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-bromoisoobutyryl 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

1H 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 D2O 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 NH₄BF₄ 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 °C. 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 (Rₜₜ) from Contin’s method:

\[ Rₜₜ = \frac{kₜ T}{6\pi \eta S D} \]  

(1)

kₜ is the Boltzmann constant, T is the temperature of the sample, ηₛ 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.](image)

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

![Figure S2. $^1$H NMR of the macroinitiator PEG-Br showing the appearance of the methyl peak (e) at 1.96 ppm in D$_2$O.](image)
was confirmed by comparing the ratios of the integral of CH\textsubscript{3}-O to the integral of C(CH\textsubscript{3})\textsubscript{2}Br (expected 3:6) and the integral ratios of CH\textsubscript{3}-O-CH\textsubscript{2}-R to C(CH\textsubscript{3})\textsubscript{2}Br (expected 2:6). Yield = 3.4 g (62.8%). \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 400 MHz) \(\delta\): 4.42 (m, 2H, CH\textsubscript{3}-O-CH\textsubscript{2}-R), 3.74 (b, 176H, R-O-CH\textsubscript{2}-CH\textsubscript{2}-O-R PEG repeating unit), 3.42 (s, 3H, CH\textsubscript{3}-O-R), 2.00 (s, 6H, C(CH\textsubscript{3})\textsubscript{2}Br).

**Synthesis of D-Mannose glycomonomer**

![Figure S3. Schematic representation of the synthesised D-Mannose glycomonomer.](image)

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\textsubscript{2}O (2:1 vol/vol, 60 mL), aqueous solution of CuSO\textsubscript{4}·5H\textsubscript{2}O (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%).

\textsuperscript{1}H NMR (D\textsubscript{2}O, 298 K, 400 MHz): \(\delta\) =8.07, 8.06 (s, overlaped, 1 H, NCH=C), 6.37 (dd, J=1.8, 15.5 Hz), 6.36 (dd, J=1.6, 15.7 Hz) (anomeric 1 H, CH\textsubscript{2}=C), 6.14 (dd,
$J=10.4, 6.9 \text{ Hz}$, 6.13(dd, $J=10.4, 7.0 \text{ Hz}$) (anomeric, 1 H, CH$_2$=CHC=O), 5.89 (dd, 1 H, $J=1.5, 8.9 \text{ Hz}$, CH$_2$=C), 4.70-5.05 (m, CH$_2$-OH, H-1 of mannose, overlap with H$_2$O), 4.64 (d, 1 H, $J=12.3 \text{ Hz}$, CH$_2$-OH), 4.55 (t, 2 H, $J=6.9 \text{ Hz}$, CH$_2$-N), 4.19 (t, 2 H, $J=6.0 \text{ Hz}$, C=O-O-CH$_2$), 3.40-3.92 (m, H residues of mannose), 2.30 (m, 2H, CH$_2$-CH$_2$-CH$_2$) ppm.

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

$^{13}$C NMR (D$_2$O, 298 K, 400 MHz): δ =146.4 (C=O), 145.4 (N-CH=C), 131.9 (CH$_2$=C), 129.2 (CH$_2$=C), 125.6 (N-CH=C), 100.8 ($\beta$ 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(CH$_2$-OH), 62.6 (C=O-O-CH$_2$), 60.7 (C-CH$_2$-O), 48.5 (CH$_2$-CH$_2$-CH$_2$-N), 28.5(CH$_2$-CH$_2$-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°C using a Cu(0)/Cu(II) and Me$_6$Tren 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.

<table>
<thead>
<tr>
<th>Polymerization Time</th>
<th>$M_n_{\text{GPC}}$ (g/mol)</th>
<th>$D$</th>
<th>$\rho$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st block 30 min</td>
<td>5600</td>
<td>1.11</td>
<td>0.57</td>
</tr>
<tr>
<td>1st block 45 min</td>
<td>6800</td>
<td>1.09</td>
<td>0.76</td>
</tr>
<tr>
<td>1st block 60 min</td>
<td>7450</td>
<td>1.11</td>
<td>0.87</td>
</tr>
<tr>
<td>1st block 120 min</td>
<td>7850</td>
<td>1.11</td>
<td>0.94</td>
</tr>
<tr>
<td>1st block 180 min</td>
<td>8300</td>
<td>1.11</td>
<td>0.98</td>
</tr>
<tr>
<td>2nd block 30 min.</td>
<td>9650</td>
<td>1.11</td>
<td>0.46</td>
</tr>
<tr>
<td>2nd block 60 min.</td>
<td>10100</td>
<td>1.11</td>
<td>0.62</td>
</tr>
<tr>
<td>2nd block 120 min</td>
<td>10350</td>
<td>1.11</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>--------</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>2nd block 180 min</td>
<td>10600</td>
<td>1.12</td>
<td>0.83</td>
</tr>
<tr>
<td>2nd block 360 min</td>
<td>10850</td>
<td>1.12</td>
<td>0.97</td>
</tr>
</tbody>
</table>

a) DMF eluent, PMMA standards; b) Conversion (\(\rho\)) 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), CuBr₂ (0.04 eq) and DMSO (6 ml) and the mixture was degassed by gentle bubbling of argon gas for 30 min. Pre-degassed Me₆TREN (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°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 (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. The end pure product was characterised by $^1$H NMR and GPC.

![Figure S8](image.png)

**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 25°C 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. Physicochemical properties of the glyconanoparticles.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Molar ratio of [MA] to [ManAc]</th>
<th>DLS</th>
<th>TEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$d_h$ (nm)</td>
<td>$\phi$</td>
</tr>
<tr>
<td>P1</td>
<td>1.17:1</td>
<td>22±1.2</td>
<td>0.144</td>
</tr>
<tr>
<td>P2</td>
<td>1.58:1</td>
<td>23±1.3</td>
<td>0.173</td>
</tr>
<tr>
<td>P3</td>
<td>2.00:1</td>
<td>26±1.1</td>
<td>0.221</td>
</tr>
<tr>
<td>P4</td>
<td>3.16:1</td>
<td>380±2.6</td>
<td>0.356</td>
</tr>
<tr>
<td>P5</td>
<td>2.64:1</td>
<td>65±1.6</td>
<td>0.376</td>
</tr>
</tbody>
</table>
Figure S13. DLS measurements 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) glycopolymymer at different concentrations was used as control for binding. It showed higher affinities during binding with DC-SIGN because of higher mannose content.