SUPPORTING INFORMATION FOR:

# Development of C-type lectin-oriented surfaces for high avidity glycoconjugates: towards mimicking multivalent interactions on the cell surface

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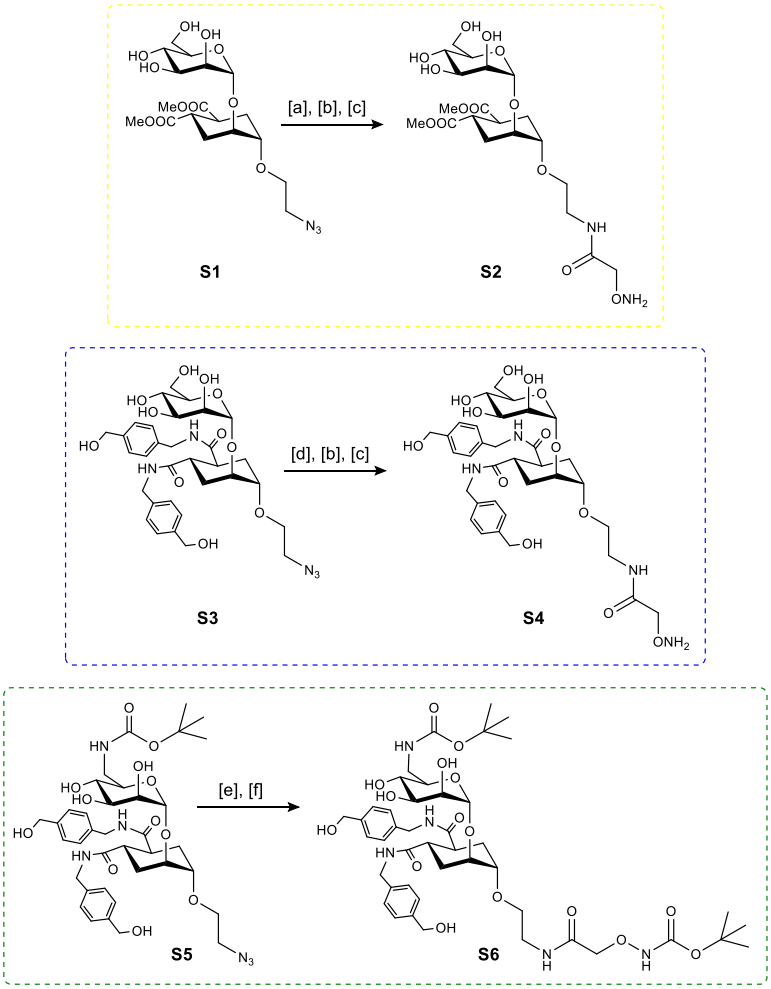
Table of contents:

1. Synthesis of glycodendrimers and characterization

1.1. Synthetic procedures for glycodendrimers **3.B**, **3.C**, **3.D**, **4.B** and **4.C**.

General procedures

All chemical reagents were purchased from Aldrich (Saint Quentin Fallavier, France) orAcros (Noisy-Le-Grand, France). All protected amino acids and Fmoc-Gly-Sasrin® resin was obtained from Advanced ChemTech Europe (Brussels, Belgium). For peptides and glycopeptides, analytical RP-HPLC was performed on Waters system equipped with a Waters 2695 separations module and a Waters 2487 Dual Absorbance UV/Visible Detector. Analysis was carried out at 1.23 mL/min (EC 125/3 nucleosil 300-5 C18) with UV monitoring at 214 nm using a linear A–B gradient (buffer A: 0.09% CF3CO2H in water; buffer B: 0.09% CF3CO2H in 90% acetonitrile). Purifications were carried out at 22.0 mL/min (VP 250/21 nucleosil 100-7 C18) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient. $^{1}H$ and $^{13}C$ NMR spectra were recorded on BrukerAvance 400 MHz spectrometer and chemical shifts (δ) were reported in parts per million (ppm). Spectra were referenced to the residual proton solvent peaks relative to the signal of CD3OD (δ 3.31 and 49.0 ppm for $^{1}H$ and $^{13}C$), assignments were done by GCOSY, DEPT-135° and HSQC experiments. Standard abbreviations s, d, t, dd, bs, m, refer to singlet, doublet, triplet, doublet of doublet, broad singlet, multiplet. $ESI^{+}$-MS spectra were recorded on ThermoFischer LCQ apparatus, at university of Milano or on Waters Acquity UPLC-MS equipped with a SQ Detector 2. HRMS spectra were measured on a Waters Xevo G2-S QTof at Mass Spectrometry facility, PCN-ICMG of Grenoble. MALDI-TOF MS were performed on a Autoflex (Bruker Daltonics) using sinapinic acid matric (Sigma, 10 mg/mL in acetonitrile/water-0.1% TFA 50:50) at the mass spectrometry plateform of Institut de Biologie Structurale of Grenoble.




**Scheme S1.** Synthesis of mannose mimics (ligand **B**, ligand **C** and ligand **D**, see Figure 5) equipped with aminooxy moieties **S2**, **S4** and **S6**. Conditions: [a] TFA, Pd/C, $H_2$, MeOH; [b] Boc-aminooxyacetic acid *N*-hydroxysuccinimide ester, DIPEA, DMF; [c] TFA:$CH_2Cl_2$:$NH_2OH$ (60:38:2, v/v/v); [d] Lindlar-Pd, $H_2$, MeOH; [e] 1,3-propanedithiol, TEA, MeOH; [f] Boc-aminooxyacetic acid *N*-hydroxysuccinimide ester, DMF.

Compound **S2**

To a solution of **S1**[1] (98.9 mg, 213 µmol) in freshly distilled MeOH (10 mL), under anhydrous conditions, TFA (19 µL, 248 µmol) was added. After 10 min stirring at room temperature, a catalytic amount of Pd/C was added, and the suspension was stirred under a $H_2$ atmosphere for 45 minutes. TLC ($CH_2Cl_2$:MeOH:$H_2O$ 8:2:0.25 v/v/v) revealed consumption of the starting material. The reaction mixture was then filtered through a Celite pad, washed with MeOH, concentrated under vacuum to give the corresponding amine as TFA salt. The reaction crude was used for the next reaction without any purification. $ESI^+$-MS m/z: calcd for $C_{18}H_{31}NO_{11}Na$ $[M+Na]^+$: 460.2, found: 460.5. The crude was dissolved in dry DMF (10 mL) containing DIPEA (45 µL, 287 µmol), then Boc-aminooxyacetic acid *N*-hydroxysuccinimide ester[2] (92.2 mg, 320 µmol) was added, and the reaction was stirred at room temperature for 30 minutes. The reaction mixture was concentrated under reduced pressure to dryness, then 10 mL of a TFA:$CH_2Cl_2$:$NH_2OH$ (60:38:2, v/v/v) cocktail were added. The reaction was stirred for 1 hour, then solvent mixture was concentrated and ice-cold Et2O (20 mL) was added to the residue to give a white precipitate. After centrifugation, the precipitate was washed with Et2O (10 mL), dried, then purified by preparative RP-HPLC (0-30% buffer B in 30 min.) to give pure compound **S2** (67.8 mg) as white powder after

lyophilisation, in a 62% yield over three steps. $^{1}$H NMR (400 MHz, $CD_3OD$): δ 4.931 (1H, d, J = 1.3 Hz, H-1'), 4.446 (2H, bs, -COCH2ONH2), 3.988 (1H, d, J = 2.6 Hz, H-2), 3.833-3.875 (2H, m, H-2', H-6'a), 3.511-3.694 (13H, m, 2 x -CH3, -OCH2-, H-1, H-3', H-4', H-5', H-6'b), 3.443 (2H, t, J = 5.3 Hz, -CH2NH-), 2.815-2.936 (2H, m, H-4, H-5), 2.023-2.105 (2H, m, H-6b, H-3a),1.738-1.825 (2H, m, H-6a, H-3b) ppm. $^{13}$C NMR (100 MHz, $CD_3OD$): δ 176.993, 176.967 (2 x C=O), 100.419 (H-1'), 75.664, 75.638 (C-5', C-1), 73.086 (-COCH2ONH2), 72.521, 72.436, 72.399 (C-2, C-2', C-3'), 68.666 (C-4'), 68.315 (-OCH2-), 63.096 (C-6'), 52.436 (2 x -OCH3), 40.406, 40.362, 40.253 (C-4, C-5, -CH2NH-), 28.900 (C-6), 28.823 (C-3) ppm. HRMS (ESI-TOF) *m/z* calcd. for $C_{20}H_{35}N_2O_{13}$ [M+H]+: 511.2139, found: 511.2146 (error = +1.4 ppm). Analytical RP-HPLC: $t_R$ = 6.72 min (C18, λ = 214 nm 0-30% B in 15 min).

