Electronic Supplementary Material (ESI) for Polymer Chemistry.

ELECTRONIC SUPPORTING INFORMATION

Glyconanoparticles with Controlled Morphologies and Their Interactions with a Dendritic Cell Lectin

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Experimental Section

Materials

α-bromoisobutyryl bromide (EBiB) (98%), triethylamine (BioUltra, ≥99.5%), poly(ethylene glycol) methyl ether (average Mn ~2000) (MeO-PEG), methyl acrylate (MA) (99%, contains ≤100 ppm MEHQ as inhibitor), 2-bromoisobutyryl bromide (BiBB) and Phosphotungstic acid (PTA) solution (10% w/v) were purchased from Sigma Aldrich. tris(2-(Dimethylamino)ethyl)amine (Me6TREN), H2SO4-silica catalyst, N-(ethyl)-2-pyridylmethanimine ligand (ethyl ligand) used were previously synthesized within the group. ManAc monomer synthesized according to a procedure reported in literature.9 (Figure S1-13, ESI). All other reagents and solvents were obtained at the highest purity available from Sigma Aldrich Chemical Company. MA was passed through a basic alumina column to remove inhibitors prior to reactions. Dialysis tubes were purchased from Spectrum Laboratories. Copper coated 3.05mm diameter square carbon film mesh grids were purchased from Agar Scientific.

Instrumental Methods

Nuclear magnetic resonance spectroscopy

^{1}H NMR spectroscopy (Bruker DPX-300 and DPX-400) was used to determine the chemical structure of compounds synthesized and the conversion of the monomers during polymerizations. Samples were dissolved at 10 mg/mL concentration in D_2O or DMSO depending on the solubility of the samples.

Gel Permeation Chromatography

Size-exclusion chromatography measurements were conducted on an Agilent 1260 infinity system operating in DMF with 5mM NH_4BF_4 and equipped with refractive index detector and variable wavelength detector, 2 PLgel 5 μm mixed-C columns (300×7.5mm), a PLgel 5 mm guard column (50x7.5mm) and an autosampler. The instrument was calibrated with linear narrow poly(methyl methacrylate) standards in range of 550 to 46890 g/mol. All samples were passed through neutral aluminium oxide and 0.2 μm PTFE filter before analysis.

Dynamic Light Scattering

The particle size distributions of the nanoparticles were determined by using a Malvern Zetasizer Nano ZS instrument at 25 ^oC. Samples were introduced into the cells after filtration through 0.45 μm PTFE microfilters to determine the size of nanoparticles in aqueous solutions. The correlation function was analyzed *via* the general purpose method to obtain the distribution of diffusion coefficients (*D*) of the solutes. The Stokes– Einstein equation allows us to obtain the apparent equivalent hydrodynamic radius (R_H) from Contin's method:

$$R_H=k_BT/6\pi\eta_S D \quad (1)$$

k_B is the Boltzmann constant, T is the temperature of the sample, η_S is the viscosity of the fluid and *D* is the translational diffusion coefficient at a finite dilution.

Transmission Electron Microscopy

The morphologies of the self-assembled structures were analysed by Transmission Electron Microscopy (TEM), using a JEOL 2100 instrument operating at an acceleration voltage of 200kV and equipped with a CCD camera from Gatan. Each TEM sample was prepared by dropping 5μL of the nanoparticle aqueous solution on a Fresh glow-discharged carbon-coated copper grid for 1 min. The residue of aqueous solution was blotted away with a strip of filter paper and the grid was subsequently dipped into 20 μL of a 0.75 % *PTA* aqueous solution, pH 7, for 10 seconds in order to positively stain the sample. After removing the excess of PTA solution by a strip of filter paper, the grid was dried under vacuum and stored at room temperature until imaging.

Surface plasmon resonance

Beckman DU Series 700 UV/Vis Scanning Spectrophotometer was used to analyse the binding ability of the nanoparticles. SPR Sensorgrams were recorded in a Biorad ProteOn XPR36 SPR biosensor (Biorad, Hercules CA). Soluble DC-SIGN was immobilized to 6000 response units (RU) on discrete channels within Biorad GMC sensor chips via amine coupling. Soluble-phase analytes were prepared in 25 mM HEPES pH 7.4, 150 mM NaCl, 5 mM CaCl2, 0.01% Tween-20 and flowed over the immobilized materials at a rate of 25 μL/min at 25˚C. Regeneration of the sensor chip surfaces was performed using 10 mM glycine pH 2.5.

Synthetic Methods

Synthesis of PEG-Br initiator

This synthesis is the typical esterification reaction. In a 250 mL round-bottom flask, 5 gr (2.5 mmol) of MeO-PEG and 1.046 mL (7.5 mmol) of TEA were stirred in 50 mL of THF and cooled down to 0°C. Then, a mixture of 0.775 mL (6.25 mmol) of BIBB and 5 mL of THF was added dropwise to the reaction mixture over a period of 1 hour. The reaction was allowed to warm to room temperature and stirred overnight. The formed salt was filtered and excess volatiles were removed from the filtrate. The filtrate was then precipitated twice in cold hexane to yield a white powder.

