Supporting information
Aggregation of a double hydrophilic block glycopolymer: the effect of block polymer ratio

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1. Material

D-Mannose, triethyl amine (TEA) (99%), 2,2'-azobis (isobutyronitrile) (AIBN) (98%), calcium chloride (95%), 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) (99%), 4,4"-azobis(4-cyanovaleric acid) (V-501) (98.0%), carbon disulfide (98.0%), toluene (99.5%), *N,N'*-dicyclohexylcarbodiimide (DCC) (95.0%), 4-dimethylaminopyridine (DMAP) (99.0%), ethanol (99.5%) and deuterium oxide (D_2O, 99.8%) were purchased from Wako. *N*,*N*-diisopropylethlamine (99%), iodine (98.0%), 1-butane thiol (97.0%), sodium hydride (60%, dispersion in paraffin liquid), methacryloyl chloride (80%), Osmium Tetroxide (4% in Water), *N,N*-Diisopropylethylamine (99%) and poly(ethylene glycol)monomethyl ether (Mw: 400, 2000 and 4000) were purchased from Tokyo Chemical Industry. Sodium azide (97%), sodium chloride (99%), magnesium chloride hexahydrate (99%), potassium chloride (99.5%), dry dichloromethane (99.5%), dry *N,N*-dimethylformamide (DMF) (99.5%), ethyl acetate (99.3%), sodium thiosulfate pentahydrate (99.0%) and diethyl ether (99.0%) and ethyl acetate (99%) were purchased from Kanto Chemical. 2-chloro-1,3-dimethylimidazolium chloride, copper (I) bromide (97.0%) were purchased from Aldrich. Dimethyl sulfoxide (DMSO, 98%) was purchased from Kishida. Propargyl alcohol (99%) and cupper bromide (I) (99.99%) was purchased from sigma Aldrich.

2. Characterization

2-1. ^{1}H NMR measurement.

^{1}H NMR spectra were recorded on a JNM-ECZ400 spectrometer (JEOL, Tokyo, Japan) using $CDCl_3$, d_6-DMSO or D_2O as a solvent.

2-2. Gel permeation chromatography measurement.

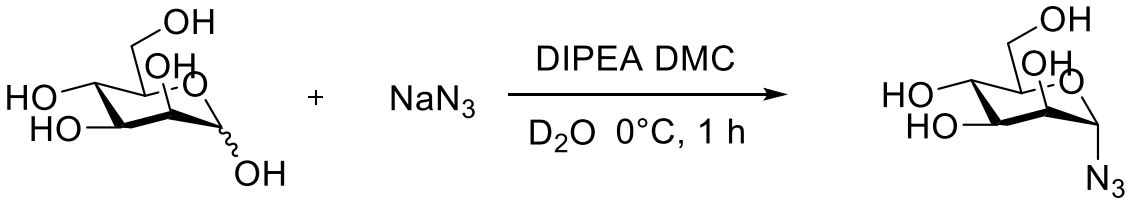
Gel permeation chromatography (GPC) with organic solvent was performed on a HLC-8320 GPC Eco-SEC system equipped with a TSKgel Super AW guard column and TSKgel Super AW (4000, 3000 and 2500) columns (Tosoh, Tokyo Japan). GPC with

water solvent was performed on a JASCO DG-980-50 degasser equipped with a JASCO PU-980 pump (JASCO Co., Tokyo, Japan), a Shodex OH pak SB-G guard column, a Shodex OH pak LB-806 HQ column (Showa Denko, Tokyo, Japan), a JASCO RI-2031 Plus RI detector. GPC analyses were performed by injecting 20 μL of a polymer solution (1 g L–1) in DMF buffer containing 10 mM LiBr or $NaNO_3$ aqueous solution (100 mM). The buffer solution was also used as the eluent at a flow rate of 0.5 mL/min. The GPC system was calibrated using a poly(methyl methacrylate) standard (Shodex).

3. Experimental

3-1. Synthesis of mannose azide.

Scheme S 1. Synthesis of mannose azide.



Mannose azide were synthesized according to the literature.[1] D-mannose (2.78 mmol), NaN_3 (27.8 mmol), *N,N*-Diisopropylethylamine (DIPEA) (25 mmol) were dissolve in D_2O (11 mL) with stirring in ice bath. After the mixture had cooled sufficiently, 2-chloro-1,3-dimethylimidazolium chloride (DMC) (8.34 mmol) were added into the mixture. Then the mixture was stirred for 1 h at 0°C. The progress of reaction was confirmed by ^1H NMR. After concentration of the reaction mixture and addition of ethanol, the solid was removed by filtration. The filtrate was concentrated *in vacuo* and the product was dissolved in water. Then mixture was washed using dichloromethane and the aqueous phase was concentrated *in vacuo*. After concentration, the product purified by Ion-exchange chromatography (activated by 1N NaOH solution overnight and washed by MilliQ). The product was concentrated and addition ethanol, the solid was removed by filtration. The filtrate was concentrated and freeze dried to give yellow solid. (yield: 2.1 mmol, 76.3%)

^1H NMR (400 MHz, D_2O), δ (ppm): 5.44 (d, J=1.6 Hz, –C(**H**)-N_3), 3.77 (d, J=10.1 Hz, -C**H**$_2$-OH), 3.73 (dd, J=2.3, 3.2 Hz, -C**H**$_2$-OH, 2H), 3.65 (t, J=6.4 Hz, -CH-C**H**-CH_2-, 1H), 3.60 (s, -C**H**-CH-CH-N_3, 1H), 3.52 (dd, J=4.1, 9.6 Hz, -CH-C**H**-CH_2, 1H), 3.49 (t, J=5.3 Hz, -CH-C**H**-CH-N_3, 1H).

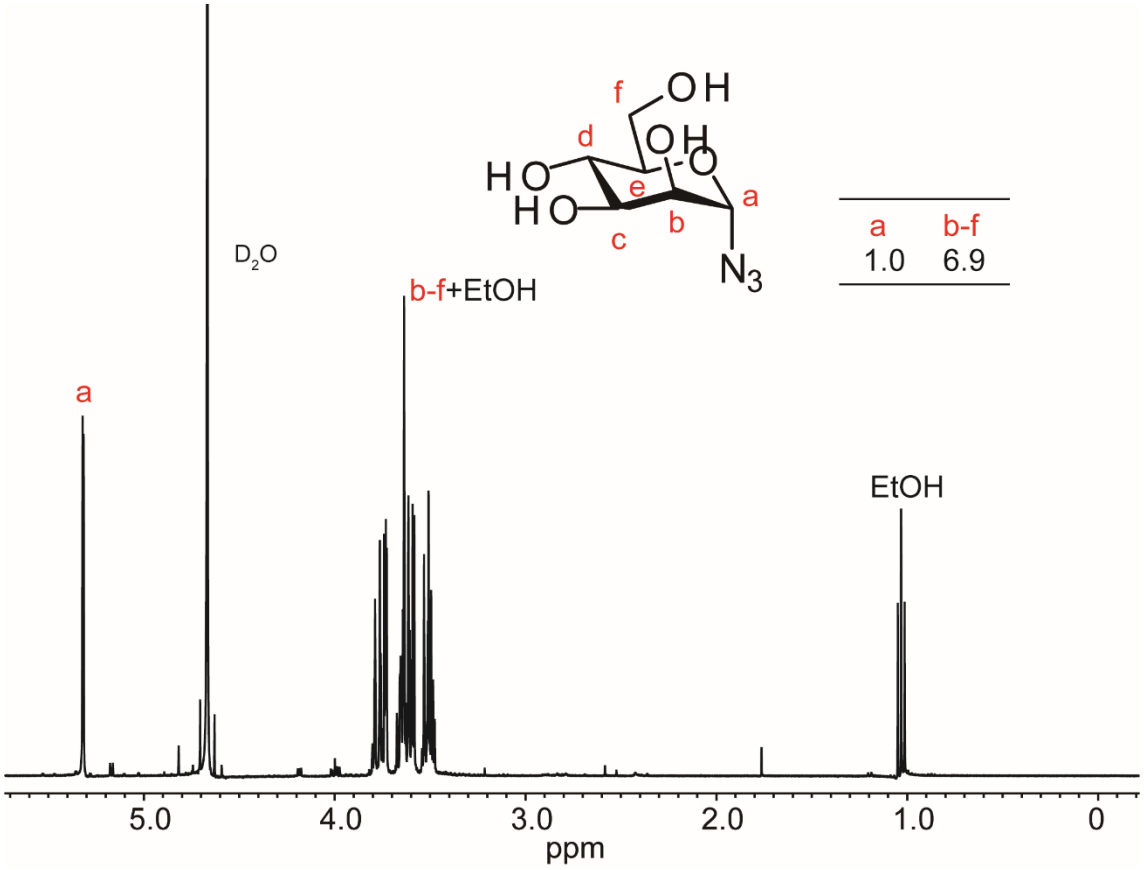
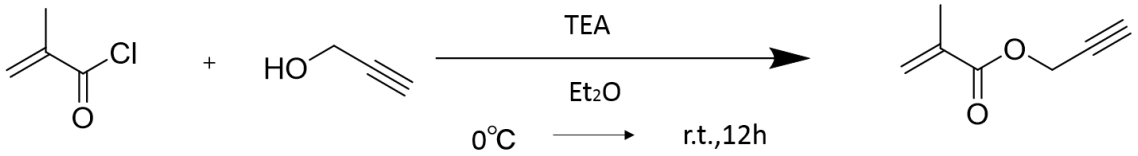


Figure S 1. ^{1}H NMR of mannose azide.

3-2. Synthesis of propargyl methacrylate.

Scheme S 2. Synthesis of propargyl methacrylate.



Propargyl methacrylate were synthesized according to the literature[2]. Propargyl alcohol (15 mL, 257 mmol) and Triethyl amine (TEA) (46.6 mL, 333 mmol) were dissolved in Diethyl ether (Et_2O, 120 mL) with stirring in ice bath for 10 minutes. Methacryloyl chloride (22.1 mL, 283 mmol) were dissolved in Et_2O (120 mL) and it was slowly dropped into the solution. Then the mixture was stirring for 12 h at room temperature. The progress of the reaction was confirmed by TLC (Ethyl acetate : Hexane = 1 : 10). The precipitate was removed by filtration. The crude product was concentrated under reduced pressure, and it was purified by reduced distillation at about 1 kPa and 60°C to give colorless oil product (yield: 40.7 %).

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) : 6.17 (s, 1H, C=C**H**$_2$, trans), 5.62 (s, 1H, C=C**H**$_2$, cis), 4.75 (d, J = 2.4 Hz, 2H, -COOC**H**$_2$-), 2.47 (t, J=2.4, 2.8 Hz CH_2-C≡C**H**), 1.96 (s, 3H, -C**H**$_3$).

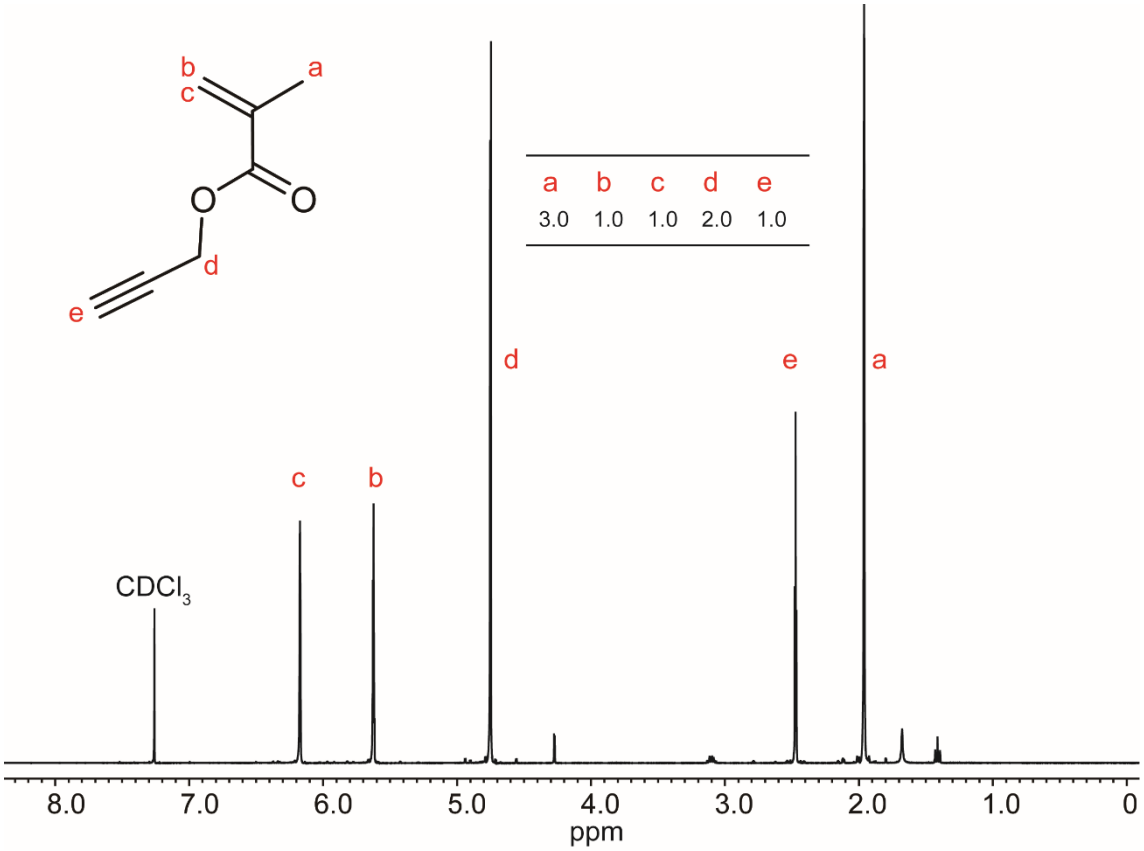


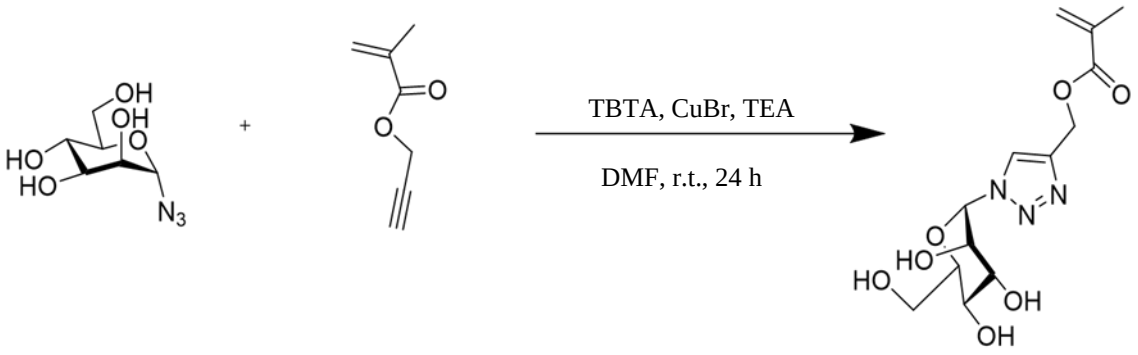
Figure S 2. ^{1}H NMR of propargyl methacrylate.

