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Supporting Information

Synthesis and comparison of linear and hyperbranched multivalent glycosides for C-type lectin binding

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1 Material and methods

All the reagents and solvents were purchased from commercial suppliers and used without further purification unless stated otherwise. Reactions requiring dry or oxygen-free conditions were carried out under argon in Schlenk glassware. NMR spectra were recorded on JEOL ECP500, BRUKER AV500 and BRUKER AV700 spectrometers at 400 MHz, 500 MHz and 700 MHz for ^{1}H NMR spectra and 125 MHz and 175 MHz for ^{13}C NMR spectra, respectively. Chemical shifts are given in parts per million (ppm) in relation to deuterated solvent peak calibration. Infrared (IR) spectra were recorded with a Nicolet AVATAR 320 FT-IR 5 SXC (Thermo Fisher Scientific, Waltham, MA, USA) with a DTGS detector from 4000 to 650 cm^{-1}. A TSQ 7000 (Finnigan Mat) instrument was used for ESI measurements and a JEOL JMS-SX- 102A spectrometer was used for the high-resolution mass spectra.

DLS measurements of the various polymers were conducted by using a Nano DLS particle sizer (Brookhaven Instruments Corp.) at 25 ^{o}C. Aqueous samples were filtered through 0.2 mm filters prior to analysis. Water of Millipore quality was used in all experiments.

NS0-derived recombinant human DC-SIGN/CD209 Fc Chimera Protein, CF and HEK293-derived recombinant human MBL Protein, CF were purchased as dimers from R & D Systems Biotechnology company, US. 2'-Fucosyllactose was purchased from Carbosynth. 2-Azidoethyl-2,3,4,6-tetra-O-acetyl-alpha-D-mannopyranoside was purchased from Apollo scientific.

2 Label-free microscale thermophoresis (MST)

Label-free microscale thermophoresis was used to measure the binding interactions between MBL and PG based glycoconjugates according to the following protocol. For each measurement, a dilution series with constant MBL concentration but varying ligand concentrations was prepared in PBS^{++}. No significant ligand-derived autofluorescence was detected at 280 nm wavelength. The final MBL concentration was 100 µM. All measurements were performed at 22 °C. The thermophoretic movement of fluorescent MBL was monitored with a laser on for 30 s and off for 5 s keeping the MST power at 20% and LED power at 20%. Fluorescence was measured before laser heating (FInitial) and after 30 s of laser irradiation (FHot). The K_d values were then calculated

from three independent thermophoresis measurements using the NanoTemper software (NanoTemper Technologies, Munich, Germany).

3 Surface Plasmon Resonance (SPR)

Experiments were performed on a Biacore X100 instrument (GE Healthcare Europe, Freiburg, Germany) at 25 °C, using HBS-Ca-Mn buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 0.1 mM $CaCl_2$ and 0.01 mM $MnCl_2$) in all cases. DC-SIGN, Fc Chimera Protein (R & D Systems Biotechnology company, US) was immobilised on a protein A sensor chip (GE Healthcare, final response 1700 RU), whereas the reference lane was left unfunctionalized. Each cycle consisted of a 120 s period of sample contact time (association phase) followed by a 600 s dissociation phase. All sample measurements were analysed with single cycle kinetics. Therefore, a concentration series of each sample was measured in triplicates. The determination of K_d values was performed with response unit (RU) data points taken at 15 s before injection stop using built-in software of the Biacore X100. Corresponding binding isotherms were plotted.

4. Synthesis and characterization of all intermediates and final compounds

2'-Fucosyllactose azide

2'-Fucosyllactose (0.07 g, 0.143 mmol) was dissolved in deuterium oxide (1 mL). Diisopropylethylamine (0.25 ml, 1.43 mmol), NaN_3 (0.092 g, 1.73 mmol) and DMC (0.071 g, 0.43 mmol) were added to the above mixture and the reaction mixture was stirred for 1 h at 0 ^oC. After 1 h, the solvent was evaporated under high vacuum, DMF was added and it was centrifuged, and supernatant was collected. This centrifugation step was repeated 3-4 times and all the supernatant were collected and concentrated in vacuo. It was then dissolved in water and passed through pre-neutralized resin column (Dowex H). All the fraction was collected and dialysed in water to give the pure product. (0.071g, 0.139 mmol, Yield = 97.41 %). **^{1}H NMR (500 MHz, D_2O)**: δ = 5.30 (d, J = 2.95 Hz, 1H, H-1'), 4.85 - 4.83 (1H, m), 4.64 (d, J = 7.55 Hz, 1H, H-1'), 4.31 (quart, J = 6.4 Hz, 1H, H-4''), 4.09 - 3.66 (m, 14H), 3.43 (t, J = 8.8 Hz, 1H, H-2), 1.32 (d, J = 6.15 Hz, 3H, -CH_3); **IR (film):** ν = 3368.07, 2930.31, 2119.39, 1251.07, 1075.12, 1040.41 cm^{-1}.

$LPG_8Propargyl_{0.40}$ 2a

Dried LPG (0.200 g, 1.08 mmol OH to be functionalized) was dissolved in dry DMF (10 mL) and cooled to 0 °C. To the stirred solution of LPG in dry DMF at 0 ºC, NaH (0.054 g, 2.15 mmol, 2 eq., 95%) was added. After addition ice bath was removed and the reaction mixture was allowed to stir at room temperature for 3 hours and cooled down again to 0 ºC. The propargyl bromide (0.278 mL, 3.22 mmol, 3 eq.) in dry DMF (1 mL) was added slowly to the reaction mixture and stirred at room temperature overnight. The excess of NaH was quenched by the dropwise addition of water while keeping the reaction flask in an ice bath. The DMF was removed under reduced pressure and the resulting mixture was dialyzed in MeOH to afford LPG-propargyl (0.180 g, 0.018 mmol, Yield = 73.17 %). Degree of propargylation was quantified by ^{1}H NMR, DF = 0.40. **^{1}H NMR (500 MHz, CD_3OD)**: δ = 4.22 (s, 2H, $OCH_2C{\equiv}CH$), 3.71 - 3.55 (m, 13H, LPG backbone), 2.90 (s, 1H, C≡CH); **IR (film)**: *ν* = 3397.96, 3281.29, 2917.77, 2874.38, 2113.6, 1713.3, 1644.98, 1460.81, 1352.82, 1072.23 cm^{-1}.

$LPG_8propargyl_{1.00}$ 2b

Similar procedure as for **2a**: LPG (0.235 g, 3.17 mmol OH to be functionalized) was propargylated using NaH (0.16 g, 6.35 mmol, 2 eq., 95%) and propargyl bromide (0.081 mL, 9.52 mmol, 3.0 eq.). DF = 1.00. (0.231 g, 0.0194 mmol, Yield = 66.57 %). 1H NMR (500 MHz, CD_3OD): **^{1}H NMR (500 MHz, $CDCl_3$)**: δ = 4.18 (s, 2H, $OCH_2C{\equiv}CH$), 3.65 - 3.57 (m, 5H, LPG backbone), 2.48 (s, 1H, C≡CH); **IR (film)**: *ν* = 3285.14, 2919.7, 2114.58, 1357.64, 1033.66 , 952.66.

$hPG_{10}Propargyl_{0.60}$ 6a

Similar procedure as for **2a**: hPG (0.198 g, 1.60 mmol OH to be functionalized) was propargylated using NaH (0.081 g, 3.2 mmol, 2 eq., 95%) and propargyl bromide (0.0.413 mL, 4.8 mmol, 3.0 eq.). DF = 0.60 (0.190 g, 0.0145 mmol, Yield: 73.64 %). **^{1}H NMR (500 MHz, CD_3OD)**: δ = 4.36 (s, 1H, sec $OCH_2C{\equiv}CH$), 4.23 (s, 1H, primary $OCH_2C{\equiv}CH$), 3.89 - 3.60 (m, 8H, hPG backbone), 2.91 (s, 1H, C≡CH); **IR (film)**: *ν* = 3420.14, 3284.18, 2919.70, 2114.56, 1092.48, 1032.69 cm^{-1}.

$hPG_{10}Propargyl_{1.00}$ 6b

Similar procedure as for **2a**: hPG (0.227 g, 3.06 mmol OH to be functionalized) was propargylated using NaH (0.155 g, 6.13 mmol, 2 eq., 95%) and propargyl bromide

(0.078 mL, 9.19 mmol, 3.0 eq.). DF = 1.00. (0.259 g, 0.017 mmol, Yield: 75.51 %). **^{1}H NMR (500 MHz, CD_3COCD_3)**: δ = 4.37 (s, 1H, sec $OCH_2C{\equiv}CH$), 4.23 (s, 1H, primary $OCH_2C{\equiv}CH$), 3.87 - 3.60 (m, 5H, hPG backbone), 2.97 (brs, 1H, C≡CH); **IR (film)**: *v* = 3287.07, 2868.59, 2114..56, 1033.66 cm^{-1}.

