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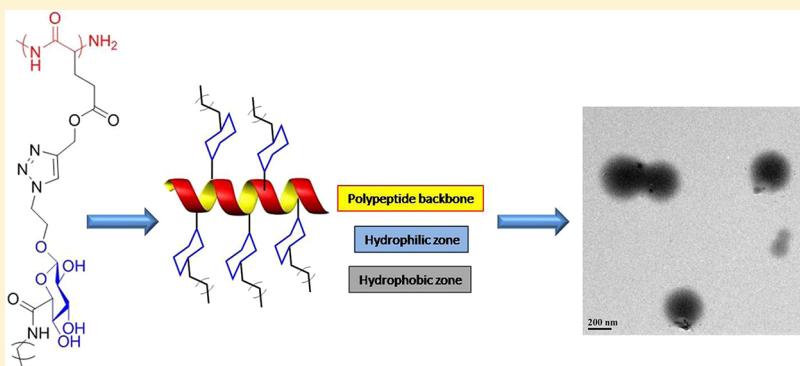
## Synthesis and Self-assembly of Amphiphilic Homoglycopolypeptide

Vinita Dhaware,<sup>†</sup> Ashif. Y. Shaikh,<sup>†</sup> Mrityunjoy Kar,<sup>†</sup> Srinivas Hotha,<sup>\*,‡</sup> and Sayam Sen Gupta<sup>\*,†</sup>

<sup>†</sup>Chemical Engineering Division, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411008

<sup>‡</sup>Department of Chemistry, Indian Institute of Science Education & Research, Pune 411008, India

### Supporting Information



**ABSTRACT:** The synthesis of the amphiphilic homoglycopolypeptide was carried out by a combination of NCA polymerization and click chemistry to yield a well-defined polypeptide having an amphiphilic carbohydrate on its side chain. The amphiphilicity of the carbohydrate was achieved by incorporation of an alkyl chain at the C-6 position of the carbohydrate thus also rendering the homoglycopolypeptide amphiphilic. The homoglycopolypeptide formed multimicellar aggregates in water above a critical concentration of  $0.9 \mu\text{M}$  due to phase separation. The multimicellar aggregates were characterized by DLS, TEM, and AFM. It is proposed that hydrophobic interactions of the aliphatic chains at the 6-position of the sugar moieties drives the assembly of these rod-like homoglycopolypeptide into large spherical aggregates. These multimicellar aggregates encapsulate both hydrophilic as well as hydrophobic dye as was confirmed by confocal microscopy. Finally, amphiphilic random polypeptides containing 10% and 20%  $\alpha$ -D-mannose in addition to glucose containing a hydrophobic alkyl chain at its 6 position were synthesized by our methodology, and these polymers were also found to assemble into spherical nanostructures. The spherical assemblies of amphiphilic random glycopolypeptides containing 10% and 20% mannose were found to be surface bioactive and were found to interact with the lectin Con-A.

### INTRODUCTION

Carbohydrates play an important role in various biological processes like signal transmission, fertilization, cell–cell recognition, inflammation, and protein folding, among others.<sup>1–3</sup> In particular, glycoproteins are seen to play a key role in these biological processes.<sup>4</sup> The increased understanding of the role carbohydrates play in biological processes has triggered the synthesis of synthetic carbohydrate-based materials, particularly, glycopolymers.<sup>2</sup> In the past decade many efforts were directed toward the synthesis of various well-defined sugar-conjugated synthetic macromolecules, so-called “glycopolymers”, which can mimic glycoproteins.<sup>5,6</sup> Since glycopolymers are typically polyvalent, they present a platform from which multiple copies of a carbohydrate can be presented simultaneously, thus enhancing their affinity and selectivity for carbohydrate binding proteins like lectins by many folds.<sup>7,8</sup> Owing to their biomimetic nature, synthetic glycopolymers have found several biomedical applications which include application in carbohydrate-based vaccines<sup>9</sup> and targeted drug delivery.<sup>10</sup> An important criteria for the applicability of these glycopolymers in biomedical applications is their biocompatibility

and biodegradability under biological condition. The majority of glycopolymers reported until recently displayed a polymer backbone made exclusively of robust carbon–carbon bond that makes them less desirable toward biomedical applications. On the other hand, synthetic glycopolypeptides<sup>11</sup> resemble natural glycoproteins and hence are expected to be more biocompatible. They are also known to fold into well-defined secondary structure (e.g.,  $\alpha$ -helix) allowing ordered display of the carbohydrate moieties. The most efficient and effective methodology to synthesize controlled well-defined polypeptides is by the ring-opening polymerization of  $\alpha$ -amino acid *N*-carboxyanhydrides (NCAs). This well developed and versatile NCA polymerization of various functional  $\alpha$ -amino acids has been extended for the synthesis of glycopolypeptides.

Two synthetic methodologies have been developed for the synthesis of glycopolypeptides: (i) direct synthetic methodology and (ii) post-synthetic grafting methodology. In direct

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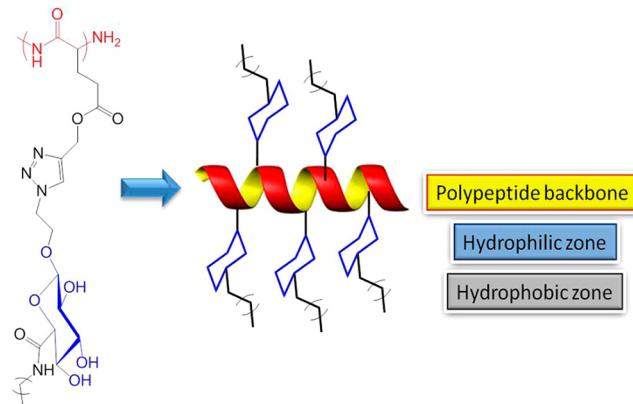


synthetic method the carbohydrates are incorporated within the monomers followed by their polymerization to yield the glycopolymers or glycopolypeptides.<sup>12–15</sup> Direct synthesis of glycopolypeptides by polymerization of glyco- $\alpha$ -amino acid NCAs has been reported by us and others. However, the synthesis of glyco amino acid NCA monomer is very challenging and requires the use of protected sugars.<sup>12,13,16</sup> In post-synthetic methodology, carbohydrate moieties are grafted into the side chains of presynthesized polypeptides by using highly efficient coupling reactions like “click chemistry”.<sup>17–21</sup> For example, Hammond et al. first described the synthesis of glycopolypeptides by attaching azide containing carbohydrates onto alkyne containing polypeptides by Cu(1) catalyzed azide–alkyne Huisgen 1,3-dipolar cycloaddition “click” reactions (CuAAC).<sup>18</sup> Recently there have been several other reports on the application of CuAAC and NCA polymerization for the synthesis of glycopolypeptides.<sup>21,22</sup> The thiol–ene coupling reaction has also been used for the synthesis of glycopolypeptides via the post-synthetic grafting method. For example, thiol–ene coupling was used by Heise group for the modification of a cysteine containing copolypeptide<sup>20</sup> while Schlaad et al. used the same methodology to couple thiol functional sugars to poly (DL-allylglycine).<sup>20</sup> Lecommandoux described the synthesis of amphiphilic block copolymers by NCA polymerization including the coupling of oligosaccharides by “click” reaction to polypeptide end-groups.<sup>23–26</sup>