3-3. Synthesis of tris[(1-benzyl-1H-1, 2, 3-triazol-4-yl)methyl]amine (TBTA).

TBTA were synthesized according to the literature.[3]

3-4. Synthesis of (*D*-mannose-1*H*-1,2,3-triazol-4-yl) methyl methacrylate (Man-MA).

Scheme S 3. Synthesis of Man-MA.



Mannose azide (5.72 mmol), propargyl methacrylate (6.86 mmol) and TBTA (0.69 mmol) were

dissolved in Dry DMF (28.6 mL). The reaction solution was degassed by N_2 bubbling for 30 mins. TEA (0.4 mmol) and cupper bromide (CuBr) (0.69 mmol) were added into the solution and degassed for another 5 mins. The reactions solution was stirred for 24 h. After 24 h, add the scavenger (2.12g) to the solution and stirred overnight in order to remove Cu. The solution was filtered to remove the scavenger and concentrate *in vacuo*. Add the water (80 mL) into the mixture to precipitate the TBTA, then the mixture was filtered to remove the TBTA. The mixture was purified by reverse-phase chromatography (gradient: water → MeOH). The solution was concentrated and freeze-dried to obtain white powder. (yield: 50.4%)

^{1}H NMR (400 MHz, CD_3OD), δ (ppm) : 8.23 (s, 1H, triazole), 6.11 (s, 1H, C=C**H**$_2$, trans), 5.64 (m, J = 2.0 and 1.2 Hz, 1H, C=C**H**$_2$, cis), 6.03 (d, J=2.8 Hz, 1H, anomer of mannose), 5.30 (d, J = 2.4 Hz, 2H, -COOC**H**$_2$-), 2.47 (t, J=2.4, 2.8 Hz CH_2-C≡C**H**), 1.93 (s, 3H, -C**H**$_3$).

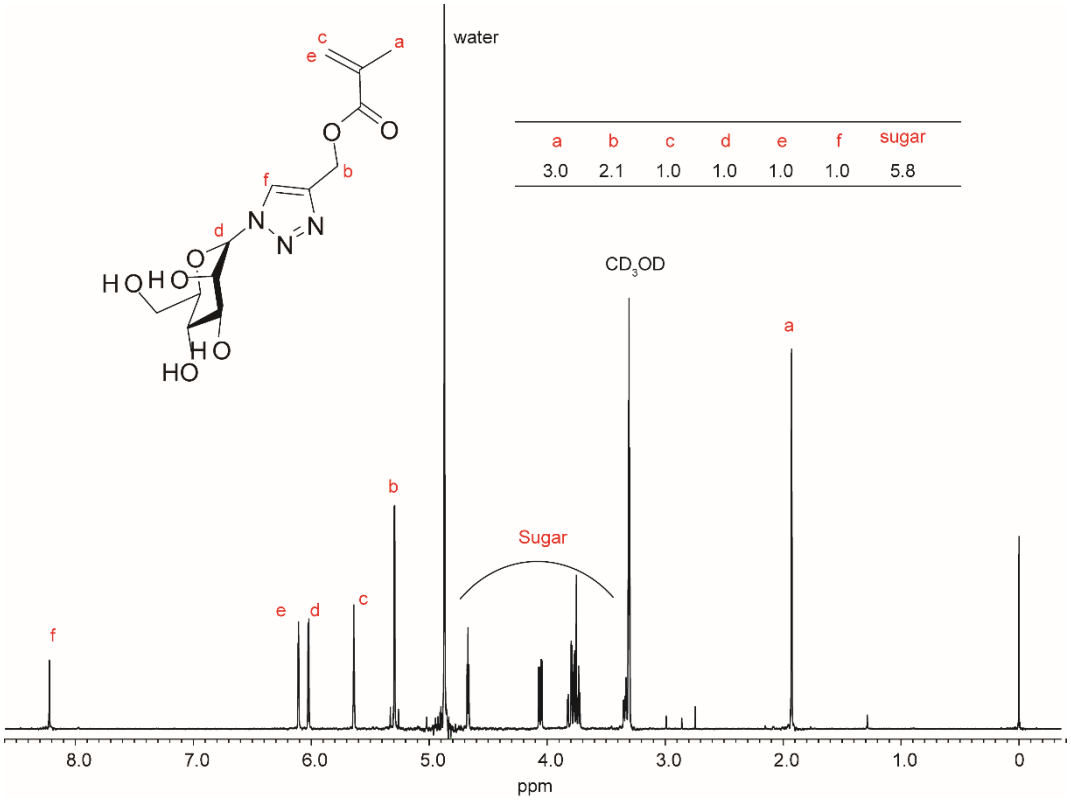
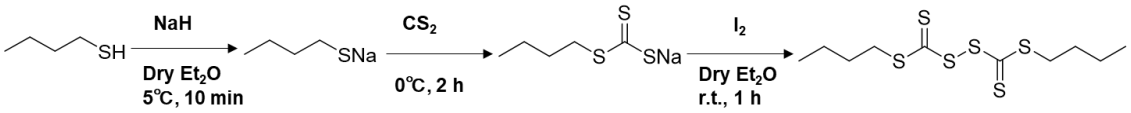


Figure S 3. ^{1}H NMR of Man-MA.

3-5. Synthesis of bis (butyl sulfanyl thio carbonyl) disulfide.

Scheme S 4. Synthesis of bis (butyl sulfanyl thio carbonyl) disulfide.



The two-necked flask was degassed and filled with nitrogen. Sodium hydride (0.15 mol) was added into the flask, then the flask was degassed and filled with nitrogen again. Diethyl ether (288 mL) and 1-butane thiol (0.09 mol) were added gently into the flask in order, and the mixture was stirred for 10 mins in ice bath. After that carbon disulfide (0.15 mol) was added dropwise using dropping funnel and stirred for 2 h. the reaction solution was concentrated by rotary evaporator to get yellow solid. The flask was degassed and filled with nitrogen, then the diethyl ether (280 mL) and iodine (45 mmol) was added. The reaction mixture was stirred for 1 h at room temperature. The reaction mixture was filtered and washed by sodium thiosulfate aq. (30 g/L, three times) and water (twice). The organic phase was dried by sodium sulfate, then filtered. The solution was concentrated by rotary evaporator to obtain yellow oil product. (Yield: 77.1%)

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 3.31 (t, J = 7.2 and 8.0 Hz, 3H, -CH_2-S-), 1.69 (m, J = 6.4, 8.4, 7.6 Hz, 2H, CH_3-C**H$_2$**-CH_2-), 1.44 (m. J = 7.2 Hz, 2H, -C**H$_2$**-S-CH_2-), 0.93 (t, J = 7.6 Hz, 3H, -C**H$_3$**)

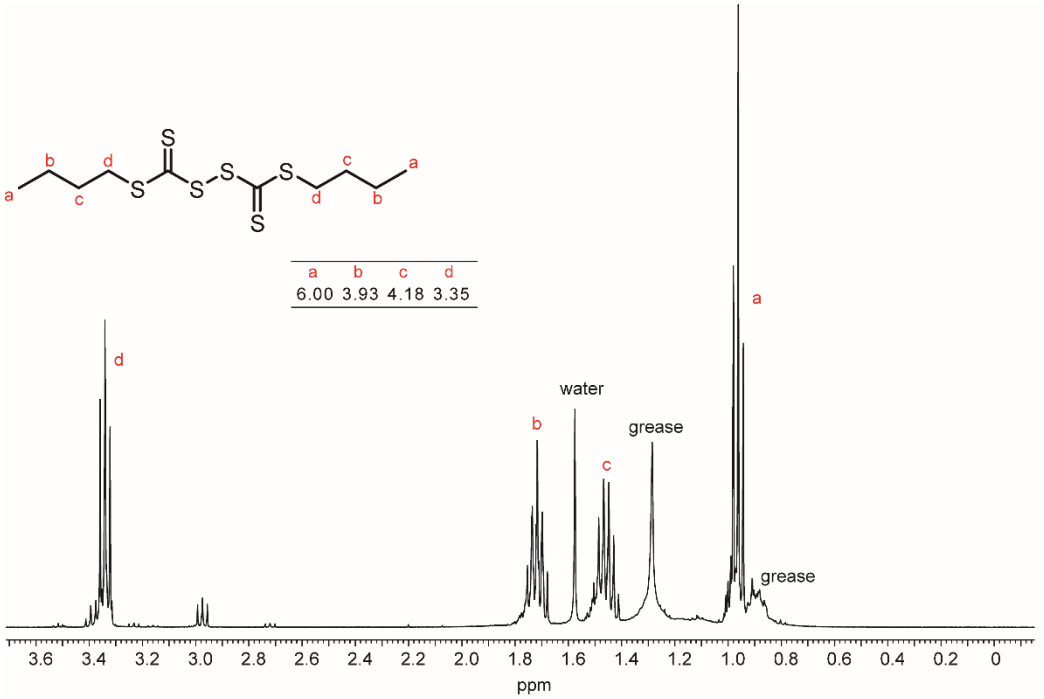
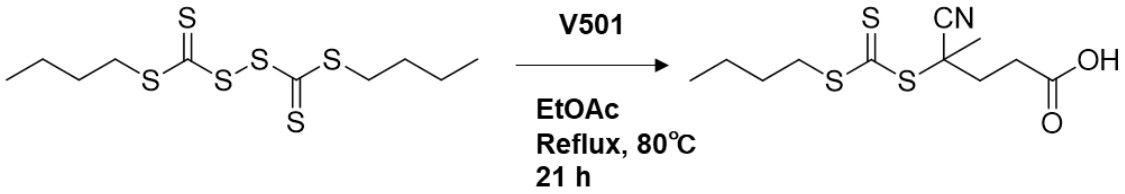


Figure S 4. ^{1}H NMR of bis (buthylsulfanyl thiocarbonyl)disulfide.

3-6. Synthesis of 4-(((butylthio)carbonothioyl)thio)-4-cyanopentanoic acid (raft agent 1).

Scheme S 5. Synthesis of raft agent 1.



