

Article

Use of a Longer Aglycon Moiety Bearing Sialyl $\alpha(2\rightarrow3)$ Lactoside on the Glycopolymer for Lectin Evaluation

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Abstract: A polymerizable alcohol having 9 PEG repeats was prepared in order to mimic an oligosaccharide moiety. Sialyl $\alpha(2\rightarrow3)$ lactose, which is known as a sugar moiety of GM3 ganglioside, was also prepared, and the polymerizable alcohol was condensed with the sialyl $\alpha(2\rightarrow3)$ lactose derivative to afford the desired glycomonomer, which was further polymerized with or without acrylamide to give water-soluble glycopolymers. The glycopolymers had higher affinities than those of glycopolymers having sialyl lactose moieties with shorter aglycon moieties.

Keywords: glycopolymers; radical polymerizations; carbohydrates; glycosides; oligosaccharides; glycoclusters; fluorescence spectroscopy; lectins; affinity constant



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1. Introduction

Sialyl $\alpha(2\rightarrow3)$ lactose [Neu5Ac $\alpha(2\rightarrow3)$ Gal $\beta(1\rightarrow4)$ Glc; SLac] is known as a saccharide chain of GM3 ganglioside, and it exhibits a variety of biological activities [1]. Although the binding specificity of carbohydrate-binding proteins for SLac is high, the typical biological response of SLac to the carbohydrate-binding proteins is not good [10^{-3} M (mM) range] [2]. A remarkable enhancement of the low affinity of carbohydrates for lectins was accomplished by means of the sugar-clustering effect presented by Lee et al. [3,4]. Glycopolymers can be used as sugar-cluster substances [5,6], and various glycopolymers have been used as multivalent-type carbohydrate substrates for determining lectin—carbohydrate interactions [7,8]. Our ongoing synthetic studies using multivalent-type carbohydrate substances such as heterofunctional derivatives [9], glycodendrimers [10], and glycopolymers [11] showed effective enhancement of protein—carbohydrate interaction. Glycopolymers having SLac moieties as epitopes for the carbohydrate chain recognition protein on the mumps virus [12] were prepared, and they showed inhibitory potency against mumps viruses [13]. In addition to the mumps virus, avian influenza viruses also recognize the *N*-acetyl-neuraminic acid (Neu5Ac) residue in the SLac moiety [14]. Figure 1 shows the synthetic assembly of SLac moieties by means of polymer support, in which SLac having a triethylene glycol unit (PEG3) or a hexaethylene glycol unit (PEG6) was used as a linker between SLac and the polymer backbone displaying an appropriate length. In addition, acrylamide (AAm) was used as a controller adjusting the densities of inter SLac distances. Glycopolymers having SLac with PEG3 aglycon, as shown in Figure 1 were used as inhibitors for the mumps virus, and a glycopolymer having a low sugar density showed the highest inhibitory potency [13].

In this study, we planned to synthesize glycopolymers having a nonaethylene glycol unit (PEG9) moiety as a longer linker arm than that of previously synthesized glycopolymers, as shown in Figure 1. Figure 2 shows an outline of the synthesis of glycopolymers

having a longer linker arm. Biological evaluation of the glycopolymers for a lectin on the basis of fluorescence changes of the lectin was also carried out.

