Synthesis and NMR analysis of Type 1 Lewis b hexasaccharide antigen structures featuring flexible incorporation of L-[U-$^{13}C_6$]-fucose for NMR binding studies

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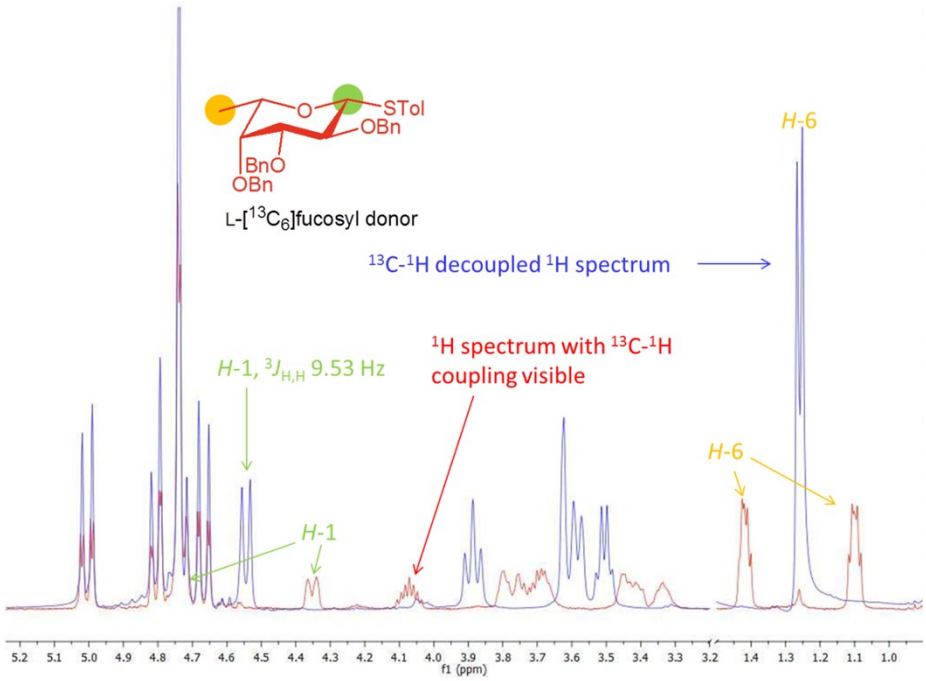

Supplementary Figure 1. Overlay of the ^{1}H NMR spectrum of L-[U-$^{13}C_6$]-fucosyl donor **4** (red), the ^{13}C-^{1}H-decoupled ^{1}H NMR spectrum (blue).

Experimental

General experimental

Solvents were supplied by Fisher Scientific. L-[U-$^{13}C_6$]Galactose was supplied by Omicron Biochemicals. All other reagents were purchased from Alfa Aesar, Fisher Scientific and Sigma Aldrich. Silica gel column chromatography was carried out using Fluorochem 6A silica gel, LiChroprep® RP-C18 (40 – 63 µm), and BIO-RAD Bio-Gel® P-2 Gel (45 – 90 µm). TLC was performed using Merck silica gel 60F_{254} glass plates and visualised under UV, sulfuric acid

($H_2SO_{4(aq)}$ 10%), ninhydrin (0.3 g in n-butanol (100 ml) an AcOH (3 ml)), iodine (absorbed onto silica powder), and Dragendorff's reagent (A. bismuth nitrate (0.17 g) dissolved in H_2O/AcOH (4:1, 10 ml) B. KI (4 g) dissolved in H_2O/AcOH (2:1, 30 ml). Solutions A and B mixed together and diluted to 100 ml with H_2O). NMR experiments were run on Bruker 400 MHz and 500 MHz spectrometers. Spectra were calibrated using TMS, $CDCl_3$, CD_3OD and D_2O as internal standards. The ^1H NMR data is reported as for the ^1H-^{13}C decoupled experiment The ^{13}C data for compound **4** and **1** is reported as ^{13}C-^{13}C coupled ^1H-^{13}C decoupled data, while compound **2** is reported for the decoupled-decoupled experiment. Mass spectrometry was carried out on a Bruker MICRO-TOF and MALDI-TOF mass spectrometers. MALDI-TOF was run using 2',4',6'-trihydroxyacetophenone (THAP) in acetonitrile/water (MeCN/H_2O 1:1) as a matrix.

1,2,3,4,6-penta-*O*-acetyl-α/β-L-[$^{13}C_6$]-galactopyranose (9): A suspension of NaOAc (0.16 g, 1.9 mmol) in Ac_2O (1.95 mL, 19.5 mmol) was stirred at 140 °C. L-[U-$^{13}C_6$]-Galactose (349 mg, 1.92 mmol) was added in small portions over a period of 6 minutes and the reaction was refluxed for a further 25 minutes after the final addition. The reaction mixture was poured onto crushed ice (50 mL) and stirred for 2 h. EtOAc (50 mL) was added to the mixture and the organic layer was washed with H_2O (2x 30 mL), sat. aq. $NaHCO_3$ (30 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was purified by flash chromatography on silica gel (toluene/EtOAc, 6:1→1:1) to give **9** as a white solid. (757 mg, 99%). R_f = 0.40 (toluene/EtOAc, 1:1); ^1H NMR (500 MHz, $CDCl_3$) δ 5.72 (d, *J* = 8.5 Hz, 1H, H-1), 5.44 (d, *J* = 3.4 Hz, 1H, H-4), 5.34 (t, *J* = 8.2 Hz, 1H, H-2), 5.09 (dd, *J* = 10.7 Hz, 1H, H-3), 4.22 - 4.12 (m, 2H, H-$6_{(A+B)}$), 4.07 (t, *J* = 6.4 Hz, 1H, H-5), 2.18 (s, 3H, C*H*$_3$C=O), 2.13 (s, 3H, C*H*$_3$C=O), 2.06 (s, 6H, 2x C*H*$_3$C=O), 2.01 (s, 3H, C*H*$_3$C=O). Data in agreement with the unlabelled enantiomer.

***p*-Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-L-[$^{13}C_6$]-galactopyranoside (10):** Compound **9** (757 mg, 1.91 mmol) was placed under N_2 and dissolved in dry CH_2Cl_2 (6 mL). *p*-Thiocresol (356 mg, 2.87 mmol) and $BF_3 \cdot Et_2O$ (0.57 mL, 3.4 mmol) were added and the reaction was stirred at room temperature for 2 hours. After completion, the solution was cooled to 0 °C and quenched with Et_3N (0.35 mL). The reaction mixture was then diluted with CH_2Cl_2 (20 mL) and washed with brine (2x 20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by flash chromatography on silica gel (toluene→toluene/EtOAc, 3:1) to give **10** as a white solid (880 mg, 98%). R_f = 0.58 (toluene/EtOAc 3:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, *J* = 7.9 Hz, 2H, Ar-*H*), 7.12 (d, *J* = 7.9 Hz, 2H, Ar-*H*), 5.41 (d, *J* = 2.5 Hz, 1H, H-4), 5.20 (t, *J* = 10.1 Hz, 1H, H-2), 5.03 (dd, *J* = 9.8, 3.0 Hz, H-3), 4.63 (d, *J* = 9.8 Hz, 1H, H-1), 4.09 – 4.20 (m, 2H, H-$6_{(A+B)}$), 3.90 (t, *J* = 6.3 Hz, 1H, H-5), 2.34 (s, 3H, PhCH_3), 2.11 (s, 3H, CH_3C=O), 2.09 (s, 3H, CH_3C=O), 2.04 (s, 3H, CH_3C=O), 1.97 (s, 3H, CH_3C=O). Data in agreement with the unlabelled enantiomer. [1]

***p*-Tolyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-L-[$^{13}C_6$]-galactopyranoside (11):** Compound **10 (**5.01 g, 11.0 mmol) was added to a solution of 1 M NaOMe/MeOH (7.5 mL, 7.5 mmol) and MeOH (100 mL). The reaction was stoppered and stirred at room temperature for 30 min. After completion, the solution was neutralised with Amberlite® IR120 (H^+ form) resin, filtered and concentrated to give crude, crystalline product (3.115 g, 10.9 mmol, 99%). R_f = 0.65 (CH_2Cl_2/MeOH, 3:1). The crude (557 mg, 1.91 mmol) was dried *in vacuo*, placed under N_2 and cooled to 0 °C. TBDMSCl (320 mg, 2.10 mmol) and pyridine (3 mL) were added and the reaction stirred for 4 h over which the reaction mixture was allowed to reach room temperature. Upon completion, the solution was co-evaporated with toluene (3x 5 mL) before the crude product was purified by flash chromatography on silica gel (toluene/Et_3N, 19:1→toluene/EtOAc, 1:3) in a column with buffered silica gel (Et_3N 0.5% in toluene) to give the triol product (736 mg, 95%). R_f = 0.69 (CH_2Cl_2/MeOH 6:1); ^{1}H NMR

