Supporting Information
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Supporting information for:

Regioselective deacetylation and glycosylation in the synthesis of the sialyl Lewis X tetrasaccharide, a key component of the recognition site of PSGL-1.

General Methods

Solvents for moisture-sensitive reactions (MeCN, THF, MeOH, CH$_2$Cl$_2$) were distilled and dried by standard procedures. Anhydrous DMF, acetic anhydride and pyridine were purchased from Acros. Reactions were monitored by thin-layer chromatography with pre-coated silica gel 60 F$_{254}$ aluminium plates (Merek KGaA, Darmstadt). Flash chromatography was performed on silica gel purchased from Fluka (0.040-0.063 mm) and Acros (0.030-0.075 mm). Optical rotations [α]$_{24}^{24}$D were measured with Perkin-Elmer 241 polarimeter. RP-HPLC analyses were performed on a Knauer HPLC system with Luna C18 column (250 x 4.6 mm, 5 µm) and pump rate 1 mL/min. Semi-preparative HPLC separations were performed on a Knauer HPLC system with Luna C18(2) (250 x 21.20 mm, 10 µm) and pump rate 10 mL/min. For both systems a gradient of H$_2$O in MeCN was used. $^1$H, $^{13}$C and 2D NMR experiments were recorded on Bruker AM 400 and ARX 400 spectrometers. Proton shifts are reported in ppm relative to residual CHCl$_3$ (δ=7.26) and methanol (δ=3.31). Multiplicities are given as: s (singlet), d (dublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet). $^{13}$C chemical shifts are reported relative to residual CHCl$_3$ (δ=77.00) and methanol (δ=49.00). Assignments of proton and carbon signals was based on COSY, TOCSY, HSQC and HMBC experiments. For $^1$H and $^{13}$C signals of the saccharide portions the following units were used: N-acetyl-d-glucosamine (GlcNAc), d-galactose (Gal), L-fucose (Fuc), and N-acetyl-neuraminic acid (Sia). ESI-mass spectra were recorded on a ThermoQuest-Navigator spectrometer, whereas HR-ESI mass spectra were obtained on a Micromass Q-TOF Ultima spectrometer.
Synthesis of tert-butyldiphenylsilyl 2-acetamido-3-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-4-O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (12)

A solution of anhydrous 7\(^1\) (4.28 g, 7.91 mmol) and 11 (3.81 g, 4.38 mmol) in CH\(_2\)Cl\(_2\) (120 mL) was stirred with activated 4 Å MS (4.70 g) at RT under an argon atmosphere for 1 h. Afterwards the mixture was cooled to –50 °C and a solution of TMSOTf in CH\(_2\)Cl\(_2\) (0.19 mL in approx. 5 mL CH\(_2\)Cl\(_2\), 1.03 mmol, 0.13 eq based on trichloroacetimidate) was added dropwise during 30 min. The reaction mixture was held at this temp. for 30 min, and afterwards was allowed to slowly attain RT, and stirred at this temperature overnight. The reaction was then neutralised with solid NaHCO\(_3\) to pH 7 at RT. Molecular sieves were filtered-off, washed with CH\(_2\)Cl\(_2\), organic phase was washed with NaHCO\(_3\) sat., water, then the combined water phases were extracted twice with CH\(_2\)Cl\(_2\), and the combined organic phases were dried over MgSO\(_4\) and concentrated. Flash chromatography of the crude product on silica gel with the gradient of C\(_6\)H:E 3:1 → 3:4 gave 4.42 g of 12 (81% yield) as a white amorphous material with \(R_f=0.14\) in C\(_6\)H:E 2:1.

