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Chemical synthesis of 6$^{(\mathrm{GlcNAc})}$- and 6$^{(\mathrm{Gal})}$-*O*-sulfated SiaLe$^{\mathrm{X}}$ tetrasaccharides in spacer-armed form

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Practical synthesis of tetrasaccharide sulfates, 6$^{(\mathrm{GlcNAc})}$-*O*-Su-SiaLe$^{\mathrm{X}}$-OCH$_2$CH$_2$CH$_2$NH$_2$ and 6$^{(\mathrm{Gal})}$-*O*-Su-SiaLe$^{\mathrm{X}}$-OCH$_2$CH$_2$CH$_2$NH$_2$ (Su=SO$_3$H), selectin ligands, and leukocyte trafficking agents is presented. Both sulfates were synthesized starting from the same precursor, protected SiaLe$^{\mathrm{x}}$, by the conventional procedures of carbohydrate chemistry. The sulfated SiaLe$^{\mathrm{x}}$ derivative was modified at the spacer group to give 6$^{(\mathrm{Gal})}$-*O*-Su-SiaLe$^{\mathrm{x}}$-OCH$_2$CH$_2$CH$_2$NH-COCH$_2$CH$_2$C≡CH, convenient for “click chemistry” mode conjugation with an azido carrier, particularly, for the synthesis of an immunogen.

Keywords: leukocyte trafficking agents/selectin ligands/sulfates of sialyl Lewis$^{\mathrm{X}}$

Introduction

SiaLe$^{\mathrm{x}}$ and its derivative 6$^{(\mathrm{GlcNAc})}$-*O*-Su-SiaLe$^{\mathrm{x}}$ (Su=SO$_3$H) are known to be ligands for selectins and to play the outstanding role in leukocyte trafficking (Rosen 2004). L-Selectin binds to *O*-glycan capped with 6$^{(\mathrm{Gal})}$-*O*-Su-SiaLe$^{\mathrm{x}}$ (Blixt et al. 2004; McEver 2005). SiaLe$^{\mathrm{x}}$ sulfated in the position 6-OH of Gal is also known to be the highest-affinity ligand for siglec-8 (Bochner et al. 2005), whereas SiaLe$^{\mathrm{x}}$ derivative sulfated at the position 6-OH of GlcNAc is a specific receptor for deadly avian influenza viruses (Gambaryan et al. 2004). In the glycobiology literature, one can find numerous other functions mediated by 6$^{(\mathrm{GlcNAc})}$-*O*-Su-SiaLe$^{\mathrm{x}}$ and 6$^{(\mathrm{Gal})}$-*O*-Su-SiaLe$^{\mathrm{x}}$; therefore, the constant interest to the synthesis of these sulfated molecules is not surprising. Enzymatic synthesis of SiaLe$^{\mathrm{x}}$ sulfates is complicated because specific transferases are not available yet. Chemical syntheses of SiaLe$^{\mathrm{x}}$ derivatives sulfated at the 6-OH position of Gal or GlcNAc and related complex carbohydrates were published by several groups, for example Jain et al. (1994) and Yamaguchi et al. (2009). However, there is a necessity in a new approach combining (i) the practical procedure allowing the synthesis of 10–100 mg amounts, (ii) the divergent strategy allowing obtaining both molecules, and (iii) the availability of appropriate spacer-arm.

As the Sia residue in composition of SiaLe$^{\mathrm{x}}$ contains the carboxyl group, the amino group seems to be the most practical function for the spacer-arm. Reasoning for a short 3-carbon spacer choice raises from minimization of potential unspecific binding in bioprobing from one hand, and from our own and Consortium for Functional Glycomics (CFG, www.functionalglycomics.org) long time experience in the application of short-spacer glycan probes evidencing the absence of negative results with them – from the other hand.

Realizing well the great complexity of full multistep synthesis of each of the target molecules, we have been looking for a simplified strategy enabling to obtain spacered sulfated tetrasaccharides starting from one and obligatory available precursor.