(500MHz, $CDCl_3$) δ 7.45 (d, *J* = 8.1 Hz, 2H, Ar-*H*), 7.10 (d, *J* = 8.2 Hz, 2H, Ar-*H*), 4.44 (d, *J* = 9.7 Hz, 1H, H-1), 4.07 (app s, H-4), 3.87 – 3.95 (m, 2H, H-$6_{(A+B)}$), 3.65 (t, *J* = 9.5 Hz, 1H, H-2), 3.58 (d, *J* = 3.5 Hz, 1H, H-3), 3.50 (t, *J* = 4.8 Hz, 1H, H-5), 0.90 (s, 9H, SiC(C$\underline{H}_3)_3$), 0.11 (s, 3H, Si(C$\underline{H}_3)_2$), 0.09 (s, 3H, Si(C$\underline{H}_3)_2$). A mixture of NaH (0.17 g, 7.1 mmol) and BnBr (0.65 mL, 6.5 mmol) in dry DMF (2 mL) were placed under N_2 and stirred at 0 °C. The triol (736 mg, 1.81 mmol) was dissolved in DMF (5 mL) and added to the reaction vessel in portions. The reaction was allowed to reach room temperature and was stirred for 2 hours. The solvent was then removed by co-evaporation with toluene (2x 5 mL) before dissolving in CH_2Cl_2 (50 mL) and washing with H_2O (50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was then purified by flash chromatography on buffered (0.5% Et_3N) silica gel (toluene→toluene/EtOAc 49:1) to give compound **11** (968 mg, 79%). R_f = 0.47 (toluene/EtOAc, 20:1); ^{1}H NMR (500MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.2 Hz, 2H, Ar-*H*) 7.18 – 7.42 (m, 15H, Ar-*H*), 7.01 (d, *J* = 7.9 Hz, 2H, Ar-*H*), 5.00 (d, *J* = 11.7 Hz, 1H, OCH$\underline{H}$Ph), 3.92 (t, *J* = 9.5 Hz, 1H, H-2), 4.73 – 4.81 (m, 4H, 4x OCH$\underline{H}$Ph), 4.64 (d, *J* = 11.4 Hz, 1H, OCH$\underline{H}$Ph) 4.60 (d, *J* = 9.8 Hz, 1H, H-1), 3.96 (m, 1H, H-4), 3.73 – 3.79 (m, 2H, H-$6_{(A+B)}$), 3.61 (dd, *J* = 9.5, 2.8 Hz, 1H, H-3), 3.44 (t, *J* = 6.8 Hz, 1H, H-5), 2.31 (s, 3H, PhCH_3), 0.90 (s, 9H, SiC(C$H_3)_3$), 0.05 (2x s, 6H, Si(C$H_3)_2$).

***p*-Tolyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-[$^{13}C_6$]-galactopyranoside (12):** Compound **11** (968 mg, 1.43 mmol) and Bu_4NF (0.45 g, 1.7 mmol) were placed under N_2 and dissolved in dry THF (2 mL). The reaction was stirred at room temperature for 16 hours and the solvent was then removed under reduced pressure. Flash chromatography on silica gel (toluene→toluene/EtOAc, 6:1) yielded product **12** (772 mg, 96%). R_f = 0.21 (toluene/EtOAc 9:1); ^{1}H NMR (500MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.3 Hz, 2H, Ar-*H*) 7.14 – 7.38 (m, 15 H, Ar-*H*) 7.00 (d, *J* = 7.9 Hz, 2H, Ar-*H*), 4.94 (d, *J* = 12.0 Hz, 1H, OCH$\underline{H}$Ph) 4.72 – 4.81 (m, 4H, 4x OCH$\underline{H}$Ph), 4.61 (d, *J* = 11.7 Hz, 1H, OCH$\underline{H}$Ph), 4.56 (d, *J* = 10.0 Hz, 1H, H-1), 3.88 (t, *J* =

9.5 Hz, 1H, H-2), 3.77 – 3.81 (m, 2H, H-$6_{(A+B)}$), 3.57 (dd, *J* = 9.1, 2.8 Hz, 1H, H-3), 3.48 (m, 1H, H-4), 3.38 (t, *J* = 6.2 Hz, 1H, H-5), 2.27 (s, 3H, PhCH_3). Data in agreement with enantiomer [2]

***p*-Tolyl 2,3,4-tri-*O*-benzyl-6-*O*-*p*-tolylsulfonyl-1-thio-β-L-[$^{13}C_6$]-galactopyranoside (13):** Compound **12** (772 mg, 1.37 mmol) and TsCl (0.34 g, 1.8 mmol) were dried together *in vacuo* and then placed under N_2. Dry pyridine (3 mL) was added and the solution was stirred at room temperature for 3 hours. Upon completion, the pyridine was removed by co-evaporation with toluene (2x 5 mL) and the mixture diluted with EtOAc (40 mL). The organic phase was washed with H_2O (2x 40 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Compound **13** was then purified by flash chromatography on silica gel (toluene→toluene/EtOAc, 3:1), yielding a white, crystalline solid (923 mg, 94%). R_f = 0.48 (toluene/EtOAc, 9:1); ^{1}H NMR (500MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.2 Hz, 2H, Ar-*H*), 7.23 – 7.39 (m, 19 H, Ar-*H*), 6.99 (d, *J* = 7.9 Hz, 2H. Ar-*H*), 4.94 (d, *J* = 11.4 Hz, 1H, OCH*H*Ph), 4.68 – 4.78 (m, 4H, 4x OCH*H*Ph), 4.51 (d, *J* = 9.8 Hz, 1H, H-1), 4.47 (d, *J* = 11.3 Hz, 1H, OCH*H*Ph), 4.04 – 4.13 (m, 2H, H-$6_{(A+B)}$), 3.89 (s, 1H, H-4), 3.82 (t, *J* = 9.5 Hz, 1H, H-2), 3.64 (t, *J* = 6.5 Hz, 1H, H-5), 3.56 (dd, *J* = 9.5, 2.5 Hz, 1H, H-3), 2.39 (s, 3H, PhCH_3), 2.30 (s, 3H, PhCH_3). Data in agreement with unlabelled enantiomer.[2]

***p*-Tolyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-[$^{13}C_6$]-fucopyranoside (4):** Compound **13** (0.923 mg, 1.29 mmol) and $LiAlH_4$ (147 mg, 38.7 mmol) were dried together *in vacuo* for 2 hours and then placed under N_2. Dry THF (2 mL) was added and the reaction mixture refluxed for 2 hours. After completion, the reaction mixture was diluted with THF (10 mL) and added dropwise to a solution of THF (10 mL) and glacial acetic acid (5 mL) at 0 °C. H_2O (30 mL) was added and 2 M aq. HCl was added dropwise until the precipitate dissolved. The product was then extracted with EtOAc (3x 40 mL). The combined organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash

chromatography on silica gel (toluene→toluene/EtOAc, 49:1) yielded compound **4** as a white solid (427 mg, 61%). R_f = 0.40 (toluene/EtOAc, 20:1); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.52 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.36 (m, 15H, ArH), 7.04 (d, *J* = 7.8 Hz, 2H, ArH), 5.03 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.83 (d, *J* = 10.1 Hz, 1H CH_2Ph), 4.79 - 4.73 (m, 3H, CH_2Ph), 4.69 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.57 (d, *J* = 9.6 Hz, 1H, H-1), 3.91 (t, *J* = 9.3 Hz, 1H, H-2), 3.65 (bs, 1H, H3/H4), 3.61 (d, *J* = 9.1 Hz, 1H, H3/H4), 3.53 (q, *J* = 6.1 Hz, 1H, H5), 2.33 (s, 3H, CH_3), 1.29 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.2, 129.5, 128.44, 128.35, 128.32, 128.2, 128.0, 127.7, 127.6, 87.9 (d, *J* = 41.3 Hz), 84.6 (t, *J* = 39.9 Hz), 77.6, 77.3, 77.1, 77.0, 76.7, 76.6, 76.2, 74.5 (t, *J* = 40.2 Hz), 17.3 (dt, *J* = 41.0, 3.9 Hz). *m/z* (MICRO-TOF) 564.2275 $[M + Na]^+$ $C_{33}{}^{13}CH_{36}NaO_4S$ requires 564.2266. Data in agreement with unlabelled enantiomer.[3]

3-Azidopropyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (14)[4]: Lactoside acceptor **6** (2.20 g, 2.28 mmol) and *N*-phthalimido-glucosyl donor **7** (1.37 g, 2.51 mmol) were placed under N_2 together and dissolved in dry CH_2Cl_2 (10 mL). 4 Å powdered molecular sieves were added and the resulting suspension was stirred at room temperature for 2 h. NIS (1.03 g, 4.56 mmol) and AgOTf (cat.) were added and stirred was continued at room temperature for 20 minutes. The reaction was then quenched with Et_3N (1 mL) and filtered through Celite®. The filtrate was diluted with EtOAc (75 mL) and washed with sat. aq. $NaHCO_3$ (70 mL), 10% aq. $Na_2S_2O_3$ (2x 70 mL) and H_2O (70 ml). The organic phase was dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography on buffered (0.5% Et_3N) silica gel (toluene→toluene/EtOAc, 9:1), giving trisaccharide **14** as an amber solid (1.82 g, 58%). R_f = 0.22 (toluene/EtOAc 6:1); ^{1}H NMR (400 MHz, Chloroform-*d*) δ7.48 -7.45 (m, 2H, ArH), 7.38 - 7.05 (m, 35H, ArH), 6.90 - 6.88 (m, 2H, ArH), 5.95 - 5.92 (m, 1H, H3''), 5.68 (d, *J* = 8.2 Hz,