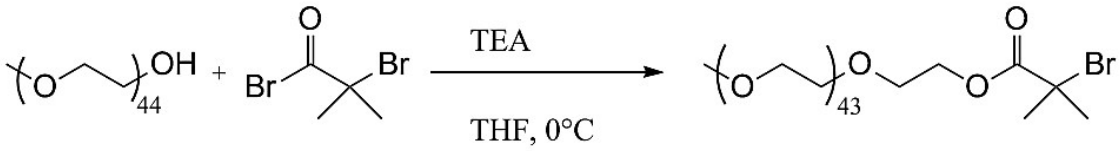

Figure S1. Schematic representation of the esterification reaction of PEG using BIBB.

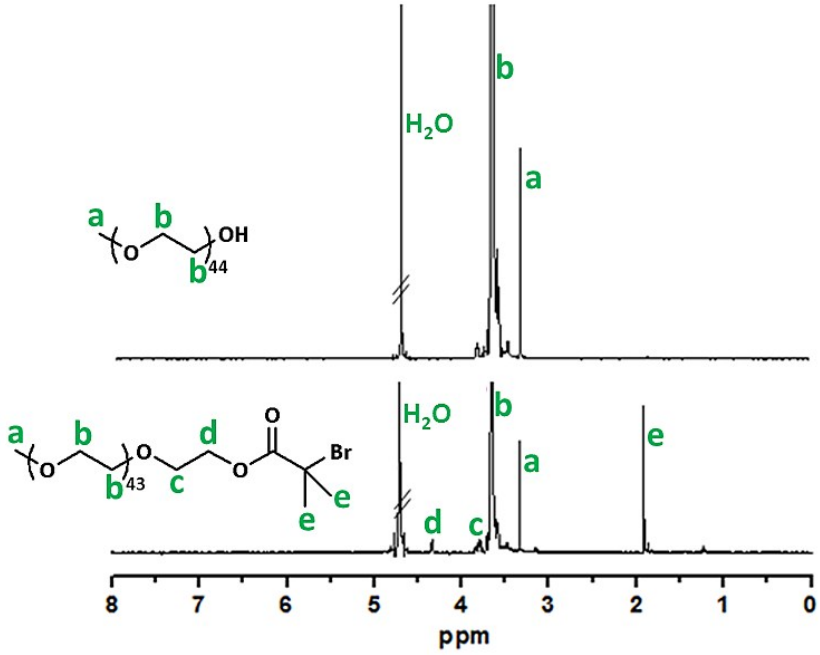

Figure S2. ^{1}H NMR of the macroinitiator PEG-Br showing the appearance of the methyl peak (e) at 1.96 ppm in D_2O.

The reaction was monitored by ^{1}H-NMR and the esterification was confirmed by the disappearance of the hydroxy peak at 1.8 ppm of PEG and the appearance of the peaks at 2.0 ppm from the methyl groups of the product. Furthermore, the structure

was confirmed by comparing the ratios of the integral of CH_3-O to the integral of $C(CH_3)_2Br$ (expected 3:6) and the integral ratios of CH_3-O-CH_2-R to $C(CH_3)_2Br$ (expected 2:6). Yield = 3.4 g (62.8%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 4.42 (m, 2H, CH_3-O-CH_2-R), 3.74 (b, 176H, R-O-CH_2-CH_2-O-R PEG repeating unit), 3.42 (s, 3H, CH_3-O-R), 2.00 (s, 6H, $C(CH_3)_2Br$).

Synthesis of D-Mannose glycomonomer

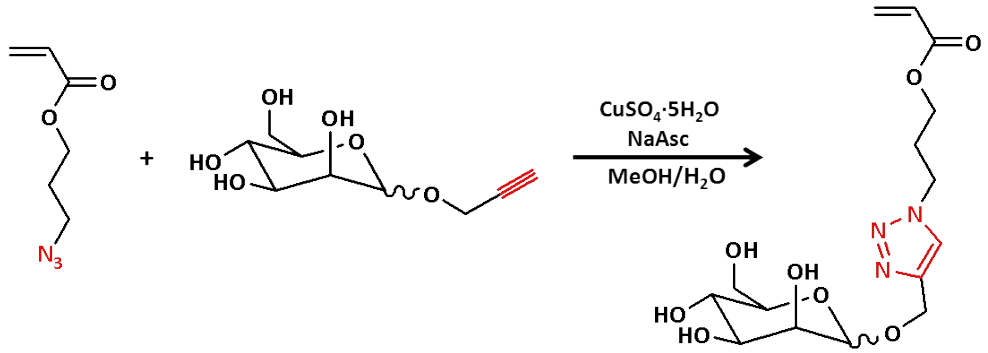

Figure S3. Schematic representation of the synthesised D-Mannose glycomonomer.