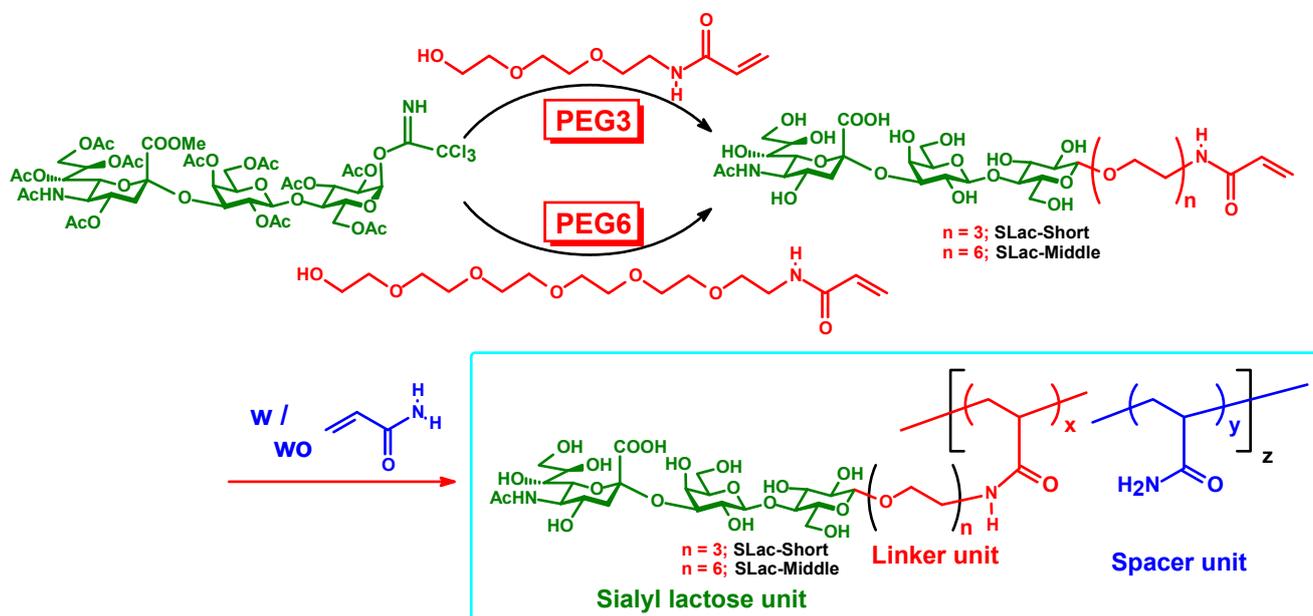


Figure 1. Synthetic assembly of glycomonomers bearing PEG3 ($n = 3$, short) or PEG6 ($n = 6$, middle) from a glycosyl imidate and polymers.

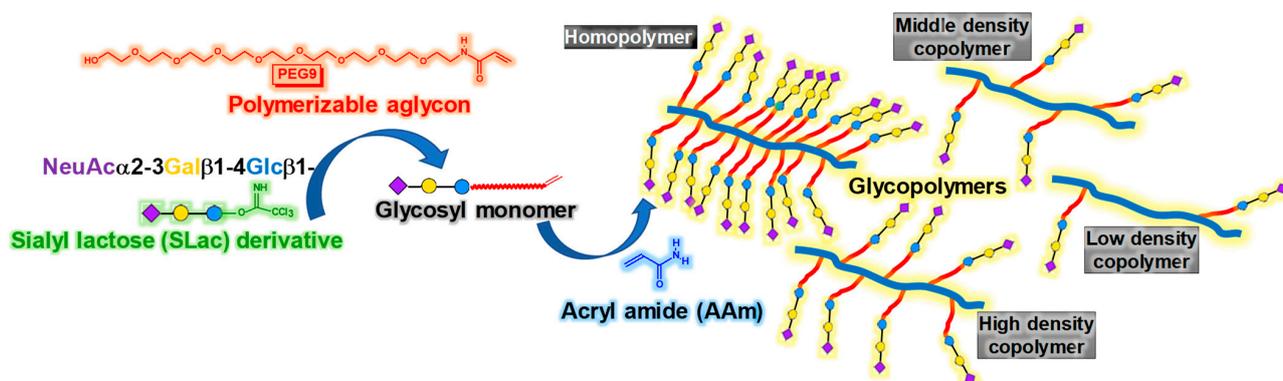


Figure 2. Synthetic plan for glycopolymers having PEG9 on the basis of various sugar densities.

2. Materials and Methods

2.1. Materials

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Methanol (MeOH) and dichloromethane (DCM) were stored over 3 Å MS, and 1,2-dichloromethane was stored over 4 Å MS before use. Acrylamide was recrystallized from chloroform (CHCl₃) before use. 17-Azido-3,6,9,12,15-pentaoxaheptadecan-1-ol (**1**) was prepared according to the previous reported method [15]. An imidate **6** used as a glycosyl donor was obtained by means of a known procedure [13]. Optical rotations were determined with a JASCO DIP-1000 digital polarimeter (JASCO Corporation, Tokyo, Japan). IR spectra were obtained using a Shimadzu IR Prestige-21 spectrometer (SHIMADZU CORPORATION, Kyoto, Japan). ¹H NMR spectra were recorded at 400 MHz for ¹H and at 101 MHz for ¹³C with a Bruker DPX-400 spectrometer (Bruker BioSpin MRI GmbH, Ettlingen, Germany) or at 500 MHz for ¹H and at 126 MHz for ¹³C with a Bruker AVANCE 500 spectrometer (Bruker BioSpin MRI GmbH, Ettlingen, Germany) in chloroform-*d* (CDCl₃), dimethyl sulfoxide-*d*₆ (DMSO-*d*₆), or deuterium oxide (D₂O). Chemical shifts

