Supporting information for

Multivalent effect of glycopolypeptide based nanoparticles for galectin binding

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1. Materials and methods

Materials. All chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted. γ-Benzyl-L-glutamate and DL-propargylglycine were supplied by Bachem. Anhydrous DMF, DMSO, CH_2Cl_2, ethyl acetate, diethyl ether, THF and methanol were used directly from the bottle under an inert and dry atmosphere. Sodium cyanoborohydride (≥ 95%), propargylamine, α-D-lactose monohydrate, acetic acid, acetyl bromide, sodium ascorbate, copper sulfate, $(PPh_3)_3CuBr$, 3-chloropropylamine hydrochloride (98%), tetrabutylammonium hydrogen sulfate and sodium azide (99%) were used as received and when necessary under inert atmosphere. Linear galactan (CAS 39300-87-3, Mn=3500 g/mol, polydispersity index: 1.6 as determined by SEC in water, see instrumentation) was purchased from Carbosynth Limited. 1-Azido-3-aminopropane was synthesized following a literature procedure[1] as well as poly(γ-benzyl-L-glutamate)$_{20}$-*block*-poly(galactosylated propargylglycine)$_{32}$.[2] Spectroscopic data were in agreement with literature data.

Instrumentation. ^{1}H NMR spectra were recorded at room temperature with a BrukerAvance 400 (400 MHz). Infrared measurements were performed on a Bruker Tensor 27 spectrometer using the attenuated total reflection (ATR) method. The molar masses of galactan and α-azidogalactan were determined by SEC equipped with two PL aquagel OH 30 and OH 40 columns (25 x 300 mm), a Wyatt OptilabrEX differential refractometer and with $NaNO_3$ 0.1 M as eluent (0.5 mL.min^{-1}). SEC analyses of (γ-benzyl-L-glutamate–*b*–DL-propargylglycine) were performed using HFIP (Biosolve, AR from supplier or redistilled) as eluent and were carried out using a Shimadzu LC-10AD pump (flow rate 0.8 ml/min) and a WATERS 2414 differential refractive index detector (at 35 °C) calibrated with poly(methyl methacrylate) (range 1000 to 2000000 g/mol). Two PSS PFG-lin-XL (7 µm, 8*300 mm) columns at 40°C were used and injections were done by a Spark Holland MIDAS injector using a 50 µL injection volume. SEC analyses of glycosylated (γ-benzyl-L-glutamate–*b*–DL-propargylglycine) were performed in N,N,-dimethylacetamide (DMAc SEC) at 60 °C on a system equipped with a Waters 2695 separation module, a Waters 2414 refractive index detector (50 °C), a Waters 486 UV detector, and a PSS GRAM guard column followed by two PSS GRAM columns in series of 100 (10 mm particles) and 3000 (10 mm particles), respectively. DMAc was used as eluent at a flow rate of 1 mL/min. The molecular weights were calculated using polystyrene standards. Before SEC analyses were performed, the samples were filtered through a 0.2 µm PTFE filter (13 mm, PP housing, Alltech). SEC analyses of galactan-based glycopeptides were accomplished by PL-GPC 50 plus Integrated GPC from Polymer laboratories-Varian having UV and RI detectors, columns oven at 80°C, an integrated on-line degasser and equipped with two PLgel 5µm MIXED-D columns (75 x 300 mm). DMSO HPLC grade SCHARLAU with 1g.L^{-1} of LiBr was used as eluent (0.6 mL.min^{-1}). Transmission Electron Microscopy (TEM) images were recorded on a Hitachi H7650 microscope working at 80 kV. Samples were prepared by spraying a 1g.L^{-1} solution of the block copolymer onto a copper grid (200 mesh coated with carbon) using a homemade spray tool and negatively stained with 1.25% uranyl acetate. The apparent hydrodynamic radius of the particles was obtained by using a Malvern ZetaSizerNanoZS instrument. The dynamic light scattering (DLS) measurements were carried out by multi-angle light-scattering

analysis using an ALV laser goniometer, with a 22 mW linearly polarized laser (632.8 nm HeNe) and an ALV- 5000/EPP multiple tau digital correlator (125 ns initial sampling time). All the measurements were performed at a constant temperature of 25°C. The accessible scattering angles range from 30 to 150°. The solutions were placed in 20 mm diameter glass cells. Data were acquired with an ALV correlator control software, and the counting time was fixed for each sample at 30s for dynamic diffusion measurements. In the DLS mode, the hydrodynamic radius was calculated from the diffusion coefficient using the Stokes-Einstein relation. Atomic Force Microscopy (AFM) images were recorded in air with a Nanoscope III in dry Tapping mode. AFM measurements were performed at room temperature using a Veeco Dimension Icon AFM system equipped with a Nanoscope V controller. Both topographic and phase images of nanoparticles were obtained in tapping mode using a rectangular silicon cantilever (AC 160-TS, Atomic Force, Germany) with a spring constant of 42 N m^{-1}, a resonance frequency lying in the 290–320 kHz range, and a radius of curvature of less than 10 nm. The scan rates were in a range of 0.6 to 0.8 Hz. Samples were prepared by adsorption onto freshly cleaved HOPG (High Oriented Pyrolitic Graphite) or Mica from solutions at 1g.l^{-1} and allowed to dry overnight at room temperature. Measurements of height and width were taken using the section analysis tool provided with the AFM software (Nanoscope Analysis V1.20 from Bruker).

2. Synthesis of reactants (monomers, saccharides)

Synthesis of γ-benzyl-L-glutamate NCA. α-pinene (31.29 g, 229.68 mmol) and γ-benzyl-L-glutamate (15.0 g, 63.3 mmol) were dissolved in 120 mL anhydrous ethyl acetate in a three-neck round-bottomed flask. The mixture was stirred and heated to reflux. Then a solution of triphosgene (10.34 g, 34.8 mmol) in anhydrous ethyl acetate (60 mL) was added drop-wise. Two-third of the solution was added within 1 h and the reaction was left at reflux for another hour. Then, the rest of the triphosgene solution was added until the γ-benzyl-L-glutamate completely disappeared. Subsequently, around 90 mL of the solvent was removed under pressure and 180ml n-heptane was added slowly to precipitate NCA. The mixture was allowed to cool down to room temperature and then placed in a freezer overnight. After filtration, the solid was recrystalized by ethyl acetate and n-heptane twice, and then washed with n-heptane. The NCA was recovered as a white powder after being dried under vacuum. Mp 94°C, Yield: 4.20 g (83%) ^{1}H-NMR (400MHz, $CDCl_3$, δ, ppm): 2.13 (m, 2H,CH_2), 2.59 (t, 2H, CH_2, J=7.09 Hz), 4.37 (t, 1H, CH, J=6.56 Hz), 5.13 (s, 2H, CH_2O), 6.75 (s, 1H, NH), 7.35 (m, 5H, ArH). ^{13}C-NMR (400MHz, $CDCl_3$, δ, ppm): 26.98 (CH_2CH), 29.87 (CH_2CO), 57.01 (CH), 67.22 (CH2O), 128.48 (ArH), 128.71 (ArH), 128.83 (ArH), 135.30 (ArH), 152.10 (NHC(O)O), 169.53 (CH2CO(O)), 172.51 (CHC(O)O). **Synthesis of DL-propargylglycine NCA.** DL-propargylglycine (2.5 g, 22.1 mmol) and α-pinene (14.88 g, 109 mmol) were dissolved in 60 mL of anhydrous THF in a three-neck round-bottom flask. The reaction mixture was heated to 50 °C under nitrogen and then triphosgene (4.92 g, 16.6 mmol) in 20 mL THF was added drop-wise over a period of 1 hour. The reaction was continued for 4 hours until the mixture became gradually clear. The mixture was concentrated under reduced pressure and the NCA precipitated by addition of 100 mL n-heptane. The mixture was then placed in a freezer overnight. After filtration, the crude product was dissolved in dry THF,

and re-crystallized twice by addition of n-heptane. The obtained solid was washed with n-heptane, yielding white crystals in 75% yield. 1H-NMR (400 MHz, Acetone-d^6, δ, ppm): 2.62 (t, J = 2.5 Hz, 1H, CH), 2.86 (dd, J = 4.5 and 2.5 Hz, 2H, -CH_2-C), 4.75 (t, J = 4.5 Hz, 1H, CH), 8.05 (s, 1H, NH), ^{13}C-NMR (400 MHz, Acetone-d^6, δ, ppm): 22.26 (-CH2-C), 57.30 (CH), 73.64 (CH), 78.23 (-COCH), 152.71 (-O(CO)NH-), 170.62 (-O(CO)CH). FTIR (neat, cm^{-1}): 3363, 3247, 1854, 1771, 1286, 1195, 1111, 1089, 934, 893, 777, 756, 723, 698, 668. Mp: 114 °C. **Synthesis of azidogalactan.** Galactan (5 g, 1.25 mmol) was solubilized in acetate buffer (pH = 5.6), at 2% w/w. 1-azido-3-aminopropane (8 g, 93.75 mmol) was added under magnetic stirring. Then, 5.9 g of $NaCNBH_3$ (93.75 mmol, 75 equiv) were added and the mixture was stirred for 2 days at 30 °C. Subsequently, the mixture was concentrated under vacuum, precipitated in cold methanol and collected by centrifugation to remove the excess of 1-azido-3-aminopropane and sodium cyanoborohydride. The reaction medium was then dialyzed 4-5 days against milliQ water (Spectra/Por®6 MWCO 2 kDa membrane) to remove the excess of reactants and lyophilized. Yield: 4.2 g (84%).

