# Synthesis of Amphiphilic Block Copolymer consisting of Glycopolymer and Poly(L-lactide) and Preparation of Sugar-coated Polymer Aggregates

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**ABSTRACT**: The block glycopolymer, poly(2-(α-D-mannopyranosyloxy)ethyl methacrylate)-*b*-poly(L-lactide) (PManEMA-*b*-PLLA), was synthesized via a coupling approach. PLLA having an ethynyl group was successfully synthesized via ring-opening polymerization using 2-propyn-1-ol as an initiator. The ethynyl functionality of the resulting polymer was confirmed by MALDI-TOF mass spectroscopy. In contrast, PManEMA having an azide group was prepared via AGET ATRP using 2-azidopropyl 2-bromo-2-methylpropanoate as an initiator. The azide functionality of the resulting polymer was confirmed by IR spectroscopy. The Cu(I)-catalyzed 1,3-dipolar cycloaddition between PLLA and PManEMA was performed to afford PManEMA-*b*-PLLA. The block structure was confirmed by $^1$H NMR spectroscopy and size exclusion chromatography. The aggregating properties of the block glycopolymer, $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$ ($M_{n,PManEMA} = 16{,}000$, $M_{n,PLLA} = 6400$) was examined by $^1$H NMR spectroscopy, fluorometry using pyrene, and dynamic light scattering. The block glycopolymer formed complicated aggregates at concentrations above 21 mg·L$^{-1}$ in water. The D-mannose presenting property of the aggregates was also characterized by turbidimetric assay using concanavalin A. © 2016 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2016**, *00*, 000–000

**KEYWORDS**: atom transfer radical polymerization (ATRP); block copolymers; ring-opening polymerization

**INTRODUCTION** Polymer micelles are promising vehicles for drug delivery system (DDS).[1–3] Amphiphilic block copolymers form nanoparticles with core-shell type microstructures. Polyethylene glycol (PEG) is the *de facto* standard, as a non-ionic hydrophilic segment used in various types of amphiphilic polymers.[4] The PEG segment affords not only steric stabilization of nanoparticles but also stealth behavior from the reticuloendothelial system to give prolonged blood circulation. However, some drawbacks and limitations of PEG have been recognized in DDS applications.[5] The PEG segment dramatically decreases the interaction with blood components. However, an immunological response to PEG segments is frequently recognized, and the mechanism is not fully understood. Non-biodegradability, a lack of homing ability, and interference in cellular uptake are also drawbacks of PEG segments in DDS applications. Therefore, PEG alternatives have been intensively explored by many researchers.

Synthetic polymers bearing carbohydrates on the side chain are known as glycopolymers, which are an important class of biomimetic materials.[6–9] Recently, glycopolymers have been examined as a hydrophilic segment of amphiphilic block copolymers for DDS applications.[9–11] Thanks to the recent development of a controlled radical polymerization (CRP) technique, various block vinyl glycopolymers having hydrophobic,[12–23] hydrophilic,[24–28] cationic,[29–41] thermosensitive,[42,43] and other functional[44,45] segments have been synthesized by a chain extension strategy in atom transfer radical polymerization (ATRP)[14,20,22,23,28,36,38] and reversible addition and fragmentation chain transfer (RAFT) polymerization.[12,13,15–19,21,24–35,37,39–45] For example, Su et al. synthesized polystyrene-*b*-poly(4-(α-D-mannopyranosyloxymethyl)styrene) via RAFT polymerization, followed by the deprotection of the carbohydrate moieties.[17] Their block glycopolymers formed micelles, with glycopolymer segments located on their outer surfaces (called glyco-outside micelles).

In contrast, block glycopolymers consisting of non-vinyl polymers, such as biodegradable polyester or polyamide segments, are relatively rare. In the synthesis of such block glycopolymers, the synthetic strategies are roughly divided into three categories: (1) macromolecular initiator or chain transfer agent (CTA) approach; (2) (orthogonal) bifunctional

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initiator for CRP and ring-opening polymerization (ROP) approach, and (3) polymer-coupling approach. Block glycopolymers having PEG,[46,47] polypeptide,[48] poly(ε-caprolactone),[49–52] and poly(ethylene-*alt*-propylene)[53] segments were synthesized by the first approach. In this approach, an initiating functionality for ATRP or a CTA functionality for RAFT polymerization is introduced to the chain end. However, it is frequently difficult or impossible to confirm and quantify the extent of post-polymerization modification by conventional spectroscopic and chromatographic techniques, due to very low concentrations of the end group. The second approach is a variation on the first approach, but has the advantage of being able to eliminate post-polymerization modification to install an initiator or CTA functionality. This approach used molecules having two functionalities to initiate two different types of polymerizations, such as CRP and ROP. Ideally, these two functional groups do not interfere in either polymerization; this feature is known as "orthogonal." If one functionality interferes with the other, one side may be inactivated by protecting groups in the first polymerization. Block glycopolymers having poly(L-lactide) (PLLA),[54,55] poly(ε-caprolactone),[56] and polystyrene[57] were synthesized by using this strategy. For example, Ganda et al. synthesized block glycopolymers having poly(ε-caprolactone) by using benzyl 2-hydroxyethyl carbonotrithioate as a bifunctional initiator.[56] The first and second approaches consist of CRP followed by ROP. Hence, the polymer synthesized in the first polymerization must dissolve in the second polymerization medium and must not interfere with the second polymerization. These requirements are sometimes difficult to satisfy in the synthesis of block glycopolymers consisting of two segments having very different solubilities, such as unprotected glycopolymer and polyester or polyamide. Even though the protected glycopolymer may have solubility similar to those of polyether or polyamide, the deprotection of the glycopolymer can degrade the biodegradable segments.

