11.1 mL, 4.2 M equiv) was added dropwise with stirring over a period of 3–4 h. The mixture was diluted with CH_2Cl_2, and then processed with a conventional aqueous extraction. Evaporation of the solvent under diminished pressure gave the crude product. Crystallization from hot CH_3OH gave the crystalline tetrabenzoate (**4**), with a yield of 70% (13.6 g), in addition to a small yield of the corresponding pentabenzoate. The obtained crude crystalline compound was used for the next step without further purification. For identification purpose, a portion was purified by chromatography on a column of silica gel using 20:1 toluene–acetone as the eluent. $[\alpha]_D$ +43 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06–7.86 (3d, 8H, *J* = 7.6 Hz, *ortho*-Ar), 7.56–7.25 (m, 12H, *meta*- and *para*-Ar), 5.37 (t, 1H, *J* = 7.6 Hz, H-2′), 5.23 (dd, 1H, *J* = 9.4, 8.2 Hz, H-2), 4.87 (dd, 1H, *J* = 12.5, 2.4 Hz, H-6$'_a$), 4.67 (d, 1H, *J* = 8.2 Hz, βH-1′), 4.65 (d, 2H, *J* = 8.2 Hz, βH-1, OH-3), 4.47–4.36 (m, 3H, H-6$_a$, H-6$'_b$, H-3′), 4.29–4.25 (m, 2H, H-4′, H-5′), 4.20 (dd, 1H, *J* = 12.2, 3.8 Hz, H-6$_b$), 4.01 (dd, 1H, *J* = 9.9, 7.7 Hz, H-3), 3.86–3.80 (m, 1H, OC*H*H–CH_2OCH_3), 3.74 (t, 1H, *J* = 9.9 Hz, H-4), 3.69–3.66 (m, 1H, H-5), 3.63–3.59 (m, 1H, OCH*H*–CH_2OCH_3), 3.39–3.36 (m, 2H, –CH_2–OCH_3), 3.10 (s, 3H, OCH_3), 1.65–1.35 (2s, 6H, C(CH_3)$_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 167.1–165.9 (4 × *C*OAr), 133.9–128.8 (Ar-*C*), 111.8 (*C*(CH$_3$)$_2$), 102.1 (C-1), 101.6 (C-1′), 82.9 (C-4), 77.6 (C-3′), 74.0 (C-3, C-4′), 73.6 (C-2′), 73.4 (C-2), 72.6 (C-5, C-5′), 72.2 (–*C*H$_2$–OCH$_3$), 69.4 (–O*C*H$_2$–CH$_2$OCH$_3$), 64.2 (C-6′), 63.2 (C-6), 59.4 (OCH$_3$), 28.2, 26.8 (C(*C*H$_3$)$_2$). HRESIMS: *m/z* calcd for $[C_{46}H_{48}O_{16}+Na]^+$, 879.2840; found, 879.2957.