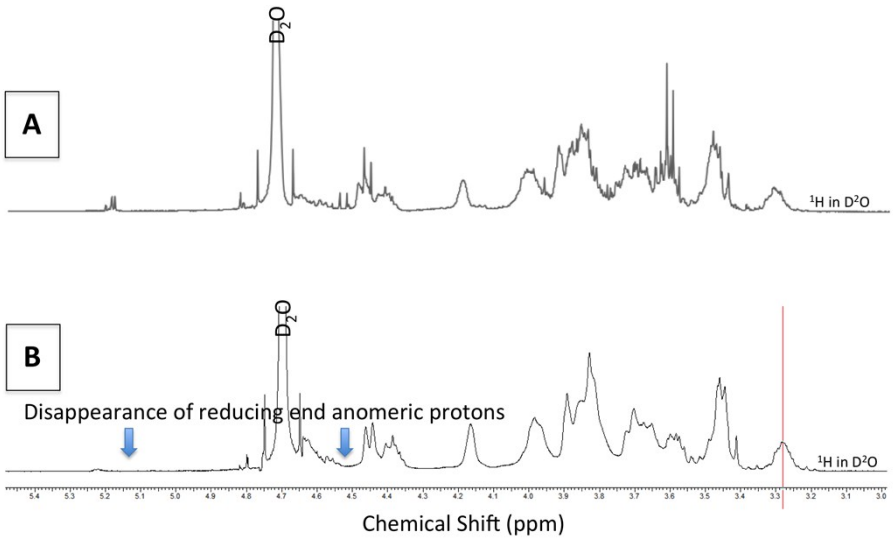


Figure S1 : ^{1}H NMR of galactan before (A) and after (B) reductive amination

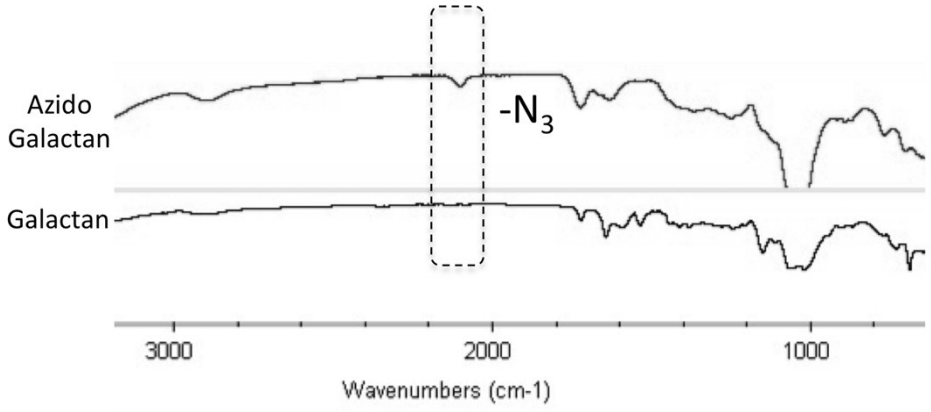
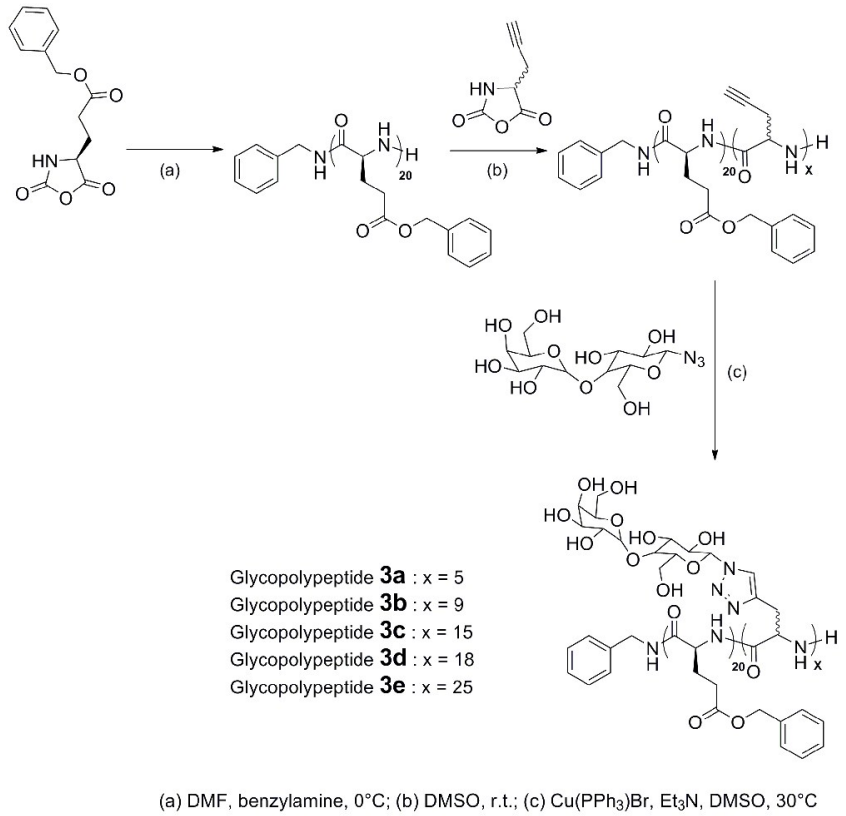


Figure S2 : FTIR of α-azidogalactan before and after purification (% transmittance).

^{1}H NMR analysis was performed in D_2O **(figure S1)** because water was the only solvent in which galactan was fully soluble. In this solvent, all the visible peaks were located at chemical shifts between 3.2 and 4.6 ppm. According to previous works,[2,3] the full disappearance of the reducing end anomeric proton peaks indicated a nearly quantitative reaction. FTIR was performed to verify the appearance of the N_3 group stretching (**figure S2**). IR (cm^{-1}): 3100-3600 (peak : 3300); 2920; 2150, 1200-1650 (multiple peaks). SEC in water (see instrumentation) was used to characterize galactan before reductive amination (Mn = 3500 $g.mol^{-1}$) and after reductive amination (Mn= 4000 $g.mol^{-1}$). Difference in molecular weight between these two analyses was attributed to the purification step involving oligosaccharide precipitation. **Synthesis of 1-Azido-1-deoxy-β-D-lactopyranoside (1-azido-β-lactose).** a) *1-Bromo-1-deoxy-2,2',3,3',4',6,6'-hepta-O-acetyl-α-D-lactose.* Acetyl bromide (10.8 mL, 133.2 mmol) was added to a solution of α-D-lactose monohydrate (3.0 g, 8.3 mmol) in acetic acid (20 mL) and the mixture was stirred at room temperature until a homogeneous solution was obtained. The mixture was concentrated under reduced pressure at 70 °C and coevaporated three times with dry toluene (20 mL) to give a foam of 1-Bromo-1-deoxy-2,2',3,3',4',6,6'-hepta-O-acetyl-α-D-lactose. This compound was used without further purification. ^{1}H-NMR (400MHz, $CDCl_3$, δ, ppm): 1.95-2.20 (m, 21H), 3.80-3.95 (m, 2H), 4.04-4.26 (m, 4H), 4.41-4.58 (m, 2H), 4.73-4.90 (m, 1H), 4.93-5.00 (m, 1H), 5.13 (m, 1H), 5.36 (d, J=3.61Hz, 1H), 5.49-5.60 (m, 1H), 6.52 (d, J=4.05Hz, 1H). b) *β-Azido-D-lactose heptaacetate.* NaN_3 (2.39 g, 36.76 mmol), tetrabutylammonium hydrogen sulfate (2.5 g, 7.37 mmol) and 50 mL of a saturated solution of $NaHCO_3$ were added to a solution of 1-Bromo-1-deoxy-2,2',3,3',4',6,6'-hepta-O-acetyl-α-D-lactose (5.15 g, 7.37 mmol) in 50 mL of CH_2Cl_2 at room temperature. The reaction mixture was stirred vigorously at room temperature for 4 hours and then diluted with 500 mL of ethyl acetate. The organic layer was washed with 200 mL of a saturated solution of $NaHCO_3$ and evaporated under reduced pressure. The residue was purified by using a silica gel column (Hexane/EtOAc, 4:6) to obtain β-Azido-D-lactose heptaacetate as white crystals (2.7 g, yield 55%). ^{1}H-NMR (400MHz, CDCl3, δ, ppm): 1.95-2.20 (m, 21H), 3.66-3.75 (m, 1H, H-5), 3.76-3.94 (m, 2H, H-4, H-6b'), 4.03-4.19 (m, 3H, H-5', H-6'a, H-6b), 4.45-4.58 (m, 2H, H-1', H-6a), 4.63 (d, J=8.80Hz, 1H, H-1), 4.86 (t, J=9.36Hz, 1H, H-2), 4.95 (dd, J=10.40Hz, J=3.53Hz, 1H, H-3'), 5.10 (dd, J=10.4Hz, J=7.82Hz, 1H, H-2'), 5.20 (t, J=9.30Hz, 1H, H-3), 5.32 (d, J=3.28Hz, 1H, H-4'). c) *1-Azido-1-deoxy-β-D-lactopyranoside*. β-Azido-D-lactose heptaacetate (2.5 g, 6.81 mmol) was dissolved in 40 mL of anhydrous methanol in a Schlenk tube. A catalytic amount of anhydrous potassium carbonate (38 mg, 0.27 mmol) was added to this solution and the reaction mixture was vigorously stirred at room temperature under a nitrogen atmosphere for 3 hours. Amberlite IR-120 ion-exchange resin was washed with methanol and then added and stirred with the reaction mixture for 1 hour. The resin was then filtered under gravity and the resulting solution was concentrated to dryness in vacuum to yield a white powder (184 mg, 90%). ^{1}H-NMR (400 MHz, D2O, δ, ppm): 3.26 (m, 1H), 3.49 (dd, J=7.83Hz, J=9.84Hz, 1H), 3.57-3.83 (m, 8H), 3.87 (d, J=3.3Hz, 1H), 3.94 (d, J=11.8Hz, 1H), 4.40 (d, J=7.83Hz, 1H, H-1'), 4.73 (d, J=8.84Hz, 1H, H-1). ^{13}C-NMR (400 MHz, D2O, δ, ppm):102.78, 89.87, 77.55,76.50, 75.27, 74.25, 72.43, 72.39, 70.85, 68.45, 60.96, 59.72.