For glycopolymers to be used as drug delivery vehicles and as biomaterials, it would be advantageous if these could be assembled into supramolecular nanostructures that can be tuned to appropriately display their carbohydrate moieties. Thus, amphiphilic block copolymers containing glycopolymers as one of their blocks represent an interesting motif to build self-assembled nanostructures. We have recently shown that amphiphilic glycopolypeptide-dendron conjugates can be self-assembled into micelles and nanorods by tuning their hydrophobicity and hydrophilicity.<sup>16</sup> Lecommandoux et al. have described the synthesis of amphiphilic block copolymers by NCA polymerization including the coupling of oligosaccharides by “click” reaction to polypeptide end-groups.<sup>23–26</sup> These amphiphilic block glycopolypeptides have also been shown to assemble into various nanostructures owing to the mutual incompatibility of the hydrophobic and the hydrophilic block along with its immiscibility in water. This has led to a number of interesting structures such as vesicles, micelles, and nanorods that have been exhaustively studied.<sup>12,13,15,24–27</sup> We hypothesized that it would be interesting if such amphiphilicity could be engineered on a smaller length scale (i.e., on a monomer scale) because this would allow great tunability in the supramolecular assemblies.<sup>28</sup> We conceived of achieving this by incorporating amphiphilic properties within a monomer unit (i.e., by synthesizing amphiphilic homoglycopolypeptide). Although the self-assembly properties of amphiphilic block glycopolypeptides have been reported, to the best of our knowledge there has been no report on the study of the self-assembly of amphiphilic homoglycopolypeptide. Recently, Dan et al. have reported pH responsive aggregation of amphiphilic acrylate based homoglycopolymers where the repeating unit consists of both hydrophilic and hydrophobic moieties.<sup>29</sup> Hence it would be interesting to understand the self-assembly properties of amphiphilic homoglycopolypeptides as these could help us in designing carriers for drug delivery.

We hereby report the synthesis of amphiphilic homoglycopolypeptide and its self-assembly in water. We have synthesized

amphiphilic homoglycopolypeptide based on a polypeptide backbone in which each side chain contains carbohydrate units having three hydrophilic –OH groups and one hydrophobic –C<sub>6</sub>H<sub>13</sub> group. Further, the polypeptide is expected to have a rodlike conformation, and each individual side chain which protrudes out of the rod is amphiphilic. The polypeptide was synthesized by polymerization of an alkyne substituted N-carboxyanhydride ( $\gamma$ -propargyl-L-glutamate NCA) followed by conjugation of a hydrophilic sugar moiety containing a hydrophobic alkyl chain at its 6 position by CuAAC (Figure 1). This amphiphilic homoglycopolypeptide was found to self-



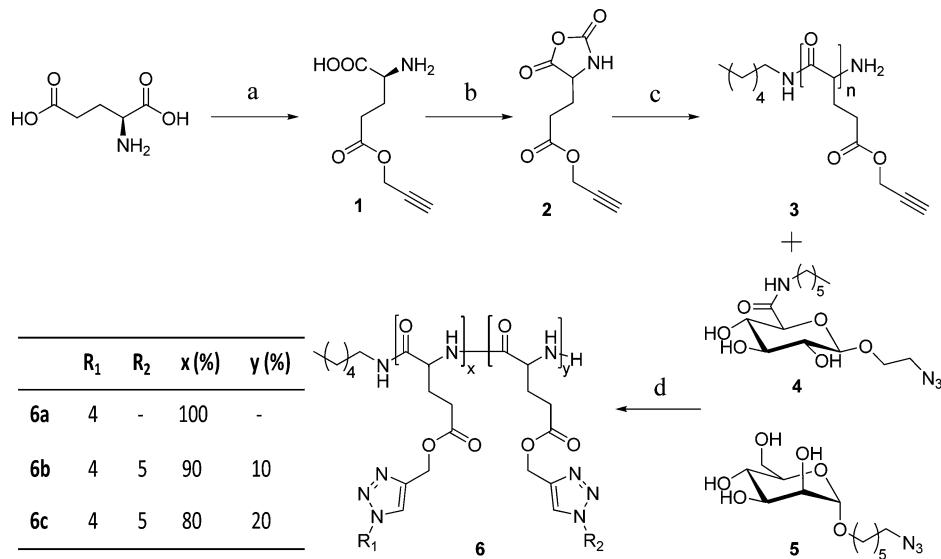
**Figure 1.** Schematic representation of amphiphilic homoglycopolypeptides.

assemble into spherical nanostructures in water. Finally, amphiphilic random polypeptides containing 10% and 20%  $\alpha$ -D-mannose in addition to glucose containing a hydrophobic alkyl chain at its 6 position were synthesized by our methodology, and these polymers were also found to assemble into spherical nanostructures. All the assemblies were characterized by TEM, AFM, and DLS. The spherical assemblies of amphiphilic random glycopolypeptides containing 10% and 20% mannose were found to be surface bioactive and were found to interact with the lectin Con-A.

## EXPERIMENTAL SECTION

**Materials.** Propargyl alcohol was obtained from Sigma Aldrich and used as received. L-Glutamic acid (puriss for biochemistry) and triphosgene were obtained from Merck, India. All other chemicals were obtained from Merck, India. Hexane and tetrahydrofuran were dried over sodium wire, and ethylacetate was dried over calcium hydride, deoxygenated, and stored in glovebox, and then used for NCA synthesis. DMF (99.99% anhydrous) obtained from Merck (Germany) was used for polymerization inside the glovebox. TLC was performed on aluminum sheets precoated with silica gel (60 F<sub>254</sub>, Merck), and spots were visualized by charring with anisaldehyde solution. Flash chromatography was carried out with silica gel 100–200 mesh (Merck), and stepwise solvent polarity gradient correlated with TLC mobility. Dialysis tubing cellulose membrane was purchased from Sigma-Aldrich.