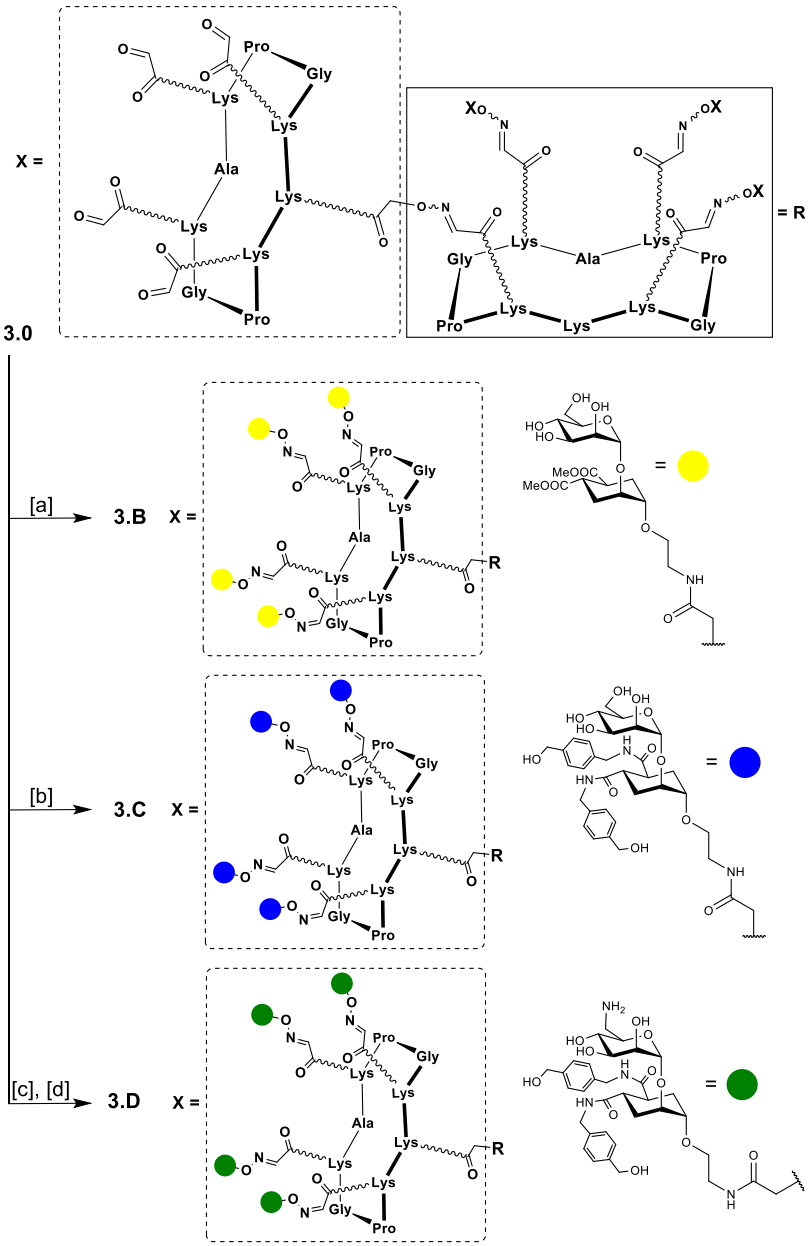
Compound **S4**

To a solution of **S3**[3] (113 mg, 168 µmol) in freshly distilled MeOH (17 mL), under anhydrous conditions, TFA (15 µL, 196 µmol) was added. After 10 min stirring at room temperature, 350 mg of Lindlar-Pd were added, and the suspension was stirred overnight under a $H_2$ atmosphere. The reaction was monitored through TLC analysis ($CHCl_3$:MeOH 7:3 v/v). The reaction mixture was then filtered through a Celite pad, washed with MeOH, concentrated under vacuum to give the corresponding amine as TFA salt. The reaction crude was used for the next reaction without any purification. ESI$^+$-MS m/z: calcd for $C_{32}H_{46}N_3O_{11}$ $[M+H]^+$: 648.3, found: 648.4; calcd for $C_{32}H_{45}N_3O_{11}Na$ $[M+Na]^+$: 670.3, found: 670.5. The crude was dissolved in dry DMF (10 mL) containing DIPEA (40 µL, 230 µmol), then Boc-aminooxyacetic acid *N*-hydroxysuccinimide ester (72.6 mg, 252 µmol) was added and the reaction was stirred at room temperature for 30 minutes. The reaction mixture was concentrated under reduced pressure to dryness, then 10 mL of a TFA:$CH_2Cl_2$:$NH_2OH$ (60:38:2, v/v/v) cocktail were added. The reaction was stirred for 1 hour, then solvent mixture was concentrated and ice-cold $Et_2O$ (20 mL) was added to the residue to give a white precipitate. After centrifugation, the precipitate was washed with $Et_2O$ (10 mL), dried, then purified by preparative RP-HPLC (0-30% buffer B in 30 min.) to give pure compound **S4** (80.5 mg) as white powder after lyophilisation, in a 66% yield over three steps. $^{1}$H NMR (400 MHz, $CD_3OD$): δ 7.204-7.287 (8H, m, Ar-H), 4.930 (1H, d, J = 1.45 Hz, H-1'), 4.560 (4H, bs, CONH-CH2-Ar), 4.414 (2H, bs, -COCH2ONH2), 4.290 (4H, bs, Ar-CH2-OH), 4.290 (4H, bs, Ar-CH2-OH), 4.003 (1H, d, J = 2.9 Hz, H-2), 3.652-3.730 (4H, m, H-5', H-3', H-6'b, 1 x -OCH2-), 3.551-3.596 (3H, m, H-1, H-4', 1 x -OCH2-), 3.448 (2H, t, J = 5.2 Hz, -CH2NH-), 2.841-2.931 (2H, m, H-4, H-5), 1.903-1.944 (4H, m, H-6a, H-6b, H-3a, H-3b) ppm. $^{13}$C NMR (100 MHz, $CD_3OD$): δ 176.987, 176.947 (2 x C=O), 141.605, 141.597, 139.020, 138.973 (4 x CAr), 128.409, 128.378, 128.176, 128.170 (8 x CHAr), 100.237 (C-1'), 75.956 (C-3'), 75.528 (C-1), 73.246 (-COCH2ONH2), 72.572, 72.373, 72.302 (C-2, C-2', C-3'), 68.824 (C-4'), 68.041 (-OCH2-), 64.911 (Ar-CH2-OH), 63.111 (C-6'), 43.703 (CONH-CH2-Ar), 41.914, 41.788, (C-4, C-5), 40.445 (-CH2NH-), 29.498 (C-6), 28.860 (C-3) ppm. HRMS (ESI-TOF) *m/z* calcd. for $C_{34}H_{49}N_4O_{13}$ [M+H]+: 721.3296, found: 721.3301 (error = +0.7 ppm). Analytical RP-HPLC: $t_R$ = 4.84 min (C18, λ = 214 nm 0-30% B in 15 min).

Compound **S6**

To a solution of **S5**[4] (60 mg, 77.5 µmol) in dry MeOH (1.3 mL), 1,3-propanedithiol (78 µL, 775 µmol) and triethylamine (108 µL, 775 µmol) were added. The reaction was stirred at 40 °C overnight under nitrogen atmosphere. The formation of a white precipitate was observed. After completion of the reaction, the crude was filtered over a cotton pad, washed with MeOH, and the filtrate was dried under reduced pressure to obtain the corresponding amine, which was used for the next step without any purification. $R_f$ = 0.1 ($CH_2Cl_2$:MeOH:$H_2O$ =

8.5:1.5:0.1). $^{1}$H NMR (400 MHz, CD3OD) δ: 7.27 (d, J = 7.9 Hz, 4H, Ar-H); 7.22 (d, J = 7.9 Hz, 4H, Ar-H); 4.91 (br s, 1H, H-1'); 4.56 (bs, 4H, HO-C*H*$_2$-Ar-); 4.30 (bs, 4H, -CONH-C*H*$_2$-Ar-); 4.00-3.94 (m, 1H, H-2); 3.91-3.87 (m, 1H, H-2'); 3.75-3.43 (m, 7H, H-1, H-3', H-4', H-5', H-6'a, -OC*H*$_2$CH$_2$NH$_2$); 3.30-3.21 (m, 1H, H-6'b); 2.99-2.84 (m, 4H, H-4, H5, -OCH$_2$C*H*$_2$NH$_2$); 2.03-1.83 (m, 4H, H-3, H6); 1.91 (s, 9H, *t*Bu) ppm. The crude was dissolved in dry DMF (1.0 mL) and Boc-aminooxyacetic acid *N*-hydroxysuccinimide ester (33.5 mg, 116 μmol) was added, then the reaction stirred at room temperature for 1 h. After reaction completion the solvent was removed under reduced pressure, and the crude was purified by automated flash chromatography (silica, $CH_2Cl_2$ with gradient of MeOH from 0 to 15%) to afford 57 mg of **S6** as a solid in 80% yield over two steps. $R_f$ = 0.3 ($CH_2Cl_2$:MeOH:$H_2O$ = 8.5:1.5:0.1). $[\alpha]_D^{25}$: +8.5 (c = 0.99 in MeOH). $^{1}$H NMR (400 MHz, $CD_3OD$) δ: 7.28 (d, J = 8.0 Hz, 4H, H-Ar); 7.22 (d, J = 8.0 Hz, 4H, H-Ar); 4.91 (br s, 1H, H-1'); 4.56 (bs, 4H, HO-C*H*$_2$-Ar-); 4.31-4.25 (m, 6H, -CONH-C*H*$_2$-Ar-, -COC*H*$_2$ONHBoc); 4.02-3.96 (m, 1H, H-2); 3.90-3.86 (m, 1H, H-2'); 3.74-3.60 (m, 4H, -OC*H*$_2$CH$_2$NH-, H-3', H-1); 3.58-3.41 (m, 5H, H-4', H-5', H-6'a, -OCH$_2$C*H*$_2$NH-); 3.27-3.18 (m, 1H, H-6'b); 3.00-2.80 (m, 2H, H-4, H-5); 2.03-1.82 (m, 4H, H-3, H-6); 1.46 (s, 9H, *t*Bu); 1.43 (s, 9H, *t*Bu) ppm. $^{13}$C NMR (100 MHz, $CD_3OD$) δ: 177.04, 176.86 (*C*ONH); 171.66 (-*C*OCH$_2$ONHBoc), 159.80, 158.59 (-*C*=OO*t*Bu), 141.51, 139.05, 139.01, 128.39, 128.36, 128.14 (C-Ar); 100.69 (C-1'); 83.07, 80.21 ($C_{quat}$-*t*Bu); 76.48 (-CO*C*H$_2$ONHBoc); 76.10 (C-1); 74.05 (C-5'); 72.93 (C-2); 72.40 (C-2'); 72.26 (C-3'); 69.77 (C4'); 68.35 (-O*C*H$_2$CH$_2$NH-); 64.92 (HO-*C*H$_2$-Ar-); 43.66 (-CONH-*C*H$_2$-Ar-); 42.63 (C-6'); 41.87, 41.75 (C-4, C-5); 40.35 (-OCH$_2$*C*H$_2$NH-); 29.87, 29.20 (C-3, C-6); 28.86, 28.54 (*t*Bu) ppm. HRMS (ESI-TOF) *m/z* calcd. for $[C_{44}H_{65}N_5O_{16}Na]^+$: 942.43240; found: 942.43344 (error = +1.1 ppm).




**Scheme S2.** Synthesis of mannose mimics-containing hexadecavalent glycodendrimers **3.B**, **3.C** and **3.D**. Conditions: [a] **S2**, 0.1% TFA in $H_2O$, 37 ºC; [b] **S4**, 0.1% TFA in $H_2O$, 37 ºC; [c] TFA/$CH_2Cl_2$ (1:1, v/v); [d] **S6**, 0.1% TFA in $H_2O$, 37 ºC.

Compound **3.B**

A solution of **3.0**[5] (5.2 mg, 0.81 µmol) and **S2** (12.2 mg, 23.9 µmol) in water (200 µL) containing 0.1% of TFA was heated at 37 °C for 45 minutes. The reaction mixture was directly purified through preparative RP-HPLC (5-100% buffer B in 30 min), to afford compound **3.B** (8.0 mg, 69% yield) as white powder after lyophilisation. MALDI-TOF m/z: calcd for $C_{603}H_{942}N_{111}O_{286}$ $[M+H]^+$: 14322.5 , found: 14328.6 (+6.1, error = 426 ppm). Analytical RP-HPLC: $t_R$ = 5.38 min (C18, λ = 214 nm 5-100% B in 15 min.).

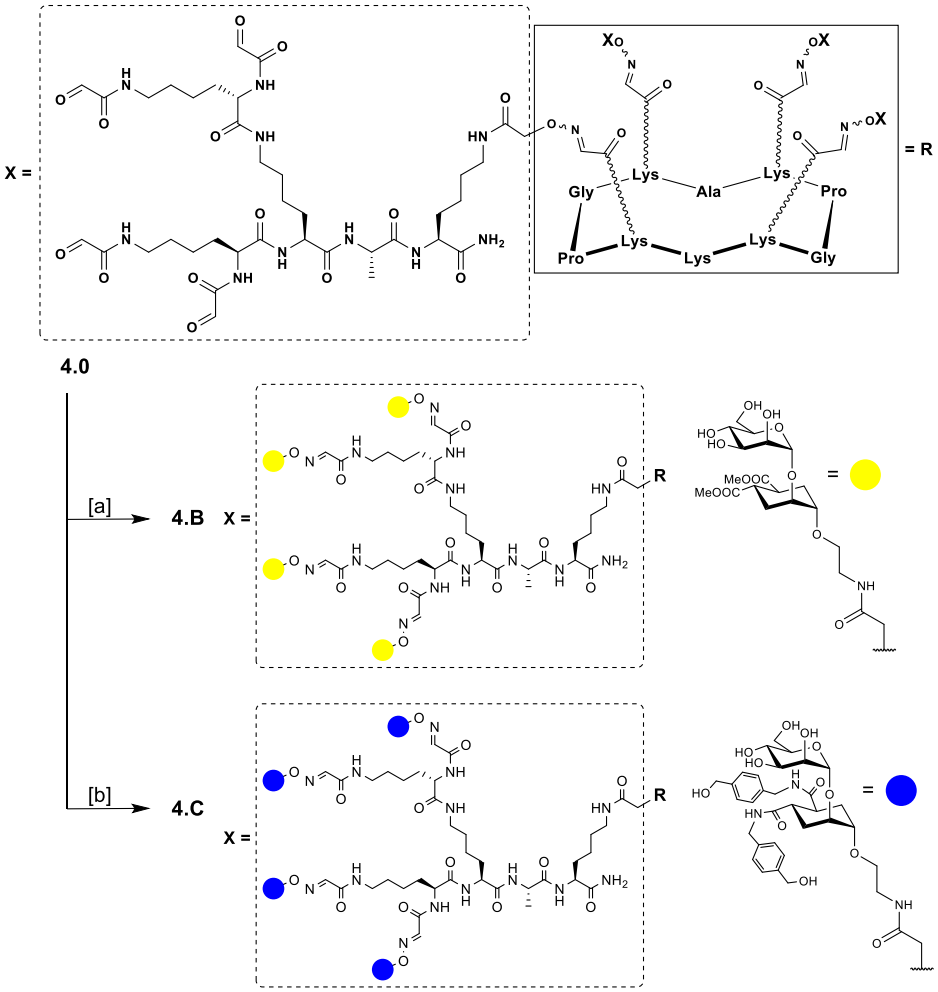
Compound **3.C**

A solution of **3.0** (3.9 mg, 0.60 µmol) and **S4** (12.1 mg, 16.8 µmol) in water (200 µL) containing 0.1% of TFA was heated at 37 °C for 45 minutes. The reaction mixture was directly purified through preparative RP-HPLC (5-100% buffer B in 30 min), to afford

compound **3.C** (6.8 mg, 64% yield) as white powder after lyophilisation. MALDI-TOF m/z: calcd for $C_{827}H_{1166}N_{143}O_{286}$ $[M+H]^+$: 17686.9 , found: 17691.4 (+4.3, error = 254 ppm). Analytical RP-HPLC: $t_R$ = 5.13 min (C18, λ = 214 nm 5-100% B in 15 min.).

Compound **3.D**

A solution of **S6** (12.3 mg, 13.4 µmol) in a TFA/$CH_2Cl_2$ (1:1, v/v, 1.0 mL) mixture was stirred at room temperature for 30 minutes, then the solvent mixture was evaporated to dryness. The crude was added of **3.0** (4.2 mg, 0.65 µmol) and water (250 µL) containing 0.1% of TFA, then the reaction mixture at 37 °C for 45 minutes. The reaction mixture was directly purified through preparative RP-HPLC (5-100% buffer B in 30 min), to afford compound **3.D** (7.2 mg, 63% yield). $ESI^+$-MS m/z: calcd for $C_{827}H_{1187}N_{159}O_{270}$ $[M+6H]^{6+}$: 2946.0, found: 2947.1; calcd for $C_{827}H_{1188}N_{159}O_{270}$ $[M+7H]^{7+}$: 2525.3, found: 2526.0; calcd for $C_{827}H_{1189}N_{159}O_{270}$ $[M+8H]^{8+}$: 2209.8, found: 2210.4; calcd for $C_{827}H_{1190}N_{159}O_{270}$ $[M+9H]^{9+}$: 1964.4, found: 1964.9; calcd for $C_{827}H_{1191}N_{159}O_{270}$ $[M+10H]^{10+}$: 1768.0, found:1768.6; calcd for $C_{827}H_{1192}N_{159}O_{270}$ $[M+11H]^{11+}$: 1607.4, found: 1607.8; calcd for $C_{827}H_{1193}N_{159}O_{270}$ $[M+12H]^{12+}$: 1473.5, found: 1474.1; calcd for $C_{827}H_{1194}N_{159}O_{270}$ $[M+13H]^{13+}$: 1360.2, found: 1359.9 (mean error = 30 ppm). Analytical RP-HPLC: $t_R$ = 5.10 min (C18, λ = 214 nm 5-100% B in 15 min.).