3. Synthesis of lactose-based glycopolypeptides 3a-e



Scheme S1. Synthesis of poly(γ-benzyl-L-glutamate)-block-poly(lactosylated propargylglycine) **3a-e**

As already reported,[4] lactose has been efficiently used to target lectins having specific recognition with β-galactosides, a class of lectins that includes human galectins. Lactose based glycopolypeptides **3a-e** have been prepared by sequential ring-opening polymerization of γ-benzyl-*L*-glutamate NCA and propargylglycine NCA followed by post-polymerization glycosylation with azido-sugar ligands (*cf scheme S1*). The preparation of the copolypeptide backbone was based on a previously reported synthesis: this involved the synthesis of a polypeptide backbone that was obtained by ring-opening polymerization of DL-propargylglycine N-carboxyanhydride (PG-NCA) macroinitiated by poly(γ-benzyl-L-glutamate) (*cf scheme 2*). This macroinitiator was obtained by reacting γ-benzyl-L-glutamate N-carboxyanhydride (BLG-NCA) monomers with benzylamine in DMF at 0°C to prevent amino end-group termination.[5] After complete consumption of the BLG-NCA as observed by NMR and FTIR, the macroinitiator was used for chain extension with PG-NCA in DMSO at room temperature.

Block Copolymer [a]	M_n [b] before glycosylation (g/mol)	M_n [c] after glycosylation (g/mol)	PDI [c] after glycosylation	Hydrophilic weight ratio *f*
3a $PBLG_{20}$-b-PLG_5	5800	9369	1.16	0.35
3b $PBLG_{20}$-b-PLG_9	7400	11383	1.18	0.49
3c $PBLG_{20}$-b-PLG_{15}	7482	13263	1.37	0.62
3d $PBLG_{20}$-b-PLG_{18}	7800	14470	1.21	0.66
3e $PBLG_{20}$-b-PLG_{25}	8200	15987	1.65	0.73

Table S1. Poly(γ-benzyl-L-glutamate)-block- poly(lactosylated propargylglicine) copolymers **3a-e** (BLG: γ-benzyl-L-glutamate, PLG: Lactosylated poly(propargylglycine)). [a] Calculated from ^{1}H NMR using the integrated peak ratios of PBLG at 5.0 ppm (-O-CH_2-C_6H_5) and the combined PLG/PBLG backbone (Cα) signals at 3.8-4.6 ppm (-CH-CO-) while using the PBLG aromatic signal at 7.3 ppm as an internal standard. [b] Determined by SEC in HFIP with PMMA standards. [c] Determined by SEC in DMAc with polystyrene standards.

While the ratio of initiator to BLG-NCA was kept constant at 1:20, the ratio of PG-NCA to BLG-NCA was successively increased from 5:20 to 20:20 to obtain a library of block copolymers with increasing ratios *f* of hydrophilic (glycosylated) to hydrophobic blocks. As indicated in Table S1, analysis of the block copolymers was achieved by size exclusion chromatography (SEC) in DMAs and by NMR spectroscopy. Glycosylation of the block copolypeptides was subsequently carried out with azide-functionalized lactose via Huisgens cycloaddition (*Scheme S2*). The synthesis of this functionalized lactose was achieved in 3 steps from α-D-lactose in 50% overall yield (*cf part 2*). The success of the click reaction and the presence of lactose in the block copolymers were monitored by SEC as well as by ^{1}H and ^{13}C NMR (*see table S1 and figures S3 and S4*). After size exclusion chromatography, lactose addition to the PG block coincided with a significant increase of the molecular weights of the block copolymers (*Table S1*). Moreover, the complete disappearance of alkyne peaks at 73 and 80 ppm in the ^{13}C NMR spectra of the block copolymers suggested a nearly quantitative glycosylation of the materials (*figure S4*). Overall, the characterizations support the full functionalization of the poly(propargyl glycine) block with lactose, the chosen disaccharide unit. **Typical synthesis of poly(γ-benzyl-L-glutamate–b–DL-propargylglycine), example of 2a.** The NCA monomer of γ-benzyl-L-glutamate (1.01 g, 3.82 mmol, see part 2 for synthesis) was dissolved in 9 mL of DMF in a Schlenk tube. A solution of benzylamine (20.44 mg, 0.191 mmol) in 2 mL of dry DMF was added after NCA was dissolved. The reaction was stirred in a cold water bath of 0°C for 4 days under an inert atmosphere. After 4 days, BLG-NCA has been completely consumed as monitored by FTIR and NMR, and the PBLG macroinitiator was added to a solution of DL-propargylglycine NCA (see part 2 for synthesis, 267 mg, 1.9 mmol for DP = 10) in DMSO at room temperature. The reaction mixture was stirred for another five days. The reaction mixture was then precipitated into an excess of diethyl ether, filtered and dried under vacuum to obtain a pale yellow solid (typical yield: 80%). The polymer was analyzed by ^{1}H NMR and by SEC in DMAc (see instrumentation). Molar masses were determined by ^{1}H NMR using the intensity of the protons of the initiator and the intensity of methylene protons of the polypeptides.

Representative ^{1}H-NMR of the polypeptide backbone (400 MHz, TFA-d6, δ, ppm): 7.22 (s broad, benzyl), 4.53-5.21 (m broad, -CH-NH- + -O-CH_2-), 3.25 (s broad, -CH_2-NH_2-), 1.68-3.12 (m broad, -CH_2-CH_2-C(O)- + -CH-CH_2- + -CH), 1.48 (s broad, -CH_2-), 1.24 (s broad, -CH_2-CH_2-CH_2-), 0.81 (m broad, CH_3-). The copolypeptides **2b-e** were prepared in a similar manner by adjusting DL-propargylglycine NCA molarity. **Typical lactose-based glycopolypeptide preparation : poly(γ-benzyl-L-glutamate)-block-poly(lactosylated propargylglycine) 3a.** Poly(γ-benzyl-L-glutamate–b–DL-propargylglycine **2a**) (200 mg, ca. 0.818 mmol of alkyne units), 1-Azido-1-deoxy-β-D-lactopyranoside (383 mg, 0.982 mmol, 1.2 equiv. to alkyne groups, see ESI synthesis) and triethylamine (57 µL, 0.409 mmol, 0.5 equiv) were dissolved in 8 mL of anhydrous DMSO in a Schlenk tube. The mixture was stirred and degassed by bubbling nitrogen for 30 min. $(PPh_3)_3CuBr$ (76 mg, 0.082 mmol, 0.1 equiv.) was then added and nitrogen was bubbled through the resulting solution for another 30 min. Then the Schlenk tube was placed in an oil bath at 30 °C for 72 h under nitrogen atmosphere. Then ion exchange resin (150 mg) was added and the suspension gently stirred at ambient temperature overnight. After filtration and centrifugation, the polymer solution was precipitated in a 2:1 THF/diethyl ether mixture and washed with THF twice. The polymer was separated by centrifugation and dialyzed against distilled water for 3 days, then lyophilized to obtain an off-white polymer (typical yield: 60%). The glycopolypeptides **3b-e** were prepared in a similar manner by adjusting glycan molarity.