The third approach is usually very inefficient because of the low concentration of polymer end groups. However, recent development of a highly efficient and selective coupling methodology, known as "click chemistry," opens this route to the synthesis of block glycopolymers. For example, Glassner et al. synthesized PEG-*b*-poly(3-*O*-acryloyl-D-glucose) by hetero Diels-Alder between cyclopentene-functionalized PEG and poly(3-*O*-acryloyl-D-glucose) having a 2-pyridylthiocarboylthio group at the terminating end, prepared by RAFT polymerization.[58] Their coupling reaction was ultra-fast (the coupling was completed within 15 min) and catalyst-free. In this article, an amphiphilic block glycopolymer consisting of PLLA as a hydrophobic segment and poly(2-(α-D-mannopyranosyloxy)ethyl methacrylate) (PManEMA) was synthesized by a polymer-coupling approach using Cu(I)-catalyzed 1,3-dipolar cycloaddition. In addition, the micellar formation and lectin-binding ability of the resulting block glycopolymer were examined.

## EXPERIMENTAL

### Materials and Analytical Techniques

All chemicals were of analytical grade. L-Lactide was purchased from Tokyo Chemical Industry (Tokyo, Japan) and recrystallized from toluene just before use. 2-Propyn-1-ol was purchased from Tokyo Chemical Industry and distilled under reduced pressure just before use. Tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) was purchased from Wako Pure Chemical Industries, (Osaka, Japan) and used as received. 2,5-Dihydroxybenzoic acid (DHBA) and polyethylene glycol monomethyl ether (MPEG) ($M_n = 2000$) were purchased from Sigma-Aldrich (MO) and used as received. 2-(α-D-Mannopyranosyloxy)ethyl methacrylate (ManEMA) was prepared according to our previous paper.[59] $^1$H and $^{13}$C{$^1$H} NMR spectra were recorded using an AVANCE III HD instrument (500 MHz; Bruker Biospin K.K., Yokohama, Japan). IR spectra were recorded on an IRAffinity-1S instrument (Shimadzu, Kyoto, Japan) using a KBr pellet for PManEMA or a NaCl disk for 2-azidopropyl 2-bromo-2-methylpropanoate. Size exclusion chromatography (SEC) was performed with an HPLC system (pump, LC-20AT; refractive index detector, RID-10A, Shimadzu Co.) using Styragel HR4 (7.8 × 300 mm) (Waters, MA), Styragel HR3 (7.8 × 300 mm), and Styragel HR1 (7.8 × 300 mm) columns as a stationary phase and *N*,*N*-dimethylformamide (DMF) containing 50 mM LiBr as a mobile phase at a flow rate of 0.5 mL min$^{-1}$. The SEC system was calibrated with 5 poly(methyl methacrylate) (PMMA) standards (Showa Denko, K.K., Tokyo, Japan) ranging in molecular weight from 2.89 to 202 kg·mol$^{-1}$. The number average molecular weights ($M_n$s) and polydispersities ($M_w/M_n$s) were calculated by PMMA calibration. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded on a Voyager-DE$^{TM}$ PRO (Applied Biosystems, CA) operated in reflection mode with a 20 kV acceleration voltage. Sample solution for the MALDI-TOF mass spectrometry were prepared by mixing 5 μL of tetrahydrofuran (THF) solution of polymer (10 mg·mL$^{-1}$) and 5 μL of THF solution of DHBA (30 mg·mL$^{-1}$). MPEG ($M_n = 2000$) was used as an internal standard. The size distribution of block glycopolymer aggregates was determined by dynamic light scattering measurements (UPA-UT151; NIKKISO, Tokyo, Japan).

### 2-Azidopropyl 2-Bromo-2-Methylpropanoate

The initiator, 2-azidopropyl 2-bromo-2-methylpropanoate, was prepared according to the literature.[60] Briefly, 3-bromo-1-propanol (6.21 g, 45 mmol), $NaN_3$ (5.05 g, 78 mmol), and water (80 mL) were placed into a 200 mL flask equipped with a thermometer and a condenser. The solution was stirred at 80 °C for 30 h. After the solution was cooled down, it was concentrated to a volume of approximately 30 mL under reduced pressure. The solution was saturated with NaCl, and then extracted with toluene (200 mL × 4). After the extract was dried over anhydrous $Na_2SO_4$, the toluene solution was concentrated to a volume of approximately 30 mL under reduced pressure to give a toluene solution (31.5 g) of 3-azido-1-propanol. The concentration of 3-azido-1-propanol was determined to be 9.2 wt % by $^1$H NMR spectroscopy.

The toluene solution of 3-azido-1-propanol (9.2 wt %, 7.70 g, 7.0 mmol) and triethylamine (1.10 g, 10 mmol) were