11: \([\alpha]_{24D}^\circ = -38\) (c=1.00, CHCl\(_3\)); \(^1\)H NMR (CDCl\(_3\)): 7.72-7.63 (4H, m, H\(_{ar}\)), 7.32-7.22 (16H, m, H\(_{ar}\)), 5.53 (1H, d, GlcNHAc, \(J_{NH,2}=7.4\) Hz), 5.32-5.29 (1H, dd, H3-Fuc, \(J_{3,4}=3.3\) Hz), 5.28-5.26 (1H, dd, H4-Fuc, \(J_{4,5}=1.1\) Hz), 5.04-5.03 (1H, d, H1-Fuc, \(J_{1,2}=3.7\) Hz), 4.98-4.96 (1H, d, H1-GlcNHAc, \(J_{1,2}=7.9\) Hz), 4.65-4.57 (2H, dd, CH\(_2\)-Bn, J=12 Hz), 4.51-4.41 (2H, dd, CH\(_2\)-Bn, J=12.2 Hz), 4.38-4.34 (1H, m, H5-Fuc), 4.00-3.96 (1H, d, H3-GlcNHAc, \(J_{2,3}=8.5\) Hz, \(J_{3,4}=10.4\) Hz), 3.87-3.82 (1H, dd, H2-Fuc, \(J_{2,3}=10.4\) Hz), 3.81 (1H, bs, HO-GlcNHAc), 3.60-3.56 (2H, m, H6a,b-GlcNHAc, \(J_{6a,6b}=4.4\) Hz), 3.55-3.50 (1H, dd, H4-GlcNHAc, \(J_{4,5}=9.3\) Hz), 3.41-3.34 (1H, ddd, H2-GlcNHAc), 3.18-3.14 (1H, ddd, H5-GlcNHAc, \(J_{5,6b}=7.6\) Hz, \(J_{4,5}=9.6\) Hz), 2.11, 1.98, 1.55.
(9H, 3 x s, CH$_3$-Ac), 1.11-1.10 (1H, d, Me(6)-Fuc, J$_{5,6}$=6.5 Hz), 1.06 (9H, s, CH$_3$-tBu); $^{13}$C NMR (100.6 MHz, CDCl$_3$): 170.37, 170.34, 169.94 (C=O), 138.34, 137.69 (C$_{\text{ipso-Ph}}$), 135.91, 135.77 (C$_{\text{0,m,p-Ph}}$), 133.18, 133.00 (C$_{\text{ipso-Ph}}$), 129.64, 129.56, 128.52, 128.20, 128.02, 127.94, 127.67, 127.51, 127.36, 127.26 (C$_{\text{0,m,p-Ph}}$), 98.48 (C1-Fuc), 94.80 (C1-GlcNAc), 83.09 (C3-GlcNAc), 74.41 (C5-GlcNAc), 73.78 (C2-Fuc), 73.71 (CH$_2$-Bn), 73.47 (CH$_2$-Bn), 71.29 (C4-Fuc), 70.73 (C4-GlcNAc), 69.87 (C3-Fuc), 69.34 (C6-GlcNAc), 65.73 (C5-Fuc), 58.45 (C2-GlcNAc), 26.70 (CH$_3$-tBu), 23.13, 20.78, 20.64 (CH$_3$-Ac), 15.85 (Me(6)-Fuc); MS (ESI): m/z: found 892.41 [M + Na]$^+$; C$_{48}$H$_{59}$NO$_{12}$SiNa caled 892.37; HR-MS (ESI-TOF): m/z: found 870.3898 [M + H]$^+$; C$_{48}$H$_{60}$NO$_{12}$Si caled 870.3885.