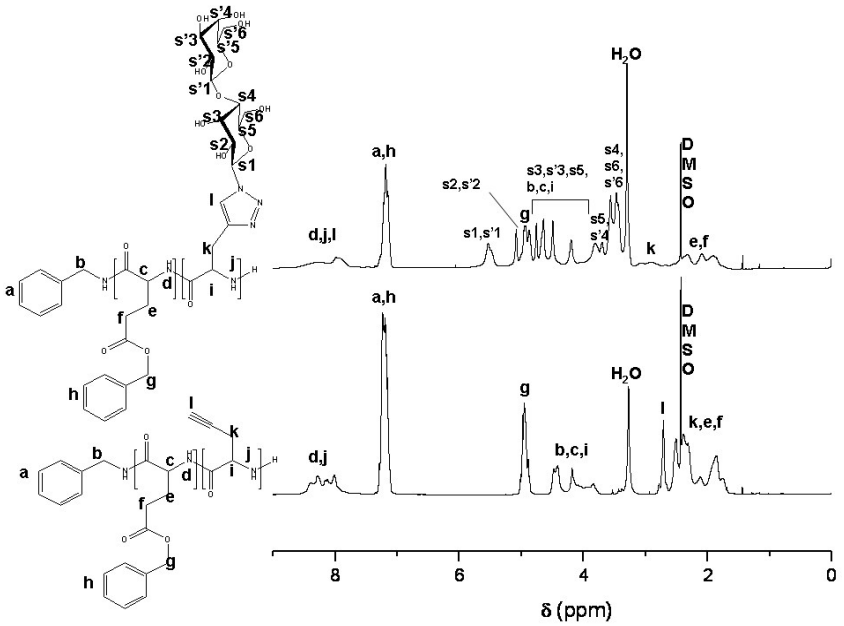


Figure S3. ^{1}H NMR of PBLG-b-PG before and after lactose coupling

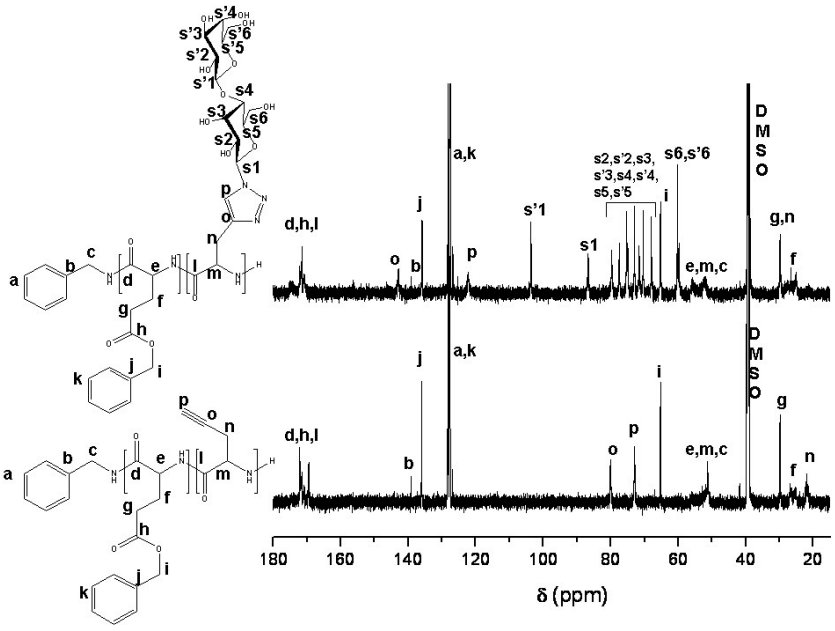
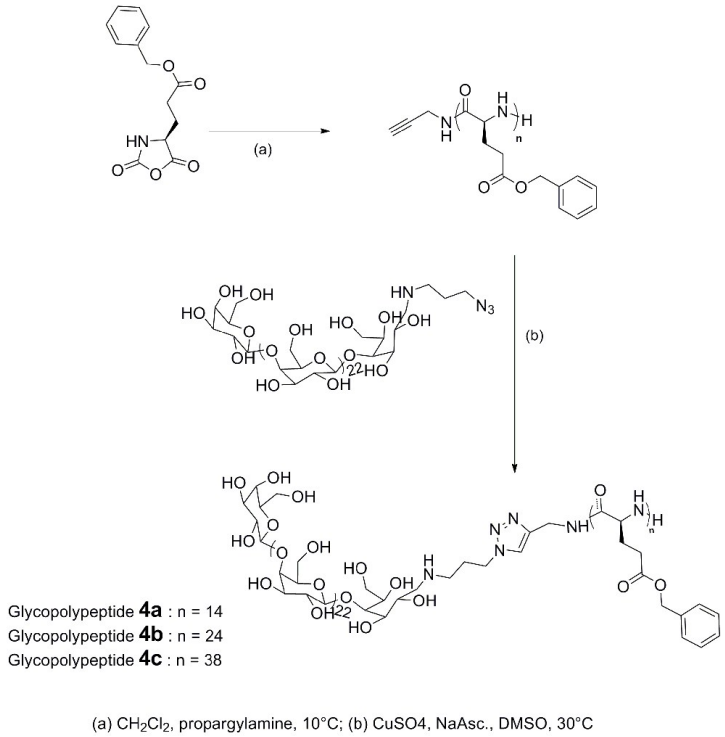


Figure S4. ^{13}C NMR of PBLG-b-PG before and after lactose coupling

4. Synthesis of galactan-based glycopolypeptides 4a-c

Oligosaccharide addition at the end-extremity of a polypeptide block is an efficient way to design amphiphilic glycopolypeptides.[6] Oligosaccharide-based glycopolypeptides are able to self-assemble in water into various glycosylated nanoassemblies including polymersomes with structures similar to viral capsids.[1] The originality of this strategy which consisted of using a biopolymer moiety that acts as a bioactive ligand and a stabilizing agent was also used to prepare hyaluronan-block-poly(γ-benzyl-*L*-glutamate) forming nanoparticles able to target cancer cells overexpressing the CD44 receptor.[7] In this work, propargylamine was used to initiate the ring-opening polymerization of γ-benzyl-*L*-glutamate N carboxyanhydride to introduce an alkyne reactive group at the C_{term} extremity of the synthetic polypeptide. Three poly(γ-benzyl-*L*-glutamate) block polymers having three different degree of polymerization were indeed obtained (Polymerization degrees of 14, 28 and 38). Galactan coupling to the polypeptides blocks was subsequently carried out via Huisgens cycloaddition (*Scheme S2*) in DMSO at 30°C by using $CuSO_4$ as catalyst in association with sodium ascorbate. Two equivalents of end-functionalized galactan were used for this coupling step in order to favor the full coupling conversion. Subsequently, the reaction medium was dialyzed against water by using a membrane with a molecular-weight cutoff of 50 kDa to ensure the full removal of the oligosaccharide excess. Both ^1H and size exclusion chromatography confirmed the block copolymer structure (*see figure S5 and S6 for* ***4a***). It is to note that α-azidogalactan is UV transparent in these SEC analysis conditions. In addition, IR spectra after coupling clearly indicated the disappearance of the signals at 2200 cm^{-1} belonging to unconjugated α-azidogalactan, thus confirming that the excess used for the coupling was removed upon dialysis.



Scheme S2. Synthesis of poly(γ-benzyl-L-glutamate)-block-galactan **4a-c.**

Typical galactan-based glycopolypeptide preparation, example of 4a : *a) Synthesis of α-propargyl PBLG.* The NCA monomer of γ-benzyl-L-glutamate (2 g, 7.6 mmol) was weighed in a glovebox under pure argon, introduced in a flame-dried schlenk, and dissolved with 5 mL of anhydrous CH_2Cl_2. The solution was stirred for 10 min, and propargylamine (32 µL, 0.5 mmol) was added with a nitrogen purged syringe. The solution was stirred for 2 h at room temperature under argon. The polymer was then recovered by precipitation in diethylether and dried under high vacuum, analyzed by ^{1}H NMR ($CDCl_3$ + 15% trifluoroacetic acid). Molar masses were determined by ^{1}H NMR using the intensity of methylene protons of the initiator at 3.9 ppm and the intensity of methylene protons of the PBLG at 5.1 ppm. Representative ^{1}H-NMR of the polypeptide backbone (400MHz, δ, ppm): 2.13 (m, 2H,CH_2), 2.59 (t, 2H, CH_2, J=7.09 Hz), 4.37 (t, 1H, CH, J=6.56 Hz), 5.13 (s, 2H, CH_2O), 6.75 (s, 1H, NH), 7.35 (m, 5H, ArH). *b) Synthesis of galactan-block-poly(γ-benzyl-L-glutamate).* α-propargyl poly(γ-benzyl-L-glutamate) (DP 14 from ^{1}H NMR, 100 mg, ca. 32 µmol of alkyne units), α-azido-galactan (258 mg, 64 µmol, 2 equiv. to alkyne groups) and sodium ascorbate (22 mg, 111 µmol, 3.5 equiv. to alkyne groups) were dissolved in 5 mL of anhydrous DMSO in a Schlenk tube. $CuSO_4$ (16 mg, 64 µmol, 2 equiv. to alkynes groups) was then added and the Schlenk tube was placed in an oil bath at 30 °C for 5 hours. The reaction medium was then dialyzed 4-5 days against milliQ water (Spectra/Por®6 MWCO 50 kDa membrane) containing EDTA the first 2 days. Aqueous solution was then purified by ultrafiltration to be sure to get ride off all the unreacted oligosaccharides (yield after lyophilization= 169 mg, 74%). All the two other galactan-based glycopolypeptides **4b** and **4c** were prepared in a similar manner.

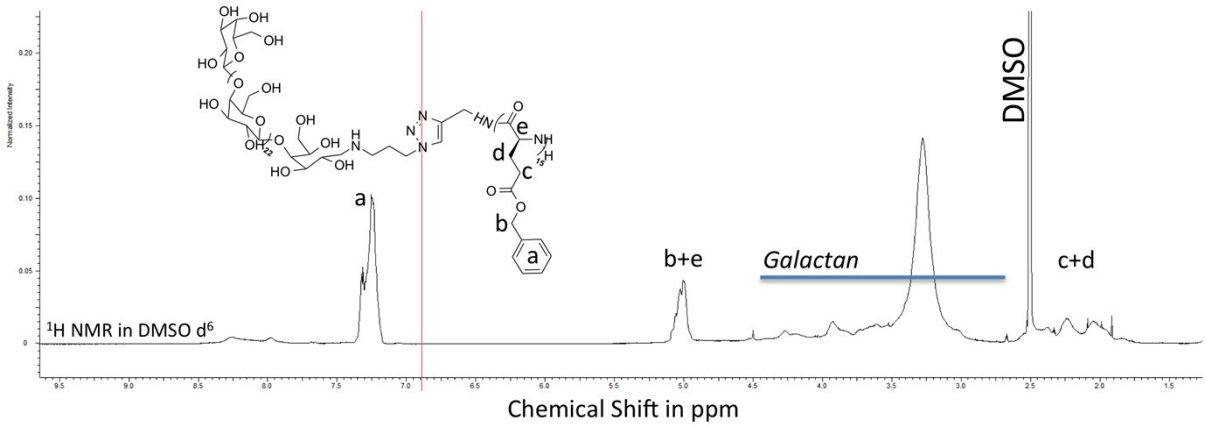


Figure S5 : ^{1}H NMR of galactan-*block*-poly(γ-benzyl-L-glutamate) **4a** in DMSO-d^{6} (solvent in which galactan alone is poorly soluble).