1.4. Methoxyethyl 2,6-di-*O*-benzoyl 3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (6)

The glycosyl acceptor **4** (3 g, 3.5 mmol) was dissolved in 5:1 dry CH_2Cl_2–DMF (35 mL), and the glycosylation promoter cupric bromide (1.4 g, 6.26 mmol), tetra-*n*-butylammonium bromide (240 mg, 0.74 mmol), and molecular sieve 4 Å (3 g) were added to the solution, which was stirred for 2 h at room temperature. A solution of methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**5**) (2.4 g, 5.16 mmol) in CH_2Cl_2 (4 mL) was slowly added over a period of 30 min. Stirring was continued for 48 h and the reaction progress was monitored by TLC (20:1 toluene–acetone) until complete absence of the acceptor and the formation of a major product were observed. CH_3OH (3 mL) was added to the reaction mixture and stirred for 30 min. The precipitates were filtered through Celite and washed with CH_2Cl_2. The filtrate was washed with aqueous $NaHCO_3$ and H_2O, followed by drying over anhydrous $MgSO_4$ and concentrated to a syrup which was purified by chromatography on a column of silica gel with (80:10:1, toluene–acetone–CH_3OH) to afford pure **6** as a syrup (3.4 g, 77%). $[\alpha]_D$ +14.6 (*c* 0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20–7.90 (4d, 8H, *J* = 7.6 Hz, *ortho-H* of 4 × Bz), 7.57–6.91 (m, 27H, Ph-*H*), 5.46 (d, 1H, $J_{1',2'}$ = 3.7 Hz, αH-1′), 5.42 (t, 1H, *J* = 8.0 Hz, H-2″), 5.24 (t, 1H, *J* = 8.5 Hz, H-2), 4.95 (d, 1H, *J* = 11.5 Hz, C*H*HPh), 4.92–4.86 (m, 1H, H-5′), 4.82–4.72 (m, 3H, H-6$''_a$, CH_2Ph), 4.66 (d, 1H, *J* = 11.3 Hz, C*H*HPh), 4.56–4.48 (m, 4H, H-1″, H-1, H-6$_a$, H-6$_b$), 4.37 (d, 1H, *J* = 11.5 Hz, C*H*HPh), 4.33–4.20 (m, 5H, H-3, H-3′, H-3″, H-6$''_b$, C*H*HPh), 4.23 (br d, 1H, H-4″), 4.08 (t, 1H, *J* = 9.3 Hz, H-4), 3.94 (dd, 1H, $J_{1',2'}$ = 3.7 Hz, $J_{2',3'}$ = 10.3 Hz, H-2′), 3.85–3.74 (m, 3H, H-4′, H-5″, OC*H*H–CH_2OCH_3), 3.58–3.46 (m, 2H, H-5, OC*H*H–CH_2OCH_3), 3.34–3.24 (m, 2H, –CH_2–OCH_3), 3.00 (s, 3H, OCH_3), 1.62–1.33 (2s, 6H, C(CH_3)$_2$), 1.35 (d, 3H, *J* = 7.3 Hz, CH_3 of fucose moiety); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 167.3–165.0 (4 × *C*OAr), 134.0–126.0 (Ar-*C*), 111.5 (*C*(CH$_3$)$_2$), 102.2, 101.0 (C-1 and C-1″), 98.0 (C-1′), 79.7–72.0 (11 *C*H of sugar, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-2″, C-3″, C-4″, C-5″), 75.5 (*C*H$_2$Ph), 73.2, 73.1 (2 × *C*H$_2$Ph), 72.4 (–*C*H$_2$–OCH_3), 69.4 (–O*C*H$_2$–CH$_2$OCH$_3$), 67.0 (C-5′), 63.2 (C-6″), 63.1 (C-6), 59.3 (OCH$_3$), 28.4, 26.5 (C(*C*H$_3$)$_2$), 17.8 (*C*H$_3$ of fucose moiety); HRESIMS: *m/z* calcd for $[C_{73}H_{76}O_{20}+Na]^+$, 1295.4828; found, 1295.4865.

1.5. Methoxyethyl 2,6-di-*O*-benzoyl 3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (7)

Compound **6** (3 g, 2.4 mmol) was dissolved in 80% acetic acid (10 mL), and stirred at 80 °C for 4 h, at which time TLC (8:2 toluene–EtOAc) showed complete conversion of the starting material into a product with lower mobility. The mixture was evaporated and coevaporated with toluene to remove the acetic acid. The crude diol syrup was purified on a column of silica gel (CH_3Cl) to give the title compound **7** (2.5 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14–7.95 (m, 8H, *ortho-H* of 4 × Bz), 7.60–6.93 (m, 27H, Ph-*H*), 5.43 (t, 1H, J = 8.0 Hz, H-2″), 5.41 (d, 1H, $J_{1',2'}$ = 3.6 Hz, αH-1′), 5.24 (t, 1H, J = 8.1 Hz, H-2), 4.95–4.86 (m, 2H, C*H*HPh, H-5′), 4.82–4.66 (m, 4H, 3 × C*H*HPh, H-6″$_a$), 4.64–4.54 (m, 4H, H-1″, H-1′, H-6$_a$, H-6$_b$), 4.36 (d, 1H, J = 11.4 Hz, C*H*HPh), 4.28–4.12 (m, 7H, H-3, H-4, H-3′, H-3″, H-4″, H-6″$_b$, C*H*HPh), 3.94–3.74 (m, 4H, H-2′, H-4′, H-5″, OC*H*H–CH_2OCH_3), 3.62–3.46 (m, 3H, H-5, OC*H*H–CH_2OCH_3, O*H*), 3.30–3.14 (m, 3H, –C*H*$_2$–OCH_3, O*H*), 3.00 (s, 3H, OC*H*$_3$), 1.33 (d, 3H, J = 7.3 Hz, C*H*$_3$ of fucose moiety); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 167.3–165.0 (4 × *C*OAr), 134.0–126.5 (Ar-*C*), 102.2 (C-1), 100.6 (C-1″), 98.2 (C-1′), 79.6–68.4 (11 *C*H of sugar, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-2″, C-3″, C-4″, C-5″), 75.8 (*C*H$_2$Ph), 73.2, 73.0 (2 × *C*H$_2$Ph), 72.4 (–*C*H$_2$–OCH_3), 69.3 (–OCH$_2$–CH_2OCH_3), 67.1 (C-5′), 63.4 (C-6″), 62.4 (C-6), 59.4 (O*C*H$_3$), 17.2 (*C*H$_3$ of fucose moiety); HRESIMS: *m/z* calcd for $[C_{70}H_{72}O_{20}+Na]^+$, 1255.4828; found, 1255.4893.

