

# Electronic Supplementary Information

for

## Expanding the scope of oxime ligation: facile synthesis of large cyclopeptide-based glycodendrimers

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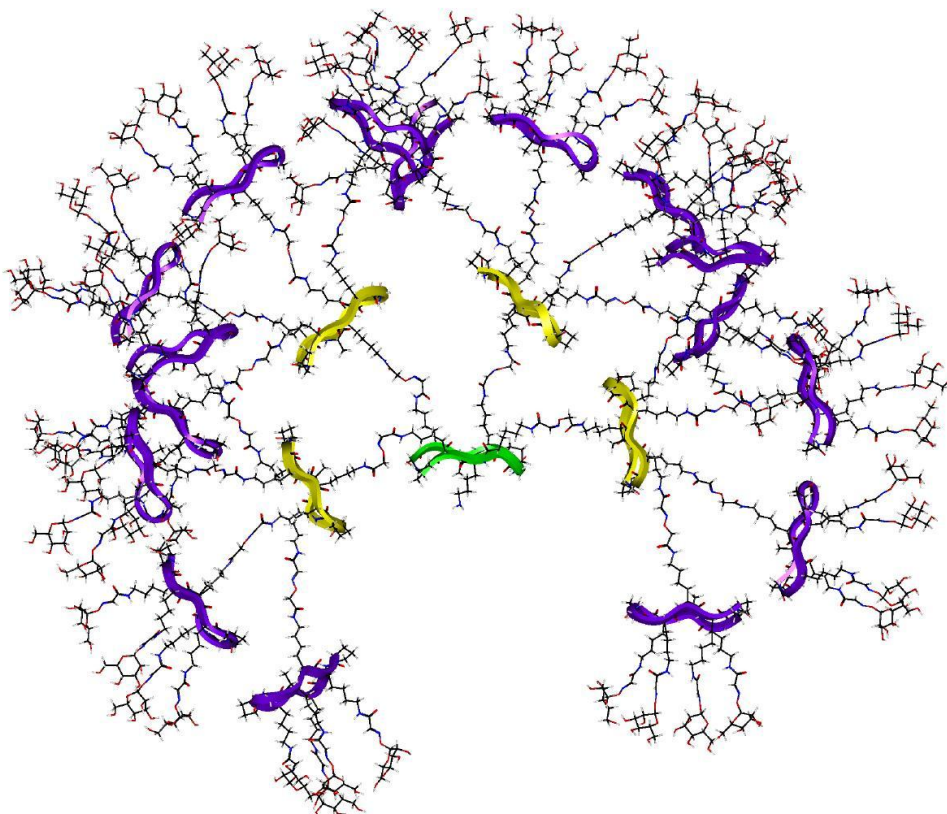
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### General details

All chemical reagents were purchased from Aldrich (Saint Quentin Fallavier, France) or Acros (Noisy-Le-Grand, France) and were used without further purification. Protected amino acids and Fmoc-Gly-Sasrin resin were obtained from Advanced ChemTech Europe (Brussels, Belgium), Bachem Biochimie SARL (Voisins-Les-Bretonneux, France) and France Biochem S.A. (Meudon, France). All amino-acids belong to the L-series. PyBOP was purchased from France Biochem. Analytical RP-HPLC was performed on Waters system equipped with a Waters 600 controller and a Waters 2487 Dual Absorbance Detector. Analysis was carried out at 1.0 mL/min (EC 125/3 nucleosil 300-5 C<sub>18</sub>) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient (buffer A: 0.09% CF<sub>3</sub>CO<sub>2</sub>H in water; buffer B: 0.09% CF<sub>3</sub>CO<sub>2</sub>H in 90% acetonitrile). Preparative HPLC was performed on Gilson GX 281 equipped with a fraction collector. Purifications were carried out at 22.0 mL/min (VP 250/21 nucleosil 100-7 C<sub>18</sub>) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient (buffer A: 0.09% CF<sub>3</sub>CO<sub>2</sub>H in water; buffer B: 0.09% CF<sub>3</sub>CO<sub>2</sub>H in 90% acetonitrile).

### Molecular modelling of compound 3b

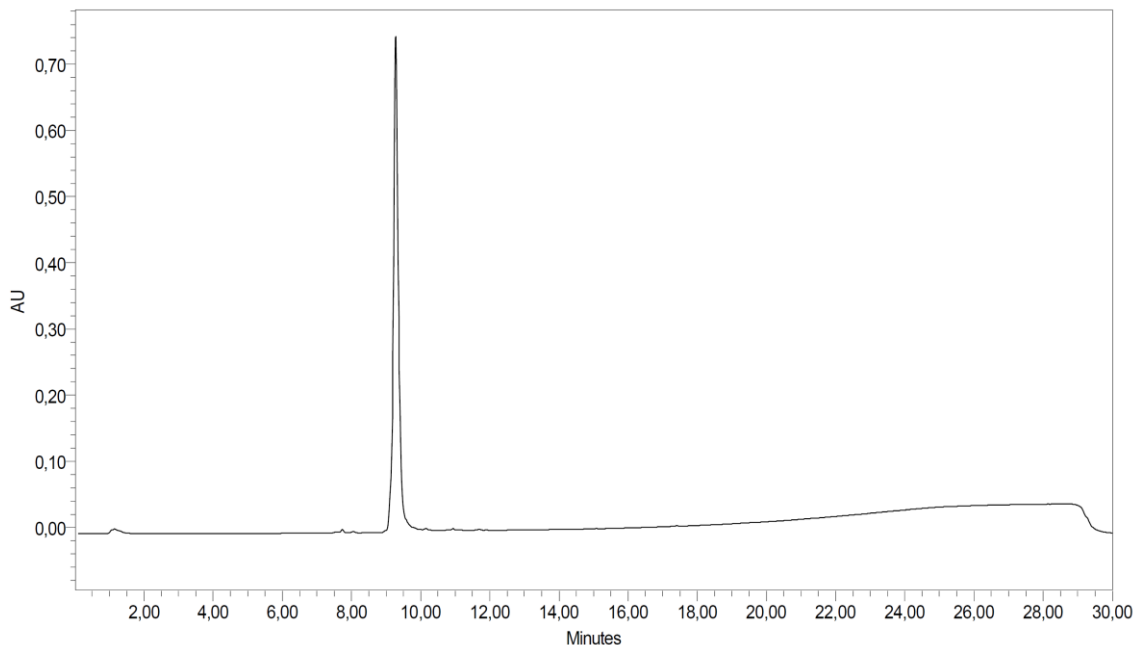
Structure calculations were performed using InsightII / Discover (Version 2005, Accelrys, SanDiego,CA,USA) software. Calculations were performed *in vacuo*, and the energy of the system was calculated by the consistent CVFF force field (version 2.3). To shorten the range of Coulomb interaction, a distance-dependent relative dielectric constant,  $\epsilon_r$ , was used ( $\epsilon_r = 4r$ ). The resulting molecule was subjected to 2500 iterations of steepest descent minimization, followed by 5000 iterations of conjugate gradient minimization and the convergence of minimization was followed until the RMS derivative was less than 0.01 kcal.mol<sup>-1</sup>.