Results and discussion

The scaled chemical synthesis of such precursor in the form of 3-aminopropyl glycoside was published earlier (Pazynina et al. 2003), it applies SiaGal + GlcNAc–spacer block glycosylation as a key step followed by fucosylation; here, we use protected tetrasaccharide **1** as a precursor for sulfation in positions 6 of galactose or *N*-acetylglucosamine moieties. Oligosaccharide **1** possessed only two types of *O*-protective groups, benzyl and acetyl ones; de-*O*-acetylation or debenzylation gave rise to partially protected derivatives convenient for the further conversion into the target sulfated SiaLe$^{\mathrm{x}}$ derivatives **6** and **10**, respectively (Schemes 1 and 2). Based on our previous experience in the selective 6-*O*-sulfation of the lactosamine derivatives under reduced temperature ($-10 \div -20^\circ$C) (Pazynina et al. 2008) due to the higher activity of the primary 6-OH groups in Gal and GlcNAc residues as compared with the secondary hydroxyls, we expected selective 6-*O*-sulfation of the partially protected SiaLe$^{\mathrm{x}}$ derivatives in selected conditions.

Carefully controlled de-*O*-acetylation of **1** with 0.04 M MeONa gave rise to the mixture of products containing two major compounds, monoacetate **2** and lactone **3** (Scheme 1), easily separable by chromatography on silica gel (yield 38% of **2** and 38% of **3**). The first of them contains six hydroxyls, the second contains five hydroxyls, and both compounds have two primary OH group. Obviously, direct *O*-sulfation has to give numerous isomeric monosulfates together with the products of more advanced substitution. Therefore, we protected Neu5Ac fragment with the isopropylidene group; not surprising that tetraol **3** gave better yield on the re-protection step with acetonation reagent, namely, 86% **5** versus 53% **4**. Notably, both polyols contain only one primary hydroxyl group, thus giving us good chances for selective *O*-sulfation. Indeed, triol **5** yielded aimed monosulfate **6**, 51%, together with 20% of disulfate **7**. Similarly, tetraol **4** also gave rise to monosulfate **6** with satisfactory yield 41% and by this route, 12% of disulfate **7** was obtained; monosulfate

