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# First Synthesis Of The 3' - Sulfated Lewis$^a$ Pentasaccharide, The Most Potent Human *E*-Selectin Ligand So Far

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**Abstract**—Tri- and pentasaccharides of Lewis$^a$-type, sulfated at position 3 of the outer galactose, have been prepared using the new 4-methoxybenzyl glycoside of *N*-acetylglucosamine **5** as starting material. The synthesis of the pentasaccharide **2** was achieved through a β-stereoselective coupling of an α-trichloroacetimidate activated form of the *N*-acetamido protected trisaccharide **18** on to a 3',4'-unprotected lactose derivative.

## Introduction

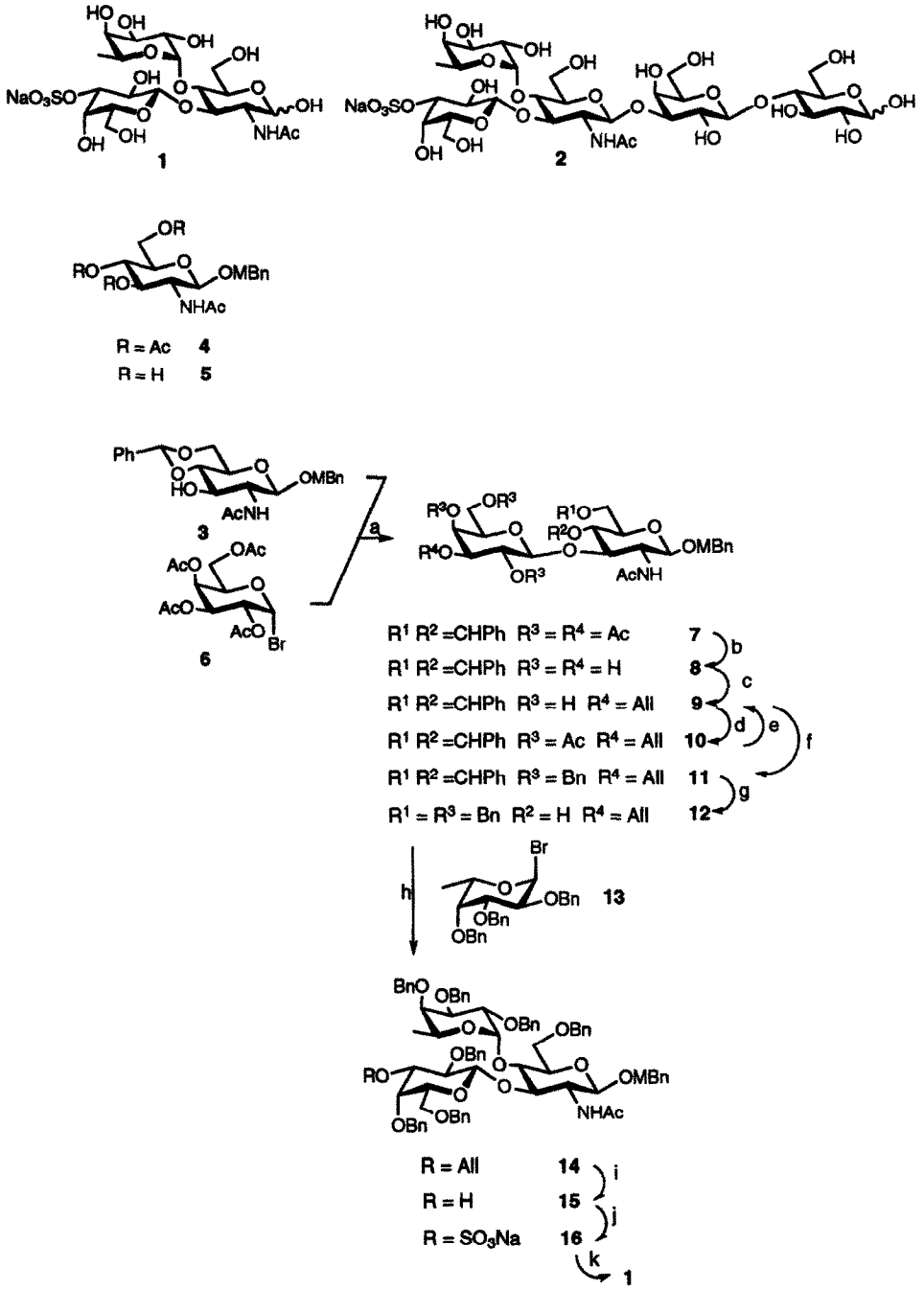
The selectins are a family of calcium dependent mammalian lectins that initiate interactions between blood cells or cancer cells and vascular endothelium.[1,2] Knowledge of their roles in the recruitment of leukocytes to inflammation or injury sites has led to intense work for the development of a new class of carbohydrate-based drugs which would inhibit the primary recognition and thus attenuate undesirable inflammatory responses. Of course, from the economical point of view, it is necessary to define the minimum carbohydrate structure with the highest affinity as a lead to chemical synthesis of oligosaccharides or analogues with selectin inhibitory properties. Moreover, this will help at the fundamental level to better understand the biological events in leukocyte recruitment and extravasation.

The established ligands for *E*-selectin expressed on cytokine-stimulated endothelial cells include a family of blood group-related fuco-oligosaccharides, Le$^a$ and Le$^x$ and their 3'-sialylated derivatives[3] as well as sulfate containing Le$^a$ and Le$^x$ -type O-linked oligosaccharides derived from an ovarian cystadenoma glycoprotein.[4] In the latter study, after conversion to neoglycolipids, the active oligosaccharide fraction with *E*-selectin binding properties was found to be an equimolar mixture of Le$^a$ and Le$^x$ -type tetrasaccharides sulfated at position 3 of the outer galactose with a binding intensity at least equal to that observed with the most widely used 3'-sialyl Le$^x$ glycolipid analog. It is worth noting that this neoglycolipids mixture was found to bind also to *l*-selectin.[5] In order to ascertain which of the two sulfated isomers is the most potent ligand for selectins, we have synthesized the sulfated tri- and penta-oligosacharides of the Le$^a$ -type structure **1** and **2**.[6]

## Results and Discussion

The strategy for the synthesis of the trisaccharide **1** is delineated in Scheme I. The starting compound **3** was prepared in three steps from the common *O*,*N*-peracetylated glucosaminyl chloride. Stereoselective glycosylation with 4-methoxy benzyl alcohol ($Hg(CN)_2$, toluene) gave **4** in 96 % yield. After a quantitative de-*O*-acetylation ($NEt_3$–MeOH–$H_2O$, 1:8:1) giving **5**, reaction with benzaldehyde dimethyl acetal in THF in the presence of TsOH afforded **3** in 93 % yield. The methoxy benzyl group of the anomeric position was chosen because of the very high yield to introduce it and the possibility to remove it selectively at the trisaccharide stage before the activation step for the pentasaccharide synthesis. Then β-stereoselective galactosylation was performed with acetobromogalactose **6** ($Hg(CN)_2$, molecular sieves 4 Å, nitromethane–toluene, 1:1) to give **7** in quantitative yield. The allyl group at *O*-3' was introduced by the stannylene procedure[7] after de-*O*-acetylation of the galactose residue. As direct purification of **9** was inextricable, silica gel chromatography was performed after peracetylation to give pure **10** which gave back **9** in 77 % overall isolated yield (85 % based on starting material recovery). Although near quantitative, the last de-*O*-acetylation step was shown to require rather drastic conditions ($NEt_3$–MeOH–$H_2O$, 1:8:1, 60 h at 80 °C) to go to completion. In fact, the same reaction run at room temperature led to the corresponding *O*-2' monoacetylated disaccharide as shown by $^1H$ NMR. This difficult deacetylation of a 2-*O*-acyl 3-*O*-substituted galactopyranoside has already been noted.[8] Perbenzylation was then performed in DMF with benzyl bromide (2 eq. per OH) in the presence of HNa (1,2 eq. per OH) added portionwise at 0 °C during 1 h to avoid *N*-benzylation. Under these conditions, compound **11** was isolated in 88 % yield. Regioselective opening[9] of the benzylidene acetal by treatment with $NaBH_3CN$-HCl in THF gave the compound **12** in 81 % yield. The fucose residue was then introduced from freshly prepared perbenzyl fucosyl bromide **13**[10] under *in situ* anomerization conditions[11] using tetrabutylammonium bromide in DMF–$CH_2Cl_2$ in the presence of diisopropylethylamine. The protected trisaccharide **14** was thus obtained in 89 % yield. Two step deallylation (1:$RhCl(PPh_3)_3$ 2:$HgCl_2$–HgO, acetone–

water) afforded compound **15** in 67 % yield (90 % based on starting material recovery). The sulfate group was then introduced using classical conditions ($SO_3$–$NMe_3$ complex in anhydrous DMF, 12 h at 55 °C) giving **16** in 82 % yield. One step complete deprotection [10 % Pd/C, $H_2$ (1 atm)] afforded in 80 % isolated yield, the sulfated $Le^a$ trisaccharide target **1** after silica gel chromatograhy ($Pr^iOH$–AcOEt–$H_2O$, 3:5:2) followed by cation exchange chromatography [AG50W-X8 ($Na^+$)], and freeze-drying of the aqueous solution. The stucture of compound **1**, obtained as a mixture of α and β anomers (α:β, 1.5 : 1) was fully ascertained by NMR using a COSY experiment. In particular, the H-3' is found at δ 0.7 downfield compared with H-3' of the $Le^a$ trisaccharide[12] showing unambiguously the presence of the sulfate on galactose at *O*-3'.