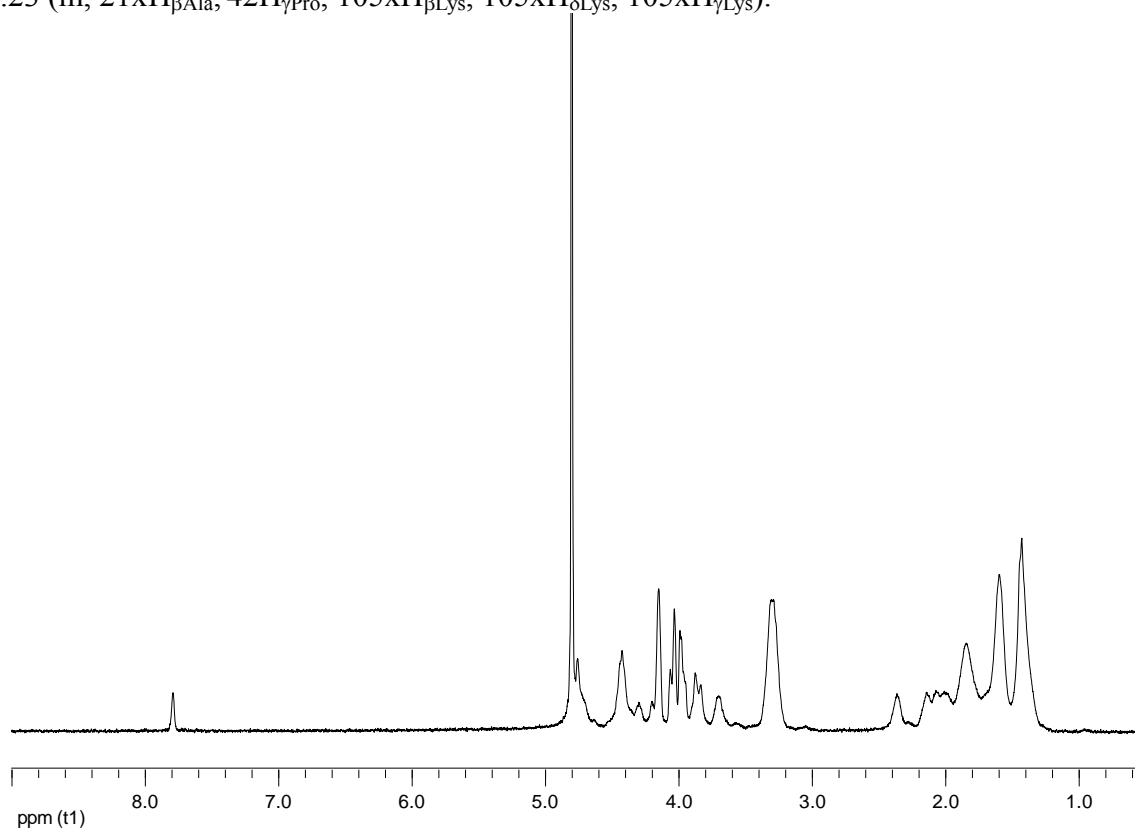
Compound **3b** was constructed by using the model of **2** that was previously proposed from NMR data.<sup>12d</sup> Mannose units were removed and replaced by 16 cyclodecapeptides containing the fucosyl groups. Energy minimisation showed that compound **3b** is stable and no steric clashes were observed. This result point out that our construct can well accommodate a large number of sugar residues. For clarity the arms have been spread out.

Experimental and analytical data for compound 7.

- Aldehyde-containing compound derived from compound **6** (5.0 mg, 0.78  $\mu\text{mol}$ ) was dissolved in 0.1% TFA in water (10 mM) and compound **5** (35.7 mg, 24.8  $\mu\text{mol}$ ) was added. The solution was stirred for 1h at 37°C. The crude mixture was purified by preparative HPLC (linear gradient 5 to 60% B in 25 min,  $R_t = 14.1$  min) to give compound **7** (20.3 mg, 0.69  $\mu\text{mol}$ , 89%).
- RP-HPLC profile (5 to 100% B in 20 min,  $\lambda = 214$  nm).



