CHEMBIOCHEM

Supporting Information

DOI: 10.1002/cbic.200900211

Supporting Information

for

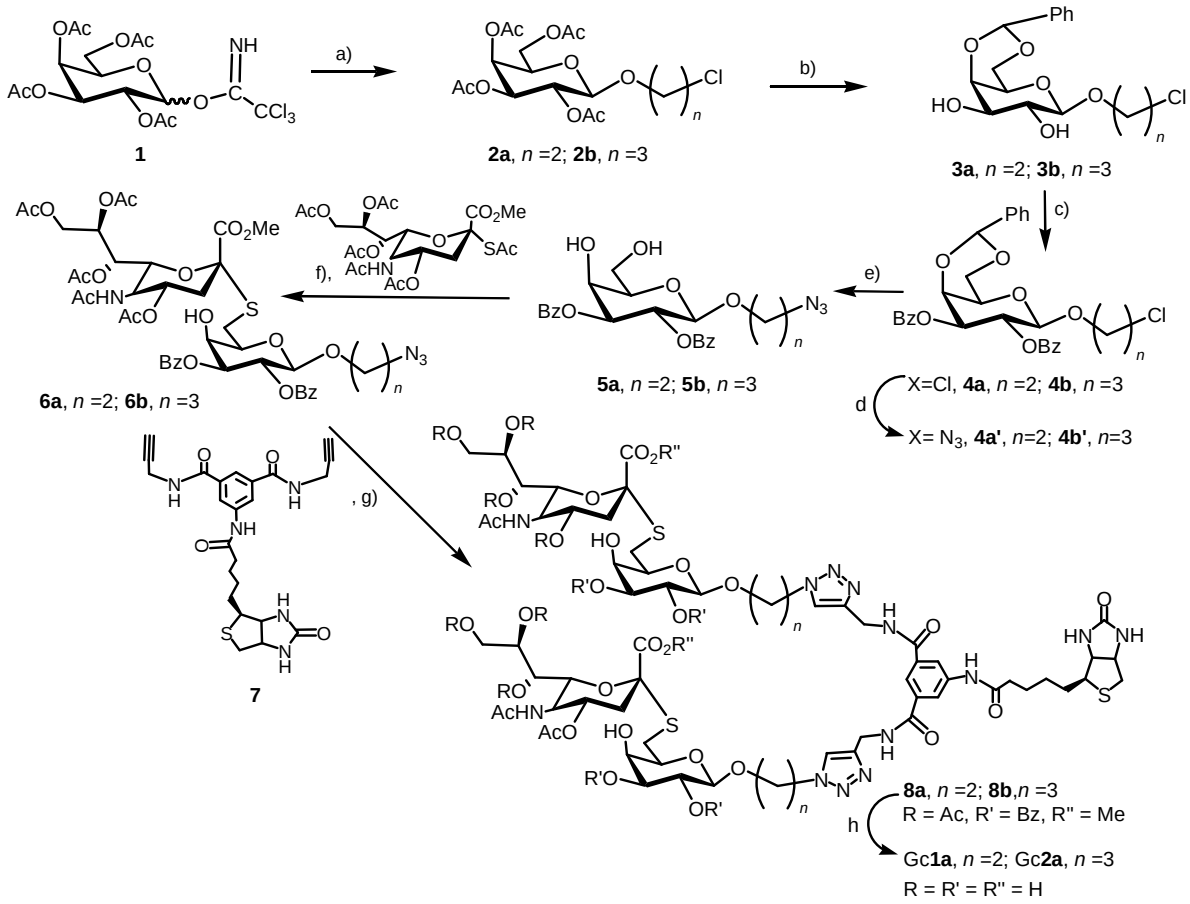
Factors Affecting Protein-Glycan Specificity: Effect of Spacers and Incubation Time

Daniel M. Lewallen, David Siler, and Suri S. Iyer*

A. Synthesis and characterization of glycans

General Methods: All chemical reagents were of analytical grade, used as supplied without further purification unless indicated. Acetic anhydride and acetyl chloride were distilled under an inert atmosphere and stored under argon. 4Å Molecular sieves were stored in an oven (>130 ºC) and cooled in vacuo. The acidic ion-exchange resin used was Dowex-50 and Amberlite. (H^+ form) Analytical thin layer chromatography (TLC) was conducted on silica gel 60-F254 (Merck). Plates were visualized under UV light, and/or by treatment with acidic cerium ammonium molybdate followed by heating. Column chromatography was conducted using silica gel (230-400 mesh) from Qualigens. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX 400 MHz spectrometer. Chemical shifts are reported in δ (ppm) units using ^{13}C and residual ^{1}H signals from deuterated solvents as references. Spectra were analyzed with Mest-Re-C Lite (Mestrelab Research) and/or XWinPlot (Bruker Biospin). Electrospray ionization mass spectra were recorded on a Micromass Q Tof 2 (Waters) and data were analyzed with MassLynx 4.0 (Waters) software.

Abbreviations: 2-chloro-4,6-dimethoxy-1,3,5-triazine, CDMT; *N*–methyl morpholine, NMM; N,N Dimethyl formamide, DMF; Ethyl acetate, EtOAc; Triisopropylsilane, TIPS; Trifluroacetic acid,TFA; Acetonitrile, CH_3CN; Dichloromethane, DCM; Dichloroethane, DCE; Trifluoromethanesulfonic anhydride, Tf_2O; Trimethylsilyltrifluoromethanesulfonate, TMSOTf; Benzaldehyde dimethyl acetal, BDA; Dichloroethane, DCE; Camphor sulfonic acid, CSA; Benzoyl chloride, BzCl; sodium azide, NaN_3; Diethylamine, DEA; Triethylamine; Et_3N.



Scheme 1. Synthesis of **Gc1a** and **Gc2a**. *Reagents and conditions:* a. TMSOTf, DCE, -25^{o}C to RT. Yield for **2a**, 52% using $HOCH_2CH_2Cl$; For **2b**, 51% using $HOCH_2CH_2CH_2Cl$. b) i: NaOMe, MeOH, 12h. ii: BDA, CSA, THF, reflux, 12 h. Yield for **3a**, 58%; for **3b**, 64%. c) BzCl, DMAP, pyridine, 50^{o}C, 12 h. Yield for 4a, 88%; for **4b**, 90%. d) NaN_3, DMF, 90^{o}C, 12 h, Yield for **4a**', 88%; for **4b'**, 86%. e) 80% AcOH, 50^{o}C, 12 h. Yield for **5a**, 80%; for **5b**, 83%. f) i: Tf_2O, pyridine, DCM, -20^{o}C to 0^{o}C, 90 min. ii: DEA, DMF, -25^{o}C to RT, 2.5 h. Yield for **6a**, 80%; for **6b**, 80%. g) $CuSO_4$, Sodium ascorbate, THF:H_2O, 24 h. Yield for **7a**, 61%; for **7b**, 67%. h) i: NaOMe, MeOH, 16 h. ii: 0.05 M NaOH, 16 h. Yield for **8a**, 20%; for **8b**, 56%.

2-Chloroethyl 2,3,4,6 tetra-*O*-acetyl-β-D-galactopyranoside (2a):

15 mL of DCE was added to a dry argon-purged flask containing 2,3,4,6 tetra-*O*-acetyl-α,β-D-galactopyranosylimidate (1.19 g, 2.47 mmol), 2-chloroethanol (0.24 g, 2.97 mmol, 1.5 equiv) and cooled to -25℃. TMSOTf (2.24 mL, 0.50 m mol, 0.2 equiv) was added dropwise and the reaction stirred for 2 h while warming to RT. Neutralization with Et_3N and concentration in vacuo yielded a white solid which was purified by flash chromatography (60:40 hexanes/ EtOAc) to give **2a** (0.53 g, 52%). NMR and mass spectral analysis matched reported values.[1]

2-Chloroethyl 4,6 *O*-benzylidene β-D-galactopyranoside (3a):

10 mL of MeOH was added to a dry argon-purged flask containing **2a** (0.43 g, 1.02 mmol). NaOMe (6.5 mL, 3.25 mmol, 3 equiv) was added dropwise and the reaction stirred for 12 h. The reaction was neutralized with H^+ resin and concentrated in vacuo. The crude residue was dissolved in 10 mL of THF in a dry argon purged flask. BDA (0.27 mL, 1.77 mmol, 1.7 equiv) and CSA (50mg) was added and the flask heated to 80℃ for 12h. The reaction was neutralized with Et_3N and concentrated in vacuo. Purification by flash chromatography (100% EtOAc) yielded **3a** (0.20 g, 58% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52 (m, 2H, ArH), 7.32 (m, 3H, ArH), 5.59 (s, 1H, C*H*Ph), 4.39 (d, 1H, H-1, $J_{1,2}$ = 7.6Hz), 4.35, (dd, 1H, H6-a/b, *J* = 1.2 Hz, *J* = 12.4 Hz), 4.26-4.21 (m, 2H, H-4, OCH_2CH$_2$Cl) 4.13 (dd, 1H, H6-a/b, *J* = 2.0Hz, *J* = 12.8Hz), 3.85-3.81 (m, 2H, OCH_2CH$_2$Cl, H-2), 3.77-3.71 (m, 3H, H-3, OCH$_2$-CH_2Cl) 3.43 (vt, 1H, H-5), 3.08 (s, 1H, C$_2$-OH, *J*=1.2 Hz), 2.53 (d, 1H, C$_3$-OH, *J* = 8Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 137.5, 129.2, 128.3, 126.4, 103.1, 101.4, 75.3, 72.5, 71.5, 69.6, 69.1, 66.8, 42.7. HRMS (ESI): *m/z* calcd for $[C_{15}H_{19}O_6ClNa]^+$: 353.0768; found 353.0774.

2-Chloroethyl 2,3 di-*O*-benzoyl 4,6-*O*-benzylidene β-D-galactopyranoside (4a):

5 mL of pyridine was added to a dry argon purged flask containing **3a** (0.19 g, 0.60 mmol). DMAP (30mg) and BzCl (0.21 mL, 1.80 mmol, 3 equiv) was added dropwise to the flask and heated at 50℃ for 12h. After cooling to RT, 2ml of MeOH was added and the reaction was concentrated in vacuo. Purification by flash chromatography (hexanes: EtOAc, 75:25 → 50: 50) yielded **4a** (0.29g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ =8.01 (m, 4H, ArH), 7.53 (m, 4H, ArH), 7.38 (m, 7H, Ar), 5.90 (dd, 1H, H-2, *J* = 10.4Hz, 8Hz), 5.58 (s, 1H, C*H*Ph), 5.39 (dd, 1H, H-3, *J* = 3.6Hz, 10.4Hz), 4.88 (d, 1H, H-1, *J* = 8Hz), 4.62 (br, d, 1H, H-4), 4.43 (d, 1H, H-6a/b, *J* = 12.4Hz), 4.19 (m, 2H, H-6a/b, OCH_2CH$_2$Cl), 3.87 (m, 1H, OCH_2CH$_2$Cl), 3.72 (m, 1H, H-5), 3.62 (m, 2H, OCH$_2$CH_2Cl).^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.2, 165.4, 137.4, 133.4, 133.1, 130.0, 129.8, 129.6, 129.1, 129.0, 128.7, 128.4, 128.3, 128.2, 127.0, 126.2, 101.4, 100.9, 73.5, 72.6, 69.1, 68.9, 68.8, 66.7, 42.5. HRMS (ESI): *m/z* calcd for $[C_{29}H_{27}O_8ClNa]^+$: 561.1292; found 561.1313.

2-Azidoethyl 2,3 di-*O*-benzoyl 4,6-*O*-benzylidene β-D-galactopyranoside (4a'):

20 mL DMF was added to a dry argon purged flask containing **4a** (0.29g, 0.53 mmol) and NaN_3 (0.06 g, 0.91mmol, 1.7 equiv). The reaction was heated to 90℃ for 12h before diluting with EtOAc and washing with H_2O and concentrated in vacuo. Purification by column chromatography (hexanes: EtOAc 75:25 → 60:40) yielded **4a'** (.25g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (m, 4H, ArH), 7.51 (m, 5H, ArH), 7.36 (m, 6H, ArH), 5.94 (dd, 1H, H-2, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 8.0 Hz), 5.59 (s, 1H, C*H*Ph), 5.41 (dd, 1H, H-3, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.6 Hz), 4.88 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.63 (br, d, 1H, H-4), 4.43 (dd, 1H, H-6a/b, *J* = 1.6 Hz, *J* =

12.4 Hz), 4.17 (dd, 1H, H-6a/b, *J* = 1.6 Hz, *J* = 12.4 Hz), 4.13 (m, 1H, OC*H*$_2$CH$_2$N$_3$), 3.78 (m, 1H, OC*H*$_2$CH$_2$N$_3$), 3.73 (m, 1H, H-5), 3.47 (1H, m, OCH$_2$C*H*$_2$N$_3$), 3.35 (m, 1H, OCH$_2$C*H*$_2$N$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.2, 165.3, 137.4, 133.4, 133.1, 130.0, 129.8, 129.7, 129.1, 129.0, 128.5, 128.3, 128.2, 126.3, 101.1, 101.0, 73.5, 72.7, 69.0, 68.8, 67.5, 66.7, 50.7. HRMS (ESI): *m/z* calcd for $[C_{29}H_{27}N_3O_8Na]^+$: 568.1696; found 568.1670.