EtOAc (100 mL) was added into the flask and degassed by N_2 bubbling for 30 mins. 4,4'-azobis(4-cyanovaleric acid) (V501) (9 mmol) was added into the flask and the mixture was stirred for 24 h at 80°C with reflux. The reaction mixture was concentrated by rotary evaporator. The reaction mixture was purified by normal-phase chromatography (Hexane : EtoAc = 30 : 1 → EtoAc only) to get yellow oil. (Yield: 22.6%)

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 3.33 (t, J = 7.2 Hz, 2H, -CH_2-C**H$_2$**-S-), 2.68 (m, J = 7.6 Hz, 2H, -C**H$_2$**-COOH), 2.35-2.57 (m, 2H, -C**H$_2$**-CH_2-COOH), 1.87 (s, 3H, C(-CN)(-C**H$_3$**)-S-), 1.68 (m, J = 8.0 Hz, 2H, -S-CH_2-C**H$_2$**-CH_2-), 1.42 (m, J = 7.2 and 8.0 Hz, 2H, -C**H$_2$**-CH_3), 0.93 (t, J = 7.6 Hz, 3H, -C**H$_3$**)

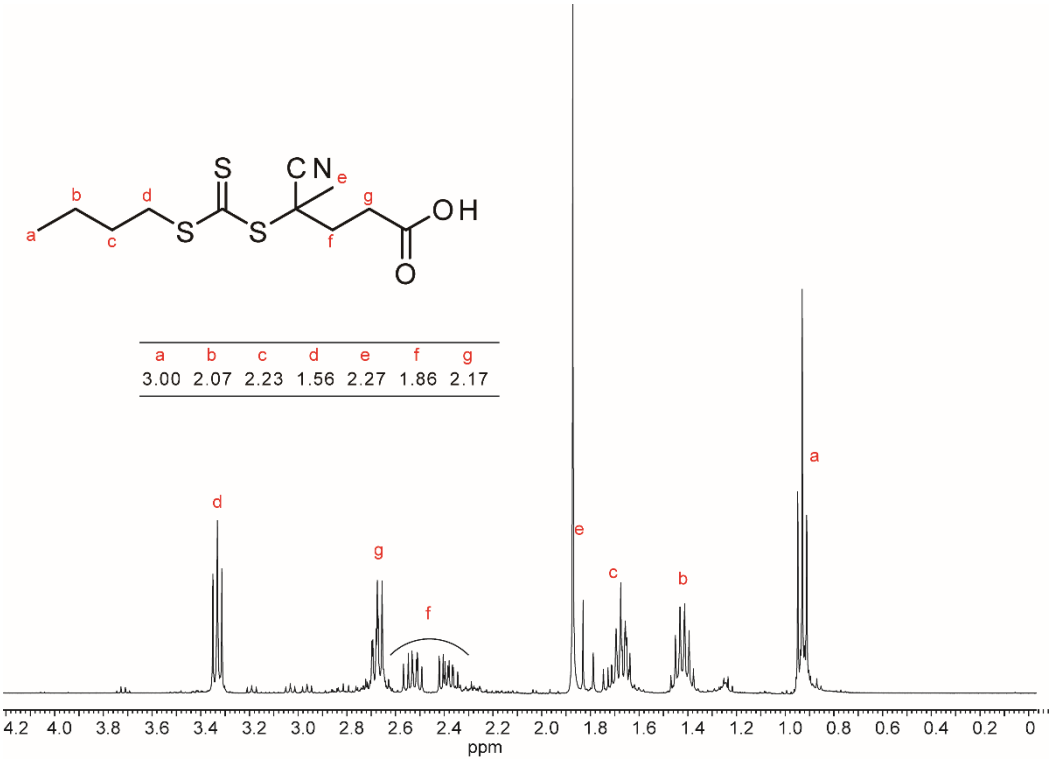
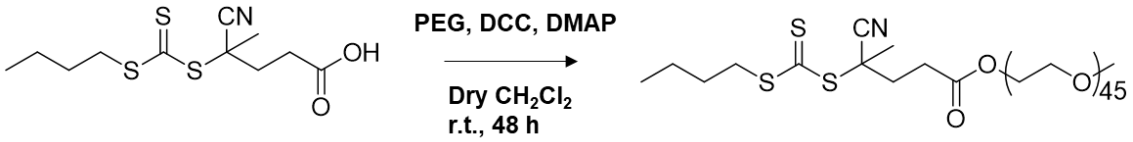


Figure S 5. ^{1}H NMR of 4-(((butylthio)carbonothioyl)thio)-4-cyanopentanoic acid.

3-7. Synthesis of peg terminated RAFT agent (PEG-CTA).

Scheme S 6. Synthesis of PEG-CTA. (molecular weight: 400, 2000 and 4000)



The poly(ethylene glycol)monomethyl ether (PEG) (0.25 mmol) was dissolved in toluene (10 mL) and azeotropic distillate the solution to remove the water. 4-(((butylthio)carbonothioyl)thio)-4-cyanopentanoic acid (0.50 mmol), DMAP (0.25 mmol) and dry CH_2Cl_2 (6.25 mL) were added. The flask was placed in an ice bath. DCC (0.5 mmol) was added into mixture and stirred for overnight (over 12 h) at 0°C. The temperature is increased to room temperature, and the mixture was stirred for another 24 h. The precipitated, which is dicyclohexylurea was removed by filtration. The solution was concentrated by rotary evaporator.

(1) PEG-CTA (molecular weight: 2000 and 4000**)**

The yellow solid product was precipitated by adding the solution into diethyl ether. the solid was collected by filtration and dried *in vacuo* to get the product. (Yield: PEG2000-CTA: 73.6%, PEG4000-CTA: 88.9%)

(2) PEG-CTA (molecular weight: 400**)**

The solution was purified by normal-phase chromatography ($CHCl_3$ only → $CHCl_3$: MeOH : 7 : 3). The solution was concentrated in vacuo to get the product. (Yield: 77.9%)

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm):4.25 (t, J = 4.4 Hz, 2H, -C**H$_2$**-COO-), 3.52-3.80 (m, -C**H$_2$**-C**H$_2$**-O-), 3.37 (s, 3H, -CH_2-O-C**H$_3$**), 3.33 (t, J = 7.2 Hz, 2H, -CH_2-C**H$_2$**-S-), 2.64 (m, J = 7.6 Hz, 2H, -C**H$_2$**-COOH), 2.35-2.55 (m, 2H, -C**H$_2$**-CH_2-CO-), 1.86 (s, 3H, C(-CN)(-C**H$_3$**)-S-), 1.68 (m, J = 8.0 Hz, 2H, -S-CH_2-C**H$_2$**-CH_2-), 1.42 (m, J = 7.2 and 8.0 Hz, 2H, -C**H$_2$**-CH_3), 0.92 ((t, J = 7.2 Hz, 3H, -C**H$_3$**)

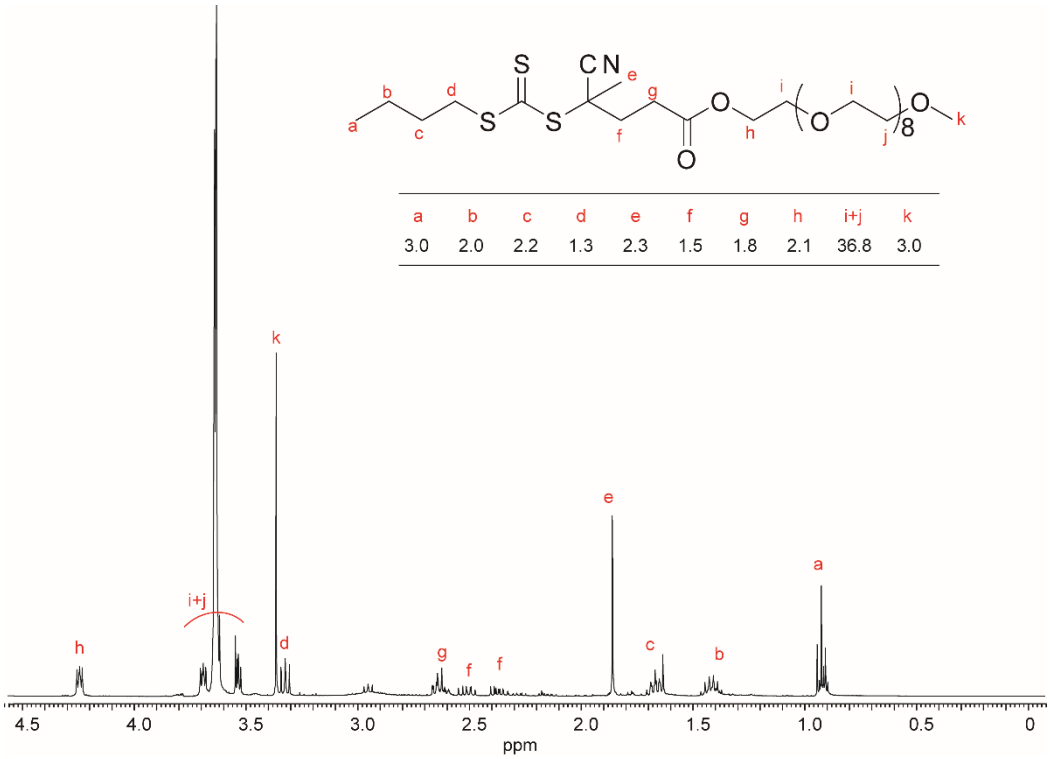


Figure S 6. ^{1}H NMR of poly (ethylene glycol) 4-(((butylthio)carbonothioyl)thio)-4-cyanopentanoate (PEG Mw: 400)

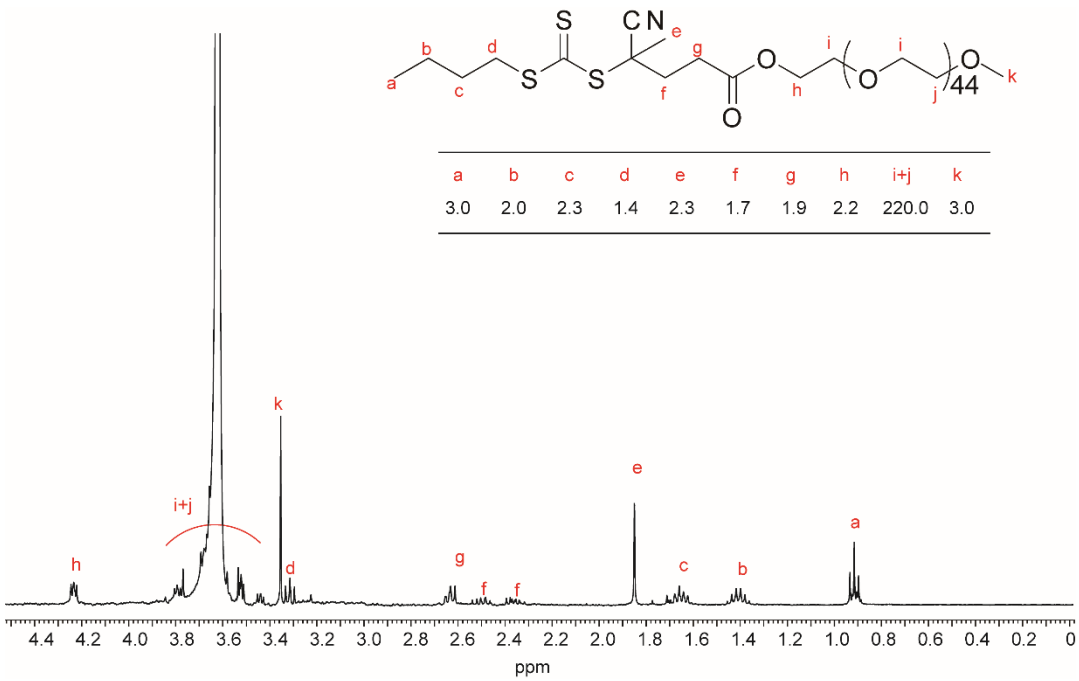


Figure S 7. ^{1}H NMR of poly (ethylene glycol) 4-(((butylthio)carbonothioyl)thio)-4-cyanopentanoate (PEG Mw: 2000)

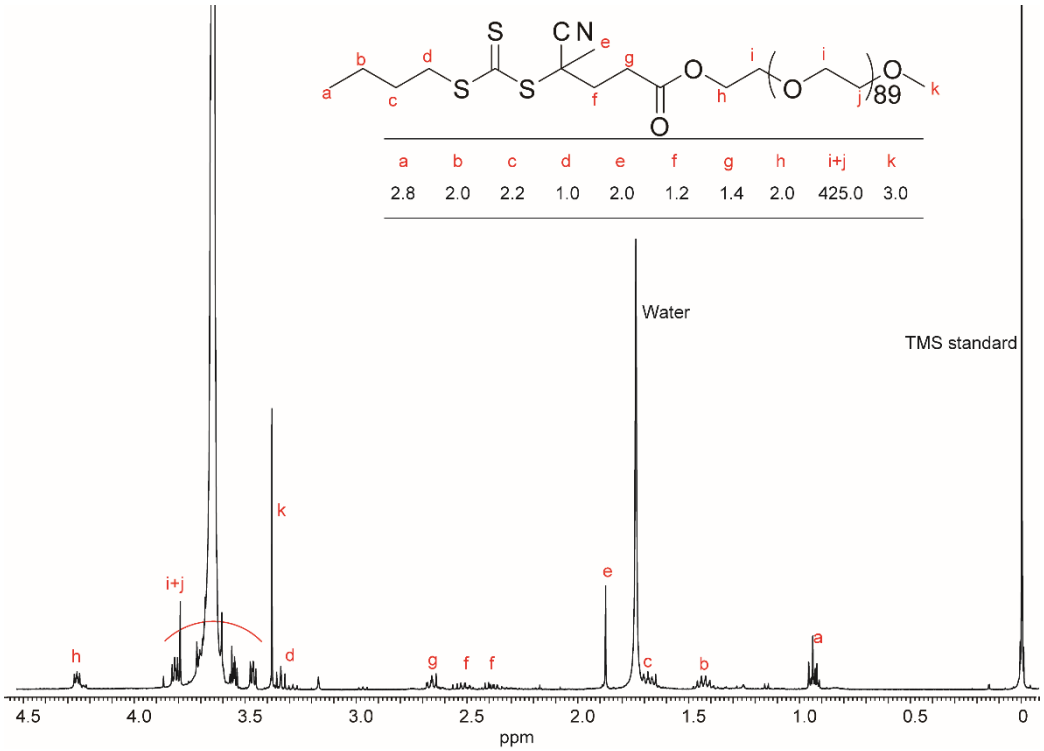


Figure S 8. ^{1}H NMR of poly (ethylene glycol) 4-(((butylthio)carbonothioyl)thio)-4-cyanopentanoate (PEG Mw: 4000).

3-8. Calculate the condensation rate by UV-Vis spectrometer.