**Scheme S3.** Synthesis of mannose mimics-containing hexadecavalent glycodendrimers **4.B** and **4.C**. Conditions: [a] **S2**, 0.1% TFA in $H_2O$, 37 ºC; [b] **S4**, 0.1% TFA in $H_2O$, 37 ºC.

Compound **4.B**

A solution of **4.0**[5] (5.0 mg, 1.05 µmol) and **S2** (15.7 mg, 30.6 µmol) in water (200 µL) containing 0.1% of TFA was heated at 37°C for 45 minutes. The reaction mixture was directly purified through preparative RP-HPLC (5-100% buffer B in 30 min.), to afford compound **4.B** (8.7 mg, 66% yield) as white powder after lyophilization. MALDI-TOF m/z: calcd for C523H826N91O266 [M+H]+: 12644.6 , found: 12651.5 (+6.9, error = 546 ppm. Analytical RP-HPLC: $t_R$ = 5.26 min (C18, λ = 214 nm 5-100% B in 15 min).

Compound **4.C**
A solution of **4.0** (3.0 mg, 0.63 µmol) and **S4** (12.6 mg, 17.5 µmol) in water (200 µL) containing 0.1% of TFA was heated at 37°C for 45 minutes. The reaction mixture was directly purified through preparative RP-HPLC (5-100% buffer B in 30 min.), to afford compound **4.C** (6.9 mg, 68% yield) as white powder after lyophilisation. MALDI-TOF m/z: calcd for $C_{747}H_{1050}N_{123}O_{266}$ $[M+H]^+$: 16009.0, found: 16015.2 (+6.2, error = 387 ppm). Analytical RP-HPLC: $t_R$ = 5.01 min (C18, λ = 214 nm 5-100% B in 15 min).

Compounds **1.A**, **2.A**, **3.A** and **4.A** were synthesized according to previously reported procedures.[5]