**Synthesis.** *Synthesis of  $\gamma$ -Propargyl-L-glutamate-N-carboxy Anhydride (PLG-NCA) (2).* A 13.5 mmol portion of  $\gamma$ -propargyl-L-glutamate hydrochloride (1) was taken in a flame-dried round-bottom flask and placed under high vacuum for 1 h. Then, 4.46 mmol of triphosgene was added followed by the addition of 25 mL dry THF. The reaction solution was refluxed under slow and steady argon flux until the cloudy reaction mixture was clear. THF was removed after stirring the reaction mixture at room temperature for another 30 min. A 30 mL portion of dry ethylacetate was added, and the flask was sealed and kept at –20 °C overnight. The organic layer was washed

**Scheme 1.** Synthesis of Glycopolypeptides<sup>a</sup>

<sup>a</sup>Conditions: (a) Propargyl alcohol, sulphuric acid, 0 °C to RT; (b) triphosgene, dry THF, 60 °C; (c) n-hexylamine, DMF; (d) CuI, Na-ascorbate, PMDETA, DMSO, 60 °C.

with ice cold 0.5% NaHCO<sub>3</sub> solution until the pH was neutral, and then with 25 mL of ice-cold brine solution. The purification of NCA with ice-cold bicarbonate is preferred when the NCA formed is isolated as an oily compound. The method is used especially for the NCAs of the  $\gamma$ -esterified-L-glutamates containing 2–18 C atoms or benzyl as the R group.<sup>30</sup> The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to give PLG-NCA (2) as viscous oil (yield: 1.71 g, 60%).

**Typical Glycosylation Procedure of Poly( $\gamma$ -propargyl-L-glutamate) by Using Click Chemistry (6).** For a typical “click” reaction poly( $\gamma$ -propargyl-L-glutamate) (3) (30 mg, 0.178 mmol), (2-azidoethyl)-hexyl glucuronic amide 4 (73.905 mg, 0.214 mmol), N,N,N',N"-pentamethylenediyethylenetriamine (PMDETA) (6.17 mg, 0.035 mmol), and sodium ascorbate (17.62 mg, 0.09 mmol) were dissolved in DMSO (3 mL) in a Schlenk tube. The resultant mixture was degassed by three freeze–pump–thaw cycles, then CuI (6.76 mg, 0.0355 mmol) was added, and argon was bubbled through the resulting solution for 10 min. The Schlenk tube was placed in oil bath at 60 °C for 36 h under argon atmosphere. Then, Dowex HCR-W2 (acidic cation exchange) resin (190 mg) was added, and the suspension was gently stirred overnight at room temperature. After filtration the reaction mixture was directly transferred in a dialysis tubing (MWCO 12 kDa) and dialyzed against deionized water for three days to afford the sugar grafted polymer (6a) as a brown solid (yield: 38.5 mg).

**General Procedure for Self Assembly of Glycopolypeptides in Water.** A 1 mg/mL sample of glycopolypeptides in DMSO was filtered through 0.22  $\mu$ m nylon filters and then transferred in dialysis tubing of 3.5 kDa membrane. It was then dialyzed against 250 mL of deionized water for 12 h, then 500 mL for another 12 h, and finally with 1000 mL of water for 2 days changing the water every 4 h.

**Characterization Methods.** **FT-IR Spectroscopy.** FT-IR spectra were recorded on Perkin-Elmer FT-IR spectrum GX instrument by either making KBr pellets or as thin films between NaCl plates. Pellets were prepared by mixing 97 mg of KBr and 3 mg of sample.

**Nuclear Magnetic Resonance.** NMR spectra were recorded on Bruker spectrometer (200, 400, and 500 MHz) and JEOL 400 MHz instrument. Chemical shifts are reported relative to either internal standard tetramethylsilane or with respect to the deuterated solvents used. <sup>13</sup>C signals for sugars were assigned with the aid of DEPT, and multiplicities are given in parentheses.

**High-Resolution Mass Spectroscopy.** Mass samples were analyzed by high resolution mass spectrometry using ESI TOF (waters Synapt G2).

**Size Exclusion Chromatography.** Gel permeation chromatography/light scattering (GPC/LS) was performed on a Viskotek TDA 305–040 triple detector array refractive index (RI), viscometer (VISC), low angle light scattering (LALS), right angle light scattering (RALS) GPC/SEC module. Separations were achieved by three columns (T6000M, general mixed org 300 mm × 7.8 mm) and one guard column (tguard, org guard col 10 mm × 4.6 mm), with 0.05 M LiBr in DMF as the eluent at 60 °C. GPC/LS samples were prepared at concentrations of 5 mg/mL. A constant flow rate of 1 mL/min was maintained. System was standardized by narrow molecular weight distribution PMMA (65 kDa, PDI = 1.07) standard.

**Laser Light Scattering Measurements.** The hydrodynamic diameters of freshly prepared dilute, polymer micelles were determined by dynamic light scattering (DLS) using a Brookhaven Instruments equipped with a HeNe laser operating at 632.8 nm. The particle size was calculated using 90Plus Particle Sizing Software Ver. 3.94.

**Transmission Electron Spectroscopy.** Transmission electron microscopy (TEM) measurements were done at 100 KV on an FEI Technai F20 instrument. One drop of sample was placed on a precoated copper grid and left for 60 s. After 60 s excess sample was blotted using filter paper and dried for 24 h in a desiccator prior to imaging.

**AFM.** Topology of the micelles was investigated in the dry state with AFM-JPK instrument with nanowizard-II setup. AFM is also attached with Zeiss inverted optical microscope. A drop of sample was placed on mica surface and dried for 24 h in a desiccator prior to imaging.

**Fluorescence Emission Spectroscopy.** The critical micelle concentration (CMC) of PPLG-Glu (6a) in deionized water was determined by fluorescence spectroscopy using nile red as a probe. The fluorescence spectra were recorded on Cary Eclipse Varian fluorescence spectrophotometer. A 0.5 mg/mL sample of polymer in DMSO was filtered through 0.22  $\mu$ m filter and then dialyzed against deionized water. The final stock solution of 15.9  $\mu$ M was serially diluted to 5, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078125, 0.039, and 0.0195  $\mu$ M of 250  $\mu$ L solutions of each. A 10  $\mu$ L portion of Nile red (0.8 mg/mL in DMSO, 2.52 mM) was added to each of the polymer solutions having different concentrations. The emission spectra were scanned from 540 to 800 nm at an excitation wavelength of 530 nm.