In order to confirm the condensation rate of PEG-CTA, the absorption of 305 nm wavelength of PEG-CTA was measured by UV-Vis spectrometer. A linear calibration line was obtained from the absorbance of 4-(((butylthio)carbonothioyl)thio)-4-cyanopentanoic acid at 305 nm against the concentration of the RAFT agent. The absorbance at 305 nm was plotted for each concentration of PEG-CTA. The condensation rate of each RAFT agent was calculated from the calibration line and was more than 99% in all cases. Thus, the unreacted 4-(((butylthio)carbonothioyl)thio)-4-cyanopentanoic acid was almost absent.

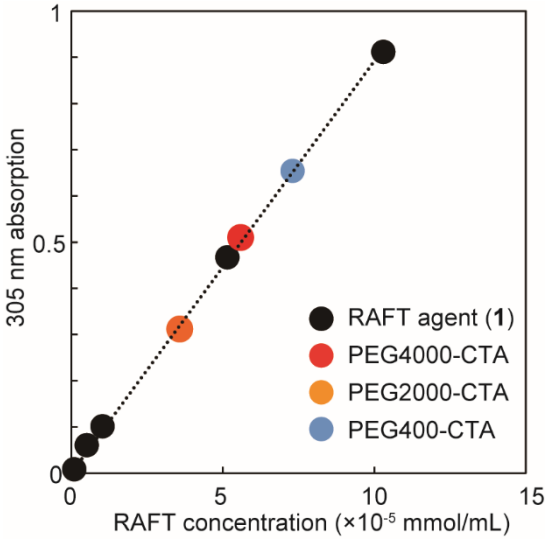
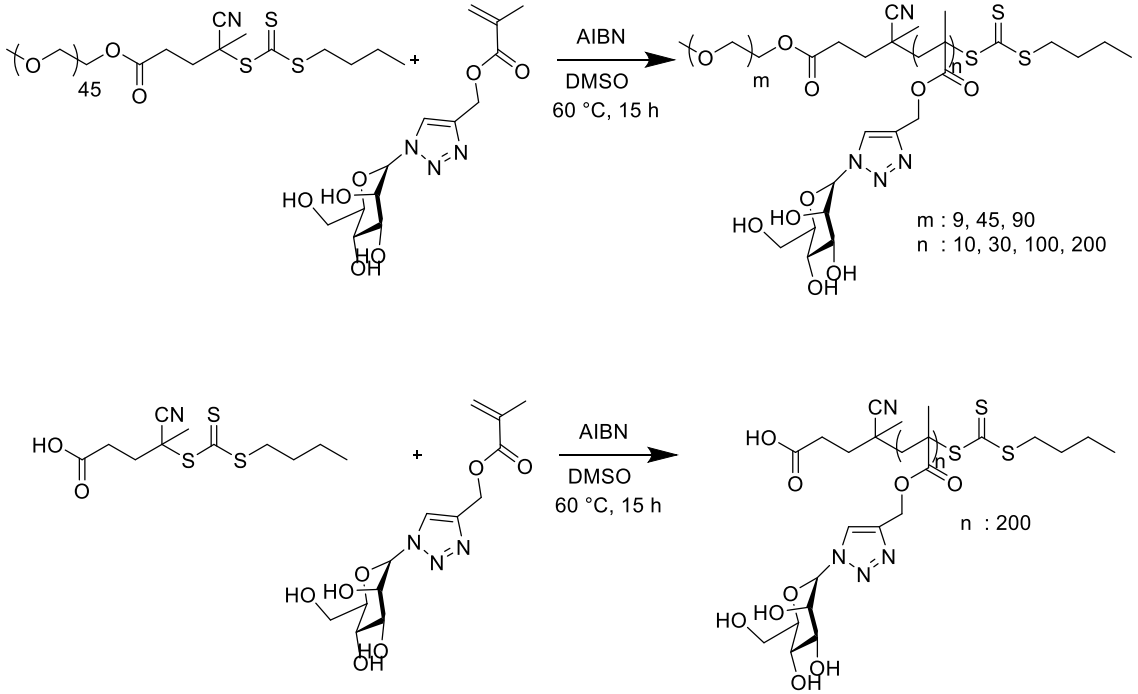


Figure S 9. The 305 nm absorption of each RAFT agent.

3-9. General procedure of RAFT polymerization.

Scheme S 6 RAFT polymerization of Man-MA.



Man-MA was introduced into a glass tube and mixed with a dimethyl sulfoxide solution of PEG-CTA or 4-(((butylthio)carbonothioyl)thio)-4-cyanopentanoic acid as the RAFT agent and AIBN as the radical initiator. The [Man-MA] : [RAFT agent] : [initiator] ratios are shown in Table 1 and Table 2. The tube was degassed with freeze–pump–thaw cycles, sealed under vacuum, and transferred to an oil bath at 60 °C. After heating for 15 h, polymerization was stopped by cooling the solution with liquid nitrogen. Then, the polymer solutions were dialyzed against DMSO (three times) and water (three times). The resultant polymer solution was then freeze-dried, and the conversion and degree of polymerization were determined by ^{1}H NMR (Table 3). The polydispersity of the polymer was determined by GPC (Table 3).

PmMn (m: 9, 45, 90, n: 10, 100, 200)

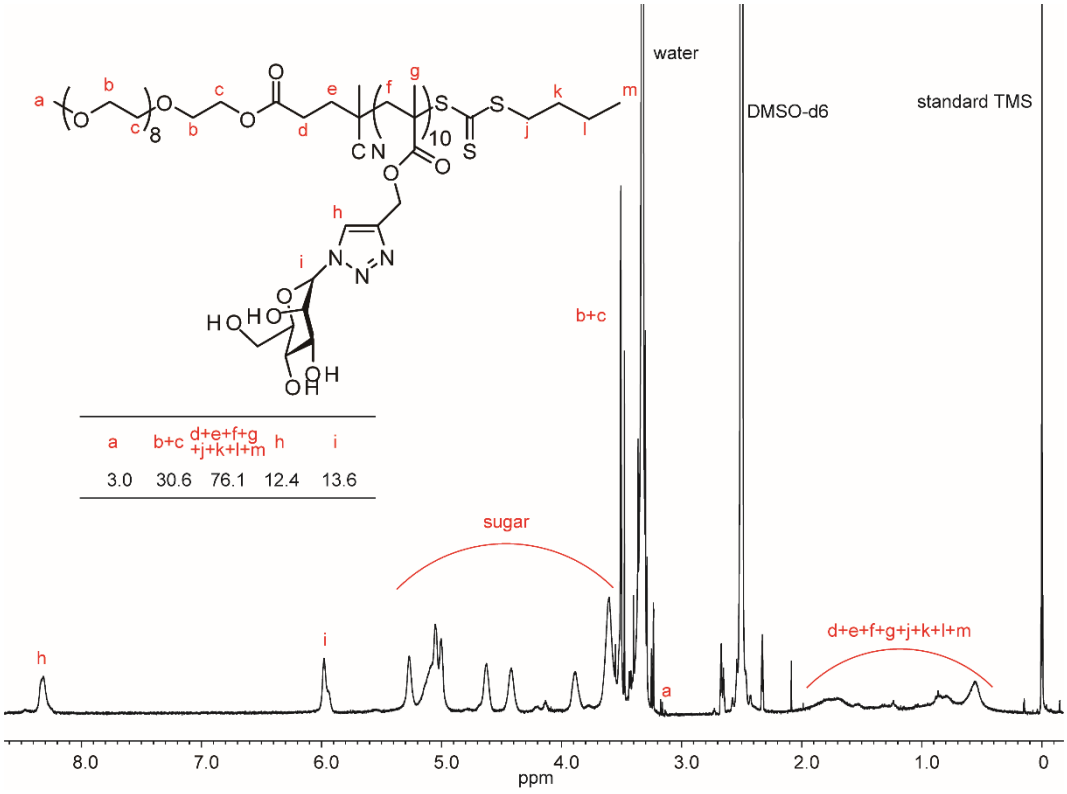
^{1}H NMR (400 MHz, DMSO-d6), δ(ppm):8.33 (s, triazole) , 5.98 (s, anomer proton of mannose), 3.60-5.28 (proton of mannose), 3.5 (s, -CH_2-CH_2-O-), 3.24 (s, -CH_2-CH_2-O-CH_3), 0.0-2.2 (brdd, -C(CH_3)-CH_2-)

M200

^{1}H NMR (400 MHz, DMSO-d6), δ(ppm):8.32 (s, triazole), 5.98 (s, anomer proton of mannose), 3.62-5.28 (proton of mannose), 0.53-2.08 (brdd, -C(CH_3)-CH_2-)

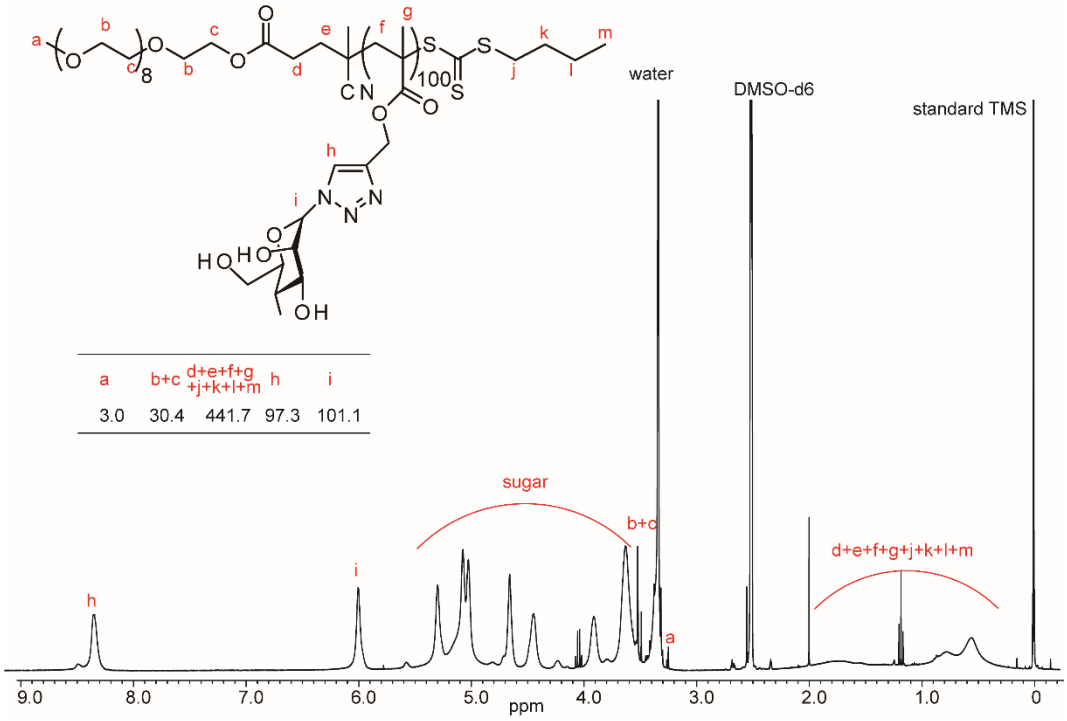
4. Additional figure.

4-1. ^{1}H NMR of each DHBG.



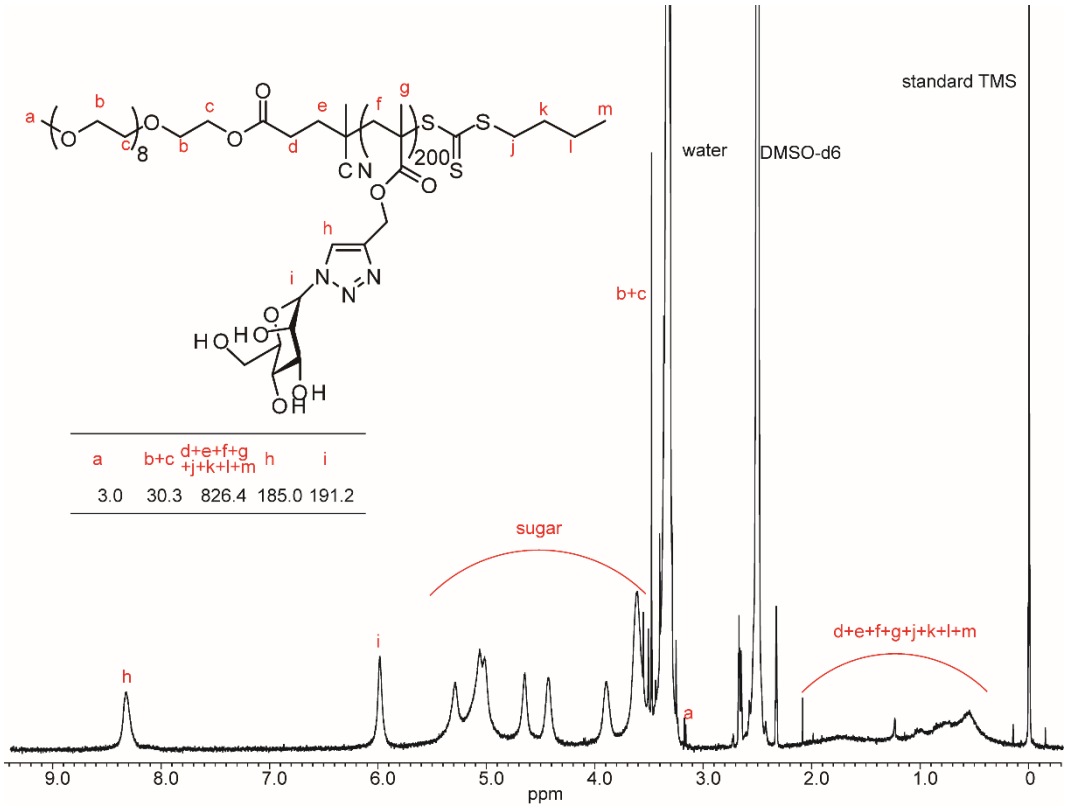
a	b+c	d+e+f+g +j+k+l+m	h	i
3.0	30.6	76.1	12.4	13.6