placed into a 50 mL flask, which was sealed tightly with a rubber septum. The solution was cooled in an ice bath, and then 2-bromopropionyl bromide (1.2 mL, 11 mmol) was added dropwise. The solution was stirred at ambient temperature overnight. To the solution was added a portion of water to quench the reaction. The resulting mixture was extracted with toluene (100 mL) and washed with 1 M NaOH (aq.), 1 M HCl (aq.), sat. $NaHCO_3$ (aq.), water, and brine. After drying over anhydrous $Na_2SO_4$, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexane = 2/8, v/v), followed by preparative gel permeation chromatography to give a colorless oil. Yield, 96.7 mg (14%). $^1$H NMR (500.1 MHz, $CDCl_3$, $Si(CH_3)_4 = 0$ ppm): $\delta$ (ppm) = 4.28 (t, $^3J = 6.0$ Hz, 2H, $CH_2$—O—), 3.45 (t, $^3J = 6.8$ Hz, 2H, $CH_2$—$N_3$) 1.97 (m, 2H, $CH_2$), 1.94 (s, 6H, $CH_3$). $^{13}$C{$^1$H} NMR (125.8 MHz, $CDCl_3$, $CDCl_3 = 77$ ppm): $\delta$ (ppm) = 171.5 (C═O), 62.7 ($CH_2$—O), 55.6 (C), 48.0 ($CH_2$), 30.7 ($CH_3$), 27.9 ($CH_2$—$N_3$). IR (NaCl disk): $\nu_{max}$ ($cm^{-1}$) = 3200–2850 (br), 2100 (s, $N_3$), 1736 (s, C═O).

### Synthesis of PLLA Bearing an Ethynyl Group at the Initiating End

A typical procedure for the ring-opening polymerization of L-lactide was as follows: L-Lactide (1.02 g, 7.0 mmol), 2-propyn-1-ol (10.2 mg, 0.18 mmol), $Sn(Oct)_2$ (72 mg, 0.18 mmol), and dry toluene (5 mL) were placed into a 10 mL flask, which was sealed tightly with a rubber septum. The flask was immersed in an oil bath preheated at 90 °C, and stirred for 20 h. After the resulting mixture was cooled down, a small amount of the polymerization mixture was taken to determine the monomer conversion by $^1$H NMR spectroscopy. The solvent was evaporated under reduced pressure, then the residue was poured into excess methanol. The resulting precipitate was collected by centrifugation and washed with methanol. After drying under vacuum, a white powder was obtained. Yield, 905 mg (89%). $M_n = 10{,}000$, $M_w/M_n = 1.13$ (PMMA std.).

### Synthesis of PManEMA Having an Azide Group at the Initiating End

A typical procedure for an aqueous activator generated by electron transfer (AGET) ATRP was as follows: Ascorbic acid (6.50 mg, 36.9 $\mu$mol) was dissolved in a mixture of 2-propanol and water (2/8, v/v) (10 mL), and nitrogen gas was bubbled through the solution for 1 h to prepare a deaerated stock solution of ascorbic acid. ManEMA (86.5 mg, 296 $\mu$mol), 2-azidoethyl 2-bromo-2-methylpropanoate (0.64 mg, 2.5 $\mu$mol), $CuCl_2$ (0.19 mg, 1.4 $\mu$mol), tris(2-pyridylmethyl)-amine (0.38 mg, 1.3 $\mu$mol), and a mixture of 2-propanol and water (2/8, v/v) (0.4 mL) were charged in a 1.5 mL auto sampler vial, which was sealed with a rubber septum and cap. Nitrogen gas was bubbled through the solution for 2 min. The vial was immersed in a preheated water bath at 30 °C. A deaerated stock solution of ascorbic acid (0.1 mL) was injected into the vial to initiate the polymerization. After 1 h, the polymerization mixture was exposed to air and a small amount of water was added to quench the reaction. The crude product was purified by dialysis with deionized water for more than 3 days. After lyophilization, PManEMA was obtained as a white powder. Yield, 62.1 mg (72%). $M_n = 16{,}000$, $M_w/M_n = 1.24$ (PMMA std.).

### Click Synthesis of PManEMA-*b*-PLLA

A typical procedure for the coupling reaction was as follows: PManEMA ($M_n = 16{,}000$) (29.2 mg, 1.83 $\mu$mol), PLLA ($M_n = 4500$) (10.8 mg, 2.40 $\mu$mol), pentamethyldiethylenetriamine (PMDETA) (11.0 mg, 63.5 $\mu$mol), and dry DMF (1 mL) were placed into a Schlenk tube. The tube was degassed with several freeze-pump-thaw cycles. CuBr (2.40 mg, 16.7 $\mu$mol) was added to the tube. The tube was immersed into a water bath thermostated at 25 °C, and stirred for 24 h. After removal of the solvent under reduced pressure, the residue was dissolved in water and dialyzed with deionized water for more than 3 days. After lyophilization, the coupling product was obtained as a white powder. Yield, 18.5 mg (46%).

### Critical Aggregation Concentration

The critical aggregation concentration (CAC) was determined by using pyrene as a hydrophobic fluorescent probe. The typical procedure was as follows: A stock solution of polymeric aggregates was diluted with distilled water in the concentration range from 0.5 to 5.12 $\times$ $10^{-7}$ mg·mL$^{-1}$. An aqueous solution of pyrene containing 0.1 vol % acetone ($2.4 \times 10^{-6}$ M) (1.6 mL) was added to the diluted polymer solution (1.6 mL), and thoroughly mixed. The solution was kept at 25 °C for 1 h in the dark. The fluorescence intensity at 390 nm was measured, with photoexcitation at 333 nm ($I_{333}$) and 338 nm ($I_{338}$) at 25 °C. The fluorescence intensity ratio ($I_{338}/I_{333}$) was plotted as a function of the logarithm of the polymer concentration. The CAC was determined as the intersection point of two tangents to the plot at higher and lower concentrations.

