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Synthesis of Sulfated Le^x-Trisaccharides

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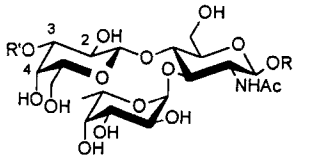
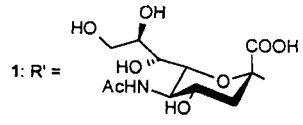
Dedicated to Prof. Dr. R. R. Schmidt on the Occasion of his 60th Birthday

Abstract. The regioselective sulfation of a Le^x-trisaccharide having free hydroxyl groups in 2-, 3- and 4- position of the galactose elucidated their different reactivities and gave mono- and disulfated Le^x derivatives.

The recognition of carbohydrate epitopes by E-, P- and L-selectin, a class of cell surface receptors, mediates rolling of leukocytes on endothelial cells. This is an early step in a cascade of events that finally leads to the adhesion of leukocytes to endothelial cells followed by their invasion into tissue. This process has been identified as an important step at the onset of inflammatory diseases [1]. The first ligand for E- and P-selectin was identified to be the saccharide sialyl Le^x, **1** [2], a carbohydrate epitope of glycoproteins and glycolipids which are present on cell surfaces. More recently sulfated Le^x, **2**, and Le^a have been isolated from an ovarian cystadenoma glycoprotein and were found to show superior binding to E-selectin [3]. These findings have stimulated a significant amount of research devoted to the synthesis of sulfated Lewis antigen derivatives [4].

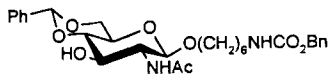




2: R' = HO_3S

Figure 1

As part of a program directed to the development of sialyl Le^x based anti adhesion molecules we were also interested in the synthesis of sulfated Le^x derivatives. With this goal a strategy was developed that leaves the 2-, 3- and 4- hydroxyl groups of the galactose unit of Le^x unprotected before the sulfation, as the 3-hydroxyl group has demonstrated superior reactivity in glycosylation reactions compared with the 2- and 4- hydroxyl groups [5]. In the synthesis of sulfated Le^x and its analogs reported so far [4a–c] the protecting group strategies applied were based on the generation of a single free hydroxyl group before the sulfation step.



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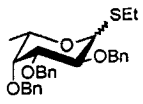
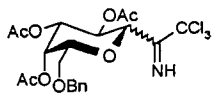