12: [a]$_{24D}$ = −42 (c=1.00, CHCl$_3$); $^1$H NMR (CDCl$_3$): 7.69-7.67 (2H, d, H$_{ar}$), 7.60-7.58 (2H, d, H$_{ar}$), 7.37-7.28 & 7.22-7.20 (21H, m, H$_{ar}$), 5.46-5.45 (1H, m, H4-Gal, J$_{3,4}$=3.5 Hz), 5.35 (1H, d, GlcN/HAc, J$_{\text{NH,2}}$=7.6 Hz), 5.29-5.25 (2H, m, H4-Fuc, H3-Fuc), 5.11-5.10 (1H, d, H1-Fuc, J$_{1,2}$=3.7 Hz), 5.07 (1H, d, H1-GlcNAc, J$_{1,2}$=7.3 Hz), 4.99-4.95 (1H, dd, H2-Gal, J$_{2,3}$=8.2 Hz), 4.93-4.91 (1H, m, H5-Fuc), 4.89-4.85 (1H, dd, H3-Gal), 4.78 (1H, d, CH$_2$-Bn, J=12.4 Hz), 4.67 (1H, d, H1-Gal, J$_{1,2}$=8.0 Hz), 4.63-4.30 (5H, m, CH$_2$-Bn, {4.62, 4.59, 4.56, 4.52, 4.32, 5 x d, J=11.8 Hz, J=12 Hz}), 4.26-4.23 (1H, m, H3-GlcNAc, J$_{3,4}$=9.0 Hz, J$_{2,3}$=8.4 Hz), 4.01 (1H, t*, H4-GlcNAc, J$_{3,4}$=J$_{4,5}$=9.0 Hz), 3.90 (1H, dd, H2-Fuc, J$_{1,2}$=3.7 Hz, J$_{2,3}$=9.9 Hz), 3.82-3.78 (1H, m, H6a-Gal), 3.73-3.69 (1H, m, H6b-Gal, J$_{6a,6b}$=9.5 Hz), 3.65-3.64 (1H, m, H5-Gal), 3.62-3.55 (2H, m, H6a-GlcNAc, H2-GlcNAc), 3.35-3.32 (1H, m, H6b-GlcNAc), 3.07-3.05 (1H, m, H5-GlcNAc), 2.13, 2.06, 1.96, 1.88, 1.62 (18H, 6 x s, CH$_3$-Ac), 1.12 (3H, d, Me(6)-Fuc, J$_{5,6}$=6.55 Hz), 1.04 (9H, s, CH$_3$-tBu); $^{13}$C NMR (100.6 MHz, CDCl$_3$): 170.76, 170.29, 169.97, 169.92, 169.48, 169.01 (C=O), 138.66, 138.17, 137.79 (C$_{\text{ipso-Ph}}$), 135.84, 135.71 (C$_{\text{0,m,p-Ph}}$), 133.35, 132.92 (C$_{\text{ipso-Ph}}$), 129.72, 129.60, 128.52, 128.45, 128.29, 128.00, 127.89, 127.78, 127.61, 127.54, 127.45, 127.29, 127.25 (C$_{\text{0,m,p-Ph}}$), 99.38 (C1-Gal), 96.98 (C1-Fuc), 94.04 (C1-GlcNAc), 74.49 (C5-GlcNAc), 74.17 (C2-Fuc), 73.71 (C4-GlcNAc), 73.67 (C3-GlcNAc), 73.55 (CH$_2$-Bn), 73.29 (CH$_2$-Bn), 72.91 (CH$_2$-Bn), 72.31 (C5-Gal), 72.03 (C4-Fuc), 71.12 (C3-Gal), 70.30 (C3-Fuc), 69.27 (C2-Gal), 67.40 (C4-Gal), 67.22 (C6-GlcNAc), 66.98 (C6-Gal, C2-GlcNAc), 64.03 (C5-Fuc), 26.73 (CH$_3$-tBu), 23.29, 20.88, 20.67, 20.61, 19.12 (CH$_3$-Ac), 15.71 (Me(6)-Fuc); MS (ESI): m/z: found 1248.54 [M + H]$^+$;
C₆₇H₈₂NO₂₀Si calcd 1248.52; HR-MS (ESI-TOF): m/z: found 1248.5197 [M + H]⁺; C₆₇H₈₂NO₂₀Si calcd 1248.5199.