are expressed as parts per million (ppm, δ) and are relative to an internal tetramethylsilane (TMS) in CDCl_3 (δ 0.0), CH_3 in $\text{DMSO-}d_6$ (δ 2.50), or HDO in D_2O (δ 4.78) for ^1H and CDCl_3 in CDCl_3 (δ 77.0), CH_3 in $\text{DMSO-}d_6$ (δ 39.51), or CH_3 in MeOD (δ 49.0) for ^{13}C . Ring-proton assignments in the ^1H NMR spectra were made by a first-order analysis of the spectra, and these are supported by the results of homonuclear decoupling experiments and H–H or HMQC experiments. Elemental analyses were performed with a Fisons EA1108 (Fisons Co., Milan, Italy) on samples extensively dried at 50–60 °C over phosphorus pentoxide for 4–5 h. The reactions were monitored by thin layer chromatography (TLC) on a precoated plate of Silica Gel 60F₂₅₄ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). For the detection of the intermediates, TLC sheets were dipped in (a) a solution of 85:10:5 (*v/v/v*) MeOH–*p*-anisaldehyde–concd H_2SO_4 and heated for a few minutes (for carbohydrate) or (b) an aq solution of 5 wt% KMnO_4 and heated similarly (for the detection of C=C double bonds). Column chromatography was performed on silica gel (Silica Gel 60; 63–200 μm , E. Merck). Flash column chromatography was performed on silica gel (Silica Gel 60, spherical neutral; 40–100 μm , E. Merck). All the extractions were concentrated below 45 °C under diminished pressure. The weight-average molecular weights (*s*) were estimated by size-exclusion chromatography in 0.3 M aq NaCl using tandem-bonded Shodex SB-803 HQ and SB-804 HQ columns (SHOWA DENKO K.K., Tokyo, Japan). Calibration curves were obtained using pullulan standards (5.9, 11.8, 22.8, 47.3, 112, 212, 404, and 788 kDa; Shodex P-82). Wheat germ agglutinin (WGA; a lectin from *Triticum vulgare*) was purchased from J-Oil Mills (J-OIL MILLS, INC., Tokyo, Japan) (Lot # 62015).

2.2. Synthesis

2.2.1. 17-Azido-3,6,9,12,15-pentaoxaheptadecyl methanesulfonate (2)

To an ice-cold solution of azidoalcohol **1** (5.01 g, 16.3 mmol) and Et_3N (2.70 mL, 19.5 mmol) in DCM (50 mL) was dropwise added methanesulfonyl chloride (1.50 mL, 19.4 mmol) at 0 °C under an Ar atmosphere. After stirring at 0 °C for 1 h, TLC showed a complete conversion of the starting alcohol, and the mixture was filtered through a pad of Celite. The filtrate was concentrated in vacuo and diluted with THF. The organic solution was successively washed with water and brine, dried over anhyd Na_2SO_4 , filtered, and evaporated at diminished pressure to afford pure yellowish mesylate (6.25 g, 99%) as a syrup; R_f 0.53 [EtOAc]; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 4.32–4.26 (m, 2 H, OCH_2), 3.69–3.63 (m, 2 H, OCH_2), 3.62–3.49 (m, 20 H, $\text{OCH}_2 \times 10$), 3.16 (s, 3 H, CH_3); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 69.86 (OCH_2), 69.82 (OCH_2), 69.78 (OCH_2), 69.74 (OCH_2), 69.71 (OCH_2), 69.28 (OCH_2), 68.32 (OCH_2), 36.87 (CH_3); IR (NEAT) 2872 ($\nu_{\text{C-H}}$), 2110 ($\nu_{\text{N=N=N}}$), 1350 ($\nu_{\text{S=O}}$), 1174 ($\nu_{\text{S=O}}$), 1113 ($\nu_{\text{C-O}}$) cm^{-1} .