$LPG_8Man_{0.40}$ 3a

To a mixture of $LPG_8Propargyl_{0.40}$ **2a** (0.023 g, 0.101 mmol of propargyl to be functionalized) and azido mannose (0.0465 g, 0.112 mmol) in DMF (15 mL), $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.040 g, 0.203 mmol) solution in H_2O (2 mL) were added dropwise. The reaction mixture was degassed thoroughly with argon for 5-10 minutes and then allowed to stir for 2 days at 40 °C. The reaction was stopped, and solvent was removed under reduced pressure. 2M NaOH (7 mL) was added to the residue and stirred at room temperature for 4-5 hrs. The reaction mixture was neutralized by adding 2M HCl solution and dialyzed first against water and aqueous EDTA solution for 2 days and again using only water for 4 days. The solvent of the dialysis was changed thrice a day. The aqueous solution obtained after dialysis was lyophilized to afford LPG_8 $Man_{0.40}$. DF = 0.36. (0.046 mg, 0.002 mmol, Yield: 93.91%). DF = 0.36. **^{1}H NMR (500 MHz, D_2O)**: δ = 8.10 (s, 1H, C=CH), 4.64 (brs, 4H, CH_2CH_2Trz, $TrzCH_2O$), 4.04 – 3.52 (m, 22H, Man: H-1, H-2, H-3, H-4, H-5, H-6, $CH\mathit{H}_aCH_2Trz$, LPG backbone) 3.02 (s, 1H, $CH\mathit{H}_bCH_2Trz$); Elemental analysis: calcd (%): N 7.83%; found: N 6.36 %.

$LPG_8Man_{1.00}$ 3b

Similar procedure as for **3a**: $LPG_8Propargyl_{1.00}$ **2b** (0.020 g, 0.141 mmol of propargyl to be functionalized) and azido mannose (0.107 g, 0.257 mmol) were coupled using $CuSO_4{\cdot}5H_2O$ (0.008 g, 0.034 mmol) and sodium ascorbate (0.068 g, 0.342 mmol) assisted click reaction. Deprotection was performed by similar procedure as **3a** using 2M NaOH. (0.051 g, 0.016 mmol, Yield: 69.86%). DF = 1.00. DF = 1.00. **^{1}H NMR (700 MHz, D_2O)**: δ = 8.13 (s, 1H, C=CH), 4.65 (brs, 4H, CH_2CH_2Trz, $TrzCH_2O$), 4.10 – 3.61 (m, 13H, Man: H-1, H-2, H-3, H-4, H-5, H-6, $CH\mathit{H}_aCH_2Trz$, LPG backbone), 3.11 (s, 1H, $CH\mathit{H}_bCH_2Trz$); Elemental analysis: calcd (%): N 10.23%; found: N 9.24 %.

$LPG_8FL_{0.40}$ 4a

Similar procedure as for **3a**: $LPG_8Propargyl_{0.40}$ (0.020 g, 0.089 mmol of propargyl to be functionalized) and 2'-fucosyllactose azide (0.054 g, 0.106 mmol) were coupled using $CuSO_4{\cdot}5H_2O$ (0.004 g, 0.178 mmol) and sodium ascorbate (0.035 g, 0.178 mmol) assisted click reaction. (0.048 g, 0.014 mmol, Yield: 72.72 %). DF = 0.39. DF = 0.39. **^{1}H NMR (700 MHz, D_2O)**: δ = 8.31 (s, 1H, C=CH), 5.80 (s, 1H, H-1), 5.35 (s, 1H, H-1"), 4.72 (brs, 2H, CH_2Trz), 4.61 (d, J = 7 Hz, 1H, H-1'), 4.26 (s, 1H, H-4"), 4.09 - 3.73 (m, 28H, FL: H-2, H-3, H-4, H-5, H-6, H-2', H-3', H-4', H-5', H-6', H-2", H-3", H-5", LPG backbone), 1.28 (s, 3H, CH_3); Elemental analysis: calcd (%): N 5.34 %; found: N 5.61 %.

$LPG_8FL_{1.00}$ 4b

Similar procedure as for **3a**: $LPG_8Propargyl_{1.00}$ (0.020 g, 0.171 mmol of propargyl to be functionalized) and 2'-fucosyllactose azide (0.105 g, 0.205 mmol) were coupled using $CuSO_4{\cdot}5H_2O$ (0.008 g, 0.034 mmol) and sodium ascorbate (0.068 g, 0.342 mmol) assisted click reaction. (0.091 g, 0.013 mmol, Yield: 75.20%). DF = 1.00. **^{1}H NMR (700 MHz, D_2O)**: δ = 8.20 (s, 1H, C=CH), 5.70 (s, 1H, H-1), 5.26 (s, 1H, H-1"), 4.52 - 3.64 ((m, 28H, FL: H-1, H-2, H-3, H-4, H-5, H-6, H-1', H-2', H-3', H-4', H-5', H-6', H-1" H-2", H-3", H-4", H-5", LPG backbone), 1.18 (s, 3H, CH_3); Elemental analysis: calcd (%): N 6.27 %; found: N 5.45 %.

$hPG_{10}Man_{0.70}$ 7a

Similar procedure as for **3a**: $hPG_{10}Propargyl_{1.00}$ (0.020 g, 0.178 mmol of propargyl to be functionalized) and azidomannose (0.089 g, 0.214 mmol) were coupled using $CuSO_4{\cdot}5H_2O$ (0.008 g, 0.035mmol) and sodium ascorbate (0.070 g, 0.356 mmol) assisted click reaction. Deprotection was performed by similar procedure as **3a** using 2M NaOH. (0.051 g, 0.016 mmol, Yield: 69.86%). DF = 0.68. **^{1}H NMR (700 MHz, D_2O)**: δ = 8.21 (s, 1H, C=CH), 4.68 (brs, 4H, CH_2CH_2Trz, TrzCH_2O), 4.10 – 3.62 (m, 16H, Man: H-1, H-2, H-3, H-4, H-5, H-6, CH$H_a$$CH_2$Trz, hPG backbone), 3.13 (s, 1H, CH$H_b$$CH_2$Trz Elemental analysis: calcd (%): N 9.29%; found: N 9.02 %.

$hPG_{10}Man_{1.00}$ 7b

Similar procedure as for **3a**: $hPG_{10}Propargyl_{1.00}$ (0.055 g, 0.495 mmol of propargyl to be functionalized) and azidomannose (0.0309 g, 0.743 mmol) were coupled using $CuSO_4{\cdot}5H_2O$ (0.025 g, 0.099 mmol) and sodium ascorbate (0.196 g, 0.99 mmol) assisted click reaction. Deprotection was performed by similar procedure as **3a** using 2M NaOH. DF = 1.00. (0.155 g, 0.003 mmol, Yield: 77.6 %). **^{1}H NMR (700 MHz, D_2O)**: δ = 8.12 (s, 1H, C=CH), 4.65 (brs, 4H, CH_2CH_2Trz, $TrzCH_2O$), 4.10 – 3.87 (m, 13H, Man: H-1, H-2, H-3, H-4, H-5, H-6, $CH\mathit{H}_aCH_2Trz$, hPG backbone), 3.13 (s, 1H, $CH\mathit{H}_bCH_2Trz$); Elemental analysis: calcd (%): N 10.36%; found: N 10.78 %.

$hPG_{10}FL_{0.60}$ 8a

Similar procedure as for **3a**: $hPG_{10}Propargyl_{0.60}$ (0.020 g, 0.124 mmol of propargyl to be functionalized) and 2'-fucosyllactose azide (0.082 g, 0.161 mmol) were coupled using $CuSO_4{\cdot}5H_2O$ (0.006 g, 0.0.024 mmol) and sodium ascorbate (0.049 g, 0.248 mmol) assisted click reaction. (0.0646 g, 0.001 mmol, Yield: 72.58 %). DF = 0.60. **^{1}H NMR (700 MHz, D_2O)**: δ = 8.21 (s, 1H, C=CH), 5.70 (s, 1H, H-1), 5.26 (s, 1H, H-1''), 4.52 - 3.65 (m, 27H, CH_2Trz , FL: H-2, H-3, H-4, H-5, H-6, H-1', H-2', H-3', H-4', H-5', H-6', H-2'', H-3'', H-5'', H-5'', hPG backbone), 1.18 (s, 3H, CH_3); Elemental analysis: calcd (%): N 5.84 %; found: N 6.21 %.

$hPG_{10}FL_{1.00}$ 8b

Similar procedure as for **3a**: $hPG_{10}Propargyl_{1.00}$ (0.015 g, 0.134 mmol of propargyl to be functionalized) and 2'-fucosyllactose azide (0.089 g, 0.173 mmol) were coupled using $CuSO_4{\cdot}5H_2O$ (0.006 g, 0.027 mmol) and sodium ascorbate (0.053 g, 0.267 mmol) assisted click reaction. DF = 1.00. (0.065 g, 0.0007 mmol, Yield: 73.03 %). **^{1}H NMR (500 MHz, D_2O)**: δ = 8.26 (s, 1H, C=CH), 5.77 (s, 1H, H-1), 5.34 (s, 1H, H-1''), 4.25 – 3.73 (m, 27H, CH_2Trz , FL: H-2, H-3, H-4, H-5, H-6, H-1', H-2', H-3', H-4', H-5', H-6', H-2'', H-3'', H-5'', H-5'', hPG backbone), 1.24 (s, 3H, CH_3); Elemental analysis: calcd (%): N 6.27 %; found: N 7.05 %.

5 ^{1}H spectra of all the intermediates and final molecules.