1.2. NMR spectra.

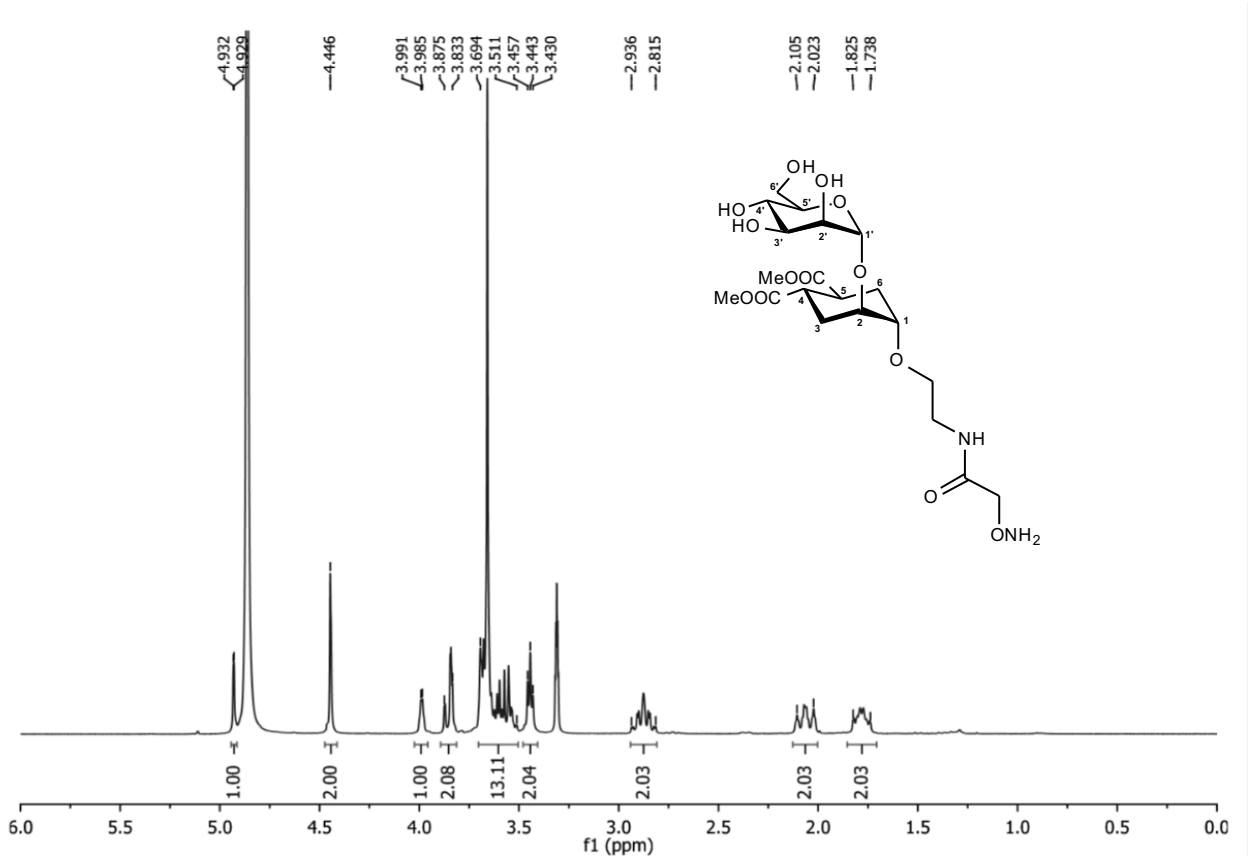



**Figure S1.** $^{1}$H spectrum of **S2** (400 MHz, $CD_3OD$).

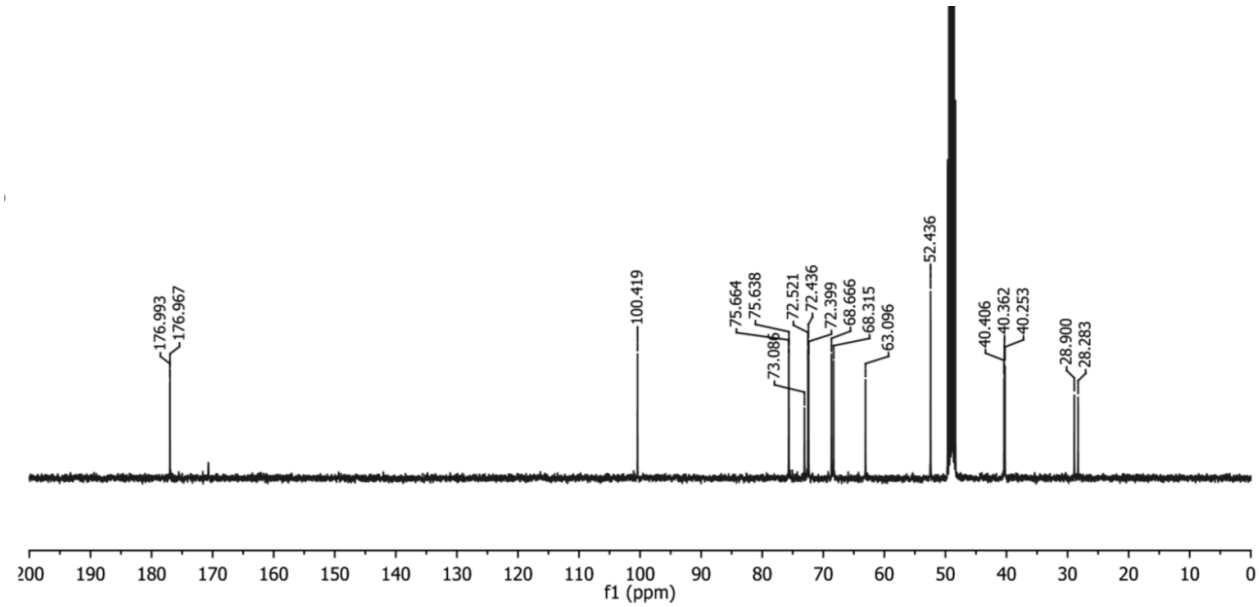



**Figure S2.** $^{13}$C spectrum of **S2** (100 MHz, $CD_3OD$).

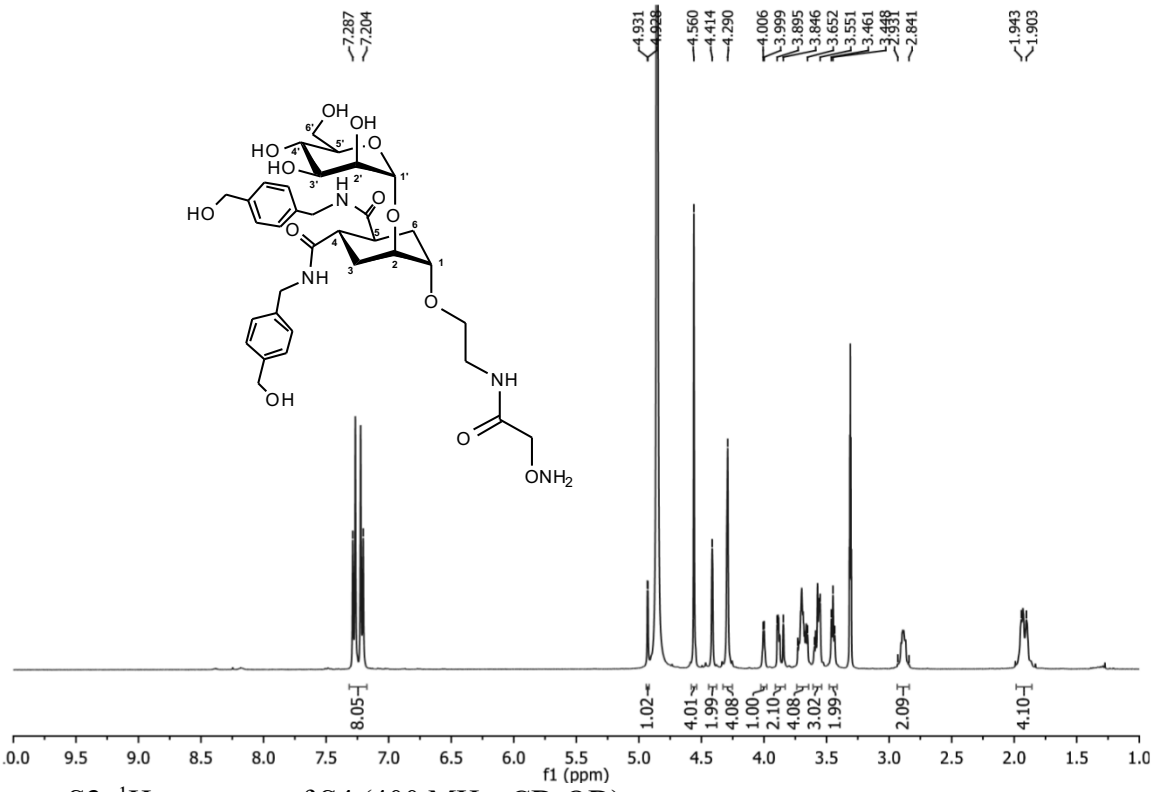



**Figure S3.** $^{1}$H spectrum of **S4** (400 MHz, $CD_3OD$).

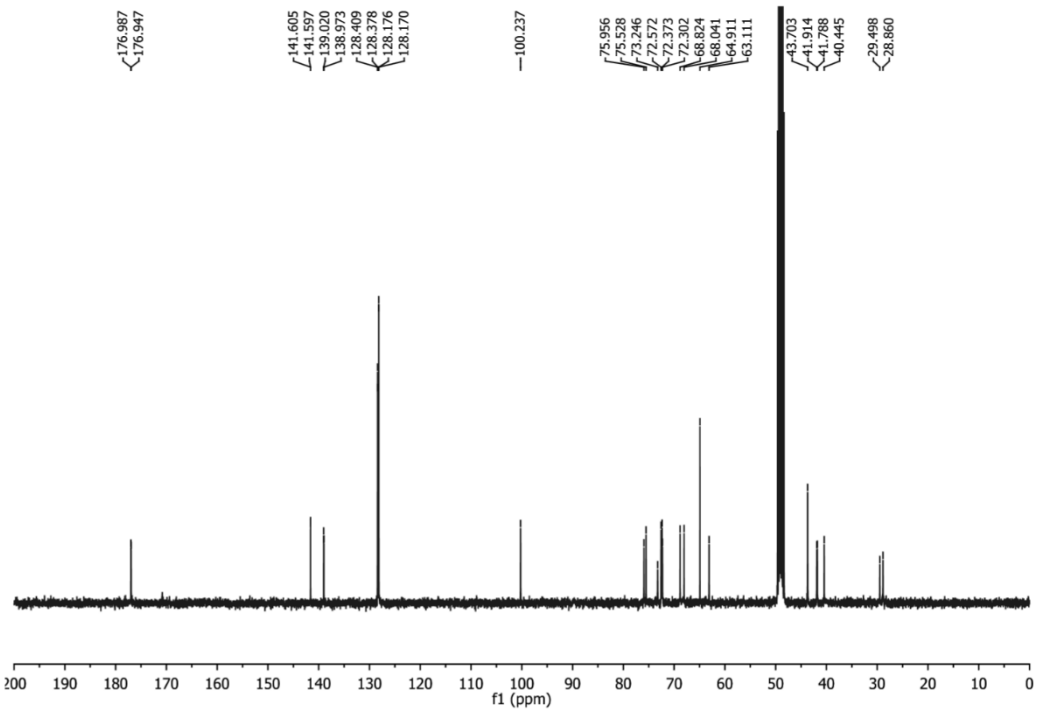



**Figure S4.** $^{13}$C spectrum of **S4** (100 MHz, $CD_3OD$).

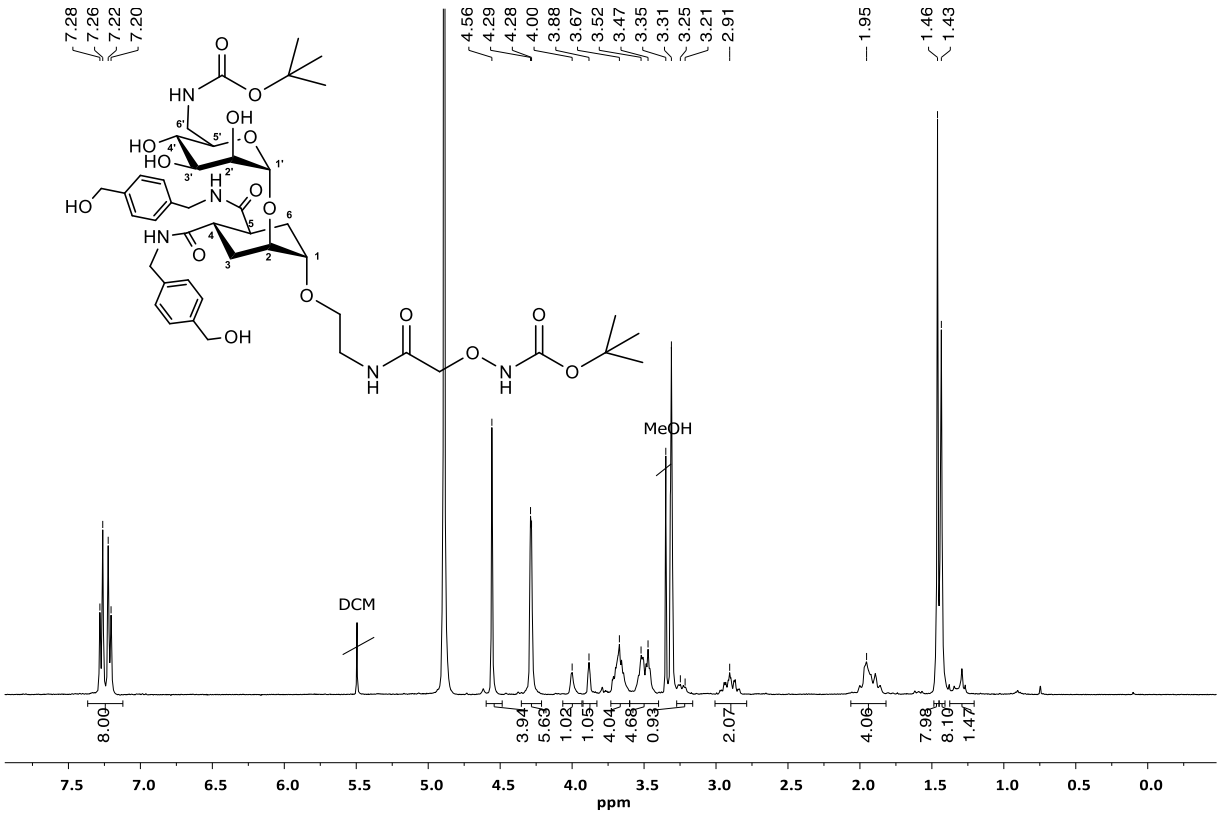



**Figure S5.** $^{1}$H spectrum of **S6** (400 MHz, $CD_3OD$).

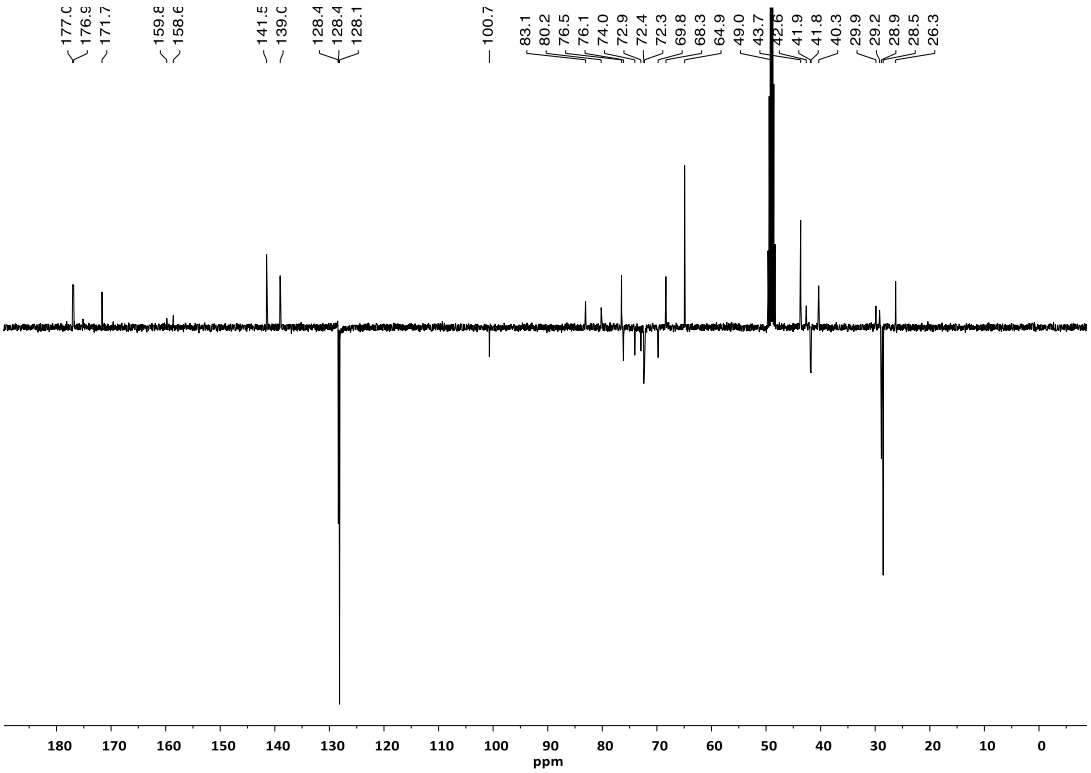



**Figure S6.** $^{13}$C spectrum of **S6** (100 MHz, $CD_3OD$).

1.3. Mass spectra.

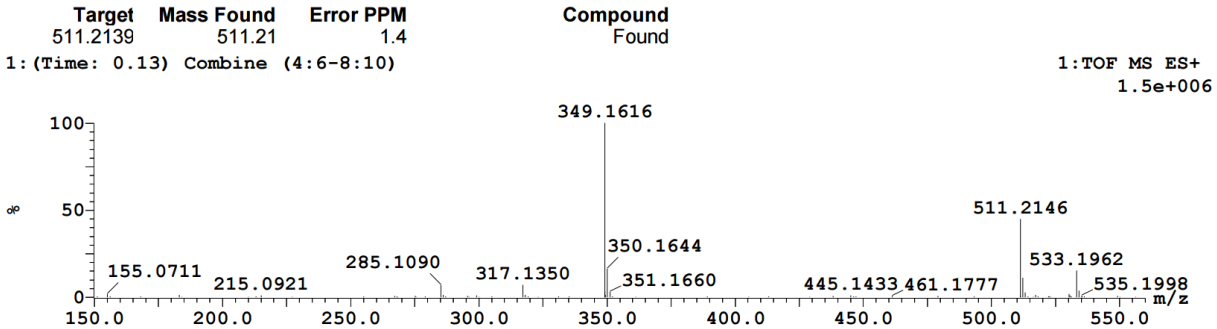



**Figure S7.** HRMS (ESI-TOF) of **S2**. *m/z* calcd for $C_{20}H_{35}N_2O_{13}$ $[M+H]^+$: 511.2139, found: 511.2146 (error = +1.4 ppm).

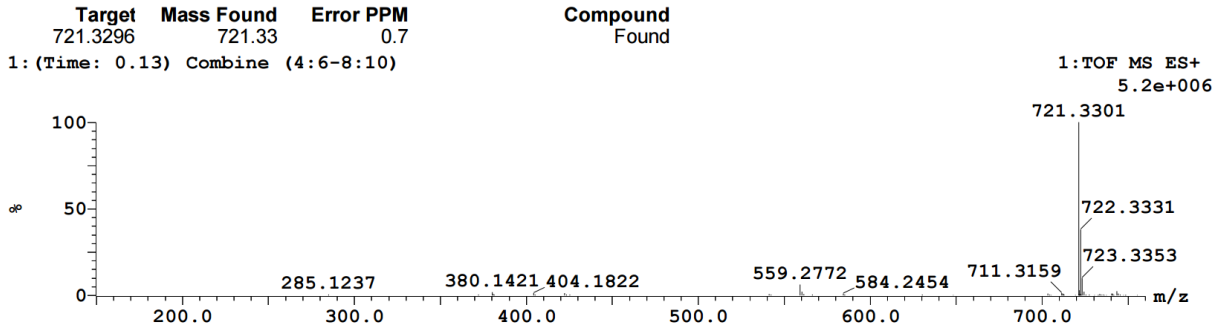



**Figure S8.** HRMS (ESI-TOF) of **S4**. *m/z* calcd for $C_{34}H_{49}N_4O_{13}$ $[M+H]^+$: 721.3296, found: 721.3301 (error = +0.7 ppm).

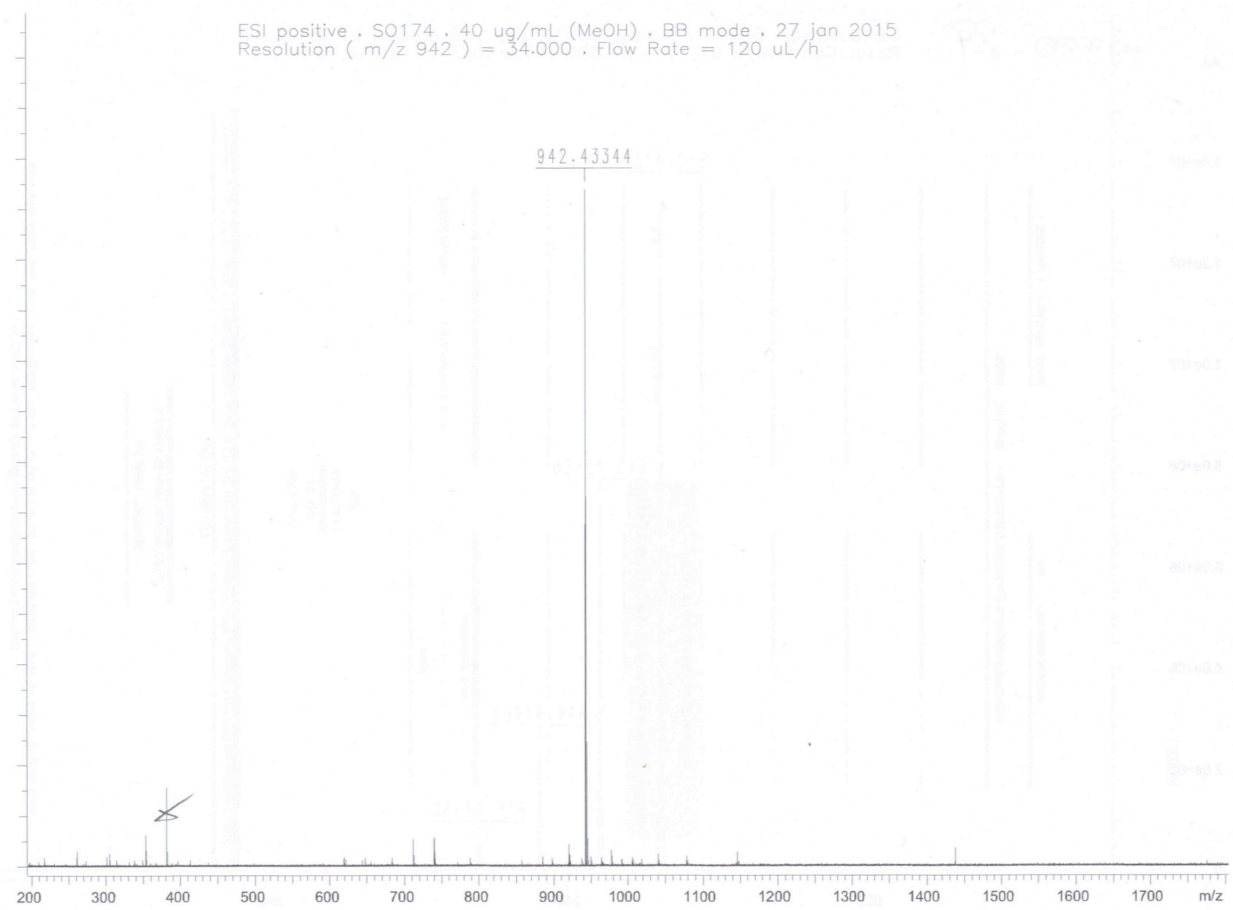



**Figure S9.** HRMS (ESI-TOF) of **S6**. *m/z* calcd. for $[C_{44}H_{65}N_5O_{16}Na]^+$: 942.43240; found: 942.43344 (error = +1.1 ppm).