1H, H1''), 5.95 (s, 1H, CHPh), 4.96 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.88 (d, *J* = 10.6 Hz, 1H, CH_2Ph), 4.80 – 4.63 (m, 2H, CH_2Ph), 4.57 - 4.43 (m, 4H), 4.38 - 4.23 (m, 6H), 4.18 (d, *J* = 7.5 Hz, 1H), 4.05 (d, *J* = 12.1 Hz, 1H), 3.92 - 3.75 (m, 6H), 3.58 (dd, *J* = 9.9, 2.9 Hz, 1H), 3.54 - 3.46 (m, 4H), 3.36 - 3.26 (m, 7H), 2.91 (d, *J* = 11.2 Hz, 1H), 1.86 (s, 3H), 1.83 - 1.77 (m, 2H, CH_2). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2 (*C*=O), 139.3, 139.1, 138.7, 138.5, 138.4, 138.3, 137.0, 134.5 (Ar-C_{quat}), 128.5 - 125.4 (Ar-*C*H), 103.6 (C1), 102.5 (C1'), 101.8 (CHPh), 100.1 (C1''), 83.1, 81.98, 81.7, 79.4, 78.9, 76.6, 75.8 (CH's), 75.5 (O*C*H_2Ph), 75.16 (O*C*H_2Ph), 75.13 (O*C*H_2Ph), 74.8 (CH), 74.1 (O*C*H_2Ph), 73.5 (O*C*H_2Ph), 73.2 (O*C*H_2Ph), 73.0, 69.7 (CHs), 68.9, 68.3, 67.8 (OCH_2), 66.5 (O*C*$H_{2(Linker)}$), 66.1 (CH), 55.9 (C2''), 48.4 ($CH_2N_{3(Linker)}$), 29.3 (CH_2-$_{(Linker)}$), 20.7 (CH_3). *m/z* (MICRO-TOF) 1409.6673 $[M + Na]^+$ $C_{80}H_{82}N_4NaO_{18}$ requires 1409.5522.

3-Azidopropyl 2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (15): Compound **14** (5.63 g, 4.06 mmol) was placed under N_2 and dissolved in absolute EtOH (40 mL). EDA (14 mL, 210 mmol) was added and the reaction was refluxed at 105 °C. After 16 hours, the volatiles were removed through co-evaporation with toluene. The crude was then placed under N_2 and dissolved in dry MeOH/toluene (40 mL, 2:1, v/v). Ac_2O (3.9 mL, 41 mmol) was added and the reaction was stirred at room temperature for 30 minutes. The mixture was then co-evaporated with toluene *in vacuo* and H_2O (120 mL) was added. The product was extracted with EtOAc (3 x 120 mL), dried over $MgSO_4$, filtered and concentrated. Compound **15** was isolated by flash chromatography on silica gel (toluene/EtOAc, 4:1→EtOAc) as a white foam (3.86 g, 76%). R_f = 0.27 (toluene/EtOAc, 3:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 - 7.14 (m, 35H, ArH), 5.58 (s, 1H, CHPh), 5.37 (d, 2H, J = 5.2 Hz), 5.04 - 4.99 (dd, 2H, J = 14.6, 11.8 Hz), 4.90 - 4.80 (m, 3H), 4.76 - 4.69 (m, 2H), 4.66 - 4.59 (m, 2H), 4.53 (d, 1H, J = 11.6 Hz), 4.46 (d, 1H, J = 7.7 Hz), 4.40 - 4.31 (m, 4H), 4.26 (d, 1H, J = 11.8 Hz), 4.04 - 3.88 (m, 3H),

3.82 - 3.73 (m, 4H), 3.64 - 3.52 (m, 7H), 3.49 - 3.45 (m, 2H), 3.42 - 3.36 (m, 4H), 3.29 (d, 1H , J = 7.74 Hz), 1.89 - 1.83 (d, 2H), 1.47 (s, 3H, CH3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.6 (*C*=O), 139.08, 139.04, 138.7, 138.6, 138.3, 138.14, 137.09 (Ar-C_{quat}), 129.3 - 126.0 (Ar-*C*H), 103.7 (C1), 102.66 (C1'), 102.65 (C1''), 102.1 (CHPh), 82.9, 81.77, 81.73, 81.6, 80.1 (C-2_{Gal}-), 76.3, 76.2 (CH's), 75.6, 75.19 (O*C*H_2Ph), 75.14 (CH), 75.06, 74.3, 73.56, 73.54 (O*C*H_2Ph), 73.4, 72.9 (CH's), 68.7, 68.22, 68.15 (C6's), 66.6 (C5'', O*C*$H_{2(Linker)}$), 59.3 (C2''), 48.4 (*C*$H_2N_{3(Linker)}$), 29.4 (*C*H_2-$_{(Linker)}$), 22.8 (CH_3). m/z (MICRO-TOF) 1279.5325 $[M + Na]^+$ $C_{72}H_{80}N_4NaO_{16}$ requires 1279.5467.

3-Azidopropyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (16): Trisaccharide acceptor **15** (1.54 g, 1.22 mmol) and donor **8**[3] (971 mg, 1.47 mmol) were placed under N_2 together and dissolved dry CH_2Cl_2 (10 mL). 4 Å powdered molecular sieves were added and the suspension was stirred at room temperature for 2 hours. NIS (550 mg, 2.44 mmol) and AgOTf (cat.) were added and the reaction was stirred at room temperature for 40 minutes. The reaction was then quenched with Et_3N (0.5 mL) and filtered through Celite®. The filtrate was diluted with EtOAc (100 mL) and washed with sat. aq. $NaHCO_3$ (100 mL), 10% aq. $Na_2S_2O_3$ (2x 100 mL) and H_2O (70 mL). The organic phase was then dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography on buffered (0.5% Et_3N) silica gel (toluene→toluene/EtOAc, 7:3) to give tetrasaccharide **16** as an amber solid (1.78 g, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 7.6 Hz, 2H), 7.59 – 7.07 (m, 76 H), 5.60 (m, 1H), 5.51 (s, 1H, C*H*$_{acetal}$), 5.31 (d, *J* = 8.1 Hz, 1H, H-1), 5.02 (m, 1H), 4.93 (t, *J* = 12.7 Hz, 2H), 4.81 (m, 2H), 4.71 – 4.55 (m, 9H), 4.53 (d, *J* = 12.1 Hz, 1H, OCH*H*Ph), 4.51 (d, *J* = 11.6 Hz, 1H, OCH*H*Ph), 4.42 – 4.28 (m, 8H), 4.26 – 4.20 (m, 3H), 3.99 – 3.82 (m, 5H), 3.78 – 3.69 (m, 4H), 3.67 – 3.52 (m, 10H), 3.51 – 3.45 (m, 4H), 3.44 – 3.28 (m, 10H), 3.19 (m,

1H), 3.00 (m, 1H), 1.96 – 1.82 (m, 2H, -C*H*$_2$-$_{(Linker)}$), 0.83 (s, 3H, C*H*$_3$C=O); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 165.3, 139.5, 139.1, 138.9, 138.7, 138.6, 138.4, 138.23, 137.8, 137.7, 133.2, 129.9 - 126.3 (Ar-*C*H), 103.6 (C-1), 102.5 (C-1), 101.4 (C_{acetal}), 101.0 (C-1), 100.6 (C-1), 83.0, 82.4, 81.7, 81.3, 80.0, 79.4, 77.2, 77.1, 76.4, 76.3, 75.4 (O*C*H$_2$Ph), 75.14 (O*C*H$_2$Ph), 75.07, 74.9 (O*C*H$_2$Ph), 74.8 (O*C*H$_2$Ph), 74.6 (O*C*H$_2$Ph), 73.5 (O*C*H$_2$Ph), 73.3 (O*C*H$_2$Ph), 73.2 (O*C*H$_2$Ph), 73.0, 72.9, 72.6, 72.1, 71.6 (O*C*H$_2$Ph), 69.0 (C-6), 68.31 (C-6), 68.25 (C-6), 68.1 (C-6), 66.51 (O*C*H$_{2(Linker)}$), 48.4 (*C*H$_2$N$_{3(Linker)}$), 29.33 (-*C*H$_{2-(Linker)}$), 22.38 (*C*H$_3$C=O); m/z (MICRO-TOF) 1815.7297 $[M + Na]^+$ $C_{106}H_{112}N_4NaO_{22}$ requires 1815.7666.