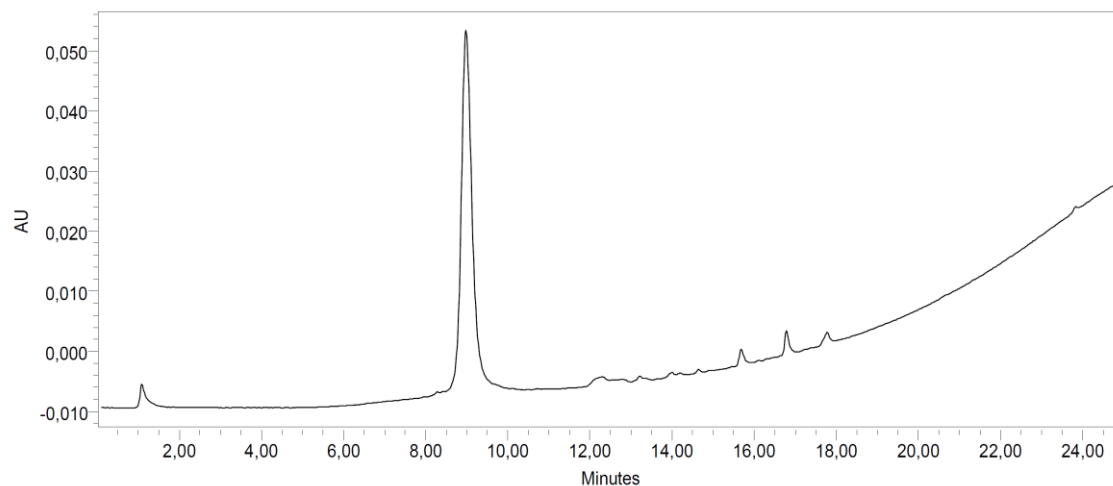
- $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 7.78$  (bs, 20xHox), 4.76-4.65 (m, 20x $\text{CH}_2\text{O}$ ), 4.49-4.13 (m, 105x $\text{H}_{\alpha\text{Lys}}$ , 21x $\text{H}_{\alpha\text{Ala}}$ , 42x $\text{H}_{\alpha\text{Gly}}$ , 42x $\text{H}_{\alpha\text{Pro}}$ , 64x $\text{H}_{\alpha\text{Ser}}$ ), 4.16-4.08 (m, 64x $\text{H}_{\beta\text{Ser}}$ ), 4.06-3.60 (m, 42x $\text{H}_{\alpha'\text{Gly}}$ , 42x $\text{H}_{\delta\text{Pro}}$ ), 3.37-3.18 (m, 104x $\text{H}_{\epsilon\text{Lys}}$ ), 3.03 (bt, 1x $\text{H}_{\epsilon\text{Lys}}$ ), 2.41-2.28 (m, 42x $\text{H}_{\beta\text{Pro}}$ ), 2.17-1.23 (m, 21x $\text{H}_{\beta\text{Ala}}$ , 42x $\text{H}_{\gamma\text{Pro}}$ , 105x $\text{H}_{\beta\text{Lys}}$ , 105x $\text{H}_{\delta\text{Lys}}$ , 105x $\text{H}_{\gamma\text{Lys}}$ ).



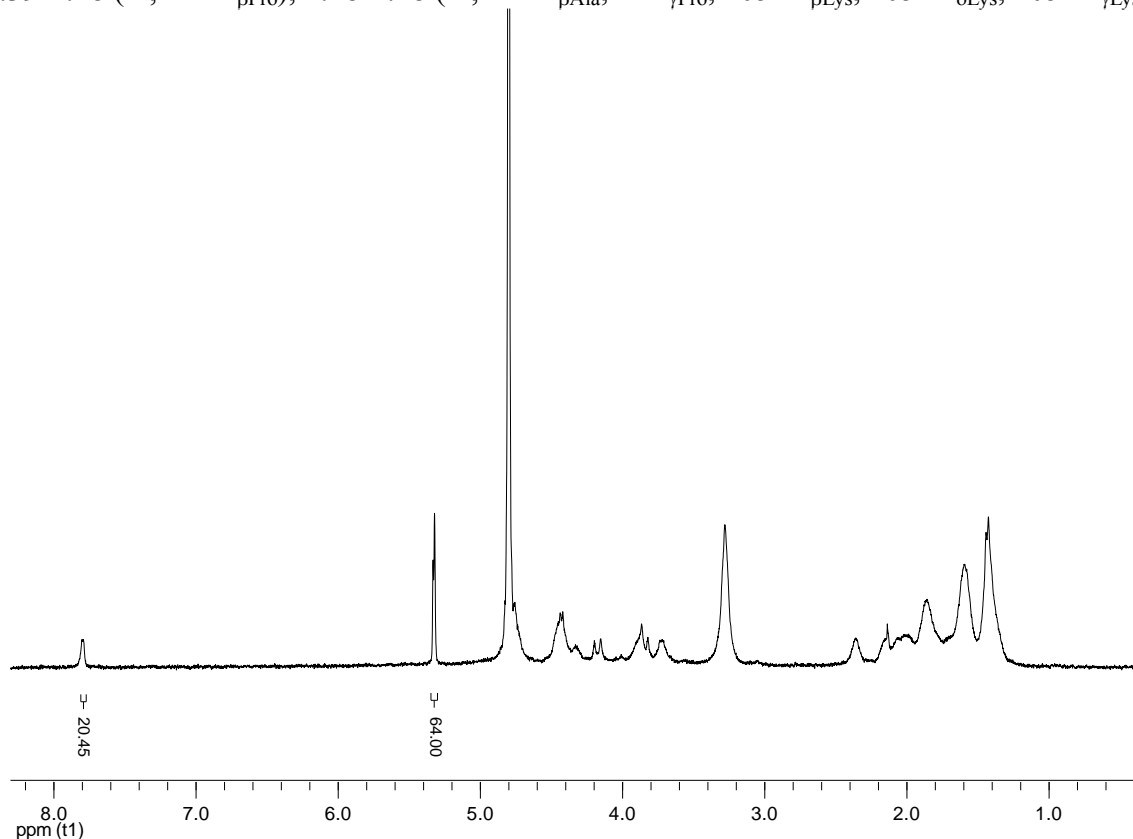
Experimental and analytical data for aldehyde-containing compound derived from 7.