1. LPG$_8$Propargyl$_{0.40}$ 2a

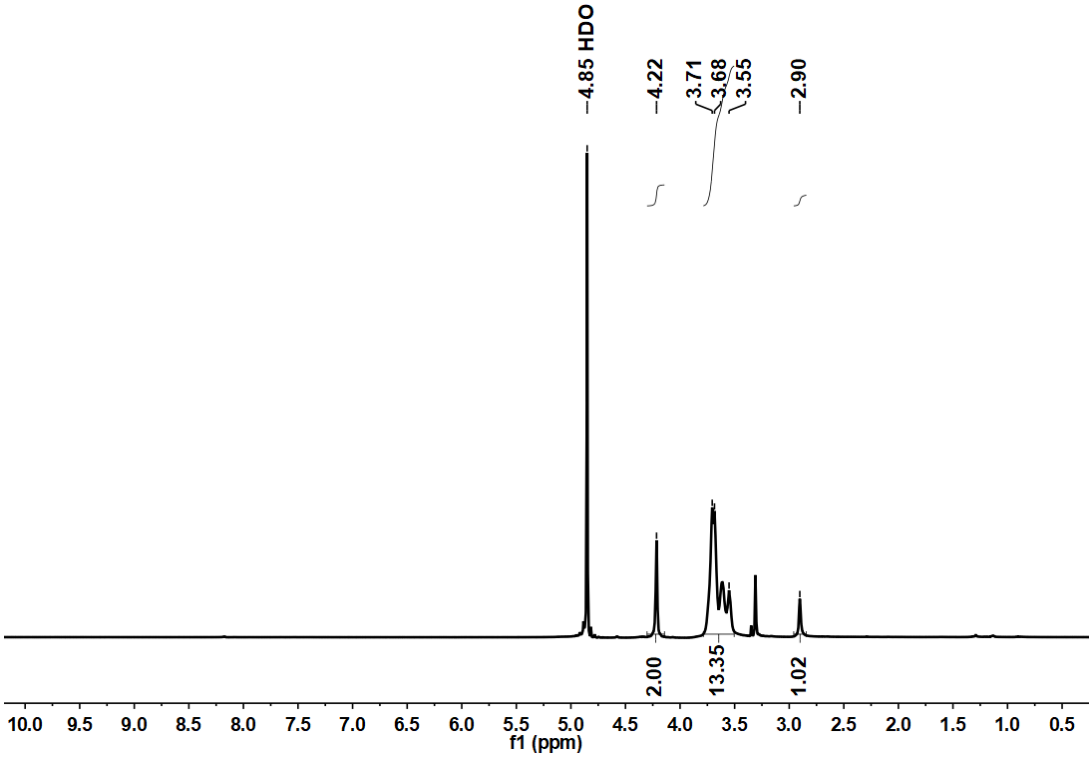

Fig. s1 ^{1}H NMR (500 MHz, CD_3OD) spectra of compound **2a**

2. LPG$_8$Propargyl$_{1.00}$ 2b

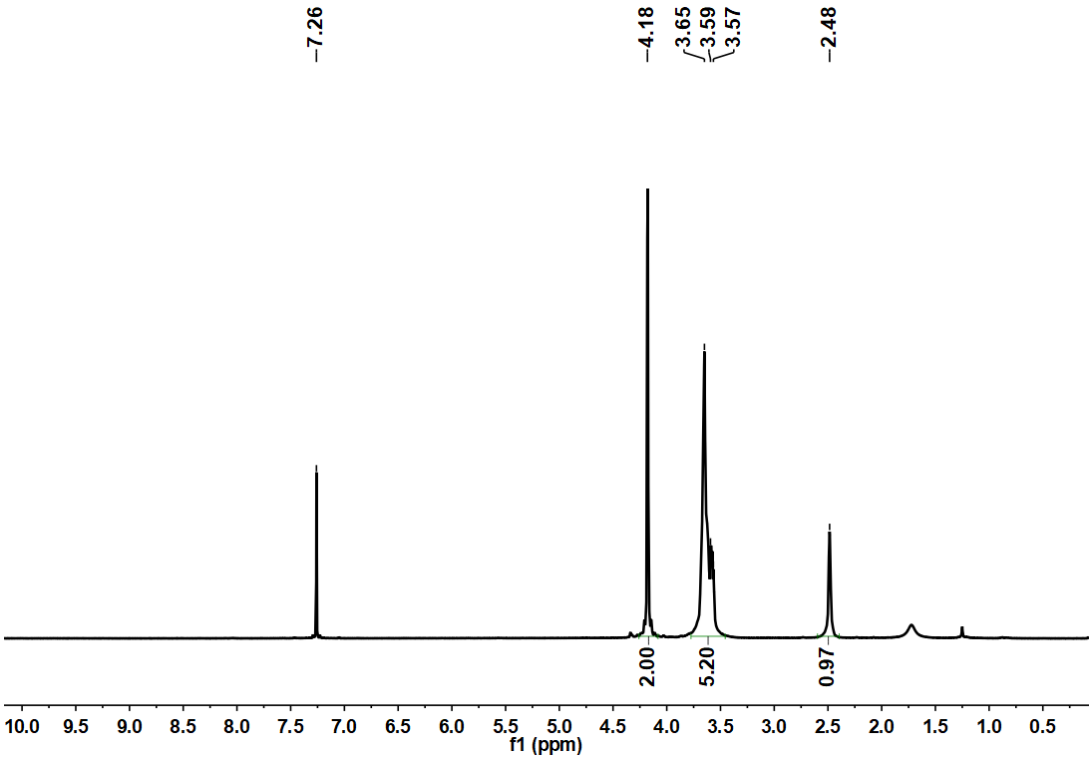

Fig. s2 ^{1}H NMR (500 MHz, $CDCl_3$) spectra of compound **2b**

3. hPG$_{10}$Propargyl$_{0.60}$ 6a

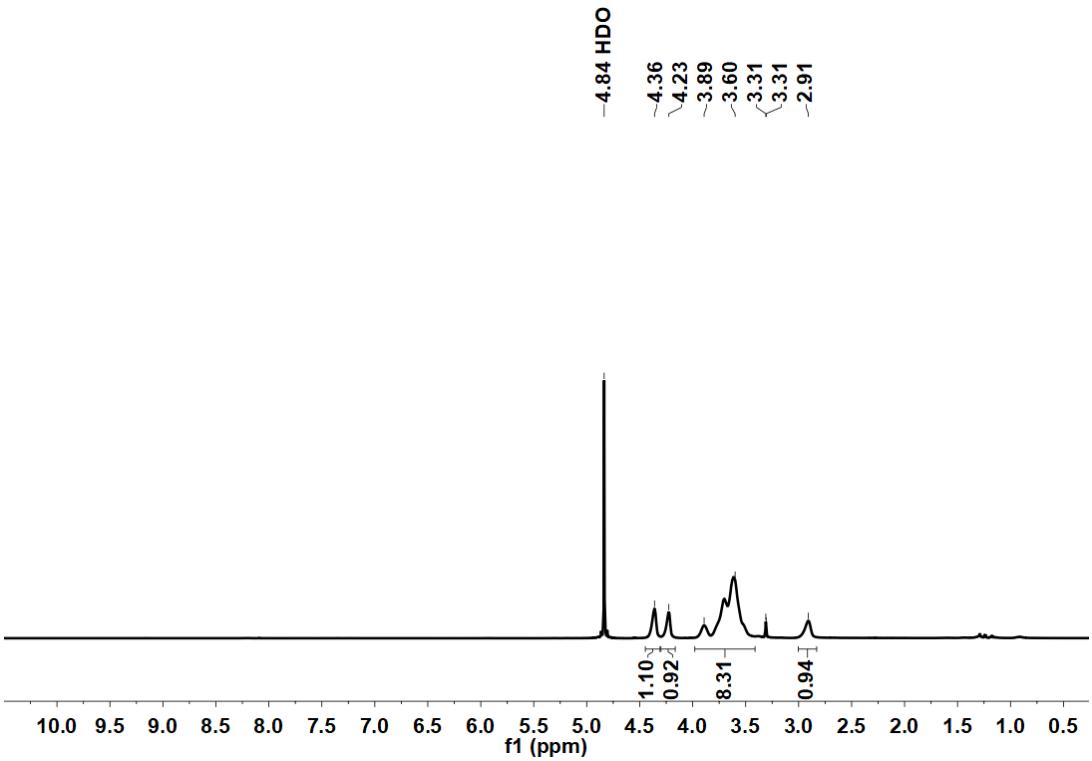

Fig. s3 ^{1}H NMR (500 MHz, CD_3OD) spectra of compound **6a**

4. hPG$_{10}$Propargyl$_{1.00}$ 6b

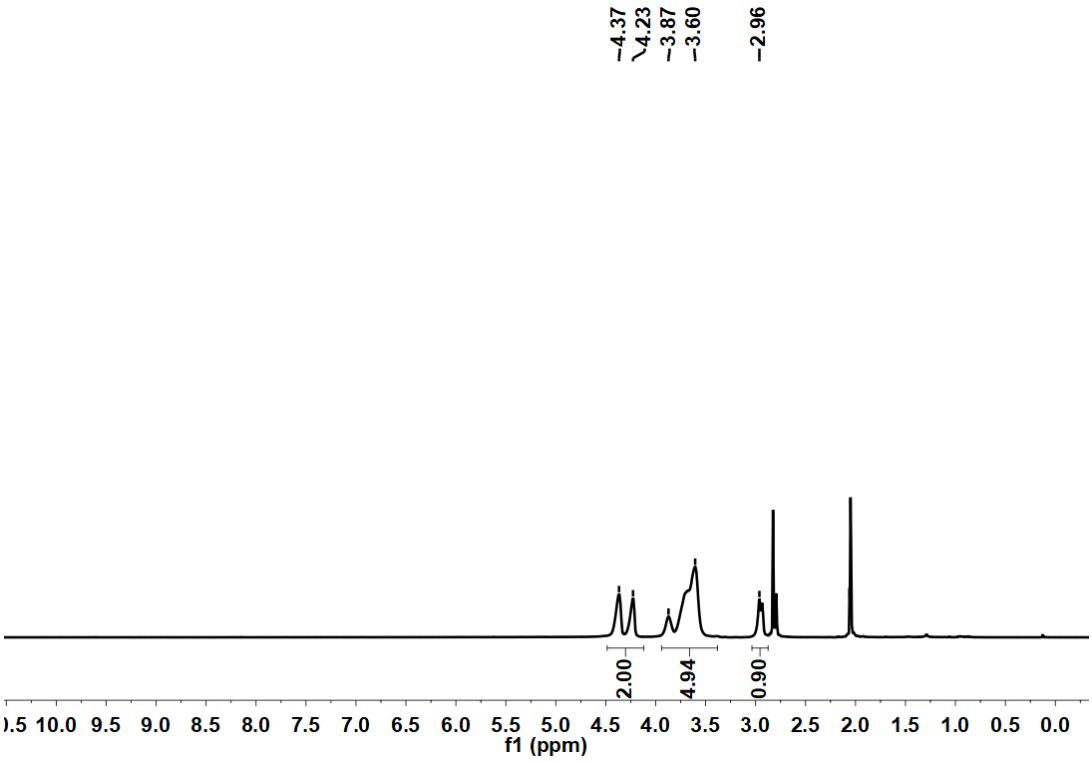

Fig. s4 ^{1}H NMR (500 MHz, CD_3COCD_3) spectra of compound **6b**

5. 2'-Fucosyllactose azide

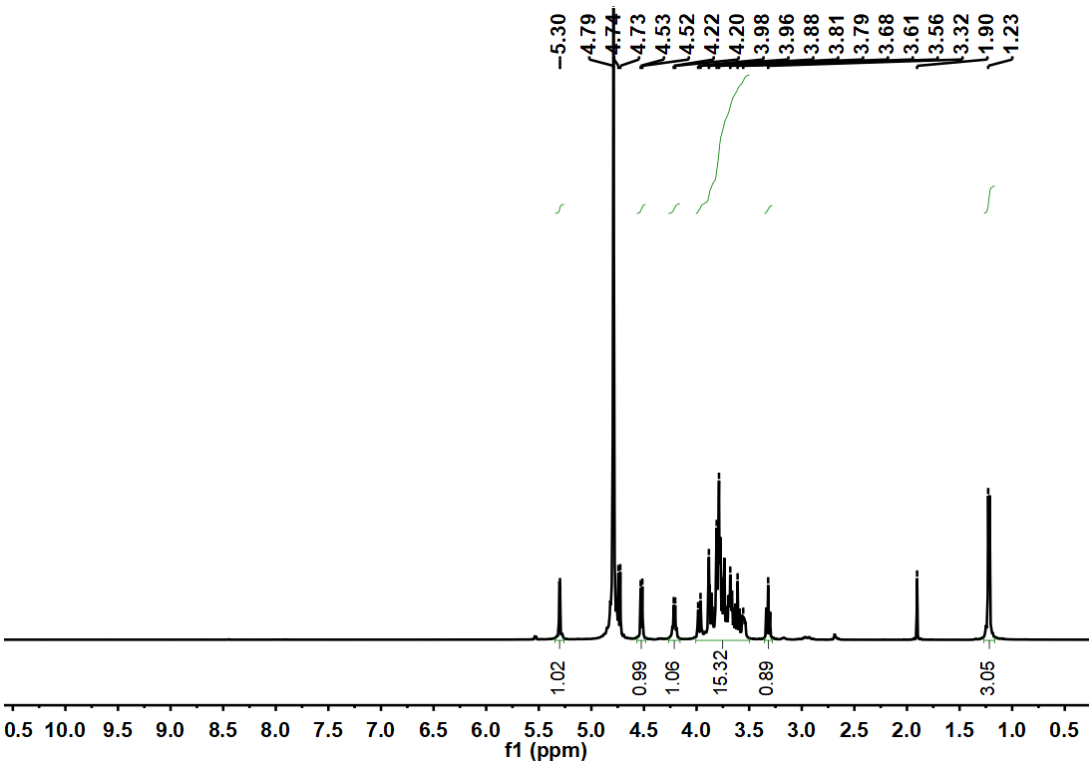