*Reagents and conditions*: (a) **6** (1.3 equiv.), $Hg(CN)_2$ (1.3 equiv.), $MeNO_2$–toluene, 1:1, 60 °C, 20 h, 96 %; (b) $Et_3N$–MeOH–$H_2O$, 1:8:1, rt, 48 h, 93 %; (c) $Bu_2SnO$ (1.1 equiv.), toluene, reflux, 14 h, then $BrNBu_4$ (0.5 equiv.), A; Br (30 equiv.), toluene, 40 °C, 24 h; (d) $Ac_2O$–pyridine, 1:1, rt, 12 h; 77 % (c and d steps); (e) $Et_3N$–MeOH–$H_2O$, 1:8:1, 80 °C, 60 h, 93 %; (f) NaH (3.6 equiv.), BrBn (6 equiv.), DMF, 0 °C, 1 h, 88 %; (g) $NaBH_3CN$ (8 equiv.), $HCl_{gas}$, THF, 0 °C, 1 h, 81 %; (h) **13** (3 equiv.), DIPEA (2.7 equiv.), $BrNBu_4$ (1.5 equiv.), DMF–$CH_2Cl_2$, 1:4, rt, 72 h, 89 %; (i) $Rh(Ph_3P)_3Cl$ (0.5 equiv.), EtOH–toluene–$H_2O$, 7:3:1, 70 °C, 17 h, then HgO (2 equiv.), $HgCl_2$ (2 equiv.), acetone–$H_2O$, 9:1, rt, 1h, 67 % (90 %); (j) $SO_3$-$NMe_3$ (4 equiv.), DMF, 55 °C, 12 h, 82 %; (k) Pd/C, $H_2$ (1 atm), EtOH–$H_2O$, 9:1, rt, 60 h, 80 %.

**Scheme I.** Total synthesis of sulfated $Le^a$ trisaccharide **1**.

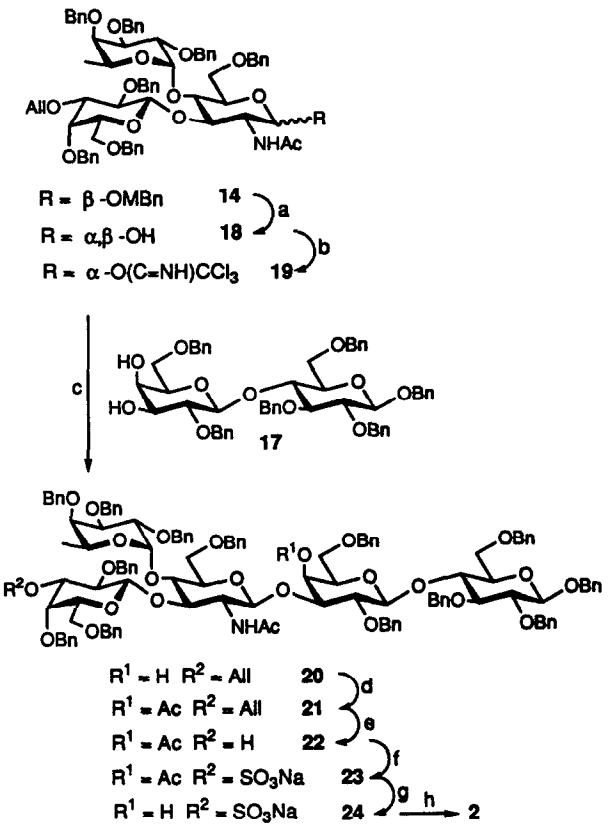
Having in hand the protected trisaccharide **14**, access to the pentasaccharide **2** required deprotection of the anomeric position, activation and coupling with the known disaccharide acceptor **17**.[13] This has been readily accomplished without problem although we were aware that a high yielding stereoselective coupling reaction of *N*-acetamido sugar has not actually been solved in the carbohydrate literature. In fact, in our case, use of acetamido group in **3** allowed the coupling of acetobromogalactose **6** in high yield[14] and avoided the sometimes problematic replacement of the *N*-phthalimido protecting group by the natural *N*-acetyl group.

The synthesis of the pentasaccharide **2** is described in Scheme II. Selective removal of the anomeric paramethoxybenzyl group in **14**, performed by treatment with cerium ammonium nitrate,[15] gave **18** (91 %) which was then converted to the α-trichloroacetimidate **19** in 75 % yield.[16] The crucial coupling of **19** with the lactose derivative **17** in the presence of $BF_3$–$Et_2O$ gave the pentasaccharide **20** in 25 % isolated yield. After acetylation of the free OH group giving **21**, the following sequences leading to **2** were a repetition of what we had done with the trisaccharide. Removal of the allyl protecting group (67 %) gave the hydroxy derivative **22** which was sulfated with the $SO_3$–$NMe_3$ complex in anhydrous DMF to give **23** in 92 % yield. Finally, deacetylation to **24** followed by hydrogenolysis gave the 3'-sulfated pentasaccharide **2** in 66 % overall yield having adequate $^1H$ and $^{13}C$ NMR and mass spectrometry spectra. These oligosaccharides were tested by Feizi and colleagues[17] for their ability to support *E*-selectin binding when converted into neoglycolipids or as inhibitors of the binding of *E*-selectin to immobilized lipid-linked sialyl-$Le^a$, sialyl $Le^x$ or sulfated $Le^a$ pentasaccharides. In these studies, compound **2** was found to be the most potent *E*-selectin ligand so far.[18]

## Experimental

### *General procedures*

Solvents were purified in the usual way: toluene, methylene chloride, dimethylformamide and pyridine over calcium hydride, tetrahydrofuran and diethylether over sodium/benzophenone. Reactions were monitored by TLC performed on Silica Gel 60 $F_{254}$ (Merck), with detection by UV light and by charring with sulfuric acid (5 % v/v in ethanol), or with orcinol (2 % m/v in sulfuric acid 5 % v/v in ethanol). Flash-chromatography was performed on Silica Gel 60AC.C. 6–35μ (SDS); the elution solvents are




*Reagents and conditions*: (a) CAN (5 equiv.), $CH_3CN$–$H_2O$, 9:1, –10 °C, 10 min, 91 %; (b) NaH (cat.), $CCl_3CN$ (5 equiv.), $CH_2Cl_2$, 0 °C, 1.5 h, 75 %; (c) **17** (4 equiv.), $BF_3.Et_2O$ (0.5 equiv.), $CH_2Cl_2$, –40 °C–rt, 2 h, 25 %; (d) $Ac_2O$–pyridine, 1:1, rt, 54 h; (e) 1-Rh($Ph_3P$)$_3$Cl (1 equiv.), EtOH–toluene–$H_2O$, 7:3:1, 70 °C, 17.5 h, 2-HgO (5 equiv.), $HgCl_2$ (5 equiv.), acetone–$H_2O$, 9:1, rt, 1.5 h, 67 %; (f) $SO_3$.-$NMe_3$ (7 equiv.), DMF, 55 °C, 4.5 h, 92 %; (g) 2M MeONa–MeOH, 60 °C, 22 h; (h) Pd/C, $H_2$ (1 atm), rt, 96 h, 66 % (g and h steps).

**Scheme II.** Synthesis of sulfated $Le^a$ pentasaccharide **2**.

indicated in brackets and are given v/v. Concentrations were performed under reduced pressure at ≤ 35 °C (bath). Melting points (uncorrected) were measured on an oil-bath Büchi apparatus. The optical rotations were determined on a Jasco DIP370 micropolarimeter. NMR Spectra were recorded using Bruker AC-250 (at 250 MHz for $^1H$ NMR or at 62.9 MHz for $^{13}C$ NMR) and AM-400 (at 400 MHz for $^1H$ NMR and at 100 MHz for $^{13}C$ NMR) spectrometers. Most of the $^1H$ NMR assignments were based on 2-D homonuclear correlation spectroscopy experiments (COSY). Data matrices of 1K × 2K points were used to digitize spectral widths of either 4000, 3500, 2000, 1900, or 1700 Hz. Sixteen scans were used per increment with a relaxation delay of 1.5 s; the 90° pulse width was 7.5 μs. When using $CDCl_3$ as deuterated solvent, the spectra were referenced to $(CH_3)_4Si$ ($\delta_H$ = 0 ppm) and to $CDCl_3$ (central line $\delta_C$ = 77 ppm). When using $CD_3OD$ as deuterated solvent, the spectra were referenced to $(CH_3)_4Si$ ($\delta_H$ = 0 ppm) and to $CD_3OD$ (central line $\delta_C$ = 49 ppm). When using $(CD_3)_2SO$ as deuterated solvent, the spectra were referenced to $(CH_3)_4Si$ ($\delta_H$ = 0 ppm) and to $(CD_3)_2SO$ (central line $\delta_C$ = 39.5 ppm). When using $D_2O$ as deuterated solvent, the spectra were referenced to $(CH_3)_2CO$ ($\delta_H$ = 2,225 ppm) and to $CD_3OD$ (central line $\delta_C$ = 49 ppm). Mass spectrum of **2** was recorded on a Finnigan MATT 95 coupled with electrospray.