2-Azido-ethyl 2,3 di-*O*-benzoyl β-D-galactopyranoside (5a):

10 mL of 80% acetic acid was added to an argon-purged flask containing **4a'** (0.25 g, 0.47 mmol). The flask was heated to 50℃ for 12h and concentrated in vacuo. Purification by column chromatography (EtOAc/hexanes 80:20 → 90:10) yielded **5a** (.17g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.97 (m, 4H, ArH), 7.48 (m, 2H, ArH), 7.36 (m, 4H, ArH), 5.83 (dd, 1H, H-2, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.4 Hz), 5.33 (dd, 1H, H-3, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.2 Hz), 4.87 (d, 1H, H-1, $J_{1,2}$ = 8 Hz), 4.45 (br,d, 1H, H-4), 4.09 (m, 1H, OC*H*$_2$CH$_2$N$_3$), 4.02 (m, 2H, H-6a/b), 3.83 (t, 1H, H-5, 5.2 Hz), 3.77 (m, 1H, OC*H*$_2$CH$_2$N$_3$), 3.58 (s, 1H, H-4-O*H*), 3.44 (m, 1H, OCH$_2$-C*H*$_2$N$_3$), 3.33 (m, 1H, OCH$_2$C*H*$_2$N$_3$), 3.11 (s, 1H, H-6-O*H*) ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.0, 165.6, 133.5, 133.2, 129.9, 129.7, 129.5, 129.1, 128.5, 128.4, 101.5, 74.5, 74.4, 69.5, 68.2, 68.0, 62.2, 50.7. HRMS (ESI): *m/z* calcd for $[C_{22}H_{23}N_3O_8Na]^+$: 480.1383 ; found 480.1341.

2-Azidoethyl-S-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→6)-2,3-di-*O*-acetyl-6-thio-β-D-galactopyranoside (6a):

3ml of DCE was added to a dry argon-purged flask containing **5a** (166 mg, 0.36 mmol), followed by pyridine (0.15 mL, 1.81 mmol, 5equiv). After cooling to -25℃, Tf_2O (0.22 M, 0.075 mL, 0.43 mmol, 1.2 equiv) was added and the flask slowly warmed to RT. After 2.5h, com-plete conversion to the triflate was observed by TLC. The reaction was diluted with 10 mL of DCM and sequentially washed with 1 M HCl, sat. $NaHCO_3$, water, dried over Na_2SO_4 and concentrated in vacuo. The crude triflate and methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-S-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-non-2-ulopyranosylonate was dissolved in 3 mL of DMF. After cooling to -25℃, DEA (0.37 mL, 3.62 mmol, 10equiv) was added dropwise and the reaction allowed to warm to RT over 2.5 h and concentrated in vacuo. Purification by flash chromatography using 100% EtOAc yielded **6a** (0.27g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.90 (m, 4H, ArH), 7.45 (m, 2H, ArH), 7.31 (m, 4H, ArH), 5.72 (dd, 1H, H-2, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 2.4 Hz), 5.42-5.31 (m, 4H, H-3,H'-7, H'-8, N-H), 4.94-4.89 (m, 2H, H'-4, H-1), 4.46 (m, 1H, H'-9a/b), 4.36 (dd, 1H, H-4, *J* = 12.4 Hz, *J* = 2.8 Hz), 4.17-4.01 (m, 1H, H'-9a/b), 3.86-3.78 (m, 7H, CO_2*Me*,H'-5, H-6a, OC*H*$_2$), 3.48 (m, 2H, H-5, H-6b), 3.09-2.96 (m, 2H, C*H*$_2$N$_3$), 2.76 (1H, H'-3e, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 8.4Hz), 2.18 (s, 3H, O*Ac*), 2.08 (s, 3H,

O*Ac*), 2.01(s, 3H, O*Ac*), 1.98 (s, 3H, O*Ac*),1.89(s, 3H, NH*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 170.8, 170.4, 170.3, 170.2, 168.5, 165.8, 165.5, 133.3, 133.0, 129.8, 129.7, 129.3, 128.4, 128.3, 108.0, 101.0, 83.6, 74.3, 74.2, 74.0, 69.5, 69.4, 68.2, 68.0, 67.7, 67.0, 62.4, 53.2, 50.6, 49.3, 42.9, 40.0, 38.0, 29.8, 23.2, 23.1, 21.4, 21.3, 20.9, 20.8, 14.2. HRMS (ESI): *m/z* calcd for $[C_{42}H_{50}N_4O_{19}SNa]^+$: 969.2687; found 969.2665.

Compound 8a:

4 mL of 1:1 THF:H_2O was added to an argon-purged flask containing **6a** (78 mg, 0.083 mmol, 2.2 equiv). Next, the dimeric scaffold bearing the biotin and the two alkynes, **7**,[2] (18 mg, 0.038 mol, 1 equiv), $CuSO_4$ (15 mg, 0.056 mmol, 1.5 equiv) and sodium ascorbate (19 mg, 0.094 mmol, 2.5 equiv) were added and the reaction stirred for 16h. Next, the reaction mixture was filtered and concentrated in vacuo. Purification by flash chromatography (5:1 DCM: MeOH) yielded **8a** (54 mg, 61%). ^{1}H NMR (400 MHz, MeOD): δ = 8.14 (s, 2H), 7.92 (m, 10H), 7.50 (m, 5H), 7.37 (m, 9H), 5.65 (m, 2H), 5.42 (dd, 4H, *J*=2.8Hz, 10Hz), 5.33 (m, 3H), 5.10 (d, 2H, *J*=8Hz), 4.60 (br, s, 6H), 4.51 (m, 2H), 4.40 (m, 5H), 4.32-4.22 (m, 14H), 4.09 (m, 7H), 3.96 (s, 6H), 3.81 (m, 3H), 3.60 (s, 1H), 3.34 (s, 3H), 3.20 (br, s, 2H), 2.89 (m, 5H), 2.80 (m, 3H), 2.72 (d, 2H, *J*=12.4Hz), 2.36 (m, 2H), 2.22 (s, 6H), 2.17 (s, 6H), 2.01 (s, 6H), 1.96 (s, 6H), 1.88 (s, 6H), 1.69 (m, 4H), 1.45 (m, 3H), 1.31 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ = 172.0, 171.0, 170.5, 170.4, 170.3, 168.3, 165.7, 133.3, 133.2, 133.1, 133.1, 129.4, 129.4, 129.3, 128.3, 128.2, 100.5, 88.1, 87.8, 84.5, 75.6, 74.6, 74.6, 73.9, 73.7, 73.4, 73.3, 70.0, 69.7, 67.8, 67.4, 66.2, 62.3, 62.2, 61.9, 60.2, 52.4, 37.8, 29.6, 28.4, 28.1, 21.4, 20.1, 19.6, 19.5, 19.4. HRMS (ESI): *m/z* calcd for $[C_{108}H_{127}N_{13}O_{42,}S_3+2H]^{2+}$: 1188.3771; found 1188.3551.

Biotinylated biantennary *S*-sialoside (Gc1a):

3 mL MeOH was added to a dry argon purged flask containing **8a** (54 mg, 0.023 mmol). NaOMe (800 µL, 0.41 mmol, 18 equiv) and the reaction stirred for 18 h before neutralization with H+ resin, filtered and concentrated in vacuo. Next, 4 mL of 0.05M NaOH was added and the reaction stirred overnight. The reaction was neutralized with H^+ resin and concentrated in vacuo. Purification by size-exclusion chromatography (Biogel-10) yielded **Gc1a** (7.0 mg, 20%). ^{1}H NMR (400 MHz, D_2O): δ = 7.94 (s, 1H), 7.90 (s, 1H), 7.86 (s, 1H), 4.56 (m, 7H), 4.46 (dd, 1H, *J*= 5.2Hz, 7.6Hz), 4.28 (m, 1H), 4.20 (d, 2H, *J*=8Hz), 4.16 (m, 2H), 4.02 (m, 2H), 3.83 (d, 2H, *J*=3.2Hz), 3.73-3.41 (m, 19H), 3.29 (t, 2H, *J*=8Hz), 3.20 (m, 1H), 2.84 (dd, 1H, *J*=4.8 Hz, 13.2 Hz), 2.70-2.60 (m, 6H), 2.34 (t, 1H, *J*=7.2Hz), 1.90 (s, 6H), 1.62-1.54 (m, 6H), 1.35 (m, 2H). ^{13}C NMR (100 MHz, D_2O): δ = 174.9, 103.1, 83.4, 73.8, 73.8, 72.7, 71.0, 70.3, 68.7, 68.4, 67.5, 63.0, 54.0, 53.6, 51.6, 50.5, 39.9, 29.0, 22.1. HRMS (ESI): *m/z* calcd for $[C_{62}H_{91}N_{13}O_{30,}S_3+2H]^{2+}$: 797.7651; found 797.7676.

3-Chloropropyl 4,6 *O*-benzylidene β-D-galactopyranoside (3b):

2b was synthesized from **1** (1.01g, 2.29 mmol) and 3-chloropropanol (0.28 g, 2.98 mmol, 1.5 equiv) following a procedure described for **2a**. Purification by flash chromatography (60: 40 hexanes/EtOAc) yielded **2b** (0.49g, 51%). Next, **3b** was synthesized from **2b** (0.49g, 1.16 mmol) in a manner described for **3a**. Purification by flash chromatography (100% EtOAc) yielded **3b** (.26 g, 64% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52 (m, 2H, ArH), 7.36 (m, 3H, ArH), 5.47 (s, 1H, C*H*Ph), 4.27-4.23 (m, 2H, H-1, $H6_{a/b}$), 4.01-3.98 (m, 3H, H-4, OCH_2CH_2Cl, $H6_{a/b}$), 3.73-3.62 (m, 5H, $OCH_2CH_2CH_2Cl$, H-2, H-3, C_3-OH, C_4-OH), 3.39-3.32 (m, 3H, H-5, $OCH_2CH_2CH_2Cl$), 2.07 (m, 2H, $OCH_2CH_2CH_2Cl$) . ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.7, 129.2, 128.2, 126.5, 103.0, 101.2, 75.4, 72.6, 71.4, 69.1, 66.6, 66.5, 42.0, 32.6. HRMS (ESI): *m/z* calcd for $[C_{16}H_{21}O_6,Cl + Na]^+$: 367.0924; found 367.0928.

3-Chloropropyl 2,3 di-*O*-benzoyl 4,6 *O*-benzylidene β-D-galactopyranoside (4b):

4b was synthesized from **3b**(0.26 g, 0.74 mmol) in a manner described for **4a**. Purification by flash chromatography (60:40 hexanes/EtOAc) yielded **4b** (0.37g,90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.02 (m, 4H, ArH), 7.53 (m, 4H, ArH), 7.40 (m, 6H, ArH), 5.88 (dd, 1H, H-2, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 8 Hz), 5.58 (s, 1H, C*H*Ph), 5.39 (dd, 1H, H-3, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.6 Hz), 4.78 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.62 (br, d, 1H, H-4), 4.43 (dd, 1H, H-6a/b, *J* = 1.2 Hz, *J* = 12.4 Hz), 4.17 (dd, 1H, H-6a/b, *J* = 1.6 Hz, *J* = 12.4 Hz), 4.11 (m, 1H, $OCH_2CH_2CH_2Cl$), 3.75 (m, 1H, $OCH_2CH_2CH_2Cl$), 3.72 (1H, m, H-5), 3.27 (m, 2H, $OCH_2CH_2CH_2Cl$), 1.81 (m, 2H, $OCH_2CH_2CH_2Cl$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.2, 165.3, 133.4, 133.2, 130.0, 129.7, 129.0, 128.4, 128.4, 128.1, 126.3, 101.6, 100.9, 73.6, 72.8, 69.2, 69.0, 66.6, 66.0, 41.6, 32.4. HRMS (ESI): *m/z* calcd for $[C_{30}H_{29}O_8Cl + Na]^+$: 575.2448; found 575.1428.

3-Azidopropyl 2,3 di-*O*-benzoyl 4,6 *O*-benzylidene β-D-galactopyranoside (4b'):

4b was synthesized from **3b** (0.37g, 0.66 mmol) in a manner described for **4a'**. Purification by flash chromatography (hexanes: EtOAc 70:30→ 60:40) yielded **4b'** (0.32g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.02 (m, 4H, ArH), 7.53 (m, 4H, ArH), 7.40 (m, 6H, ArH), 5.88 (dd, 1H, H-2, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 8 Hz), 5.58 (s, 1H, C*H*Ph), 5.39 (dd, 1H, H-3, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.6 Hz), 4.77 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.62 (br, d, 1H, H-4), 4.43 (dd, 1H, H-6a/b, *J* = 1.2 Hz, *J* = 12.4 Hz), 4.17 (dd, 1H, H-6a/b, *J* = 1.6 Hz, *J* = 12.4 Hz), 4.06 (m, 1H, $OCH_2CH_2CH_2N_3$), 3.71 (1H, m, H-5), 3.62 (m, 1H, $OCH_2CH_2CH_2N_3$), 3.27 (m, 2H, $OCH_2CH_2CH_2N_3$), 1.81 (m, 2H, $OCH_2CH_2CH_2N_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.2, 165.3, 133.2, 130.0, 129.7, 129.0, 128.4, 128.2, 126.3, 101.4, 100.9, 73.6, 72.7, 69.1, 69.0, 66.6, 66.1, 48.0, 29.0. HRMS (ESI): *m/z* calcd for $[C_{30}H_{29}N_3O_8 + Na]^+$: 582.1852; found 582.1831.