Figure S 10 ^{1}H NMR of P_9M_{10}



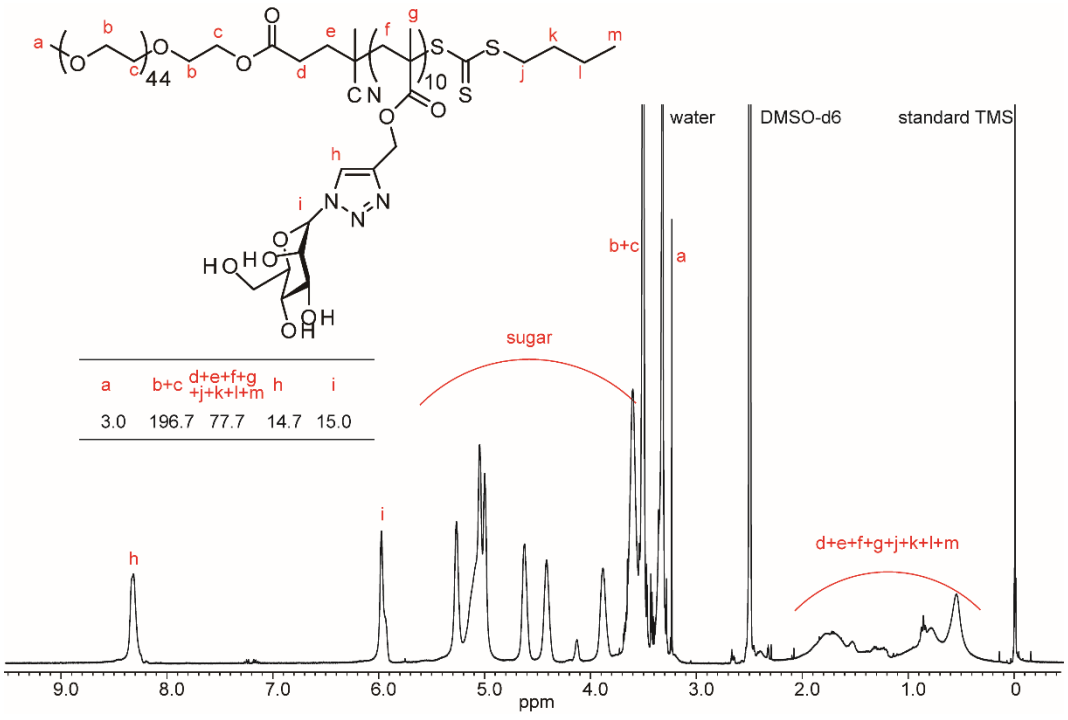
a	b+c	d+e+f+g +j+k+l+m	h	i
3.0	30.4	441.7	97.3	101.1