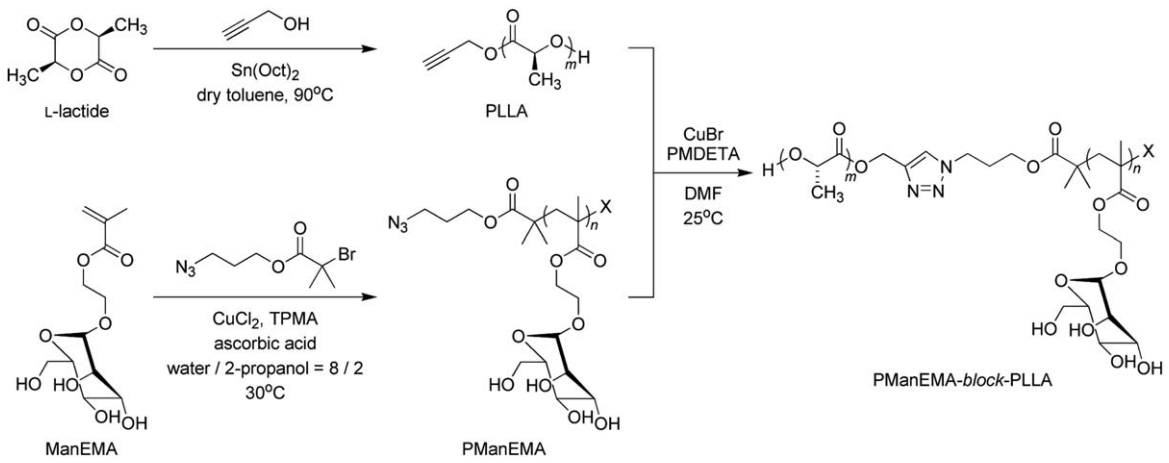
### Turbidimetric Assay

The binding behavior of $PManEMA_{16k}$ and $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$ with concanavalin A (ConA) was examined by turbidity assay according to a previous paper, with minor modifications. ConA was diluted to 1 $\mu$M (based on ConA tetramer) with 10 mM HEPES-buffered (pH = 7.4) saline containing 150 mM NaCl and 1 mM $CaCl_2$ (referred to HBS). The ConA solution (450 $\mu$L) was transferred to a 1 cm micro cuvette, which was settled into a thermostated cell holder at 25 °C. Into the cuvette was rapidly injected 50 $\mu$L of distilled water solution of $PManEMA_{16k}$ or $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$. The final concentration of the ManEMA unit in $PManEMA_{16k}$ was varied from 6.3 to 100 $\mu$M. The final concentration of $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$ was varied from 5 to 80 mg·L$^{-1}$.

## RESULTS AND DISCUSSION

### Synthesis of PLLA Having an Ethynyl Group at the Initiating End

Ring-opening polymerization of L-lactide was carried out using 2-propyn-1-ol as an initiator and tin(II) 2-ethylhexanoate as a catalyst in dry toluene at 90 °C (Scheme 1). Table 1 summarizes the polymerization conditions and




**SCHEME 1** Synthesis of PManEMA-*b*-PLLA.

results. Figure 1 shows the SEC traces and MALDI-TOF mass spectrum of the resulting PLLA. The mass spectrum shows peaks corresponding to the sodium and potassium adducts of the PLLA bearing an ethynyl group and hydrogen at the initiating and terminating ends, respectively. However, minor peaks are also found and are plausibly attributable to PLLA initiated with water impurities. All SEC traces show relatively narrow molecular weight distributions in which the polydispersities fall between 1.05 and 1.12. The number average molecular weight ($M_n$) of PLLA increased from 4500 to 10,000 with an increase in the monomer-to-initiator ratio. It should be noted that the $M_n$ values were obviously overestimated due to the PMMA calibration. Unfortunately, no technique was available to determine the absolute $M_n$ values of the resulting PLLA.

### Synthesis of PManEMA Having Azide Group at the Initiating End

ManEMA and 2-azidopropyl 2-bromo-2-methylpropanoate were prepared according to the literature.[59,60] Aqueous polymerization of ManEMA was hard to control by means of the normal ATRP technique due to the high polymerization rate in aqueous media. Hence, the AGET technique was applied to the aqueous ATRP of ManEMA. The AGET ATRP of ManEMA was carried out using 2-azidopropyl 2-bromo-2-methylpropanoate as an initiator, $CuCl_2$ and PMDETA as a copper source and a ligand, respectively, and ascorbic acid as a reducing agent in a mixture of water and 2-propanol (8/2, v/v) at 30 °C for 1 h. Figure 2 shows an SEC trace and IR spectrum of the resulting polymer, PManEMA. The $M_n$ and $M_w/M_n$ values were determined to be 16,000 and 1.24, respectively, on the basis of PMMA calibration. The relatively narrow molecular weight distribution indicated that the polymerization proceeded in a controlled fashion. The IR spectrum shows the characteristic peak at approximately

**TABLE 1** Ring-opening polymerization of L-lactide in toluene at 90 °C

| Run no. | $[M]_0/[I]_0$[a] | Time | Conv.[b] | Yield[c] | $M_n$ ($M_w/M_n$)[d] |
|---|---|---|---|---|---|
| | | (h) | (%) | (%) | |
| 1 | 10 | 3 | 93 | 55 | 4,500 (1.05) |
| 2 | 20 | 7 | 93 | 85 | 6,400 (1.10) |
| 3 | 40 | 20 | 98 | 88 | 10,000 (1.12) |

[a] M, L-lactide; I, 2-propyn-1-ol; $[M]_0 = 1.4$ M; $[I]_0/[Sn(Oct)_2] = 1$.
[b] Monomer conversion determined by $^1$H NMR spectroscopy.
[c] Isolated yield.
[d] Number average molecular weights ($M_n$s) and polydispersities ($M_w/M_n$s) were determined by SEC using PMMA calibration.