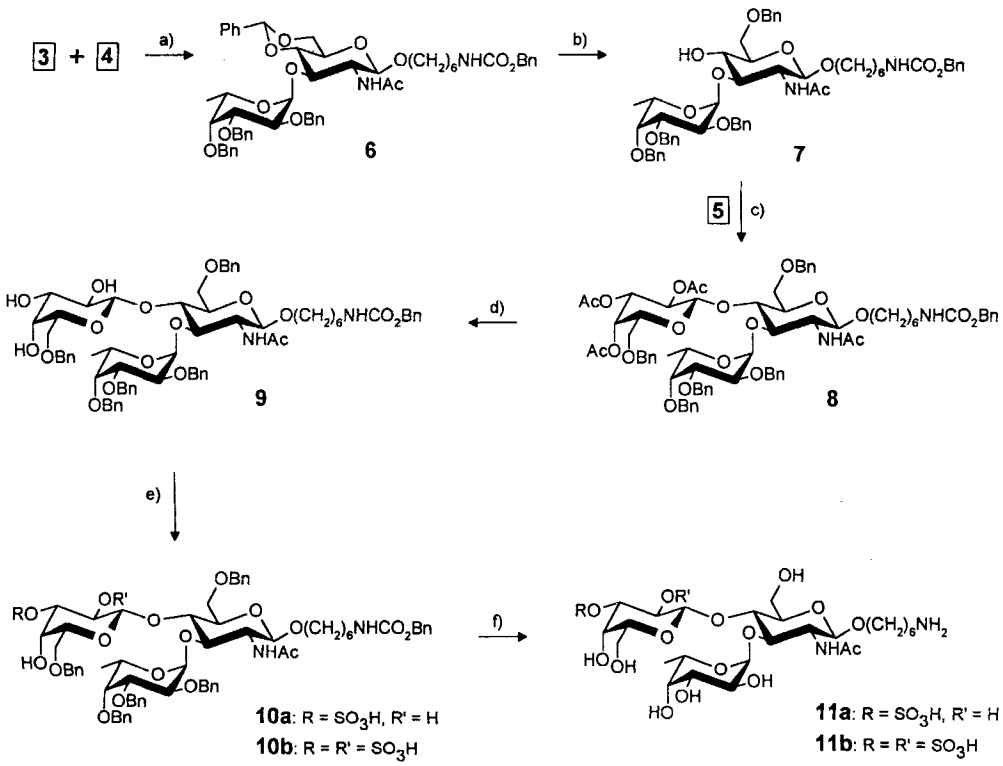
4 **5**

Figure 2

For the preparation of sulfated Le^x we used the strategy, that has proven to be very efficient in the synthesis of deoxy sialyl Le^x analogs [6]. A spacer was incorporated at C-1 of N-acetyl-glucosamine which gives the option to conjugate the synthesized compounds to a carrier [7]. Key building blocks of the synthesis are the N-acetyl-glucosamine acceptor **3** [8], the fucose donor **4** [9] and the galactose donor [10] **5**. As we have shown before [6] connecting **3** and **4** in the

first glycosylation step and coupling the galactose to this disaccharide (versus galactosylation of the N-acetyl-glucosamine in the first and fucosylation of the disaccharide in the second step) was more efficient because it saves protection and deprotection of the 3-hydroxyl group of the N-acetyl glucosamine. The glycosylation of **3** with **4** was carried out in a 1:1 mixture of dimethylformamide and dichloromethane using copper bromide and tetrabutylammonium bromide as promotors. The α-fucoside **6**, the only detectable isomer, was isolated in 95 % yield. The deprotection of the 4-hydroxyl group of N-acetyl-glucosamine was achieved by regioselective reductive opening of the acetal [11] using sodium cyanoborohydride in tetrahydrofuran and hydrogenchloride in ether to give the desired product **7** in 90 % yield. The galactose building block **5**, activated as trichloroacetimidate [12], was coupled to the disaccharide **7** using boron trifluoride-ether as a promotor in dichloromethane. The β-glycoside **8** was isolated as the only detectable isomer in 73 % yield. The acetyl protecting groups were removed by treatment with sodium methoxide in methanol generating **9**, the substrate for the sulfation reaction, in 97 % yield. The sulfate was introduced using the sulfur trioxide-trimethylamine complex in dimethylformamide. The reaction mixture contained two products, **10a** and **10b**, with different polarity carrying one or two sulfate groups, respectively. Purification and structure elucidation were more favourably achieved after removal of the benzyl and benzyloxycarbonyl protecting groups. The usual deprotection conditions (methanol, Pd/C, H_2) revealed to be ineffective [13]. Catalytic transfer hydrogenation turned out to be the deprotection method of choice. The reaction conducted in dioxane resulted in complete desulfation [14]. With 10 % formic acid in methanol a mixture of mono- and disulfated products was obtained. The purification of the two products was successfully achieved by column chromatography on Biogel P2. 25 % of monosulfated trisaccharide **11a** and 37 % of disulfated **11b** were isolated.

The positions of sulfation were identified based on chemical shift differences for the signals of protons and carbons at the 2-, 3- and 4-positions of the galactose unit in **11a** and **11b** compared to the corresponding Le^x derivatives [15]. The sulfate group in the monosulfate **11a** was introduced into position 3. This is proven by chemical shift differences of 0.68 ppm for the signals of the corresponding protons and 9.31 ppm for the signals of the corresponding carbons (table 1). **11b** carried the sulfates in positions 2 and 3 of the galactose which was indicated by chemical shift differences of 0.92 and 0.77 ppm for the signals of the corresponding protons and 6.92 and 7.54 ppm for the signals of the corresponding carbons (table 1).