3-Azidopropyl 2,3 di-*O*-benzoyl β-D-galactopyranoside (5b):

5b was synthesized from **4b'** (0.16 g, 0.29 mmol) in a manner similar to the synthesis of **5a**. Purification by column chromatography (80:20 EtOAc: hexanes) yielded **5b** (0.11 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99 (m, 4H, ArH), 7.53 (m, 2H, ArH), 7.40 (m, 4H, ArH), 5.80 (dd, 1H, H-2, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.4 Hz), 5.32 (dd, 1H, H-3, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.2 Hz), 4.74 (d, 1H, H-1, $J_{1,2}$ = 8 Hz), 4.43 (br,d, 1H, H-4, $J_{3,4}$ = 3.2 Hz), 4.02 (m, 3H, H-6a/b, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.81 (t, 1H, H-5, 4.8 Hz), 3.67 (m, 1H, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.29 (m, 2H, OCH$_2$CH$_2$-C*H*$_2$N$_3$), 2.95 (s, 1H, H-4-O*H*), 2.45 (s, 1H, H-6-O*H*), 1.81 (m, 2H, OCH$_2$C*H*$_2$CH$_2$N$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ =165.9, 133.5, 133.3, 129.9, 129.7, 128.5, 128.4, 101.6, 76.7, 74.3, 74.2, 69.6, 68.5, 66.3, 62.7, 47.8, 29.0. HRMS (ESI): *m/z* calcd for [$C_{23}H_{25}N_3O_8$+ Na]$^+$: 494.1539 ; found 494.1531.

3-Azidopropyl-S-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→6)-2,3-di-*O*-acetyl-6-thio-β-D-galactopyranoside (6b):

6b was synthesized from **5b** (109 mg, 0.23 mmol) in a manner similar to the synthesis of **6a**. Purification by column chromatography (100% EtOAc) yielded **5b** (177 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (m, 4H, ArH), 7.53 (m, 2H, ArH), 7.40 (m, 4H, ArH), 5.72 (dd,1H, H-2, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 10.4 Hz), 5.41-5.31 (m, 4H, H-3, H'-7, H'-8, N-H), 4.95 (ddd, 1H, H'-4, Hz, $J_{4,3a}$ = 7.2 Hz, $J_{4,5}$ = 4.4 Hz, $J_{4,3e}$ = 1.6 Hz), 4.79 (d, 1H, H-1, $J_{1,2}$ = 8 Hz), 4.45 (dd, 1H, H'-9a/b, *J* = 4Hz, *J* = 5.2 Hz), 4.37 (dd, H-4, *J* = 12.4 Hz, *J* = 2.4 Hz), 4.15 (dd, 1H, H'-9a/b, *J* = 5.2 Hz, *J* = 12.4 Hz), 3.86-3.78 (m, 7H, CO$_2$*Me*,H'-5, H-6a/b, OC*H*$_2$CH$_2$), 3.63-3.59 (m, 2H, H-5, H-6a/b), 3.48 (vt, 2H, OCH$_2$C*H*$_2$, *J* = 5.2 Hz), 3.09-2.96 (m, 4H, OC*H*$_2$C*H*$_2$N$_3$), 2.76 (1H, H'-3e, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 8.4Hz), 2.18 (s, 3H, O*Ac*), 2.08 (s, 3H, O*Ac*), 2.01(s, 3H, O*Ac*), 1.98 (s, 3H, O*Ac*),1.89(s, 3H, NH*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 129.8, 129.7, 128.5, 128.4, 101.2, 74.0, 69.5, 69.4, 68.2, 66.3, 62.3, 53.3, 48.0, 29.9, 29.1, 23.3, 21.3, 20.9, 20.9, 20.8. HRMS (ESI): *m/z* calcd for [$C_{43}H_{52}N_4O_{19}S$+ Na]$^+$: 983.2844; found 983.2843.

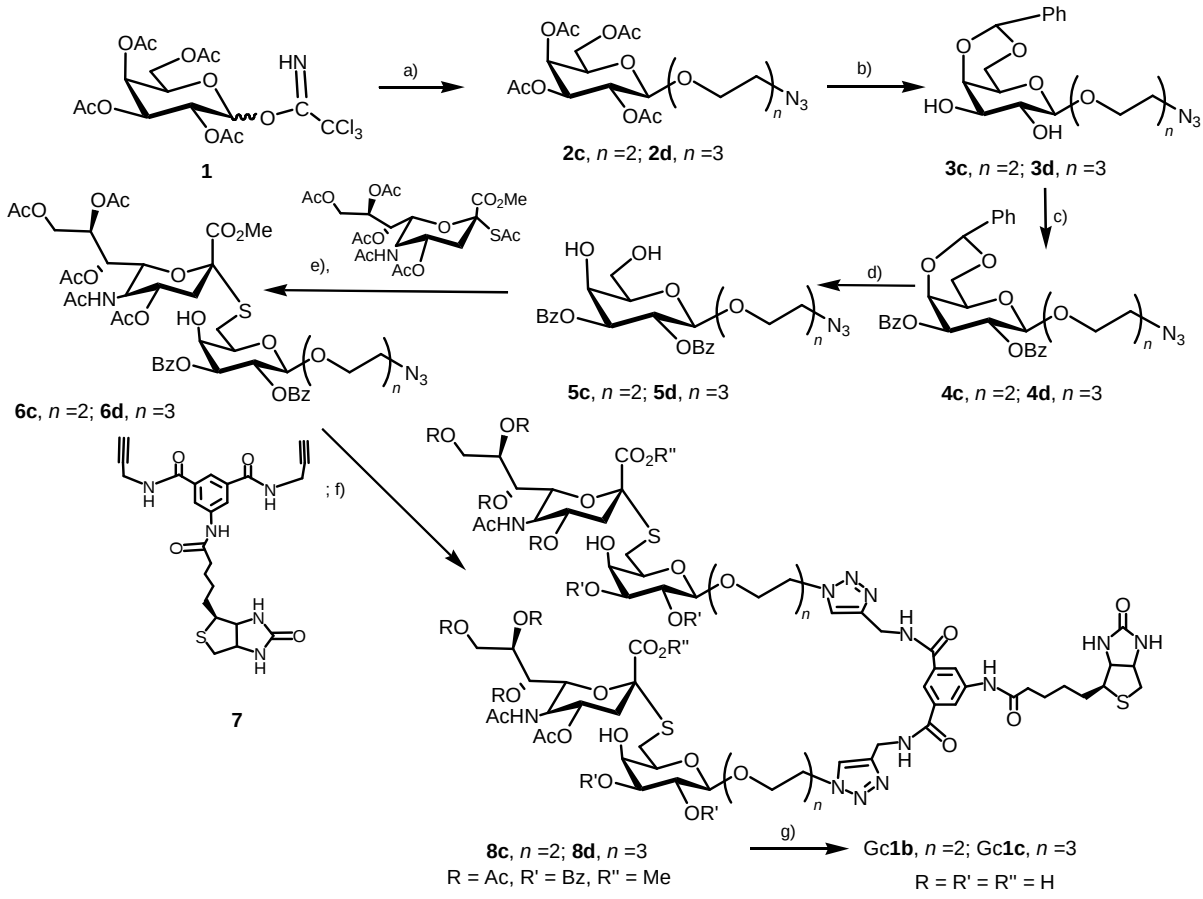
Compound 8b:

8b was synthesized from **6b** (86 mg, 0.09 mmol) in a manner similar to the synthesis of **8a**. Purification by column chromatography (DCM:MeOH 10:1 → 5:1) yielded **8b** (68 mg, 67%). ^{1}H NMR (400 MHz, MeOD): δ = 8.16 (br,s, 2H), 7.95 (m, 8H), 7.80 (br, s, 2H), 7.50 (m, 6H), 7.38 (m, 10H), 5.68 (dd, 2H, *J*= 8Hz, 10Hz), 5.43 (m, 4H), 5.35 (m, 2H), 5.05 (d, 2H, *J*= 7.6Hz), 4.61 (m, 6H), 4.50 (m, 2H), 4.38 (m, 8H), 4.21 (dd, 2H, *J*=4.8, 12.4Hz), 4.07 (vt, 2H, *J*=6Hz), 3.96 (m, 7H), 3.86 (s, 6H), 3.73 (m, 2H), 3.69 (m, 2H), 3.21 (m, 1H), 2.93 (d, 2H, *J*=6.8Hz), 2.80 (dd, 2H, *J*=4.4, 12.8Hz), 2.72 (d, 1H, *J*=12.4Hz), 2.58 (br, s, 2H), 2.39 (br,s,

3H), 2.20 (s, 6H), 2.16 (s, 6H), 2.00 (s, 6H), 1.98 (s, 6H), 1.88 (s, 6H), 1.74 (m, 4H), 1.47 (m, 3H). ^{13}C NMR (100 MHz, MeOD): δ = 172.0, 171.0, 168.4, 165.8, 165.8, 135.1, 133.2, 133.0, 129.3, 129.2, 128.3, 128.1, 100.6, 84.3, 74.7, 73.8, 73.7, 70.2, 69.7, 67.9, 67.0, 66.4, 65.7, 65.6, 62.1, 61.9, 60.2, 54.6, 52.4, 39.9, 37.9, 36.2, 34.9, 29.9, 29.7, 28.3, 28.1, 25.1, 21.4, 20.1, 19.6, 19.5, 19.4. HRMS (ESI): *m/z* calcd for $[C_{110}H_{131}N_{13}O_{42}S_3+ 2Na]^{2+}$: 1224.3746; found 1224.3835.

Biotinylated biantennary *S*-sialoside (Gc2a):

Gc2a was synthesized from **8b** (68 mg, 0.03 mmol) in a manner similar to the synthesis of **Gc1a**. Purification by size exclusion chromatography (Biogel-10) yielded **8b** (25 mg, 56%). ^{1}H NMR (400 MHz, D_2O): δ = 7.97 (m, 5H), 4.59 (m, 2H), 4.43 (br,s, 5H), 4.24 (br, s, 1H), 4.16 (d, 1H, *J*=5.6Hz), 3.85 (s, 2H), 3.68 (m, 9H), 3.50 (m, 15H), 3.30 (m, 2H), 3.12 (br, s, 1H), 2.78 (m, 4H), 2.63 (m, 3H), 2.27 (m, 2H), 2.09 (m, 3H), 1.91 (s, 6H), 1.55 (m, 6H), 1.27 (m, 5H).^{13}C NMR (100 MHz, D_2O): δ = 175.0, 168.6, 165.2, 102.9, 100.0, 74.9, 74.2, 72.8, 72.2, 70.6, 69.0, 68.4, 68.0, 66.5, 66.5, 62.5, 62.0, 60.2, 55.3, 51.7, 40.8, 39.7, 29.5, 29.5, 29.4, 28.0, 27.9, 27.7, 24.9, 24.8, 22.0. HRMS calculated $[C_{64}H_{95}N_{13}O_{30}S_3+Na]^+$: 1644.5367; found 1644.5287.



Scheme 2. Synthesis of **Gc1b** and **Gc1c**. Reagents and conditions: a) TMSOTf, DCE, -25^{o}C to RT. Yield for **2c**, 57% using $H(OCH_2CH_2)_2N_3$; for **2d**, 65% using $H(OCH_2CH_2)_3N_3$. b) i: NaOMe, MeOH, 12 h. ii: BDA, CSA, THF, reflux, 12 h, Yield for **3c**, 50%; for **3d**, 65%. c) BzCl, DMAP, pyridine, 50^{o}C, 12 h, Yield for **4c**, 91%; for **4d**, 80%. d) 80% AcOH, 50^{o}C, 12 h, Yield for **5c**, 91%; for **5d**, 71%. e) i: Tf_2O, pyridine, DCM, -20^{o}C to 0^{o}C, 1.5 h, ii: DEA, DMF, -25^{o}C to RT, 2.5 h Yield for **6c**, 67%; for **6d**, 70%. f) $CuSO_4$, Sodium ascorbate, THF/H_2O, 24 h, Yield for **8c**, 72%; for **8d**, 47%. g) NaOMe, MeOH, 16 h. ii: 0.05 M NaOH, 16 h, Yield for **Gc1b**, 73%; for **Gc1c**, 99%.

2-(2-Azido-ethoxy) ethyl 2,3,4,6 tetra-*O*-acetyl-β-D-galactopyranoside (2c):

2c was synthesized from **1** (0.86g, 1.79 mmol) and 2-(2-azido)ethanol (0.35 g, 2.68 mmol, 1.5 equiv) following a procedure described for **2a**. Purification by flash chromatography (60: 40 hexanes/EtOAc) yielded **2c** (0.47g, 57%). NMR and mass spectral analysis matched reported values.[3]

2-(2-Azido-ethoxy)-ethyl 4,6 *O*-benzylidene β-D-galactopyranoside (3c):

3c was synthesized from **2c** (.47g, 1.02 mmol) in a manner described for **3a**. Purification by flash chromatography (100% EtOAc) yielded **3c** (.19 g, 50% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53 (m, 2H, Ar), 7.38 (m, 3H, Ar), 5.58 (s, 1H, C*H*Ph), 4.41 (d, 1H, H-1, *J*= 7.6Hz), 4.35 (dd, 1H, H6a/b *J*= 1.2Hz, 12.4Hz), 4.25 (dd, 1H, H-4 *J* = 0.8Hz, 3.6Hz), 4.15-4.10 (m, 2H), 3.82-3.72 (m, 8H), 3.53 (m, 1H, H-5), 3.43 (vt, 2H, *J*= 5.2Hz, CH_2N_3), 2.97 (d, 1H, C_2-OH, *J*= 1.6Hz), 2.53 (d, 1H, C_3-OH, *J* = 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.5, 129. 2, 128.2, 126.4, 103.2, 101.4, 75.3, 72.6, 71.5, 70.4, 70.1, 69.2, 68.6, 66.8, 50.7. HRMS (ESI): *m/z* calcd for $[C_{17}H_{23}N_3O_7 + Na]^+$: 404.1434; found 404.1439.