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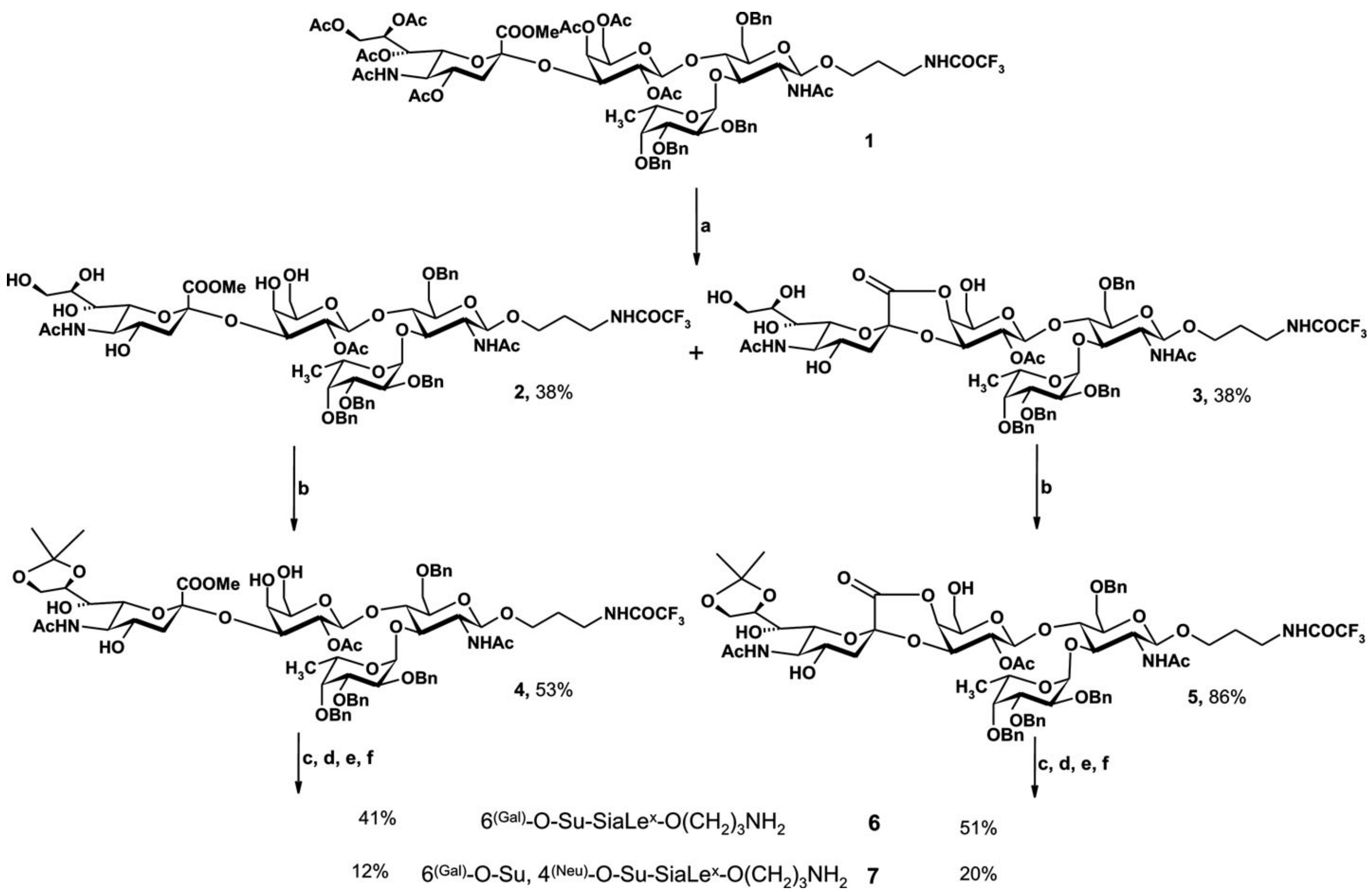


Scheme 1. (**A**) 0.04 M MeONa/MeOH, 20 min; (**B**) $(CH_3)_2C(OCH_3)_2$, TsOH, MeCN, 1 h; (**C**) Py·SO_3/Py, −10 ÷ −20°C, 5 h; (**D**) 80% aq. AcOH, 40°C, 2 h; (**E**) H_2-Pd/C, MeOH, 2 h; (**F**) 0.1N NaOH/H_2O, 3 h; DEAE, HPLC ($LiClO_4$), LH-20, Dowex Na^{+}.

$4^{(Neu)}$-*O*-Su-SiaLe^{x} was also isolated (∼1%). Importantly, when all mixed fractions of chromatography procedures (tetraol and lacton routes) were combined, deprotected, and separated, 30% of SiaLe^{x} tetrasaccharide (sulfate free) was obtained; thus, total efficacy of synthetic scheme, taking into account recovered SiaLe^{x}, is considered to be 36% calculated per starting **1**.