Fig. s5 ^{1}H NMR (500 MHz, D_2O) spectra of 2'-fucosyllactose azide

6. LPG$_8$Man$_{0.40}$ 3a

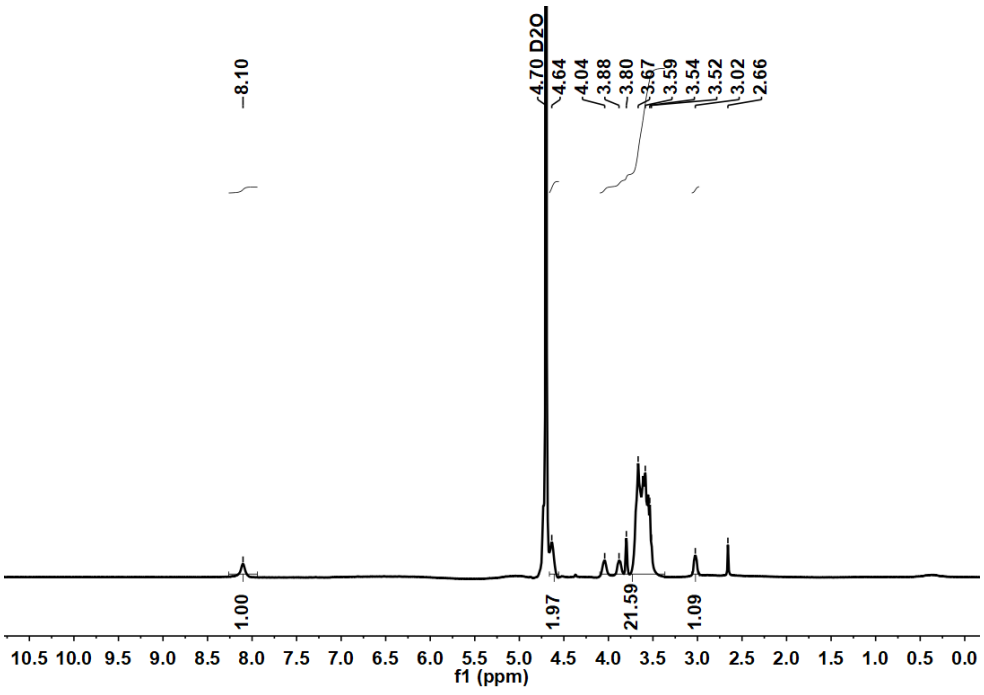

Fig. s6 ^{1}H NMR (500 MHz, D_2O) spectra of compound **3a**

7. LPG$_8$Man$_{1.00}$ 3b

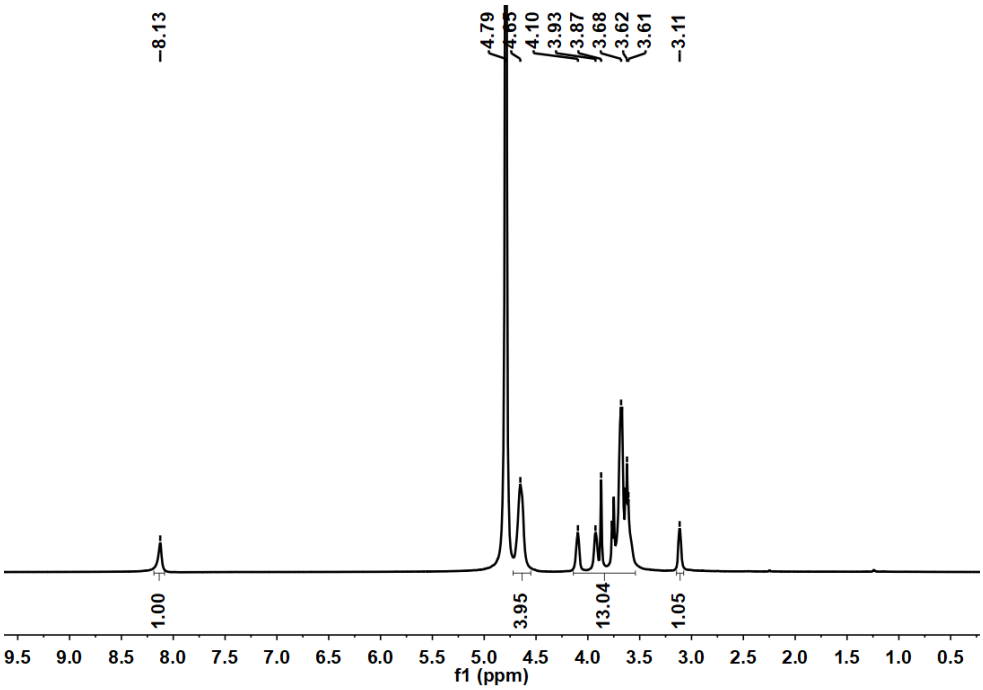

Fig. s7 ^{1}H NMR (700 MHz, D_2O) spectra of compound **3b**

8. LPG$_8$FL$_{0.40}$ 4a

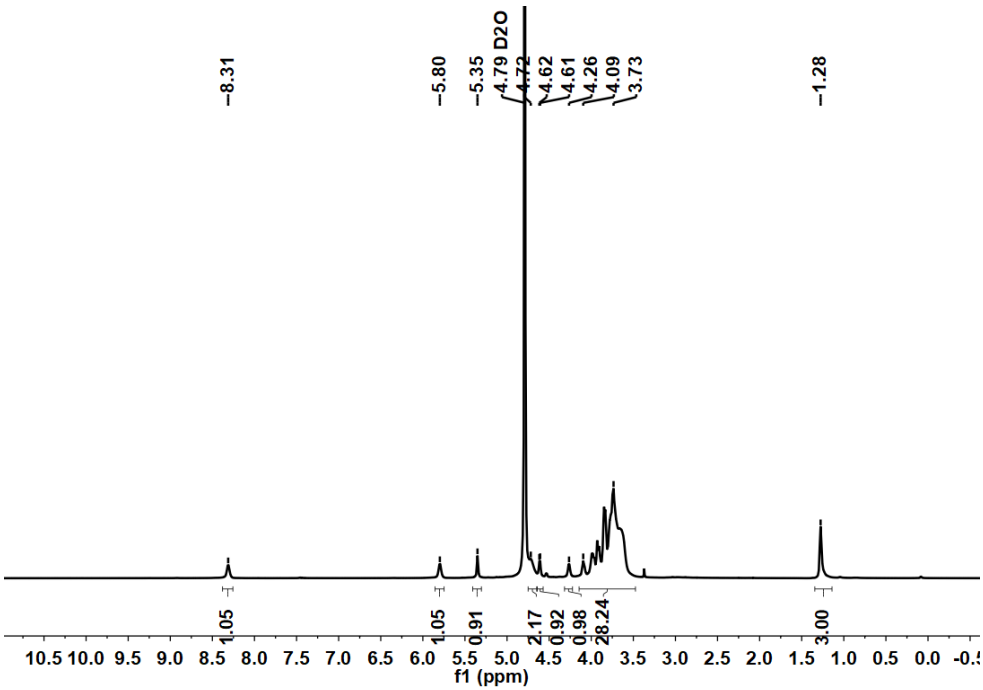

Fig. s8 ^{1}H NMR (700 MHz, D_2O) spectra of **4a**

9. $LPG_8FL_{1.00}$ 4b

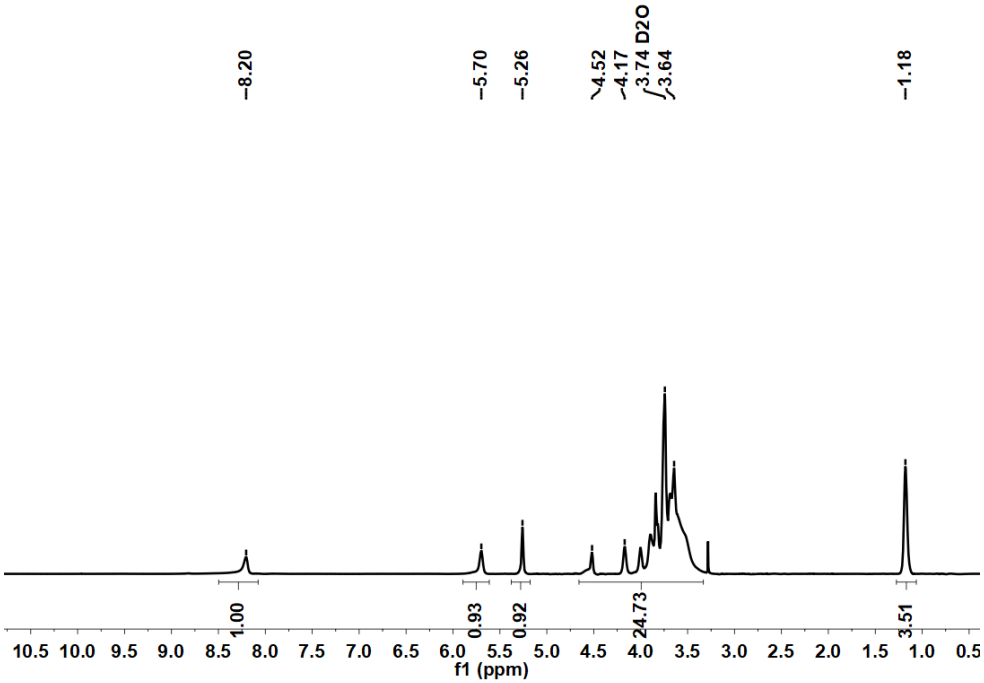

Fig. s9 ^{1}H NMR (700 MHz, D_2O) spectra of compound **4b**

10. $hPG_{10}Man_{0.70}$ 7a

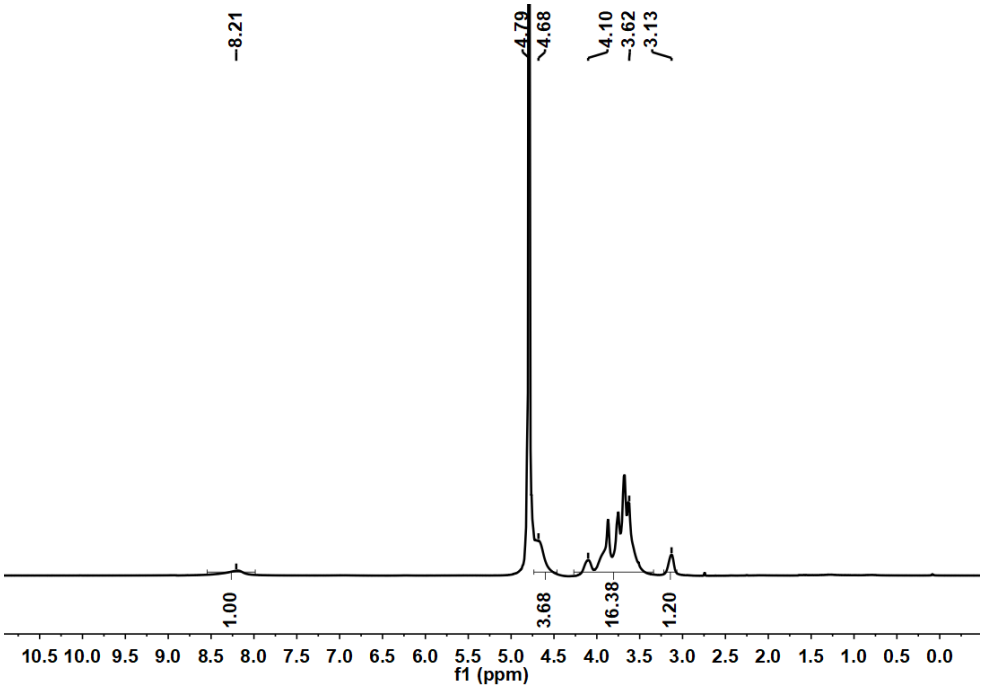

Fig. s10 ^{1}H NMR (700 MHz, D_2O) spectra of compound **7a**