*4-Methoxybenzyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (4)*

A mixture of 4-methoxybenzyl alcohol (6.8 g, 49 mmol) and mercuric cyanide (13 g, 52 mmol) in toluene (100 mL) was stirred for 1 h at room temperature in the presence of 4 Å molecular sieves (7 g) Then *O*,*N*-peracetylated glycosaminyl chloride (9 g, 24.6 mmol) was added and the mixture was stirred at 60 °C. After 5 days the mixture was diluted with $CH_2Cl_2$, filtered and washed successively with a saturated solution of potassium hydrogencarbonate, a saturated solution of potassium iodide and finally with water. After evaporation, the crude residue was recrystallized in ethyl acetate to give **4** (10.8 g, 94 %). Flash chromatography (toluene–acetone, 8:2) of the residue obtained by evaporation of the ethyl acetate solution gave 0.3 g more (total yield 96 %). Mp 164–165 °C. $[\alpha]_D^{20}$ –49 (c 0.9, $CH_2Cl_2$). $^1H$ NMR $CDCl_3$, 250 MHz δ 1.9 (s, 3H, NH-Ac), 2.03 (s, 6H, 2OAc), 2.12 (s, 3H, OAc), 3.66 (s, 3H, $OCH_3$) 3.82 (ddd, 1H, *J* = 2.5, 4.5, 9.5 Hz, H-5), 3.95 (ddd, 1H, *J* = 8.5, 9.5, 11 Hz, H-2), 4.18 (dd, 1H, *J* = 2.5, 12 Hz, H-6), 4.29 (dd, 1H, *J* = 4.5, 12 Hz, H-6'), 4.54 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 4.60 (d, 1H, *J* = 8.5 Hz, H-1β), 4.83 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 5.08 (t, 1H, *J* = 9.5 Hz, H-4), 5.20 (t, 1H, *J* = 9.5 Hz, H-3), 5.35 (d, 1H, *J* = 11Hz, NH), 6.88 (m, 2H, arom.), 7.23 (m, 2H, arom.). $^{13}C$ NMR ($CDCl_3$, 62.9 MHz) δ 20.52, 20.57, 20.66, 23.15, 54.31, 55.15, 62.09, 70.19, 68.59, 71.64, 72.37, 99.01, 113.73, 128.78, 129.59, 159.34, 169.29, 170.09, 170.64, 170.74. Anal. calcd for $C_{22}H_{29}NO_{10}$ : C , 56.53; H , 6.25; N, 3.00; O, 34.22 . Found : C, 56.73; H, 6.10; N, 2.98; O, 34.04.

*4-Methoxybenzyl 2-acetamido-2-deoxy-β-D-glucopyranoside (5)*

A solution of **4** (10 g, 21.4 mmol) in a mixture of triethylamine–methanol–water (1:8:1, 100mL) was left for 16 h at room temperature. After several evaporations with toluene, the crystalline residue (7.39 g, 100 %) which looked pure in TLC can be directly used for the preparation of **3**. It could be recrystallized from EtOH, mp 210–211 °C, $[\alpha]_D^{20}$ –41 (*c* 1, $H_2O$).$^1H$ NMR δ ($CD_3OD$, 250 MHz) 1.9 (s, 3H, NHAc), 3.23–3.34 (m, 2H, H-3, H-4), 3.42 (ddd, 1H, *J* = 8.5, 9 Hz, H-2), 3.70–3.75 (m, 2H, H-5, H-6), 3.78 (s, 3H, $OCH_3$), 3.91 (dd, 1H, *J* = 2.5, 12 Hz, H-6'), 4.42 (d, 1H, *J* = 8.5 Hz, H-1), 4.54 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 4.79 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 6.87–7.25 (m, 4H, arom.). $^{13}C$ NMR ($CD_3OD$, 62.9 MHz) δ 22.98, 55.66, 57.32, 62.85, 71.20, 72.16, 75.95, 78.01, 101.38, 114.69, 130.56, 131.02, 160.82, 173.90. Anal. calcd for $C_{16}H_{23}NO_7$ : C, 56.30; H, 6.79; N, 4.10; O, 32.81. Found : C, 56.42; H, 6.81; N, 4.29; O, 33.01.

*4-Methoxybenzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (3)*

To a solution of compound **5** (10.5 g, 30.7 mmol) in THF (200 mL) were added benzaldehyde dimethylacetal (23 mL, 154 mmol) and *para*-toluene sulfonic acid monohydrate (100 mg) and the mixture was stirred at 50 °C. After 24 h, triethylamine (1 mL) was added and the reaction mixture evaporated. The residue was then triturated with water (70 mL) and filtered. Recrystallization from EtOH afforded 12.5 g (93 %) of pure **3**, mp 285 °C, $[\alpha]_D^{20}$ –54 (*c* 1, pyridine). $^1H$ NMR ($CDCl_3$, 250 MHz). δ 1.9 (s, 3H, NHAC), 3.4–3.55 (m, 2H, H-2, H-5), 3.58 (dd, 1H, *J* = 2.5, 10 Hz, H-6), 3.82 (s, 3H, $OCH_3$), 3.84 (t, 1H, *J* = 10 Hz, H-4), 4.00 (t, 1H, *J* = 10 Hz, H-3), 4.38 (dd, 1H, *J* = 5, 10 Hz, H-6'), 4.54 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 4.70 (d, 1H, *J* = 8 Hz, H-1β ) 4.84 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 5.56 (s, 1H, CHPh), 6.90–7.49(m, 9H, arom.). $^{13}C$ NMR ($CDCl_3$, 62.9 MHz) δ 23.58, 55.31, 50.10, 66.34, 70.76, 71.48, 75.89, 81.56, 98.98, 101.98, 114.10, 126.31, 128.28, 128.65, 129.21, 129.96, 136.95, 159.71, 171.05. Anal. calcd for $C_{23}H_{27}NO_7$ : C, 64.32; H, 6.34; N, 3.26; O, 26.08. Found : C, 64.37; H, 6.67; N, 3.23; O, 25.68.

*4-Methoxybenzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl β-D-galactopyranoside)-β-D-glucopyranoside (7)*

To a solution of compound **3** (9 g, 21 mmol) in a mixture of nitromethane and toluene (200 mL, 1:1) were added mercuric cyanide (7 g, 28 mmol) and 4 Å molecular sieves (4 g). After 1 h at reflux, the reaction was cooled and acetobromogalactose **6** (11.5g, 28 mmol) was added. After 20 h at 40 °C, the mixture was diluted with $CH_2Cl_2$, washed with a saturated solution of potassium hydrogencarbonate and filtered. The filtered solution was washed with saturated solution of potassium iodide and evaporated. The residue was crystallized from a mixture of

ethyl acetate–hexane to give 14 g (87 %) of **7**. Flash chromatography (toluene–acetone 7:3) of the filtered solution gave an additional crop (2 g) and raised the yield to 99 %; mp 136–137 °C, $[\alpha]_D^{20}$ –39 (*c* 1, $CH_2Cl_2$. $^1H$ NMR ($CDCl_3$, 400 MHz). δ 1.93 (2), 1.97 (2), 2.11 (5s, 15H, 4-OAc, NHAc), 3.09 (m, 1H, H-2a) 3.68 (t, 1H, *J* = 9 Hz, H-4a), 3.53–3.61 (m, 2H, H-5a, H-5b), 3.79 (dd, 1H, *J* = 6, 11 Hz, H-6a ax.), 3.80 (s, 3H, $OCH_3$), 3.91 (dd, 1H, *J* = 6.5, 11Hz, H-6b), 4.06 (dd, 1H, *J* = 7.5, 11 Hz, H-6'b), 4.37 (dd, 1H, *J* = 4.5, 11 Hz, H-6a eq.), 4.50 (d, 1H, *J* = 11.5 Hz, $CH_2Ph$), 4.70 (t, 1H, *J* = 9 Hz, H-3a), 4.73 (d, 1H, *J* = 8 Hz, H-1a), 4.81 (d, 1H, *J* = 11.5 Hz, $CH_2Ph$), 4.91 (dd, 1H, *J* = 3, 10 Hz, H-3b), 5.15 (dd, 1H, *J* = 8, 10 Hz, H-2b), 5.24 (d, 1H, *J* = 8 Hz, H-1b), 5.28 (d, 1H, *J* = 3 Hz, H-4b), 5.52 (s, 1H, CHPh), 5.71 (d, 1H, *J* = 7 Hz, NH), 6.82–7.50 (m, 9H, arom.). $^{13}C$ NMR ($CDCl_3$, 62.9 MHz) δ 20.50, 20.58, 20.67, 23.57, 55.20, 58.21, 60.94, 66.09, 71.37, 65.80, 66.74, 69.21, 70.40, 70.97, 76.61, 80.54, 98.53, 100.14, 101.42, 113.79, 126.01, 128.29, 128.97, 129.22, 129.77, 136.97, 159.43, 169.39, 170.07, 170.17, 170.52. Anal. calcd for $C_{37}H_{45}NO_{16}$ : C, 58.49; H, 5.97; N,1.85; O, 33.69. Found : C, 58.03; H, 5.92; N, 1.77; O, 34.02.