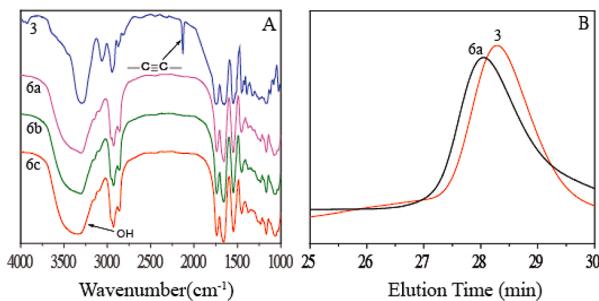
**Calcein Dye Incubation.** Calcein solution (500  $\mu$ L, 1 mg/mL in H<sub>2</sub>O) was added to a solution of PPLG-Glu (**6a**; 2 mg/mL, 500  $\mu$ L in DMSO) and stirred overnight. This was then directly transferred to dialysis membrane (MWCO 12 kDa) and dialyzed against deionised water for 4 days.

**Confocal Imaging.** The dye incorporated samples were directly spotted on glass slides, air-dried, and imaged using LSM 710 Carl Zeiss laser scanning confocal microscope.

**Recognition of Mannose Residues with Lectins.** Con A binding interactions with glycopeptides were studied in 100 mM PBS buffer pH 7.2 containing 0.1 mM MnCl<sub>2</sub>, 0.1 mM CaCl<sub>2</sub>, and 0.1 M NaCl. Con A was dissolved at 0.5 mg/mL and sterile filtered. Turbidimetry measurements were carried out by taking 200  $\mu$ L of Con A solution in a dry quartz cuvette (350  $\mu$ L, 1 cm path length). A 10  $\mu$ L portion of functionalized polymer solution (1 mg/mL) was added gradually. Upon addition, the solution was mixed vigorously for 10 s in the cuvette using a micropipet, and then absorbance was recorded at 490 nm on JASCO V-570 UV-vis spectrophotometer using 10 mm quartz cuvettes at 25 °C.

## RESULTS AND DISCUSSIONS

**Synthesis and Characterization of Glycosylated Amphiphilic Homoglycopolypeptides.** Poly- $\gamma$ -propargyl-L-glutamate (PPLG) was synthesized following a procedure reported by Chen et al.<sup>21</sup> PPLG was prepared by the ring-opening polymerization of  $\gamma$ -propargyl-L-glutamate NCA in dimethylformamide (DMF) at room temperature using hexylamine as the initiator (Scheme 1). The degree of polymerization ( $M_n$  9400 and DP = 56) was calculated from the <sup>1</sup>H NMR spectra of the polymer from the ratio of the relative intensities of the –CH<sub>3</sub> peak of hexyl amine group at 0.83 ppm denoted as (a) to the characteristic methylene protons of glutamic acid at 4.67 ppm denoted as (g) (Figure 3A). The molecular weight distribution ( $M_w/M_n$ ) was found to be 1.27 from gel permeation chromatography (Figure 2B).



**Figure 2.** (A) FT-IR spectra of (3) PPLG, (6a) PPLG-Glu, (6b) PPLG-Glu-90-Man-10, and (6c) PPLG-Glu-80-Man-20. (B) Size exclusion chromatogram of (3) PPLG, (6a) PPLG-Glu.

Grafting of PPLG with (2-azidoethyl)-hexyl glucuronic amide (**4**) was carried out using click chemistry, with a molar ratio of alkyne/azide 1/1.2. The azido derivative of glucuronic acid was synthesized following modification of a reported procedure.<sup>31</sup> The C-6 position of glucuronic can be easily modified with different amines. The commercially available D-glucuronic acid was acetylated in the presence of iodine followed by treatment with hexyl amine to give hexyl-1,2,3,4-tetra-O-acetyl glucuronic amide. This was Fischer glycosidated using BF<sub>3</sub>·Et<sub>2</sub>O as catalyst with 2-bromoethanol at 0 °C which gives (2-bromoethyl)-hexyl-2,3,4-tri-O-acetyl glucuronic amide. Treatment of bromo derivative with NaN<sub>3</sub> in DMF at 70 °C afforded (2-azidoethyl)-hexyl-2,3,4-tri-O-acetyl glucuronic amide. The deprotection was carried out using Zemplén

condition (catalytic amount of sodium methoxide in methanol) to give (2-azidoethyl)-hexyl glucuronic amide (Supporting Information, Scheme S1). The progress of click reaction was monitored by FT-IR spectroscopy by observing the complete disappearance of signal at 2130 cm<sup>-1</sup> characteristic of alkyne stretch and appearance of broad peak of the –OH group at 3430 cm<sup>-1</sup> due to the presence of the sugars (Figure 2A). After completion of reaction ion-exchange resin was added to remove Cu, and then the solution was dialyzed against deionized water to purify the polymer. The successful click conjugation of the sugar azides with nearly quantitative yields was further confirmed by <sup>1</sup>H NMR spectroscopy (Figure 3A,B). <sup>1</sup>H NMR reveals presence of new resonances from the sugar in addition to the formation of the triazole ring (denoted as j) resonating at 8.2 ppm. Further, the shift of the ester peak at 4.67 ppm denoted as (g) to 5.1 ppm (which overlaps with the signals of the OH group of the sugar) in addition to the complete disappearance of the alkyne peak at 3.2 ppm denotes successful completion of the click reaction. To quantify the extent of click reaction the peak at 1.96 ppm (e) from the –(CONH)CH-CH<sub>2</sub>– group of the glutamic acid was selected since it does not overlap with the other peaks from the sugar moieties. The relative intensity of (e) with respect to the anomeric proton (k) of the glucuronic at 4.3 ppm confirms that almost 100% of the sugar was clicked (Figure 3A,B).