1-(2′-propargyl) D-mannose (2.46 g, 12.6 mmol) and 3-azidopropyl acrylate (2.85 g, 11.8 mmol) were dissolved in MeOH/H_2O (2:1 vol/vol, 60 mL), aqueous solution of $CuSO_4 \cdot 5H_2O$ (246 mg, 0.9 mmol) and (+)-sodium L-ascorbate (284 mg, 1.2 mmol) were added into the reaction solution. The reaction mixture was stirred at ambient temperature for 24 h and then the methanol was removed under vacuum and residue mixture was freeze dried to remove water. The purification of the obtained product was done by silica gel column chromatography using dichloromethane-MeOH (8:1) as eluent. After the removing of solvent, the product was obtained as white (1.62 g, yield: 58.2%).

^{1}H NMR (D_2O, 298 K, 400 MHz): δ =8.07, 8.06 (s, overlaped, 1 H, NC*H*=C), 6.37 (dd, J=1.8, 15.5 Hz), 6.36 (dd, J=1.6, 15.7 Hz) (anomeric 1 H, CH_2=C), 6.14 (dd,

J=10.4, 6.9 Hz), 6.13(dd, J=10.4, 7.0 Hz) (anomeric, 1 H, CH_2=C*H*C=O), 5.89 (dd, 1 H, J=1.5, 8.9 Hz, C*H*$_2$=C), 4.70-5.05 (m, C*H*$_2$-OH, H-1 of mannose , overlap with H_2O), 4.64 (d, 1 H, J=12.3 Hz, C*H*$_2$-OH), 4.55 (t, 2 H, J=6.9 Hz, C*H*$_2$-N), 4.19 (t, 2 H, J=6.0 Hz, C=O-O-C*H*$_2$), 3.40-3.92 (m, H residues of mannose), 2.30 (m, 2H, CH_2-C*H*$_2$-CH_2) ppm.

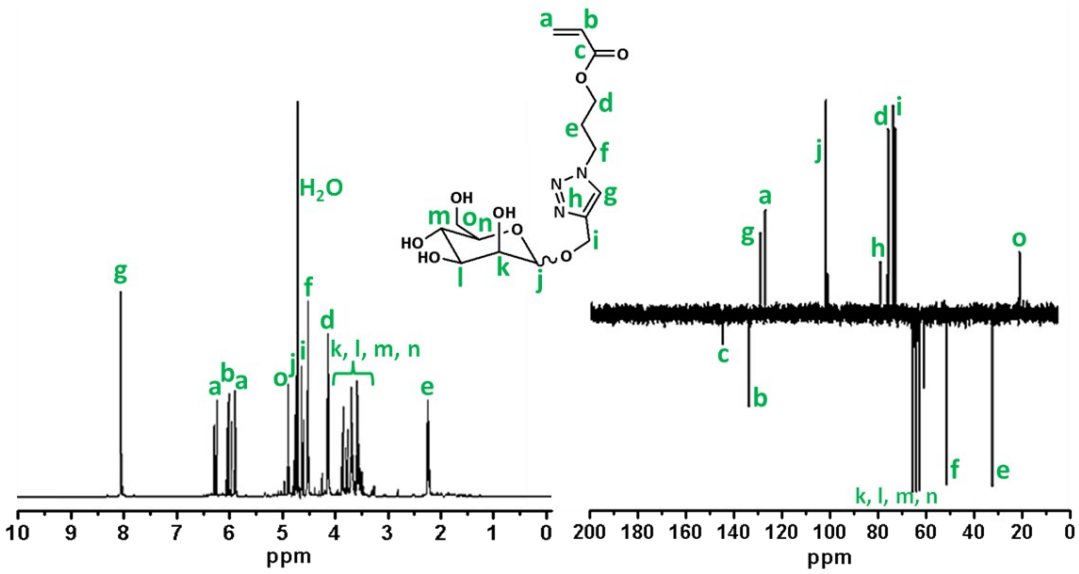

Figure S4. ^{1}H and ^{13}C NMR spectra of D-Mannose glycomonomer.

^{13}C NMR (D_2O, 298 K, 400 MHz): δ =146.4 (*C*=O), 145.4 (N-CH=*C*), 131.9 (*C*H_2=C), 129.2 (CH_2=*C*), 125.6 (N-*C*H=C), 100.8 (β anomeric, C 1 of mannose), 100.7 (α anomeric, C 1 of mannose), 78.4,75.2, 75.0, 72.5, 72.3, 72.0, 68.6, 68.4 (carbons of anomeric mannose), 63.0(*C*H_2-OH), 62.6 (C=O-O-*C*H_2), 60.7 (C-*C*H_2-O), 48.5 (CH_2-*C*H_2-N), 28.5(CH_2-*C*H_2-CH_2) ppm.