Figure S 11 ^{1}H NMR of P_9M_{100}



a	b+c	d+e+f+g +j+k+l+m	h	i
3.0	30.3	826.4	185.0	191.2

Figure S 12 ^{1}H NMR of P_9M_{200}



a	b+c	d+e+f+g +j+k+l+m	h	i
3.0	196.7	77.7	14.7	15.0

Figure S 13 ^{1}H NMR of $P_{45}M_{10}$

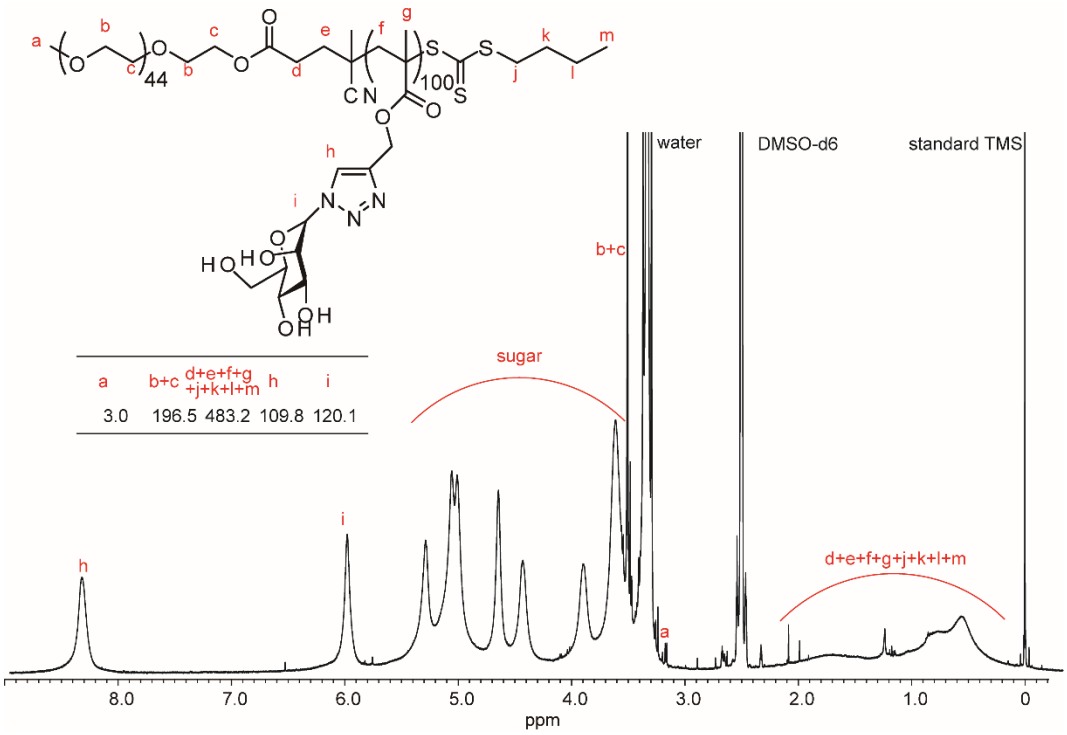


Figure S 14 ^{1}H NMR of $P_{45}M_{100}$

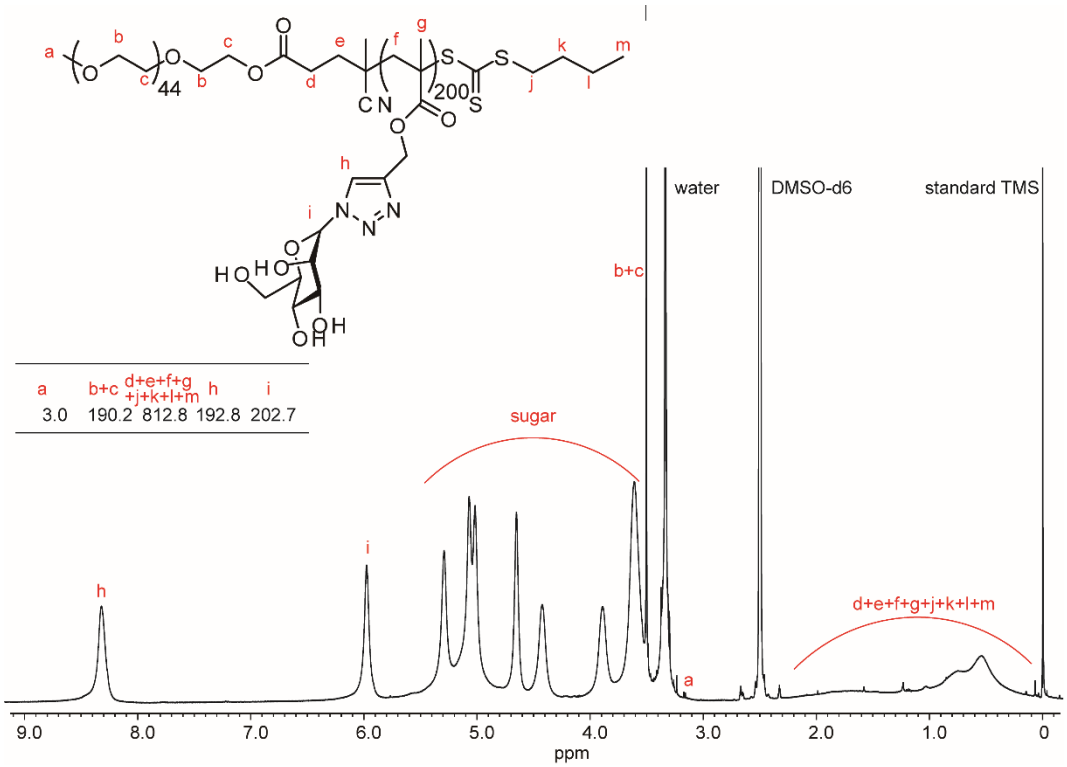


Figure S 15 ^{1}H NMR of $P_{45}M_{200}$

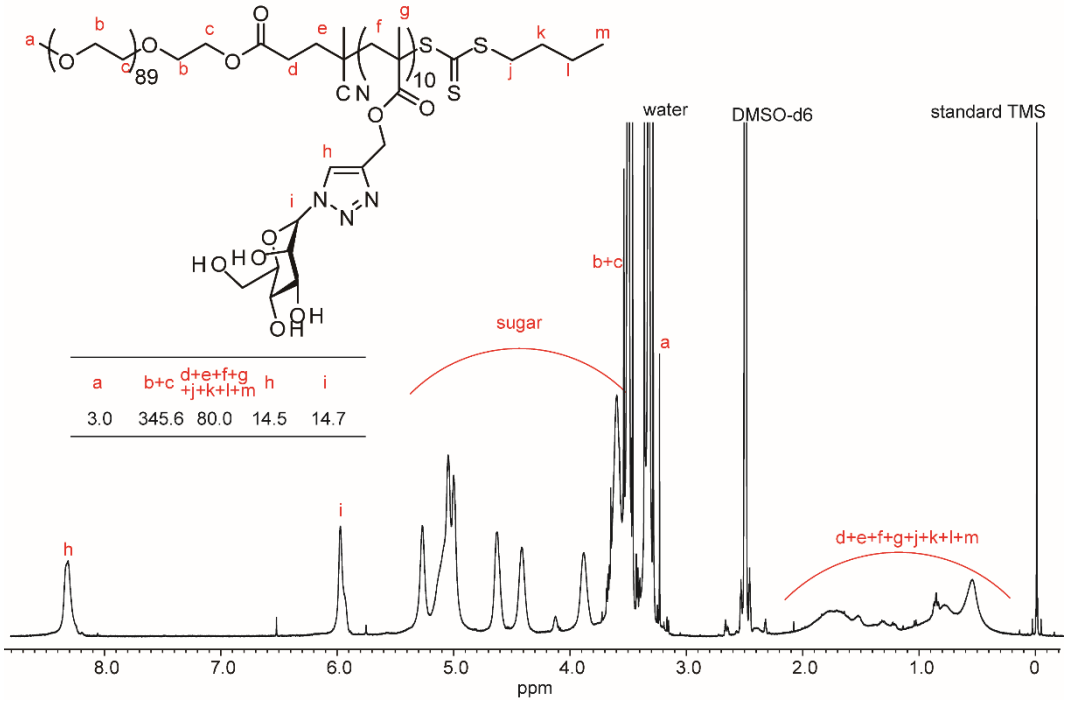


Figure S 16 ^{1}H NMR of $P_{90}M_{10}$

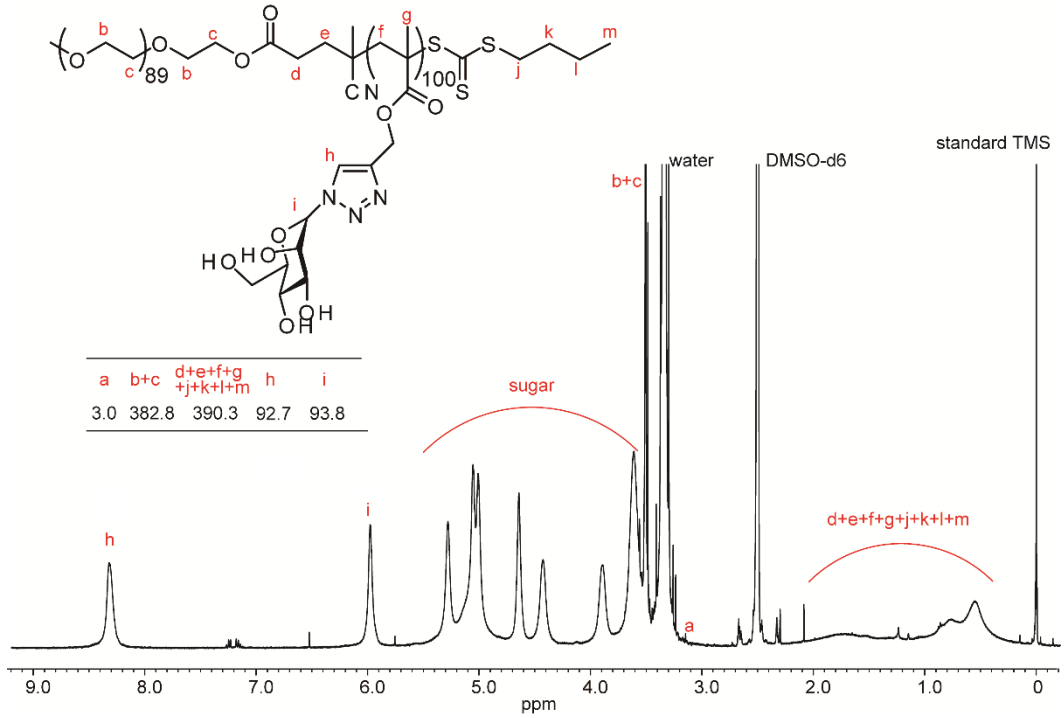


Figure S 17 ^{1}H NMR of $P_{90}M_{100}$

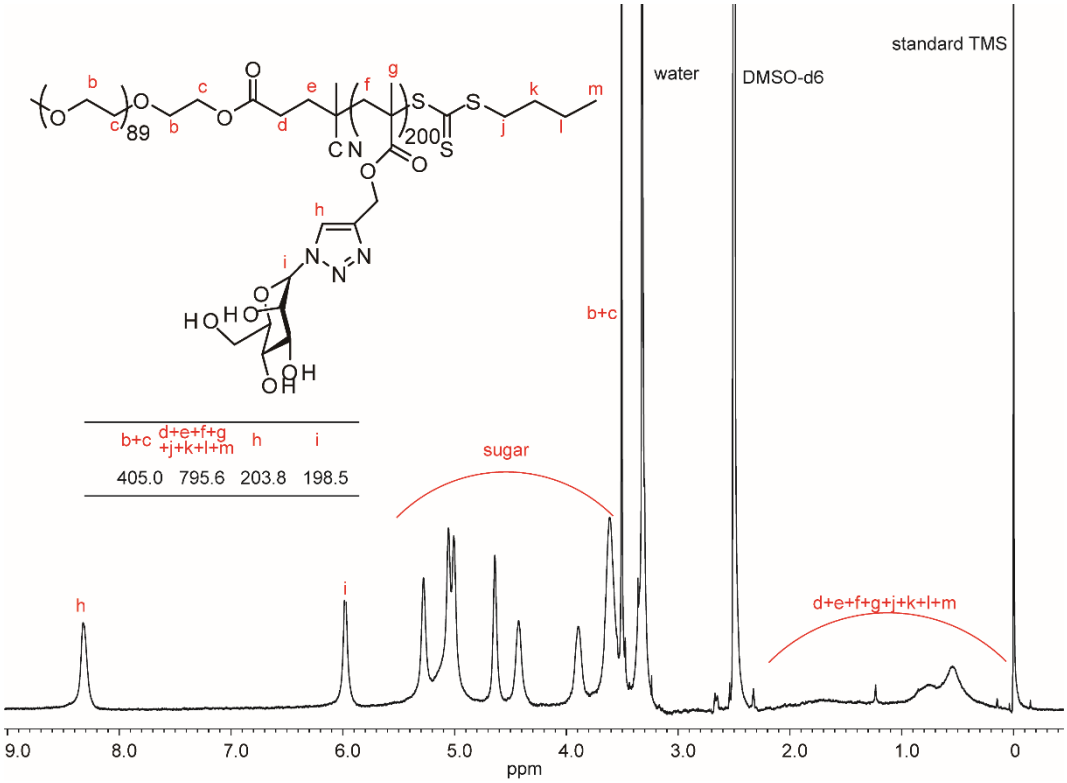


Figure S 18 ^{1}H NMR of $P_{90}M_{200}$.

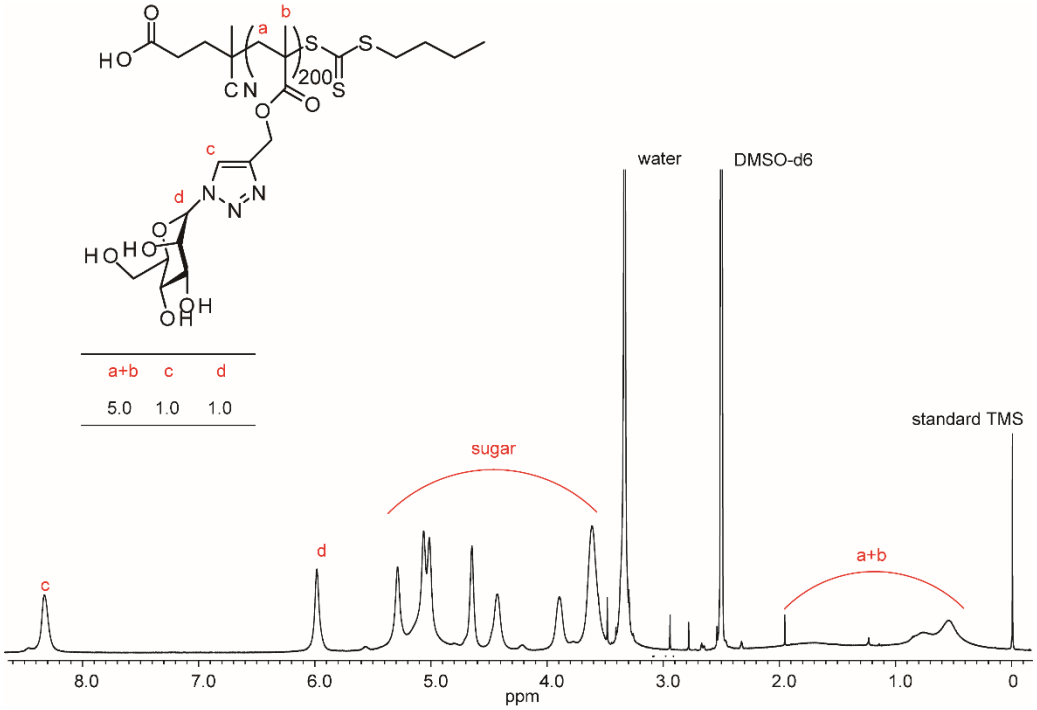


Figure S 19 ^{1}H NMR of M_{200}.

4-2. GPC measurement

From the GPC traces, **$P_{90}M_{200}$** and **$P_{90}M_{100}$** contained 6 and 3% of unreacted PEG.

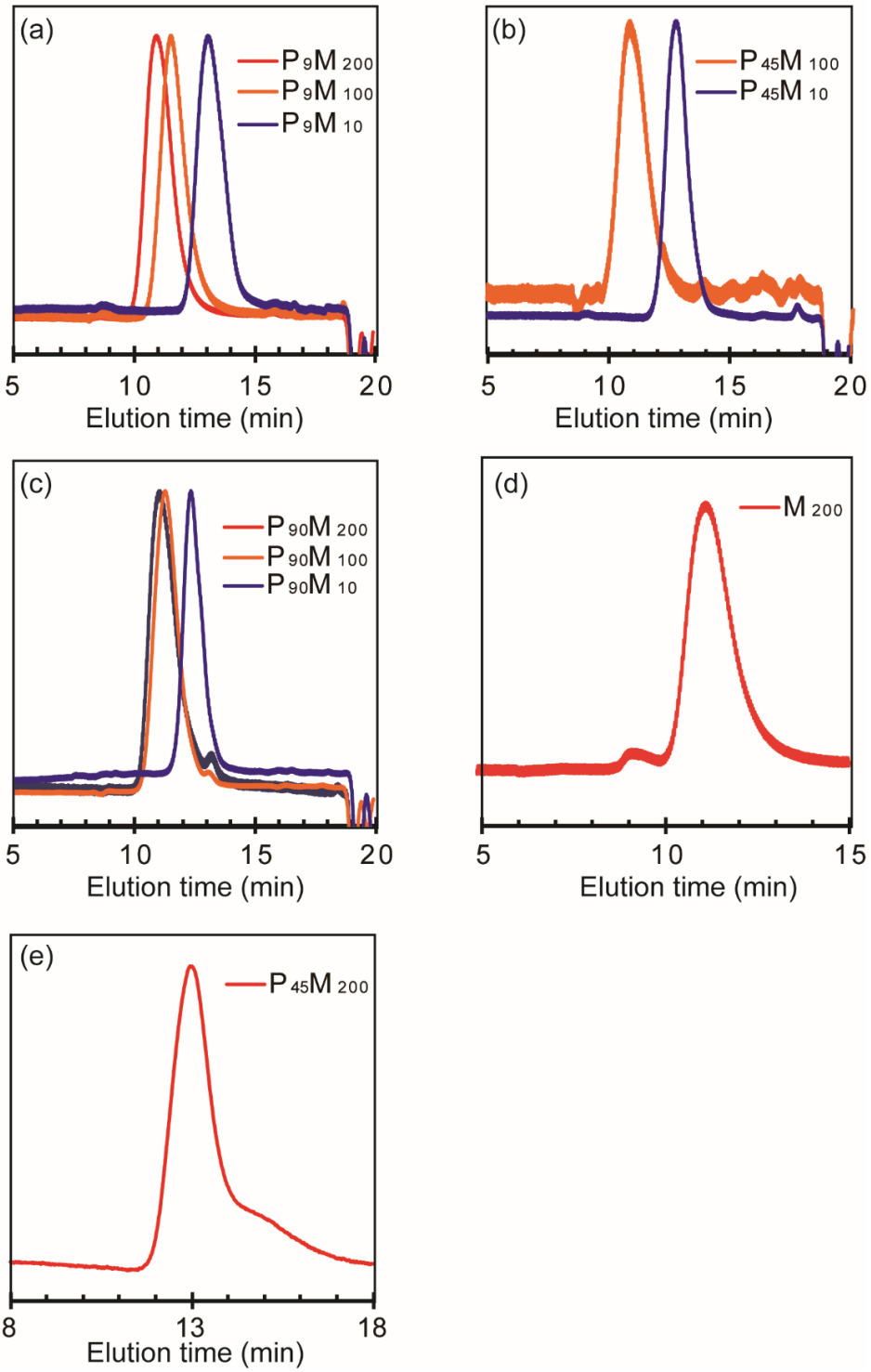


Figure S 20 GPC traces of each polymer. (a)-(d): DMF with LiBr (10 mM) was used as an eluent. (e): $NaNO_3$ aq. (100 mM) was used as an eluent, because the polymer couldn't be dissolved in DMF solution.

4-2. Turbidity measurement by UV-Vis spectrometer

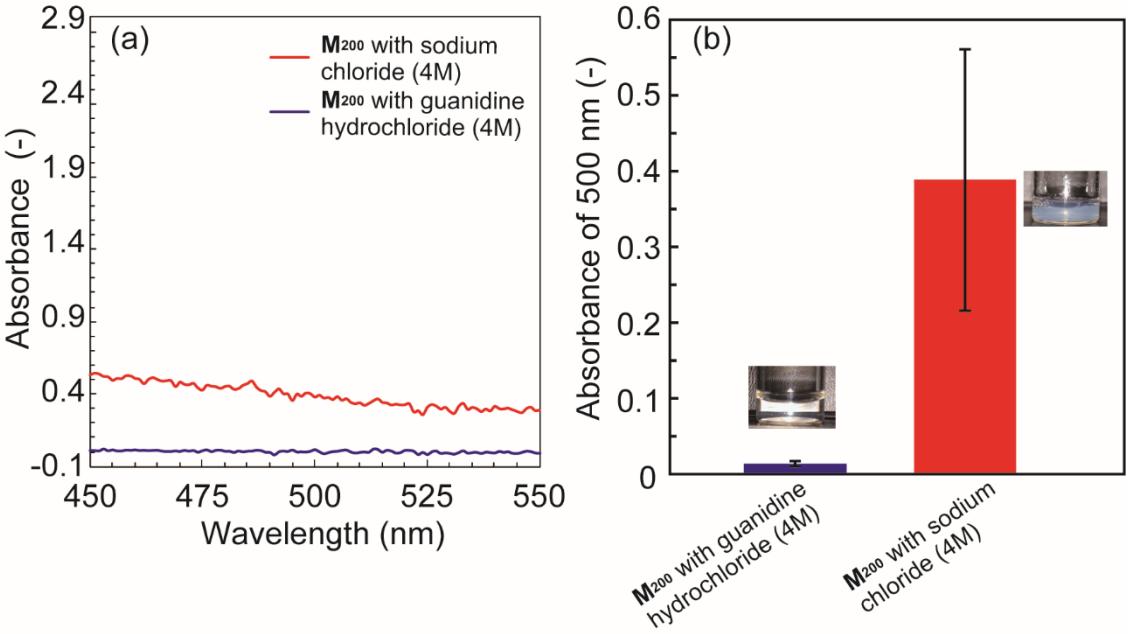


Figure S 21 turbidity measurement of mannose homopolymer (M_{200}) with guanidine hydrochloride (4M) and sodium chloride (4M). (a) raw data (b) bar graph of absorbance at 500 nm.

4-2. DLS result after incubation at 5°C.