3-Azidopropyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (17): Tetrasaccharide **16** (569 mg, 0.317 mmol) was placed under N_2 and dissolved in dry THF (5 mL). 3 Å molecular sieves were added and the resulting suspension was stirred at room temperature for 3 hours. $NaBH_3CN$ (199 mg, 3.17 mmol) was added to the mixture along with an extra portion of 3 Å molecular sieves and stirring was continued for 1 hour. 2 M HCl/Et_2O (1.6 mL, 3.2 mmol) was added dropwise until gas ceased evolving. The reaction was stirred at room temperature for 40 min and was then quenched with Et_3N. The solvent was removed *in vacuo* and the product was purified by flash chromatography on silica gel (toluene/EtOAc, 9:1→1:1), yielding compound **17** as an off-white solid (481 mg, 85%). R_f = 0.66 (toluene/EtOAc, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (1 H, d, *J* 7.9), 7.56 (1 H, d, *J* 7.3), 7.42 (2 H, t, *J* 7.8), 7.35 – 7.06 (38 H, m), 5.62 (1 H, t, J 9.0), 5.10 (1 H, d, *J* 8.2), 5.00 (1 H, d, *J* 10.7), 4.94 (1 H, d, *J* 11.3), 4.92 (1 H, d, *J* 9.1), 4.81 (1 H, d, *J* 11.0), 4.73 (1 H, d, *J* 11.2), 4.66 (1 H, d, *J* 10.7), 4.62 (1 H, d, *J* 12.8), 4.58 (1 H, d, *J* 12.1), 4.54 (1 H, d, *J* 9.2), 4.51 – 4.43 (7 H, m), 4.42 (1 H, d, *J* 12.0), 4.36 – 4.27 (3 H, m, *J* 19.4, 8.4), 4.29 (1 H, d, *J* 11.2), 4.26 (1 H, d, *J* 7.8), 4.18 (1 H, d, *J* 12.1), 3.98 (1 H, d, *J* 2.2), 3.96 – 3.89 (2 H, m, *J* 15.9, 5.9), 3.92 (1 H, s), 3.91 – 3.85 (1 H, m), 3.85 (1 H,

d, *J* 10.0), 3.73 – 3.69 (1 H, m), 3.68 – 3.62 (4 H, m), 3.61 – 3.54 (5 H, m), 3.53 – 3.42 (8 H, m), 3.40 – 3.31 (6 H, m), 3.21 – 3.15 (1 H, m), 2.78 (1 H, dd, *J* 16.8, 8.6), 1.86 (2 H, dd, *J* 11.0, 5.8), 0.87 (3 H, s); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 165.1, 139.8, 139.2, 138.9, 138.7, 138.6, 138.4, 138.2, 138.1, 137.6, 137.4, 133.4, 130.0 - 127.1 (Ar-*C*H), 103.6 (C-1), 102.6 (C-1), 101.8 (C-1), 99.9 (C-1), 83.1, 83.0, 81.9, 81.7, 79.8, 79.5, 77.16, 77.16, 76.8, 75.4 (O*C*H_2Ph), 75.2 (O*C*H_2Ph), 75.09, 75.04, 74.8 (O*C*H_2Ph), 74.7 (O*C*H_2Ph), 74.6 (O*C*H_2Ph), 73.9, 73.8 (O*C*H_2Ph), 73.6 (O*C*H_2Ph), 73.37 (O*C*H_2Ph), 73.35, 73.2 (O*C*H_2Ph), 72.27 (O*C*H_2Ph), 72.24, 72.15, 70.3 (C-6), 70.1, 68.8 (C-6), 68.5 (C-6), 68.0 (C-6), 66.5 (O*C*$H_{2(Linker)}$), 58.7, 48.4 (*C*$H_2N_{3(Linker)}$), 29.4 (-*C*H_2-$_{(Linker)}$), 22.8 (*C*H_3C=O); m/z (MICRO-TOF) 1817.6311 $[M + Na]^+$ $C_{106}H_{114}N_4NaO_{22}$ requires 1817.7822.

3-Azidopropyl 3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (18): Tetrasaccharide **16** (5.9 g, 3.43 mmol) was dissolved in CH_2Cl_2 (15 mL). Methanol (15 mL) was added and the solution was heated to 50 C. A solution of 1M NaOMe in methanol (35 mL) was added and the reaction temperature was maintained at 50 °C for 30 min. The reaction was reduced to dryness. Water (50 mL) was added to the residue followed by CH_2Cl_2 (50mL) and the biphasic mixture was vigorously stirred. The water layer was acidified with 2M HCl (16 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The organic phase was dried over MgSO4, filtered and reduced to dryness. The product **18** was isolated as a white foam (4.92 g, 86%). Rf = 0.2 toluene/ethyl acetate 7/3 v/v. ^{1}H NMR (500 MHz, $cdcl_3$) δ 7.50 - 7.11 (m, 50H, ArH), 5.55 (s, 1H, CHPh), 5.53 (d, 1H, NH), 5.03 - 4.99 (m, 2H), 4.92 (d, 1H, J = 11.5 Hz), 4.88 (d, 1H, J - 8.3 Hz), 4.82 - 4.73 (m, 3H), 4.68 - 4.58 (m, 5H), 4.54 (d, 1H, J = 8.2 Hz), 4.52 (d, 1H, J = 8.6 Hz), 4.41 - 4.20 (m, 9H), 4.06 (t, 1H, J - 9.7 Hz), 3.95 - 3.91 (m, 3H), 3.88- 3.86 (m, 2H), 3.79 - 3.74 (m, 2H), 3.72 - 3.65 (m, 3H), 3.63 - 3.54 (m, 4H), 3.52 - 3.44 (m, 4H), 3.42 - 3.35 (m, 6H), 3.31 (dd, 1H, J = 9.7, 2.8

Hz), 3.26 - 3.24 (m 1H), 1.88 - 1.82 (m, 2H, CH_2), 1.54 (CH_3). ^{13}C NMR (126 MHz, cdcl$_3$) δ 171.2 (C=O), 139.4, 139.1, 138.8, 138.7, 138.5, 138.4, 138.2, 137.7, 136.8 (ArC), 129.3, 128.5, 128.5, 128.2, 128.6, 128.4, 128.39, 128.3, 128.28, 128.2, 128.1, 128.04, 128.0, 127.97, 127.9, 127.8, 127.78, 127.7, 127.71, 127.69, 127.6, 127.61, 127.3, 127.2, 126.3 (ArCH), 103.6, 103.3, 103.0, 102.7, 101.7 (C1, C1', C1'', C1''', CHPh), 82.9, 81.7, 81.5, 80.3, 80.0, 76.5, 76.2, 75.9, 75.5, 75.1, 74.8, 74.7, 74.6, 73.8, 73.4, 73.4, 73.3, 73.2, 72.6, 70.6, 68.8, 68.4, 68.3, 68.2, 66.5, 66.4, 56.4 (C2''), 48.4 (CH_2N_3), 29.3 (CH_2), 23.3 (CH_3).

3-Azidopropyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (19): Compound **17** (1 eq, 0.557 g, 0.310 mmol) was dissolved in DCM (3 ml) and MeOH (18 ml) added dropwise with stirring while ensuring the starting material remained in solution. A solution of NaOMe in MeOH (3 ml, 1 moldm^{-3}) was added dropwise and the mixture left to stir at rt. After 16 h had elapsed, starting material was still observed by TLC (toluene/ EtOAc 1:1). The temperature was raised to 40 ^oC, with the reaction appearing to be complete after 2 days. Solvent was removed under reduced pressure before adding EtOAc (60 ml) and washing with water (2 x 60 ml), before drying the organic layer over $MgSO_4$. The solvent was removed under vacuum and the product purified by silica gel column chromatography (toluene/EtOAc 5% → 50%) to give compound **19** as a colourless solid (0.461 g, 0.273 mmol, 88%). R_f = 0.48 (toluene/EtOAc 1:1). δ_H (400 MHz, $CDCl_3$) 7.42 – 7.09 (1 H, m), 5.11 – 4.86 (1 H, m), 4.84 – 4.64 (1 H, m), 4.56 (1 H, dd, *J* 21.8, 11.2), 4.37 (1 H, m), 4.20 (1 H, d, *J* 12.0), 4.04 (1 H, d, *J* 7.5), 3.94 (1 H, dd, *J* 20.1, 12.7), 3.80 – 3.63 (1 H, m), 3.61 – 3.46 (1 H, m), 3.39 (1 H, dd, *J* 16.2, 5.0), 3.26 (1 H, d, *J* 8.8), 1.85 (2 H, d, *J* 4.4), 1.47 (3 H, s). δ_C (101 MHz, $CDCl_3$) 171.60, 139.23, 139.11, 139.02, 138.60, 138.54, 138.38, 138.32, 138.26, 138.24, 138.13, 137.51, 128.66, 128.43,

128.37, 128.33, 128.28, 128.21, 128.13, 128.08, 128.05, 127.98, 127.91, 127.73, 127.65, 127.58, 127.52, 127.23, 127.15, 126.03, 104.82, 103.49, 102.48, 101.70, 87.35, 82.84, 81.63, 81.58, 81.38, 80.04, 76.32, 75.45, 75.40, 75.05, 75.02, 74.89, 74.59, 74.20, 74.14, 73.70, 73.61, 73.42, 73.34, 73.30, 72.91, 71.24, 70.09, 69.52, 68.89, 68.34, 67.97, 66.43, 55.70, 48.31, 29.24, 23.10. m/z (MICRO-TOF) 1713.7589 $[M + Na]^+$ $C_{99}H_{110}N_4NaO_{21}$ requires 1713.7560.