*4-Methoxybenzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (**8**)*

A solution of compound **7** (5.3 g, 7 mmol) in a mixture of methanol–triethylamine–water (50 mL, 8:1:1) was left for 48 h at room temperature. Several co-evaporations with toluene afforded 3.7 g (93 %) after crystallization from ethanol; mp 251 °C, $[\alpha]_D^{20}$ –67 (*c* 1, DMF). $^1H$ NMR (DMSO-$d_6$, 250 MHz). δ 1.8, (s, 3H, NHAc), 3.2–3.7 (m, 8H, H-2a, H-2b, H-3b, H-4b, H-5a, H-5b, H-6b, H-6' b), 3.71 (m, 1H, H-6a ax.), 3.72 (s, 3H, $OCH_3$), 3.80 (t, 1H, *J* = 9 Hz, H-4a), 3.96 (t, 1H, *J* = 9 Hz, H-3a), 4.03 (d, 1H, *J* = 3 Hz, OH), 4.24 (m, 2H, H-6a eq. H-1b), 4.32 (d, 1H, *J* = 3 Hz, OH), 4.44 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 4.47 (t, 1H, *J* = 3 Hz, OH), 4.61 (d, 1H, *J* = 8 Hz, H-1a), 4.67 (d, 1H, *J* = 12 Hz, $CH_2Ph$) 4.70 (d, 1H, *J* = 4 Hz, OH), 5.60 (s, 1H, CHPh), 6.90–7.45 (m, 9H, arom.), 7.83 (d, 1H, *J* = 9 Hz, NH). $^{13}C$ NMR (DMSO $d_6$, 62.9 MHz) δ 23.10, 55.08, 60.11, 67.77, 69.82, 65.93, 67.77, 70.96, 73.26, 75.25, 77.43, 78.90, 99.76, 100.77, 103.31, 113.61, 126.15, 127.84, 128.61, 129.01, 129.61, 137.64, 158.76, 169.91. Anal. calcd for $C_{29}H_{37}NO_{12}{\cdot}H_2O$: C, 57.14; H, 6.45; N, 2.30; O, 34.12. Found : C, 57.31; H, 6.26; N, 2.36; O, 34.03.

*4-Methoxybenzyl 2-acetamido-3-O-(3-O-allyl-β-D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**9**)*

A solution of **10** (3.6 g, 4.75 mmol) in a mixture of methanol–triethylamine–water (50 mL, 8:1:1) was left for 60 h at 80 °C. Several co-evaporations with toluene afforded 2.8 g (93 %) after crystallization from methanol, mp 247 °C, $[\alpha]_D^{20}$ –74 (*c* 0.5, DMF). $^1H$ NMR (DMSO $d_6$, 250 MHz). δ 1.82, (s, 3H, NHAc), 3.10 (dd, 1H, *J* = 3, 10 Hz, H-3b), 3.21 (t, 1H, *J* = 6 Hz, H-5b), 3.27–4.32 (m, 11H, H-34b), 3.21 (t, 1H, *J* = 6 Hz, H-5b), 3.27–4.32 (m, 11H, H-4b, 2H-6b, 2H-6a, H-2b, H-4a, H-5a, H-3a, $OCH_2$-$CH{=}CH_2$), 3.74 (s, 3H, $OCH_3$), 4.27 (d, 1H, *J* = 8 Hz, H-1b), 4.45 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 4.62 (d, 1H, *J* = 8 Hz, H-1a) 4.70 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 5.10 (m, 1H, $OCH_2$–$CH{=}CH_2$), 5.30 (m, 1H, $OCH_2$–$CH{=}CH_2$, 5.63 (s, 1H, CHPh), 5.90 (m, 1H, $OCH_2$–$CH{=}CH_2$ ), 6.91 (d, 2H, *J* = 8.5 Hz, arom.), 7.22 (d, 2H, *J* = 8.5 Hz, arom.), 7.30–7.53 (m, 5H, arom.), 7.83 (d,1H, *J* = 9 Hz, NH). $^{13}C$ NMR (DMSO $d_6$, 62.9 MHz) δ 23.09, 55.06, 59.96, 67.79, 69.34, 69.81, 64.24, 65.90, 69.81, 75.08, 77.30, 78.96, 80.90, 99.78, 100.77, 103.17, 113.60, 115.99, 126.15, 127.83, 128.59, 128.99, 129.61, 135.94, 137.63, 158.75, 169.81. Anal. calcd for $C_{32}H_{41}NO_{12}$, $CH_3OH$: C, 59.72; H, 6.83; N, 2.11; O, 31.34. Found : C, 59.45; H, 6.51; N, 2.67; O, 31.36.

*4-Methoxybenzyl 2-acetamido-3-O-(3-O-allyl-2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**10**)*

A mixture of **8** (0.8 g, 1.35 mmol), dibutyltin oxide (0.370 g), 1.487 mmol) in toluene (20 mL) was heated under reflux for 14 h with continuous removal of water using a Dean–Stark apparatus. After cooling to 40 °C, tetrabutylammonium bromide (0.217 g, 0.675 mmol) and allyl bromide (3.51 mL, 40.5 mmol) were added and the mixture was stirred for 24 h at 40 °C. Evaporation gave a residue which was treated with a mixture of acetic anhydride–pyridine (10 mL, 1:1) for 12 h at room temperature. After several co-evaporations with toluene, flash chromatography of the residue (toluene–acetone, 85:15) afforded 0.635 g (77 %) of **10** along with 0.156 g of **8**. **10**: mp 175 °C (ethyl acetate–hexane), $[\alpha]_D^{20}$ –14 (*c* 1.5, $CHCl_3$). $^1H$ NMR ($CDCl_3$, 400 MHz). δ 1.90–2.12, (4s, 12H, 3 OAc, NHAc), 3.05 (ddd, 1H, *J* = 7, 8, 10 Hz, H-2a), 3.41 (dd, 1H, *J* = 3.5, 10 Hz, H-3b), 3.49 (t, 1H, *J* = 6 Hz, H-5b), 3.57 (ddd, 1H, *J* = 4.5, 9, 10 Hz, H-5a), 3.69 (t, 1H, *J* = 9 Hz, H-4a), 3.76–3.88 (m, 5H, $OCH_3$, H-6a, $OCH_2$-$CH{=}CH_2$), 3.91 (dd, 1H, *J* = 6, 11 Hz, H-6b), 4.03–4.12 (m, 2H, H-6'b, $OCH_2$-$CH{=}CH_2$) 4.36 (dd, 1H, *J* = 4.5, 10.5 Hz, H-6'a), 4.50 (d, 1H, *J* = 11 Hz, $CH_2Ph$), 4.68 (d, 1H, *J* = 8 Hz, H-1b), 4.70 (t, 1H, *J* = 9 Hz, H-3a), 4.81 (d, 1H, *J* = 11 Hz, $CH_2Ph$), 5.02 (dd, 1H, *J* = 8, 10 Hz, H-2b), 5.14 (m, 1H, $OCH_2$–$CH{=}CH_2$), 5.21(m, 1H, $OCH_2$–$CH{=}CH_2$), 5.30 (d, 1H, *J* = 8 Hz, H-1a), 5.31 (d, 1H, *J* = 3.5 Hz, H-4b) 5.54 (s, 1H, CHPh), 5.74 (m, 1H, $OCH_2$–$CH{=}CH_2$), 5.78 (d, 1H, *J* = 7 Hz, NH), 6.88 (d, 2H, *J* = 8.5 Hz, arom.), 7.23 (d, 2H, *J* = 8.5 Hz, arom.), 7.34–7.52 (m, 5H, arom.). $^{13}C$ NMR ($CDCl_3$, 62.9 MHz) δ 20.59, 20.76, 20.88, 23.68, 55.26, 61.64, 68.83, 70.48, 71.51, 58.56, 65.76, 66.96, 70.73, 71.09, 76.79, 80.79, 98.53, 100.14, 101.45, 113.85, 117.28, 126.12, 128.27, 129.16, 129.81, 133.98, 137.18, 159.49, 169.47, 170.27, 170.62. Anal. calcd for $C_{38}H_{47}NO_{15}$ : C, 60.23; H, 6.25; N, 1.85; O, 31.67. Found : C, 59.46; H, 6.05; N, 1.65; O, 31.57.