11. hPG$_{10}$Man$_{1.00}$ 7b

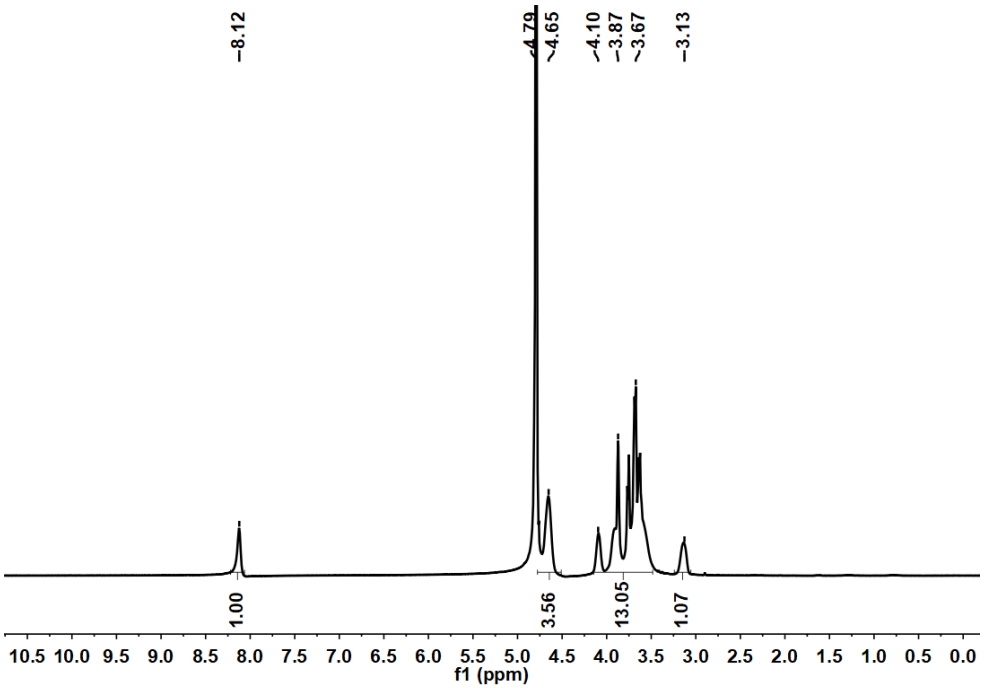

Fig. s11 ^{1}H spectra of compound **7b**

12. hPG$_{10}$FL$_{0.60}$ 8a

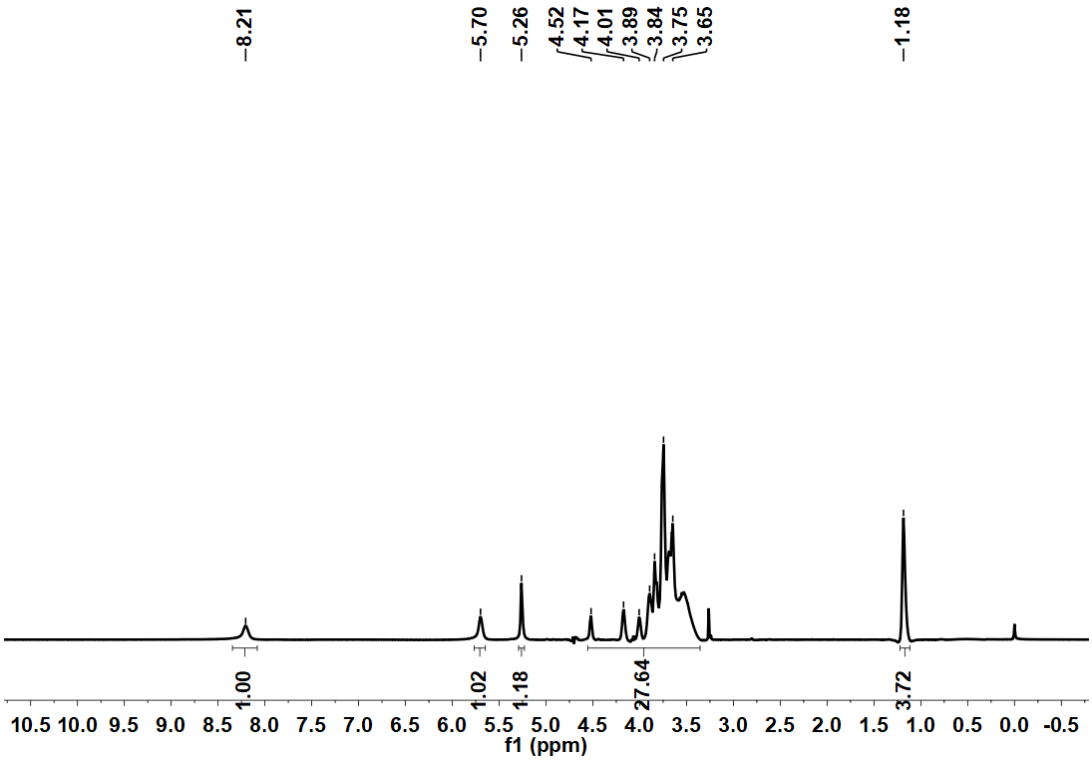

Fig. s12 ^{1}H NMR (700 MHz, D_2O) spectra of compound **8a**

13. $hPG_{10}FL_{1.00}$ 8b

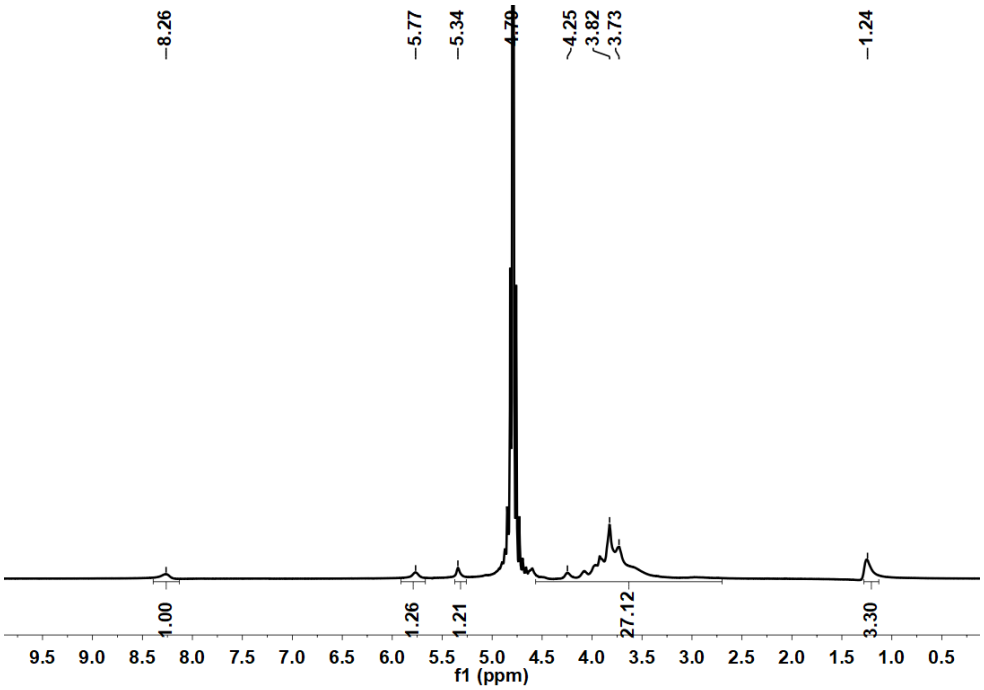

Fig. s13 ^{1}H NMR (500 MHz, D_2O) spectra of compound **8b**

6 Resulting binding isotherms derived from single-cycle kinetic measurements

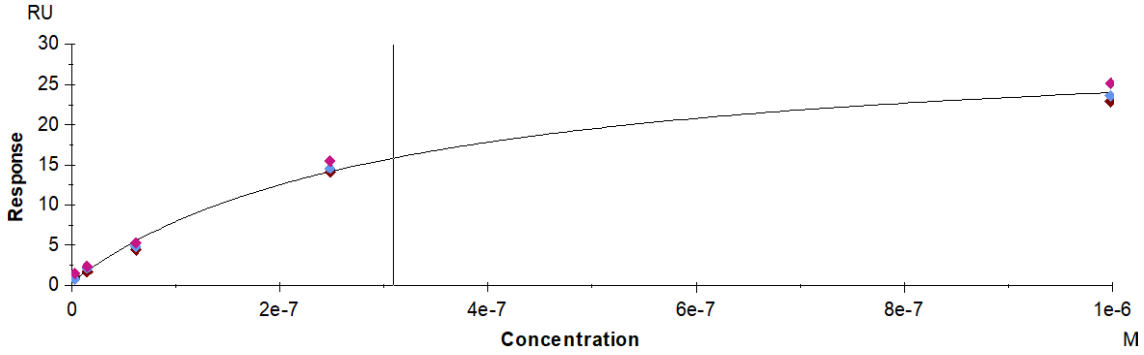

Fig. s14 Binding isotherm of compound **2a**

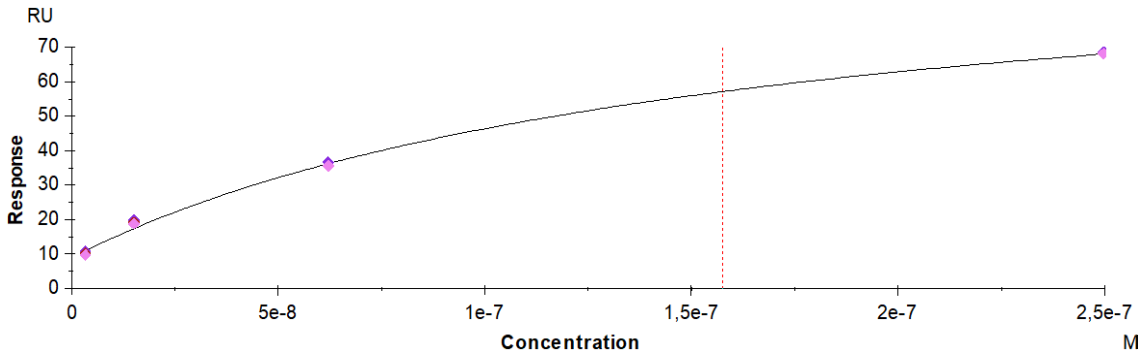

Fig. s15 Binding isotherm of compound **2b**