- Compound **7** (4.4 mg, 0.15  $\mu\text{mol}$ ) was dissolved in water ( $10^{-2}\text{M}$ ) and  $\text{NaIO}_4$  (20.6 mg, 96.4  $\mu\text{mol}$ ) was added. The solution was stirred at room temperature for 30 min. The product was directly purified by RP-HPLC (0% B for 10 min, then linear gradient 0 to 100% B in 15 min,  $R_t = 18.3$  min) affording pure aldehyde-containing cyclodecapeptide as a white powder after freeze-drying (3.6 mg, 0.13  $\mu\text{mol}$ , 88%).

- RP-HPLC profile (5 to 100% B in 20 min,  $\lambda = 214$  nm).

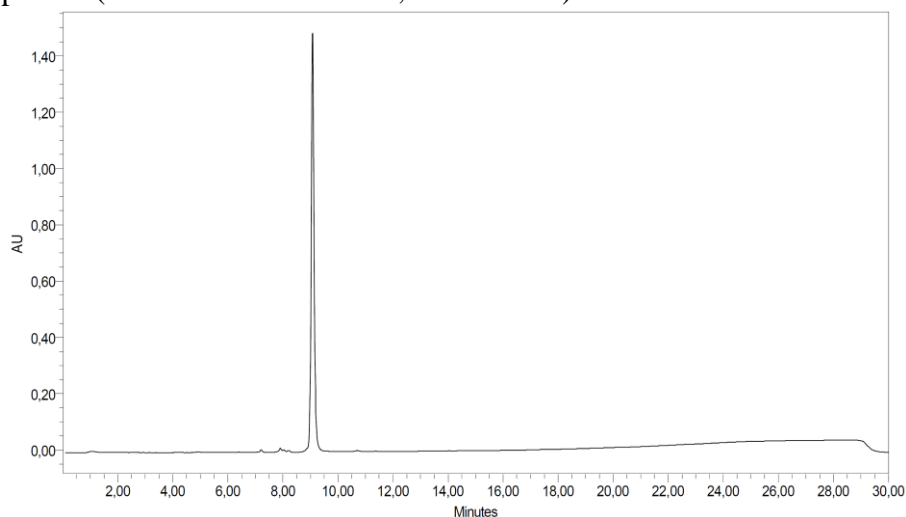


- $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 7.78$  (bs, 20xH $\alpha$ ), 5.33-5.30 (m, 64xCH(OH) $_2$ ), 4.77-4.68 (m, 20xCH $_2$ O), 4.49-4.27 (m, 105xH $_{\alpha}$ Lys, 21xH $_{\alpha}$ Ala, 42xH $_{\alpha}$ Pro), 4.20-4.10 (bd,  $^2J_{\text{H}\alpha,\text{H}\alpha'} = 16.7$  Hz, 42xH $_{\alpha}$ Gly), 3.95-3.62 (m, 42xH $_{\alpha'}$ Gly, 42xH $_{\delta}$ Pro), 3.35-3.18 (m, 104xH $_{\epsilon}$ Lys), 3.03 (bt, 1xH $_{\epsilon}$ Lys), 2.39-2.28 (m, 42xH $_{\beta}$ Pro), 2.18-1.28 (m, 21xH $_{\beta}$ Ala, 42H $_{\gamma}$ Pro, 105xH $_{\beta}$ Lys, 105xH $_{\delta}$ Lys, 105xH $_{\gamma}$ Lys).

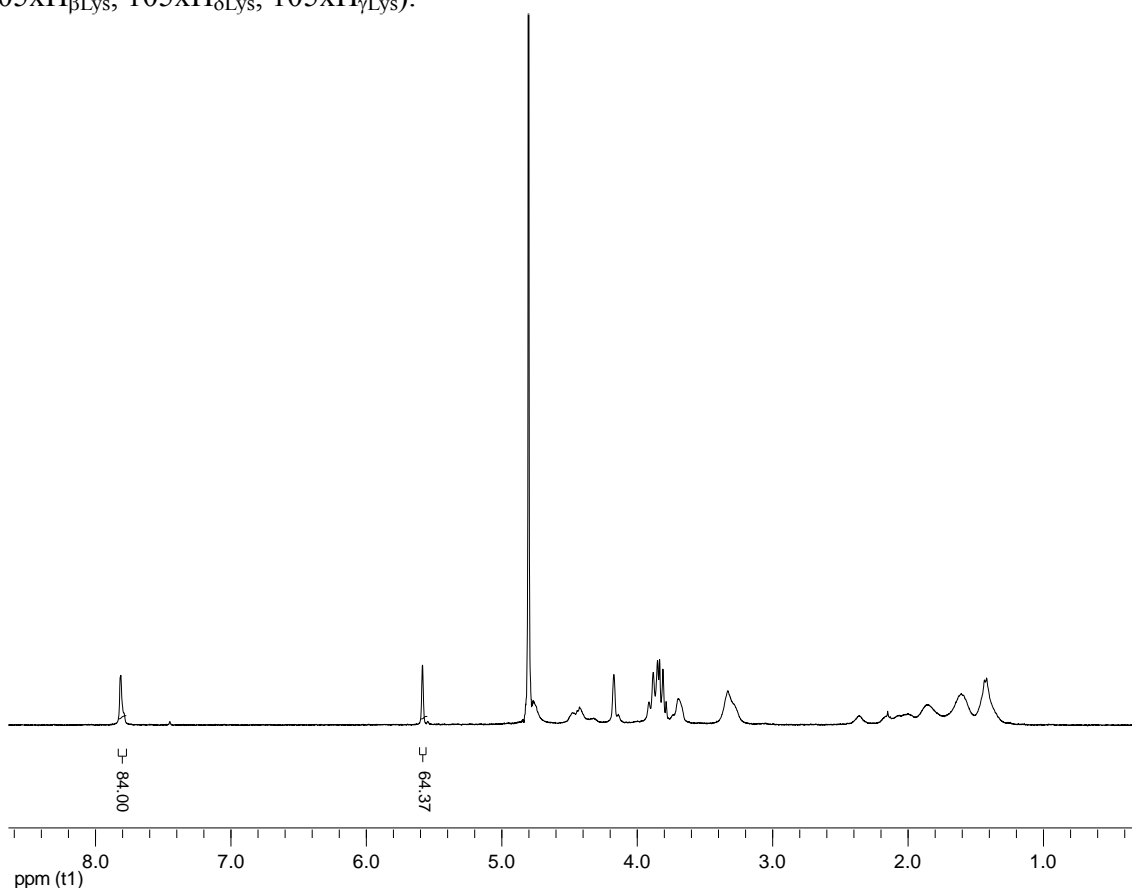


Experimental and analytical data for mannosylated glycodendrimer 3a.