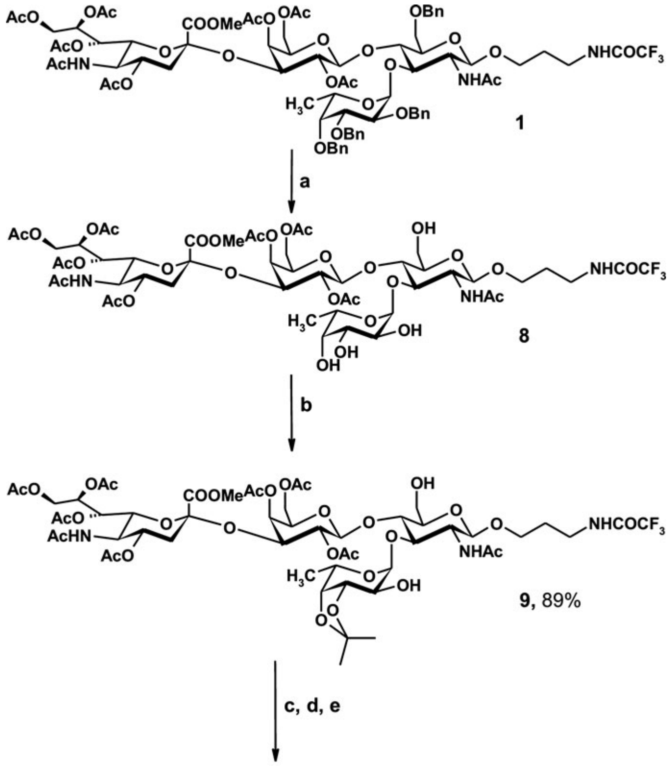
Debenzylation of **1** (Scheme 2) led to tetraol **8**, a compound with one primary and three secondary hydroxyls that seemed to be promising for the synthesis of the second aimed monosulfate, $6^{(GlcNAc)}$-*O*-Su-SiaLe^{x} **10**. However, *O*-sulfation under the same conditions gave rise mainly to disulfates (47%). SiaLe^{x} monosulfate mixture consisted of the following derivatives: 9% 6-*O*-substituted at glucosamine, 6% 3-*O*-substituted at fucose, and 3% 2-*O*-substituted at fucose. So, 3-OH and 4-OH of Fuc moiety were blocked by isopropylidene groups and diol **9**, obtained with 89% yield per **1**, was mono-*O*-sulfated at position 6-OH of GlcNAc moiety (**10**) with the yield 32%. We failed to avoid formation of di-sulfated product **11** (23%). Interestingly, isomeric monosulfate **12** with Su in position 2-OH of Fuc moiety was isolated with 3% yield, so, this position in **9** seems to demonstrate reactivity comparable to that of 6-OH^{GlcNAc}. Sulfate-free SiaLe^{x} tetrasaccharide (8%) was also isolated from the reaction mixture.

Target sulfated products were isolated at the final step of the synthesis after removal of all protective groups. Omitting intermediate chromatographic separations allowed us to maximize the yield of the target compounds.

Noteworthy, the choice of $-OCH_2CH_2CH_2NH_2$ as spacer-arm proved to be fortunate: we observed neither lactam formation with the carboxyl group during deprotection nor encumbrance of sulfate in respect of the amino group reactivity when coupled with chip, polymer (Blixt et al. 2004; Bochner et al. 2005; Rapoport et al. 2006; Klopocki et al. 2008) or additional tag (see below).

Thus, $6^{(GlcNAc)}$-*O*-Su-SiaLe^{x} and $6^{(Gal)}$-*O*-Su-SiaLe^{x} as spacer-armed tetrasaccharides were chemically synthesized with satisfactory yields, starting from available protected SiaLe^{x} by the simple procedure in the four stages: deacetylation (or debenzylation), acetonation, selective sulfation, and final deprotection. One of them, $6^{(Gal)}$-*O*-Su-SiaLe^{x} was also modified by the spacer group giving rise to $6^{(Gal)}$-*O*-Su-SiaLe^{x}-$OCH_2CH_2CH_2NH\text{-}COCH_2CH_2C{\equiv}CH$, convenient for “click chemistry” fashion conjugation with an azido-carrier.

The structures of final compounds were confirmed by ^{1}H NMR spectroscopy data. Sulfation of the hydroxyl function resulted in the downfield shift of the signals of neighboring protons compared to SiaLe^{x} (δ 3.65–4.12 ppm): δ 4.14 and 4.29 ppm for H-6′ and H-6″ Gal; δ 4.43 ppm for H-6′ and H-6″ GlcNAc; δ 4.44 ppm for H-2 Fuc and 4.34 ppm for H-4 Neu (see details in spectral data).