3-Azidopropyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (20): Tetrasaccharide **17** (268 mg, 0.149 mmol), fucosyl donor **5**[5] (121 mg, 0.224 mmol) and Et_4NBr (47 mg, 0.22 mmol) were placed under N_2 together and dissolved in dry CH_2Cl_2/DMF (2 mL, 9:1, v/v). 4 Å molecular sieves were added and the suspension was stirred at room temperature for 2 hours. Br_2 (2.25 eq, 54 mg, 0.34 mmol) was then added and the reaction was stirred at room temperature for 16 hours. Upon completion, Et_3N was added and the mixture was filtered through Celite®. The filtrate was diluted with EtOAc (25 mL) and washed with 10% aq. $Na_2S_2O_4$ (2x 25 mL). The organic layer was then dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography on silica gel (toluene/EtOAc, 19:1→3:2) to give product **20** as a colourless solid (147 mg, 45%). R_f = 0.73 (toluene/EtOAc, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (t, *J* = 7.7 Hz, 2H), 7.54 (dd, *J* = 12.9, 6.9 Hz, 1H), 7.46 – 7.07 (m, 76H), 5.53 (m, 1H), 5.19 (d, *J* = 7.1 Hz, 1H), 5.05 – 4.98 (m, 2H), 4.96 (d, *J* = 5.0 Hz, 1H), 4.94 (d, *J* = 11.4 Hz, 1H), 4.88 (d, *J* = 11.5 Hz, 1H), 4.85 (s, 1H), 4.81 (d, *J* = 10.9 Hz, 1H), 4.80 (d, *J* = 11.8 Hz, 1H), 4.75 (s, 1H), 4.75 (d, *J* = 11.5 Hz, 2H), 4.72 (d, *J* = 8.3 Hz, 2H), 4.68 (d, *J* = 12.3 Hz, 2H), 4.66 (d, *J* = 10.1 Hz, 2H), 4.64 (d, *J* = 11.1 Hz, 2H), 4.62 (d, *J* = 11.9 Hz, 2H), 4.61 – 4.56 (m, 4H), 4.53 (dd, *J* = 7.8, 4.8 Hz, 2H), 4.49 (t, *J* = 6.5 Hz, 3H), 4.45 (s, 1H), 4.44 (s, 1H), 4.40 (d, *J* = 11.6 Hz), 4.35 – 4.27 (m, 4H), 4.30 (d, *J* = 7.9 Hz, 2H), 4.26 – 4.16 (m, 3H), 4.12 (d, *J* = 11.8

Hz, 1H), 4.06 (d, *J* = 11.3 Hz, 1H), 4.05 (m, 1H), 4.01 (d, *J* = 11.3 Hz, 1H), 4.02 – 3.90 (m, 4H), 3.89 (s, 1H), 3.87 (s, 1H), 3.82 (m, 1H), 3.80 – 3.74 (m, 2H), 3.71 (m, 1H), 3.67 – 3.58 (m, 4H), 3.62 – 3.55 (m, 3H), 3.58 – 3.47 (m, 5H), 3.46 (s, 1H), 3.43 (d, *J* = 2.9 Hz, 1H), 3.42 – 3.27 (m, 8H), 3.25 (m, 1H), 3.16 (m, 1H), 1.91 – 1.82 (m, 2H), 1.54 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.8, 139.5, 139.2, 139.1, 139.0, 138.9, 138.8, 138.7, 138.6, 138.5, 138.2, 138.1, 137.7, 129.0 - 127.3 (Ar-*C*H), 103.7 (C-1), 102.6 (C-1), 101.1 (C-1), 100.4 (C-1), 100.0 (C-1), 97.8 (C-1), 83.2, 83.1, 81.7, 80.95, 80.93, 80.89, 80.84, 80.51, 80.46, 80.38, 79.8, 79.6, 78.6, 78.2, 77.4, 77.2, 76.5, 76.4, 76.3, 75.8, 75.64, 75.58, 75.4, 75.27, 75.23, 75.18, 75.0, 74.9, 74.71, 74.65, 74.57, 73.7, 73.64, 73.61, 73.59, 73.53, 73.50, 73.46, 73.38, 73.34, 73.1, 72.8, 72.4, 72.03, 71.96, 68.4, 68.1, 68.01, 67.95, 67.8, 67.0, 66.8, 66.6, 48.5, 29.4, 23.3; m/z (MICRO-TOF) 2234.1930 $[M + Na]^+$ $C_{133}H_{142}N_4NaO_{26}$ requires 2233.9810.

3-Azidopropyl 3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (21): Pentasaccharide **20** (125 mg, 56.5 μmol) was dissolved in CH_2Cl_2 (1 mL) and MeOH (6 mL) was added dropwise while ensuring the starting material remained in solution. 1 M NaOMe/MeOH (1 mL, 1 mmol) was added dropwise and the mixture stirred at room temperature. After 20 hours, starting material was still visible by TLC (toluene/ EtOAc, 3:2). The temperature was raised to 40 °C and the reaction was stirred for a further 6 hours. The solvent was then removed under reduced pressure and purification by flash chromatography on silica gel (toluene/EtOAc, 19:1→4:1) yielded product **21** as a white solid (110 mg, 93%). R_f = 0.65 (toluene/EtOAc, 3:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.06 (m, 28H), 5.04 (t, *J* = 9.4 Hz, 2H), 4.94 (t, *J* = 11.2 Hz, 1H), 4.83 (m, 3H), 4.75 – 4.59 (m, 4H), 4.59 – 4.48 (m, 2H), 4.46 (s, 1H), 4.36 (m, 3H), 4.28 – 4.17 (m, 3H), 4.07 – 3.87 (m, 5H), 3.87 – 3.68 (m, 3H), 3.67 – 3.47 (m, 5H), 3.47 – 3.33 (m, 4H), 3.28 (d, *J* = 8.9 Hz, 1H), 2.07 (s, 3H, C*H*$_3$C=O), 2.00 –

1.82 (m, 2H), 1.29 (d, J = 7.1, 3H, H-6_{Fuc}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 139.3, 139.23, 139.15, 139.05, 138.86, 138.83, 138.7, 138.5, 138.3, 137.8, 129.1 - 126.7 (Ar-*C*H), 103.6 (C-1), 102.8 (C-1), 102.5 (C-1), 101.1 (C-1), 98.0 (C-1), 82.9, 81.8, 81.7, 81.0, 80.4, 80.2, 78.2, 78.1, 77.2, 76.6, 76.3, 75.8, 75.5, 75.3, 75.1, 75.1, 74.59, 74.54, 74.1, 73.7, 73.6, 73.45, 73.42, 73.35, 73.26, 73.1, 72.9, 72.1, 71.2, 68.6, 68.3, 68.0, 67.9, 66.8, 66.5, 56.4, 48.4, 29.3, 21.1, 14.3; m/z (MICRO-TOF) 2129.8046 $[M + Na]^+$ $C_{126}H_{138}N_4NaO_{25}$ requires 2129.9547.

3-Azidopropyl 2,3,4-tri-*O*-benzyl-α-L-[$^{13}C_6$]fucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (22): Pentasaccharide **21** (97 mg, 46 μmol), ^{13}C-labelled fucosyl donor **4** (38 mg, 69 μmol) and Bu_4NBr (22 mg, 69 μmol) were placed under Ar together and dissolved in dry CH_2Cl_2/DMF (1.5 mL, 9:1, v/v). 4 Å molecular sieves were added and the suspension was stirred at room temperature for 2 hours. Br_2 (2.25 eq, 16.6 mg, 104 μmol) was added and the reaction was stirred at room temperature for 16 hours. Upon completion, Et_3N was added and the reaction mixture was filtered through Celite®. The filtrate was diluted with EtOAc (25 mL) and washed with 10% aq. $Na_2S_2O_4$ (2x 25 mL). The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by flash chromatography on silica gel (toluene/EtOAc, 19:1→4:1), yielding product **22** as a colourless solid (92 mg, 79%). R_f = 0.66 (toluene/EtOAc, 2:1). Decoupled NMR data as for unlabelled material. [6]

3-Aminopropyl (α-L-[U-$^{13}C_6$]fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[α-L-fucopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl hydrochloride (1): Hexasaccharide **23** (1 eq, 89.1 mg, 35.0 μmol) was dissolved in 1,4-dioxane (9 ml) and stirred while adding water (3 ml) dropwise. The mixture was degassed and flushed through with $N_{2(g)}$ before adding Pd/C (10

wt. %, cat), degassing and flushing through with $H_{2(g)}$. After 15 min had elapsed, $HCl_{(aq)}$ (1 eq, 0.1 moldm^{-3}, 352 μl) and additional Pd/C was added and the reaction stirred under $H_{2(g)}$. The reaction was monitored by TLC (EtOH/H_2O/NH_4OH 1:1:1) and after 16 h had not gone to completion. Fresh Pd/C was added and the mixture stirred for a further 24 h until complete. The reaction mixture was degassed and flushed through with $N_{2(g)}$ repeatedly before exposing to the air during TLC sampling or addition of reagents. Additional water was added to the mixture which was then filtered through celite. The solvent was removed by lyophilisation before purification using a size exclusion column. The column was run with a solution of butanol (1%) in ultra-pure water as the mobile phase. The solvent was eluted at a rate of 36 ml/h and collected using a Gilson fraction collector (9 min, or ~5.4 ml per test tube). A solution of the hexasaccharide in the mobile phase was added to the top of the column before carefully adding more of the mobile phase via a reservoir. The fractions were checked with TLC and MALDI TOF and the test tubes containing the product were combined and lyophilised to give the deprotected hexasaccharide **1** as a fine, white solid (37.3 mg, 33.9 μmol, 97%). R_f = 0.35 (EtOH/H_2O/NH_4OH 1:1:1) δ_H (400 MHz, D_2O) 5.18 (1 H, s, Fuc-*H*-1), 5.06 (1 H, d, *J* 3.6, Fuc-*H*-1), 4.90 (1 H, dd, *J* 12.8, 6.2), 4.69 (1 H, d, *J* 7.6, *H*-1), 4.64 (1 H, d, *J* 8.3, *H*-1), 4.54 (1 H, d, *J* 8.0, *H*-1), 4.45 (1 H, d, *J* 7.8, *H*-1), 4.37 (1 H, d, *J* 6.1), 4.17 (2 H, t, *J* 9.7), 4.08 (1 H, dd, *J* 10.9, 5.6), 4.03 – 3.74 (17 H, m), 3.70 – 3.53 (8 H, m), 3.36 (1 H, t, *J* 8.3), 3.19 (2 H, t, *J* 6.9), 2.10 (3 H, s, COCH_3), 2.07 – 1.99 (2 H, m), 1.30 (6 H, t, *J* 5.7, Fuc-*H*-6). ^{13}C NMR (101 MHz, D_2O) δ 99.5 (d, *J* = 44.4 Hz, C-1), 72.0 (t, *J* = 37.2 Hz), 69.4 (t, *J* = 37.7 Hz), 68.2 (t, *J* = 41.8), 66.2 (t, *J* = 39.5 Hz), 15.3 (d, *J* = 41.5 Hz, C6). m/z (MALDI-TOF) 1085.78 $[M + Na]^+$ $^{12}C_{35}{}^{13}C_6H_{72}N_2NaO_{29}$ requires 1085.43.