Kinetic Study of Homopolymerisation of MA using PEG-Br initiator

PEG-Br was synthesized as described above and employed as initiator for homopolymerization of MA to make an optimization of the reaction. The SET-LRP of MA was carried out in the presence of PEG-Br in DMSO at 25^{o}C using a Cu(0)/Cu(II) and Me_6Tren derived catalyst. To figure out best system, the polymerization reaction

and chain extension were done. (Table S1) In order to obtain targeted M_n values and low polydispersities, different ratios were investigated by kinetic measurements at periodic intervals.

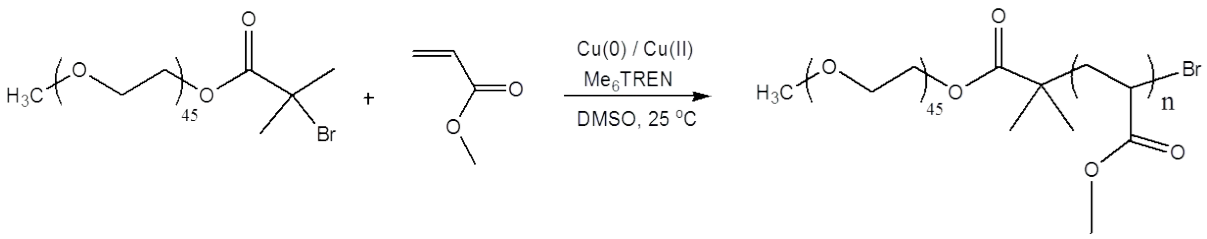

Figure S5. Schematic representation of SET-LRP of MA using PEG-Br.

The conversions of each sample were measured by ^{1}H NMR and Gas Chromatography (GC). The molecular weight and polydispersity index were determined by Gel Permeation Chromatography (GPC). The reaction displayed livingness and full conversion with a final M_n of 8800 g.mol^{-1} and polydispersity of 1.11 in 3 hours. After this, the chain extension of MA with DP=30 was done and it worked pretty well. After 6 hours, the conversion reached 97%.

Table S1. Kinetic data obtained for the SET-LRP of MA and chain extension.

Polymerization Time	Mn_{GPC}[a] (g/mol)	$Đ$[a]	ρ[b] (%)
1st block 30 min	5600	1.11	0.57
1st block 45 min	6800	1.09	0.76
1st block 60 min	7450	1.11	0.87
1st block 120 min	7850	1.11	0.94
1st block 180 min	8300	1.11	0.98
2nd block 30 min.	9650	1.11	0.46
2nd block 60 min.	10100	1.11	0.62
2nd block 120 min	10350	1.11	0.76

2nd block 180 min	10600	1.12	0.83
2nd block 360 min	10850	1.12	0.97

[a)]DMF eluent, PMMA standards; [b)]Conversion (ρ) measured by ^{1}H NMR.

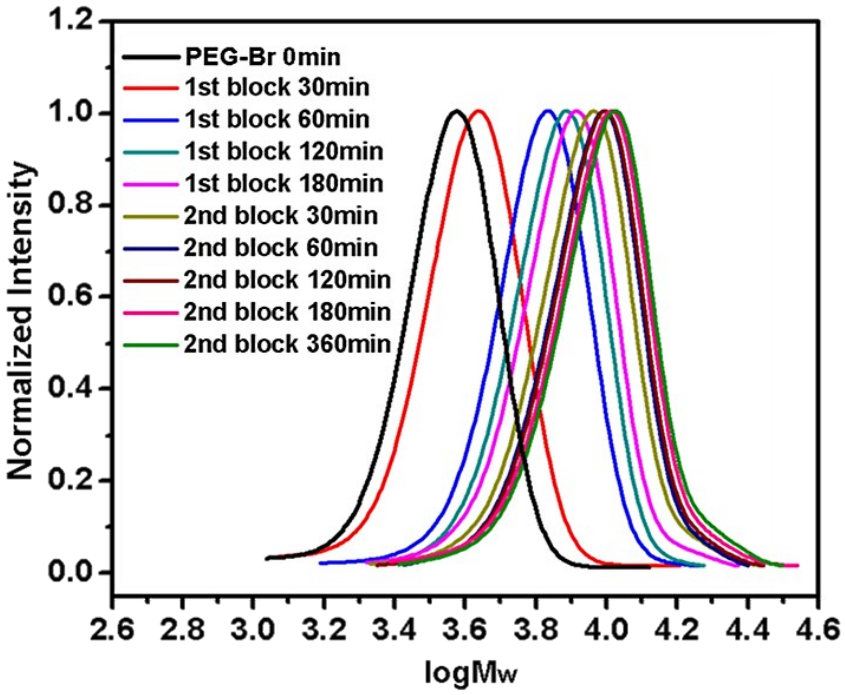

Figure S6. GPC traces of the SET-LRP of MA and chain extension using PEG-Br initiator.