- Aldehyde-containing compound derived from **7** (1.9 mg, 69.7 nmol) and aminoxy mannose **8a** (1.74 mg, 8.9  $\mu$ mol) were dissolved in 0.1% TFA in water (10 mM). After stirring for 1h at 37°C, the crude mixture was purified by preparative HPLC (linear gradient 5 to 100% B in 25 min,  $R_t = 11.3$  min) to give after freeze-drying the glycodendrimer **3a** as a flocculent powder (2.1 mg, 57.9  $\mu$ mol, 83%).
- RP-HPLC profile (5 to 100% B in 20 min,  $\lambda = 214$  nm).



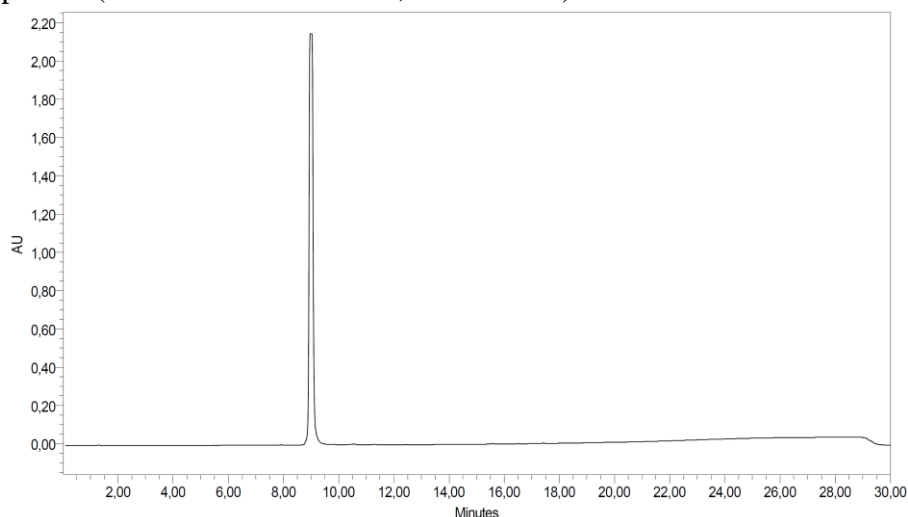
- $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 7.82-7.76$  (m, 84xHox), 5.59-5.50 (m, 64xH<sub>1Man</sub>), 4.77-4.70 (m, 20xCH<sub>2</sub>O), 4.50-4.27 (m, 105xH <sub>$\alpha$</sub> Lys, 21xH <sub>$\alpha$</sub> Ala, 42xH <sub>$\alpha$</sub> Pro), 4.18-4.10 (m, 42xH <sub>$\alpha$</sub> Gly, 64xH<sub>2Man</sub>), 3.93-3.60 (m, 42xH <sub>$\alpha'$</sub> Gly, 42xH <sub>$\delta$</sub> Pro, 64xH<sub>3Man</sub>, 64xH<sub>4Man</sub>, 64xH<sub>5Man</sub>, 64xH<sub>6Man</sub>, 64xH<sub>6'Man</sub>), 3.38-3.20 (m, 104xH <sub>$\epsilon$</sub> Lys), 3.03 (bt, 1xH <sub>$\epsilon$</sub> Lys), 2.39-2.28 (m, 42xH <sub>$\beta$</sub> Pro), 2.18-1.27 (m, 21xH <sub>$\beta$</sub> Ala, 42xH <sub>$\gamma$</sub> Pro, 105xH <sub>$\beta$</sub> Lys, 105xH <sub>$\delta$</sub> Lys, 105xH <sub>$\gamma$</sub> Lys).