$6^{(GlcNAc)}$-O-Su-SiaLe^x-O$(CH_2)_3NH_2$ **10** 32%

$6^{(GlcNAc)}$-O-Su, $2^{(Fuc)}$-O-Su-SiaLe^x-O$(CH_2)_3NH_2$ **11** 23%

$2^{(Fuc)}$-O-Su-SiaLe^x-O$(CH_2)_3NH_2$ **12** 3%

Scheme 2. (**A**) H_2-Pd/C, MeOH, 2 h; (**B**) $(CH_3)_2C(OCH_3)_2$, TsOH, MeCN, 1 h; (**C**) Py·SO_3/Py, −10 ÷ −20°C, 1.5 h; (**D**) 80% aq. AcOH, 40°C, 2 h; (**E**) 0.1 M MeONa/MeOH, 0.5 h; 0.1N NaOH/H_2O, 3 h; DEAE, HPLC ($LiClO_4$), LH-20, Dowex Na^+.

Material and methods

Two hundred microliters of 2 M MeONa/MeOH was added to a solution of 300 mg (0.183 mmol) **1** in 10 mL dry methanol. In 20 min, the solution was neutralized with 30 mL AcOH. The resulting solution was evaporated and co-evaporated with toluene (3 × 50 mL). Dry residue was chromatographed on silica gel (elution with gradient $CHCl_3$:MeOH 9:1 → 6:1) resulting in isolation of 96 mg (38%) tetrasaccharide **2** and 96 mg lactone **3**. Twenty-five microliters of dimethoxypropane and 5 mg toluenesulfonic acid were added to solutions of obtained compounds in 5 mL dry MeCN (resulting pH 2–3). In 1 h, solutions were neutralized with 100 μL pyridine and co-evaporated with toluene (3 × 25 mL). Chromatography on silica gel (elution with mixture $CHCl_3$:MeOH:Py 100:8:1 → 60:10:1) led to isolation of 86 mg (86%) compound **5** and 52 mg (53%) compound **4**.

A mixture of compound **4** or **5** with 125 mg Py·SO_3 in 2 mL dry pyridine was kept upon stirring at −10 ÷ −20°C for 5 h. $NaHCO_3$ (150 mg) was added followed by reaction mixture stirring for 15 min, addition of 20 mL MeOH, filtration after 30 min, and washing of the residue on filter with MeOH (5 × 10 mL). Combined filtrate was concentrated in vacuo and co-evaporated several times with toluene. The main part of non-carbohydrate admixtures was removed from reaction mixture by gel filtration on Sephadex LH-20 (elution with MeOH, 0.5% Py). The reaction mixture was dissolved in 2 mL 80% AcOH and kept at 40°C for 2 h. After co-evaporation with toluene (3 × 50 mL), hydrogenolysis on 10% Pd/C (30 mg) was performed in methanol (10 mL) at atmospheric pressure for 2 h. After evaporation and co-evaporation with toluene, dry residue was dissolved in 3 mL 0.1 M followed by neutralization with AcOH in 4 h. Ion-exchange chromatography on DEAE-Sephadex A-25 (OAc^--form) was performed: nonsulfated SiaLe^x was eluted with 0.01 M Py·AcOH and sulfated substances – with 1 M Py·AcOH. Then, the substances were subjected to HPLC on Phenomenex Luna 5 u C18 100 A (5 μm, 4.6 × 250 mm, 30°C, flow rate 0.5 mL/min) using 50 mM $LiClO_4$ in H_2O as an eluent (SiaLe^x – without $LiClO_4$). The substances were dried by evaporation and purified with gel filtration chromatography on Sephadex LH-20 (elution with H_2O). Na^+-salts of the synthesized sulfated derivatives were prepared by ion-exchange chromatography on cationite Dowex 50WX4 (Na^+-form). Target products were obtained as white powders after freeze-drying. Treatment of **4** led to 14.6 mg (41%) monosulfate **6** and 4.6 mg (12%) disulfate **7**. Treatment of **5** led to 31.1 mg (51%) **6** and 12.7 mg (20%) **7**. Treatment of combined waste after deprotection allowed isolating of 47 mg (30%) of the nonsulfated SiaLe^x.