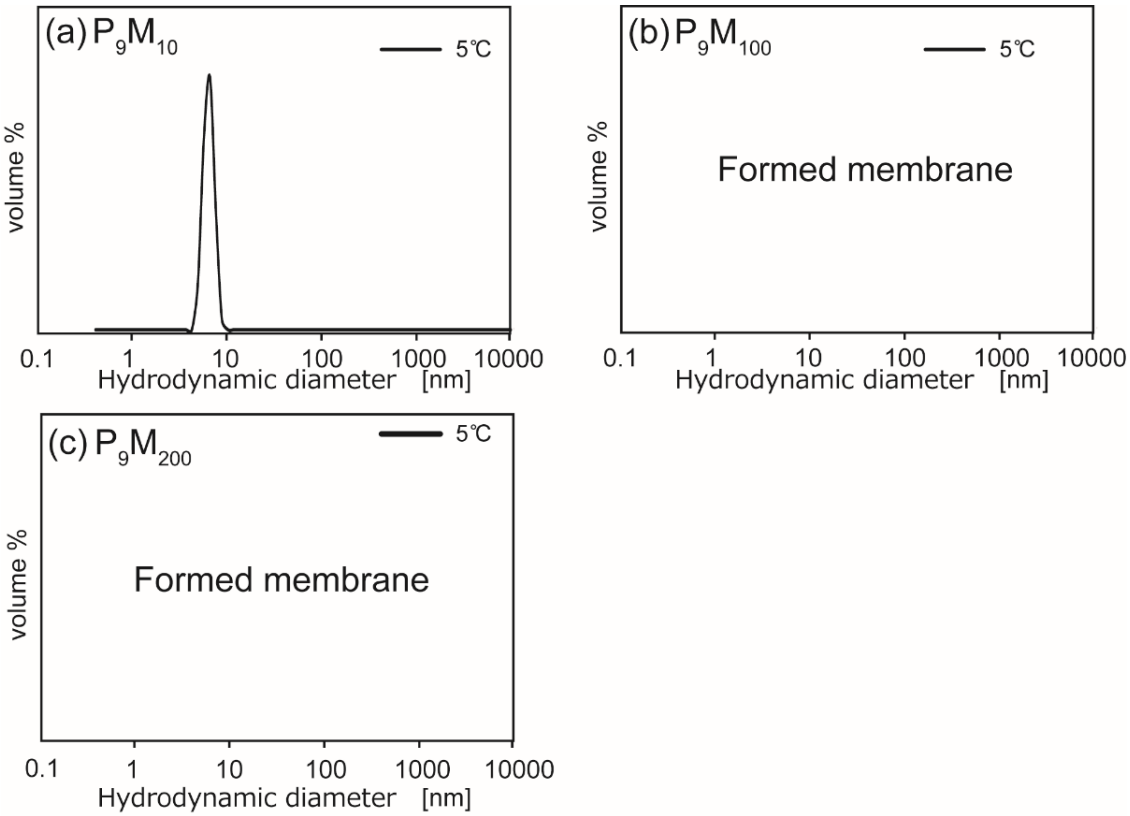


Figure S 22 hydrodynamic diameter of P_9Mn at polymer concentration 10 g/L by DLS measurement (a: n=10, b: n=100, c: n=200) The measurement was carried out after incubation at 5ºC for 16h. Solvent condition; HEPES, 10 mM; NaCl, 136.9 mM; KCl, 2.68 mM; $CaCl_2$, 1.80 mM; $MgCl_2 \cdot 6H_2O$, 0.49 mM.

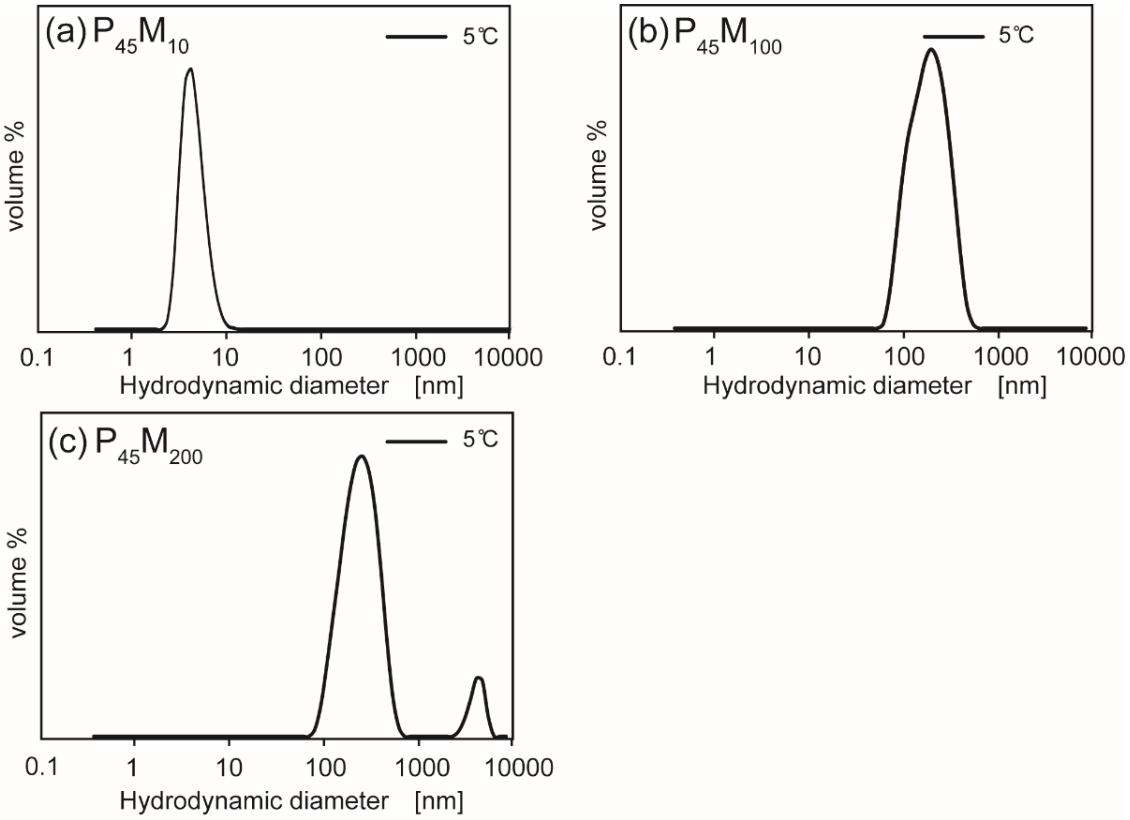


Figure S 23 hydrodynamic diameter of $P_{45}Mn$ at polymer concentration 10 g/L by DLS measurement (a: n=10, b: n=100, c: n=200). The measurement was carried out after incubation at 5ºC for 16h. Solvent condition; HEPES, 10 mM; NaCl, 136.9 mM; KCl, 2.68 mM; $CaCl_2$, 1.80 mM; $MgCl_2 \cdot 6H_2O$, 0.49 mM.

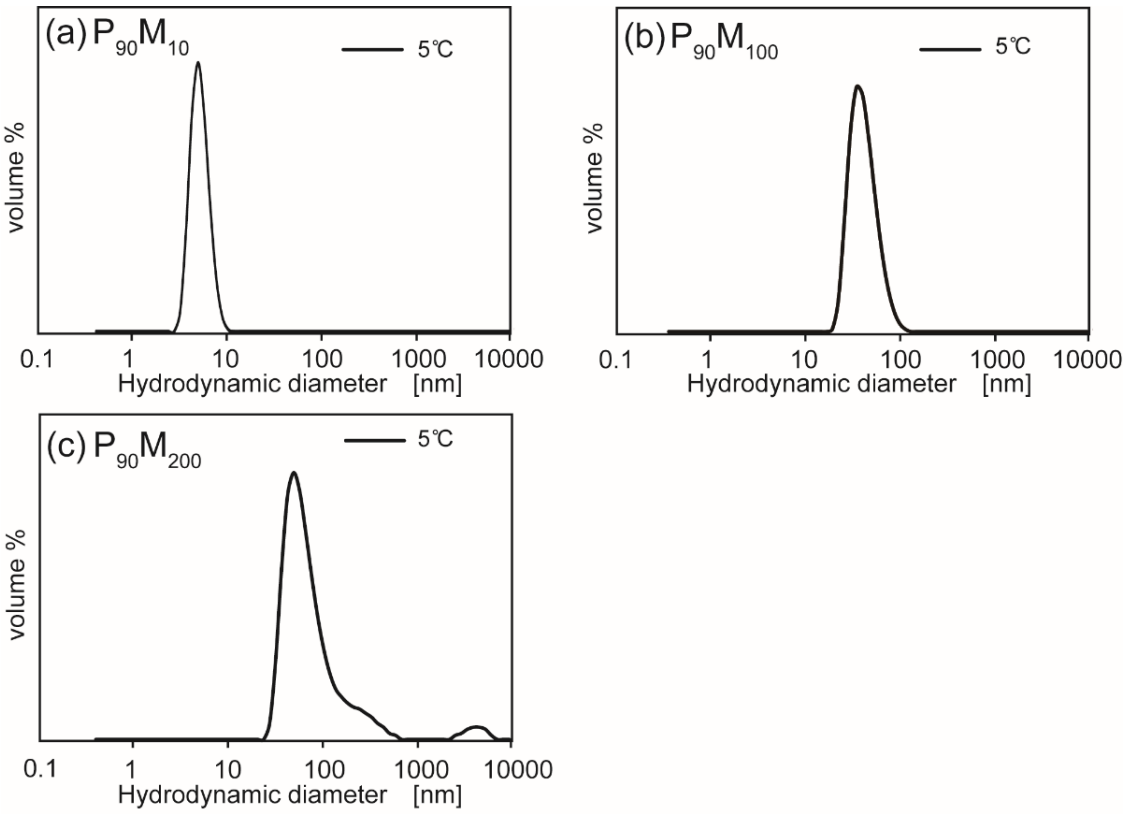


Figure S 24 hydrodynamic diameter of $P_{90}Mn$ at polymer concentration 10 g/L by DLS measurement (a: n=10, b: n=100, c: n=200). The measurement was carried out after incubation at 5ºC for 16h. Solvent condition; HEPES, 10 mM; NaCl, 136.9 mM; KCl, 2.68 mM; $CaCl_2$, 1.80 mM; $MgCl_2 \cdot 6H_2O$, 0.49 mM.

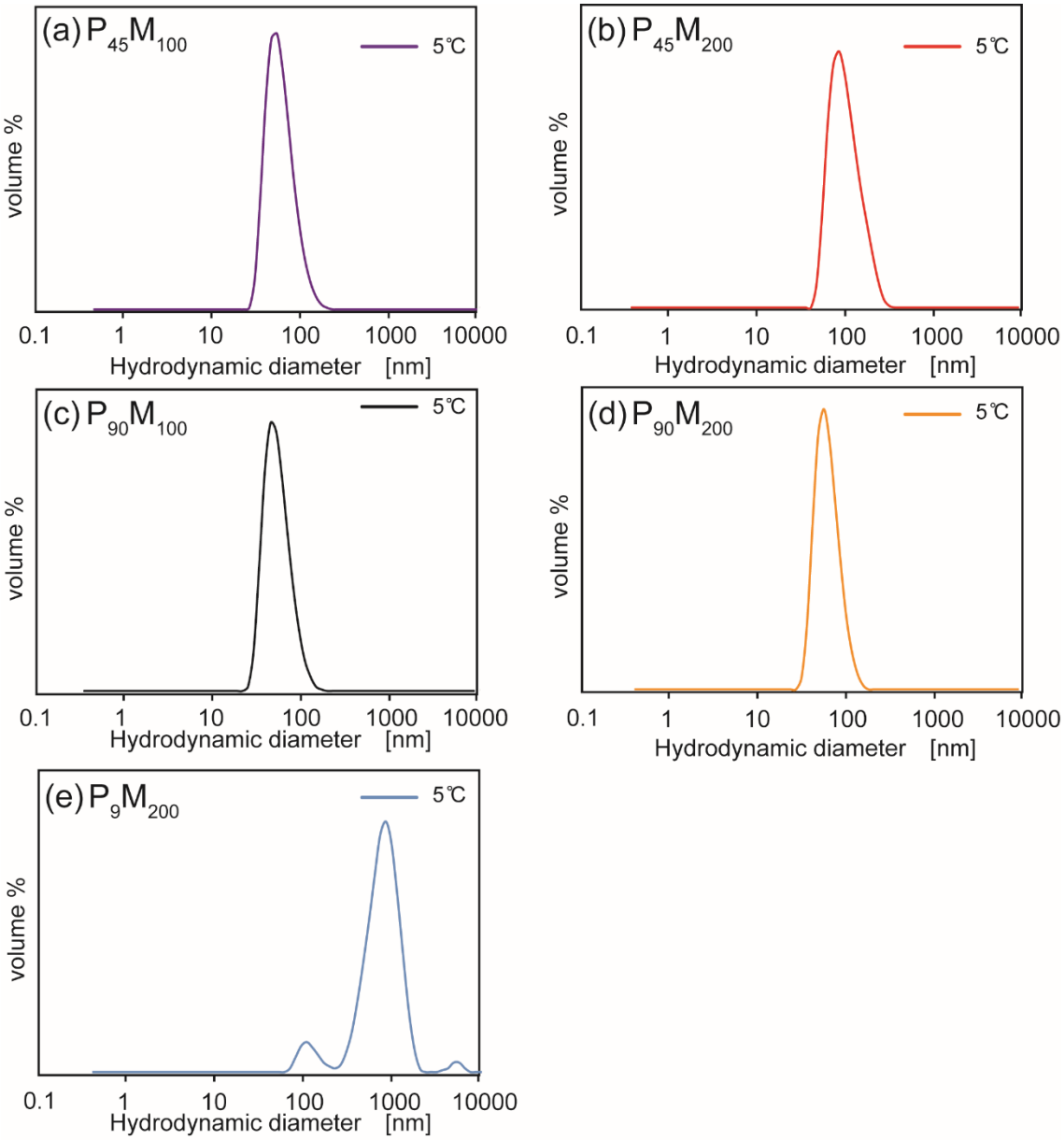


Figure S 25 hydrodynamic diameter of P_mM_n (m: 9, 45 and 90, n: 100 and 200) at polymer concentration 1 g/L obtained by DLS measurement. Solvent condition; HEPES, 10 mM; NaCl, 136.9 mM; KCl, 2.68 mM; $CaCl_2$, 1.80 mM; $MgCl_2 \cdot 6H_2O$, 0.49 mM.