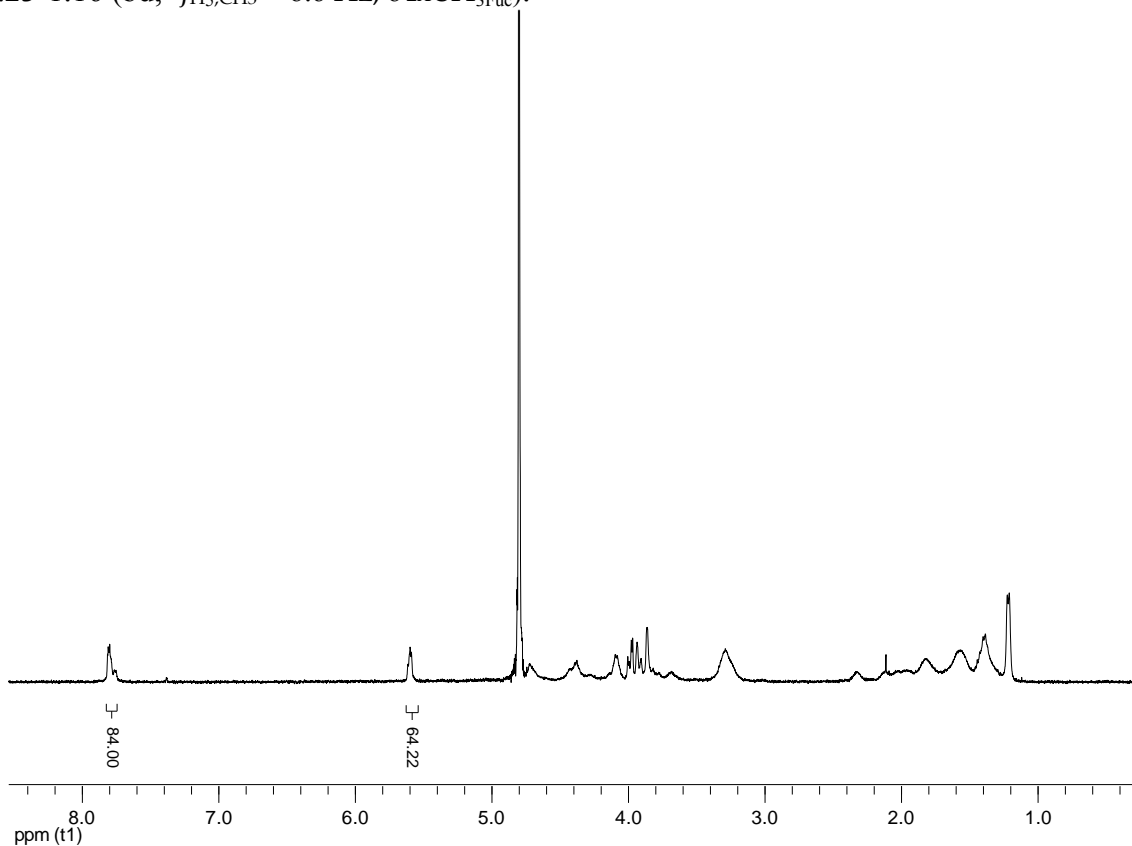
Experimental and analytical data for fucosylated glycodendrimer 3b.

- Aldehyde-containing compound derived from **7** (2.1 mg, 77.1 nmol) and aminoxy fucose **8b** (1.8 mg, 9.87  $\mu$ mol) were dissolved in 0.1% TFA in water (10 mM). After stirring for 1h at 37°C, the crude mixture was purified by preparative HPLC (linear gradient 5 to 100% B in 25 min,  $R_t = 11.7$  min) to give after freeze-drying the glycodendrimer **3b** as a flocculent powder (2.4 mg, 65.5 nmol, 85%).

- RP-HPLC profile (5 to 100% B in 20 min,  $\lambda = 214$  nm).



- $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 7.82\text{-}7.73$  (m,  $84\times\text{H}_{\text{ox}}$ ),  $5.61\text{-}5.55$  (m,  $64\times\text{H}_{1\text{Fuc}}$ ),  $4.73\text{-}4.63$  (m,  $20\times\text{CH}_2\text{O}$ ),  $4.47\text{-}4.20$  (m,  $105\times\text{H}_{\alpha\text{Lys}}$ ,  $21\times\text{H}_{\alpha\text{Ala}}$ ,  $42\times\text{H}_{\alpha\text{Pro}}$ ),  $4.15\text{-}3.59$  (m,  $42\times\text{H}_{\alpha\text{Gly}}$ ,  $42\times\text{H}_{\alpha'\text{Gly}}$ ,  $42\times\text{H}_{\delta\text{Pro}}$ ,  $64\times\text{H}_{2\text{Fuc}}$ ,  $64\times\text{H}_{3\text{Fuc}}$ ,  $64\times\text{H}_{4\text{Fuc}}$ ,  $64\times\text{H}_{5\text{Fuc}}$ ),  $3.35\text{-}3.16$  (m,  $104\times\text{H}_{\epsilon\text{Lys}}$ ),  $3.00$  (bt,  $1\times\text{H}_{\epsilon\text{Lys}}$ ),  $2.35\text{-}2.24$  (m,  $42\times\text{H}_{\beta\text{Pro}}$ ),  $2.13\text{-}1.24$  (m,  $21\times\text{H}_{\beta\text{Ala}}$ ,  $42\times\text{H}_{\gamma\text{Pro}}$ ,  $105\times\text{H}_{\beta\text{Lys}}$ ,  $105\times\text{H}_{\delta\text{Lys}}$ ,  $105\times\text{H}_{\gamma\text{Lys}}$ ),  $1.23\text{-}1.16$  (bd,  $^2J_{\text{H}_5,\text{CH}_3} = 6.0$  Hz,  $64\times\text{CH}_3\text{Fuc}$ ).



### Circular dichroism spectroscopy.

Circular dichroism (CD) spectra were acquired with signal averaging on a Jasco J-810 Spectropolarimeter equipped with a Jasco Peltier PTC-423S temperature controller, and a baseline was recorded separately and subtracted. Far-UV spectra were recorded from 340 to 185 nm, in a quartz cell with 1 mm pathlength. Spectra are the averages of 4 scans and ellipticities are reported as molar ellipticity. To reduce noise on the curves the data were smoothed with Savitzky-Golay smoothing algorithm using a convolution window of 7 data points. Stock concentrations of glycodendrimers were determined by NMR using trimethylsilyl-2,2,3,3-tetra-deuterio-propionate (TSP) as an internal concentration standard.

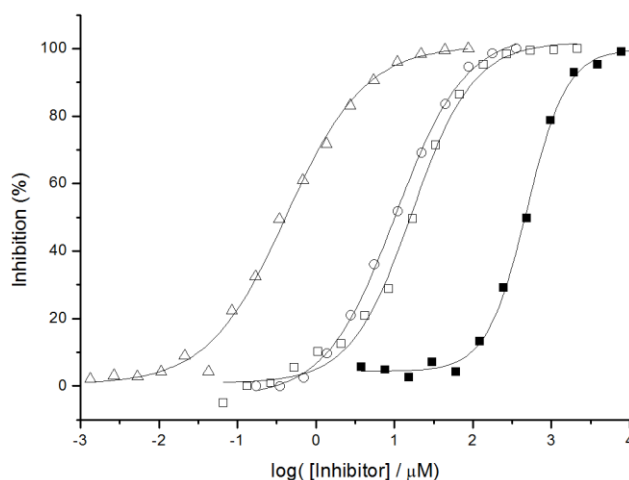
### Competitive enzyme-linked lectin assays

HRP-labelled ConA, biotinylated UEA-1, Bovine Serum Albumin and SIGMA FAST OPD were purchased from Sigma-Aldrich. Polymeric  $\alpha$ -D-Mannose (PAA- $\alpha$ -D-Man) and  $\alpha$ -L-Fucose (PAA- $\alpha$ -L-Fuc) were purchased from Lectinity Holding, Inc., Moscow. Optical density was measured with a microtiter plate reader (SPECTRAMax, model PLUS384, Molecular Devices).