*4-Methoxybenzyl 2-acetamido-3-O-(3-O-allyl-2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**11**)*

Sodium hydride (60 %) in oil (0.729 g, 18.23 mmol) was added portionwise within 1 h to a cooled stirred solution (0

°C) of compound **9** (3.20g, 5.06 mmol) and benzyl bromide (3.6 mL, 30.36 mmol) in DMF (20 mL). Water was then added, and the mixture extracted with ether. Flash chromatography (hexane–ethyl acetate, 65:35) of the residue obtained after evaporation of the extracts afforded 4.014g (88 %) of **11** which crystallized from EtOH, mp 137 °C, $[\alpha]_D^{20}$ –37 (*c* 1.5, $CHCl_3$). $^1H$ NMR ($CDCl_3$, 400 MHz). δ 1.52 (s, 3H, NHAc), 3.04 (dt, 1H, *J* = 7, 8 Hz, H-2a), 3.32 (dd, 1H, *J* = 2.5, 10 Hz, H-3b), 3.37–3.44 (m, 2H, 2H-6b) 3.52 (dt, 1H, *J* = 5,10 Hz, H-5a), 3.58 (t, 1H, *J* = 10 Hz, H-5b), 3.65 (t, 1H, *J* = 9 Hz, H-4a), 3.72–3.82 (m, 2H, H-2b, H-6a), 3.80 (s, 3H, $OCH_3$), 3.85 (d, 1H, *J* = 2.5 Hz, H-4b), 4.13 (m, 2H, $OCH_2$–$CH$=$CH_2$), 4.30 (s, 2H, $CH_2Ph$), 4.31 (dd, 1H, *J* = 5, 10 Hz, H-6'a) 4.35 (d, 1H, *J* = 8 Hz, H-1a), 4.56 (t, 1H, *J* = 9 Hz, H-3a), 4.41–4.93 (6d, 6H, *J* = 11 Hz, 6 $CH_2Ph$), 5.16 (m, 1H, $OCH_2$–$CH$=$CH_2$), 5.24 (d, 1H, *J* = 8 Hz, H-1a), 5.30 (m , 1H, $OCH_2$–$CH$=$CH_2$ ), 5.42 (d, 1H, *J* = 7 Hz, NH), 5.51 (s, 1H, CH Ph), 5.90 (m, 1H, $OCH_2$–$CH$=$CH_2$ ), 6.85 (d, 2H, *J* = 8.5 Hz, arom.), 7.12–7.50 (m, 22H, arom.). $^{13}C$ NMR ($CDCl_3$, 62.9MHz) δ 23.24, 55.25, 59.29, 68.23, 68.69, 71.38, 73.37, 74.42, 75.27, 66.07, 72.75, 72.91, 77.51, 79.71, 79.95, 82.47, 99.02, 100.83, 103.60, 113.70, 116.57, 126.18–128.67 (arom.), 129.42, 134.79, 137.47–138.93 (arom)., 159.29, 170.69. Anal. calcd for $C_{53}H_{59}NO_{12}$ : C, 70.57; H, 6.59; N, 1.55; O, 21.28. Found : C, 70.36; H, 6.59; N, 1.65; O, 21.36.

*4-Methoxybenzyl 2-acetamido-3-O-(3-O-allyl-2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-6-O- benzyl -2- deoxy- β- D-glucopyranoside (12)*

A saturated solution of HCl in ether (about 2 mL) was added into a cooled stirred mixture of compound **11** (4 g, 4.43 mmol) and $NaBH_3CN$ (2.23 g, 35.48 mmol) in THF (20 mL) until the reaction mixture became acidic as judged from an aliquot on paper pH indicator. After 1 h at 0 °C, the reaction mixture was diluted with $CH_2Cl_2$ and washed with a saturated solution of $KHCO_3$. After extraction of the aqueous phase with $CH_2Cl_2$, the combined organic phases were evaporated. Flash chromatography (hexane–ethyl acetate, 1:1) of the residue afforded 3.248 g (81 %) of **12** which crystallized from a hexane–ethyl acetate mixture, mp 167 °C, $[\alpha]_D^{20}$ –11 (*c* 1, $CHCl_3$). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 1.65 (s, 3H, NHAc), 2.95 (ddd, 1H, *J* = 7, 8, 9 Hz, H-2a), 3.40 (dd, 1H, *J* = 2.5, 9 Hz, H-3b), 3.42–3.60 (m, 3H, H-5a, 2H-6b), 3.62 (t, 1H, *J* = 6 Hz, H-5b), 3.70 (dd, 1H, *J* = 6, 11 Hz, H-6a), 3.73–3.80 (m, 2H, H-4a, H-2b), 3.78 (s, 3H, $OCH_3$), 3.84 (d, 1H, *J* = 2.5 Hz, H-4b), 3.89 (dd, 1H, *J* = 1, 11 Hz, H-6'a), 4.19 (m, 2H, $OCH_2$–$CH$=$CH_2$), 4.25 (d, 1H, *J* = 8 Hz, H-1b), 4.27 (dd, 1H, *J* = 8, 10 Hz, H-3a), 4.32–4.60 (4d, 4H, *J* = 11 Hz, 4 $CH_2Ph$), 4.62 (s, 2H, $CH_2Ph$), 4.33–4.97 (4d, 4H, *J* = 11 Hz, 4 $CH_2Ph$), 5.09 (d, 1H, *J* = 8 Hz, H-1a), 5.19 (m, 1H, $OCH_2$–$CH$=$CH_2$), 5.19 (d, 1H, *J* = 7 Hz, NH), 5.32 (m, 1H, $OCH_2$–$CH$=$CH_2$), 5.92 (m, 1H, $OCH_2$–$CH$=$CH_2$), 6.83 (d, 2H, *J* = 9 Hz, arom.), 7.21 (d, 2H, *J* = 9 Hz, arom.), 7.25–7.41 (m, 20H, arom.). $^{13}C$ NMR ($CDCl_3$, 62.9 MHz) δ 23.03, 55.09, 58.09, 68.23, 69.66, 70.81, 71.68, 73.29, 73.45, 74.42, 75.20, 69.65, 72.92, 73.29, 75.20, 79.07, 82.05, 83.27, 98.26, 103.96, 113.61, 116.69, 127.27–128.28(arom.), 129.54, 134.55, 137.46, 138.23, 138.51, 138.94, 159.14, 170.87. Anal. calcd for $C_{53}H_{61}NO_{12}$: C, 70.41; H, 6.80; N,1.55; O, 21.24. Found: C, 70.11; H, 6.68; N, 1.46; O, 21.08.

*4-Methoxybenzyl 2-acetamido-3-O-(3-O-allyl-2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-4-O-(2,3,4-tri-O-benzyl-α-L-fucopyanosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (14)*