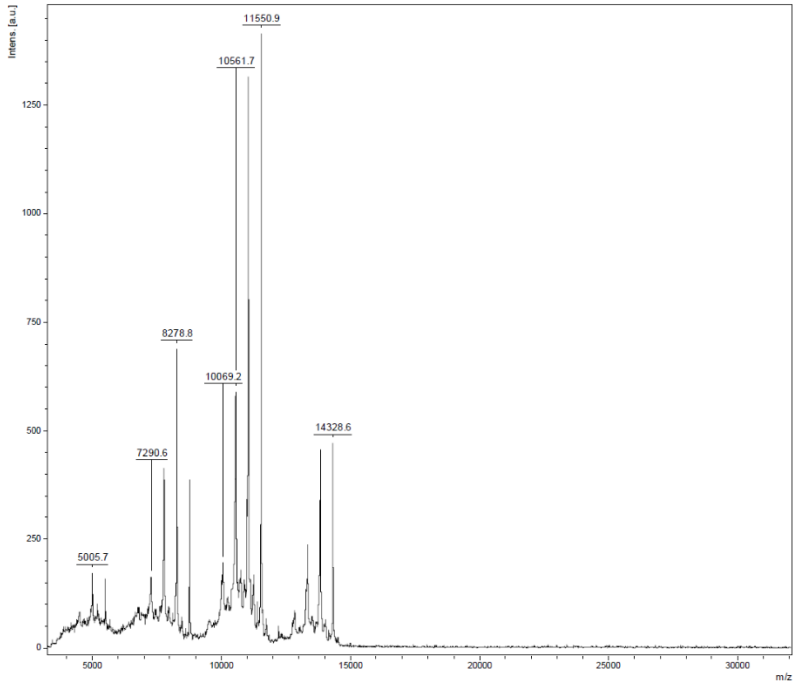



**Figure S10.** MALDI-TOF of **3.B**. *m/z* calcd for $C_{603}H_{942}N_{111}O_{286}$ [M+H]+: 14322.5 , found: 14328.6 (+6.1, error = 426 ppm).

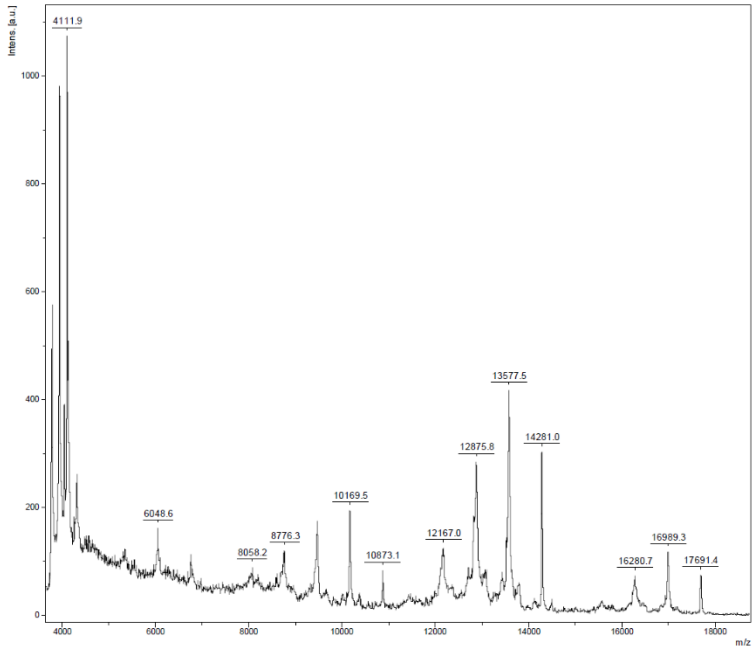



**Figure S11.** MALDI-TOF of **3.C**. *m/z* calcd for C827H1166N143O286 [M+H]+: 17686.9 , found: 17691.4 (+4.3, error = 254 ppm).

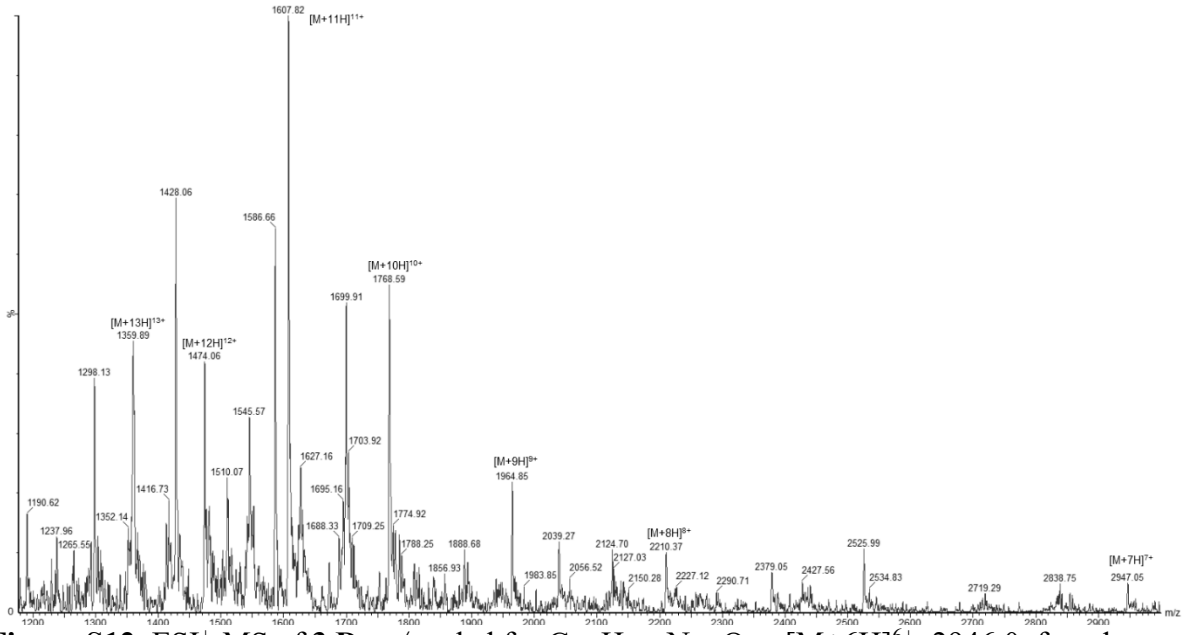



**Figure S12.** ESI$^+$-MS of **3.D**. *m/z* calcd for $C_{827}H_{1187}N_{159}O_{270}$ $[M+6H]^{6+}$: 2946.0, found: 2947.1; calcd for $C_{827}H_{1188}N_{159}O_{270}$ $[M+7H]^{7+}$: 2525.3, found: 2526.0; calcd for $C_{827}H_{1189}N_{159}O_{270}$ $[M+8H]^{8+}$: 2209.8, found: 2210.4; calcd for $C_{827}H_{1190}N_{159}O_{270}$ $[M+9H]^{9+}$: 1964.4, found: 1964.9; calcd for $C_{827}H_{1191}N_{159}O_{270}$ $[M+10H]^{10+}$: 1768.0, found:1768.6; calcd for $C_{827}H_{1192}N_{159}O_{270}$ $[M+11H]^{11+}$: 1607.4, found: 1607.8; calcd for $C_{827}H_{1193}N_{159}O_{270}$ $[M+12H]^{12+}$: 1473.5, found: 1474.1; calcd for $C_{827}H_{1194}N_{159}O_{270}$ $[M+13H]^{13+}$: 1360.2, found: 1359.9

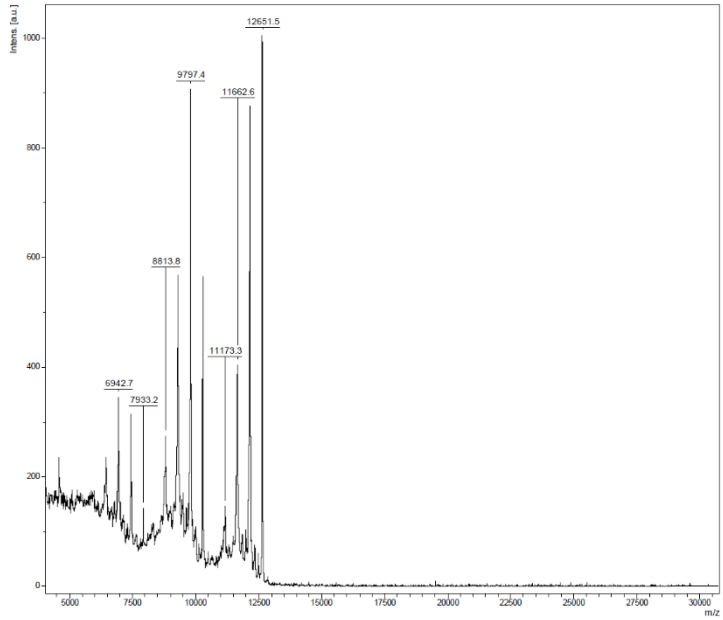



**Figure S13.** MALDI-TOF of **4.B**. *m/z* calcd for $C_{523}H_{826}N_{91}O_{266}$ $[M+H]^+$: 12644.6 , found: 12651.5 (+6.9, error = 546 ppm).

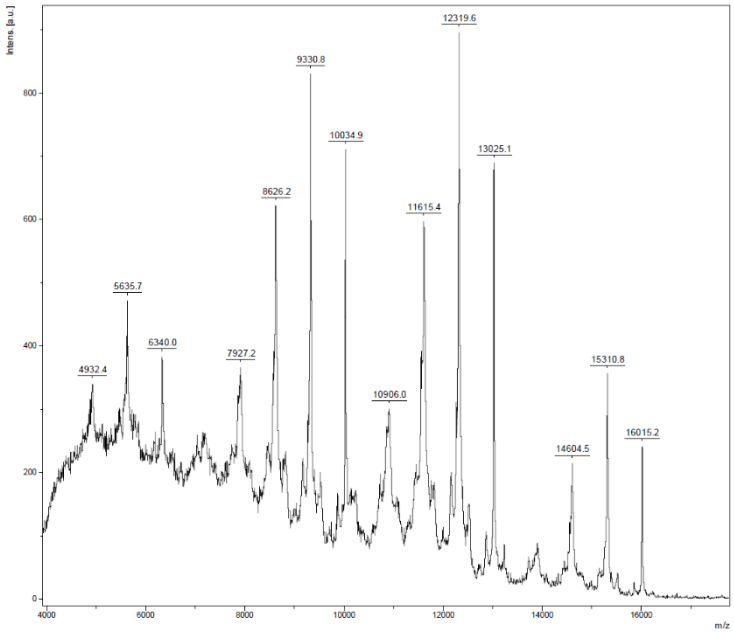



**Figure S14.** MALDI-TOF of **4.C**. *m/z* calcd for $C_{747}H_{1050}N_{123}O_{266}$ $[M+H]^+$: 16009.0, found: 16015.2 (+6.2, error = 387 ppm).