2-(2-Azido-ethoxy)-ethyl 2,3 di-*O*-benzoyl 4,6 *O*-benzylideneβ-D-galactopyranoside (4c):

4c was synthesized from **3c**(0.19 g, 0.50 mmol) in a manner described for **4a**. Purification by flash chromatography (75:25 → 50:50 hexanes/EtOAc) yielded **4c** (.14g, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (m, 4H, ArH), 7.51 (m, 5H, ArH), 7.36 (m, 6H, ArH), 5.87 (dd, 1H, H-2, $J_{2,3}$ = 10Hz, $J_{1,2}$ = 7.6 Hz), 5.55 (s, 1H, C*H*Ph), 5.37 (dd, 1H, H-3, $J_{2,3}$ = 10 Hz, $J_{3,4}$ = 3.6 Hz), 4.87 (d, 1H, H-1, $J_{1,2}$ = 7.6 Hz), 4.60 (br, d, 1H, H-4), 4.40 (dd, 1H, OC$H_2$$CH_2$O, *J* = 12.4 Hz, *J* = 1.2 Hz), 4.15 (dd, 1H, OC$H_2$$CH_2$O, *J* = 12.4 Hz, *J* = 1.2 Hz), 4.05 (m, 1H, H-6a/b), 3.80 (m, 1H, H-6a/b), 3.69 (1H, br, s, $OCH_2C$$H_2$O), 3.62 (1H, m, H-5), 3.61 (1H, br, s, $OCH_2C$$H_2$O), 3.47 (vt, 2H, OC$H_2$$CH_2N_3$, *J* = 4.8 Hz), 3.04 (m, 2H, $OCH_2C$$H_2N_3$). ^{13}C NMR (100 MHz, $CDCl_3$): HRMS (ESI): *m/z* calcd for $[C_{31}H_{31}N_3O_9 + Na]^+$: 612.1958 ; found 612.1954.

2-(2-Azido-ethoxy)-ethyl 2,3 di-*O*-benzoyl β-D-galactopyranoside (5c):

5c was synthesized from **4c** (0.14 g, 0.24 mmol) in a manner similar to the synthesis of **5a**. Purification by column chromatography (100% EtOAc) yielded **5c** (0.11 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.97 (m, 4H, ArH), 7.48 (m, 2H, ArH), 7.36 (m, 4H, ArH), 5.80 (dd, 1H, H-2, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 10.4 Hz), 5.30 (dd, 1H, H-3, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.2 Hz), 4.87 (d, 1H, H-1, $J_{1,2}$ = 8 Hz), 4.42 (br,d, 1H, H-4, *J* = 2.8 Hz), 3.78 (m, 3H, H-6a/b, OCH_2CH_2O), 3.58 (m, 2H, H-5, H-6a/b), 3.46 (m, 2H, OCH_2C$H_2$$N_3$), 3.08 (m, 2H, OC$H_2CH_2$$N_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.0, 165.5, 133.4, 133.2, 129.9, 129.7, 129.6, 129.1, 128.5, 128.4, 101.7, 74.4, 74.2, 70.5, 70.1, 69.8, 69.4, 68.2, 62.4, 50.6. HRMS (ESI): *m/z* calcd for [$C_{24}H_{27}N_3O_9$+ Na]$^+$: 524.1645 ; found 524.1645.

2-(2-Azido-ethoxy)-ethyl S-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→6)-2,3-di-*O*-acetyl-6-thio-β-D-galactopyranoside (6c):

6c was synthesized from **5c** (54 mg, 0.11 mmol) in a manner similar to the synthesis of **6a**. Purification by column chromatography (100% EtOAc) yielded **6c** (72 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.90 (m, 4H, ArH), 7.45 (m, 2H, ArH), 7.31 (m, 4H, ArH), 5.72 (dd,1H, H-2, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 2.4 Hz), 5.34-5.31 (m, 4H, H-3,H'-7, H'-8, N-H),4.91 (ddd, 1H, H'-4, Hz, $J_{4,3a}$ = 7.2 Hz, $J_{4,5}$ = 4.4 Hz, $J_{4,3e}$ = 1.6 Hz), 4.85 (d, 1H, H-1, $J_{1,2}$ = 8 Hz), 4.42 (m, 1H, H'-9a/b), 4.35 (dd, H-4, *J* = 12.4 Hz, *J* = 2.4 Hz), 4.12 (m, 1H, H'-9a/b), 3.86-3.78 (m, 7H, CO_2*Me*,H'-5, H-6a/b, OC$H_2$$CH_2$), 3.63-3.59 (m, 2H, H-5, H-6a/b), 3.48 (vt, 2H, OCH_2CH_2, *J* = 5.2 Hz), 3.09-2.96 (m, 4H, OCH_2C$H_2$$N_3$), 2.76 (1H, H'-3e, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 8.4Hz), 2.18 (s, 3H, O*Ac*), 2.08 (s, 3H, O*Ac*), 2.01(s, 3H, O*Ac*), 1.98 (s, 3H, O*Ac*),1.89(s, 3H, NH*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.0, 170.9, 170.5, 170.3, 170.2, 168.7, 165.8, 165.5, 133.3, 133.2, 129.8, 129.7, 129.7, 129.3, 128.4, 128.4, 108.0, 101.2, 83.3, 76.8, 74.3, 74.1, 74.0, 70.6, 70.3, 70.0, 69.6, 69.6, 69.3, 68.2, 67.9, 67.0, 62.4, 53.3, 50.6, 49.2, 42.9, 40.0, 37.9, 29.8, 23.2, 23.1, 21.4, 21.3, 20.9, 20.8, 20.8, 20.8, 14.2, 13.1. HRMS (ESI): *m/z* calcd for [$C_{44}H_{54}N_4O_{20}$S+ Na]$^+$: 1013.2950; found 1013.2971.

Compound 8c:

8c was synthesized from **6c** (72 mg, 0.073 mmol) in a manner similar to the synthesis of **8a**. Purification by column chromatography (DCM:MeOH 10:1 → 5:1) yielded **8c** (58 mg, 72%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.17(m, 3H), 8.00 (m, 2H) 7.93 (d, 8H, *J* = 7.2 Hz), 7.49 (q, 4H, *J* = 7.2 Hz), 7.36 (vt, 8H, *J* = 7.6 Hz), 5.69 (vt, 2H, *J* = 8.4 Hz), 5.43 (m, 5H), 5.37 (vt, 2H, *J* = 10 Hz), 5.07 (d, 2H, *J* = 8 Hz), 4.83 (m, 2H), 4.69 (br, s, 3H), 4.60 (d, 2H, *J* = 2.0 Hz), 4.49 (ddd, 2H, $J_{4,3a}$ = 7.2 Hz, $J_{4,5}$ = 4.4 Hz, $J_{4,3e}$ = 1.6 Hz), 4.40 (m, 2H), 4.35-4.24 (br, m, 7H), 4.21 (dd, 2H, *J* = 12.4 Hz, *J* = 4.4 Hz), 4.07 (vt, 2H, *J* = 7.2 Hz), 4.01-3.95 (br, m,

7H), 3.87 (s, 6H, CO_2*Me*), 3.82-3.77 (m, 4H), 3.72-3.66 (m, 5H), 3.58 (br, s, 5H), 2.93 (d, br, 4H, *J* = 5.6 Hz), 2.81 (dd, 2H, H-3e, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.4 Hz), 2.73 (br, 2H), 2.38 (br, s, 2H), 2.21 (s, 6H, O*Ac*), 2.17 (s, 6H, O*Ac*), 2.00 (s, 6H, O*Ac*), 1.99 (s, 6H, O*Ac*), 1.88 (s, 6H, NH*Ac*), 1.75 -1.67 (br,m, 8H), ^{13}C NMR (100 MHz, CD_3OD): 172.0, 171.0, 170.4, 170.2, 168.4, 165.8, 133.2, 133.1, 129.6, 129.4, 129.3, 129.2, 128.3, 128.2, 100.8, 100.0, 84.3, 74.7, 73.8, 73.7, 70.2, 69.8, 69.7, 68.9, 68.8, 68.7, 67.9, 66.9, 62.1, 60.2, 52.4, 37.9, 21.4, 20.1, 19.6, 19.5, 19.4. HRMS (ESI): *m/z* calcd for $[C_{112}H_{135}N_{13}O_{44}S_3 + 2H]^{2+}$: 1232.4032; found 1232.4188.

Biotinylated biantennary *S*-sialoside (Gc1b):

8c was synthesized from **7c** (32 mg, 0.013 mmol) in a manner similar to the synthesis of **Gc1a**. Purification by size exclusion chromatography (Biogel-10) yielded **8c** (22 mg, 73%). ^{1}H NMR (400 MHz, D_2O): 8.10-7.85 (m, 5H), 4.57-4.51 (m, 10H), 4.31-4.28 (m, 2H), 4.14-4.12 (m, 5H), 3.88-3.82 (m, 11H), 3.74-3.46 (m, 31H), 3.44-3.34 (m, 5H), 3.23-3.21 (m, 2H), 2.80 - 2.65 (m, 10H), 2.36 (br, 2H) 1.98 (m, 2H), 1.92 (s, 6H, NH*Ac*), 1.65-1.59 (m, 4H), 1.48 (m, 2H), 1.36 (br, m, 2H). ^{13}C NMR (100 MHz, D_2O): 175.0, 102.9, 100.0, 74.8, 72.7, 72.2, 70.5, 69.6, 69.0, 68.9, 68.8, 68.7, 68.4, 68.0, 67.9, 66.7, 62.5, 62.0, 60.1, 55.3, 51.6, 50.0, 40.8, 39.6, 21.9, 21.9. HRMS (ESI): *m/z* calcd for $[C_{66}H_{99}N_{13}O_{32}S_3 + 2H]^{2+}$: 841.7913; found 841.7980.

2-(2-(2-Azido-ethoxy)) ethyl 2,3,4,6 tetra-*O*-acetyl-β-D-galactopyranoside (2d):

2c was synthesized from **1** (0.86g, 1.79 mmol) and 2-(2-azidoethoxy)ethanol (0.35 g, 2.68 mmol, 1.5 equiv) following a procedure described for **2a**. Purification by flash chromatography (60: 40 hexanes/EtOAc) yielded **2c**(0.47g, 57%). NMR and and mass spectral analysis matched reported values.[4]

2-(2-(2-Azido-ethoxy)) ethyl 4,6 *O*-benzylidene β-D-galactopyranoside (3d):

3d was synthesized from **2d** (0.46g, 0.91 mmol) in a manner described for **3a**. Purification by flash chromatography (100% EtOAc) yielded **3c** (0.25 g, 65% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53-7.51 (m, 2H, Ar-H), 7.39-7.36 (m, 3H, Ar-H), 5.56 (s, 1H, C*H*Ph), 4.37 (d, 1H, H-1, *J*=7.6 Hz), 4.32 (dd, 1H, *J*= 4 Hz, *J*= 12.4 Hz), 4.13-4.06 (m, 2H), 3.82-3.78 (m, 2H), 3.76-3.64 (m, 11H), 3.54 (s, 1H), 3.47 (d, 1H, *J*= 1.2 Hz), 3.39 (td, 2H, *J*= 1.2 Hz, *J*= 4.4 Hz), 2.80 (d, 1H, *J*= 8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.6, 129.2, 128.2, 126.4, 103.3, 101.4, 75.4, 72.7, 71.2, 70.6, 70.5, 70.4, 70.0, 69.2, 68.4, 66.8, 50.7. HRMS (ESI): *m/z* calcd for $[C_{19}H_{27}N_3O_8 + Na]^+$: 448.1696; found 448.1691.

2-(2-(2-Azido-ethoxy)) ethyl 2,3 di-*O*-benzoyl 4,6 *O*-benzylideneβ-D-galactopyranoside (4d):

4d was synthesized from **3d** (0.25 g, 0.59 mmol) in a manner described for **4a**. Purification by flash chromatography (50:50 hexanes/EtOAc) yielded **4d** (0.30g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ =8.03-8.00 (m, 4H, Ar-H), 7.56-7.50 (m, 4H, Ar-H), 7.43-7.36 (m, 7H, Ar-H), 5.90 (dd, 1H, H-2, *J*= 10.4 Hz, *J*= 8 Hz), 5.58 (s, 1H, C*H*Ph), 5.40 (dd, 1H, H-3, *J*= 7.2 Hz, *J*= 10.4 Hz), 4.88 (d, 1H, H-1, *J*= 8Hz), 4.62 (d, 1H, H-4, *J*=3.2 Hz), 4.42 (dd, 1H, *J* =1.2 Hz, *J*= 12.4 Hz), 4.16 (dd, 1H, *J*= 1.6 Hz, *J*= 12.4 Hz), 4.06 (m, 1H), 3.83 (m, 1H), 3.71 (s, 1H,), 3.65 (m, 2H) 3.56-3.50 (m , 4H), 3.40 (m, 2H), 3.32 (br, 2H, *J*=5.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.2, 165.2, 137.5, 133.4, 133.1, 130.0, 129.8, 129.2, 129.0, 128.5, 128.4, 128.4, 128.1, 126.3, 101.3, 100.8, 73.6, 72.8, 70.7, 70.5, 70.4, 69.9, 69.1, 69.0, 68.8, 66.5, 50.6. HRMS (ESI): *m/z* calcd for $[C_{33}H_{35}N_3O_{10}+ Na]^+$: 656.2220; found 656.2225.