3-Azidopropyl (2,3,4-tri-*O*-benzyl-α-L-[$^{13}C_6$]fucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-[$^{13}C_6$]fucopyranosyl)-(1→4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-

galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (23): Tetrasaccharide **19** (1 eq, 50.3 mg, 29.7 μmol), radiolabelled fucosyl donor **4** (3 eq, 48.3 mg, 89.2 μmol) and Bu_4NBr (3 eq, 28.7 mg, 89.2 μmol) were dissolved in a solution of DCM/DMF (9:1, 1 ml) and stirred at rt with 4 Å molecular sieves under N_2. After 2 h, bromine (4.5 eq, 21 mg, 130 μmol) was added and the now yellow solution allowed to stir under N_2 for 16 h. Upon completion, Et_3N was added and the reaction mixture was diluted in EtOAc (25 ml) and washed with $Na_2S_2O_{4(aq)}$ (10%, 2 x 25 ml) before drying the organic layer over $MgSO_4$ and removing the solvent under vacuum. The product was purified by silica gel column chromatography (toluene/EtOAc 2% → 20%) to give the product **23** as a colourless solid (39.2 mg, 15.5 μmol, 52%). R_f = 0.30 (toluene/EtOAc 3:1) m/z (MALDI-TOF) 2547.44 $[M + Na]^+$ $C_{153}H_{167}N_4NaO_{29}$ requires 2547.16.

3-Aminopropyl (α-L-[U-$^{13}C_6$]fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[α-L-[U-$^{13}C_6$]fucopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl hydrochloride (2): Hexasaccharide **23** (1 eq, 39.2 mg, 15.5 μmol) was dissolved in 1,4-dioxane (5 ml) and stirred while adding water (1.6 ml) dropwise. The mixture was degassed and flushed through with $N_{2(g)}$ before adding Pd/C (10 wt. %, cat), $HCl_{(aq)}$ (1 eq, 0.1 moldm^{-3}, 155 μl) and stirring under $H_{2(g)}$. The reaction was monitored by TLC ($EtOH/H_2O/NH_4OH$ 1:1:1) and after 16 h had not gone to completion. Fresh Pd/C was added and the mixture stirred for a further 24 h until only 1 product spot was visible. Additional water was added to the mixture which was then filtered through celite. The solvent was removed by lyophilisation before purification using a size exclusion column. Column was run with a solution of butanol (1%) in ultra-pure water as the mobile phase. The solvent was eluted at a rate of 36 ml/h and collected using a Gilson fraction collector (9 min, or ~5.4 ml per test tube). A solution of the hexasaccharide in the mobile phase was added to the top of the column before carefully adding more of the mobile phase via a reservoir. TLC and MALDI

TOF showed the presence of an impurity in some of the fractions containing the product. These fractions were combined and a further purification performed using a RP-C18 column. This column had been prewashed with a step-gradient using 5 ml for each step (MeOH in H_20, 100%, 80%, 60%, 40%, 20%, 10%MeOH). The compound mixture was applied to the top of the column and eluted with a MeOH/H_2O solvent mixture in a step-gradient with 5 ml for each step (MeOH in H_2O, 10%, 20%, 30%, 40%). The fractions containing pure product were combined and lyophilised to give the deprotected hexasaccharide **2** as a fine, white solid (4.50 mg, 4.07 µmol, 26%). R_f = 0.35 (EtOH/H_2O/NH_4OH 1:1:1). δ_H (400 MHz, D_2O) 5.19 (1 H, s, Fuc-*H*-1), 5.06 (1 H, s, Fuc-*H*-1), 4.89 (1 H, q, Fuc-*H*-5), 4.69 (1 H, d, *J* 7.7), 4.64 (1 H, d, *J* 8.4), 4.54 (1 H, d, *J* 8.0), 4.45 (1 H, d, *J* 7.8), 4.37 (1 H, d, *J* 6.2, Fuc-*H*-5), 4.20 – 4.11 (2 H, m), 4.12 – 4.05 (1 H, m), 4.03 – 3.81 (14 H, m), 3.79 - 3.74 (10 H, m), 3.69 – 3.55 (8 H, m), 3.36 (1 H, t, *J* 8.3), 3.19 (2 H, t, *J* 6.9), 2.10 (3 H, s, COCH_3), 2.07 – 1.99 (2 H, m), 1.30 (6 H, t, *J* 6.1, Fuc-*H*-6). ^{13}C NMR (101 MHz, D_2O, ^{13}C-^{13}C decoupled) δ 99.76, 99.32, 97.99, 97.54, 72.33, 71.97, 71.60, 69.82, 69.44, 69.08, 68.72, 68.62, 68.17, 67.77, 67.38, 66.99, 66.60, 66.20, 65.80, 15.51, 15.10. m/z (MALDI-TOF) 1092.05 $[M + Na]^+$ $^{12}C_{29}{}^{13}C_{12}H_{72}N_2NaO_{29}$ requires 1091.88.

3-Azidopropyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galacto-pyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (24): Tetrasaccharide **19** (1 eq, 43.9 mg, 25.9 µmol), fucosyl donor **5** (3 eq, 42.1 mg, 77.8 µmol) and Et_4NBr (3 eq, 16.0 mg, 77.8 µmol) were dissolved in a solution of DCM/DMF (9:1, 1 ml) and stirred at rt with 4 Å molecular sieves under N_2. After 2 h, bromine (4.5 eq, 19 mg, 117 µmol) was added and the now yellow solution allowed to stir for 16 h. Upon completion, Et_3N was added and the reaction mixture was diluted in EtOAc (25 ml) and washed with $Na_2S_2O_{4(aq)}$ (10%, 2 x 25 ml) before

drying the organic layer over $MgSO_4$ and removing the solvent under vacuum. The product was purified by silica gel column chromatography (toluene/EtOAc 2% → 20%) to give the product **24** as a colourless solid (52 mg, 20.6 μmol, 80%). R_f = 0.28 (toluene/EtOAc 3:1). δ_H (400 MHz, $CDCl_3$) 7.41 – 7.03 (99 H, m), 6.97 (2 H, d, *J* 7.3), 5.56 (1 H, d, *J* 3.2), 5.08 – 4.94 (4 H, m), 4.81 (9 H, dt, *J* 19.8, 10.8), 4.73 – 4.42 (23 H, m), 4.42 – 4.17 (12 H, m), 4.06 – 3.78 (16 H, m), 3.71 (6 H, dd, *J* 25.3, 11.4), 3.65 – 3.51 (8 H, m), 3.41 (10 H, dt, *J* 13.7, 9.3), 3.24 (2 H, d, *J* 11.3), 1.85 (2 H, dd, *J* 9.5, 6.0), 1.61 (3 H, s), 1.20 (6 H, d, *J* 5.8). δ_C (101 MHz, $CDCl_3$) 139.45, 139.42, 139.32, 139.14, 138.95, 138.82, 138.81, 138.61, 138.58, 138.38, 138.35, 138.34, 138.22, 138.20, 138.15, 138.14, 137.64, 128.66, 128.56, 128.43, 128.38, 128.35, 128.32, 128.21, 128.18, 128.12, 128.02, 127.98, 127.91, 127.88, 127.85, 127.76, 127.71, 127.68, 127.60, 127.56, 127.51, 127.45, 127.43, 127.39, 127.35, 127.29, 127.15, 127.11, 127.07, 127.01, 126.93, 126.31, 126.22, 103.50, 102.55, 102.11, 101.53, 98.43, 97.92, 83.80, 82.84, 81.64, 80.37, 79.20, 78.09, 76.37, 76.28, 75.60, 75.55, 75.42, 74.93, 74.86, 74.76, 74.44, 74.00, 73.63, 73.41, 73.34, 73.20, 73.13, 73.01, 72.92, 72.70, 71.77, 71.68, 71.24, 68.64, 68.30, 68.10, 67.44, 66.90, 66.60, 66.44, 60.39, 48.30, 29.24, 23.43, 16.34, 16.20.