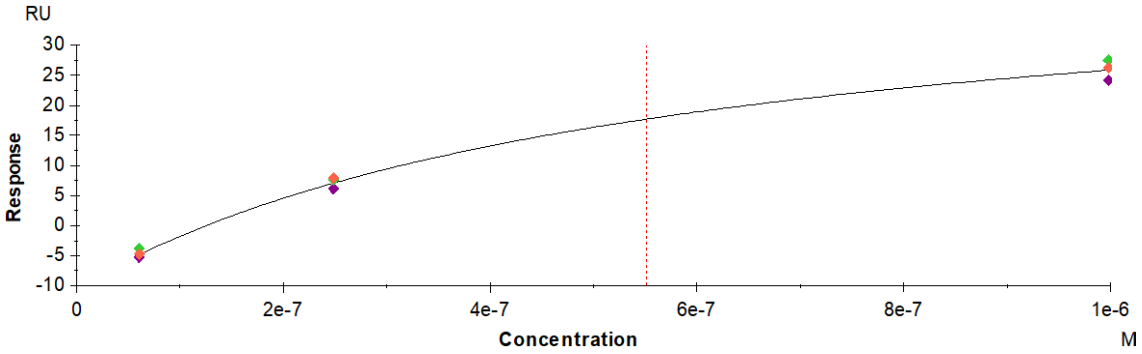

Fig. s16 Binding isotherm of compound **4a**

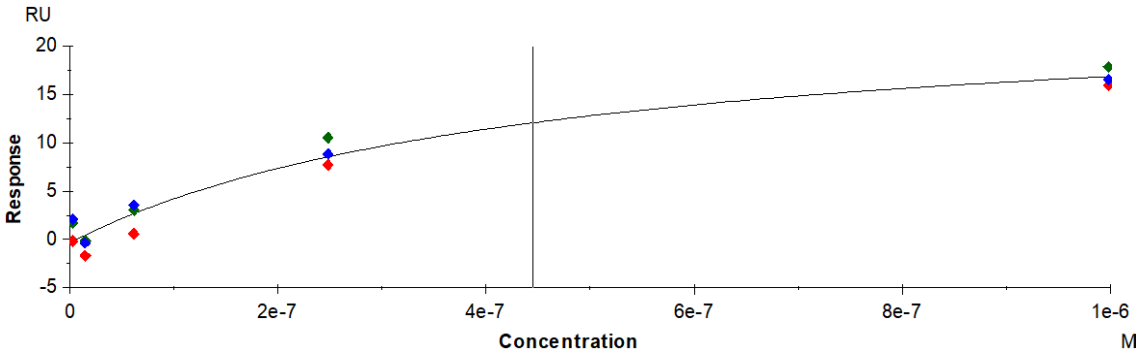

Fig. s17 Binding isotherm of compound **4b**

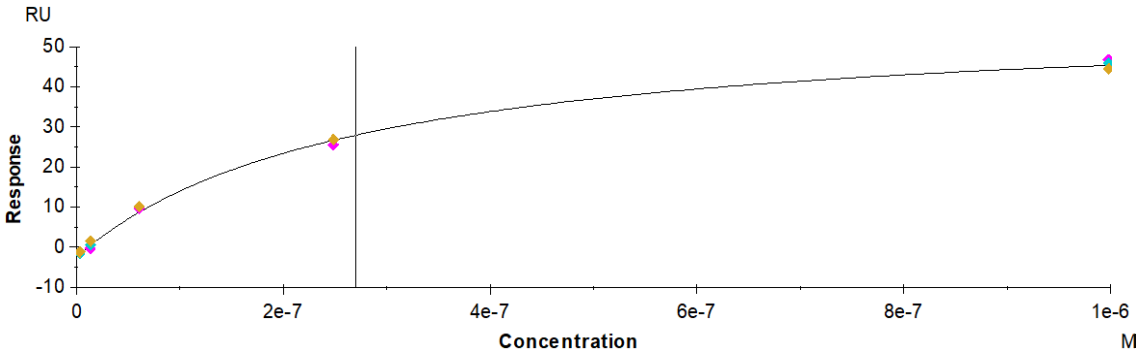

Fig. s18 Binding isotherm of compound **7a**

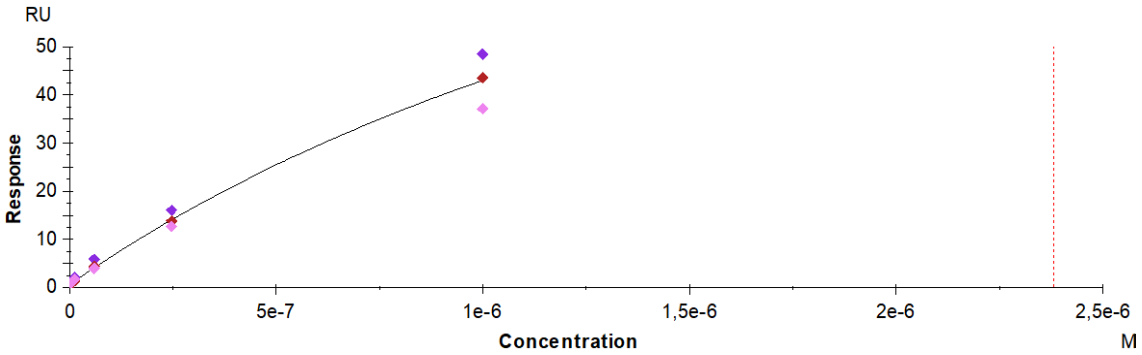

Fig. s19 Binding isotherm of compound **7b**

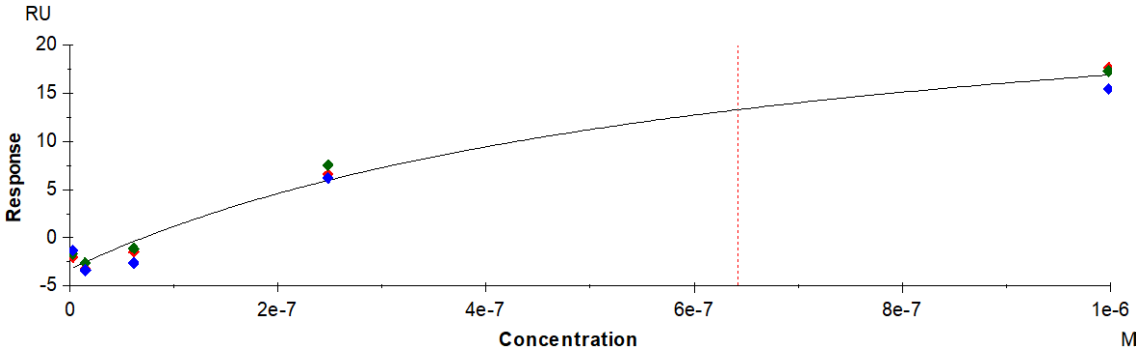

Fig. s20 Binding isotherm of compound **8a**

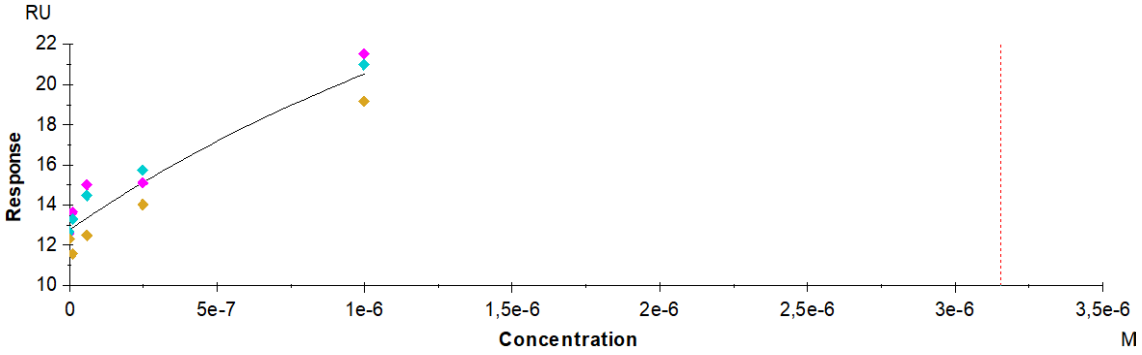

Fig. s21 Binding isotherm of compound **8b**

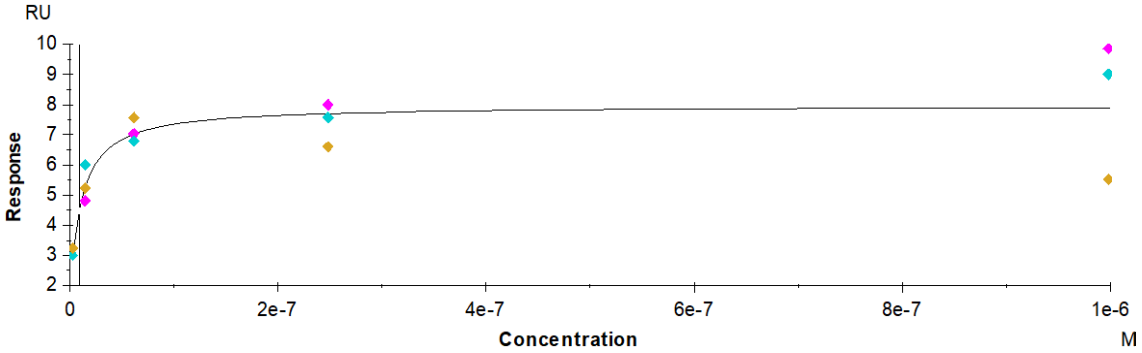

Fig. s22 Binding isotherm of compound **Man_9Glycan**

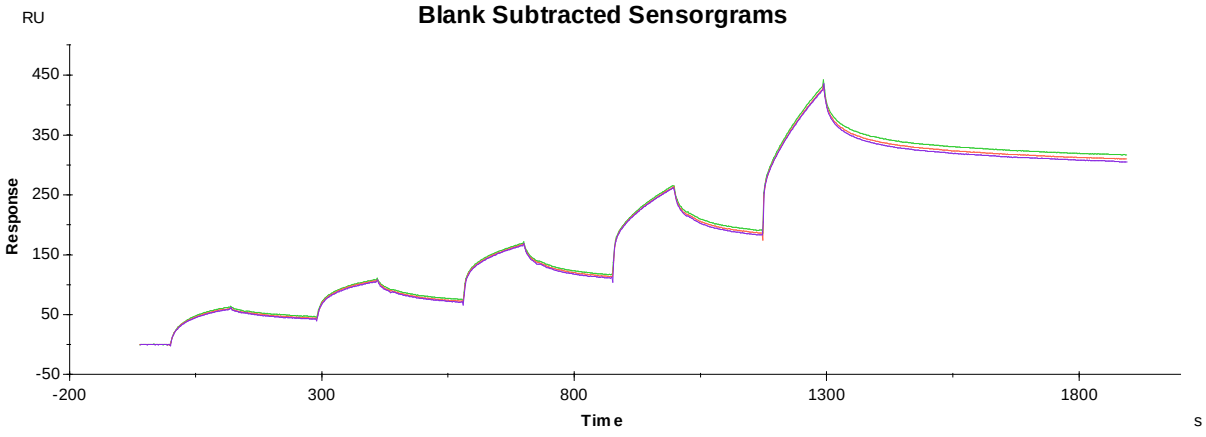