Diisopropylethylamine (0.286 mL, 1.645 mmol) and a solution of perbenzylated fucosyl bromide (0.91 g, 1.83 mmol) in $CH_2Cl_2$ (4 mL) were succesively added to a solution of compound **12** (0.550 g, 0.608 mmol) and tetrabutylammonium bromide (0.294 g, 0.912 mmol) in DMF (1 mL). The reaction mixture was strirred at room temperature for 72 h then diluted with $CH_2Cl_2$ and washed successively with 10 % $KHCO_3$ solution and water. Flash-chromatography (hexane–ethyl acetate 55:45) of the residue obtained after evaporation of the organic phase afforded 0.717 g (89 %) of **14** as an amorphous mass, $[\alpha]_D^{20}$ –40 (*c* 1.5, $CHCl_3$). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 1.03 (d, 4H, *J* = 6.5 Hz, $CH_3$c), 1.65 (s, 3H, NHAc), 3.30–3.37 (m, 2H, H-2a, H-4c), 3.37 (dd, 1H, *J* = 3, 10 Hz, H-3b), 3.50 (dd, 1H, *J* = 5, 9 Hz, H-6b), 3.57 (dd, 1H, *J* = 4.5, 9 Hz, H-6'b), 3.60–3.72 (m, 4H, H-2b, H-5b, H-5a, H-6a), 3.75 (s, 3H, $OCH_3$), 3.82 (dd, 1H, *J* = 2.5, 10 Hz, H-3c), 3.90 (dd, 1H, *J* = 4.5, 11 Hz, H-6'a), 3.94 (d, 1H, *J* = 3 Hz, H-4b), 3.95 (dd, 1H, *J* = 8, 9 Hz, H-4a), 4.00 (dd, 1H, *J* = 3.5, 10 Hz, H-2c), 4.17 (d, 1H, *J* = 11 Hz, $CH_2Ph$), 4.22 (m, 2H, $OCH_2$–$CH$=$CH_2$), 4.28 (t, 1H, *J* = 8 Hz, H-3a), 4.36–4.42 (2s, 4H, $2CH_2Ph$), 4.43 (2d, 2H, *J* = 8 and 11.5 Hz, H-1b, $CH_2Ph$), 4,48 (d, 1H, *J* = 11 Hz, $CH_2Ph$), 4.54 (q, 1H, *J* = 6.5 Hz, H-5c), 4.74 (d, 1H, *J* = 11.5 Hz, $CH_2Ph$), 4.58–4.91 (8d, 8H, *J* = 11 Hz, $8CH_2Ph$), 5.02 (d, 1H, *J* = 6.5 Hz, H-1a), 5.07 (d, 1H, *J* = 3.5 Hz, H-1c), 5.18 (m, 1H, $OCH_2$–$CH$=$CH_2$), 5.33 (m, 1H, $OCH_2$–$CH$=$CH_2$), 5.80 (d, 1H, *J* = 7 Hz, NH), 5.94 (m, 1H, $OCH_2$–$CH$=$CH_2$), 6.78 (d, 2H, *J* = 8.5 Hz, arom.), 7.10 (d, 2H, *J* = 8.5 Hz, arom.), 7.10–7.43 (m, 35H, arom.). $^{13}C$ NMR ($CDCl_3$, 62.9 MHz) δ 16.45, 23.12, 55.07, 57.20, 67.70, 68.17, 70.22, 71.70, 72.11, 72.94, 73.24, 74.01, 74.86, 75.13, 66.16, 71.58, 72.35, 73.24, 75.58, 77.51, 77.99, 79.43, 79.98, 82.05, 95.92, 98.31, 103.31, 113.52, 116.49, 127.11–129.47 (arom.), 134.80, 137.68, 138.19, 138.69, 138.77, 138.88, 139.00, 159.02, 170.15. Anal. calcd for $C_{80}H_{89}NO_{16}$ : C, 72.76; H, 6.79; N, 1.06; O, 19.38. Found: C, 72.58; H, 7.04; N, 1.19; O, 19.33.

*4-Methoxybenzyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-4-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside (15)*

Wilkinson catalyst ($(PPh_3)_3RhCl$) (0.139 g, 0.15 mmol) was added to a solution of compound **14** (0.4 g, 0.3 mmol) in a mixture of ethanol–toluene–water (2 mL, 7:3:1). After stirring for 17 h at 70 °C, the reaction mixture was evaporated and a mixture of acetone–water (2 mL, 2:1) was added to the residue. Mercuric oxide (0.130 g) and a 2 M solution of mercuric chloride (1.5 mL) were then added successively to the stirred solution. After 1 h, the reaction

mixture was diluted with $CH_2Cl_2$ and washed with a saturated solution of KI. After extraction of the aqueous phase with $CH_2Cl_2$, the combined organic phases were evaporated. Flash chromatography (hexane–ethyl acetate 4:6) of the residue afforded 0.260 g (67 %) of **15** as an amorphous mass along with 0.107 g of the starting material **14** giving a 90 % yield of **15** based on starting material recovery, $[\alpha]_D^{20}$ –54 (c 0.1, $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 1.05 (s, 3H, $CH_3$c), 1.66 (s, 3H, NHAc), 2.28 (d, 1H, *J* = 3 Hz, OH), 3.40 (d, 1H, *J* = 2.5 Hz, H-4c), 3,48 (dd, 1H, *J* = 8, 9.5 Hz, H-2b), 3.52–3.66 (m, 5H, H-2a, H-3b, H-5b, H-6b, H-6'b), 3.70 (dd 1H, *J* = 3, 11 Hz, H-6a), 3.73 (s, 3H, $OCH_3$), 3.78 (m, 1H, H-5a), 3.82 (dd, 1H, *J* = 2.5, 10 Hz, H-3c), 3.90 (d, 1H, *J* = 3 Hz, H-4b), 3.95 (dd, 1H, *J* = 4.5, 11 Hz, H-6'a), 4.04 (dd, 1H, *J* = 7, 8 Hz, H-4a), 4.18 (t, 1H, *J* = 7 Hz, H-3a), 4.43 (q, 1H, *J* = 6.5 Hz, H-5c), 4,44 (s, 2H, $CH_2Ph$), 4.88 (d, 1H, *J* = 6 Hz, H-1a), 4.26–4.92 (14d, 14H, *J* = 11 Hz, $14CH_2Ph$), 5.12 (d, 1H, *J* = 3.5 Hz, H-1c), 5.90 (d, 1H, *J* = 7 Hz, NH), 6.75 (d, 2H, *J* = 9 Hz, arom.), 7.12 (d, 2H, *J* = 9 Hz, arom.), 7.16–7.40 (m, 35H, arom.). $^{13}C$ NMR ($CDCl_3$, 62.9 MHz) δ 16.60, 23.07, 55.09, 54.40, 67.86, 68.56, 69.99, 72.36, 73.02, 73.18, 73.99, 74.52, 74.90, 66.32, 71.18, 72.74, 74.20, 75.36, 75.74, 75.85, 77.86, 79.60, 79.96, 95.37, 98.18, 103.10, 113.58, 127.11–128.45 (arom.), 129.40, 129.60, 137.74–138.87 (arom.), 159.07, 170.05. Anal. calcd for $C_{77}H_{85}NO_{16}$: C, 72.22; H, 6.69; N, 1.09; O, 19.99. Found: C, 71.20; H, 6.65; N, 1.08; O, 19.16.

*4-Methoxybenzyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(4-O- sulfo- 2,4,6- tri- O- benzyl -β-D-galactopyranosyl)-4-O-(2, 3, 4-tri-O-benzyl- α-L-fucopyranosyl)- β-D-glucopyranoside sodium salt (16)*

Sulfur trioxide–trimethylamine complex (0.2 mL of a 1.44 M solution in DMF) was added to **15** (0.09 g, 0.07 mmol). After stirring for 12 h at 55 °C, the reaction mixture was diluted with $CH_2Cl_2$, washed successively with saturated solution of $KHCO_3$ and water. After extraction of the aqueous phase with $CH_2Cl_2$, the combined organic phases were evaporated. Flash chromatography of the residue (ethyl acetate–methanol, 9:1) followed by cation exchange chromatography (AG50W-X8, $Na^+$ form, elution with MeOH) afforded 0.08 g (82 %) of **16** as an amorphous mass, $[\alpha]_D^{20}$ –42 (c 1, $CH_2Cl_2$). $^1H$ NMR ($CD_3OD$–$CDCl_3$, 9:1, 400 MHz) δ 0.87 (d 3H, *J* = 6.5 Hz, $CH_3$c), 1.83 (s, 3H, NHAc), 3.25 (bs, 1H, H-4c), 3.33 (m, 1H, H-5a), 3.57–3.80 (m, 4H, H-2b, H-5b, H-6a, H-6'a), 3.80 (t, 1H, *J* = 9 Hz, H-4a), 3.80–3.90 (m, 4H, H-2c, H-3c, H-6b, H-6'b), 3.90 (t, 1H, *J* = 2.5, 10 Hz, H-3a), 3.97 (d, 1H, *J* = 11 Hz, $CH_2Ph$), 4.01 (t, 1H, *J* = 9 Hz, H-2a), 4.34 (d, 1H, *J* = 8 Hz, H-1a), 4.36–4.37 (2d, 2H, *J* = 12 Hz, $CH_2Ph$), 4.39–4.57 (m, 9H, $7CH_2Ph$, H-3b, H-4b), 4.59 (d, 1H, *J* = 11 Hz, $CH_2Ph$), 4.62–4.70 (m, 3H, $CH_2Ph$, H-1b, H-5c), 4.73 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 4.76 (d, 1H, *J* = 12.5 Hz, $CH_2Ph$), 4.90 (d, 1H, *J* = 12 Hz, $CH_2Ph$), 4.97 (d, 1H, *J* = 3 Hz, H-1c), 5.05 (d, 1H, *J* = 11 Hz, $CH_2Ph$), 6.83 (d, 2H, *J* = 9 Hz, arom.), 7.10–7.60 (m, 37H, arom.). $^{13}C$ NMR ($CD_3OD$, 62.9 MHz) δ 16.86, 23.02, 55.59, 56.78, 68.72, 69.48, 70.88, 72.80, 74.06, 74.12, 75.07, 75.28, 75.99, 76.17, 67.45, 73.61, 76.00, 76.51, 76.74, 77.25, 77.73, 79.26, 80.78, 81.82, 98.16, 100.76, 104.26, 114.47, 127.84–129.21(arom.), 129.97, 130.39, 139.06–140.37(arom.), 160.39, 172.89. Anal. calcd for $C_{77}H_{84}NO_{19}SNa{\bullet}H_2O$: C, 66.03; H, 6.19; N, 1.00; S, 2.29. Found: C, 65.66; H, 6.01; N, 1.14; S, 2.20.