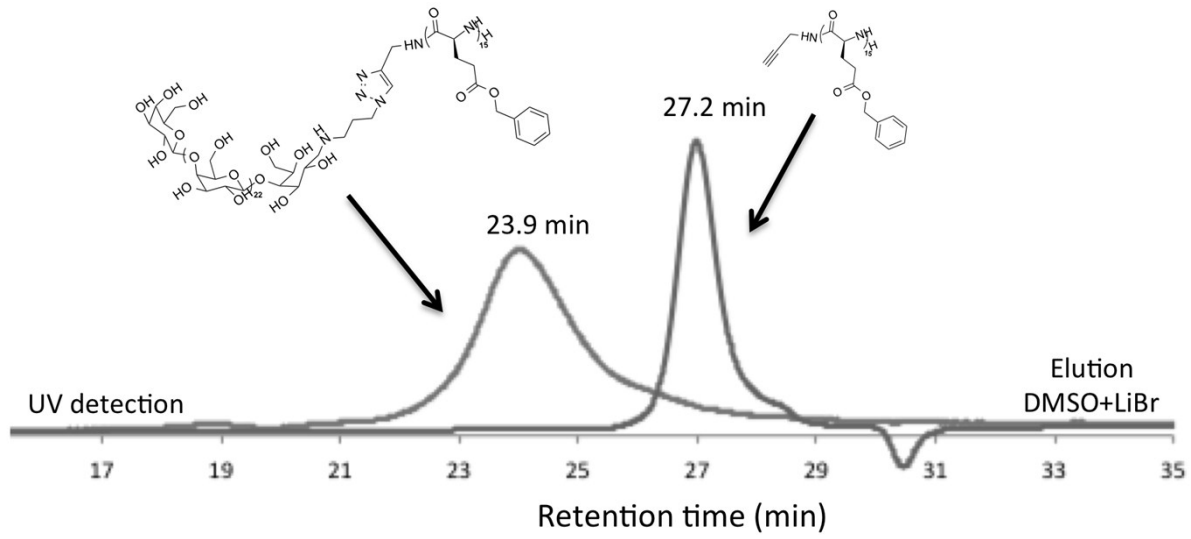


Figure S6 : SEC traces *of α-propargyl PBLG$_{14}$* (Tr = 27.2 min) and **4a** (Tr = 23.9 min). Elution in DMSO containing 1% LiBr, UV detection)

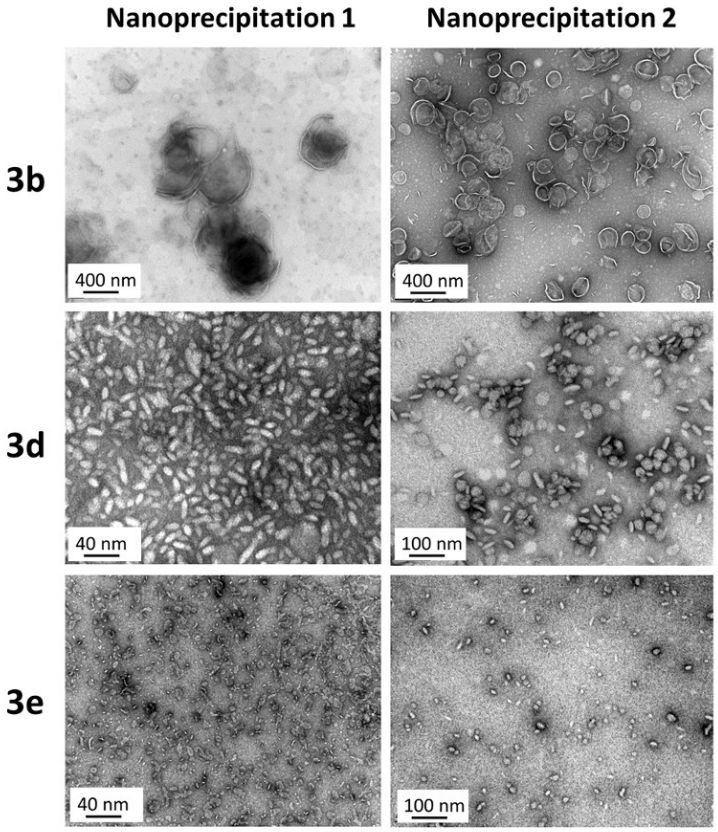
Block Copolymer [a]	Molecular weight of PBLG from ^{1}H NMR (DP)	Coupling conversion (SEC in DMSO)	Hydrophilic weight ratio f
4a PBLG$_{14}$-b-galactan	3100 (14)	> 95%	0.57
4b PBLG$_{24}$-b-galactan	5300 (24)	> 95%	0.43
4c PBLG$_{38}$-b-galactan	8400 (38)	> 95%	0.32

Table S2. Poly(γ-benzyl-L-glutamate)-block-galactan copolymers **4a-c** (PBLG: poly(γ-benzyl-L-glutamate),DP: polymerization degree).

5. Self-assembly of the synthetic glycopolypeptides in aqueous solutions

Self-assembly by nanoprecipitation *Method 1*: 0.5 mL of block copolymer solution in DMSO (10 mg/mL, filtered with 0.22 µm polypropylene membrane) were placed into a glass vial and 4.5 mL of ultrapure water were added instantaneously (1 s) under magnetic stirring (500 rpm). The mixture was dialyzed 24 h against water (Spectra/Por® MWCO 50kDa membrane) to remove DMSO. *Method 2*: 0.5 mL of block copolymer solution in DMSO (10 mg/mL, filtered with 0.22 µm polypropylene membrane) were added instantaneously (1 s) into a glass vial containing 4.5 mL of ultrapure water under magnetic stirring (500 rpm). The mixture was dialyzed 24 h against water (Spectra/Por® MWCO 50kDa membrane) to remove DMSO.

Self-assembly of lactose-based glycopolypeptides. All the lactose-based block copolymers were self-assembled using the nanoprecipitation method, which consists of adding a nonsolvent for the hydrophobic segment [here deionized (DI) water] to a copolymer solution (10 mg/mL) in a common solvent for both blocks (here DMSO). During this process, DMSO diffused into the water phase, leading to aggregation of the hydrophobic chains and driving the self-assembly process of the amphiphilic block copolymers. Dynamic light scattering (DLS) analysis were performed after removal of DMSO by dialysis, except for samples which underwent macroscopic aggregation and transmission electron microscopy (TEM) was used to probe the morphology directly after self-assembly. As shown in figure S7, polymeric nanoparticles were obtained after self-assembly of copolymers **3b-e** by using the nanoprecipitation methods presented above. Copolymer **3a** underwent macroscopic aggregation evidencing that a hydrophilic weight ratio of 35% was too low for self-assembly, a ratio that was in a range of the limiting *f* ratio determined studying the self-assembly of galactose-based glycopolypeptides.[2] As expected, the morphology probed by TEM was strongly influenced by this *f* ratio: vesicular assemblies were observed for the lower *f* ratio whereas micellar assemblies were formed for *f* ratio above 0.6. Overall, the size polydispersities of the nanoparticles, determined by dynamic light scattering, revealed that our formulation poorly controlled the self-assembly process. Lowering the addition speed from few seconds to 2h and/or changing the copolymer concentration from 0.1 to 10 g/L did not significantly modify this control. As already noticed with galactose-based glycopolypeptides,[2] changing the order of addition was critical (DMSO in water instead of water in DMSO, *figure 3*, nanoprecipitation 2). A quick diffusion of the DMSO solution promoted the formation of more defined polymeric nanostructures and samples having hydrophilic weight ratio above 65% (copolymer **3d** and **3e**) were found forming small micelles of about 30 nm with polydispersities values below 0.2.



TEM Imaging (Uranyl acétate 1.2%)

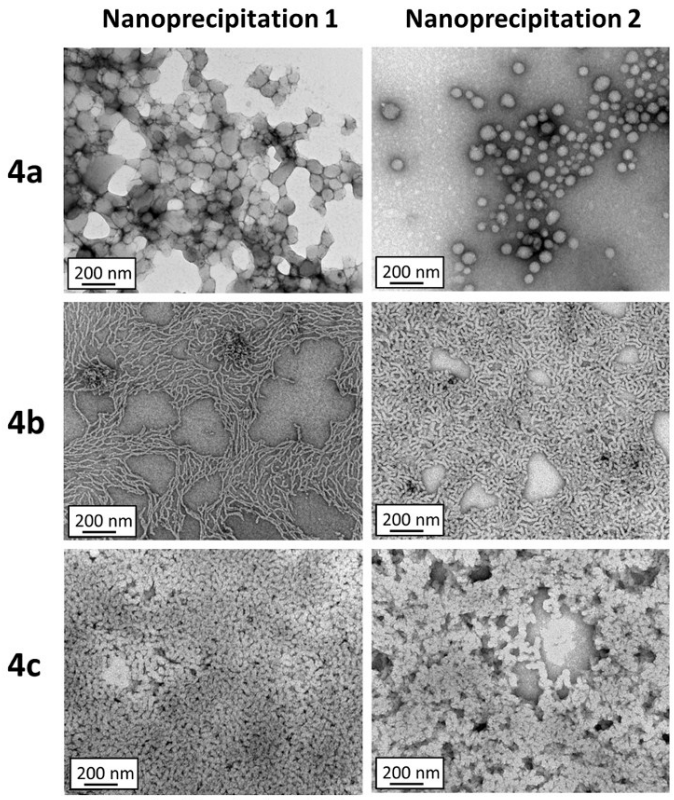
	Nanoprecipitation 1		Nanoprecipitation 2	
	D_H(nm)	PDI	DH(nm)	PDI
3a	Macroscopic aggregates			
3b	1660	0.25	569	0.4
3c	33	0.40	73	0.49
3d	38	0.48	31	0.15
3e	52	0.55	29	0.12

DLS data after self-assembly

Figure S7. TEM images of polymeric nanoparticles made of lactose-based glycopolypeptides depending on the nanoprecipitation method used.

Self-assembly of galactan-based glycopolypeptides. As shown in figure S8, self-assembly of PBLG-*block*-galactan copolymers **4a-c** was also envisaged via the two methods of nanoprecipitation used for copolymers **3a-e** : adding water to a DMSO copolymer solution (nanoprecipitation 1) or the contrary (nanopreciptation 2). TEM imaging was used to probe the influence of the hydrophilic weight ratio upon dialysis. It clearly evidenced the formation of spherical nanoparticles in the case of **4a** copolymers. Dynamic light scattering (DLS) analysis was performed after removal of DMSO by dialysis and it was evidenced that the second nanoprecipitation method was at the origin of smaller micellar assemblies (DH = 51 nm, PDI = 0.21). For the two other molecular weights of PBLG (28 and 38, **4b** and **4c**

respectively), TEM imaging evidenced the formation of worm-like morphologies upon dialysis. DLS analysis of these micellar assemblies revealed sizes comprised between 100 and 200 nm associated to polydispersities comprised between 0.3 and 0.4.