Fig. s23 Sensogram of compound **hPG$_{10}$Man$_{1.00}$**

7.1 Gel permeation chromatography of hyperbranched and linear polyglycerol

M_n = 10642 g/mol, M_w = 16633 g/mol, M_z = 24882 g/mol, D = 1.56

Detecter: RI, Eluent = H_2O, Flow rate = 1mL/min, GPC Colum = Suprema, Reference = Pullulan

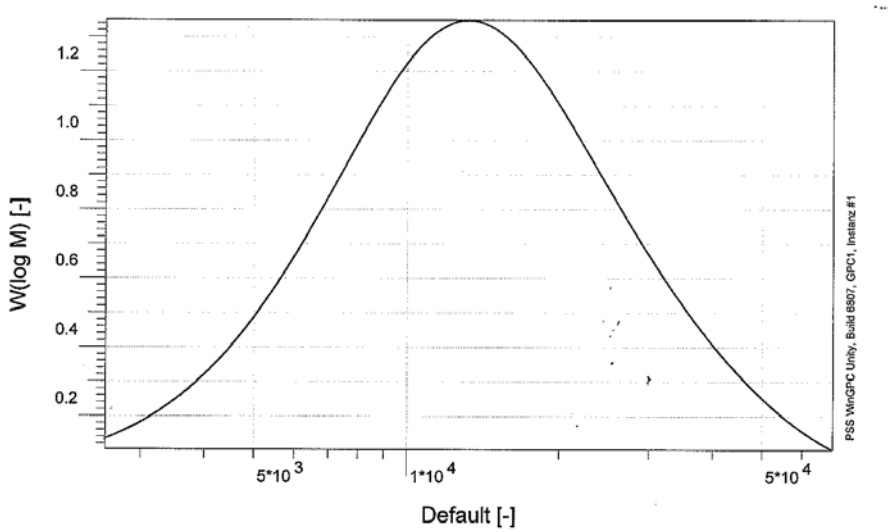

Fig. s24 GPC of compound **hPG$_{10}$OH**

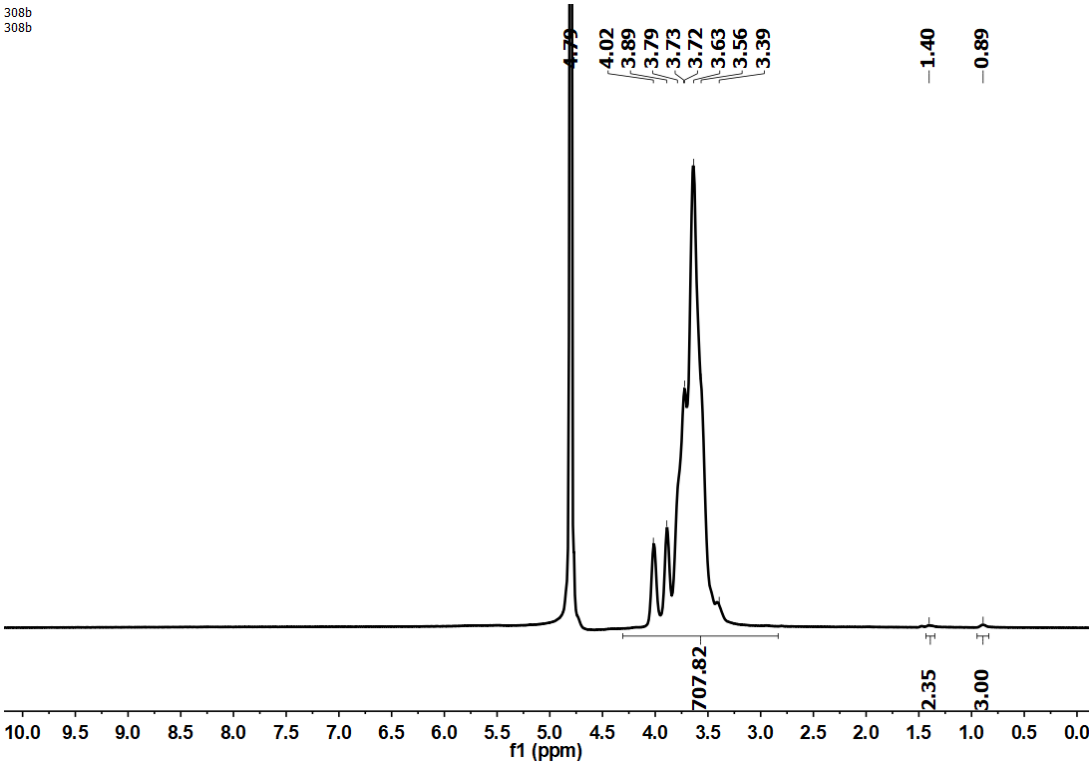

Fig. s25 ^{1}H NMR spectra of compound $dPG_{10}OH$

Calculation for M.Wt of hPG:

Integration value for the PG backbone with respect to five protons of TMP = 707

MWt. Of hPG = (Integration of PG backbone/No. of protons in glycidol unit) * M.Wt of glycidol