4-3. TEM observation.

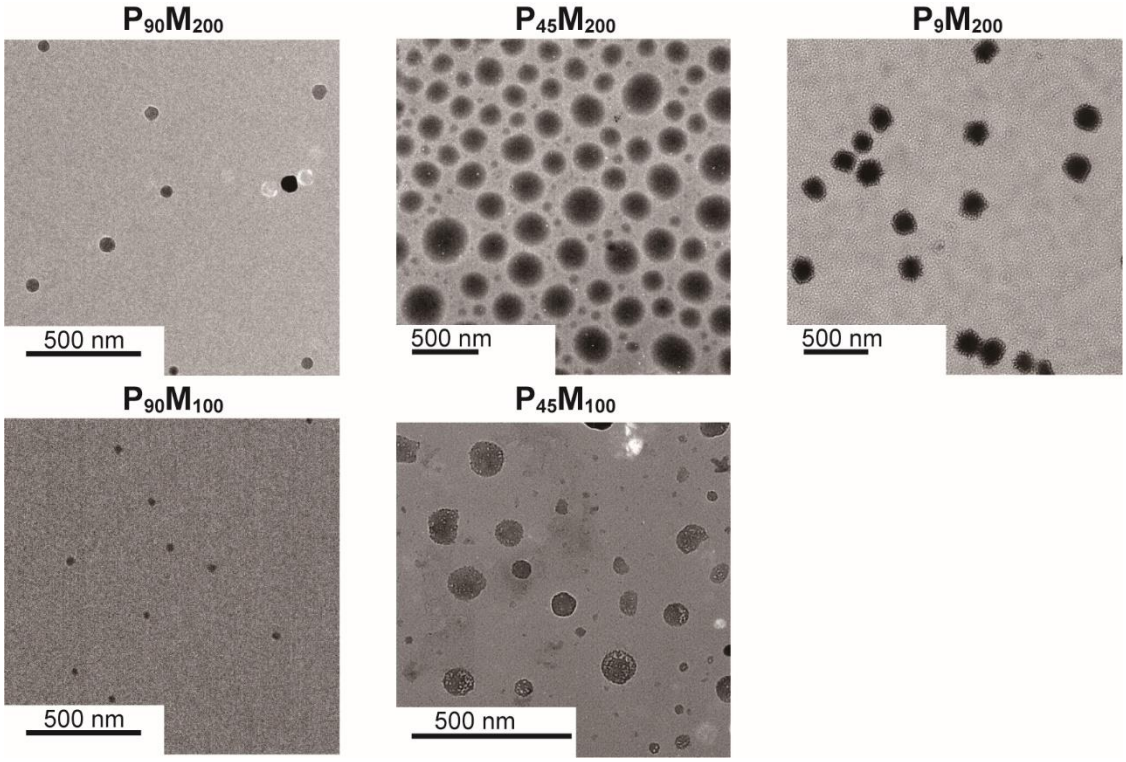


Figure S 26 TEM image of each DHBG stained by Osmium Tetroxide (4% in Water). Solvent condition; HEPES, 10 mM; NaCl, 136.9 mM; KCl, 2.68 mM; $CaCl_2$, 1.80 mM; $MgCl_2 \cdot 6H_2O$, 0.49 mM.

4-4. DLS measurement with temperature swing.

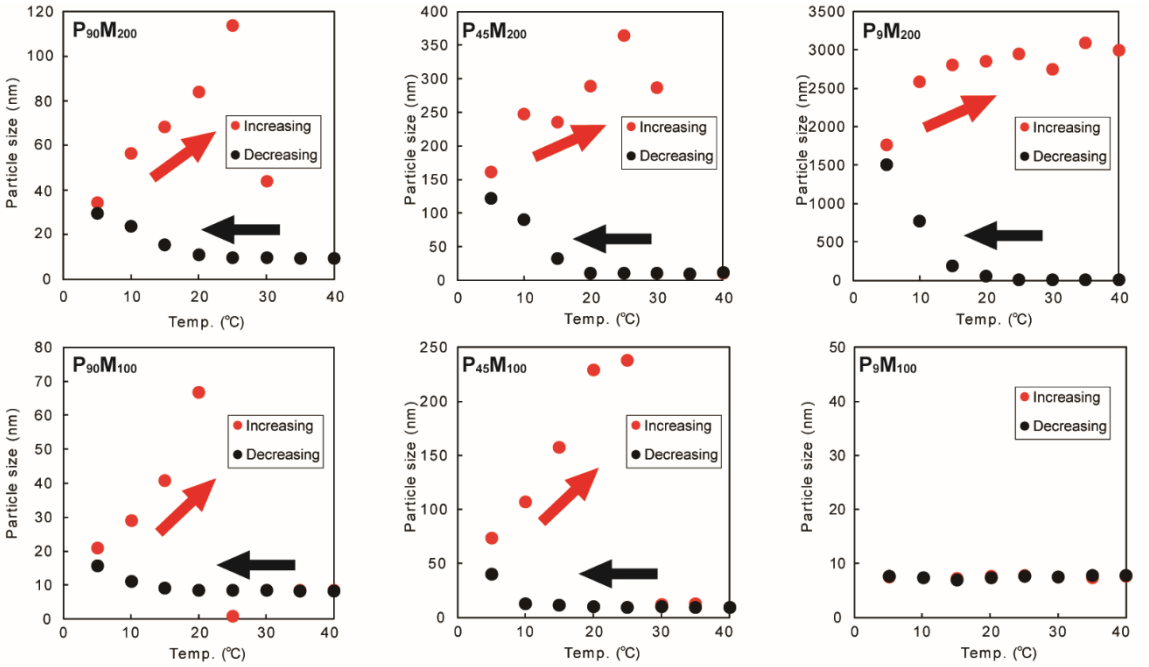


Figure S 27. DLS result with different temperature of each DHBG. Solvent condition; HEPES, 10 mM; NaCl, 136.9 mM; KCl, 2.68 mM; $CaCl_2$, 1.80 mM; $MgCl_2 \cdot 6H_2O$, 0.49 mM.

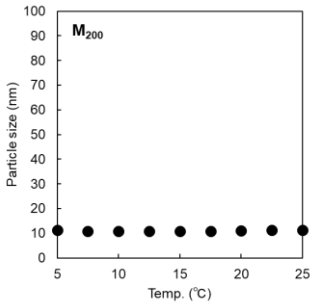


Figure S 28. DLS result with different temperature of mannose homopolymer (M_{200}). Solvent condition; HEPES, 10 mM; NaCl, 136.9 mM; KCl, 2.68 mM; $CaCl_2$, 1.80 mM; $MgCl_2 \cdot 6H_2O$, 0.49 mM.

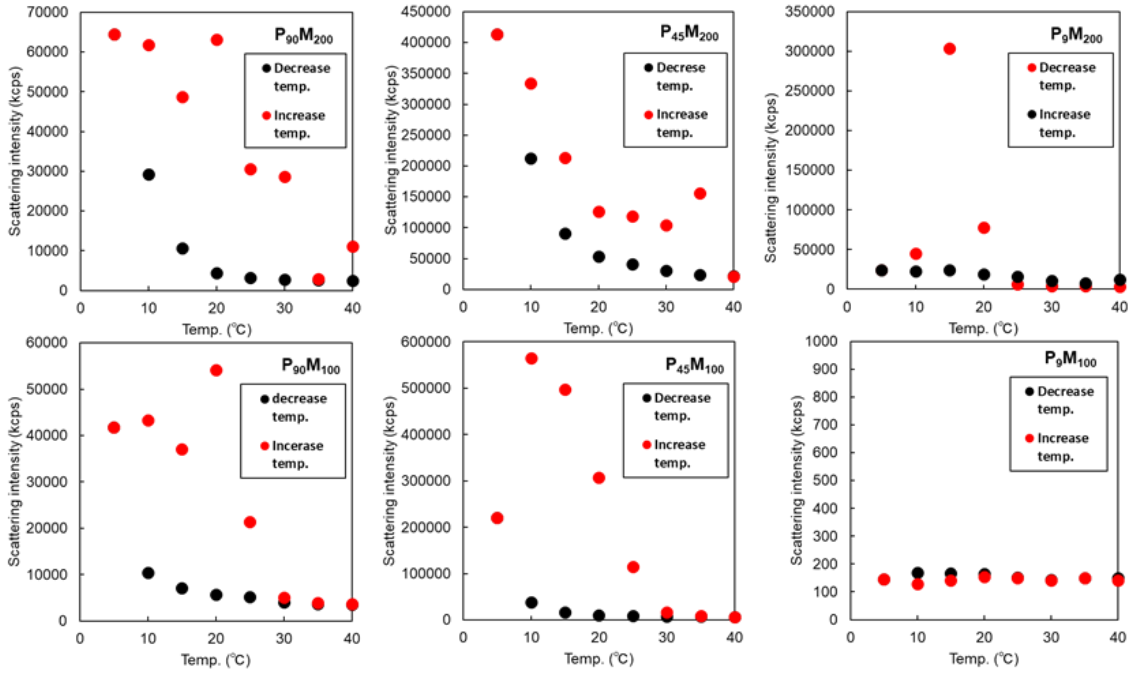


Figure S 29. The scattering intensity of each DHBG aqueous solution by DLS measurement. Solvent condition; HEPES, 10 mM; NaCl, 136.9 mM; KCl, 2.68 mM; $CaCl_2$, 1.80 mM; $MgCl_2 \cdot 6H_2O$, 0.49 mM.

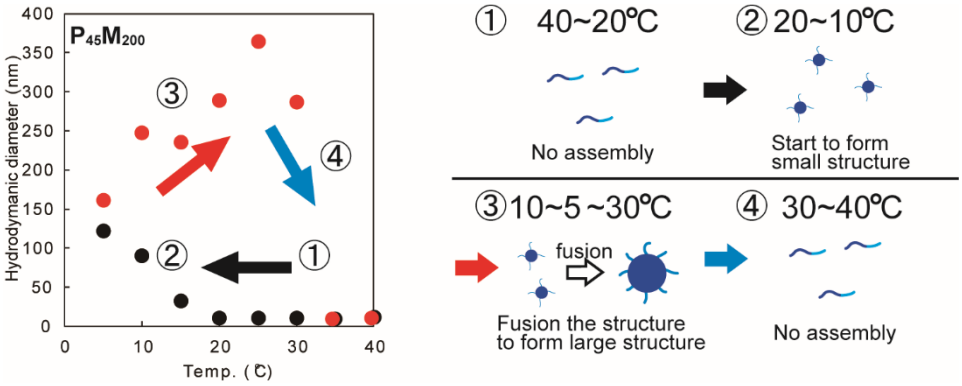


Figure S 30. Schematic illustration of the self-assembly process of $P_{45}M_{200}$ with temperature dependence.

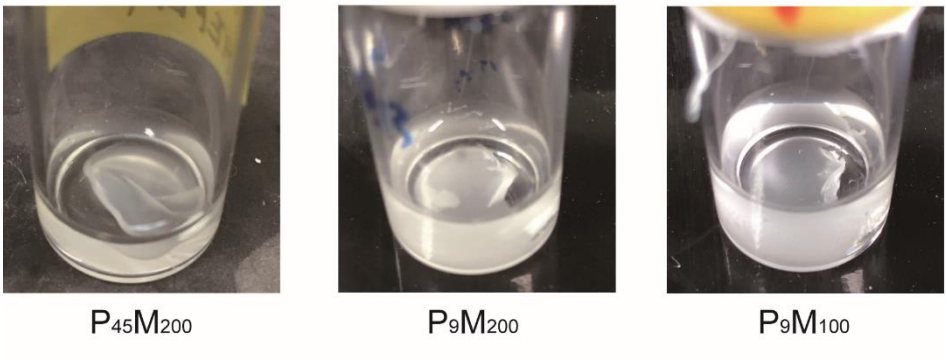


Figure S 31. The image of the membrane of $P_{45}M_{200}$, P_9M_{200} and P_9M_{100} after incubation at 4°C for over 2 days.

Reference

(1) T.Tanaka, H. Nagai, M. Noguchi, A. Kobayashi, S. Shoda, *Chem. Commun.* 2009, **23**, 3378–3379.

(2) N. Vinson, Y. Gou, C. R. Becer, D. M. Haddleton, M. I. Gibson, *Polym. Chem.* 2011, **2**, 107–113.

(3) M. Nagao, Y. Kurebayashi, H. Seto, T. Tanaka, T. Takahashi, T. Suzuki, Y. Hoshino, Y. Miura, *Polym. J.* 2016, **48**, 745–749.