1.6. Methoxyethyl 2,6-di-*O*-benzoyl 3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-carbobenzyloxymethyl-β-D-galactopyranosyl)-β-D-glucopyranoside (10)

A stirred mixture of compound **7** (1.5 g, 1.2 mmol) and dibutyltin oxide (0.36 g, 1.2 M equiv) in CH_3OH (20 mL) was heated at reflux for 1 h. The CH_3OH was evaporated and the residual solid was dried under vacuum for 1 h. The residue of 3′,4′-*O*-dibutylstannylene derivative **8** in benzene (15 mL), benzyl-2-bromoacetate (0.58 mL, 3 M equiv), tetra-*n*-butylammonium bromide (196 mg, 0.5 M equiv), and molecular sieves 4 Å (0.5 g) were heated for 3 h at 70 °C. Most of the solvent was removed by evaporation under reduced pressure and the product was extracted with CH_2Cl_2. After evaporation of the solvent, the residue was purified by chromatography on a column of silica gel (CH_3Cl) to give *methoxyethyl 2,6-di-O-benzoyl 3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-(2,6-di-O-benzoyl-3-O-carbobenzyloxymethyl-β-D-galactopyranosyl)-β-D-glucopyranoside* (**9**) (1.33 g, 79%) as a syrup.

Acetylation of compound **9** with acetic anhydride in pyridine at room temperature afforded the title compound **10**. $[\alpha]_D$ +2.3 (*c* 9.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14–7.92 (m, 8H, *ortho-H* of 4 × Bz), 7.60–7.00 (m, 27H, Ph-*H*), 5.47 (d, 1H, J = 2.9 Hz, H-4″), 5.45 (d, 1H, $J_{1',2'}$ = 3.8 Hz, αH-1′), 5.42 (t, 1H, J = 8.4 Hz, H-2), 5.35 (t, 1H, J = 9.3, 8.4 Hz, H-2″), 5.01–4.89 (m, 4H, H-5′, OC*H*$_2$–COOBn, C*H*HPh), 4.83, 4.72 (2d, J = 12.3 Hz, C*H*$_2$Ph), 4.78 (d, 1H, J = 12.3 Hz, C*H*HPh), 4.62 (d, 1H, J = 8.4 Hz, βH-1″), 4.56 (d, 1H, J = 8.4 Hz,βH-1′), 4.55–4.51 (m, 2H, C*H*HPh, H-6$_b$), 4.38 (d, 1H, J = 11.5 Hz, C*H*HPh), 4.30 (t, 1H, J = 9.2 Hz, H-3), 4.27–4.22 (m, 2H, C*H*HPh, H-6″$_a$), 3.12–4.03 (m, 5H, H-4, H-3′, H-6$_a$, H-6″$_b$, C*H*HPh), 3.98 (dd, 1H, $J_{1',2'}$ = 3.8 Hz, $J_{2',3'}$ = 10.0 Hz, H-2′), 3.80–3.76 (m, 1H, OC*H*H–CH_2OCH_3), 3.75–3.73 (m, 2H, H-4′, H-3″), 3.65 (br t, 1H, H-5″), 3.57–3.53 (m, 1H, OC*H*H–CH_2OCH_3), 3.50–3.47 (m, 1H, H-5), 3.33–3.25 (m, 2H, –C*H*$_2$–OCH_3), 2.98 (s, 3H, O*CH*$_3$), 1.80 (s, 3H, C*H*$_3$CO), 1.40 (d, 3H, J = 6.9 Hz, C*H*$_3$ of fucose moiety); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.0 (*C*OOBn and CH_3CO), 167.3–165.0 (4 × *C*OAr), 134.0–126.5 (Ar-*C*), 102.2 (C-1), 101.3 (C-1″), 98.1 (C-1′), 80.1–67.1 (11 *C*H of sugar, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-2″, C-3″, C-4″, C-5″), 74.7 (*C*H$_2$Ph), 73.6, 73.3 (2 × *C*H$_2$Ph), 72.4 (–*C*H$_2$–OCH_3), 69.4 (–OCH_2–CH_2OCH_3), 67.5 (O*C*H$_2$–COOBn), 68.5 (OCH$_2$–COO*C*H$_2$Ph), 66.5 (C-5′), 63.1 (C-6″), 61.5 (C-6), 59.4 (O*C*H$_3$), 21.0 (*C*H$_3$CO), 17.8 (*C*H$_3$ of fucose moiety); HRESIMS: *m/z* calcd for $[C_{81}H_{82}O_{23}+Na]^+$, 1445.5145; found, 1445.5275.