2-(2-(2-Azido-ethoxy)) ethyl 2,3 di-*O*-benzoyl β-D-galactopyranoside (5d):

5d was synthesized from **4d** (0.14 g, 0.24 mmol) in a manner similar to the synthesis of **5a**. Purification by column chromatography (100% EtOAc) yielded **5d** (0.11 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98 (m, 4H, Ar-H), 7.51 (m, 2H, Ar-H), 7.36 (m, 4H, Ar-H), 5.78 (dd, 1H, H-2, *J*= 8 Hz, *J*= 10.4 Hz), 5.32 (dd, 1H, H-3, *J*= 3.2 Hz, *J*= 10 Hz), 4.83 (d, 1H, H-1, *J*= 8 Hz), 4.43 (d, 1H, H-4, *J*= 3.2 Hz), 4.04-3.98 (m, 3H), 3.83-3.77 (m, 2H) 3.58-3.55 (m, 4H), 3.50 (m, 2H), 3.42 (m, 3H), 3.34 (vt, 2H, *J*= 5.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.0, 165.4, 133.4, 133.2, 129.9, 129.7, 129.6, 129.1, 128.5, 128.4, 101.6, 74.5, 74.3, 70.6, 70.5, 70.5, 69.9, 69.8, 69.3, 68.2, 62.4, 50.6. HRMS (ESI): *m/z* calcd for $[C_{33}H_{35}N_3O_{10}+ Na]^+$: 568.1907; found 568.1924.

2-(2-(2-Azido-ethoxy))-ethyl S-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→6)-2,3-di-*O*-acetyl-6-thio-β-D-galacto pyranoside (6d):

6d was synthesized from **5d** (86 mg, 0.16 mmol) in a manner similar to the synthesis of **6a**. Purification by column chromatography (100% EtOAc) yielded **6d** (113 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99 (d, 4H, Ar-H, *J*= 8Hz), 7.51 (m, 2H, Ar-H), 7.38 (t, 4H, Ar- H, *J*= 6.8Hz), 5.72 (dd, 1H, H-2, *J*= 8 Hz, *J*= 10.4 Hz), 5.52 (m, 1H) {5.43-5.3 (m, 6H)}, 4.93 (td, 1H, *J*= 4.8 Hz, *J*= 8 Hz), 4.85 (d, 1H, H-1, *J*= 8 Hz), 4.41 (m, 2H), 4.36 (dd, 1H, *J*= 2.8 Hz, *J*= 8 Hz), {4.23-4.01 (m, 4H)}, 3.86 (s, 3H), 3.81 (m, 4H), 3.62 (m, 2H, 3.52 (m, 4H), 3.38 (m, 2H), 3.30 (m, 2H), 2.99 (d, 1H, *J*= 5.6 Hz), 2.76 (dd, 1H, *J*= 4.8 Hz, *J*= 12.8 Hz), {acetates: 2.18 (6H), 2.07 (m, 8H), 2.02 (m, 5H), 1.87 (s, 3H, NHAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 170.5, 170.3, 170.2, 170.1, 168.6, 165.8, 165.3, 133.3, 133.0, 129.9, 129.8, 129.7, 129.3, 128.4, 128.3, 101.2, 83.3, 74.2, 74.0, 70.7, 70.5, 70.2, 69.9, 69.5, 69.4, 69.2, 68.3,

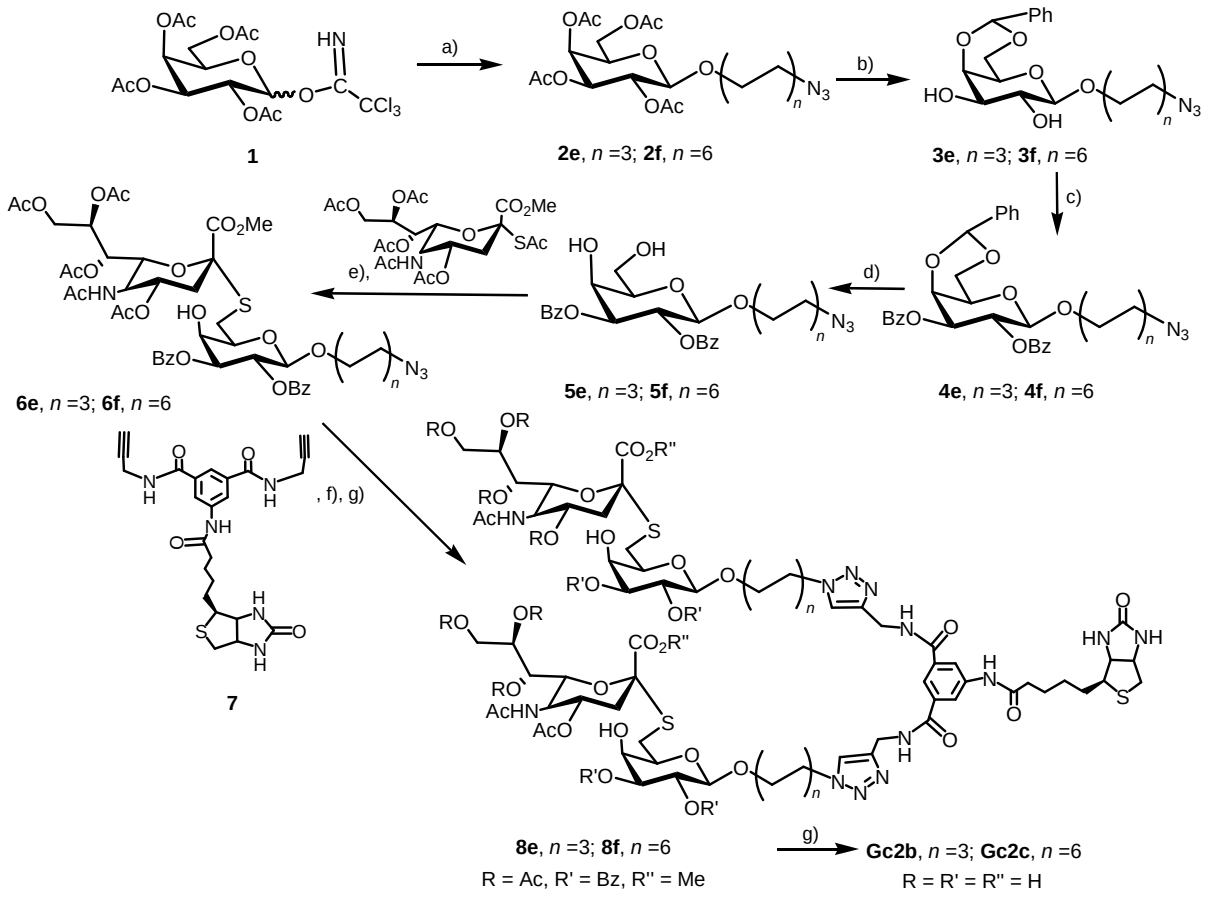
68.1, 67.8, 67.6, 66.9, 62.3, 53.2, 50.6, 46.6, 37.9, 29.9, 23.2, 21.3, 20.9, 20.8, 20.8. HRMS (ESI): *m/z* calcd for $[C_{46}H_{58}N_4O_{21}+ Na]^+$: 1057.3212; found 1057.3241.

Compound 8d:

8d was synthesized from **6d** (68 mg, 0.066 mmol) in a manner similar to the synthesis of **7a**. Purification by column chromatography (DCM:MeOH 10:1 → 5:1) yielded **8d** (36mg, 47%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.14 (m, 1H), 8.06 (m, 3H), 8.00 (m, 8H), 7.50 (m, 5H), 7.36 (m, 8H), 5.67 (dd, 2H, *J*=8.4Hz, 9.2 Hz), 5.43 (m, 4H), 5.37 (m, 3H), 5.07 (d, 2H, *J*=8Hz), 4.64 (m, 8H), 4.59 (m, 3H), 4.51-4.39 (m, 8H), 4.35 (m, 1H), 4.21 (dd, 2H, *J*=4Hz, 12.4Hz), 4.06 (vt, 3H, *J*=6.8 Hz), 3.96 (m, 7H), 3.87 (s, 6H), 3.79 (m, 5H), 3.68 (m, 5H), 3.54 (m, 5H), 3.28 (m, 4H), 2.93 (dd, 4H, *J*=0.8Hz, 5.6Hz), 2.83-2.72 (m, 4H), 2.37 (br,s, 2H), 2.22 (s, 6H), 2.16 (s, 6H), 2.00 (s, 6H), 1.99 (s, 6H), 1.88 (s, 6H), 1.67-1.62 (m, 4H), 1.46 (m, 2H), 1.31 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ = 172.1, 171.1, 170.4, 170.3, 168.4, 165.8, 165.8, 133.1, 129.6, 129.5, 129.3, 129.3, 128.3, 128.3, 128.2, 100.8, 84.3, 74.8, 74.8, 73.8, 73.8, 73.7, 73.6, 70.2, 70.1, 69.8, 69.8, 69.7, 69.0, 69.0, 68.0, 66.9, 66.5, 62.1, 52.4, 29.7, 28.4, 28.1, 21.4, 20.1, 19.6, 19.5, 19.4. HRMS (ESI): *m/z* calcd for $[C_{116}H_{143}N_{13}O_{46}S_3+ 2Na]^{2+}$: 1297.9104; found1297.9186.

Biotinylated biantennary *S*-sialoside (Gc1c):

Gc1c was synthesized from **8d** (22 mg, 0.009 mmol) in a manner similar to the synthesis of **Gc1a**. Purification by size exclusion chromatography (Biogel-10) yielded **Gc1c** (16mg, 99%). ^{1}H NMR (400 MHz, D_2O): δ = 7.93 (m, 4H), 7.86 (s, 1H), 4.58 (s, 5H), 4.51 (m, 5H), 4.29 (m, 1H), 4.15 (d, 2H, *J*=8Hz), 3.90-3.66 (m, 20H), 3.61-3.32 (m, 40H), 3.21 (m, 2H), 2.82 (m, 9H), 2.68 (m, 5H), 2.61 (s, 3H), 2.34 (m, 2H), 1.92 (s, 6H), 1.65-1.57 (m, 6H), 1.48 (m, 2H), 1.36 (m, 3H). ^{13}C NMR (100 MHz, D_2O): δ = 175.6, 175.0, 174.0, 136.3, 134.8, 124.4, 123.1, 102.9, 74.8, 74.1, 72.7, 72.2, 70.5, 69.6, 69.5, 69.4, 69.0, 68.8, 68.7, 68.5, 68.4, 68.0, 62.5, 60.2, 55.3, 55.2, 51.7, 50.0, 40.8, 39.7, 29.4, 27.9, 24.9, 21.9. HRMS (ESI): *m/z* calcd for $[C_{70}H_{107}N_{13}O_{34}S_3+ 2H]^{2+}$: 885.8176; found 885.8208.



Scheme 3. Synthesis of **Gc2b** and **Gc2c**. Reagents and conditions: a9 TMSOTf, DCE, -25^{o}C to RT. Yield for **2e**, 46% using $HO(CH_2)_6N_3$; for **2f**, 88% using $HO(CH_2)_{12}N_3$. b) i: NaOMe, MeOH, 12 h. ii: BDA, CSA, THF, reflux, 12 h, Yield for **3e**, 50%; for **3f**, 52%. c) BzCl, DMAP, pyridine, 50^{o}C, 12 h, Yield for **4e**, 75%; for **4f**, 75%. d) 80% AcOH, 50^{o}C, 12 h, Yield for **5e**, 80%; for **5f**, 79%. e) i: Tf_2O, pyridine, DCM, -20^{o}C to 0^{o}C, 1.5 h, ii: DEA, DMF, -25^{o}C to RT, 2.5h Yield for **6e**, 60%; for **6f**, 78%. f. $CuSO_4$, Sodium ascorbate, THF/H_2O, 24 h, Yield for **8e**, 75%; for 8f, 55%. g) NaOMe, MeOH, 16h, ii: 0.05M NaOH, 16 h, Yield for **Gc1b**, 83%; for **Gc1c**, 61%.

6-Azido-hexyl 2,3,4,6 tetra-*O*-acetyl-β-D-galactopyranoside (2e):

2e was synthesized from **1** (1.03g, 2.16 mmol) and 6-azidohexanol (0.46 g, 3.24 mmol, 1.5 equiv) following a procedure described for **2a**. Purification by flash chromatography (70: 30 hexanes/EtOAc) yielded **2c** (0.47g, 46%). NMR and and mass spectral analysis matched reported values.[5]

6-Azido-hexyl 4,6 *O*-benzylidene β-D-galactopyranoside (3e):

3e was synthesized from **2e** (0.47g, 0.98 mmol) in a manner described for **3a**. Purification by flash chromatography (100% EtOAc) yielded **3e** (.13 g, 50% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52 (m, 2H, ArH), 7.39 (m, 3H, ArH), 5.58 (s, 1H, C*H*Ph), 4.36 (dd,

1H, *J*= 1.6 Hz, *J*= 12.4 Hz), 4.30 (d, 1H, H-1, *J*= 7.6 Hz), 4.24 (dd, 1H, *J*= 0.8 Hz, 3.6 Hz), 4.10 (dd, 1H, *J*= 2Hz, *J*= 12.8 Hz), 3.99 (m, 1H), 3.74 (m, 2H), 3.54 (m, 1H), 3.51 (m, 1H), 3.29 (t, 2H, *J*= 6.8 Hz), 2.55 (d, 1H, *J*= 8.8 Hz), 2.53 (d, 1H, *J*= 1.6 Hz), 1.65 (m, 3H), 1.43 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.5, 129.3, 128.2, 126.4, 102.8, 101.5, 75.3, 72.8, 71.9, 69.7, 66.7, 51.4, 29.4, 28.8, 26.5, 25.6. HRMS (ESI): *m/z* calcd for $[C_{19}H_{27}N_3O_6I + Na]^+$: 416.1797; found 416.1784.