#### • Enzyme-Linked Lectin Assay (ELLA) with ConA

96-well microtiter Nunc-Immuno plates (Maxi-Sorp) were coated with PAA- $\alpha$ -D-Man (100  $\mu$ L per well, diluted from a stock solution of 5  $\mu$ g.mL<sup>-1</sup> in 50 mM carbonate buffer pH 9.6) for 1h at 37°C. The wells were then washed with T-PBS (3x100  $\mu$ L well<sup>-1</sup>, PBS pH 7.4 containing 0.05% (v/v) Tween 20). This washing procedure was repeated after each incubation step. The coated microtiter plates were then blocked with BSA in PBS (3% w/v, 1h at 37°C, 100  $\mu$ L per well). Serial two-fold dilutions of each inhibitor was pre-incubated 1h at 37°C in 0.01 M PBS (pH 7.4) containing 0.1 mM Ca<sup>2+</sup>, 0.1 mM Mn<sup>2+</sup> and BSA (0.3% w/v) (60  $\mu$ L per well) in the presence of ConA-HRP (60  $\mu$ L) at the desired concentration. The above solutions (100  $\mu$ L) were then transferred to the blocked microtiter plates which were incubated for 1h at 37°C. After incubation, the plates were washed with T-PBS (3x100  $\mu$ L per well) then the colour was developed using OPD (100  $\mu$ L per well, 0.4 mg.mL<sup>-1</sup> in 0.05 M phosphate-citrate buffer) and urea hydrogen peroxide (0.4 mg.mL<sup>-1</sup>). The reaction was stopped after 10 min by adding H<sub>2</sub>SO<sub>4</sub> (30% v/v, 50  $\mu$ L per well) and the absorbance was measured at 490 nm. The percentage of inhibition was plotted against the logarithm of the concentration of the sugar derivatives. The sigmoidal curves showed below were fitted and the concentration at 50% inhibition of binding of ConA-HRP to PAA- $\alpha$ -D-Man coated plates were determined (IC<sub>50</sub>). The percentages of inhibition were calculated as given in equation (1), where A=absorbance.

$$\% \text{ inhibition} = [(A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})}) / A_{(\text{no inhibitor})}] \times 100 \quad (1)$$

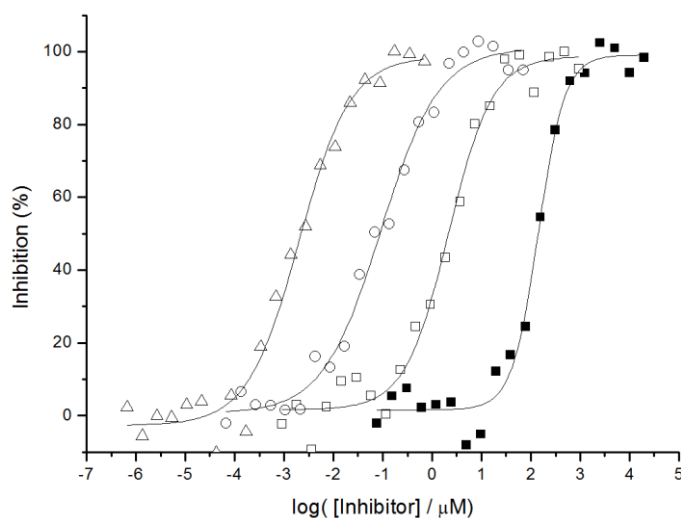


Legend:  $\alpha$ MeMan (■) and 4-valent **1a** (□), 16-valent **2a** (○) and 64-valent **3a** (Δ) glycodendrimers.

- Enzyme-Linked Lectin Assay (ELLA) with UEA-1

After 96-well microtiter Nunc-Immuno plates (Maxi-Sorp) were coated with PAA- $\alpha$ -L-Fuc (100  $\mu$ L per well, diluted from a stock solution of 5  $\mu$ g.mL<sup>-1</sup> in 50 mM carbonate buffer pH 9.6) for 1h at 37°C. The wells were then washed with T-PBS (3x100  $\mu$ L well<sup>-1</sup>, PBS pH 7.4 containing 0.05% (v/v) Tween 20). This washing procedure was repeated after each incubation step. The coated microtiter plates were then blocked with BSA in PBS (3% w/v, 1h at 37°C, 100  $\mu$ L per well). Serial two-fold dilutions of each inhibitor was pre-incubated 1h at 37°C in PBS (60  $\mu$ L per well) in the presence of UEA-biotin (60  $\mu$ L) at the desired concentration. The above solutions (100  $\mu$ L) were then transferred to the blocked microtiter plates which were incubated for 1h at 37°C. After washing with T-PBS (PBS + 0.05% Tween, 3x100  $\mu$ L.well<sup>-1</sup>) 100  $\mu$ L of streptavidin-peroxidase conjugate (dilution 1:5000 in PBS + 3% BSA w/v) was added and left for 1 h at 37°C. The wells were washed with T-PBS (3x100  $\mu$ L.well<sup>-1</sup>) then the colour was developed using 100  $\mu$ L per well of 0.05 M phosphate/citrate buffer containing OPD (0.4 mg.mL<sup>-1</sup>) and urea hydrogen peroxide (0.4 mg.mL<sup>-1</sup>). The reaction was stopped after 10 min by the addition of 50  $\mu$ L of 30% aqueous H<sub>2</sub>SO<sub>4</sub>. The absorbance was read at 490 nm using a microtitre plate reader. The percentage of inhibition was plotted against the logarithm of the concentration of the sugar derivatives. The sigmoidal curve showed below was fitted and the concentration at 50% inhibition of binding of the biotinylated UEA-1 to PAA- $\alpha$ -L-Fuc coated microtitre plates was determined. The percentage of inhibition were calculated as given in equation (1), where A=absorbance.