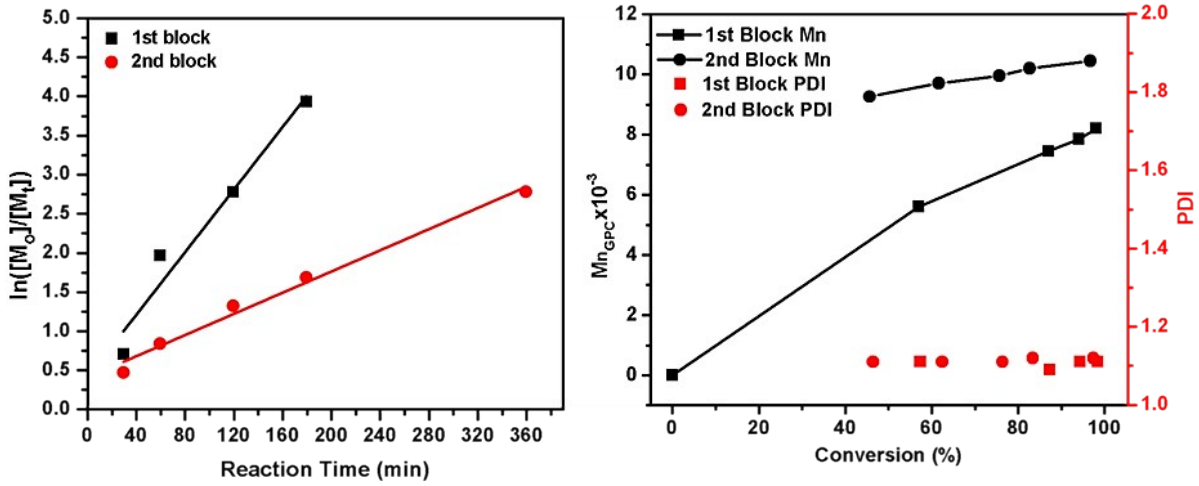

Figure S7. Kinetic and molecular weight/dispersity data of the SET-LRP of MA and chain extension using PEG-Br.

Semi-logarithmic kinetic plot displays a linear increase of conversion with time as expected, showing the livingness of the polymerization. The increase in the molecular weight with conversion over time is hence also linear. From the above data, it is obvious that the polymerization of MA has been achieved with a good control. It was also shown that the polymerization shows living characteristics throughout the whole polymerization. To retain chain end fidelity of the living polymers, chain extension was carried out at 97% conversion.

General procedure of synthesis of P((MA)$_m$-b-(ManAc)$_n$)

A Schlenk tube was charged with different ratio of MA monomer (relevant eq), pre-activated Cu(0) wire (5 cm), $CuBr_2$ (0.04 eq) and DMSO (6 ml) and the mixture was degassed by gentle bubbling of argon gas for 30 min. Pre-degassed Me_6TREN (0.19 eq) and α-bromoisobutyryl bromide initiator (1 eq) were then added *via* gas tight syringe sequentially. The Schlenk tube was sealed and the mixed solution was allowed to polymerize at 25^{o}C for 3 h according to polymerization kinetics' study of MA before. Sample of the

reaction mixture was then removed for analysis. The sample for ^{1}H NMR was directly diluted with DMSO, which confirmed >96% conversion according to integral of vinyl groups with that of the O-CH_3 groups at 3.50-3.60 ppm. After waiting for the NMR result, a solution of glycomonomer (15 eq) in 1 mL DMSO, previously degassed by argon sparging for 20 min, was directly transferred via cannula to the Schlenk tube under argon protection and polymerization for another 18 h. A sample was taken for ^{1}H NMR and SEC analysis. The ^{1}H NMR result confirmed >98% conversion according to integral of vinyl groups with that of the triazole groups (NC*H*=C) at 8.06-8.07 ppm. Catalyst residues were removed by filtering through a column of neutral alumina prior to DMF SEC analysis. The reaction was stopped via exposure to the air and then the reaction mixture was dialysed against to a mixture of distilled water and methanol for 3 days, while changing the water at least three times. Finally, it was freeze dried to get the product. The end pure product was characterised by ^{1}H NMR and GPC.