Scheme 1 a) **3** (1.0 eq.), **4** (1.4 eq.) CH_2Cl_2/DMF (1:1), 3 Å molecular sieves, Bu_4NBr (3 eq.), $CuBr_2$ (2.8 eq.), 36 h, 25 °C, 95 %; b) $NaCNBH_4$ (20 eq.), HCl_g in Et_2O, THF, 25 °C, 90 %; c) **7** (1.0 eq.), **5** (1.7 eq.) CH_2Cl_2, $BF_3 \cdot Et_2O$, (0.005 eq.), 4h, 25 °C, 75 %; d) NaOMe (1.0 eq.), MeOH, 3h, 25 °C, 97 %; e) $SO_3 \cdot NEt_3$, DMF, 25 °C; f) Pd-black, 10 % HCO_2H in MeOH, 25 °C, 25 % **11a** and 37 % **11b** (2 steps).

Table 1 Chemical shifts of the positions 2–4 of galactose in Le^x, **11a** and **11b**

Galactose	Le^x		**11a**		**11b**	
	^{13}C [ppm]	^{1}H [ppm]	^{13}C [ppm]	^{1}H [ppm]	^{13}C [ppm]	^{1}H [ppm]
Pos. 2	72.36	3.50	72.00	3.63	79.28[a)]	4.42
Pos. 3	73.80	3.65	83.11	4.33	81.34[a)]	4.42
Pos. 4	69.66	3.90	69.56	4.27	69.76	4.35

[a)] The assignment is uncertain and the data may be exchangable for these two positions.

In conclusion, the sulfation of **9** leads to only one monosulfated product, **10a**, carrying the sulfate group at the 3-position of galactose. This result illustrates the superior reactivity of the equatorial 3- over the equatorial 2- and the axial 4-hydroxyl group of the galactose unit of the Le^x derivative. This principle of regioselective modification of galactosides, which has previously been reported for glycosylations [5], has now been proven to be very useful for the sulfation reaction. The monosulfation in **10a** seems to activate the neighboring hydroxyl group in 2-position for an additional sulfation. Interestingly, neither 3,4-disulfated nor 2,3,4-trisulfated product could be isolated under the applied reaction conditions.

The biological activities of **11a** and **11b** are currently under investigation. The amino group at the spacer terminus will be used to couple the sulfated trisaccharides **11a** and **11b** to a suitable carrier in order to facilitate further investigations of the multivalency of the carbohydrate-selectin interaction [7].

Experimental

General methods. NMR spectra were recorded on a Bruker WT 300. The following abbreviations were used to explain multiplicities: s (singulet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were recorded on a Finnigan MAT 95 Q-MR. Melting points were obtained on a Gallenkamp apparatus. Optical rotations were recorded using a Perkin Elmer 241 Polarimeter.

All reaction were monitored by thin-layer chromatography carried out on 0.25 mm silica gel plates (60F-254; E. Merck, Darmstadt, Germany) using UV light, iodine or p-anisaldehyde solution and heat as developing agent. Silica gel (60, particle size 0.040–0.063 mm; E. Merck, Darmstadt, Germany) was used for flash column chromatography. All reactions were carried out under an argon atmosphere with anhydrous solvents. Yields refer to chromatographically and spectroscopically (^{1}H-NMR) homogeneous materials.

(6-N-Benzyloxycarbonylamino)hexyl O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**6**)