3-Aminopropyl (α-L-fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[α-L-fucopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl hydrochloride (3): Hexasaccharide **24** (1 eq, 24.0 mg, 9.51 μmol) was dissolved in 1,4-dioxane (3 ml) and stirred while adding water (1 ml) dropwise. A precipitate appeared, which was re-dissolved by adding a little more 1,4-dioxane. The mixture was degassed and flushed through with $N_{2(g)}$ before adding Pd/C (10 wt. %, cat), $HCl_{(aq)}$ (1 eq, 0.1 $moldm^{-3}$, 95 μl) and stirring under $H_{2(g)}$. The reaction was monitored by TLC ($EtOH/H_2O/NH_4OH$ 1:1:1) until, after 20 h, only 1 product spot was visible. Additional water was added to the mixture which was then filtered through celite. The solvent was removed by lyophilisation before purification using a size exclusion column. Column was run with a

solution of butanol (1%) in ultra-pure water as the mobile phase. The solvent was eluted at a rate of 36 ml/h and collected using a Gilson fraction collector (9 min, or ~5.4 ml per test tube). A solution of the hexasaccharide in the mobile phase was added to the top of the column before carefully adding more of the mobile phase via a reservoir. TLC showed the presence of the hexasaccharide in fractions 11-16. These were combined and lyophilised to give the deprotected hexasaccharide **3** as a fine, white solid (8.50 mg, 7.77 μmol, 82%). R_f = 0.35 (EtOH/H_2O/NH_4OH 1:1:1). δ_H (500 MHz, D_2O) 5.12 (1 H, d, *J* 3.9, Fuc-*H*-1), 5.00 (1 H, d, *J* 3.8, Fuc-*H*-1), 4.63 (1 H, d, *J* 7.7, *H*-1), 4.57 (1 H, d, *J* 8.4, *H*-1), 4.48 (1 H, d, *J* 8.0, *H*-1), 4.38 (1 H, d, *J* 7.9, *H*-1),4.34 - 4.29 (1H, m), 4.11 (2 H, dd, *J* 11.6, 8.2), 4.05 – 4.00 (1 H, m), 3.96 (1 H, s), 3.94 (1 H, s), 3.93 - 3.89 (5 H, m), 3.86 - 3.76 (10 H, m), 3.74 - 3.67 (12 H, m), 3.65 - 3.59 (6 H, m), 3.56 – 3.48 (4 H, m), 3.30 (1 H, t, *J* 8.5), 3.13 (2 H, t, *J* 7.0), 2.03 (3 H, s), 1.98 (2 H, m), 1.24 (6 H, app t, *J* 7.2 ^{13}C NMR (101 MHz, D_2O) δ 174.1 (NC=O), 103.2 (C-1), 102.9 (C-1), 102.0 (C-1), 100.6 (C-1), 99.5 (C-1), 97.7 (C-1), 81.5, 78.1, 76.4, 75.1, 74.8, 74.4, 74.2, 73.6, 72.7, 71.9, 71.7, 70.1, 69.4, 69.0, 68.7, 68.5, 68.2, 67.7, 67.0, 66.2 (CH's), 61.6, 60.9, 60.4, 59.9, 59.4 (C-6, C-6', C-6'', C-6''', link-OCH_2) 55.7 (C-2''), 37.5 (link-NCH_2), 26.60 (link-CH_2), 22.1 ($COCH_3$), 15.30 (Fuc-C-6), 15.26 (Fuc-C-6). m/z (MALDI-TOF) 1079.33 $[M + Na]^+$ $C_{41}H_{73}N_2NaO_{29}$ requires 1080.42.

Per-*O*-acetyl-α/β-D-galactopyranose (S-24): A suspension of NaOAc (0.46 g, 5.5 mmol) in Ac_2O (5.25 mL, 55.2 mmol) was stirred at 140 °C. D-Galactose (994 mg, 5.52 mmol) was added in small portions over a period of 6 minutes and the reaction was refluxed for a further 25 minutes after the final addition. The reaction mixture was then poured onto crushed ice (50 mL) and stirred for 2 hours. EtOAc (50 mL) was added to the mixture and the organic layer was washed with H_2O (2x 30 mL), sat. aq. $NaHCO_3$ (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by flash chromatography on silica gel (toluene/EtOAc, 6:1→1:1), yielding **S-24** as a white solid (2.14 g, 99%). R_f = 0.44 (toluene/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.70 (1 H, d, *J* 8.2, *H*-1), 5.42 (1 H, d, *J* 3.5, *H*-4), 5.33 (1 H, t, *J* 8.2, *H*-2), 5.08 (1 H, dd, *J* 10.4, *H*-3), 4.11 - 4.20 (2 H, m, H_2-6), 4.05

(1 H, t, *J* 6.7, *H*-5), 2.16 (3 H, s, COCH_3), 2.12 (3 H, s, COCH_3), 2.04 (6 H, s, COCH_3), 1.99 (3 H, s, COCH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.3, 170.1, 170.0, 169.4, 169.0, 92.2, 71.7, 70.8, 67.8, 66.8, 61.0, 20.8, 20.6, 20.5.

***p*-Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (S-25):** Compound **S-24** (1.10 g, 2.56 mmol) was placed under N_2 and dissolved in dry CH_2Cl_2 (10 mL). *p*-Thiocresol (0.48 g, 3.8 mmol) and $BF_3{\cdot}Et_2O$ (0.57 mL, 4.6 mmol) were added and the reaction was stirred at room temperature for 2 hours. After completion, the reaction was cooled to 0 °C and quenched with Et_3N (0.45 mL). The solution was then diluted with CH_2Cl_2 (30 mL) and the organic layer was washed with brine (2x 30 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (toluene→toluene/EtOAc, 3:1) furnished **S-25** as a white, crystalline solid (1.05 g, 90%). R_f = 0.53 (toluene/EtOAc, 3:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (2 H, d, *J* 7.9, Ph*H*), 7.12 (2 H, d, *J* 7.9, Ph*H*), 5.40 (1 H, d, *J* 2.5, *H*-4), 5.21 (1 H, t, *J* 10.1, *H*-2), 5.03 (1 H, dd, *J* 3.2, 9.8, *H*-3), 4.64 (1 H, d, *J* 9.8, *H*-1), 4.09 – 4.20 (2 H, m, H_2-6), 3.90 (1 H, t, *J* 6.3, *H*-5), 2.34 (3 H, s, PhCH_3), 2.11 (3 H, s, COCH_3), 2.09 (3 H, s, COCH_3), 2.04 (3 H, s, COCH_3), 1.97 (3 H, s, COCH_3); ^{13} C NMR(126 MHz, $CDCl_3$) δ 170.3, 170.2, 170.0, 169.4, 138.5, 133.2, 129.6, 128.6, 87.0, 74.4, 72.1, 67.3, 61.6, 21.1, 20.8, 20.6.

***p*-Tolyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside (S-26):** Compound **S-25** (5.01 g, 11.0 mmol) was added to a solution of 1 M NaOMe/MeOH (7.5 mL, 7.5 mmol) and MeOH (100 mL). The reaction was stoppered and stirred at room temperature for 30 min. After completion, the solution was neutralised with Amberlite® IR120 (H^+ form) resin, filtered and concentrated to give crystalline deacetylated product (3.21 g, 100%). R_f = 0.63 (CH_2Cl_2/MeOH, 3:1); ^{1}H NMR (500 MHz, D_2O) δ 7.47 (2 H, d, *J* 7.9, Ph*H*), 7.24 (2 H, d, *J* 7.6, Ph*H*), 4.67 (1 H, d, *J* 9.8, *H*-1), 3.97 (1 H, s, *H*-4), 3.59 – 3.75 (5 H, m, *H*-2, *H*-3, *H*-5, H_2-6), 2.32 (3 H, s, PhCH_3) . *p*-Tolyl 1-thio-β-D-galactopyranoside (492 mg, 1.72 mmol) was dried *in vacuo*, placed under N_2 and cooled to 0 °C. TBDMSCl (286 mg, 1.90