A solution of 300 mg **1** (0.18 mmol) in 15 mL MeOH was subjected to hydrogenolysis over 300 mg 10% Pd/C for 2 h. After filtration, the solution was evaporated, co-evaporated with MeCN, and dried. The residue was dissolved in 10 mL dry MeCN followed by the addition of 60 μL dimethoxypropane and 5 mg toluenesulfonic acid. In 1 h, the reaction mixture was neutralized with 100 μL Py, evaporated, co-evaporated with toluene (3 × 25 mL). Chromatography on silica gel (elution with the mixture $CHCl_3$:MeOH:Py 80:8:0.5) led to isolation of 214 mg (89%) diol **9**. Sulfation, deprotection, and isolation of reaction products were carried out as described above with the exception that double amount of reagents was taken for sulfation, reaction was carried on for 1.5 h and deacetylation with 6 mL 0.1 N MeONa/MeOH for 30 min followed by evaporation of reaction mixture was performed instead of hydrogenolysis. The total yields were 48 mg (32%) of **10**, 40.5 mg (23%) of **11**, 4.5 mg (3%) of **12**, and 10.8 mg (8%) of SiaLe^x.

A mixture of equimolar amounts (0.01 mmol) of NEt_3, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), and 4-pentynoic acid in 200 μL DMSO was added to a solution of 6 mg (0.006 mmol) of sulfate **6** in 300 μL DMSO. In 1.5 h, the reaction mixture was purified on Sephadex LH-20 (elution with MeCN:H_2O, 1:1) and DEAE-Sephadex A-25 (OAc^--form) (the product is eluted with 1 M Py·AcOH). Na^+-salts were prepared using cationite Dowex 50WX4 (Na^+-form) after evaporation and lyophilization. The yield was 3.0 mg (46%) **13** and 1.5 mg of starting **6**. TLC data: R_f **13** 0.22 (i-PrOH:EtAc:H_2O 4:3:2); 0.71; R_f **6** 0.0 (i-PrOH:MeCN:H_2O, 4:3:2).

^{1}H NMR spectra of the obtained compounds are given below. Spectra were recorded in D_2O on spectrometer Varian 600 MHz at 30°C. Chemical shift values (δ, ppm) are given with the use of HOD (δ = 4.750) as a reference; constants of the spin–spin interaction are given in Hz.

$6^{(Gal)}$-*O*-Su-SiaLe^x-O$(CH_2)_3NH_2$ **6**, 1.138 (d, 3H, $J_{5,6}$ 6.6, H-6 Fuc); 1.763 (dd, 1H, $J_{3e,3a}$ 12.4, $J_{3a,4}$ 11.8, H-3a Neu); 1.914 (m, 2H, CH_2 sp); 2.002 (s, 2 × 3H, NCOMe); 2.737 (dd, 1H, $J_{3e,4}$ 4.7, $J_{3a,3e}$ 12.4, H-3e Neu); 3.057 (m, 2H, NCH_2 sp); 3.515

(dd, 1H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.7 H-2 Gal); 3.55–4.03 (m, 20H); 4.04–4.09 (m, 2H, H-3, H-6″ Gal); 4.120 (dd, 1H, $J_{5,6'}$ 4.5, $J_{6',6''}$ 10.5, H-6′ Gal); 4.489 (d, 1H, $J_{1,2}$ 8.3, H-1 GlcNAc); 4.512 (d, 1H, $J_{1,2}$ 7.9, H-1 Gal); 4.754 (br. q, 1H, $J_{5,6}$ 6.6, $J_{4,5} \leq 1$, H-5 Fuc); 5.077 (d, 1H, $J_{1,2}$ 3.9, H-1 Fuc).