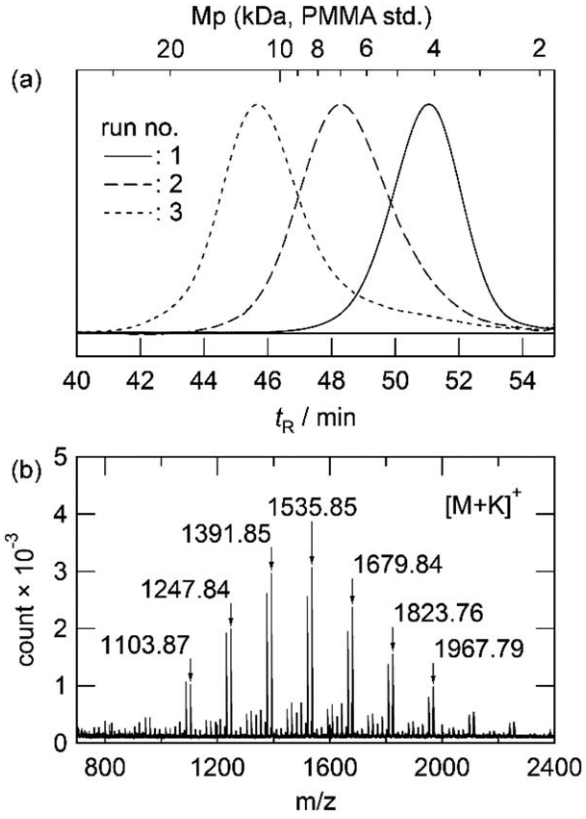



**FIGURE 1** SEC traces of PLLAs (a) and MALDI-TOF mass spectrum of $PLLA_{4.5k}$ (run no. 1 in Table 1) (b).

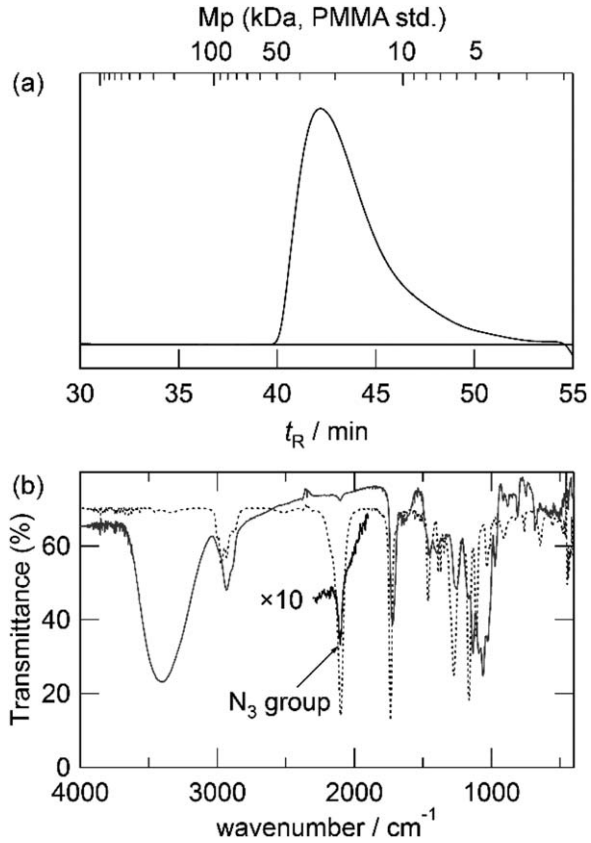



**FIGURE 2** SEC trace (a) and IR spectra (b) of $PManEMA_{16k}$ bearing an azido group at the initiating end (gray and black solid lines) and 3-azidopropyl 2-bromo-2-methylpropanoate (dotted line).

2100 $cm^{-1}$, which is attributable to the stretching mode of the azide group. This experimental evidence indicates that the resulting PManEMA has an azido group at the initiating end.

### Click Synthesis of PManEMA-*b*-PLLA

Synthesis of PManEMA-*b*-PLLA was performed by Cu(I)-catalyzed 1,3-dipolar cycloaddition of PLLA bearing an ethynyl group and PManEMA bearing an azido group at the initiating end. Usually, the amount of the two components should be adjusted, or an excess of one component, which is easily removed after reaction, may be applied for successful polymer coupling. However, as no technique was available for us to determine the absolute $M_n$ values, we used the $M_n$ values determined by SEC, calibrated by PMMA standards, to adjust the apparent molarities of PManEMA and PLLA.[61] Because the $M_n$ values of PLLA were obviously overestimated, excess PLLA was applied in the coupling reaction. The coupling reaction was carried out using CuBr and PMDETA as a catalyst and a ligand, respectively, in DMF at 25 °C for 24 h. The crude product was dialyzed against water, followed by lyophilization to afford a white powder. Figure 3 shows the $^1H$ NMR spectra of $PLLA_{10k}$, $PManEMA_{14.2k}$, and the coupling product in DMSO-$d_6$. The $^1H$ NMR spectrum of the coupling product shows peaks attributable to both $PLLA_{10k}$ and $PManEMA_{14.2k}$ segments. Figure 4 shows the SEC traces of $PLLA_{10k}$, $PManEMA_{14.2k}$ and the coupling product. The SEC trace of the coupling product clearly shifted toward a shorter retention time compared to those of $PLLA_{10k}$ and $PManEMA_{14.2k}$. This indicates that the resulting polymer is a block copolymer consisting of $PManEMA_{14.2k}$ and $PLLA_{10k}$, namely, $PManEMA_{14.2k}$-*b*-$PLLA_{10k}$. In the SEC trace of the coupling products, however, a significant amount of impurities was found on both sides of the main peak. The impurity appearing in the higher retention region is unreacted PLLA, because excess PLLA was used, as mentioned above. The impurity found in the lower retention region is still unknown. The main peak was fitted with an empirically transformed Gaussian function,[62] and the area percent of the main peak was estimated to be 92% (see Supporting Information). The $M_n$ and $M_w/M_n$ values were calculated using the best-fit curve and PMMA calibration, and are listed in Table 2.