A solution of **3** [8] (167 mg, 0.307 mmol) and **4** [9] (206 mg, 0.430 mmol) in a mixture of dichloromethane (7 ml) and dimethylformamide (7 ml) was stirred for 30 minutes with 3 Å molecular sieves and was then treated under exclusion of light with tetrabutylammonium bromide (415 mg, 1.290 mmol) and copper dibromide (268 mg, 1.204 mmol). After stirring for 36 h at 25 °C the residue was filtered off and carefully washed twice with dichloromethane (2×20 ml). The combined filtrate and washings were extracted with hydrochloric acid, saturated aqueous sodium hydrogencarbonate solution and brine. The solution was dried ($MgSO_4$), concentrated in vacuo and the reminder subjected to flash chromtography (10-30 % ethyl acetate in toluene) to give pure **6** (280 mg, 0.292 mmol, 95 %).
R_f = 0.56 (toluene/ethylacetate 1:1); $[\alpha]_D^{20}$ = –88.0 (c = 1, CH_2Cl_2); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.81 (d, $J_{6,5}$ = 6.6 Hz, 3 H, 6-H^{Fuc}), 1.3 (m, CH_2^{spacer}), 1.47 (m, CH_2^{spacer}), 1.65 (s, 3 H, NHAc), 3.17 (m, CH_2^{spacer}), 3.40 (dd, 1 H, 2-H^{GlcNAc}), 3.58 (dd, 1 H, 4-H^{Fuc}), 3.94 (dd, 1 H, 3-H^{Fuc}), 4.06 (dd, 1 H, 2-H^{Fuc}), 4.09 (dd, 1 H, 5-H^{Fuc}), 4.27 (dd, 1 H, 3-H^{GlcNAc}), 4.86 (d, 1 H, 1-H^{GlcNAc}), 5.09 (d, 1 H, 1-H^{Fuc}), 5.48 (s, 1 H, CH^{benzylidene}), 5.77 (d, $J_{1,NH}$ = 7.2 Hz, 1 H, NHAc^{GlcNAc}), 7.1–7.4 (m, 20 H, Ph) ppm; FAB-MS m/z: 957 ($M+H^+$); 979 ($M+Na^+$).

(6-N-Benzyloxycarbonylamino)hexyl O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**7**)

A suspension of **6** (260 mg, 0.271 mmol) and sodium cyanoborohydride (171 mg, 2.71 mmol) in dry tetrahydrofuran (10 ml) was slowly treated at 25 °C with a saturated solution of hydrogen chloride in ether. Close monitoring of the reaction by TLC was necessary to determine the completion of the reaction. The reaction mixture was neutralized with solid sodium hydrogencarbonate, diluted with ethyl acetate (30 ml) and extracted with saturated aqueous sodium hydrogencarbonate solution and brine. The solution was dried ($MgSO_4$), concentrated in vacuo and then submitted to flash chromtography (15–55 % ethyl acetate in toluene) to give pure **7** (240 mg, 0.249 mmol, 90 %).
R_f = 0.39 (toluene/ethylacetate 1:1); $[\alpha]_D^{20}$ = –55.5 (c = 1, CH_2Cl_2); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.14 (d, $J_{6,5}$ = 6.6 Hz, 3 H, 6-H^{Fuc}), 1.31 (m, CH_2^{spacer}), 1.5 (m, CH_2^{spacer}), 1.60 (s, 3 H, NHAc), 3.16 (m, CH_2^{spacer}), 3.31 (dd, $J_{2,1}$ = 8.3 Hz, 1 H, 2-H^{GlcNAc}), 3.68 (dd, 1 H, 4-H^{Fuc}), 3.93 (dd, 1 H, 3-H^{Fuc}), 4.07 (dd, 1 H, 2-H^{Fuc}), 4.10 (dd, 1 H, 5-H^{Fuc}), 3.85 (dd, 1 H, 3-H^{GlcNAc}), 4.83 (d, J1.2 = 8.4 Hz, 1 H, 1-H^{GlcNAc}), 4.98 (d, $J_{1,2}$ = 4.1 Hz, 1 H, 1-H^{Fuc}), 5.53 (d, 1 H, NHAc^{GlcNAc}), 7.1–7.4 (m, 20 H, Ph) ppm; FAB-MS m/z: 959 ($M+H^+$); 981 ($M+Na^+$).

(6-N-Benzyloxycarbonylamino)hexyl O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)]-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**8**)