6-Azido-hexyl 2,3 di-*O*-benzoyl 4,6 *O*-benzylidene β-D-galactopyranoside (4e):

4e was synthesized from **3e** (0.09 g, 0.23 mmol) in a manner described for **4a**. Purification by flash chromatography (75:25 → 50:50 hexanes: EtOAc) yielded **4e** (.10g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98 (m, 4H, ArH), 7.51 (m, 4H, ArH), 7.35 (m, 7H, ArH), 5.86 (dd, 1H, H-2, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 8 Hz), 5.55 (s, 1H, C*H*Ph), 5.36 (dd, 1H, H-3, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.6 Hz), 4.78 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.59 (br, d, 1H, H-4), 4.41 (dd, 1H, H-6a/b, *J* = 1.2 Hz, *J* = 12.4 Hz), 4.14 (dd, 1H, H-6a/b, *J* = 1.6 Hz, *J* = 12.0 Hz), 3.98 (m, 1H, OC*H*$_2$CH$_2$-CH$_2$N$_3$), 3.67 (1H, m, H-5), 3.51 (m, 1H, OC*H*$_2$CH$_2$CH$_2$), 3.04 (m, 2H, CH$_2$CH$_2$C*H*$_2$N$_3$), 1.22 (m, 8H, OCH$_2$C*H*$_2$C*H*$_2$ C*H*$_2$C*H*$_2$CH$_2$N$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.3, 165.2, 137.5, 133.4, 133.2, 130.0, 129.8, 129.7, 129.2, 129.0, 128.5, 128.4, 128.1, 126.3, 101.3, 100.9, 73.6, 72.8, 69.4, 69.2, 69.0, 66.5, 51.2, 29.2, 28.6, 26.3, 25.5. HRMS (ESI): *m/z* calcd for $[C_{33}H_{35}N_3O_8 + Na]^+$: 624.2322; found 624.2328.

6-Azido-hexyl 2,3 di-*O*-benzoyl β-D-galactopyranoside (5e):

5e was synthesized from **4e** (0.10 g, 0.17 mmol) in a manner similar to the synthesis of **5a**. Purification by column chromatography (100% EtOAc) yielded **5e** (0.07 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98 (m, 4H, Ar-H), 7.53 (m, 2H, Ar-H), 7.39 (m, 4H, Ar-H), 5.76 (dd, 1H, H-2, *J*= 8 Hz, *J*= 10.4 Hz), 5.30 (dd, 1H, H-3, *J*= 3.2 Hz, *J*= 10.4 Hz), 4.70 (d, 1H, H-1, *J*= 8 Hz), 4.39 (m, 1H, H-4), 4.15- 4.02 (m, 2H), 3.95 (m, 2H), 3.78 (m, 1H), 3.53 (m, 1H), 3.06 (m, 2H), 2.72 (d, 1H, *J*= 4.0 Hz), 2.14 (dd, 1H, *J*= 5.6 Hz, *J*= 7.6 Hz), 1.33-1.31 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.0, 165.4, 133.5, 133.2, 129.9, 129.7, 129.6, 129.1, 128.5, 128.4, 101.7, 74.4, 74.2, 70.0, 69.7, 68.3, 62.5, 51.2, 29.3, 28.6, 26.3, 25.4. HRMS (ESI): *m/z* calcd for $[C_{26}H_{31}N_3O_8 + Na]^+$: 536.2009; found 536.2020.

6-Azido-hexyl-S-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→6)-2,3-di-*O*-acetyl-6-thio-β-D-galactopyranoside (6e):

6e was synthesized from **5e** (70 mg, 0.136mmol) in a manner similar to the synthesis of **6a**. Purification by column chromatography (100% EtOAc) yielded **6e** (82 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98 (m, 4H), 7.50 (m, 2H), 7.37 (m, 4H), 5.71 (dd, 1H, *J*= 8 Hz, *J*=

10.4 Hz), 5.42-5.30 (m, 5H), 4.93 (td, 1H, *J*= 4.4Hz, *J*= 11.6 Hz), 4.75 (d, 1H, *J*= 8 Hz), 4.41 (m, 1H), 4.36 (dd, 1H, *J*= 2.8 Hz, *J*= 12.4 Hz), 4.14 (dd, 1H, *J*=5.2 Hz, *J*= 12.8 Hz), 4.02 (m, 1H), 3.95 (m, 1H), 3.86 (s, 3H), 3.79 (m, 2H), 3.55 (m, 1H), 3.05 (m, 3H), 2.94 (m, 2H), 2.76 (dd, 1H, *J*= 4.4 Hz, *J*= 12.8 Hz), 2.18 (m, 6H), 2.06 – 2.01 (m, 12H), 1.89 (s, 3H), 1.53 (m, 2H), 1.34-1.20 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.0, 170.9, 170.4, 170.3, 170.2, 170.1, 168.5, 165.8, 165.3, 133.3, 133.1, 129.9, 129.8, 129.7, 129.3, 128.4, 128.3, 101.1, 83.4, 74.2, 74.2, 74.0, 69.6, 69.4, 68.3, 68.0, 66.9, 62.3, 53.2, 51.2, 49.4, 38.0, 30.0, 29.3, 28.6, 26.3, 25.5, 23.2, 21.3, 20.9, 20.8, 20.8, 20.7. HRMS (ESI): *m/z* calcd for $[C_{46}H_{58}N_4O_{19}S + Na]^+$: 1025.3314; found 1025.3341.

Compound 8e:

8e was synthesized from **6e** (50 mg, 0.050 mmol) in a manner similar to the synthesis of **8a**. Purification by column chromatography (DCM:MeOH 10:1 → 5:1) yielded **8e** (43 mg, 75%). ^{1}H NMR (400 MHz, CD_3OD): 8.18 (m, 1H), 7.95 (m, 8H), 7.52 (m, 2H), 7.45 (m, 2H), 7.36 (m, 8H), 5.65 (dd, 2H, *J*=8Hz, 10Hz), 5.42 (m, 4H), 5.35 (m, 2H), 5.00 (d, 2H, *J*=8Hz), 4.68 (m, 3H), 4.60 (d, 2H, *J*= 2.8 Hz), 4.51 (m, 1H), 4.42 (m, 2H), 4.30 (m, 1H), 4.24 (m, 2H), 4.09 (m, 6H), 3.96 (d, 4H, *J*=4.8 Hz), 3.88 (s, 6H), 3.80 (m, 3H), 3.58 (m, 2H), 3.33 (m, 9H), 2.92 (d, 4H, *J*=6.8Hz), 2.82 (dd, 2H, *J*=4.8Hz, *J*=12.8Hz), 2.72 (d, 1H, *J*=12Hz), 2.40 (m, 1H), 2.22 (s, 6H), 2.16 (s, 6H), 2.00 (s, 6H), 1.99 (s, 6H), 1.88 (s, 6H), 1.74 (m, 3H), 1.46 (m, 10H), 1.30 (s, 1H), 1.17 (m, 9H), 0.9 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): 172.0, 171.0, 170.4, 170.2, 168.4, 165.8, 165.7, 133.2, 133.0, 129.6, 129.5, 129.3, 129.2, 128.3, 128.1, 100.8, 87.8, 84.4, 75.6, 74.7, 73.7, 70.3, 69.1, 69.2, 68.0, 66.9, 66.4, 62.0, 61.9, 52.4, 37.9, 29.8, 29.7, 29.0, 28.1, 25.7, 25.1, 21.4, 20.1, 19.6, 19.5, 19.4. HRMS (ESI): *m/z* calcd for $[C_{116}H_{143}N_{13}O_{42}S_3 + 2Na]^{2+}$: 1265.9200; found 1265.9213.

Biotinylated biantennary *S*-sialoside (Gc2b):

Gc2b was synthesized from **7e** (43 mg, 0.017 mmol) in a manner similar to the synthesis of **Gc1a**. Purification by size exclusion chromatography (Biogel-10) yielded **Gc2b** (24 mg, 83%). ^{1}H NMR (400 MHz, D_2O): 7.84 (m, 5H), 4.74 (m, 12H), 4.53 (br, s, 3H), 4.45 (m, 1H), 4.26 (vt, 5H), 4.14 (d, 2H, *J*=8Hz), 3.87 (br, s, 2H), 3.70 (m, 9H), 3.61-3.44 (m, 15H), 3.32 (t, 2H, *J*=8.4Hz), 2.80 (m, 6H), 2.69 (dd, 2H, *J*=4.4Hz, 12.4Hz), 2.61 (d, 1H, *J*=12.8Hz), 2.27 (m, 2H), 1.92 (s, 6H), 1.74 (m, 4H), 1.63 (m, 5H), 1.42 (m, 5H). ^{13}C NMR (100 MHz, D_2O): δ = 175.2, 175.0, 174.9, 137.4, 122.8, 102.2, 74.9, 74.2, 72.9, 72.1, 70.4, 69.6, 67.9, 65.8, 62.9, 62.1, 61.6, 51.6, 50.3, 45.2, 40.7, 30.3, 28.6, 26.1, 25.2, 24.0, 21.0. HRMS (ESI): *m/z* calcd for $[C_{70}H_{107}N_{13}O_{30}S_3 + 2H]^{2+}$: 853.8277; found 853.8312.

12-azido-dodecyl 2,3,4,6 tetra-*O*-acetyl-β-D-galactopyranoside (2f):

2f was synthesized from **1** (1.03g, 2.16 mmol) and 12-azidododecanol (0.46 g, 3.24 mmol, 1.5 equiv) following a procedure described for **2a**. ^{1}H NMR (400 MHz,$CDCl_3$): 5.40 (dd, 1H, *J*= 0.8Hz, 3.2 Hz), 5.22 (dd, 1H, *J*= 7.6Hz, 10.4Hz), 5.03 (dd, 1H, *J*=3.2Hz, 10.4Hz), 4.46 (d, 1H, *J*= 8.0Hz) 4.18 (m, 2H), 3.90 (m, 2H), 3.47 (m, 1H), 3.27 (t, 2H, *J*= 6.8Hz), 2.16 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.61 (m, 4H), 1.28 (m, 16H). ^{13}C NMR (100 MHz, $CDCl_3$): δ =170.4, 170.3, 170.2, 169.4, 101.4, 71.0, 70.6, 70.3, 68.9, 67.1, 61.3, 51.5, 29.6, 29.5, 29.5, 29.5, 29.4, 29.3, 29.1, 28.8, 26.7, 25.8, 20.8, 20.7, 20.6.

12-azido-dodecyl 4,6 *O*-benzylidene β-D-galactopyranoside (3f):

3f was synthesized from **2f** (0.44g, 0.79 mmol) in a manner described for **3a**. Purification by flash chromatography (100% EtOAc) yielded **3f** (0.20 g, 52% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53 (m, 2H, Ar-H), 7.38 (m, 3H), 5.58 (s, 1H), 4.36 (dd, 1H, *J*= 1.2 Hz, *J*= 12.4 Hz), 4.30 (d, 1H, *J*= 7.2 Hz), 4.24 (d, 1H, *J*= 2.8 Hz), 4.11 (dd, 1H, *J*= 2.0 Hz, 12.8 Hz), 3.99 (m, 1H), 3.80- 3.69 (m, 2H), 3.55-3.49 (m, 2H), 3.27 (t, 2H, *J*= 6.8 Hz), 2.50 (d, 1H, *J*= 8.8 Hz), 2.45 (m, 1H), 1.69-1.58 (m, 4H), 1.37-1.29 (m, 16H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 129.3, 128.2, 126.4, 102.8, 101.5, 75.4, 72.8, 71.9, 70.1, 69.2, 66.7, 51.5, 29.6, 29.5, 29.5, 29.2, 28.9, 26.7, 26.0. HRMS (ESI): *m/z* calcd for $[C_{25}H_{39}N_3O_6 + Na]^+$: 500.2737; found 500.2735.

12-azido-dodecyl 2,3 di-*O*-benzoyl 4,6 *O*-benzylidene β-D-galactopyranoside (4f):

4f was synthesized from **3f** (0.196 g, 0.41 mmol) in a manner described for **4a**. Purification by flash chromatography (75:25 → 50:50 hexanes/EtOAc) yielded **4f** (. 22 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (m, 4H, Ar), 7.53 (m, 4H, Ar), 7.38 (m, 7H, Ar), 5.88 (dd, 1H, H-2, *J* = 10.0 Hz, 8.0Hz), 5.57 (s, 1H, C*H*Ph), 5.37 (dd, 1H, H-3, *J* = 3.6Hz, 10.4 Hz), 4.75 (d, 1H, H-1, *J* = 8Hz), 4.61 (br, d, 1H, H-4), 4.43 (d, 1H, H-6a/b, *J*= 12Hz), 4.15 (d, 1H, H-6a/b, *J*=12Hz), 3.98 (m, 1H, OCH_2R), 3.69 (m, 1H, H-5), 3.53 (m, 1H, OCH_2R), 3.27 (m, 2H, RCH_2N_3), 1.63-1.14 (m, 16H, R-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.5, 137.4, 133.3, 129.9, 129.7, 128.8, 128.4, 128.3, 128.1, 128.1, 126.3, 126.2, 103.1, 100.7, 74.1, 73.7, 72.9, 70.1, 69.10, 68.8, 66.6, 51.5, 29.5, 29.5, 29.4, 29.1, 28.8, 26.7, 25.9. HRMS (ESI): *m/z* calcd for $[C_{39}H_{47}N_3O_8 + Na]^+$: 708.3261; found 708.3259.