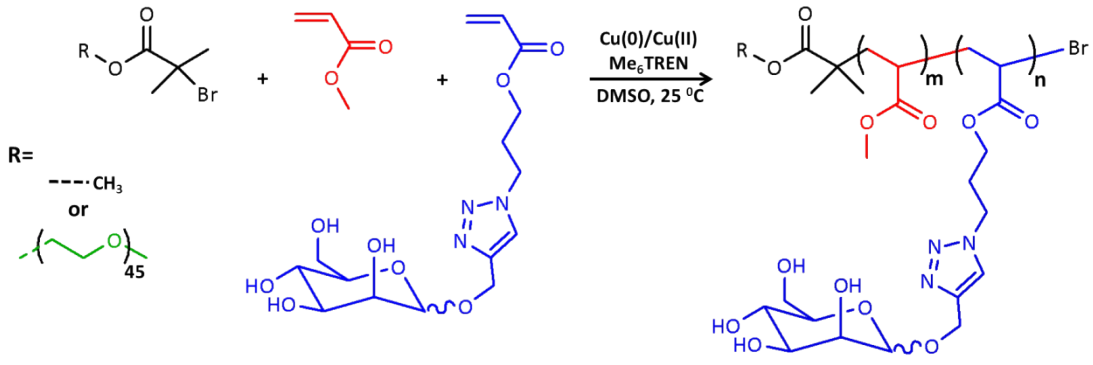

Figure S8. Schematic representation of copolymerization of MA and ManAc *via* SET-LRP with EBiB or PEG-Br initiator.

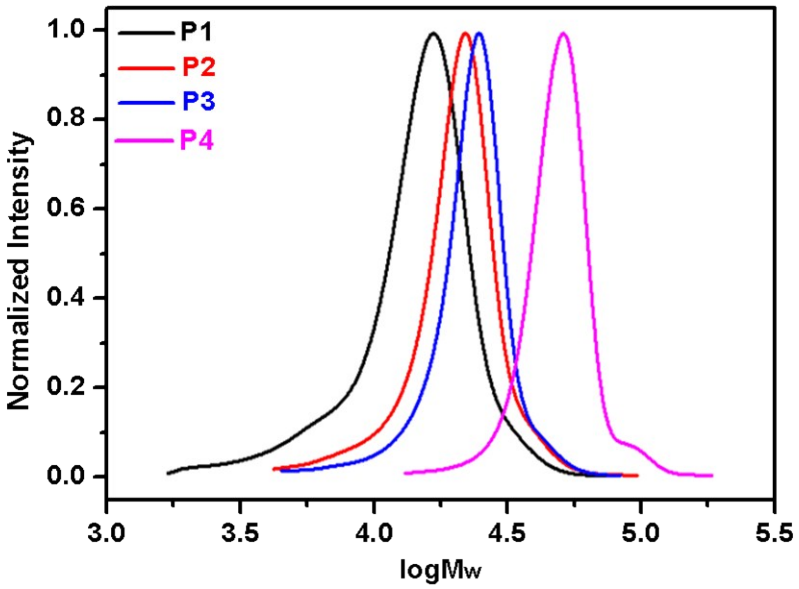

Figure S9. GPC traces of all amphiphilic block co-glycopolymers, P((MA)$_m$-b-(ManAc)$_n$) (P1, P2, P3, P4).

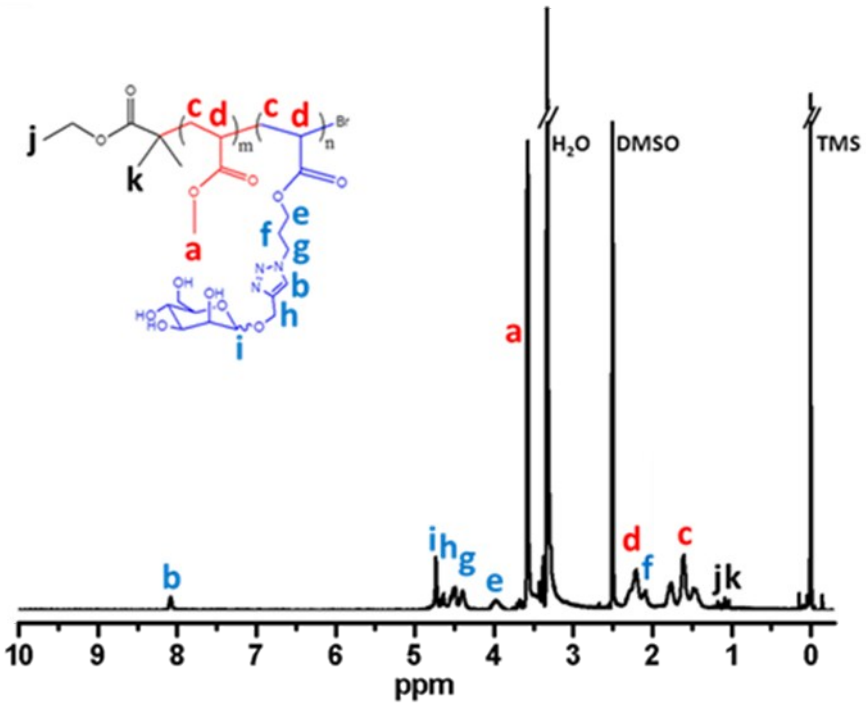

Figure S10. ^{1}H NMR spectra of the purified P((MA)$_m$-b-(ManAc)$_n$).