### Sugar-Coated Aggregate Formation and Lectin-Binding Properties

The water solubility of the resulting block glycopolymer depended on the balance of the hydrophobic and the hydrophilic segments. $PManEMA_{16k}$-*b*-$PLLA_{4.5k}$ and $PManEMA_{16k}$-

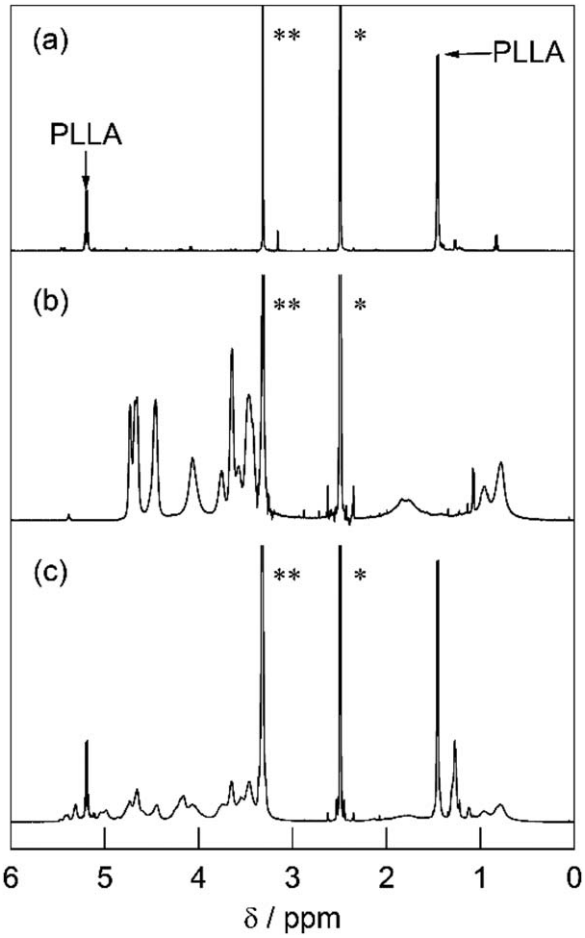



**FIGURE 3** $^1H$ NMR spectra of $PLLA_{10kDa}$ (a), $PManEMA_{14.2kDa}$ (b), and the coupling product (c) in DMSO-$d_6$. Symbols * and ** represent solvent and water peaks, respectively.

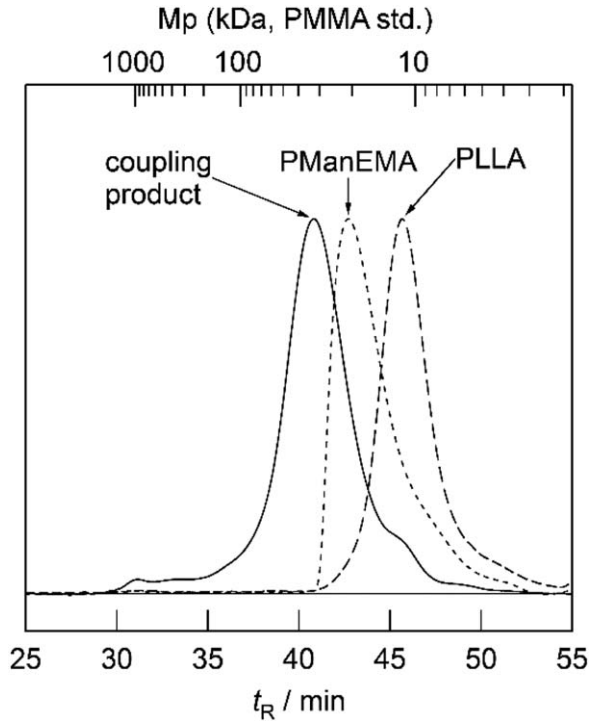



**FIGURE 4** SEC traces of $PLLA_{10k}$ (broken line), $PManEMA_{14.2k}$ (dotted line) and the coupling product ($PManEMA_{14.2k}$-*b*-$PLLA_{10k}$, solid line) (c) in DMF containing 50 mM LiBr.

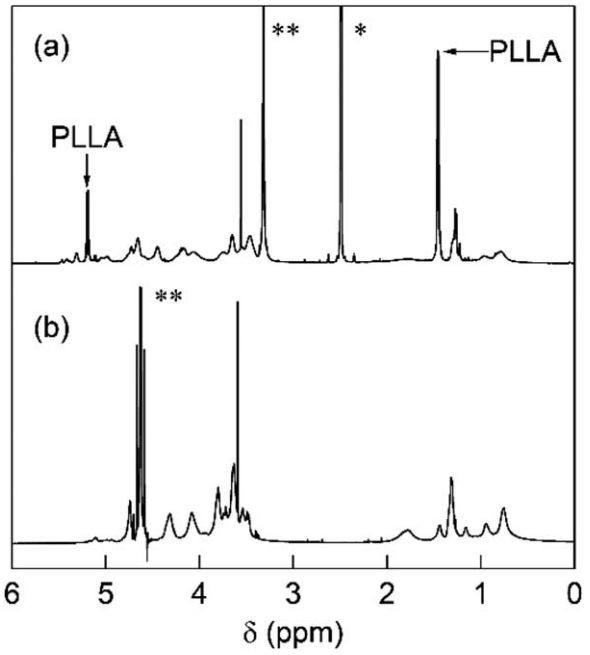



**FIGURE 5** $^1$H NMR spectra of $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$ in DMSO-$d_6$ (a) and $D_2O$ (b). Symbols * and ** represent solvent and water peaks, respectively.