TEM Imaging (Uranyl acétate 1.2%)

	Nanoprecipitation 1		Nanoprecipitation 2	
	D_H(nm)	PDI	DH(nm)	PDI
4a	96	0.27	51	0.21
4b	161	0.37	156	0.35
4c	190	0.38	207	0.37

DLS data after self-assembly

Figure S8. TEM images of polymeric nanoparticles made of galactan-based glycopolypeptides depending on the nanoprecipitation method used.

Preparation of polymer micelles functionalized with lactose. Micelles made of the copolymer **3e** were studied in more detail (*see figure S9*). Multi-angle light scattering was used to determine accurately the hydrodynamic radius which was measured from the slope of the dynamic scattering analysis by using the Stockes-Einstein equation. This radius was 13.4 nm and was associated to a polydispersity of 0.17 at 90°. High resolution TEM imaging and AFM imaging were both used to further probe the 3D structures of the polymeric micelles : they were in both cases slightly elongated.

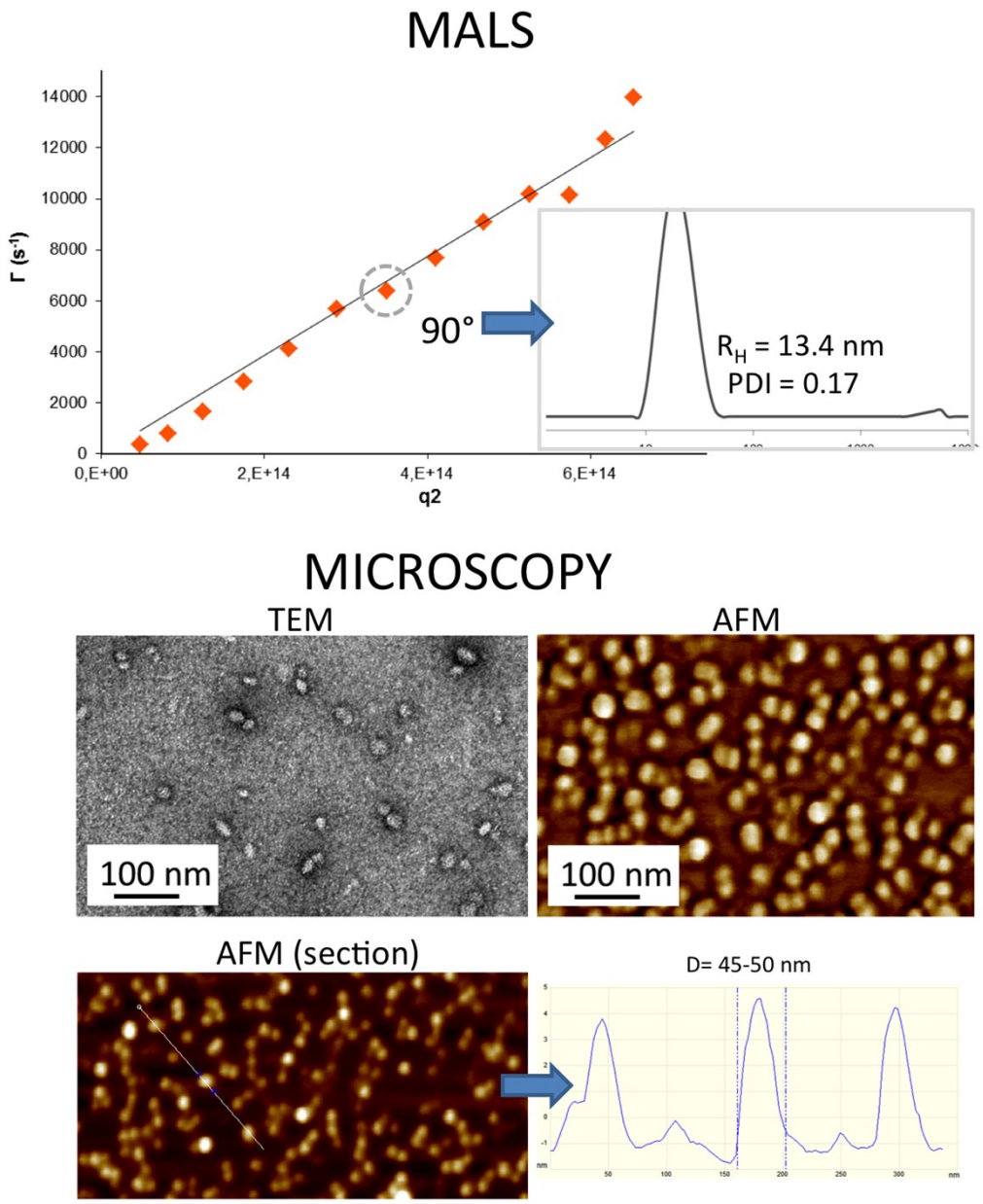


Figure S9. Polymeric micelles made of **3e** via the nanoprecipitation 2 method.

Preparation of polymer micelles functionalized with galactan. Micelles made of the copolymer **4a** were studied in more detail (*see figure S10*). Multi-angle light scattering was used to better determine the hydrodynamic radius which was measured from the slope of the multi-angle analysis by using the Stockes-Einstein equation. This radius was 26.1 nm associated to a polydispersity of 0.25 at 90°. High resolution TEM imaging and AFM imaging were both used to reveal the 3D shape structures of the micelles : the diameter observed by using these two technics were in agreement with the light scattering measurement.

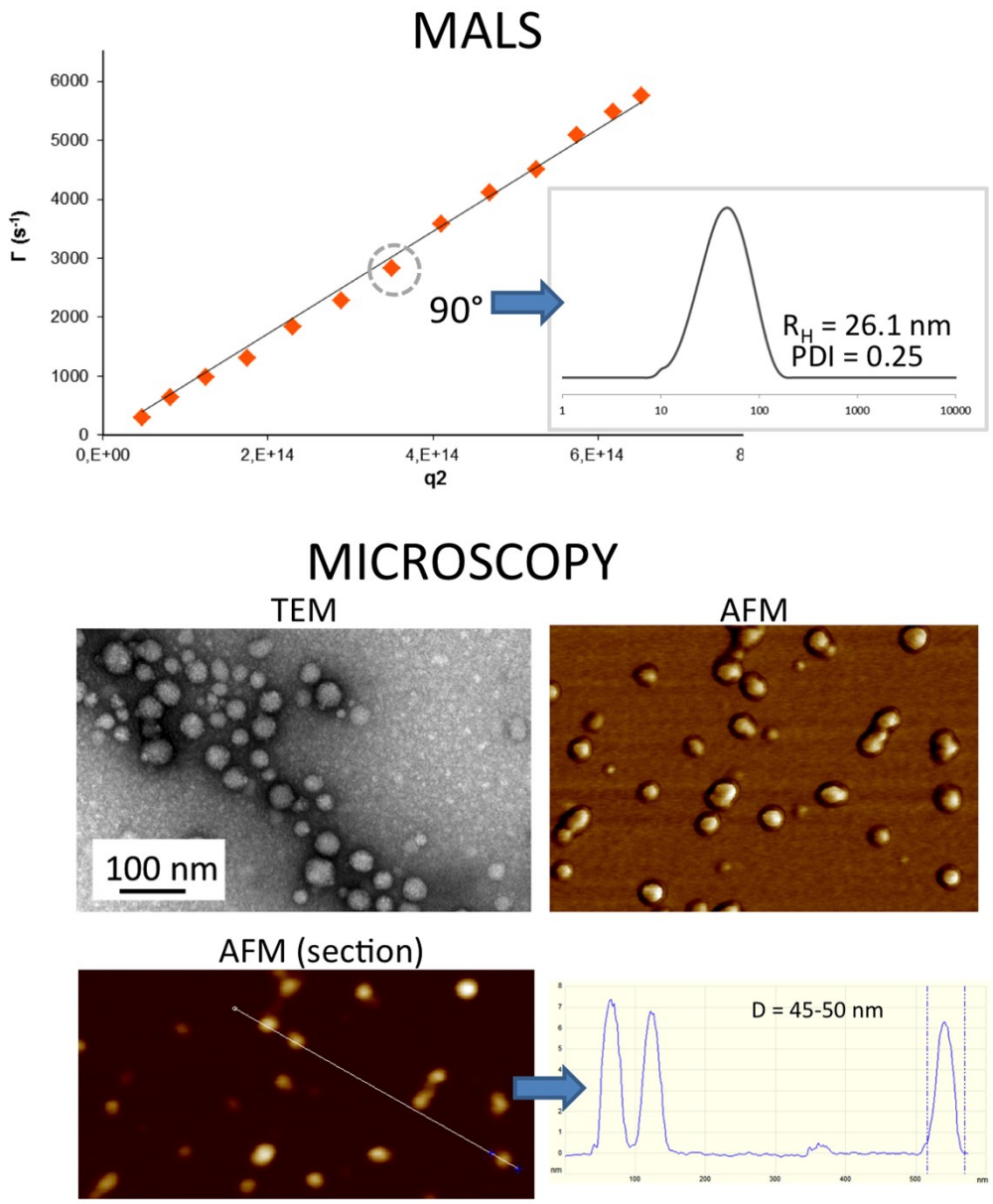


Figure S10. Polymeric micelles made of **4a** via the nanoprecipitation 2 method.