Synthesis of P($(PEG)_{45}$-b-$(MA)_{172}$-b-$(ManAc)_{15}$)

After the confirmation of the chain fidelity of P((PEG)-b-(MA)) by making chain extension, triblock co-glycopolymer. P($(PEG)_{45}$-b-$(MA)_{172}$-b-$(ManAc)_{15}$)) (**P5**) was synthesized via using same polymerization procedure. A Schlenk tube was charged with different ratio of MA monomer (180 eq, 3.8 ml), pre-activated Cu(0) wire (5 cm), CuBr2 (0.04 eq, 2.1 mg) and DMSO (6 ml) and the mixture was degassed by gentle bubbling of argon gas for 30 min. Pre-degassed Me6TREN (0.19 eq, 11.8 µl) and PEG-Br initiator (1 eq, 0.5 gr) were then added via gas tight syringe sequentially. The Schlenk tube was sealed and the mixed solution was allowed to polymerize at 25oC for 3 h according to polymerization kinetics' study of MA before. Sample of the reaction mixture was then removed for analysis. After the confirmation of >96% conversion of MA, a solution of glycomonomer (15 eq, 0.2 gr) in 1 mL DMSO, previously degassed by argon sparging for 20 min, was directly transferred via cannula to the Schlenk tube under argon protection and polymerization for another 18 h again. The ^{1}H NMR result confirmed >99% conversion according to integral of vinyl groups with that of the triazole groups (NCH=C) at 8.06-8.07 ppm. Catalyst residues were removed by filtering through a column of neutral alumina prior to DMF SEC analysis. The reaction was stopped via exposure to the air and then the reaction mixture was dialysed against to a mixture of distilled water and methanol for 3 days, while changing the water at least three times. Finally, it was freeze dried to get the product.

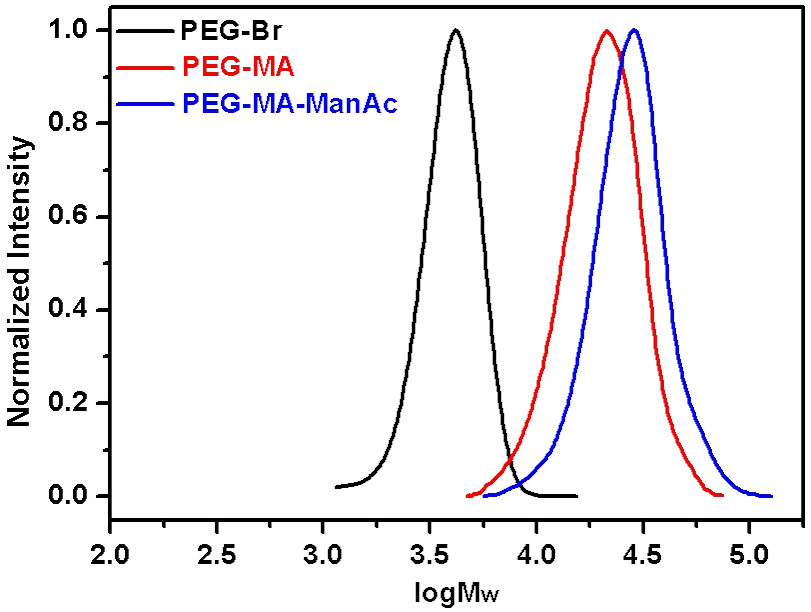

Figure S11. GPC traces of triblock co-glycopolymer, P((PEG)$_{45}$-b-(MA)$_{76}$-b-(ManAc)$_{15}$).