$6^{(Gal)}$-*O*-Su, $4^{(Neu)}$-*O*-Su-SiaLe^x-$O(CH_2)_3NH_2$ **7**, ^{1}H NMR, δ: 1.214 (d, 3H, $J_{5,6}$ 6.6, H-6 Fuc); 1.995 (m, 3H, H-3a Neu, CH_2 sp); 2.057and 2.077 (2s, 2 × 3H, NCOMe); 3.071 (dd, 1H, $J_{3e,4}$ 5.0, $J_{3e,3a}$ 12.4, H-3e Neu); 3.137 (m, 2H, NCH_2 sp); 3.595 (dd, 1H, $J_{2,1}$ 7.9, $J_{2,3}$ 9.7, H-2 Gal), 3.64–4.10 (m, 19H), 4.140 (dd, 1H, $J_{5,6''}$ 7.9, $J_{6',6''}$ 10.5, H-6″ Gal); 4.160 (dd, 1H, $J_{3,4}$ 3.1, $J_{2,3}$ 9.7, H-3 Gal); 4.206 (dd, 1H, $J_{5,6'}$ 4.3, $J_{6',6''}$ 10.5, H-6′ Gal); 4.423 (ddd, 1H, $J_{4,3e}$ 5.0, $J_{4,3a}$ 11.5, $J_{4,5}$ 10.2, H-4 Neu); 4.570 (d, 1H, $J_{1,2}$ 8.3, H-1 GlcNAc); 4.591 (d, 1H, $J_{1,2}$ 7.9, H-1 Gal); 4.826 (br. q, 1H, $J_{5,6}$ 6.6, $J_{4,5} \leq 1$, H-5 Fuc); 5.153 (d, 1H, $J_{1,2}$ 3.9, H-1 Fuc).

$6^{(GlcNAc)}$-*O*-Su-SiaLe^x-$O(CH_2)_3NH_2$ **10**, ^{1}H NMR, δ: 1.214 (d, 3H, $J_{5,6}$ 6.6, H-6 Fuc); 1.838 (dd, 1H, $J_{3a,4}$ 12.1, $J_{3a,3e}$ 12.3, H-3a Neu); 1.994 (m, 2H, CH_2 sp); 2.075 (s, 2 × 3H, NCOMe), 2.795 (dd, 1H, $J_{3e,4}$ 4.6, $J_{3e,3a}$ 12.3, H-3e Neu); 3.145 (m, 2H, NCH_2 sp); 3.555 (dd, 1H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.7, H-2 Gal); 3.62–4.09 (m, 22H); 4.138 (dd, 1H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.3, H-3 Gal); 4.430 (m ≈ d, 2H, H-6′, H-6″ GlcNAc); 4.615 (d, 1H, $J_{1,2}$ 8.3, H-1 GlcNAc); 4.644 (d, 1H, $J_{1,2}$ 7.9, H-1 Gal); 4.833 (br. q. 1H, $J_{5,6}$ 6.6, $J_{5,4} \leq 1$, H-5 Fuc); 5.155 (d, 1H, $J_{1,2}$ 4.0, H-1 Fuc).