A solution of **5** [10] (197 mg, 0.384 mmol) and **7** (222 mg, 0.226 mmol) in dry dichloromethane (2 ml) was stirred for 30 minutes with 3 Å molecular sieves and then treated with boron trifluoride-ether (0.24 ml, 0.002 mmol). After stirring for 4 hours at 25 °C the reaction was quenched by addition of solid sodium hydrogencarbonate. After dilution with dichloromethane (25 ml) the solution was extracted with saturated aqueous sodium hydrogencarbonate solution and brine. The solution was dried ($MgSO_4$), concentrated in vacuo and

the reminder subjected to flash chromtography (25–50 % ethyl acetate in toluene) to give pure **8** (221 mg, 0.165 mmol, 73 %). R_f = 0.61 (dichloromethane/methanol 20:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.11 (d, 3 H, 6-H^{Fuc}), 1.25 (m, CH_2^{spacer}), 1.46 (m, CH_2^{spacer}), 1.77 (s, 3 H, NHAc); 1.87, 1.96, 2.0 (3 s, 9 H, OAc); 3.14 (m, 2 H, CH_2^{spacer}), 5.41 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H^{Fuc}), 5.96 (d, $J_{1,NH}$ = 8.0 Hz, 1 H, $NHAc^{GlcNAc}$), 7.1–7.4 (m, 25 H, Ph) ppm; FAB-MS m/z: 1340 ($M+H^+$); 1362 ($M+Na^+$).

(6-N-Benzyloxycarbonylamino)hexyl O-(6-O-benzyl-β-D-galactopyranosyl)-(1-4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)]-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**9**)

Sodium methoxide (1.6 ml of a 0.1 N solution in methanol) was added to a solution of **8** (204 mg, 0.152 mmol) in dry methanol (6 ml) and stirred at 25 °C for 3h. Amberlyst 15 ion-exchange resin (1 g) was added and the mixture was stirred until it was neutral. The ion-exchange resin was filtered off, the filtrate was concentrated in vacuo and submitted to flash chromatography (5–20 % methanol in dichloromethane) to yield pure **9** (180 mg, 0.148 mmol, 97 %).
R_f = 0.44 (dichloromethane/methanol 20:1); $[\alpha]_D^{20}$ = –49.9 (c = 1, CH_3OH); ^{1}H-NMR (300 MHz, CD_3OD): δ = 1.05 (d, $J_{6,5}$ = 6.6 Hz, 3 H, 6-H^{Fuc}), 1.22 (m, CH_2^{spacer}), 1.4 (m, CH_2^{spacer}), 1.82 (s, 3 H, NHAc), 2.98 (t, 2 H, CH_2^{spacer}), 5.19 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H^{Fuc}), 7.1–7.4 (m, 25 H, Ph) ppm; FAB-MS m/z: 1217 ($M+H^+$); 1239 ($M+Na^+$).

6-Aminohexyl O-(3-O-sulfoxy-β-D-galactopyranosyl)-(1-4)-O-[α-L-fucopyranosyl-(1-3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (**11a**)
and
6-Aminohexyl O-(2,3-O-disulfoxy-β-D-galactopyranosyl)-(1-4)-O-[α-L-fucopyranosyl-(1-3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (**11b**)

A mixture of **9** (180 mg, 0.148 mmol) and sulfur trioxide-triethylamine complex (5 equiv.) was stirred in dimethylformamide (2 ml) for 36h at 25 °C. After concentration a first purification was achieved by chromatography (dichloromethane/methanol/pyridine 100:4:1 – 60:4:1) giving a mixture of **10a** and **b** (160 mg).