We also synthesized random copolymers containing both hexylglucuronic amides and  $\alpha$ -D-mannose so that these polymers would be able to bind to the  $\alpha$ -D-mannose binding lectin concanavalin-A (since the hexyl  $\beta$ -glucoronic amide moieties are not expected to bind to concanavalin-A). Two random copolymers were synthesized having a ratio of hexylglucuronic amides to  $\alpha$ -D-mannose of 9:1 and 8:2. The synthesis of these polymers was carried out by reaction of PPLG with a mixture of (2-azidoethyl)-hexyl glucuronic amide and (6-azidohexyl)- $\alpha$ -D-mannose using CuAAC as has been described before. The total amount of the azido sugars used (9:1 and 8:2) was 1.2 equiv of the total number of alkyne groups present in the parent PPLG polypeptide. The polymers formed after click reaction (**6b** and **6c**) were analyzed by <sup>1</sup>H NMR to investigate the incorporation of both the carbohydrate moieties. The anomeric protons of both glucuronic acid (k) and mannose (m) were well differentiated (appeared at 4.3 ppm and 4.7 ppm), and this allowed us to estimate their relative incorporation into PPLG (Figure 3A–C). From <sup>1</sup>H NMR, the ratio of hexylglucuronic amides and  $\alpha$ -D-mannose was determined to be 9:1 and 8:2 in PPLG-Glu-90-Man-10 (**6b**) (Figure 3C) and PPLG-Glu-80-Man-20 (**6c**), respectively (SI, Figure S12).

**Assembly Studies.** Assembly of the amphiphilic homoglycopolypeptide **6a** was attempted by slow exchange of a solution of **6a** in DMSO with water by dialysis (**6a** is very poorly soluble in water). We envisaged that if a polymer solution in DMSO is dialyzed against deionized water, it might lead to the clustering of the hydrophobic segment by phase separation which in turn would drive the self-assembly. Hence, a 1 mg/mL of glycopolypeptide in DMSO was dialyzed against deionized water for four days. The appearance of a turbid solution without any precipitation indicated formation of assembled structures. In order to probe the nature of these assemblies, this solution was subjected to transmission electron microscopy analysis. TEM analysis revealed the formation of large micellar aggregates with an average diameter of 250–450 nm. The hydrodynamic diameter of the micellar aggregates was

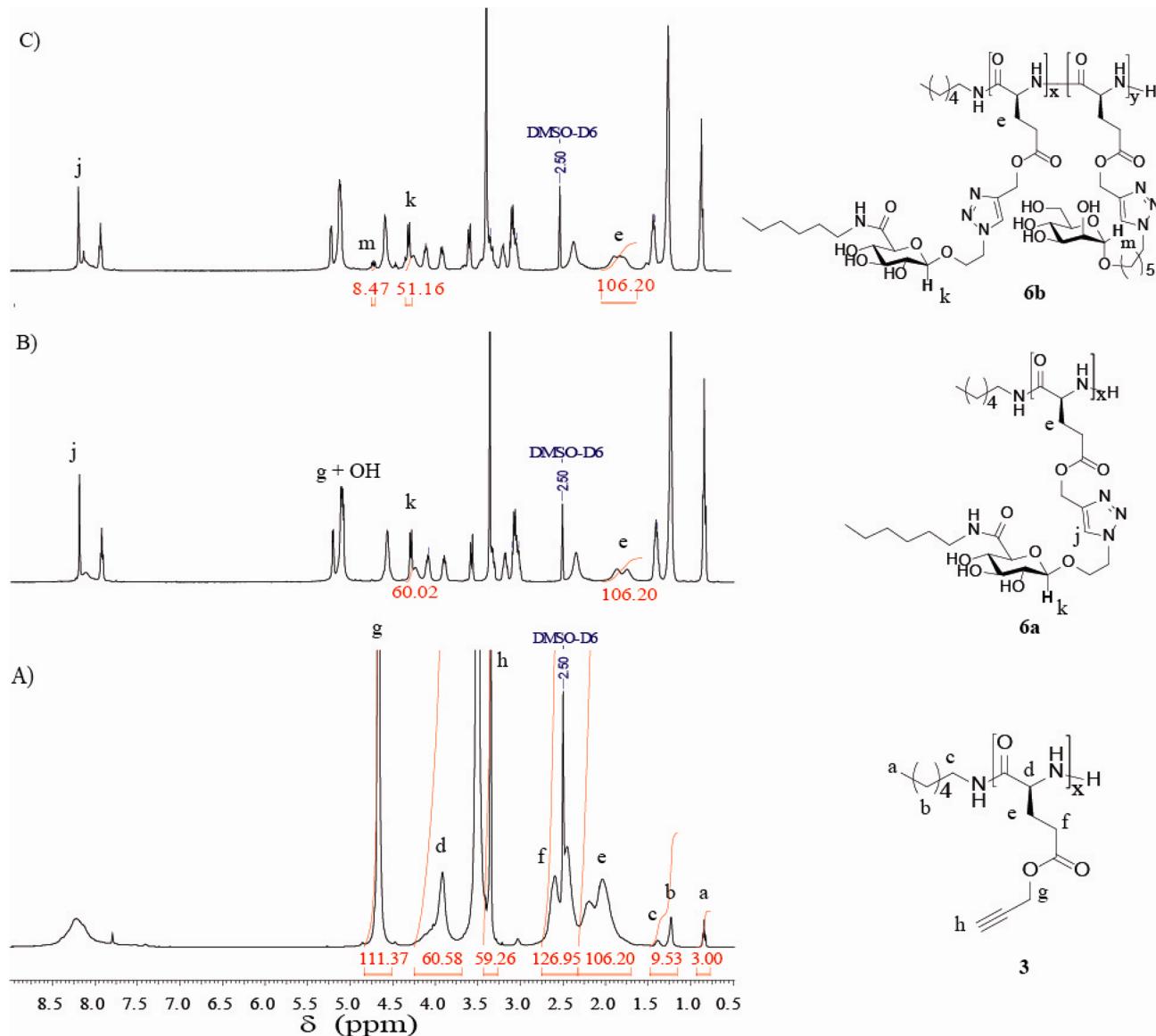
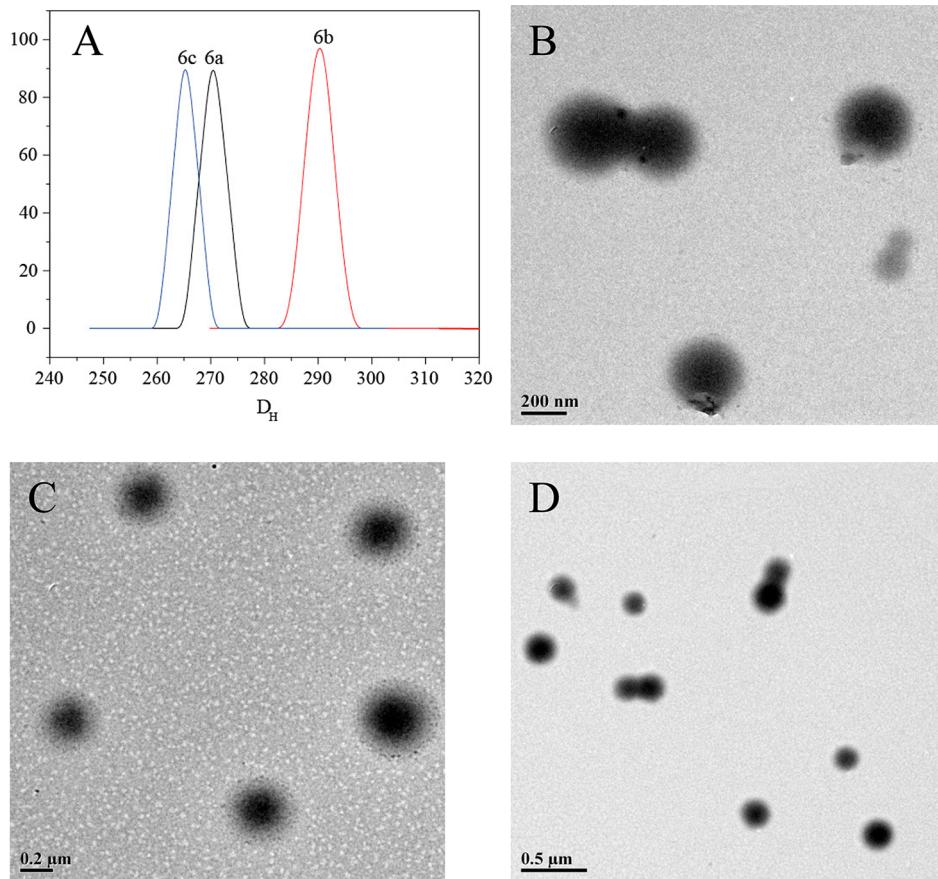