*b*-$PLLA_{6.4k}$ were soluble in water, while $PManEMA_{14k}$-*b*-$PLLA_{10k}$ was insoluble in water. Figure 5 shows the $^1$H NMR spectra of $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$ in DMSO-$d_6$ and $D_2O$. The peaks at 1.45 and 5.20 ppm, attributable to the PLLA segment, completely disappeared in the $^1$H NMR spectrum recorded in $D_2O$, indicating that the PLLA segments condensed to form a core region. The critical aggregate concentration (CAC) was determined by fluorometry, using pyrene as a hydrophobic fluorescent probe. Figure 6 shows the fluorescence intensity ratio between 333 and 338 nm in the excitation spectrum, in which the detection wavelength was 390 nm, as a function of the concentration of $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$. The plots were fitted with a sigmoid function and the CAC was determined to be 21 mg·L$^{-1}$ for $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$ from the crossing point of the base line and the tangent at the inflection point. Suriano et al. synthesized a block glycopolymer, poly(ε-caprolactone)-*b*-poly[(polyethylene glycol mono methyl ether methacrylate)-*co*-(6-*O*-methacryloyl-D-galactose)], in which the $M_n$ values of the PLLA segments and the overall block glycopolymer were 5700 and 25,300, respectively, via a macromolecular initiator approach using the ATRP technique.[49] The CAC value of their block glycopolymer was estimated to be 8.4 mg·L$^{-1}$, which is slightly lower than that of $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$. Figure 7 shows the size distribution of $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$ aggregates measured in aqueous solution at a concentration of 1 mg·mL$^{-1}$. The main peak was found at approximately 30 nm, and the median radius was determined to be 41 nm. However, minor peaks were found at 340 and 1400 nm. The multimodal size distribution suggests a non-uniform structure for the aggregates, but details of these remain to be solved.

**TABLE 2** Click synthesis of PManEMA-*b*-PLLA in DMF at 25 °C[a]

| | PManEMA | PLLA | PManEMA-*b*-PLLA | | |
|---|---|---|---|---|---|
| Run no. | $M_n$[b] | $M_n$[b] | Yield (%)[c] | $M_n$ ($M_w/M_n$)[d] | Water-solubility |
| 1 | 16,000 | 4,500 | 46 | 19,400 (1.66) | ○ |
| 2 | 16,000 | 6,400 | 63 | 28,300 (1.28) | ○ |
| 3 | 14,200 | 10,000 | 68 | 27,100 (1.34) | × |

[a] $[PManEMA]_0/[PLLA]_0/[CuBr]_0/[PMDETA]_0 = 1/1/10/25$; $[PManEMA]_0 =$ approximately 2 mM (based on the $M_n$ value calculated using PMMA calibration); time, 24 h.
[b] Number average molecular weights ($M_n$s) determined by SEC using PMMA standards.
[c] Isolated yield.
[d] Number average molecular weights ($M_n$s) and polydispersities ($M_w/M_n$s) were calculated on the basis of the best-fitted curve to the SEC traces using PMMA calibration.

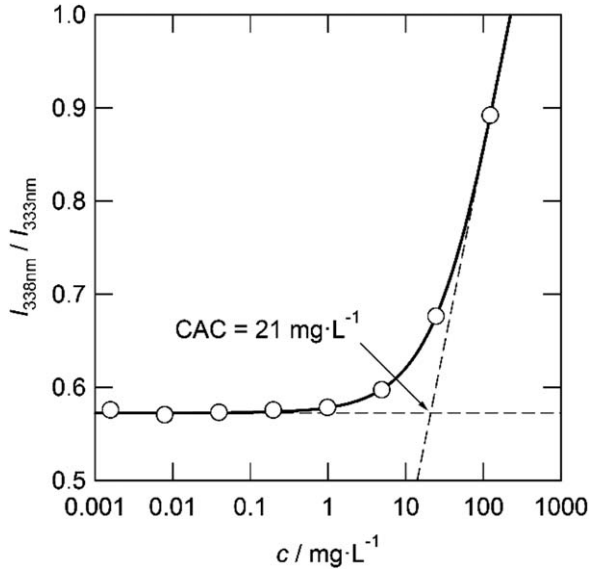



**FIGURE 6** The $I_{338nm}/I_{333nm}$ values as a function of the concentration of PManEMA$_{16k}$-*b*-PLLA$_{6.4k}$.

Even though the structure of the aggregates is unclear, the highly water-soluble PManEMA segments must be located on the outer surface of the aggregates. The functionality of the D-mannose residue presented on the aggregate surfaces was evaluated by the binding ability to concanavalin A (ConA), which is a lectin recognizing D-mannose. ConA forms a homotetramer at neutral pH, and each unit has one carbohydrate recognition domain. Hence, a material presenting multiple D-mannose residues forms aggregates by mixing with ConA. In order to evaluate the kinetics of the aggregate formation, we employed a turbidimetric assay in HEPES buffer (pH = 7.4) containing 150 mM NaCl and 1 mM $CaCl_2$.[59] Figure 8 shows the time course of the optical density at 420 nm in a mixture of ConA with PManEMA$_{16k}$ and