12-azido-dodecyl 2,3 di-*O*-Benzoyl β-D-galactopyranoside (5f):

5f was synthesized from **4f** (0.10 g, 0.17 mmol) in a manner similar to the synthesis of **5a**. Purification by column chromatography (100% EtOAc) yielded **5f** (0.07g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99 (m, 4H), 7.52 (m, 2H), 7.38 (m, 4H), 5.78 (dd, 1H, *J*= 8 Hz, *J*= 10.4 Hz), 5.32 (dd, 1H, *J*= 3.2 Hz, *J*= 10.4 Hz), 4.72 (d, 1H, *J*= 8 Hz), 4.42 (vt, 1H, *J*= 3.6 Hz),

4.04-3.97 (m, 2H), 3.93 (m, 1H), 3.80 (t, 1H, *J*= 5.2 Hz), 3.53 (m, 1H), 3.27 (t, 2H, *J*= 7.2 Hz), 3.08 (d, 1H, *J*= 4.4 Hz), 2.53 (s, 1H), 1.60 (m, 2H), 1.50 (m, 2H), 1.39-1.12 (m, 16H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.0, 165.4, 133.4, 133.1, 129.9, 129.7, 129.6, 129.1, 128.5, 128.3, 101.6, 74.4, 74.2, 70.3, 69.7, 68.3, 62.6, 51.5, 29.5, 29.3, 29.2, 28.9, 26.7, 25.8. HRMS (ESI): *m/z* calcd for $[C_{32}H_{43}N_3O_8 + Na]^+$: 620.2948; found 620.2972.

12-azido-dodecyl-S-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→6)-2,3-di-*O*-acetyl-6-thio-β-D-galacto-pyranoside (6f):

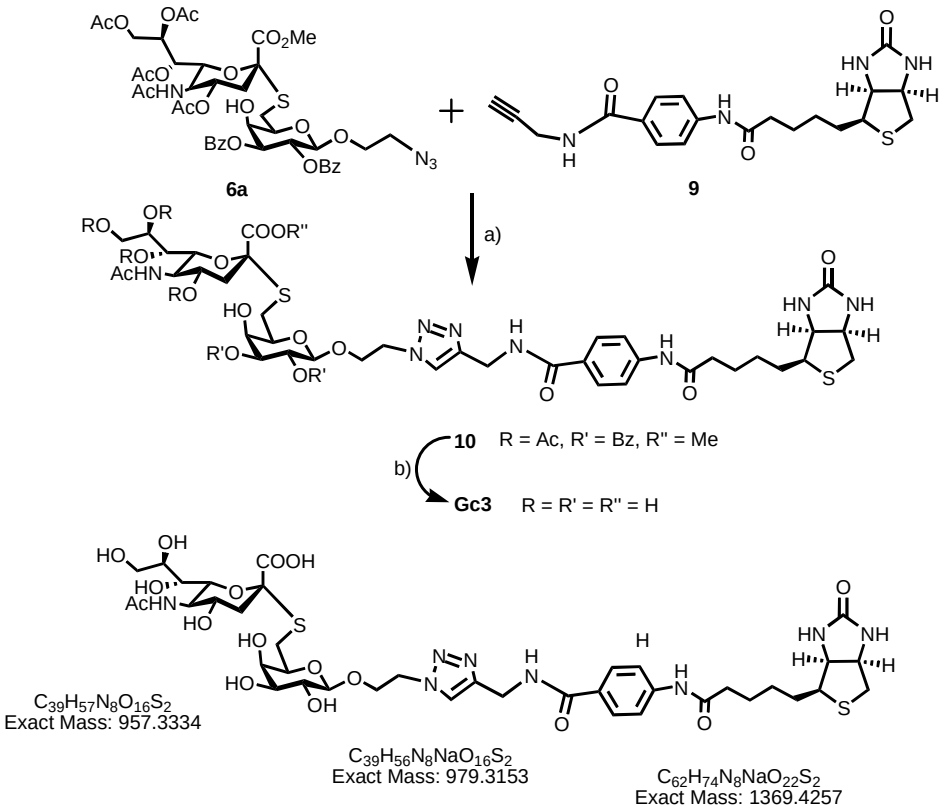
6f was synthesized from **5f** (72 mg, 0.120mmol) in a manner similar to the synthesis of **6a**. Purification by column chromatography (100% EtOAc) yielded **6f** (102 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ =7.99(m, 4H), 7.50 (m, 2H), 7.38 (m, 4H), 5.70 (dd, 1H, *J*= 8Hz, *J*= 10.4 Hz), 4.94 (td, 1H, *J*= 4.4 Hz, *J*= 11.2 Hz), 4.76 (d, 1H, *J*= 8 Hz), 4.42 (m, 1H), 4.36 (dd, 1H, *J*=2.8 Hz, *J*= 12.4 Hz), 4.15 (m, 1H), 4.02 (m, 1H), 3.93 (m, 2H), 3.87 (s, 3H), 3.79 (m, 3H), 3.55 (m, 1H), 3.26 (t, 2H, *J*= 7.2 Hz), 3.10 (m, 1H), 2.97 (m, 1H), 2.91 (d, 1H, *J*= 5.6 Hz), 1.63-1.48 (m, 4H), 1.37-1.04 (m, 16H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 170.8, 170.4, 170.3, 170.2, 168.6, 165.8, 165.4, 133.3, 132.9, 129.8, 129.7, 129.3, 128.4, 128.2, 101.1, 83.4, 74.3, 74.1, 74.0, 70.6, 69.9, 69.6, 69.4, 68.4, 68.1, 67.6, 67.0, 62.3, 61.9, 53.2, 52.6, 51.5, 49.5, 46.6, 38.0, 30.0, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.8, 26.7, 25.9, 23.2, 21.3, 20.9, 20.8, 20.8. HRMS (ESI): *m/z* calcd for $[C_{52}H_{70}N_4O_{19}S + Na]^+$: 1109.4253; found 1109.4296.

Compound 7f:

8f was synthesized from **6f** (102 mg, 0.095 mmol) in a manner similar to the synthesis of **8a**. Purification by column chromatography (DCM:MeOH 10:1 → 5:1) yielded **8f** (63 mg, 75%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.17 (br,s, 2H), 8.10 (m, 2H), 7.97 (m, 9H), 7.52 (m, 5H), 7.39 (m, 8H), 5.66 (dd, 2H, *J*=8Hz, 10Hz), 5.43 (m, 4H), 5.35 (m, 2H), 5.02 (d, 2H, *J*=8Hz), 4.61 (m, 8H), 4.50 (m, 1H), 4.40-4.30 (m, 9H), 4.23 (dd, 2H, *J*=4.4Hz, 12Hz), 4.07 (vt, 2H, *J*=7.2Hz), 3.98 (m, 4H), 3.92 (m, 1H), 3.89 (s, 6H), 3.84 (s, 1H), 3.62 (m, 2H), 3.22 (m, 1H), 2.92 (d, 4H, *J*=7.2Hz), 2.82 (dd, 2H, *J*=4.8Hz, 12.8Hz), 2.72 (d, 2H, *J*=12.8Hz), 2.42 (m, 2H), 2.23 (s, 6H), 2.17 (s, 6h), 2.06 (s, 6H), 2.00 (s, 6H), 1.88 (s, 6H), 1.73 (m, 4H), 1.63 (m, 2H), 1.49 (m, 7H), 1.26 (m, 12H), 1.19-0.980 (m, 32H). ^{13}C NMR (100 MHz, CD_3OD): δ = 172.0, 171.0, 170.4, 170.3, 170.3, 168.4, 165.7, 133.0, 129.5, 129.3, 129.2, 128.2, 128.1, 100.8, 84.4, 74.8, 73.6, 70.3, 69.7, 69.4, 68.0, 66.9, 66.4, 62.1, 60.2, 55.6, 52.3, 39.8, 37.9, 29.9, 29.3, 29.2, 29.1, 29.0, 28.7, 26.1, 25.7, 21.4, 20.0, 19.6, 19.5, 19.4. HRMS (ESI): *m/z* calcd for $[C_{128}H_{167}N_{13}O_{42}S_3 + 2Na]^{2+}$: 1351.0165; found 1351.0010

Biotinylated biantennary *S*-sialoside (Gc2c):

Gc2c was synthesized from **7f** (33 mg, 0.012 mmol) in a manner similar to the synthesis of **Gc1a**. Purification by size exclusion chromatography (Biogel-10) yielded **Gc2c** (23 mg, 61%). ^{1}H NMR (400 MHz, D_2O): δ = 8.04 (m, 4H), 7.87 (m, 4H), 4.22 (m, 5H), 3.82 (m, 3H), 3.69 (m, 7H), 3.51 (m, 14H), 3.08 (m, 2H), 2.83 (m, 4H), 2.66 (m, 4H), 2.34 (m, 3H), 1.92 (s, 6H), 1.61 (m, 7H), 1.36 (m, 7H), 1.16 (m, 4H), 0.93 (m, 4H), 0.80 (m, 21H). HRMS (ESI): *m/z* calcd for $[C_{82}H_{131}N_{13}O_{30}S_3 + 2H]^{2+}$: 938.4232; found 938.4401.



Scheme 4. Synthesis of **Gc3**. Reagents and conditions: a) $CuSO_4$, Sodium ascorbate, THF/ H_2O, 24h, 75%. 33%. b) NaOMe, MeOH, 16 h; ii: 0.05 M NaOH, 16h, 31% over 2 steps.

4-[5-(2-*O*xo-hexahydro-thieno[3,4]imidazol-4-yl)-pentanoylamino]-N-prop-2-ynyl-benzamide (9): CDMT (119 mg, .67 mmol) was dissolved in 5 mL of anhydrous THF under argon and cooled to 0 ℃, followed by addition of NMM (85 µL, 0.67 mmol). The resulting solution was stirred at 0 ℃ for 1 h and D-biotin (157 mg, 0.61 mmol) in 2 mL of DMF was added to it and stirred for 7 h at 0 ℃. 4-Amino-N-prop-2-ynyl- benzamide (53 mg, 0.31 mmol) in 5 mL of THF was added to this solution, followed by an additional 2 mL of THF and 40 µL of NMM. The resulting solution was stirred for 12 h at RT. After removal of solvent in vacuo, the crude material was purified by column chromatography (10:90 MeOH:DCM → 20:80 MeOH:DCM) to yield **9** (75 mg, 62%). ^{1}H NMR (400 MHz, DMSO- d_6): δ = 7.80 (d, 2H), 7.67 (d, 2H), 6.43 (d, 1H), 4.31 (m, 1H), 4.14 (m, 1H), 4.03 (m, 2H), 3.59 (m, 1H), 3.09 (m, 1H), 2.82 (m, 1H),

2.58 (m, 1H), 2.32 (m, 1H), 2.26 (m, 1H), 1.63-1.37 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6):δ = 172.0, 165.8, 163.2, 142.5, 128.6, 128.4, 118.5, 81.9, 73.2, 66.0, 61.4, 59.7, 55.7, 45.8, 36.3, 33.6, 28.9, 25.5. HRMS (ESI): *m/z* calcd for $[2[C_{20}H_{24}N_4O_3S] + Na]^+$: 823.3036; found 823.3012.

Compound 10: 10 was synthesized from **6a** (60 mg, 0.063 mmol) in a manner similar to the synthesis of **8a**. Purification by column chromatography (DCM:MeOH 10:1 → 5:1) yielded **10** (23 mg, 33 %) ^{1}H NMR (400 MHz, CD_3OD): δ = 7.92-7.96 (m, 5H), 7.82-7.86 (m, 4H), 7.68-7.70 (m, 2H), 7.53-7.57 (m, 2H), 7.39-7.42 (m, 5H), 5.67 (dd, 1H, J = 8 Hz, 10.4 Hz), 5.40-5.43 (m, 2H), 5.33-5.35 (m, 1H), 5.10 (d, 1H, J = 8 Hz), 4.59-4.62 (m, 6H), 4.50-4.52 (m, 2H), 4.39-4.43 (m, 2H), 4.30-4.34 (m, 5H), 4.19-4.23 (m, 2H), 4.08-4.10 (m, 3H), 3.96-4.01 (m, 3H), 3.86 (s, 3H), 3.88-2.93 (m, 4H), 2.82 (dd, 2H, J = 4.8 Hz, 12.8 Hz), 2.70-2.73 (m, 2H), 2.41-2.45 (m, 3H), 2.23 (s, 3H), 2.18 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.89 (s, 3H), 1.73-1.79 (m, 5H), 1.49-1.67 (m, 8H), 1.31 (s, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ = 129.2, 128.3, 128.1, 128.0, 100.5, 84.5, 73.7, 73.6, 70.0, 67.8, 67.4, 67.0, 66.2, 62.0, 60.2, 55.6, 52.3, 39.7, 36.3, 29.9, 29.6, 28.4, 28.1, 25.2, 21.3, 20.0, 19.5, 19.5, 19.4. HRMS (ESI): *m/z* calcd for $[C_{62}H_{74}N_8O_{22}S_2Na]^+$: 1369.4257; found 1369.4243.