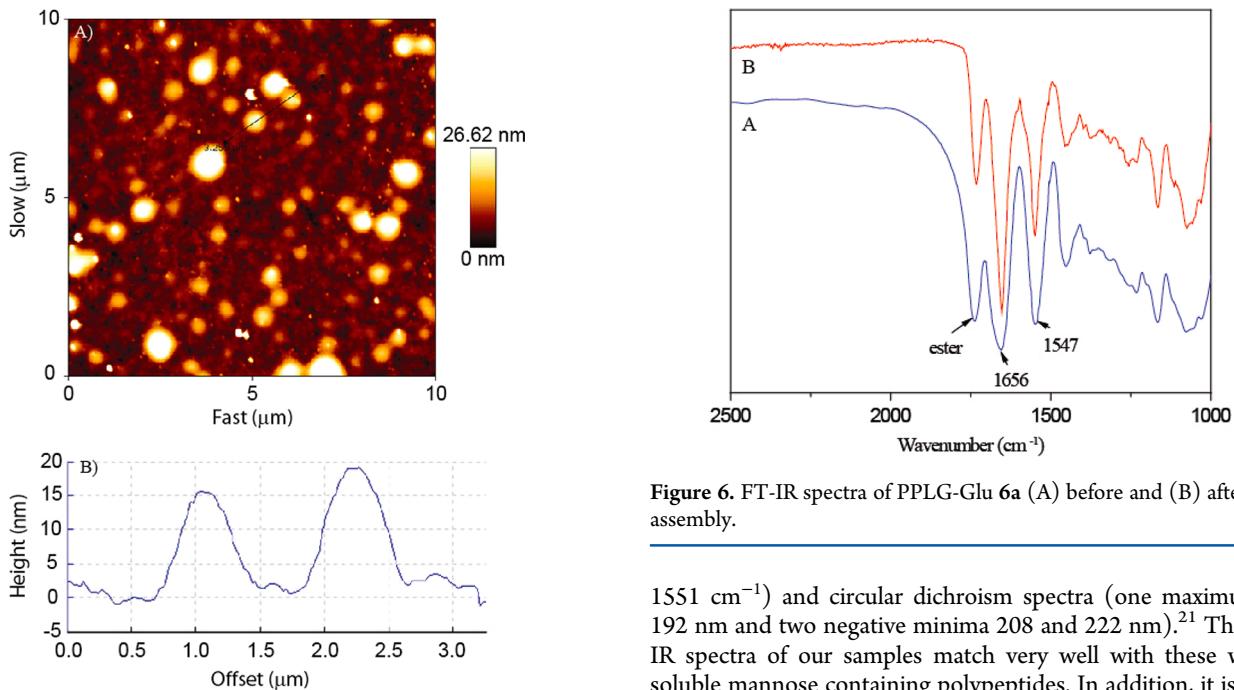
Figure 3. NMR spectra of (A) PPLG (3), (B) PPLG-Glu (6a), (C) PPLG-90-Glu-Man-10 (6b).

determined from dynamic light scattering (DLS) measurements that were performed immediately after dialysis. DLS measurements (Figure 4A) showed that these large micellar aggregates had diameter between 260 and 275 nm (PDI: 0.118) which is smaller than that obtained from TEM (Figure 4B; Figure S2). The higher values for the nanoparticle diameter observed in TEM are probably due to aggregation of the nanoparticles upon drying. To further probe the exact nature of these assemblies, AFM analysis of these assemblies was carried out. AFM analysis also showed spherical aggregates, and height was determined to be around 20 nm (Figure 5A,B). The higher height-to-width ratio (>1:15) is a result of the removal of water from the micellar aggregates during preparation of the samples. Such high height-width ratio has also been observed for nanogels and vesicles.<sup>32,33</sup> Similarly, PPLG-Glu-90-Man-10 (6b) and PPLG-Glu-80-Man-20 (6c) were also found to self-assemble into large micellar aggregates with average diameter of 250–450 nm (Figure 4C,D). The large size and absence of hollow structures indicate that these are possibly multimicellar aggregates.