1.7. Methoxyethyl 3-*O*-(α-L-fucopyranosyl)-4-*O*-(3-*O*-carbobenzyloxymethyl-β-D-galactopyranosyl)-β-D-glucopyranoside (12)

The tetrabenzoate **9** (330 mg, 0.24 mmol) was suspended in CH_3OH (30 mL), and sodium metal (100 mg) was added. The solution was warmed and debenzoylation was monitored by TLC (1:3 CH_3OH–CH_2Cl_2), which indicated the disappearance of the starting material. The solution was cooled for 1 h and neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered and rinsed with CH_3OH and the combined filtrate and washings were concentrated to afford *methoxyethyl 3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-(3-O-carbobenzyloxymethyl-β-D-galactopyranosyl)-β-D-glucopyranoside* (**11**) as syrup in almost quantitative yield. *n*-Hexane was added and then decanted to remove methyl benzoate.

A solution of compound **11** (150 mg, 0.17 mmol) in CH_3OH (15 mL) was treated with 10% palladium–charcoal catalyst (0.8 g), and the suspension was stirred overnight under hydrogen at 1 atmosphere. TLC (3:3:1, EOAc–2-propanol–H_2O) showed the disappearance of the starting material and formation of one new product, the mixture was filtered to remove the catalyst and the filtrate was evaporated under diminished pressure to give compound **12** (98 mg, 95%) as an amorphous solid. $[\alpha]_D$ −31.3 (*c* 1.8, CH_3OH), ^{1}H NMR (500 MHz, D_2O): δ 5.26 (d, 1H, $J_{1',2'}$ = 3.8 Hz, αH-1′), 4.31, 4.27 (2d, 2H, J = 8.4 Hz, J = 7.7 Hz, βH-1″ and βH-1), (m, 22H, C*H* and C*H*$_2$ of sugar, OC*H*$_2$–CO, and OC*H*$_2$–C*H*$_2$O), 3.20 (s, 3H, O*CH*$_3$), 1.00 (d, 3H, J = 6.9 Hz, C*H*$_3$ of fucose moiety); ^{13}C NMR (125.77 MHz, D_2O): δ 102.2, 101.6 (C-1, C-1″), 98.3 (C-1′), 81.7-65.0 (12 *C*H of sugar, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″), 72.5–68.0 (3 *C*H$_2$, OCH$_2$–CH_2OCH_3, OCH$_2$–COOH), 61.6 (C-6″), 59.6 (C-6), 58.0 (O*C*H$_3$), 15.2 (*C*H$_3$ of fucose moiety); HRESIMS: *m/z* calcd for $[C_{23}H_{40}O_{18}+Na]^+$, 627.2112; found, 627.2022.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.11.019.

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