1 ml of formic acid was added dropwise over 5h at 25 °C to a mixture of **10a** and **b** (140 mg) and Pd-black (100 mg) in methanol (10 ml). After removing the palladium by filtration the solution was concentrated in vacuo, coevaporated with toluene (3×20 ml) in vacuo and purified by chromatography on Biogel P2. The monosulfate **11a** (26 mg, 0.0367 mmol, 25 %) and the disulfate **11b** (43 mg, 0.0545 mmol, 37 %) were isolated as colourless, amorphous solids.
11a: R_f = 0.75 (ethylacetate/methanol/water/acetic acid = 5:3:3:0.5); $[\alpha]_D^{20}$ = –36.8 (c = 1, H_2O); mp. = > 200 °C degradation; ^{1}H-NMR (300 MHz, D_2O): δ = 1.19 (d, $J_{6,5}$ = 6.7 Hz, 3 H, 6-H^{Fuc}), 1.38 (m, 4 H, CH_2^{spacer}), 1.57 (m, 2 H, CH_2^{spacer}), 1.66 (m, 2 H, CH_2^{spacer}), 2.03 (s, 3 H, NHAc), 3.0 (t, 2 H, CH_2^{spacer}), 3.61 (1 H, CH_2^{spacer}), 3.63 (dd, 1 H, 2-H^{Gal}), 3.69 (dd, 1 H, 2-H^{Fuc}), 3.81 (dd, $J_{4,5}$ = 3.5 Hz, 1 H, 4-H^{Fuc}), 3.94 (1 H, CH_2^{spacer}), 4.27 (dd, $J_{4,3}$ = 3.2 Hz, 1 H, 4-H^{Gal}), 4.33 (dd, $J_{3,2}$ = 9.8 Hz, $J_{3,4}$ = 3.2 Hz, 1H, 3-H^{Gal}), 4.58 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1-H^{Gal}), 4.83 (dd, 1 H, 5-H^{Fuc}), 5.12 (d, $J_{1,2}$ = 3.9 Hz, 1 H, 1-H^{Fuc}) ppm; ^{13}C-NMR (75.4 MHz, D_2O): δ = 18.12 (6-C^{Fuc}), 25.12 (NHAc); 27.47, 28.07, 29.50, 31.22 (CH_2^{spacer}); 42.3 (NCH_2^{spacer}), 69.56 (5-C^{Fuc}, 4-C^{Gal}), 72.00 (2-C^{Gal}), 74.75 (4-C^{Fuc}), 83.11 (3-C^{Gal}), 101.4 (1-C^{Fuc}), 103.84 (1-C^{GlcNAc}), 104.31 (1-C^{Gal}), 176.96 ($NHAc^{GlcNAc}$) ppm; FAB-MS m/z: 707 $(M-H^+)^-$; 735 $(M+Na^+-2H^+)^-$.

11b: R_f = 0.55 (ethylacetate/methanol/water/acetic acid = 5:3:3:0.5); $[\alpha]_D^{20}$ = –42.8 (c = 1, H_2O); mp. = > 200 °C degradation; ^{1}H-NMR (300 MHz, D_2O): δ = 1.23 (d, $J_{6,5}$ = 6.7 Hz, 3 H, 6-H^{Fuc}), 1.38 (m, 4 H, CH_2^{spacer}), 1.57 (m, 2 H, CH_2^{spacer}), 1.67 (m, 2 H, CH_2^{spacer}), 2.03 (s, 3 H, NHAc), 3.0 (t, 2 H, CH_2^{spacer}), 3.6 (1 H, CH_2^{spacer}), 3.71 (dd, 1 H, 2-H^{Fuc}), 3.82 (dd, $J_{4,5}$ = 2.9 Hz, 1 H, 4-H^{Fuc}), 3.9 (1 H, CH_2^{spacer}), 4.35 (dd, 1 H, 4-H^{Gal}), 4.42 (2 dd, 2 H, 3-H^{Gal}, 2-H^{Gal}), 4.53 (d, $J_{1,2}$ = 7.3 Hz, 1 H, 1-H^{GlcNAc}), 4.7 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1-H^{Gal}), 4.89 (dd, 1 H, 5-H^{Fuc}), 5.11 (d, $J_{1,2}$ = 4.2 Hz, 1 H, 1-H^{Fuc}) ppm; ^{13}C-NMR (75.4 MHz, D_2O): δ = 18.12 (6-C^{Fuc}), 25.11 (NHAc); 27.40, 28.03, 29.46, 31.17 (CH_2^{spacer}); 42.3 (NCH_2^{spacer}), 69.69 (5-C^{Fuc}), 69.76 (4-C^{Gal}), 73.24 (CH_2^{spacer}), 74.84 (4-C^{Fuc}), 79.28, 81.34 (2-C^{Gal}, 3-C^{Gal}), 101.46 (1-C^{Fuc}), 102.88 (1-C^{Gal}), 103.84 (1-C^{GlcNAc}), 176.94 ($NHAc^{GlcNAc}$) ppm; FAB-MS m/z: 787 $(M-H^+)^-$; 809 $(M+Na^+-2H^+)^-$.

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