*2-Acetamido-2-deoxy-3-O-(3-O-sulfo-β-D-galactopyranosyl)-4-O-(α-L-fucopyranosyl)-α,β-D-glucopyranose sodium salt (1)*

A solution of **16** (0.11 g, 0.0795 mmol) in a mixture of ethanol–water (2 mL, 9:1) was stirred for 60 h under hydrogen (1 atm) in the presence of palladium on charcoal (10 %, 0.110 g). The mixture was then filtered on Celite and evaporated. Flash chromatography (isopropanol–ethyl acetate–water, 3:5:2) of the residue afforded 40 mg (80 %) of **1** as an amorphous mass. Freeze-drying of water solution of **1** afforded a white hygroscopic powder, $[\alpha]^{30}_D$ –38 (*c* 0.5, MeOH) [Lit.[6b] $[\alpha]_D^{25}$ –43 (*c* 0.42, MeOH)]. $^1H$ NMR ($D_2O$, 250 MHz) δ α-GlcNAc : 3.76 (H-4), 3.95 (H-5), 4.13 (H-2), 4.16 (H-3), 5.11 (*J* = 2.5 Hz, H-1) ; β-GlcNAc : 3.61 (H-5), 3.74 (H-4), 3.86 (H-2), 4.07 (H-3), 4.62 (H-6), 4.71 (*J* = 8.5 Hz, H-1) ; β-Gal : 3.55 (H-5), 3.61 (H-2), 4.29 (H-3 and H-4), 4.58 (*J* = 7.5 Hz, H-1(β) ), 4.62 (*J* = 7.5 Hz, H-1(α)) ; α-Fuc : 1.18 ($CH_3$), 3.79 (H-4), 3.89 (H-3), 4.86 (H-2 and H-5), 5.02 (*J* = 3.5 Hz, H-1). $^{13}C$ NMR ($D_2O$–$CD_3OD$, 9 : 1) δ 16.38, 23.03, 23.30, 55.07, 58.01, 60.76, 62.48, 67.65, 67.80, 68.99, 69.69, 70.25, 72.41, 73.03, 73.30, 73.45, 75.41, 75.54, 76.61, 77.32, 81.35, 92.06, 95.86, 99.10, 103.76, 175.14. Anal. calcd for $C_{20}H_{34}NO_{18}SNa{\bullet}3.5\ H_2O$: C, 34.54; H, 5.91; N, 2.01; S, 4.61. Found : C, 34.54; H, 5.63; N, 1.96; S, 4.64.

*2-Acetamido-6-O-benzyl-2-deoxy-3-O-(3-O-allyl-2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2-deoxy-4-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- α,β-D-glucopyranose (18)*

Cerium ammonium nitrate (0.107 g, 0.189 mmol) was added to a cooled (–10 °C) solution of compound **14** (0.05 g, 0.0379 mmol) in a mixture of acetonitrile–water (0.25 mL). After vigorous stirring for 10 min, the reaction mixture was diluted with $CH_2Cl_2$ then washed successively with a saturated solution of $KHCO_3$ and water. The aqueous phase was extracted with $CH_2Cl_2$ and the combined organic phases were evaporated. Flash chromatography (hexane–ethyl acetate 3:7) of the residue afforded 41 mg (91 %) of **18**, $[\alpha]_D^{30}$ –18 (*c* 0.92, $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 1.02 (d, 0,75 H, *J* = 6.5 Hz, CH3cβ), 1.19 (d, 2.25H, *J* = 6.5 Hz, CH3cα), 1,47 (s, 0.75H, NHAcβ), 1.76 (s, 2.25H, NHAcα), 5.33 (m, 1H, $OCH_2$–CH=$CH_2$), 5.94 (m, 1H, $OCH_2$–C*H*=$CH_2$), 7.04–7.47 (m, 35H, arom.). $^{13}C$ NMR ($CDCl_3$, 62.9MHz) δ 16.45, 23.13, 54.97, 59.23, 91.85, 95.92, 103.56, 113.52, 116.32, 116.76, 126.89–129.15 (arom.), 134.70, 134.92, 137.14–139.00 (arom), 171.07. Anal. calcd for $C_{72}H_{81}NO_{15}{\bullet}H_2O$: C, 70.97; H, 6.87; N, 1.15; O, 21.01. Found: C, 71.09; H, 6.89; N, 1.11; O, 21.18.

*Benzyl O-(3-O-allyl-2,4,6-tri-O-benzyl-β-D-galactopyranosyl) (1→3)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl) (1→4)]-O-(2-acetamido-6-O-benzyl-2-O-deoxy-β-D-glucopyranosyl) (1→3)-O-(2,6-di-O-benzyl-β-D-galactopyranosyl) (1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (20)*

Sodium hydride (60 % in oil, ~5 mg) was added to a cooled (0 °C) and stirred solution of **18** (0.69 g, 0.573 mmol) and trichloroacetonitrile (0.288 mL, 2.874 mmol) in $CH_2Cl_2$. After 1.5 h at 0 °C, the reaction mixture was filtered through silica gel and evaporated. Flash chromatography (hexane–ethyl acetate–triethylamine, 6:4:0.05) of the residue afforded 0.576 g (75 %) of the α-trichloroacetimidate **19** used directly for the next step. [$^1$H NMR ($CDCl_3$, 400 MHz) δ 6.34 (d, 1H, $J$ = 3.5 Hz, H-1a), 8.63 (s, 1H, C=NH).] A solution of **19** (0.5 g, 0.372 mmol) in $CH_2Cl_2$ (0.5 mL) was added dropwise within 0.5 h to a cooled (–40 °C) and stirred mixture of compound **17** (1.314 g, 1.49 mmol) and $BF_3$ - $Et_2O$ (23 μL, 0.186 mmol) in $CH_2Cl_2$ (0.75 mL). After 2 h, during which the temperature was slowly raised to room temperature, the reaction mixture was diluted with $CH_2Cl_2$ and washed successively with saturated solution of $KHCO_3$ and water. The mixed aqueous phases were extracted with $CH_2Cl_2$ and the combined organic phases were evaporated. Flash chromatography (toluene–acetone, 9:1) of the residue afforded 0.191 g (25 %) of **20**, $[\alpha]_D^{26}$ –246 (*c* 1, $CH_2Cl_2$). $^1$NMR ($CDCl_3$, 400 MHz) δ 1.04 (d, 3H, $J$ = 6.5 Hz, $CH_3$e), 1.39 (s, 3H, NHAc), 2.88 (bs, 1H, OH), 2.96 (m, 1H, H-2c), 3.26 (m, 1H, H-5a), 3.31 (d, 1H, $J$ = 3 Hz, H-4b), 3.32 (dd, 1H, $J$ = 3, 10 Hz, H-3d), 3.37 (dd, 1H, H-5d (or b)), 3.41–3.76 (m, 16H), 3.78 (t, 1H, $J$ = 9 Hz, H-4c), 3.83 (dd, 1H, $J$ = 2, 10 Hz, H-3e), 3.91 (dd, 1H, $J$ = 8, 9 Hz, H-4a), 3.80 (d, 1H, $J$ = 3 Hz, H-4d), 3.90 (dd, 1H, $J$ = 3.5, 10 Hz, H-2e), 4.08 (d, 1H, $J$ = 11 Hz, $CH_2$Ph), 4.20–4.65 (m, 22H), 4.67–4.80 (m, 5H, 4 $CH_2$Ph, H-5e), 4.90 (d, 1H, $J$ = 3.5 Hz, H-1e), 4.85–4.95 (4d, 4H, 4$CH_2$Ph), 5.00 (d, 1H, $J$ = 11 Hz, $CH_2$Ph), 5.20 (m, 1H, O–$CH_2CH$=C$H_2$), 5.25 (d, 1H, $J$ = 7.5 Hz, H-1c), 5.29 (d, 1H, $J$ = 7 Hz, NH), 5.36 (m, 1H, O–$CH_2CH$=C$H_2$), 5.97 (m, 1H, O–$CH_2CH$=$CH_2$), 7.10–7.45 (m, 65H, arom.). $^{13}$C NMR ($CDCl_3$, 62.9 MHz) δ 16.47, 22.97, 59.60, 67.59, 67.80, 68.19, 68.73, 70.87, 71.60, 71.97, 73.01, 73.29, 73.37, 74.42, 74.99, 75.39, 66.23, 66.81, 67.22, 72.32, 72.39, 72.75, 73.62, 74.70, 75.60, 76.49, 78.17, 78.70, 79.82, 80.17, 81.82, 82.42, 82.94, 83.33, 97.27, 98.97, 102.09, 102.41, 103.36, 116.69, 127.21–128.72 (arom.), 134.75, 137.55–139.12 (arom.), 170.78. Anal. calcd for $C_{126}H_{137}NO_{25}$ : C, 73.27; H, 6.69; N, 0.68; O, 19.37. Found : C, 72.66; H, 6.71; N, 0.65; O, 19.69.