To probe the nature of the aggregates further, FT-IR studies and dye encapsulation experiments were performed. FT-IR of glycopolypeptides can provide valuable information on the secondary structures. FT-IR of the glycopolypeptide in the solid state (KBr pellet) displayed the amide I and II bands at 1656 and 1547  $\text{cm}^{-1}$ , respectively (Figure 6A). Amide bands in this region are indicative of either an  $\alpha$ -helix or a random coil conformation.<sup>34</sup> However, we were unable to differentiate between these two conformations by CD since 6a was only soluble in DMSO. We were also unable to record the circular dichroism spectra of the nanostructures formed from the self-assembly of 6a in water since the solutions were turbid and this resulted in severe back scattering. Hence, to understand the secondary structure of the polypeptide in the assemblies, we resorted to recording the FT-IR of the assembly after air drying the micellar assembly on a Zn–Se film (Figure 6B). The FT-IR spectrum of the micellar assembly was exactly similar to spectra of the polypeptide in the solid state (Figure 6). It should be noted that the water-soluble fully mannose containing glycopolypeptide synthesized by reaction of PPLG with (2-azidoethyl)- $\alpha$ -D-mannose using CuAAC has been reported to

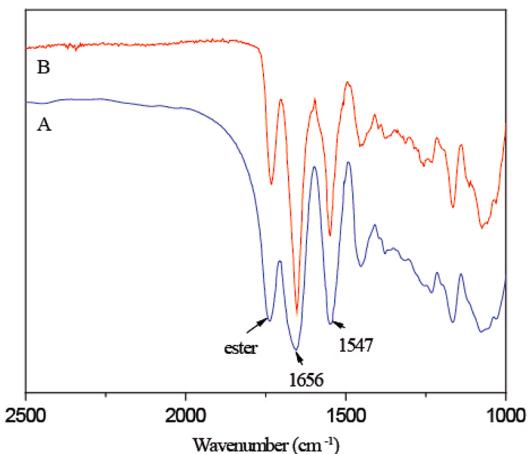


**Figure 4.** (A) DLS size distribution of PPLG-Glu (**6a**), PPLG-Glu-90-Man-10 (**6b**), PPLG-Glu-80-Man-20 (**6c**). TEM images of (B) PPLG-Glu (**6a**), (C) PPLG-Glu-90-Man-10 (**6b**), (D) PPLG-Glu-80-Man-20 (**6c**).



**Figure 5.** (A) AFM images of PPLG-Glu (**6a**). (B) Section profile of the AFM image along the black line.

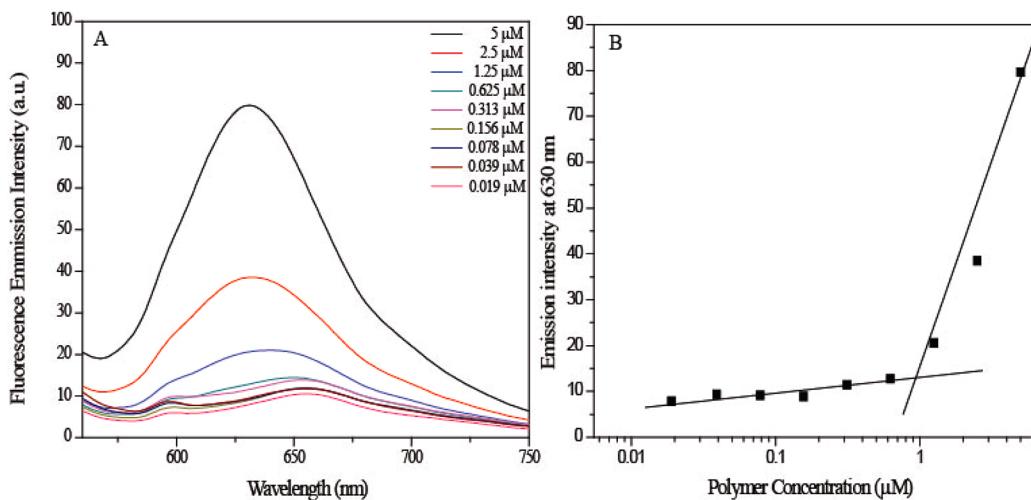
have an  $\alpha$ -helical conformation in water as was observed by both solid state FT-IR (amide I and amide II bands at 1655 and



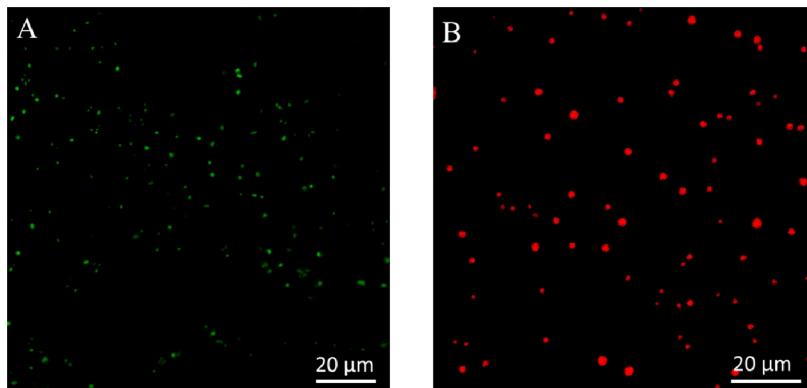
**Figure 6.** FT-IR spectra of PPLG-Glu **6a** (A) before and (B) after self-assembly.

1551  $\text{cm}^{-1}$ ) and circular dichroism spectra (one maximum at 192 nm and two negative minima 208 and 222 nm).<sup>21</sup> The FT-IR spectra of our samples match very well with these water-soluble mannose containing polypeptides. In addition, it is well-known that PPLG backbone of this polypeptide imparts helical nature. We therefore believe that, in the absence of any charged side chain, **6a** has an  $\alpha$ -helical conformation in both the solid state and in the multimicellar assembly.

These large multimicellar aggregates are expected to have both hydrophobic and hydrophilic domains. Hence, incorpo-



**Figure 7.** (A) Variation of the emission spectra with different concentration of **6a**. (B) Plot of the emission intensity versus polymer concentration ( $\mu\text{M}$ ).