Preparation of polymer micelles functionalized with galactose. Micelles made of galactose-based glycopolypeptides were prepared according to the litterature from poly(γ-benzyl-L-glutamate)$_{20}$-block-poly(lactosylated propargylglycine)$_{32}$.[2] Multi-angle light scattering was used to determine accurately the hydrodynamic radius which was measured from the slope of the dynamic scattering analysis by using the Stockes-Einstein equation. This radius was 26.5 nm and was associated to a polydispersity of 0.11 at 90°. High resolution TEM imaging and AFM imaging were both used to further probe the 3D structures of the polymeric micelles : they were in both cases perfectly spherical (*see figure S11*).

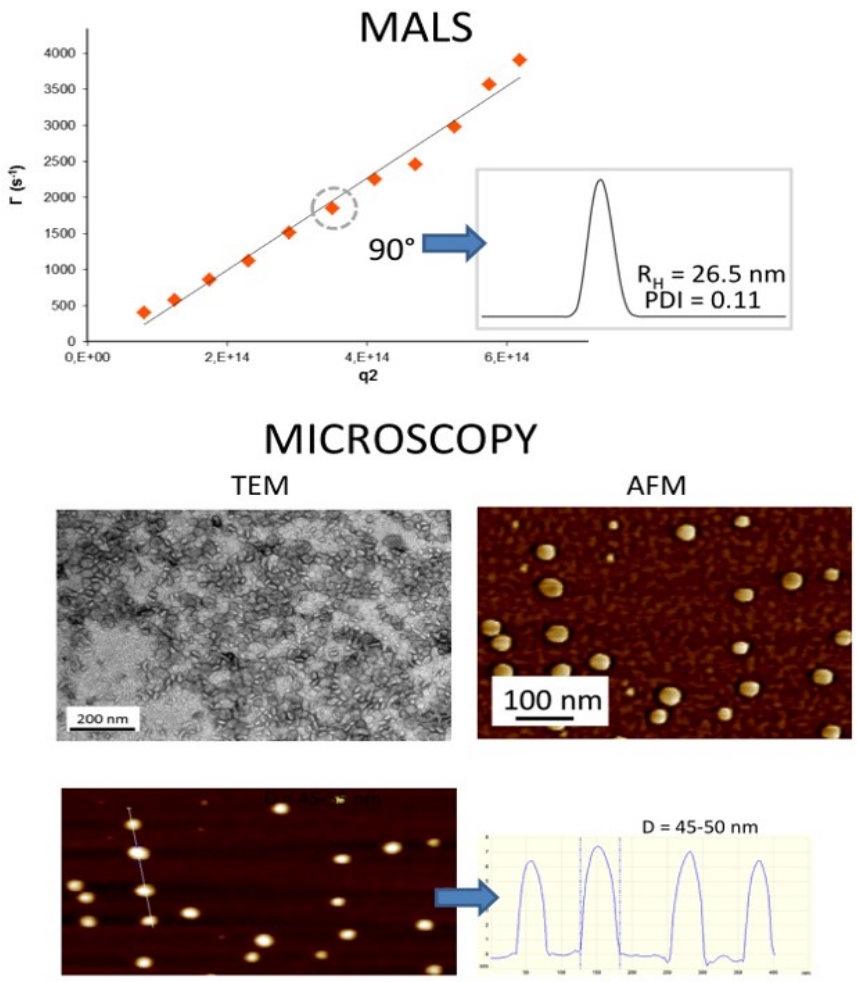


Figure S11. Polymeric micelles made of poly(γ-benzyl-L-glutamate)$_{20}$-block-poly(lactosylated propargylglycine)$_{32}$ via the nanoprecipitation 2 method.

6. Determination of the aggregation number of micelles

Static light scattering. The static light scattering (SLS) measurements were carried out to determine the aggregation number of micelles by multi-angle light-scattering analysis. All the measurements were performed at a constant temperature of 25°C. The accessible scattering angles range from 30 to 150°, in 5° increments. The solutions were placed in 20 mm diameter glass cells. Data were acquired with an ALV correlator control software, and the counting time was fixed for each sample at 5s for static diffusion measurements. In the SLS mode, the aggregation number was calculated from the Mw of nanoparticles, obtained by a Guinier plot at different angles and copolymer concentrations (Nagg = $Mw_{particles}/Mn_{copolymer}.Đ$). The differential refractive index increments dn/dc of copolymers in MilliQ water was measured by means of a differential refractometer (Wyatt Optilab rEX) operating at a wavelength of 658 nm at 25°C. A single copolymer concentration was used to determine the dn/dc coefficient through the 'dn/dc from peak' calculation module implemented in the Astra 6.1 software.

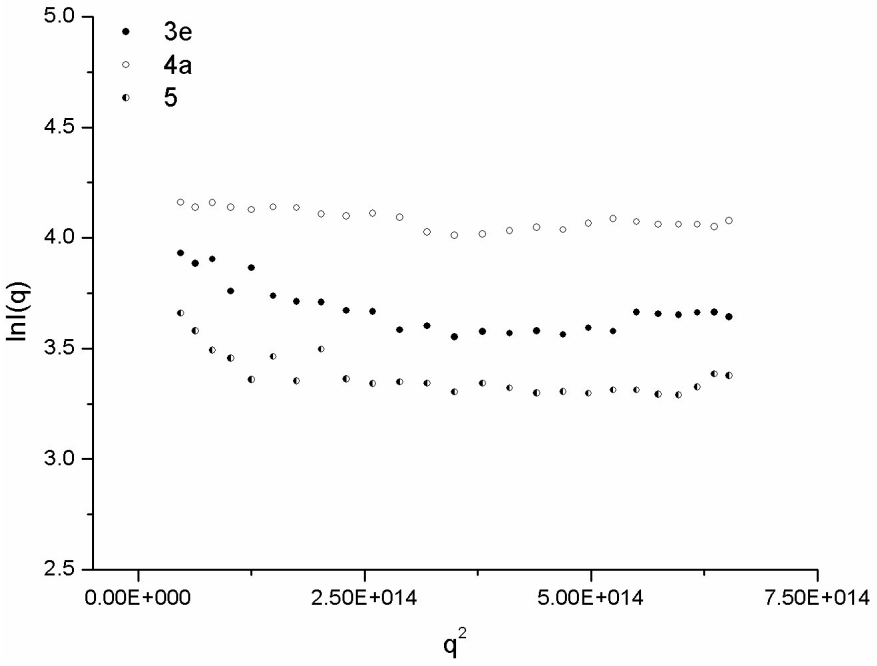


Figure S12. Guinier plots from SLS intensities of polymeric micelles made of **3e**, **4a and 5** via the nanoprecipitation 2 method (1mg.mL^{-1}).

Parameters	**Polymeric micelles**		
	3e	**4a**	**5**
dn/dc (mL.g^{-1})	0.0749	0.0944	0.0561
$Mw_{copolymer}$ (g.mol^{-1})	26,378	10,720	17,460
Đ	1.65	1.6	1.2
Rg (nm)	23.86	32.63	35.39
A_2 (mol.dm^3/g^2)	-1.496e-06	-6.965e-10	-1.042e-08
$Mw_{micelle}$ (g.mol^{-1})	6.706e+06	1.143e+07	7.981e+06
Nagg	254	1066	457

Table S3 – Molecular parameters (Rg, second virial coefficient - A_2, $Mw_{micelle}$, Nagg) calculated from the Guinier plot at different angles and concentrations of polymeric micelles **3e, 4a** and **5**.

7. Interactions with Lectins

Turbidimetric assays with RCA120. The bioactivity of the micellar assemblies made of galactose-based glycopolypeptides, **3e** and of **4a** was first assessed by turbidimetric assay. Micellar assemblies made of galactose-based glycopolypeptides was used as a control as this system has already been shown to interact with model lectins.[2] In this work, two models

lectins have been used, Ricinus communis agglutinin (RCA120) for its selectivity towards galactosyl residues, and Concanavilin A (ConA) for its selectivity towards glucosyl or mannosyl residues.

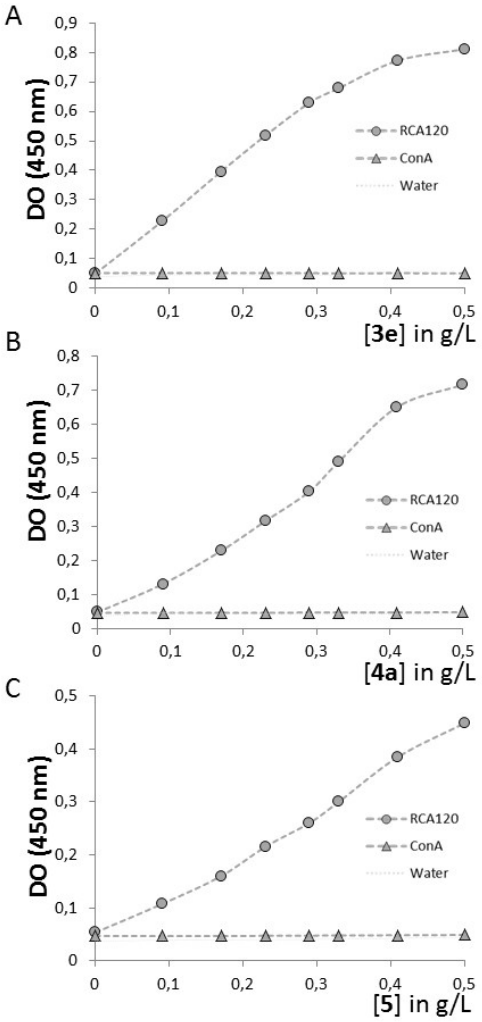


Figure S13. Turbidimetric assay with RCA120. A) Lactose-based glycopolypeptide nanoparticles; B) Galactan-based glycopolypeptides nanoparticles; C) Galactose-based glycopolypeptides nanoparticles. DO measured after 5 minutes.