**Synthesis of tert-butyldiphenylsilyl 2-acetamido-3-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-4-O-(6-O-benzyl-3-O-{methyl (5-acetamido-4,7,8,9-tetra-O-3,5-dideoxy-D-glycero-α-D-galacto-non-2- ulopyranosyl)onate} -β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (14)**

Compound 13 (1.78 g, 1.59 mmol) and 6² (2.19 g, 2.32 mmol) were dried under vacuum before dissolving in MeCN/CH₂Cl₂ (105 mL/27 mL) over activated 4 Å MS and were stirred at RT for 30 min. The reaction mixture was then cooled to – 50 °C and then AgOTf in MeCN (4 mL) was added dropwise at this temp. After 30 min, a solution of Me-S-Br³ in 1,2-dichloroethane (2.31 mL of 1.6 M, prepared from: 5.97 mmol Me₂S₂ and 5.97 mmol Br₂ in 7.5 mL dry 1,2-dichloroethane, 1-day old reagent was used for the reaction ) was added dropwise over 10 min at – 50 °C and the reaction was stirred at this temp for 2 h. Afterwhich the reaction was stirred overnight at – 28 °C (19 h in total) and then quenched at this temp. with 1.2 mL DIPEA, diluted with CH₂Cl₂, filtered through Hyflo and concentrated. The crude product was chromatographed on silica gel with CH₂Cl₂:MeOH 100:1 to give 1.92 g of 14 as slightly yellow amorphous material (76% yield) (TLC, Rₜ=0.16-0.2 in CH₂Cl₂:MeOH 50:1). For analytical purposes a small amount of 14 was purified on semi-preparative HPLC, using a gradient of H₂O in MeCN (80-100% MeCN in 80 min (0% TFA), Rₜ=19-25 min; analytical HPLC: 75-100% MeCN in 40 min (0% TFA), Rₜ=21 min).
14: [α]24D= − 17.1 (c=2.94, CHCl₃); ¹H NMR (CDCl₃): 7.72-7.60 (4H, m, H₉ar), 7.36-7.28 (21H, m, H₂ar), 5.55 (1H, d, GlcNHAc, J₁₉H₂=8 Hz), 5.48-5.44 (1H, m, H8-Sia), 5.35-5.32 (1H, dd, H7-Sia, J₆,₇=1.6 Hz, J₇,₈=8.8 Hz), 5.31-5.28 (2H, m, NH-Sia {5.30}, H1-Fuc {5.29, d, J₁₂=3.6 Hz}), 5.27-5.25 (2H, m, H3-Fuc, H4-Fuc), 4.96-4.89 (1H, m, H4-Sia, J₄,₅=10.4 Hz), 4.77-4.64 (2H, m, H5-Fuc {4.73}, H1-Gal {4.66, d, J₁₂=7.6 Hz}), 4.63-4.42 (6H, m, 3 x CH₂-Bn), 4.22-4.02 (7H, m, H1-GlcNAc {4.20}, H4-GlcNAc {4.19}, H9a-Sia {4.19}, H6-Sia {4.13}, H3-GlcNAc {4.10}, H9b-Sia {4.06}, H5-Sia {4.04}), 3.96-3.93 (1H, dd, H3-Gal, J₂₃=9.6 Hz, J₃,₄=3.2 Hz), 3.90-3.87 (1H, dd, H2-Fuc, J₁₂=3.6 Hz, J₃,₄=10.4 Hz), 3.84-3.73 (6H, m, H6a-Gal {3.83}, H6a-GlcNAc {3.79}, COome-Sia {3.76}, H6b-Gal {3.74}), 3.69 (1H, d, H4-Gal, J₃,₄=2.8 Hz), 3.67-3.61 (1H, m, H2-GlcNAc), 3.57 (1H, t*, H2-Gal, J₁₂=J₂₃=7.6 Hz), 3.56 (1H, t*, H5-Gal, J₄₅=J₅,₆=7.2 Hz), 3.46-3.43 (1H, m, H6b-GlcNAc), 3.18-3.15 (1H, m, H5-GlcNAc), 2.72 (1H, dd, H3eq-Sia, J₃eq,₃ax=12.8 Hz, J₃eq,₄=4.4 Hz), 2.65-2.40 (2H, bs, 2 x HO-Gal), 2.13-2.06 (10H, m, CH₃-Ac, 3 x s {2.12, 2.11, 2.07}, H₃ax-Sia {2.07}), 2.05, 1.98, 1.95, 1.91, 1.67 (15H, 5 x s, CH₃-Ac), 1.05-1.03 (12H, m, Me(6)-Fuc, CH₃-βBu); ¹³C NMR (100.6 MHz, CDCl₃): 170.87, 170.56, 170.48, 170.29, 170.10, 169.88, 169.83, 169.55 (C=O), 168.07 (C1-Sia), 138.64, 138.40, 138.16 (Cipso-Ph), 135.86, 135.75 (Cₒ,m,p-Ph), 133.40, 132.98 (Cipso-Ph), 129.62, 129.50, 128.53, 128.26, 128.18, 128.06, 128.00, 127.74, 127.58, 127.50, 127.37, 127.20, 127.07 (Cₒ,m,p-Ph), 100.98 (C1-Gal), 97.61 (C2-Sia), 96.91 (C1-Fuc, C1-GlcNAc), 77.50 (C3-Gal), 75.41 (C3-GlcNAc), 74.61 (C2-Fuc, C5-GlcNAc), 73.19 (C5-Gal, 2 x CH₂-Bn), 72.74 (C4-GlcNAc, CH₂-Bn), 72.61 (C6-Sia), 72.02 (C4-Fuc), 70.50 (C3-Fuc), 70.03 (C2-Gal), 68.50 (C4-Sia), 68.07 (C6-Gal), 67.78 (C₈-Sia, C₆-GlcNAc), 67.46 (C4-Gal), 66.74 (C7-Sia), 64.58 (C5-Fuc), 62.10 (C9-Sia), 60.37 (C2-GlcNAc), 53.02 (COOME-Sia), 49.43 (C5-Sia), 37.78 (C3-Sia), 26.70 (CH₁₋₇Bu), 23.26, 23.13, 21.02, 20.90, 20.79, 20.71, 20.60, 19.05 (CH₃-Ac), 15.87 (Me(6)-Fuc); MS (ESI): m/z: found 1617.5 [M + Na]+; C₈₁H₁₀₂N₂O₂₇SiNa calcd 1617.6; HR-MS (ESI-TOF): m/z: found 1617.6224 [M + Na]+; C₈₁H₁₀₂N₂O₂₇SiNa calcd 1617.6235.