mmol) and dry pyridine (2 mL) were added and the reaction was stirred for 4 hours over which the reaction mixture was allowed to reach room temperature. Upon completion, the pyridine was removed by co-evaporation with toluene (3x 5 mL). Flash chromatography on silica gel (toluene→toluene/EtOAc, 1:3) in a column with buffered silica gel (Et_3N 5% in toluene) yielded the triol product as a syrup (635 mg, 92%). R_f = 0.62 (CH_2Cl_2/MeOH 6:1); ^{1}H NMR (500MHz, $CDCl_3$) δ 7.45 (2 H, d, *J* 8.2, Ph*H*), 7.10 (2 H, d, *J* 8.2, Ph*H*), 4.45 (1 H, d, *J* 9.5, *H*-1), 4.07 (1 H, s, *H*-4), 3.87 – 3.95 (2 H, m, H_2-6), 3.65 (1 H, t, *J* 9.5, *H*-2), 3.58 (1 H, d, *J* 3.5, *H*-3), 3.50 (1 H, t, *J* 4.8, *H*-5), 0.90 (9 H, s, SiC(CH_3)$_3$), 0.10 (6 H, d, *J* 7.3, Si(CH_3)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.2, 133.0, 129.8, 128.4, 88.8, 78.1, 75.0, 69.9, 69.4, 63.2, 25.8, 21.2, 18.2 A mixture of NaH (0.14 g, 5.9 mmol) and BnBr (0.65 mL, 5.5 mmol) in dry DMF (2 mL) were placed under N_2 and cooled to 0 °C. The triol (611 mg, 1.52 mmol) was dissolved in DMF (5 mL) and was added to the reaction vessel in portions. The reaction was allowed to reach room temperature and was stirred for 2 hours. The solvent was removed by co-evaporation with toluene (2x 5 mL) before dissolving in CH_2Cl_2 (50 mL). The organic phase was washed with H_2O (25 mL), brine (25 mL), dried over $MgSO_4$, filtered and concentrated. The product was then purified by flash chromatography on silica gel (toluene→toluene/EtOAc, 49:1) in buffered silica (Et_3N 0.5% in toluene) to give compound **S-26** as a colourless syrup (898 mg, 88%). R_f = 0.46 (toluene/EtOAc, 20:1); ^{1}H NMR (500MHz, $CDCl_3$) δ 7.48 (2 H, d, *J* 8.2, Ph*H*) 7.18 – 7.42 (15 H, m, Ph*H*), 7.01 (2 H, d, *J* 7.9, Ph*H*), 5.00 (1 H, d, *J* 11.7, PhCH_2), 3.92 (1 H, t, *J* 9.5, *H*-2), 4.73 – 4.81 (4 H, m, PhCH_2), 4.64 (1 H, d, *J* 11.4, PhCH_2) 4.60 (1 H, d, *J* 9.8, *H*-1), 3.96 (1 H, m, *H*-4), 3.73 – 3.79 (2 H, m, H_2-6), 3.61 (1 H, dd, *J* 2.8, 9.5, *H*-3), 3.44 (1 H, t, *J* 6.8, *H*-5), 2.31 (3 H, s, PhCH_3), 0.90 (9 H, s, SiC(CH_3)$_3$), 0.05 (6 H, s, Si(CH_3)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.6, 136.1, 136.0, 134.7, 129.6, 128.0, 127.1, 126.4, 126.0, 125.9, 125.8, 125.7, 125.4, 125.3, 125.2, 125.2, 124.9, 122.9, 85.6, 81.9, 76.4, 73.2, 72.1, 71.1, 70.4, 69.7, 59.1, 23.6, 23.5, 19.1, 18.7, 15.8.

***p*-Tolyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (S-27)[2]:** Compound **S-26** (859 mg, 1.28 mmol) and Bu_4NF (0.41 g, 1.5 mmol) were placed under N_2 and dissolved in dry THF (1.6 mL). The reaction was stirred at room temperature for 16 hours before the volatiles were removed by co-evaporation with toluene (2x 3 mL). The yellow oil obtained was purified by flash chromatography on silica gel (toluene→toluene/EtOAc, 6:1) to give product **S-27** (682 mg, 96%). R_f = 0.15 (toluene/EtOAc, 9:1); ^{1}H NMR (500MHz, $CDCl_3$) δ 7.42 (2 H, d, *J* 8.2, Ph*H*) 7.14 – 7.38 (15 H, m, Ph*H*) 7.00 (2 H, d, *J* 7.9, Ph*H*), 4.94 (1 H, d, *J* 12.0, PhCH_2) 4.72 – 4.81 (4 H, m, PhCH_2), 4.60 (1 H, d, *J* 11.7, PhCH_2), 4.56 (1 H, d, *J* 9.8, *H*-1), 3.89 (1 H, t, *J* 9.5, *H*-2), 3.78 – 3.82 (2 H, m, H_2-6), 3.57 (1 H, dd, *J* 2.8, 9.1, *H*-3), 3.48 (1 H, dd, *J* 5, 11.3, *H*-4), 3.38 (1 H, t, *J* 6.2, *H*-5), 2.27 (3 H, s, PhCH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.4, 138.3, 138.2, 137.9, 137.4, 132.3, 130.1, 129.6, 129.1, 128.5, 128.4, 128.4, 128.3, 128.2, 127.8, 127.8, 127.7, 125.3, 88.1, 84.3, 78.7, 75.7, 74.2, 73.3, 73.1, 62.3, 21.1.

***p*-Tolyl 2,3,4-tri-*O*-benzyl-6-tosyl-1-thio-β-D-galactopyranoside (S-28)[2]:** Compound **S-27** (655 mg, 1.18 mmol) and TsCl (0.34 g, 1.8 mmol) were dried together *in vacuo* and then placed under N_2. Dry pyridine (2.5 mL) was added and the solution was stirred at room temperature for 3 hours. Upon completion, the pyridine was removed by co-evaporation with toluene (2x 5 mL) followed by addition of EtOAc (40 mL). The organic phase was washed with H_2O (2x 40 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was then purified by flash chromatography on silica gel (toluene→toluene/EtOAc, 3:1) to give compound **S-28** as a white, crystalline solid (783 mg, 94%). R_f = 0.51 (toluene/EtOAc, 9:1); ^{1}H NMR (500MHz, $CDCl_3$) 7.74 (2 H, d, *J* 8.2, Ph*H*). 7.22 – 7.39 (19 H, m, Ph*H*) 6.99 (2 H, d, *J* 7.9, Ph*H*), 4.93 (1 H, d, *J* 11.4, PhCH_2), 4.68 – 4.77 (4 H, m, PhCH_2), 4.51 (1 H, d, *J* 9.8, *H*-1), 4.47 (1 H, d, *J* 11.3, PhCH_2), 4.03 – 4.13 (2 H, m, H_2-6), 3.89 (1 H, s, *H*-4), 3.82 (1 H, t, *J* 9.5, *H*-2), 3.62 (1 H, t, *J* 6.5, *H*-5), 3.56 (1 H, dd, *J* 2.5, 9.2, *H*-3), 2.39 (3 H, s, PhCH_3), 2.30 (3 H, s, PhCH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.1, 138.2, 138.2, 138.0, 137.5, 132.4, 132.2,

129.9, 129.8, 129.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.8, 127.6, 88.0, 83.0, 75.6, 75.4, 74.4, 73.0, 73.0, 67.9, 21.7, 21.1.

***p*-Tolyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-fucopyranoside (S-29)[3]:** Compound **S-28** (115 mg, 0.162 mmol) and $LiAlH_4$ (157 mg, 4.15 mmol) were dried together *in vacuo* for 2 hours and then placed under N_2. Dry THF (2 mL) was added and the reaction mixture refluxed for 2 hours. After completion, the reaction mixture was diluted by adding THF (10 mL) and added dropwise to a solution of THF (10 mL) and glacial acetic acid (5 mL) at 0 °C. H_2O (30 mL) was added and 2 M aq. HCl was added dropwise until the precipitate dissolved. The product was then extracted with EtOAc (3x 40 mL) and the combined organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was then purified by flash chromatography on silica gel (toluene→toluene/EtOAc, 49:1) to give compound **S-29** as a white solid (51 mg, 58%). R_f = 0.42 (toluene/EtOAc, 20:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (2 H, d, *J* 7.9, Ph*H*), 7.29 – 7.41 (15 H, m, Ph*H*), 7.02 (2 H, d, *J* 7.9, Ph*H*), 5.01 (1 H, d, *J* 11.4, PhCH_2), 4.72 - 4.82 (4 H, m, PhCH_2), 4.67 (1 H, d, *J* 11.7, PhCH_2), 4.55 (1 H, d, *J* 9.8, *H*-1), 3.90 (1 H, t, *J* 9.5, *H*-2), 3.63 (1 H, d, *J* 2.6, *H*-4), 3.59 (1 H, dd, *J* 2.9, 9.2, *H*-3), 3.51 (1 H, q, *J* 6.3, *H*-5), 2.3 (3 H, s, PhCH_3), 1.27 (3 H, d, *J* 6.3, H_3-6); ^{13}C NMR (126 MHz, $CDCl_3$); 138.8, 138.5, 138.4, 137.1, 132.2, 130.5, 130.1, 129.8, 129.5, 129.1, 128.6, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.0, 127.8, 127.7, 127.6, 127.4, 87.9, 84.6, 75.5, 74.6, 72.9, 30.9, 21.1, 17.3.

1. Escopy, S.; Singh, Y.; Demchenko, A. V., Triflic acid-mediated synthesis of thioglycosides. *Organic & Biomolecular Chemistry* **2019,** *17* (36), 8379-8383.
2. Li, C.; Sun, Y.; Zhang, J.; Zhao, Z.; Yu, G.; Guan, H., Synthesis of 6'-acylamido-6'-deoxy-α-d-galactoglycerolipids. *Carbohydrate Research* **2013,** *376*, 15-23.
3. Wang, Z.; Zhou, L.; El-Boubbou, K.; Ye, X.-s.; Huang, X., Multi-Component One-Pot Synthesis of the Tumor-Associated Carbohydrate Antigen Globo-H Based on Preactivation of Thioglycosyl Donors. *The Journal of Organic Chemistry* **2007,** *72* (17), 6409-6420.
4. Sundgren, A.; Lahmann, M.; Oscarson, S., Synthesis of 6-PEtN-alpha-D-GalpNAc-(1 -> 6)-beta-D-Galp-(1 -> 4)-beta-D-GlcpNAc-(1 -> 3)-beta-D-Galp-(1 -> 4)-beta-D-Glcp, a Haemophilus influenzae lipopolysacharide structure, and biotin and protein conjugates thereof. *Beilstein Journal of Organic Chemistry* **2010,** *6*, 704-708.

5. Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H., Programmable One-Pot Oligosaccharide Synthesis. *J. Am. Chem. Soc.* **1999,** *121* (4), 734-753.
6. Lahmann, M.; Buelow, L.; Teodorovic, P.; Gybaeck, H.; Oscarson, S., Synthesis of the Lewis b hexasaccharide and HSA-conjugates thereof. *Glycoconjugate J* **2004,** *21* (5), 251-256.