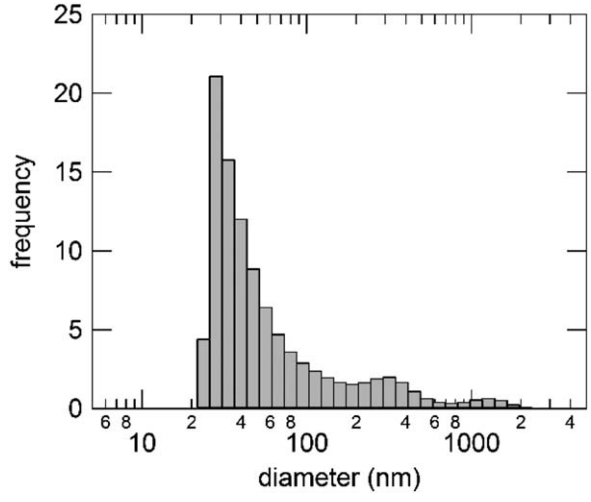



**FIGURE 7** Size distribution of PManEMA$_{16k}$-*b*-PLLA$_{6.4k}$ aggregates in aqueous solution. The concentration of the block glycopolymer was 1 mg·mL$^{-1}$.

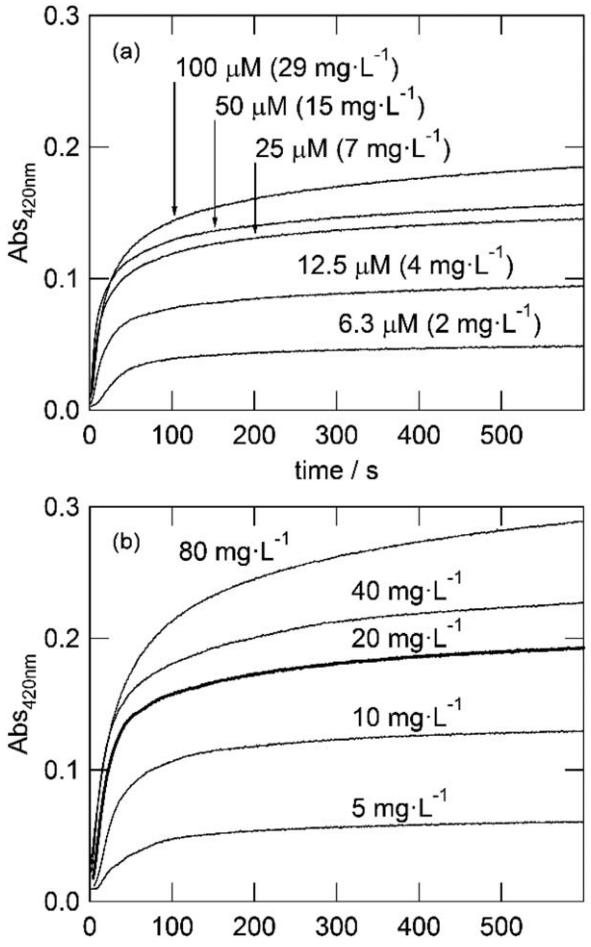



**FIGURE 8** Time course of optical density at 420 nm in a mixture of ConA and PManEMA$_{16k}$ (a) and PManEMA$_{16k}$-*b*-PLLA$_{6.4k}$ aggregate solution (b) at 25 °C. The concentration of ConA homotetramer was 1 $\mu$M. The concentration of the ManEMA unit in PManEMA$_{16k}$ was varied from 6.3 to 100 $\mu$M. The concent ration of PManEMA$_{16k}$-*b*-PLLA$_{6.4k}$ was varied from 5 to 80 mg·L$^{-1}$.

PManEMA$_{16k}$-*b*-PLLA$_{6.4k}$ at 25 °C. The resulting curve for PManEMA$_{16k}$ at a concentration of ManEMA units of 50 $\mu$M was quite similar to that reported previously,[59] indicating the good reproducibility of this technique. The initial increment rate of the optical density was increased with an increase in ManEMA units from 6.3 to 25 $\mu$M, and was almost constant above 25 $\mu$M. For PManEMA$_{16k}$-*b*-PLLA$_{6.4k}$, the initial increment rate of the optical density increased when the polymer concentration increased from 5 to 20 mg·L$^{-1}$, and was almost constant above a polymer concentration of 20 mg·L$^{-1}$. Unfortunately, the effect of aggregate formation on ConA binding cannot be separated from the contribution of the increase in the concentration of ManEMA units.[63] However, the increment rate of the optical density did not decrease even above a polymer concentration of 20 mg·L$^{-1}$, suggesting that the aggregate structure did not interfere with the interaction between D-mannose residue and ConA. These results indicate that PManEMA$_{16k}$-*b*-

$PLLA_{6.4k}$ forms aggregates that efficiently represent D-mannose residues.

## CONCLUSIONS

The block glycopolymer, PManEMA-*b*-PLLA, was synthesized by Cu(I)-catalyzed 1,3-dipolar cycloaddition between PLLA bearing an ethynyl group and PManEMA bearing an azide group at the initiating end. The block architecture was confirmed by SEC and $^1$H NMR spectroscopy. The water-solubility of the block glycopolymer depends on the balance of the two segments. The aggregate formation of the block glycopolymer, $PManEMA_{16k}$-*b*-$PLLA_{6.4k}$, was examined by $^1$H NMR spectroscopy using selective solvents, fluorometry using pyrene as a hydrophobic probe, and DLS measurement. The block glycopolymer forms a complicated aggregate in water at concentrations above 21 $mg{\cdot}mL^{-1}$. The aggregates present D-mannose units on their outer surfaces, and can interact with the lectin, concanavalin A.

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