1.4. HPLC chromatograms.

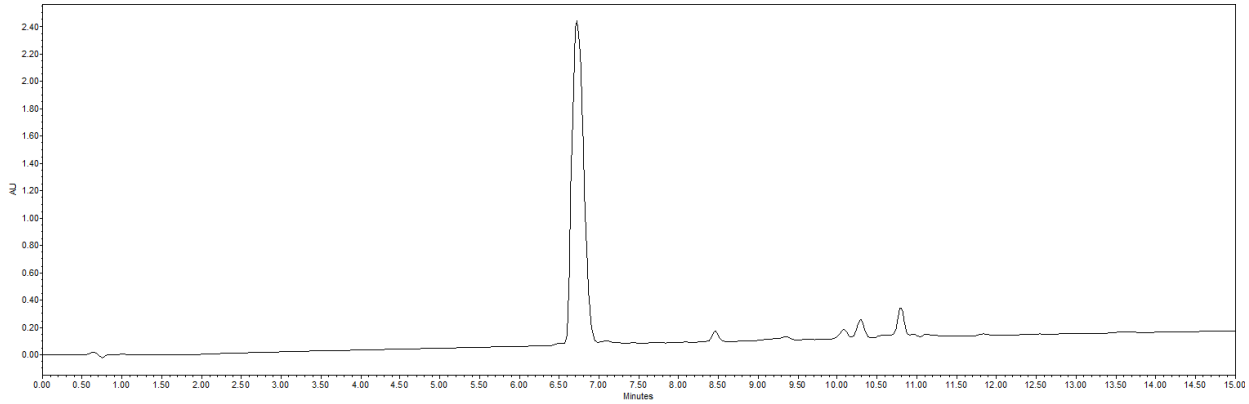



**Figure S15.** Analytical RP-HPLC of **S2**. $t_R$ = 6.72 min (C18, λ = 214 nm 0-30% B in 15 min).

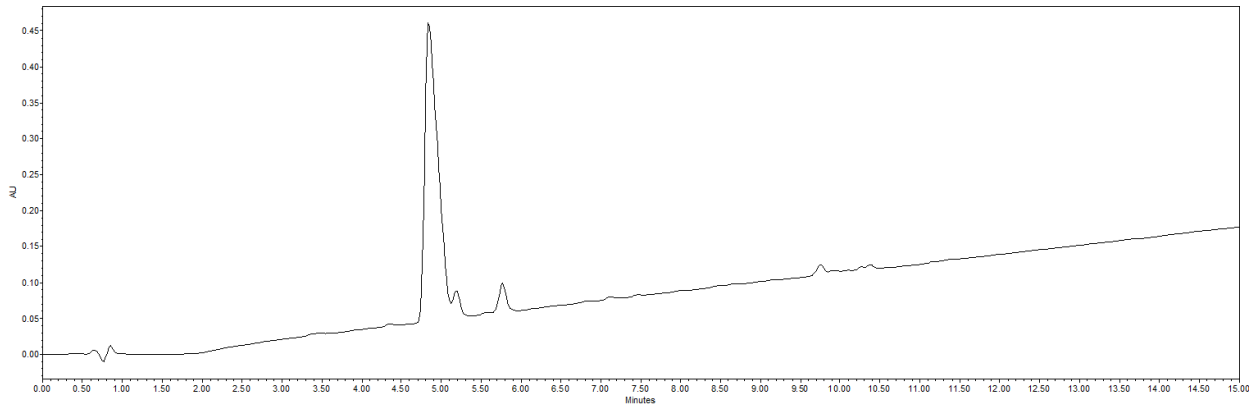



**Figure S16.** Analytical RP-HPLC of **S4**. $t_R$ = 4.84 min (C18, λ = 214 nm 0-30% B in 15 min).

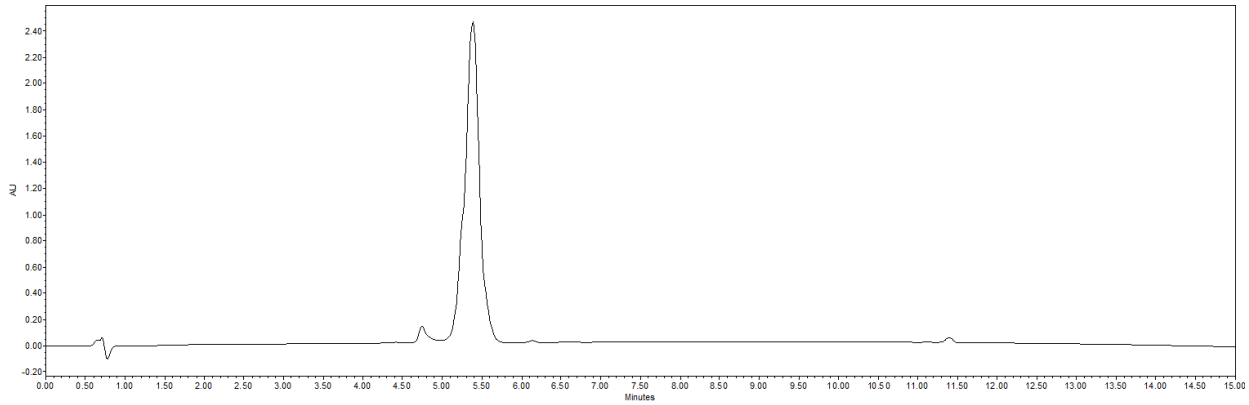



**Figure S17.** Analytical RP-HPLC of **3.B**. $t_R$ = 5.38 min (C18, λ = 214 nm 5-100% B in 15 min).

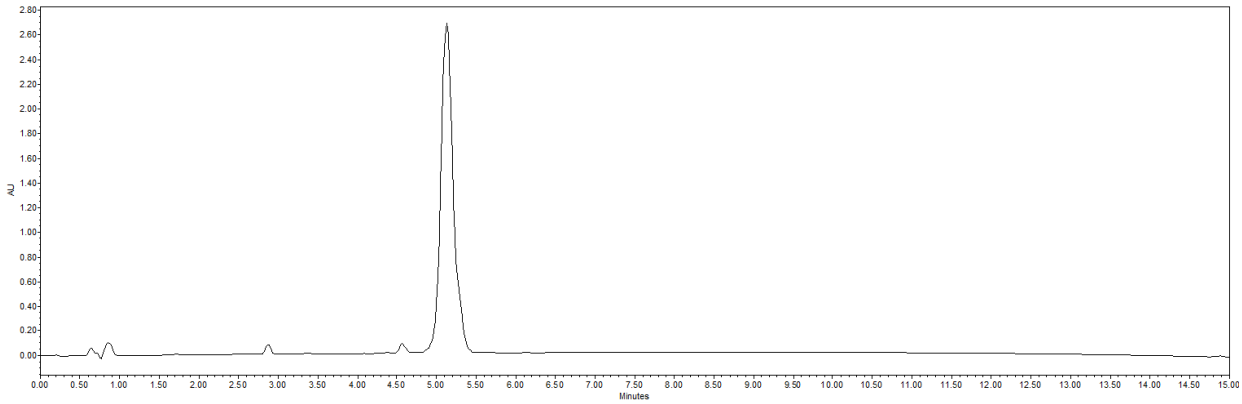



**Figure S18.** Analytical RP-HPLC of **3.C**. $t_R$ = 5.13 min (C18, λ = 214 nm 5-100% B in 15 min).

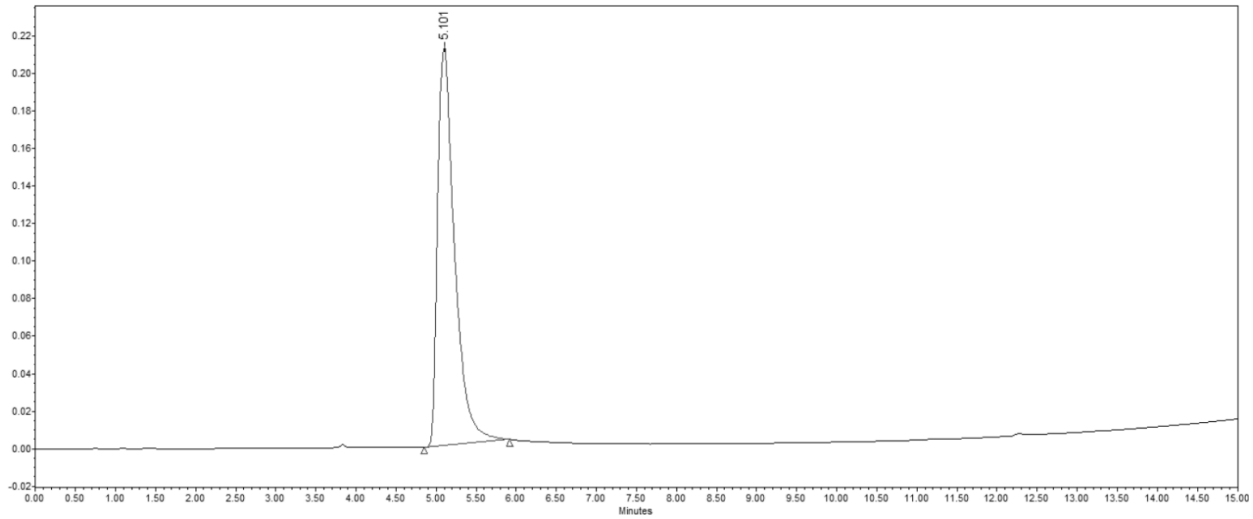



**Figure S19.** Analytical RP-HPLC of **3.D**. $t_R$ = 5.10 min (C18, λ = 214 nm 5-100% B in 15 min.).

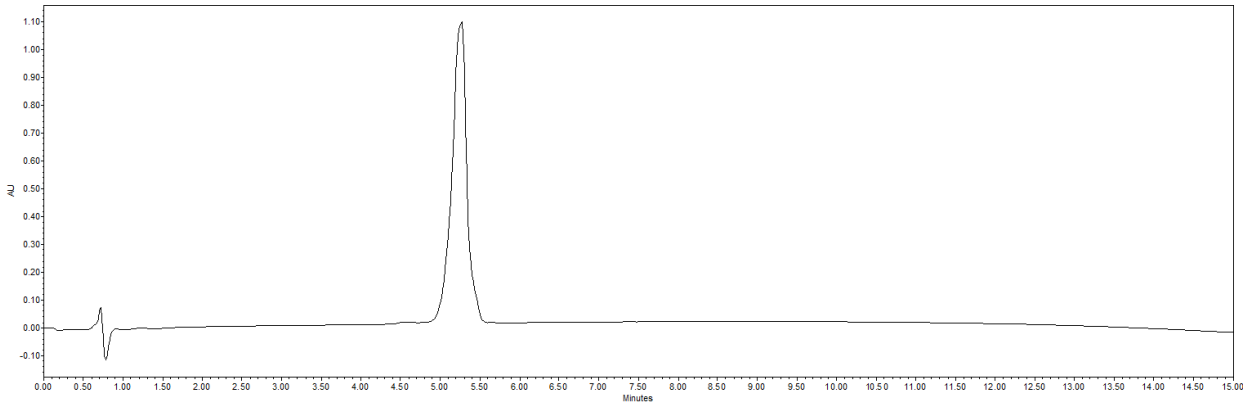



**Figure S20.** Analytical RP-HPLC of **4.B**. $t_R$ = 5.26 min (C18, λ = 214 nm 5-100% B in 15 min).

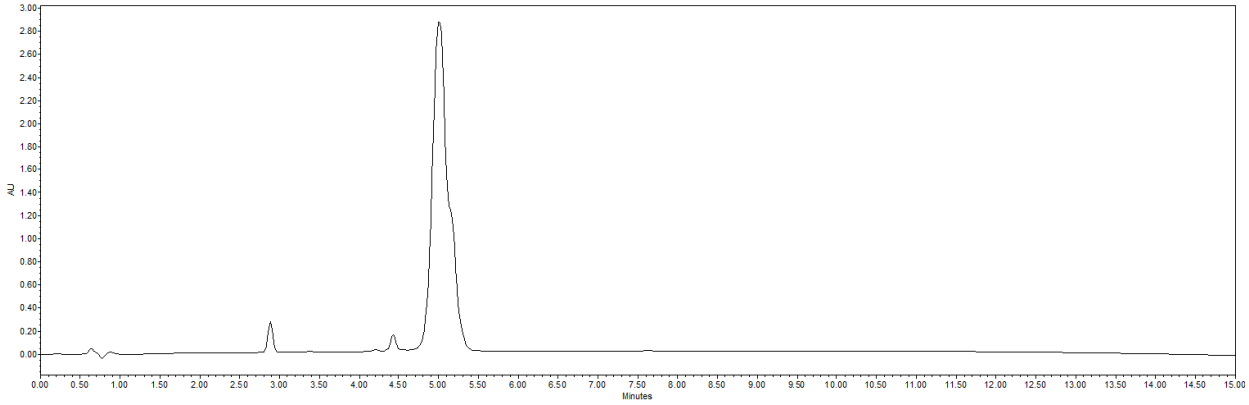



**Figure S21.** Analytical RP-HPLC of **4.C**. $t_R$ = 5.01 min (C18, λ = 214 nm 5-100% B in 15 min).

## 1.5. Bibliography.

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(3) Varga, N.; Sutkeviciute, I.; Guzzi, C.; Mcgeagh, J. Selective Targeting of Dendritic Cell-Specific Intercellular Adhesion Glycomimetics : Synthesis and Interaction Studies of Bis ( Benzylamide ) Derivatives of a Pseudomannobioside. **2013**, 4786–4797.

(4) Porkolab, V.; Chabrol, E.; Varga, N.; Ordanini, S.; Sutkevičiūtė, I.; Thépaut, M.; García-Jiménez, M. J.; Girard, E.; Nieto, P. M.; Bernardi, A.; et al. Rational-Differential Design of Highly Specific Glycomimetic Ligands: Targeting DC-SIGN and Excluding Langerin Recognition. *ACS Chem. Biol.* **2018**, *13* (3), 600–608.

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## 2. Development of stable DC-SIGN S-ECD surface

### 2.1. DC-SIGN S-ECD affinity determination over an amine-coupled *Strep*Tactin surface

In order to generate a stable DC-SIGN S ECD oriented surface, we have chosen to use the bio-specific capturing approach through the *Strep*tagII tag localized to the N-terminus of DC-SIGN. In this SPR assay, the *Strep*Tactin was covalently immobilized over a CM dextran sensor. Then, for the estimation of the appropriate concentration for capturing assay, DC-SIGN S ECD was titrated as analyte over a immobilized *Strep*Tactin surface (Fig S22). In order to determine the suitability of DC-SIGN S-ECD capturing to immobilized *Strep*Tactin surface, the affinity of this DC-SIGN construct to *Strep*Tactin was first estimated and resulted in a reasonable apparent $K_D$ value of 57 nM. This is a higher value than the manufacturer for *Strep*Tag II/*Strep*Tactin interaction, which likely resulted due to the avidity effect: because DC-SIGN ECD is tetrameric and display 4 *Strep*Tag II, and on the other side, immobilized StrepTactin, is also a tetrameric protein.

#### 2.1. Material and method of the DC-SIGN S-ECD titration over an amine-coupled Streptactin surface

SPR experiments were performed on a Biacore T200. The CM3 chip was equilibrated in HBS-P buffer (GE Lifescience). *Strep*Tactin (IBA company) was immobilized on flow cell 2 (Fc2) using a standard amine-coupling method. Using a flow rate of 5 $\mu$L/min, Fc2 were activated with 50 $\mu$L of a 0.2M EDC/ 0.05 M NHS mixture. After this step, Fc2, was functionalized with 170 µg/mL *Strep*Tactin,in 10 mM acetate buffer pH 4 and then remaining activated groups were blocked with 80 $\mu$L of 1 M ethanolamine pH 8. After blocking, Fc2 was treated with 5 $\mu$L of 10 mM HCl to remove no-specific bound proteins and 5 $\mu$L of 50 mM NaOH/ 1M NaCl to expose surface to regeneration protocol. Finally, an average of 2300 RU of *Strep*Tactin was immobilized. The same amine-coupling procedure was repeated for Fc1 excluding the *Strep*Tactin immobilization step. Fc1 is considered as the reference surface.
For DC-SIGN S-ECD titration, a 2-fold serial dilution was prepared in HBS-P buffer (SPR running buffer) and injected for 180 seconds at 5 $\mu$L/min. After 200 seconds of dissociation with the SPR running buffer, the surface was fully regenerated with 1M NaOH/50 mM NaCl at 100 $\mu$L/min for 30 seconds.