Upon the addition of different concentrations of the three different micellar assemblies to the RCA120 solution, instantaneous precipitation was observed in each case (*figure S12*). As expected, this aggregation was only observed with this lectin compared to Concanavalin A or to the assay without water. Overall, this turbidimetric assay showed that the carbohydrate groups present at the surface of the nanoassemblies were available to mediate a specific interaction with a biological target macromolecules. *Typical procedure :* The lectin recognition activity of the glycopolypeptide solution before and/or after self-assembly was analyzed by the change of the turbidity at 450 nm at room temperature. 2 mg/mL of lectin (RCA120 or Concanavalin A for the control) was solubilized in DI water. 100 µL lectin

solution were transferred into a hole of a 96-well plate cuvette and a baseline measured before adding the solution containing glycan units. A solution of 10 µL glycopeptide with different concentrations in DI water was added into the holes containing the lectin solution and the absorbance was measured after 10 minutes using a Microplate reader SpectraMax M2e (Molecular Devices).

Galectin affinity evaluation by hemmaglutination assay. Human galectins 1 and 3 (R&D Systems, USA, #1152-GA and #1154-GA, respectively) were solubilized in PBS (Invitrogen) containing 0.1% BSA at a final stock concentration of 6.67 µM, aliquoted, snap frozen and kept at -20ºC until further use. ***Inhibiting Dose Determination.*** The hemagglutination assay is based on the capacity of some molecules to agglutinate red blood cells (RBCs), through their affinity for glycans at the surface of RBC, thus preventing them from settling out of suspension, and allowing the determination of the critical agglutination concentration by visual observation of the precipitate. Relevant examples of such molecules, in the scope of human disease and tissue targeting, are galectins 1 and 3, able to agglutinate RBCs. In this sense we performed a competition assay where the developed nanoconstructs or the sugars alone were mixed with galectins 1 or 3 and then the mixture exposed to a RBCs suspension. The critical concentration that allowed the complete inhibition of hemagglutination is then the minimum concentration necessary to titrate the respective galectin. As such, molecules or architectures with lower critical concentrations present higher affinities for the respective galectin. Briefly, the protocol for competitive hemagglutination was performed as follows. Human galectins 1 or 3 (7.5 µL at 6.67µM in PBS containing 0.1% BSA) were mixed with 90µL of the tested sugar/glycopeptide formulations, during 15 minutes. Then 2.5µL of rabbit red blood cells (10%(w/v), #88R-R001, Fitzgerald, USA) were added and the mixture well homogenized. The suspension was then left to sediment at room temperature until a clear pattern could be observed. Different sugar conformations (i.e. simple or formulated in nanoparticle backbone) were compared at equimolar concentrations. A positive control (PC) containing solely the galectin 1 or 3 and RBCs was equally performed. Negative control (NC) consisted in a red blood cell suspension at the same final concentration. ***Relative binding affinity determination.*** Saccharide solutions were prepared to cover the range of 2 mM to 2 uM concentrations of glycan units, following serial dilutions (*cf figure S13*). Polymeric micelles made of **3e** or **4a** as well as the corresponding glycans (lactose or galactan) were prepared as aqueous solution and mixed with rabbit RBCs (#88R-R001, Fitzgerald, USA, at a final concentration of 0.25% (w/v) and galectin-1 or 3 (at a final 0.5 µM). Galactose in solution was used as a standard to define the relative affinity of 1 and micelles made of galactose- based glycopolypeptides used as a less efficient control. After having mixed galectins and respective saccharide solutions for 15 minutes at room temperature, the RBCs were added and solutions homogenized and left to sediment at room temperature until clear pattern could be observed. Taking into account the characteristics of each glycan-based copolymer (see table S3) we determined the equimolar concentration between the different formulations and the glycan moieties alone. The minimum concentration causing inhibition, and therefore no agglutination, was then determined and defined the limiting dose of saccharide that was necessary to inhibit galectin interaction with RBCs. Each result was performed in duplicate. From the studies of hemagglutination we were able to determine the critical concentration of nanoconstructs or glycan alone to inhibit the agglutination action of both galectin 1 (table S4) and galactin 3 (table S5). Based on the critical inhibitory

concentration we could calculate the molar equivalent of the glycan moieties (taking into account each glycan ration and number of repeats in the copolymer). Then, taking galactose alone as normalizer we could establish a comparative ranking of each glycan moieties and architectures. As depicted in tables S4 and S5 (for galectins 1 and 3, respectively) the relative binding affinity corresponds to the relative potency of each glycan moieties to bind to the respective galectin.

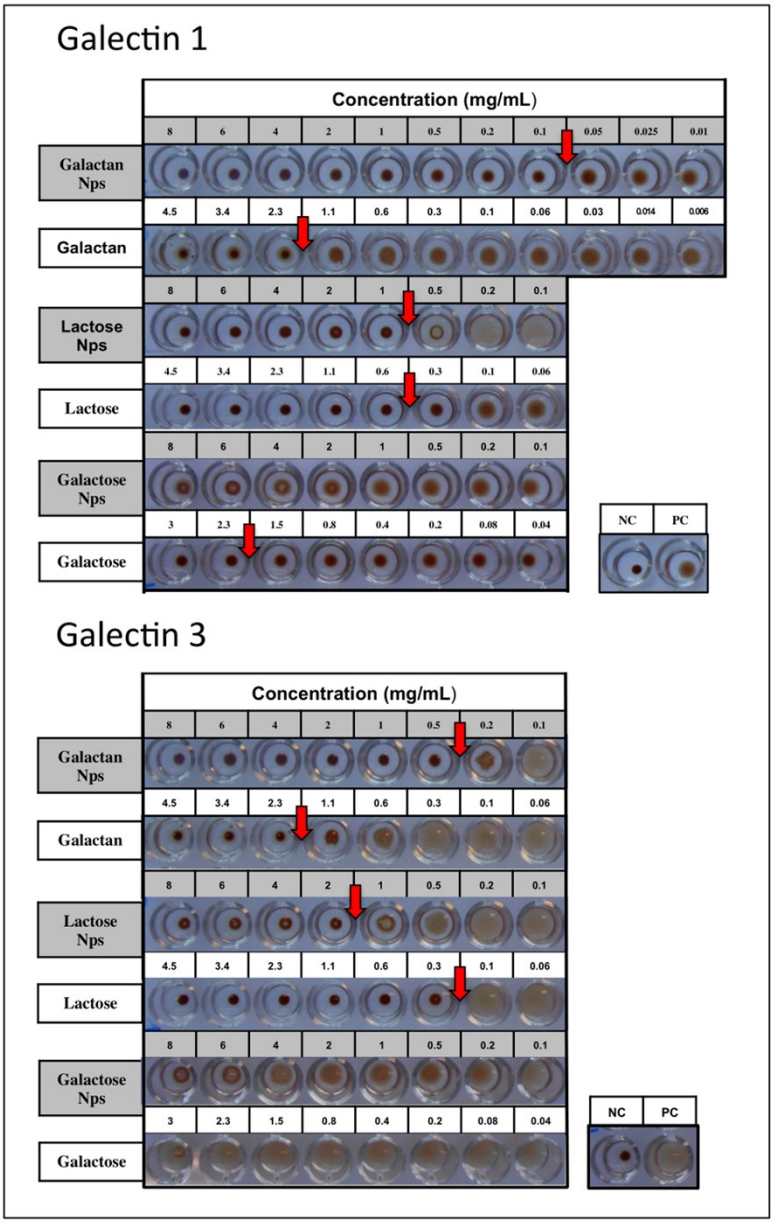


Figure S14. Competitive hemagglutination assay allowing the assessment of the different affinity of sugar moieties to galectins 1 or 3. Different sugar conformations (i.e. simple or formulated in nanoparticle backbone) were compared at equimolar concentrations (i.e. vertical adjacent wells for the same sugar have the same final sugar molar concentration, although here expressed as mass concentrations (mg/mL)). Red arrows indicate critical concentration to inhibit agglutination. Positive control (PC), negative control (NC).

Sugar moieties	PBLG (units)	PG (units)	Glycan units per block	PBLG block Mw (g/mol)	Sugar block Mw (g/mol)	Copolymer Mw (g/mol)	Glycan Mw (g/mol)	Glycan mass %
Galactose based	20	40	40	4400	12700	16300	7200	0.44
Lactose based	20	25	25	4400	12000	15600	8550	0.55
Galactan based	14	-	1	3100	4000	7100	4000	0.56

Table S4- Overview on the chemical characterization of the glycan based copolymers (i.e. galactose, lactose and galactan-based).

Sample	Critical concentration to inhibit agglutination (g/L)	Copolymer concentration to inhibit agglutination (mM)	Glycan units per copolymer	Maximum glycan concentration that inhibits agglutination (mM)	Relative binding affinity (relative to free glycan)
Galactan	2.3	-	-	0.58	**1**
Galactan Nps	0.1	0.014	1	0.014	**41.4**
Lactose	0.6	-	-	1.75	**1**
Lactose Nps	1	0.064	25	1.60	**1.1**
Galactose	2.3	-	-	12.78	**1**
Galactose Nps	>8	0.49	40	19.6	**0.65**

Table S5- Determination of the relative binding affinity to galectin 1 of the different glycan moieties.

Sample	Critical concentration to inhibit agglutination (g/L)	Copolymer concentration to inhibit agglutination (mM)	Glycan units per copolymer	Maximum glycan concentration that inhibits agglutination (mM)	Relative binding affinity (relative to free glycan)
Galactan	2.3	-	-	0.58	**1**
Galactan Nps	0.5	0.07	1	0.07	**8.3**
Lactose	0.3	-	-	0.87	**1**
Lactose Nps	2	0.128	25	3.21	**0.27**
Galactose	>3	-	-	16.67	**1**
Galactose Nps	>8	0.49	40	19.6	**0.85**

Table S6- Determination of the relative binding affinity to galectin 3 of the different glycan moieties.

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