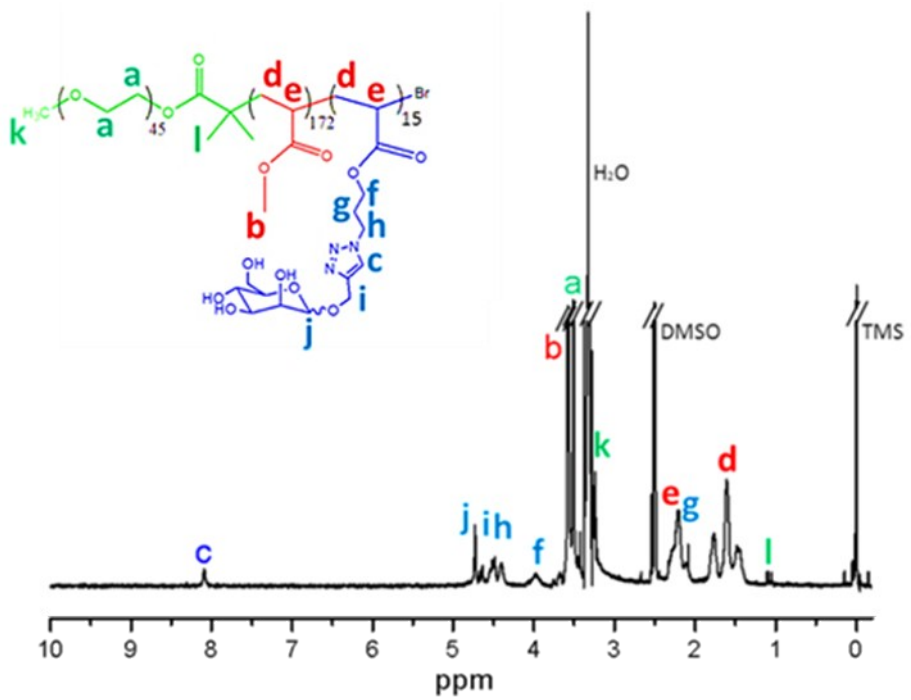

Figure S12. ^{1}H NMR of the obtained amphiphilic triblock co-glycopolymer after purification.

Preparation and characterization of glyconanoparticles

Glyconanoparticles were prepared using the nanoprecipitation (solvent-switch) method. The same procedure was used for all amphiphilic glycopolymers. Briefly, each glycopolymer (7 mg) was dissolved in 1mL DMF to yield an initial concentration of 0.5 μM. To this solution, 2 mL of ultra-pure water was progressively added at a rate of 5 μL /min using a master dual pump from Worked Precision Instruments. This procedure takes 6 hours, thus avoiding forming any kinetically trapped self-assembled structures. The final mixture was diluted with 7 mL of ultrapure water to freeze the self-assembled structures (final ratio DMF/H2O of 1/10; [glycopolymer] =0.05 μM). Excess of DMF was removed through 3 days dialysis against ultra-pure water, using membranes with a MW cut off 500-1000 kDa. The final suspensions were characterized by DLS and TEM.

Table S2. Physicohemical properties of the glyconanoparticles.

Polymer	**Molar ratio of [MA] to [ManAc]**	**DLS**		**TEM**	
		D_h **(nm)**	$Đ$	**Size (nm)**	**Observed Morphology**
P1	1.17:1	22±1.2	0.144	17±3	spherical micelles
P2	1.58:1	23±1.3	0.173	18±5	spherical micelles
P3	2.00:1	26±1.1	0.221	25±6	non-spherical micelles
P4	3.16:1	380±2.6	0.356	420±25	vesicles
P5	2.64:1	65±1.6	0.376	35±8	worm-like micelles

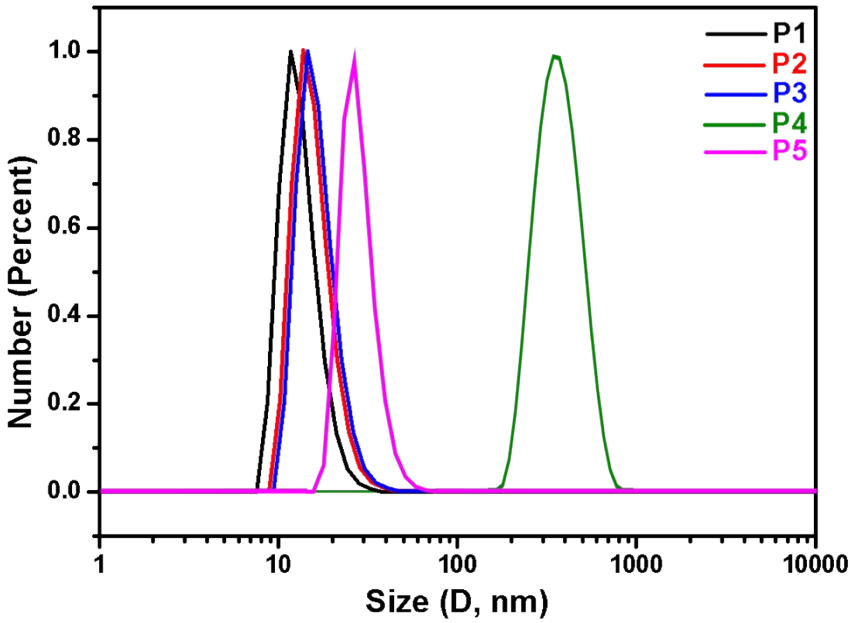

Figure S13. DLS measuraments of all glyconanoparticles.

Determination of Binding Ability of Glyconanoparticles by SPR

Interactions between the glyconaoparticles and DC-SIGN were measured using SPR in a high-throughput multichannel mode. In the concentration experiments, all glyconanoparticles were measured at different concentrations, in which the buffer was flowed over the chip alone before (90 s) and after (240 s) injection of the analyte (120 s). Buffer solution was prepared in 25 mM HEPES pH 7.4, 150 mM NaCl, 5 mM $CaCl_2$, 0.01% Tween-20. Regeneration of the sensor chip surfaces was performed using 10 mM glycine pH 2.5. Before measurements, star-shaped (5 arms) glycopolymer at different concentrations was used as control for binding. It showed higher affinities during binding with DC-SIGN because of higher mannose content.