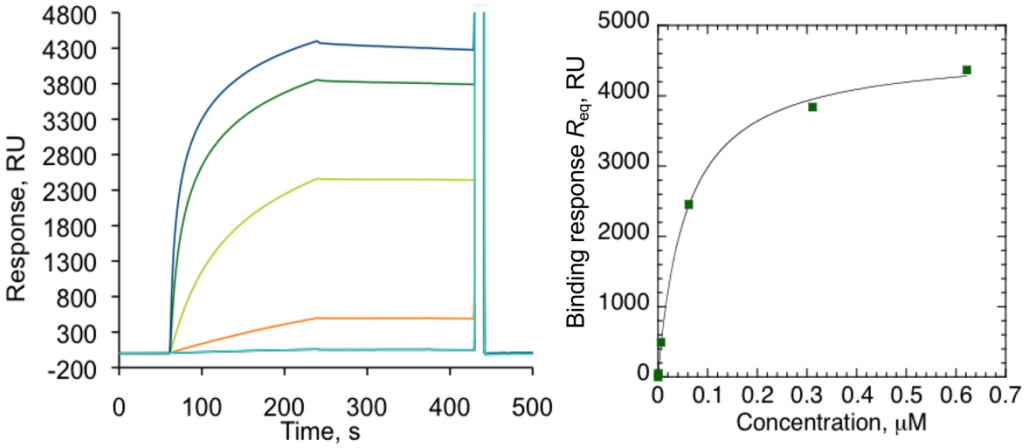



**Figure S22**. DC-SIGN S-ECD titration over a *Strep*Tactin amine-coupled surface. Left panel shows the sensorgram of DC-SIGN S-ECD (concentrations from 0.62 nM to 622 nM) over immobilized *Strep*Tactin (2700 RU). The surface was regenerated with 1M NaOH/ 50 mM. NaC.l The right panel represents the corresponding binding responses as a function of DC-SIGN S-ECD concentration. The $K_D$ extracted from *steady state affinity* model is equal to 57 nM.

### 2.2. Assessment of the covalent bio-capture DC-SIGN S-ECD through its *Strep*Tag II

To control the binding specificity, DC-SIGN S-ECD and DC-SIGN ECD, *i.e.* a construct without *Strep*TagII, were injected over a pre-activated *Strep*Tactin surface (Figure 2) following this procedure: The CM3 chip was equilibrated in HBS-P buffer (GE Lifescience). *Strep*Tactin (IBA company) was immobilized on flow cell 2 (Fc2) and Fc4 using a standard amine-coupling method. Using a flow rate of 5 $\mu$L/min, Fc2 and Fc4 were activated with 50 $\mu$L of a 0.2M EDC/ 0.05 M NHS mixture. After this step, Fc2 and Fc4 were functionalized with 170 µg/mL *Strep*Tactin,in 10 mM acetate buffer pH 4 and then remaining activated groups were blocked with 80 $\mu$L of 1 M ethanolamine pH 8. After blocking, Fc2 and Fc4 were treated with 5 $\mu$L of 10 mM HCl to remove no-specific bound proteins and 5 $\mu$L of 50 mM NaOH/ 1M NaCl to expose surface to regeneration protocol. Finally, an average of 2300-2400 RU of *Strep*Tactin were immobilized. The same amine-coupling procedure was repeated for Fc1 and Fc3 excluding the *Strep*Tactin immobilization step. Fc1and Fc3 are considered as the reference surface.

A second amine-coupling procedure was performed on the fresh-made *Strep*Tactin surfaces (Fc2 and Fc4). At 5 $\mu$L/min, active surfaces were activated with 50 $\mu$L of a 0.2M EDC/ 0.05 M NHS mixture. Then, 150 $\mu$L DC-SIGN S-ECD, prepared at 60 $\mu$g/mL in the running buffer HBS-P, was injected at a flow rate of 5 $\mu$L/min on Fc2 (Figure 2A). Then, 150 $\mu$L DC-SIGN ECD, prepared at 60 $\mu$g/mL in the running buffer HBS-P, was injected at a flow rate of 5 $\mu$L/min

on Fc4 (Figure 2B) Finally, remaining activated -COOH groups were blocked by 30 $\mu$L injection of 1 M ethanolamine pH 8 on Fc2 and Fc4.

DC-SIGN S-ECD was covalently immobilized via its *Strep*Tag II on Fc2. On the other hand, DC-SIGN ECD was not immobilized over the *Strep*Tactin surface on Fc4.

### 2.3. Assessment of DC-SIGN S-ECD surface stability

To assess the binding stability and the surface activity, a DC-SIGN S-ECD surface was made following the protocol described in the manuscript (*Lectin S-ECD surface functionalization and compound titration)*. The final amount of DC-SIGN S-ECD immobilized over the *Strep*Tactin surface (Fc2) is 3000 RU. Then, 10 consecutive cycles of 0.56 µM BSA-Man injection, diluted in HBS-P buffer (100 seconds at 5 $\mu$L/min ) followed by 75 seconds dissociation time in HBS-P buffer were performed on Fc2 and Fc1 (reference surface containing only *Strep*Tactin surface). Finally, the surface was fully regenerated with the injection of 8 $\mu$L of optimized regeneration buffer (50 mM Gly-NaOH pH 11.9 / 0.15% TritonX100 / 25 mM EDTA pH 8) at 100 $\mu$L/min flow rate. The resulting subtracted-sensorgam is depicted on Figure 3A. Binding signals, at t=140 seconds, are similar for 10 injections of BSA-Man. This experiment is presented here as qualitative data. Kinetic/affinity analyses were done and showed on Figure 4B and in SI. Part 3.(see below), by extending the association time of BSA-Man..

## 3. Binding analysis of BSA-Man over a DC-SIGN S-ECD surface

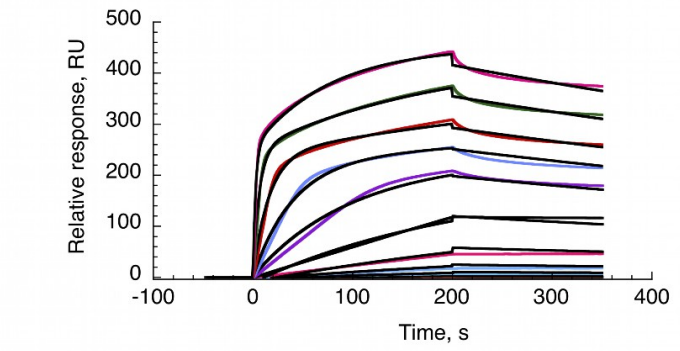



**Figure S23**: Kinetic analysis of BSA-Man titration over a DC-SIGN S-ECD surface. The fit (black curves) derives from the *heterogeneous ligand* model. The extracted values from the fit are: $K_{D1}$=6.48 nM and $K_{D2}$= 0.27 nM. The respective Rmax are 209.9 RU and 233.8 RU.

The BSA-Man interaction with DC-SIGN S-ECD was evaluated first quantitatively by kinetic. As indicated, the interaction study is complex to analyse because multivalent interactions occur between 4 available CRDs of DC-SIGN and 12 glycosylated sites of BSA-Man The first examined approach and the extinctive one is the *1:1 binding* fit that could be represent the avidity generation at the surface. Unfortunately, the corresponding model is not applicable in that case but also with different DC-SIGN S-ECD immobilization levels (200 RU, 1532 RU, 2300 RU). Interestingly, the *heterogeneous ligand* model is considered as relevant because the analyte could interact with 4 identical binding sites. Even if the model is limited to 2 ligand sites, this kinetic model shows a better fit than 1:1 binding (Figure S22). However, the kinetic using this model is working only with BSA-Man or other glycoproteins but not with rationally designed multivalent compounds.

Regarding the *fitting steady state affinity* model, the apparent $R_{eq}$ values (apparent response at e*quilibrium*) at the end of association time were plotted against compound concentration.

No equilibrium is observed at the end of the association time revealing that additional binding mechanisms are involved in the overall binding. Thus, the diversity of binding modes increases with the compound concentration.

To take in account the complexity of multivalent interactions, for each compound titration, a large concentration compound titration was performed. Then, based on the estimated theoretical $R_{max}$.(as described in equation S1), the experimental $R_{eq}$ that has the theoretical $R_{max}$ ±5% value will be considered as the experimental $R_{max}$. This requirement was made based on the strategy of the DC-SIGN S-ECD surface preparation. In this particular assay, the theoretical $R_{max}$ has to be equivalent to the expected experimental $R_{max}$ because the surface activity is 100% active with the oriented design of DC-SIGN S-ECD surface, functionalized at physiological pH.

Even if the lowest concentrations don't reach the equilibrium, the *fitting steady state affinity* model was applied here and the $K_{Ds}$ reported in this manuscript are denoted as $K_{D\ apparent}$ for

their respective surfaces. The same experimental conditions are maintained for all experiments and this model appears to be the most appropriate model to discriminate the best binders.

$$Rmax = RL \times n \times \frac{MW\ analyte}{MW\ ligand}$$

Equation S1: Theoretical analyte binding capacity of the surface in R, given by the theoretical $R_{max.}$ The molecular weight of the analyte (MW analyte) and ligand (MW ligand) corresponds respectively to the molecular weight of the analyte injected (Da) and the molecular weight of DC-SIGN S-ECD (Da). RL value is the amount of immobilized DC-SIGN-S ECD in response units (RU). S is the stoichiometry of the reaction. With the avidity effect, the average is S=1 between a multivalent compound and the DC-SIGN S-ECD surface.

4. SPR inhibition assays: sensorgrams of DC-SIGN - BSAMan surface binding and inhibition curves of glycodendrimers

Surface plasmon resonance (SPR) experiments were performed on a Biacore 3000 using a CM4 chip. Flow cells (Fc) 1 and 2 are functionalized at 5 µL/min. Fc were activated with 50 µL of a 0.2 M EDC/ 0.05 M NHS mixture. After this step, Fc1 and Fc2-3-4 were respectively functionalized with bovine serum albumine (BSA) and mannosylated bovine serum albumine (BSA-Man, BSA-manα1-3[manα1-6]man, Dextra laboratories, 60 µg.mL$^{-1}$). Then remaining activated groups of both cells were blocked with 30 µL of 1 M ethanolamine. After blocking, the four Fc were treated with 5 µL of 10 mM HCl to remove unspecific bound protein and 5 µL of 50 mM EDTA to expose surface to regeneration protocol. Finally, the final immobilization level of BSA and BSA-Man are respectively 1542 RU and 1478. For inhibition studies, 20 µM of DC-SIGN ECD are mixed with increasing concentrations of inhibiting compounds in a running buffer composed of 25 mM Tris pH8, 150 mM NaCl, 4 mM $CaCl_2$, 0.005% P20 surfactant. 13 µL of each sample was injected onto the surfaces at a 5 µL/min flow rate. The resulting sensorgrams were reference surface corrected.

$$y = R_{hi} - \frac{R_{hi} - R_{lo}}{1 + \left(\frac{Conc}{A_1}\right)^{A_2}} \quad (1) \qquad IC_{50} = A_1 \cdot \left( \left( \frac{R_{hi} - R_{lo}}{R_{hi} - 50} \right)^{\frac{1}{A_2}} - 1 \right) \quad (2)$$

The DC-SIGN binding responses were extracted from sensorgrams, converted to percent residual activity values (y), which were plotted against corresponding compound concentration. The 4-parameter logistic model (equation 1), available in the BiaEval software, was fitted to the plots, and the $IC_{50}$ values were calculated, from equation 2, using the values of fitted parameters ($R_{hi}$, $R_{lo}$, $A_1$ and $A_2$).

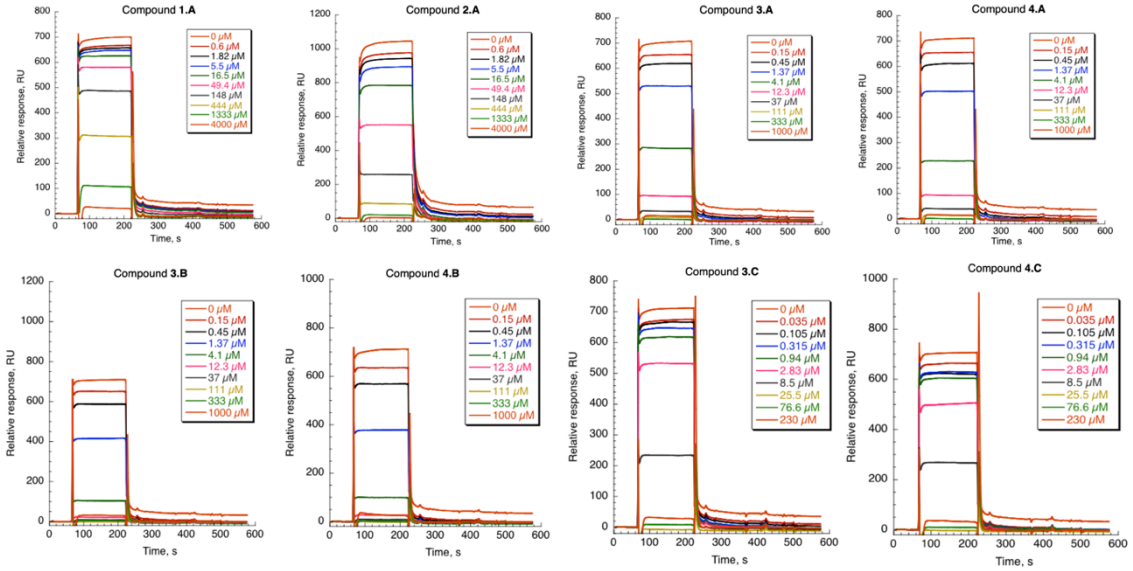



**Figure S24**: Inhibition of DC-SIGN interaction over a BSA-Man surface. 20 µM of DC-SIGN ECD and increasing concentrations of compounds were co-injected over the surface.