Biotinylated monoantennary *S*-sialoside (Gc2c):

Gc3 was synthesized from **10** (23 mg, 0.017 mmol) in a manner similar to the synthesis of **Gc1a**. Purification by size exclusion chromatography (Biogel-10) yielded **Gc3** (5 mg, 31%). ^{1}H NMR (400 MHz, D_2O): δ = 7.93 (s, 1H), 7.70 (d, 2H), 7.48 (d, 2H), 4.56 (s, 4H), 4.49 (dd, 1H, J = 4.8 Hz, 7.6 Hz), 4.29-4.32 (m, 1H), 4.21 (d, 1H, J = 8 Hz), 4.12-4.16 (m, 1H), 4.01-4.06 (m, 1H), 3.83-3.89 (m, 1H), 3.71-3.75 (m, 1H), 3.62-3.69 (m, 2H), 3.44-3.61 (m, 6H), 3.31 (dd, 1H, J = 8 Hz, 9.6 Hz), 3.20-3.25 (m, 1H), 2.88 (dd, 1H, J = 4.8 Hz, 12.8 Hz), 2.65-2.73 (m, 4H), 2.35 (t, 2H, J = 7.2 Hz), 1.92 (s, 3H), 1.48-1.66 (m, 5H), 1.35-1.40 (m, 2H). ^{13}C NMR (100 MHz, D_2O): δ = 175.8, 175.0, 165.4, 144.7, 140.6, 129.4, 128.3, 124.5, 121.0, 112.1, 103.1, 85.9, 81.9, 74.9, 74.2, 72.7, 72.3, 70.4, 68.8, 68.5, 68.4, 68.0, 62.6, 62.0, 60.2, 55.3, 51.8, 51.7, 51.5, 50.4, 40.8, 39.7, 36.3, 35.0, 29.4, 27.9, 27.7, 25.0, 22.0. HRMS (ESI): *m/z* calcd for $[C_{39}H_{57}N_8O_{16}S_2]^+$: 957.3334; found 957.3314.

B. ELISA Studies, Figure S1 and Table S1.

Microwells of 96-well, high-binding-capacity, streptavidin-coated microtiter plate (Pierce) were incubated with excess of biotinylated synthetic glycans (100 µL of 750 µM solution) overnight at 0 ^{0}C to achieve complete saturation. (>125 pm/microwell as per the manufacturer's instructions). The microwells were washed extensively with PBS to remove excess glycans. For comparison studies (Figure 2a of manuscript), the glycan-coated wells were incubated with HA (A/New Caledonia/20/99, Protein Sciences) at a concentration of 12 µg/ mL in PBS containing 0.05% Tween for 2 h with shaking, followed by washing to remove any unbound protein. Next, 100 µL of anti-HA polyclonal rabbit antibody (Protein Sciences) in PBS containing 0.1% BSA was added and incubated for 1 h at RT with shaking. Unbound antibody was removed with excessive washing with PBS containing 0.05% Tween followed by PBS. 100 µL HRP conjugated anti-rabbit antibody (Vector laboratories) was added and incubated for 30 min. at RT with shaking. Unbound antibody was removed with excessive washing with PBS containing 0.05% Tween followed by PBS. The binding signal was determined based on HRP activity using QuantaBlu peroxidase assay (Pierce) as per the manufacturer's instructions. The fluorescence was measured on a Synergy™ 4 Multi-Mode microplate reader (Biotek Instruments) with excitation of 325nm and emission of 420nm. All assays were performed in duplicate on three separate days and appropriate negative controls were included. For the protein dose dependent studies (Figure 2b of manuscript), a stock solution containing appropriate amounts of HA protein was used and a similar procedure was followed to obtain the fluorescence signals. To quantify the binding affinity, we used a linear-ized Hill equation as described previously.[6] The equation used is $\log(y/1-y) = n * \log([HA]) - \log K_d'$, where y is the fractional saturation of the glycan binding sites in the HA units, n is the cooperativity factor and K_d' is the apparent binding constant. It is important to note that the values of n and K_d' are not absolute values. The Hill plots are given in Figure S1 and the values of n, the cooperativity factor, and K_d' are given in Table S1. To study the effect of decreasing glycan surface densities (Figure 2c of manuscript) on the binding of HA to the glycans, varying ratios of glycan: PEG from appropriate stock solutions were added to high binding capacity streptavidin coated microtiter wells. Appropriate amounts of HA was added and a similar procedure was followed to obtain the fluorescence signals. To study the effect of contact time on HA binding (Figure 2d of manuscript), the synthetic glycans were added to strep-tavidin-coated microtiter wells and incubated with 10 µg/mL of HA for different time periods. a similar procedure was followed to obtain the fluorescence signals. All assays were performed in duplicate on three separate days and appropriate negative controls were included.

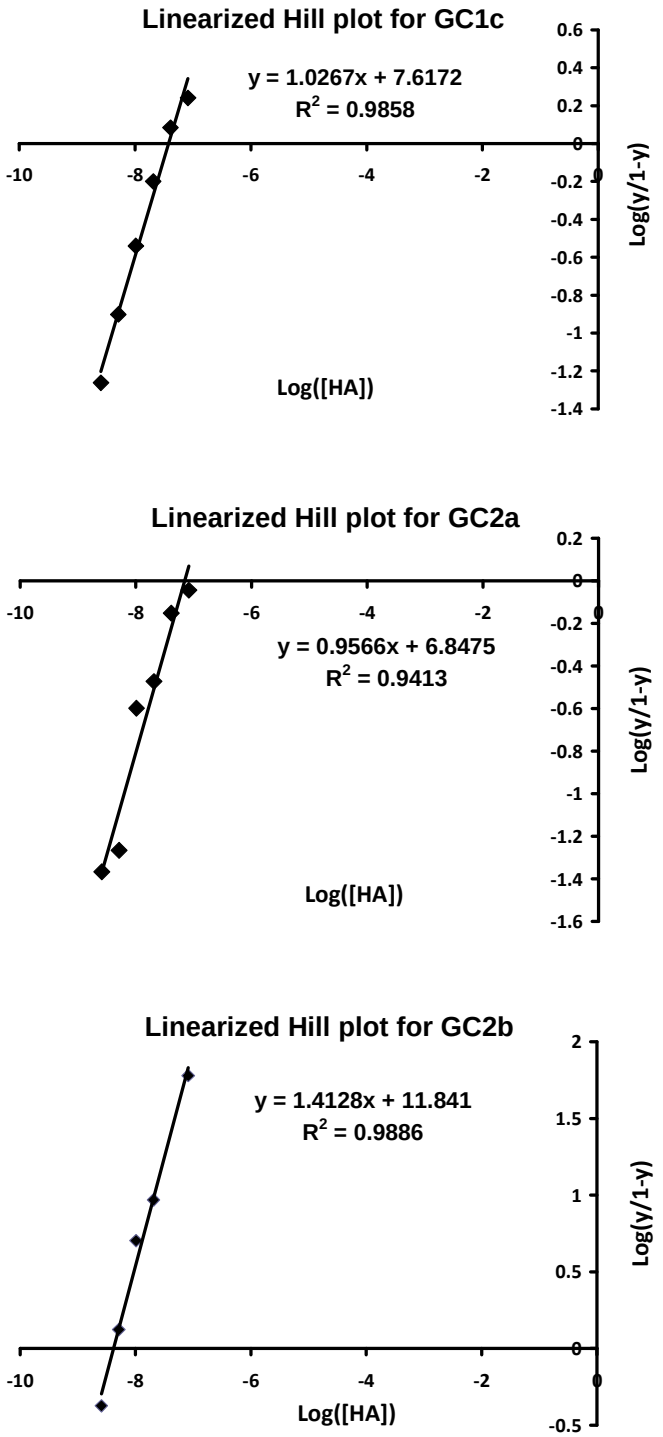


Figure S1. Linearized hill plot for **Gc2b**, **Gc2a** and **Gc1c** obtained by the dose dependent HA study. The assumptions and the equations used have the described previously. The y intercept is - log (K_d') and the slope is the cooperativity factor *n*. The values of n and the apparent K_d' are given in Table S1.

Table S1. Relative binding of HA to **Gc2b**, **Gc2a** and **Gc1c**. The values of *n* and the apparent K_d' were obtained from the plot of log([HA]) versus log(*y*/1-*y*), where *y* is the fractional saturation of the glycan binding sites in the HA units.

Glycan	*n*	K_d' (M)
Gc2a	0.96	$1.42 * 10^{-7}$
Gc1c	1.03	$0.24*10^{-9}$
Gc2b	1.41	$1.44*10^{-12}$

C. Surface Plasmon Resonance (SPR) studies, Figure S2 and Table S2.

Chip Preparation: Streptavidin coated chips (Biacore) were rinsed thoroughly with PBS. Solutions containing the biotinylated ligand were injected independently into each flow cell until saturation was reached (typically 250-350 response units (RU)) then followed by an injection of 50mM NaOH to remove any unbound ligand. This was repeated four times for each flowcell to ensure complete glycan coverage. Biotinylated PEG was used as the reference.

HA binding experiments: Appropriate amounts of HA in PBS containing 0.05% Tween were injected at a flow rate of 20 µL/min for 2.5 min. PBS containing 0.05% Tween flowed for 13 min before regeneration of the surface using two 30 s pulses of 50 mM NaOH at a flow rate of 50 µL/min. The baseline was allowed to stabilize for 3 min before the next cycle. The signal was analyzed by subtraction of the reference flow cell (biotinylated PEG) followed by subsequent analysis was carried out using BiaEval® software. The best fit of the experimental data was the conformational charge model, which was used to obtain association and dissociation constants. (Table S2). All the experiments were repeated twice to obtain identical values.

Table S2: Apparent binding constants for **Gc2b** and **Gc2c** obtained using SPR. Biaeval® software was used and the experimental data was fitted to the best bivalent model

Glycan	k_{a1} ($M^{-1}s^{-1}$)	k_{d1} (s^{-1})	k_{a2} (s^{-1})	k_{d2} (s^{-1})	K_d* (M)
Gc2b	$6.36 * 10^{4}$	$1.44 * 10^{-3}$	$5.25 * 10^{-3}$	$6.69 * 10^{-4}$	$2.56* 10^{-9}$
Gc2c	$6.33 * 10^{4}$	$1.92 * 10^{-3}$	$4.27 * 10^{-3}$	$4.60 * 10^{-4}$	$2.95* 10^{-9}$

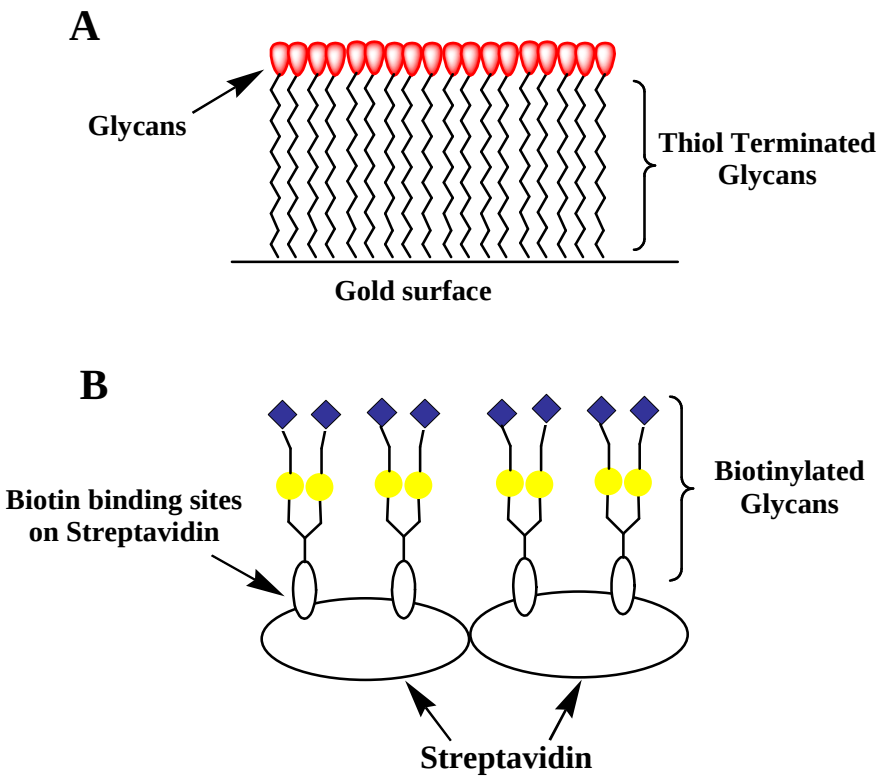


Figure S2. Representation of the two different glycan presentations. A) When thiol terminated glycans are used, a dense glycan surface coverage can be achieved. B) The biotinylated glycans used in this study cannot be packed as closely as the thiol terminated glycans as the number of biotin binding sites are a function of the packing of the streptavidin molecules.

References:

[1] A. Y. Chernyak, G. V. M. Sharma, L.O. Kononov, P.R. Krishna, A. B. Levinskii, N. K. Kochetkov, A. V. R. Rao, *Carb. Res.***1992***, 223*, 303.

[2] R. R. Kale, H. Mukundan, D. N. Price, J. F. Harris, D. M. Lewallen, B. I. Swanson, J. G. Schmidt, S. S. Iyer, *J Am Chem Soc* **2008**, *130*, 8169.

[3] F. Gonzalez Nunez, M. T. Campos Valdes, E. Aruca, R. R. Schmidt, V. B. Verez, *J. Carb. Chem.* **2003**, *22*, 395.

[4] A. Sasaki, N. Murahashi, H. Yamada, A. Morikawa, *Biol. & Pharm. Bull.* **1995**, *18*, 740.

[5] S. Deng, U. Gangadharmath, and C-W. T. Chang, *J. Org. Chem.* **2006**, **71**, 5179.

[6] A. Srinivasan, K. Viswanathan, R. Raman, A. Chandrasekaran, S. Raguram, T. M. Tumpey, V. Sasisekharan, R. Sasisekharan, *Proc Natl Acad Sci U S A* **2008**, *105*, 2800.