$6^{(GlcNAc)}$-*O*-Su, $2^{(Fuc)}$-*O*-Su-SiaLe^x-$O(CH_2)_3NH_2$ **11**, ^{1}H NMR, δ: 1.214 (d, 3H, $J_{5,6}$ 6.5, H-6 Fuc); 1.816 (dd, 1H, $J_{3e,3a}$ 12.4, $J_{3a,4}$ 12.0, H-3a Neu); 1.982 (m, 2H, CH_2 sp); 2.040 and 2.053 (2s, 2 × 3H, NCOMe); 2.775 (dd, 1H, $J_{3a,3e}$ 12.4, $J_{3e,4}$ 4.8, H-3e Neu); 3,151 (m, 2H, NCH_2 sp); 3.516 (dd, 1H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.7, H-2 Gal); 3.60–4.11 (m, 19H); 4.116 (dd, 1H, $J_{2,3}$ 9.7, $J_{3,4}$ 3.4, H-3 Gal); 4.402 (m, 2H, H-6′ and H-6″ GlcNAc); 4.425 (dd, 1H, $J_{2,3}$ 10.3, $J_{1,2}$ 3.8, H-2 Fuc); 4.631 (d, 1H, $J_{1,2}$ 7.9, H-1 Gal); 4.692 (d, 1H, $J_{1,2}$ 8.3, H-1 GlcNAc); 4.848 (br. q., 1H, $J_{5,6}$ 6.5, $J_{4,5} \leq 1$, H-5 Fuc); 5,459 (d, 1H, $J_{1,2}$ 3.8, H-1 Fuc).

$2^{(Fuc)}$-*O*-Su-SiaLe^x-$O(CH_2)_3NH_2$ **12**, ^{1}H NMR, δ: 1.233 (d, 3H, $J_{5,6}$ 6.6, H-6 Fuc); 1.828 (dd ≈ t, 1H, J 12.2, H-3a Neu); 1.982 (m, 2H, CH_2 sp); 2.064 and 2.075 (2s, 2 × 3H, NCOMe); 2.812 (dd, 1H, $J_{3a,3e}$ 12.4, $J_{3e,4}$ 4.6, H-3e Neu); 3.129 (m, 2H, NCH_2 sp); 3.551 (dd, 1H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.9, H-2 Gal); 3.60–4.14 (m, 22H); 4.441 (dd, 1H, $J_{2,3}$ 10.3, $J_{1,2}$ 3.6 H-2 Fuc); 4.570 (d, 1H, $J_{1,2}$ 7.7, H-1 Gal); 4.637 (d, 1H, $J_{1,2}$ 8.3, H-1 GlcNAc); 4.886 (br. q., 1H, $J_{5,6}$ 6.6, $J_{4,5} \leq 1$, H-5 Fuc); 5.459 (d, 1H, $J_{1,2}$ 3.7, H-1 Fuc).

$6^{(Gal)}$-*O*-Su-SiaLe^x-$OCH_2CH_2CH_2NH\text{-}COCH_2CH_2C{\equiv}CH$ **13**, ^{1}H NMR, δ: 1.138 (d, 3H, $J_{5,6}$ 6.5, H-6 Fuc); 1.743 (m, 2H, CH_2 sp); 1.763 (dd, 1H, $J_{3e,3a}$ 12.4, $J_{3a,4}$ 11.8, H-3a Neu); 2.002 (s, 2 × 3H, NCOMe); 3.352 (m ≈ t, 1H, $C{\equiv}CH$); 2.401 (t, 2H, CH_2COOH); 2.463 (m, 2H, $C\underline{H_2}\text{-}C{\equiv}CH$); 2.737 (dd, 1H, $J_{3e,4}$ 4.5, $J_{3a,3e}$ 12.4, H-3e Neu); 3.163 and 3.249 (2 m, 2 × H, NCH_2 sp); 3.508 (dd, 1H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.7, H-2 Gal); 3,55–4.03 (m, 20H); 4.04–4.09 (m, 2H, H-3, H-6″ Gal); 4.108 (dd, 1H, $J_{5,6'}$ 4.5, $J_{6',6''}$ 10.5, H-6′ Gal); 4.487 (d, 1H, $J_{1,2}$ 8.3, H-1 GlcNAc); 4.508 (d, 1H, $J_{1,2}$ 7.9, H-1 Gal); 4.781 (br. q, 1H, $J_{5,6}$ 6.5, $J_{4,5} \leq 1$, H-5 Fuc); 5.063 (d, 1H, $J_{1,2}$ 3.8, H-1 Fuc).

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Conflict of interest statement

None declared.

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