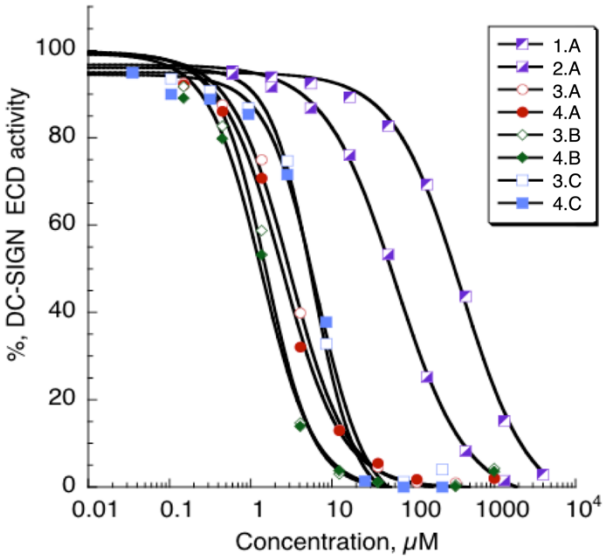



**Figure S25:** Inhibition curves of DC-SIGN interaction with BSA-Man surface. $IC_{50}$ values are extracted from the *4-parameter equation* model.

5. Binding analysis of glycodendrimers onto an oriented DC-SIGN surface. Sensorgrams and $K_{Dapp}$ determination by *Steady state affinity* model.

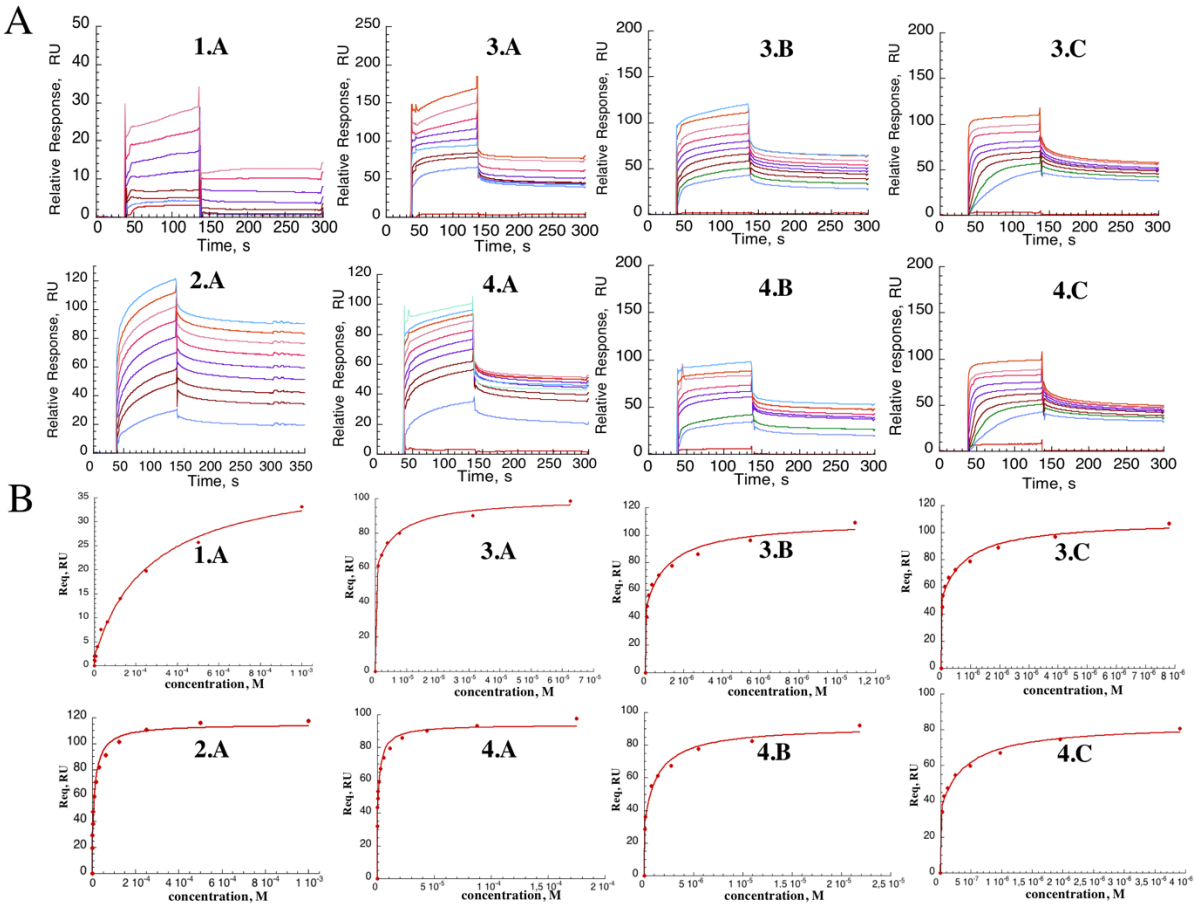



**Figure S26:** Binding analysis of glycodendrimers over an oriented DC-SIGN surface. (A) Reference surface corrected sensorgrams showing the binding of indicated compound over a DC-SIGN S-ECD surface. (B) Binding responses were plotted against their concentrations and fitted by a *steady state affinity* model.

6. Direct interaction of glycomimetic **D** over a Langerin S-ECD surface.

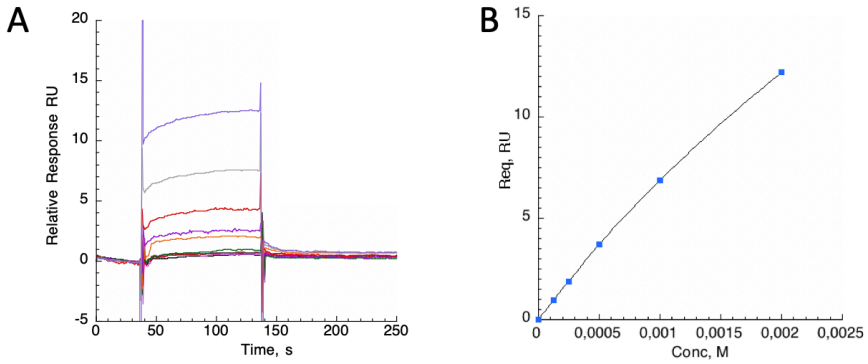
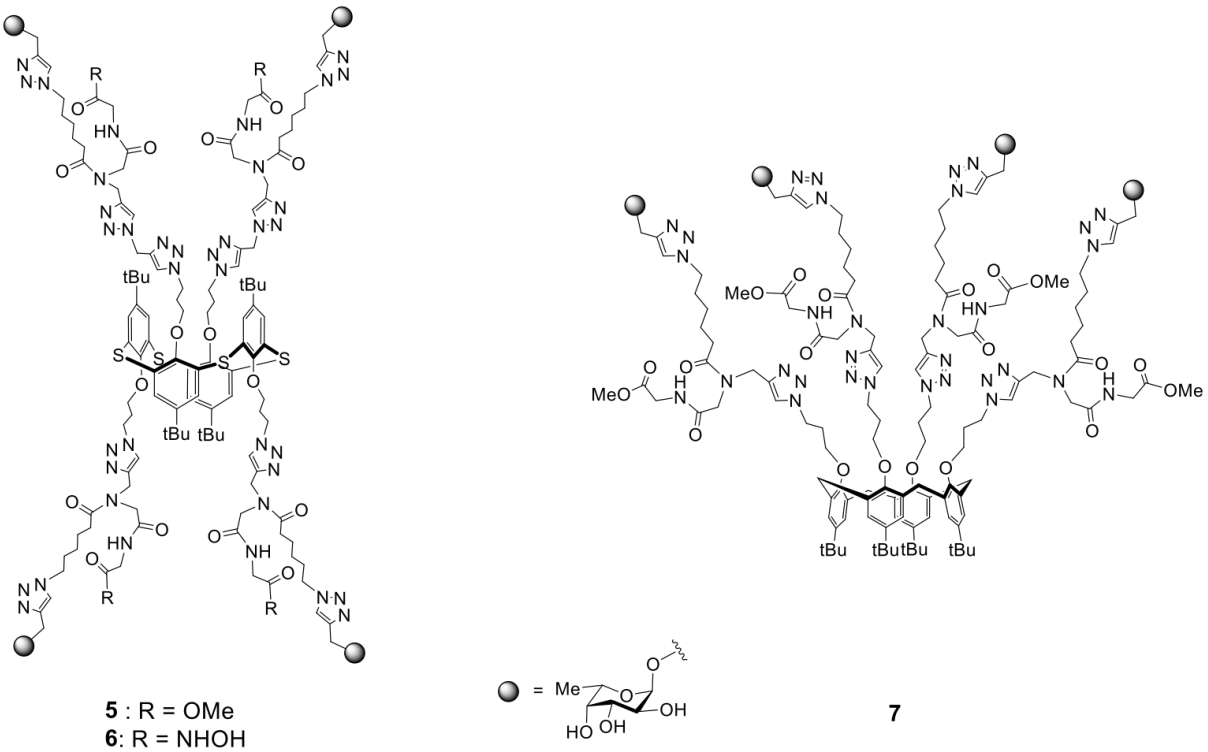



**Figure S27**: (A) Reference-subtracted sensorgram of increasing concentrations (0, 4, 8, 16, 31, 62, 125, 250, 500, 1000, 2000 µM) of glycomimetic **D** over Langerin S-ECD surface. B) Steady state binding analysis (n=1) for ligand **D** (blue dots).

7. Titration of thiacalixarene fucoclusters onto a DC-SIGN S-ECD surface by SPR direct interaction.




**Scheme S4 :** Thiacalixarene fucoclusters structures

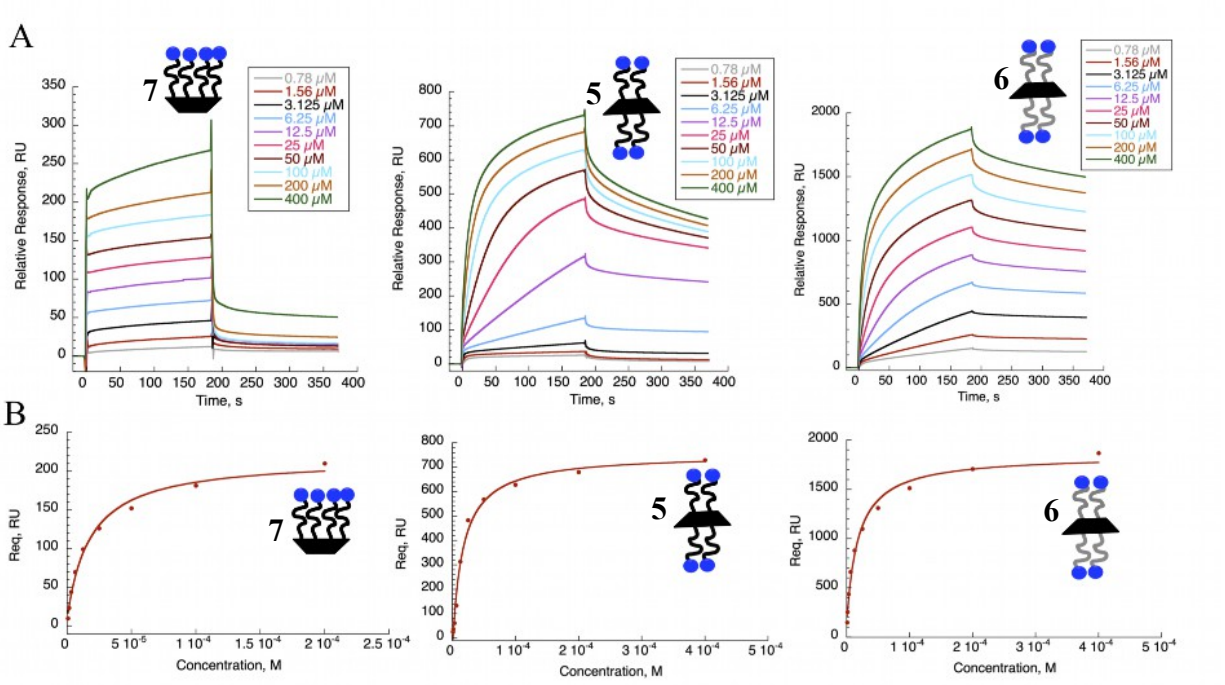



**Figure S28:** Binding analysis of thiacalixarene fucoclusters over a DC-SIGN S-ECD surface. (A) Reference surface corrected sensorgrams showing (from left to right) the binding of compound **7, 5** and **6** over a DC-SIGN surface. (B) Compound binding responses (red dots) were plotted against their concentrations. Curves (red lines) are fitted by a *steady state affinity* model (binding n=1).