*Benzyl O-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl) (1→3)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl) (1→4)]-O-(2-acetamido-6-O-benzyl-2-O-deoxy-β-D-glucopyranosyl) (1→3)-O-(4-O acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl) (1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (22)*

A solution of compound **20** (72 mg, 0.0348 mmol) in a mixture of acetic anhydride–pyridine (5 mL, 1 : 1) is left for 54 h at room temperature. The mixture was then co-evaporated several times with toluene to give 73 mg of **21** which was directly used for the next step. To the residue, were successively added a mixture of ethanol–toluene–water (0.3 mL, 7:3:1) and $(PPh_3)_3RhCl$ (32 mg, 0.0346 mmol). After stirring at 70 °C for 17.5 h, the reaction mixture was evaporated and diluted with a mixture of acetone–water (0.25 mL, 9:1). Mercuric oxide (37 mg, 0.171 mmol) was then added followed by a 0.686 M solution of mercuric chloride in a mixture of acetone–water (0.25 mL, 9:1). After 1.5 h, the reaction mixture was diluted with $CH_2Cl_2$ and washed with a saturated solution of potassium iodide. The aqueous phase was extracted with $CH_2Cl_2$, and the combined organic phases were evaporated. Flash chromatography (hexane–ethyl acetate, 6:4) of the residue afforded 49 mg (67 %) of **22**, $[\alpha]_D^{27}$ –201 (*c* 1.18, $CH_2Cl_2$). $^1$H NMR ($CDCl_3$, 400 MHz) δ 1.04 (d, 1H, $CH_3$e), 1.49 (s, 3H, NHAc), 1.99 (s, 3H, OAc), 2.21 (d, 1H, $J$ = 2.5 Hz, OH), 5.04 (d, 1H, $J$ = 3.5 Hz, H-1e), 5.07 (d, 1H, $J$ = 8 Hz, NH), 5.38 (d, 1H, $J$ = 3 Hz, H-4b), 7.10–7.45 (m, 65 H, arom.). $^{13}$C NMR ($CDCl_3$, 62.9 MHz) δ 16.72, 20.57, 23.01, 57.97, 67.80, 67.88, 68.32, 69.78, 70.83, 72.00, 73.07, 73.22, 73.40, 74.41, 74.67, 74.91, 74.97, 75.07, 75.68, 66.03, 72.16, 72.67, 72.80, 74.41, 75.19, 75.57, 75.95, 76.26, 78.17, 78.45, 79.85, 80.11, 81.59, 82.56, 100.17, 101.96, 102.36, 103.04, 126.63–128.51 (arom.), 137.45–139.12 (arom.), 169.82, 170.14. Anal. calcd for $C_{125}H_{135}NO_{26}$, $H_2O$: C, 71.99; H, 6.62; N, 0.67. Found : C, 71.59; H, 6.84; N, 0.65.

*Benzyl O-(3-O-sulfo-2,4,6-tri-O-benzyl-β-D-galactopyranosyl) (1→3)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl) (1→4)]-O-(2-acetamido-6-O-benzyl-2-O-deoxy-β-D-glucopyranosyl) (1→3)-O-(4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl) (1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside sodium salt (23)*

A solution of compound **22** (90 mg, 0.0435 mmol) and sulfur trioxide–trimethylamine complex (42 mg, 0.305 mmol) in DMF (0.2 mL) was stirred at 55 °C for 4.5 h. The reaction mixture was then diluted with $CH_2Cl_2$, washed successively with a saturated solution of $KHCO_3$ and water and then evaporated. Flash chromatography (ethyl acetate–methanol, 95:5) of the residue followed by cation exchange chromatography (AG50W-X8, $Na^+$ form, elution MeOH) gave 86 mg (92 %) of **23**, $[\alpha]_D^{28}$ –244 (*c* 0.86, $CH_2Cl_2$). $^1$H NMR ($CDCl_3$, 400 MHz) δ 0.97 (d, 3H, $J$ = 6 Hz, $CH_3$c), 1.67 (s, 3H, NHAc), 2.03 (s, 3H, OAc), 4.21 (d, 1H, $J$ = 11.5 Hz, $CH_2$Ph), 4.28 (d, 1H, $J$ = 11.5 Hz, $CH_2$Ph), 5.47 (d, 1H, $J$ = 3.5 Hz, H-4b), 7.14–7.51 (m, 65H, arom.). $^{13}$C NMR ($CD_3OD$, 62.9 MHz) δ 16.99, 21.18, 23.22, 57.51, 68.87, 69.18, 69.79, 72.17, 73.16, 74.25, 74.52, 74.59, 75.20, 75.47, 75.95, 76.32, 76.86, 67.79, 69.79, 72.17, 74.10, 74.14, 74.25, 75.75, 76.48, 76.64, 77.23, 77.63, 77.89, 79.73, 80.61, 81.04, 82.31, 82.79, 83.74, 98.53, 103.17, 103.71(2), 104.59, 128.07–130.35 (arom.), 138.99–140.82 (arom.), 171.95, 172.97. Anal. calcd for $C_{125}H_{134}NO_{29}NaS{\cdot}3H_2O$: C, 67.52; H, 6.34. Found : C, 67.37; H, 6.44.

*O-(3-O-Sulfo-β-D-galactopyranosyl) (1→3)-O-[(α-L-fucopyranosyl) (1→4)]-O-(2-acetamido-2-O-deoxy-β-D-glucopyranosyl) (1→3)-O-(β-D-galactopyranosyl) (1→4)-α,β-D-glucopyranose sodium salt (2)*

A solution of compound **23** (49 mg, 0.0226 mmol) in 2 M sodium methoxide in methanol (0.4 mL) was heated under reflux for 22 h. After cooling to room temperature, AG50W-X8 cation exchange resin ($H^+$) was added until the solution became neutral. The resin was filtered off and washed thoroughly with methanol. The combined filtrates and washings were concentrated. Flash chromatography (ethyl acetate–methanol, 95:5) of the residue gave 35 mg of **24** which was directly used for the last deprotection step. A mixture of methanol–water (2 mL, 8:2) was added, and the resulting mixture was stirred under $H_2$ atmosphere (1 atm) in the presence of Pd on charcoal (10 %, 60 mg). After 96 h at room temperature, the mixture was filtered and evaporated. Flash chromatography (isopropanol–ethyl acetate–water, 3:3:2) of the residue followed by cation exchange chromatography (AG50W-X8, $Na^+$ form, elution $H_2O$) afforded 15 mg (66 % overall yield) of **2**. Freeze-drying of its aqueous solution gave a white hygroscopic powder, $[\alpha]_D^{30}$ –19 (*c* 1.42, $H_2O$). $^1H$ NMR ($D_2O$, 500 MHz), the values in brackets indicate the difference in chemical shift if compared to the non-sulfated pentasaccharide Le$^a$ (LNFPII); δ 1.194 ($CH_3$e), 2.053 (NHAc), 3.278 (–0.001) (H-2a β), 4.435 (0.003) ($J$ = 7.90 Hz, H-1 β), 4.606 (0.103) ($J$ = 7.70 Hz, H-1d), 4.662 (0.003) ($J$ = 8.04 Hz, H-1a β), 4.716 (0.015) ($J$ = 8.44 Hz, H-1c), 4.854 (–0.021) (H-5e), 5.023 (0.000) ($J$ = 3.69 Hz, H-1e), 5.218 (0.001) ($J$ = 3.56 Hz, H-1a α). $^{13}C$ NMR ($D_2O$–$CD_3OD$ (9:1), 62.9 MHz) δ 16.33, 23.29, 56.86, 60.60, 60.93, 61.06, 61.76, 62.50, 67.61, 67.81, 68.83, 69.33, 69.60, 70.14, 70.99, 71.10, 72.16, 72.41, 72.96, 74.81, 75.37, 75.50, 75.80, 76.23, 76.98, 79.27, 79.39, 81.20, 83.03, 92.82, 96.76, 98.53, 103.58 (2), 103.93, 175.76. MS calcd for $C_{32}H_{54}NO_{28}NaS$: 955.25. Found : 978.3 (M + $Na^+$), 500.7 (M + 2$Na^+$), 932.2 (M – $Na^+$).

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18. Indeed a very similar inhibitory activity was found[17] for the corresponding tetrasaccharide without the glucose unit at the reducing end prepared by Nicolaou *et al.*[6] but binding of *E*-selectin-expressing SC2 cells to the immobilized lipid-linked pentasacharide **2** was stronger.

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