$$\% \text{ inhibition} = [(A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})}) / A_{(\text{no inhibitor})}] \times 100 \quad (1)$$

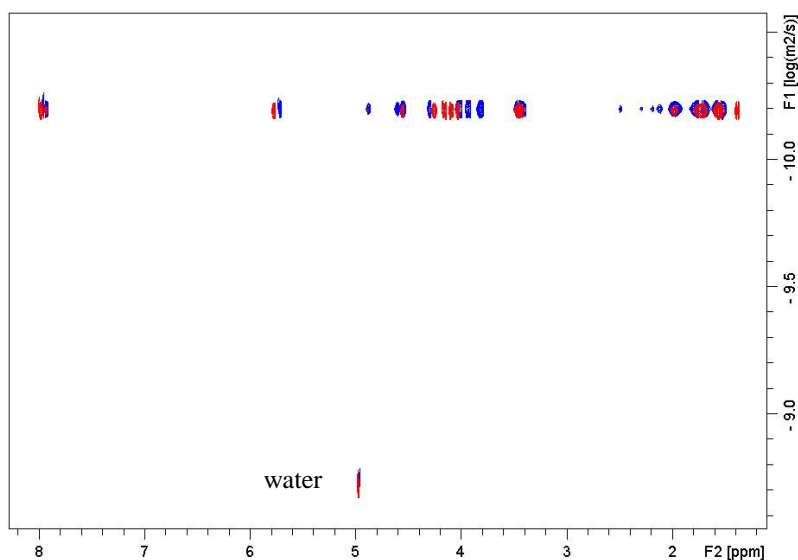


Legend:  $\alpha$ MeFuc (■) and 4-valent **1b** (□), 16-valent **2b** (○) and 64-valent **3b** (Δ) glycodendrimers.

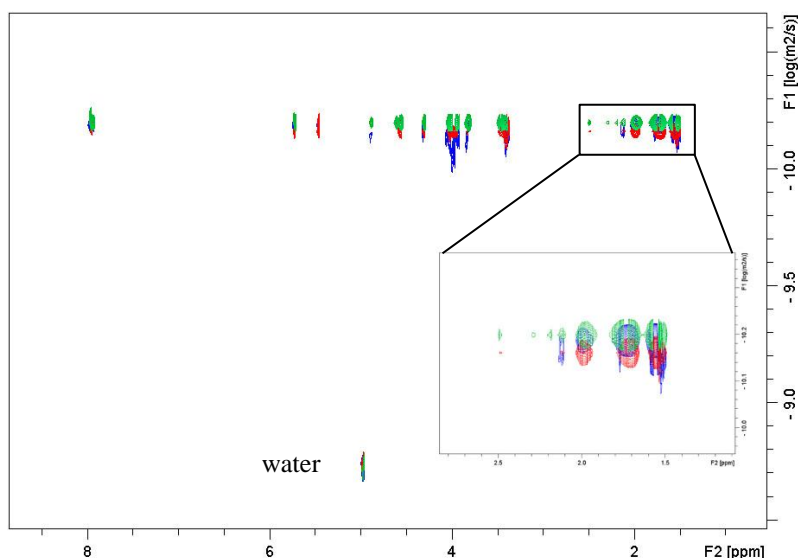


### Diffusion-ordered spectroscopy (DOSY) experiments

• This experiment which allows the separation of multiple solute species is classically used to determine the diffusion coefficients of compounds of different sizes. Pulse Gradient Spin Echo (PGSE) diffusion measurements were performed on a Bruker Avance III 500 MHz NMR spectrometer equipped with a direct Broadband cryo probe Prodigy 5 mm. The pulse sequence used was the Bruker ledbpgp2s (D. Wu, A. Chen and C. S. Johnson, Jr., *J. Magn. Reson. A*, 1995, **115**, 260) sequence that incorporates bipolar gradients, stimulated echo and a longitudinal eddy current delay as the z filter. The four 2.1 ms gradient pulses with sine-bell shapes were incremented linearly from 2 to 60 G/cm in 64 steps. The diffusion time ( $\Delta$ ) was set to 140 ms with 32K data points in t2, a sweep width of 5000 Hz and 128 transients. The data were processed using the Bruker topspin 3 package. The diffusion constant of water recommended by Bruker ( $1.9 \times 10^{-9} \text{ m}^2\text{s}^{-1}$ ) was used to calibrate the instrument. The molecular sizes were estimated from the Stokes-Einstein equation. Considering the molecules as a hard sphere, hydrodynamic radius  $R_H$  can be determined using equation  $R_H = \frac{k_B T}{6\pi\eta D}$  where  $k_B$  is the Boltzmann constant (in  $\text{J K}^{-1}$ ),  $T$  is the temperature in kelvin,  $\eta$  is the viscosity (in Pa.s) and  $D$  the diffusion coefficient (in  $\text{m}^2\text{s}^{-1}$ ).



DOSY spectrum of compounds **3a** (in blue) and **3b** (in red). **3a** and **3b** showed similar diffusion coefficient  $D = 10.19 \times 10^{-11} \text{ m}^2\text{s}^{-1}$  which corresponds to a hydrodynamic radius  $R_H$  of 68Å for both compounds.



DOSY spectrum of partially functionalized structures bearing an average of 30% (in blue) and 70% of mannose (in red) and of the fully mannosylated glycodendrimer **3a** (in green).