Synthesis of tert-butyldiphenylsilyl 2-acetamido-3-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-4-O-(2,4-di-O-acetyl-6-O-benzyl-3-O-methyl (5-acetamido-4,7,8,9-tetra-O-3,5-dideoxy-d-glycero-α-d-
galacto-non-2-ulopyranosyl)onate]-β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (15)

Compound 14 (1.82 g, 1.14 mmol) and DMAP (0.13 g, 1.03 mmol) were dissolved in a AcO/pyridine mixture (15.4 mL/31 mL) at RT, and the reaction mixture was stirred overnight under an argon atmosphere (22 h). The mixture was evaporated under high vacuum, co-evaporated three times with toluene, and the resultant residue chromatographed on silica gel with CH2Cl2:MeOH 100:1 to give 1.72 g (90% yield) of 15 as a pale yellow amorphous material with Rf=0.21-0.27 in CH2Cl2:MeOH 50:1.

15: [α]D = -32.2 (c=1.00, CHCl3); 1H NMR (CDCl3): 7.68-7.57 (4H, m, H8-Sia), 7.38-7.21 (21H, m, H6-Sia), 5.56-5.52 (1H, m, H8-Sia), 5.39-5.35 (2H, m, H7-Sia, J=5.38, dd, J=2.5 Hz, J=13.6 Hz), 5.10-5.07 (2H, m, NH-Sia, J=5.09), 4.92-4.80 (4H, m, H4-Sia, J=4.90), H5-Fuc (4.88), H2-Gal (4.85), H1-Gal (4.82, d, J=13.6 Hz), 4.74-4.33 (7H, m, 3 x CH2-Bn, 3 x CH2-Gal), 4.24 (1H, d, H1-GlcNAc, J=7.8 Hz), 4.20 (1H, dd, H9a-Sia, J=2.7 Hz, J=13.6 Hz), 4.07-3.98 (4H, m, H4-GlcNAc, J=4.05), H9b-Sia (4.04), H5-Sia (4.03), H3-GlcNAc (4.02), 3.87 (1H, dd, H2-Fuc, J=10.2 Hz, J=3.8 Hz), 3.84 (3H, s, COOMe-Sia), 3.78-3.72 (2H, m, H5-Gal, J=3.76), H6a-Gal (3.74), 3.68-3.62 (2H, m, H6a-GlcNAc, J=3.65), H6b-Gal (3.63, t*, J=8.7 Hz), 3.61-3.57 (2H, m, H6-Sia, J=10.7 Hz, J=2.6 Hz), H2-GlcNAc (3.59), 3.49 (1H, dd, H6b-GlcNAc, J=2.2 Hz, J=11.1 Hz), 3.18-3.14 (1H, m, H5-GlcNAc), 2.55 (1H, dd, H3eq-Sia, J=12.6 Hz, J=4.6 Hz), 2.10, 2.08, 2.07, 2.06, 2.04, 1.99, 1.93, 1.84 (27H, 8 x s, CH3-Ac), 1.69 (1H, t*, H3ax-Sia, J=12.5 Hz), 1.64 (3H, s, CH3-Ac), 1.12 (3H, d, Me(6)-Fuc, J=6.5 Hz), 1.02 (9H, s, CH3-Ph); 13C NMR (100.6 MHz, CDCl3): 170.96, 170.85, 170.71, 170.52, 170.38, 170.27, 170.23, 169.86, 169.48, 169.37 (C=O), 167.70 (C1-Sia), 138.64, 138.62, 138.59 (Cipso-Ph),
135.86, 135.74 (C-o,m,p-Ph), 133.37, 133.07 (C-ipso-Ph), 129.66, 129.55, 128.45, 128.13, 128.09, 127.80, 127.71, 127.62, 127.51, 127.44, 127.37, 127.29, 127.10 (C-o,m,p-Ph), 99.18 (C1-Gal), 96.91 (C2-Sia), 96.83 (C1-Fuc, C1-GlcNAc), 74.75 (C5-GlcNAc), 74.02 (C2-Fuc), 73.33 (C4-GlcNAc), 73.27 (C3-GlcNAc), 73.13 (CH2-Bn), 72.78 (2 x CH2-Bn), 72.29 (C5-Gal), 72.11 (C4-Fuc), 71.93 (C6-Sia), 71.83 (C3-Gal), 71.29 (C2-GlcNAc), 70.51 (C2-Gal), 70.34 (C3-Fuc), 69.45 (C4-Sia), 67.91 (C6-GlcNAc), 67.84 (C4-Gal), 67.41 (C6-Gal), 67.17 (C8-Sia), 66.94 (C7-Sia), 64.10 (C5-Fuc), 62.08 (C9-Sia), 53.15 (COO-Me-Sia), 49.07 (C5-Sia), 37.59 (C3-Sia), 26.75 (CH3-tBu), 23.34, 23.16, 21.29, 20.86, 20.82, 20.75, 20.72, 20.66, 20.61, 19.11 (CH3-Ac), 15.82 (Me(6)-Fuc); MS (ESI): m/z: found 1701.66 [M + Na]+; C85H106N2O31SiNa calcd 1701.64; HR-MS (ESI-TOF): m/z: found 1701.6420 [M + Na]+; C85H106N2O31SiNa calcd 1701.6447.

**Notes and references**