= (707/5)*74

= 10463 g/mol

7.2 Gel permeation chromatography of linear polyglycerol

M_n = 6530 g/mol, M_w = 9298 g/mol, M_z = 12589 g/mol, D = 1.42

Detecter: RI, Eluent = H_2O, Flow rate = 1mL/min, GPC Colum = Suprema, Reference = Pullulan

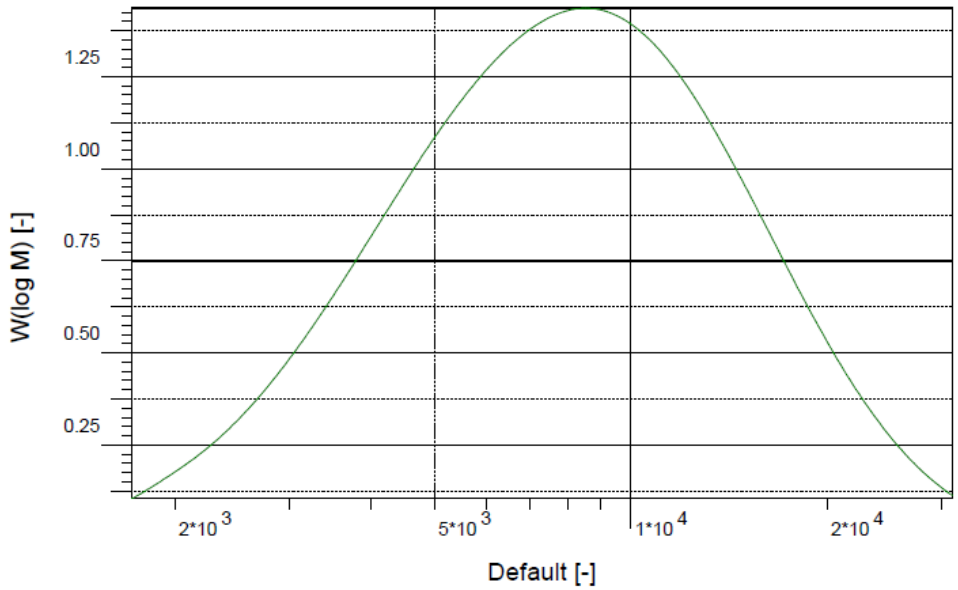

Fig. s26 GPC of compound **LPG_8OH**

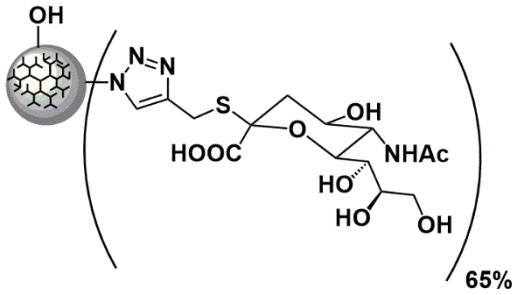

Fig. s27 Structure of **hPG$_{10}$SA$_{0.65}$**[1]

[1] S. Bhatia, D. Lauster, M. Bardua, K. Ludwig, S. Angioletti-Uberti, N. Popp, U. Hoffmann, F. Paulus, M. Budt, M. Stadtmüller, T. Wolff, A. Hamann, C. Böttcher, A. Herrmann, R. Haag, *Biomaterials* **2017**, *138*, 22-34