**Figure 8.** Confocal microscope image of PPLG-Glu (**6a**) nanoassembly encapsulated with (A) calcein and (B) nile red.

ration of a hydrophilic dye calcein and hydrophobic dye nile red into the multimicellar aggregate was probed. A solution of calcein (1 mg/mL) was incubated with a solution of **6a** (2 mg/mL) in DMSO and then was dialyzed against deionized water for three days (MWCO 12 kDa) to ensure removal of excess calcein. For the encapsulation of nile red, a solution of nile red (0.8 mg/mL) in DMSO was added to the preformed multimicellar aggregate of **6a** (1 mg/mL) and allowed to equilibrate for 1 h. The absorbance and emission spectra for calcein were recorded (SI, Figure S3). The calcein incorporated samples were found to be fluorescent, and calcein emission was observed at 510 nm indicating that it was incorporated into the hydrophilic domains of the multimicellar aggregates. For nile red encapsulated samples, an emission at 630 nm was observed indicating that nile red was incorporated into hydrophobic domains in the multimicellar aggregates. Since emission of nile red is strongly influenced by solvent polarity, this was used to calculate the apparent critical aggregation concentration values of **6a**. Solutions of different concentrations of the assemblies of **6a** in water were treated with nile red (2.52 mM) and equilibrated for half an hour to allow the dye to get encapsulated in the hydrophobic pocket of the micellar aggregates. From the plot of fluorescence emission intensity versus wavelength (Figure 7A), it was observed that excitation of the hydrophobic nile red at 530 nm results in a relatively small intensity peak at  $I_{\max}$  660 nm suggesting the dye is in

hydrophilic environment. However, with increasing polymer concentration a pronounced blue shift with increase in the emission intensity by 20-fold at  $I_{\max}$  630 nm reveals the sequestration of the dye in the hydrophobic pocket of the spherical aggregates. A plot of emission intensity at 630 nm versus polymer concentration (Figure 7B) gives a nonlinear relationship suggesting the formation of aggregates. The inflection point observed corresponds to the apparent critical aggregation concentration of 0.9  $\mu\text{M}$ . Finally, the encapsulation of both calcein and nile red was further confirmed by confocal microscope image as is seen below (Figure 8).

We believe that self-assembly of this amphiphilic homoglycopolypeptide is driven by the hydrophobic interactions of the aliphatic chains at the 6-position of the sugar moieties. Amphiphilic rods that are around 9 nm in length (60 units; 0.15 nm/unit for  $\alpha$ -helix) have both the  $-\text{OH}$  groups and the hexyl chain protruding out of the backbone making them amphiphilic. Exchange of DMSO by water during dialysis leads to clustering of the hexyl chains from adjacent rods to form micellar structures. The likely mechanism is that the individual small micelles are not stable enough due to incomplete phase separation (which is common for amphiphilic homopolymers),<sup>28</sup> and therefore to stabilize the curvature as water content increases they continue to aggregate till a stable size is reached, 275–300 nm. As water content increases these micelles cluster to form multimicellar aggregates having both

hydrophobic domains formed due to clustering of the hexyl chains and hydrophilic domains due to the water interacting with the  $-OH$  groups of the sugar molecules. This also leads to encapsulation of both hydrophilic and hydrophobic dyes in these multimicellar aggregates.

**Interaction with Lectins.** Carbohydrates play a major role in biological recognition events mediated by the specific carbohydrate-lectin interaction.<sup>1–3</sup> The low affinity and broad specificity of the monovalent carbohydrate interaction is very well-known. In biological systems these events are, therefore, amplified by the multivalency of the carbohydrates referred as the glycoside-cluster effect.<sup>35</sup> Con A is a tetrameric protein that specifically recognizes the D-(+)-glucopyranoside and D-(+)-mannopyranoside residues with free 3-, 4-, and 6-hydroxyl groups. Hence, the carbohydrates displayed on the surface of the multimicellar aggregates formed after self-assembly are expected to interact with the carbohydrate binding site of the tetrameric Con A. Therefore, the biorecognition of the multimicellar aggregates formed from self-assembly of **6a–c** with the lectin Con A was assessed by the turbidimetry assay.<sup>36</sup> Figure 9 represents the turbidity variation in the interaction of

domain as was evidenced by the incorporation of hydrophobic and hydrophilic dyes like calcein and nile red. Amphiphilic random polypeptides containing 10% and 20%  $\alpha$ -D-mannose in addition to the amphiphilic glucose unit containing the hydrophobic alkyl chain were also synthesized by our methodology, and these polymers were also found to assemble into spherical nanostructures similar to the amphiphilic homoglycopolyptide. The spherical assemblies of amphiphilic random glycopolyptides containing 10% and 20% mannose were found to interact with the lectin Con-A. The presence of both hydrophobic and hydrophilic domain together with their bioactive surface seems to provide an opportunity to encapsulate drugs inside these multimicellar clusters for targeted drug delivery. Such efforts seem especially promising for future investigations.

## ■ ASSOCIATED CONTENT

### S Supporting Information

Experimental procedures and spectral data for all the synthesized compounds. SEC, TEM histogram, and absorbance and emission spectra for calcein encapsulation. This material is available free of charge via Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*E-mail: ss.sengupta@ncl.res.in (S.S.G.).

### Notes

The authors declare no competing financial interest.

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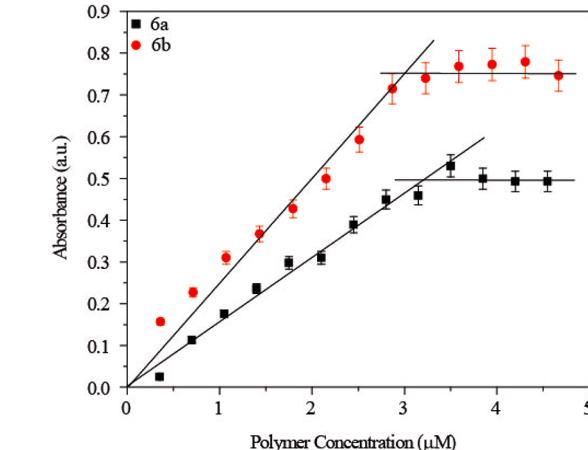


Figure 9. Turbidimetric variation of Con A as a function of glycopolymer concentration.

Con A with the multimicellar aggregates as a function of the glycopolyptide concentration. For **6b** and **6c** the turbidity increases initially with increase in the polymer concentration and then finally reaches a plateau. This point can be considered as the optimum value for the binding of the glycoconjugates and ConA since on further increasing the polymer concentration the turbidity does not increase. Turbidimetric experiments with **6a** did not show any binding to Con A since the presence of  $\beta$ -glucose will inhibit it from binding to the lectin Con-A.

## ■ CONCLUSION

We have successfully synthesized amphiphilic homoglycopolyptide by a combination of NCA polymerization and “click chemistry”. Each side-chain carbohydrate moiety in the rodlike poly-L-glutamate backbone is amphiphilic resulting from the presence of multiple  $-OH$  groups and a  $C_6H_{13}$  alkyl chain. This amphiphilic polypeptide self-assembles in water to form multimicellar clusters with diameters between 250 and 300 nm as was observed by TEM, AFM, and DLS. The helicity of the polypeptide is retained in the multimicellar clusters. These multimicellar clusters had both hydrophobic and hydrophilic

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