2.2.2. 26-Azido-3,6,9,12,15,18,21,24-octaoxahexacosan-1-ol (3)

A solution of *KOt*-Bt (5.26 g, 46.9 mmol) and triethylene glycol (11.0 mL, 82.6 mmol) in *t*-BuOH (60 mL) was refluxed for 1 h under an Ar atmosphere. Mesylate **2** (6.02 g, 15.6 mmol) was added to the mixture, and the mixture was further stirred for 2.5 h at refluxed temperature under an Ar atmosphere. TLC indicated the consumption of the starting materials and Celite filtration was performed for the reaction mixture. The filtrate was evaporated in vacuo; this was followed by chromatographic purification on silica gel with 20:1 (*v/v*) CHCl_3 –MeOH as the eluent to yield the desired azide alcohol **3** (6.03 g, 88%) as yellowish liquid; R_f 0.43 [4:1 EtOAc–MeOH (*v/v*)]; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 4.54 (t, 1 H, $J = 5.5$ Hz, OH), 3.60 (dd, 2 H, $J = 5.6$ & 4.4 Hz, OCH_2), 3.58–3.45 (m, 30 H, OCH_2), 3.45–3.36 (m, 4 H, OCH_2); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 72.32 (OCH_2), 69.80 (OCH_2), 69.76 (OCH_2), 69.67 (OCH_2), 69.22 (OCH_2), 60.19 (HOCH_2), 49.98 (OCH_2); IR (NEAT) 3482 ($\nu_{\text{O-H}}$), 2870 ($\nu_{\text{C-H}}$), 2110 ($\nu_{\text{N=N=N}}$), 1121 ($\nu_{\text{C-O}}$) cm^{-1} .

2.2.3. 26-Amino-3,6,9,12,15,18,21,24-octaoxahexacosan-1-ol (4)

A solution of azide alcohol **3** (5.33 g, 12.1 mmol) in MeOH (50 mL) was stirred for a few minutes at RT under an Ar atmosphere. $\text{Pd}(\text{OH})_2/\text{C}$ (1.64 g) was added to the

solution under an Ar atmosphere. The Ar gas was immediately replaced by H₂ gas, and the suspension was stirred for 6.5 h at RT under a H₂ atmosphere. TLC showed disappearance of the azide **3**, and the H₂ gas was changed to Ar gas. The suspension was filtered through a pad of Celite, followed by concentration at diminished pressure to give the corresponding amine **4** (3.93 g) in 78% yield; *R_f* 0.08 [4:1 EtOAc—MeOH (*v/v*)].

2.2.4. N-(26-Hydroxy-3,6,9,12,15,18,21,24-octaoxahehexacosyl)acrylamide (**5**)

Acryloyl chloride (0.40 mL, 4.92 mmol) was dropwise added to a solution of amine **4** (1.01 g, 2.45 mmol), hydroquinone (6 mg, 50 μmol), and diisopropyl ethylamine (1.3 mL, 7.46 mmol) in MeOH (10 mL) at ice-cold temperature under an Ar atmosphere. The reaction mixture was stirred for 1.5 h at the same temperature and for 1 h at RT. The reaction mixture was concentrated in vacuo, and silica gel chromatography with 4:1 (*v/v*) EtOAc—MeOH of the resulting syrup afforded desired acryl amide **5** (0.350 g, 31%) as a yellow syrup; *R_f* 0.39 [2:1 EtOAc—MeOH (*v/v*)]; ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.15 (br, 1 H, NH), 6.24 (dd, 1 H, *J*_{trans} = 17.1 and *J*_{cis} = 10.2 Hz, =CH=), 6.07 [dd, 1 H, *J*_{gem} = 2.2 Hz, =CH₂ (trans)], 5.57 [dd, 1 H, =CH₂ (cis)], 4.57 (br, 1 H, OH), 3.51 (m, 32 H, OCH₂), 3.44 (t, 2 H, *J* = 5.8 Hz), 3.41 (dd, 4 H, *J* = 5.6 and 4.6 Hz), 3.28 (q, 2 H, *J* = 5.8 Hz); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 164.64 (C=O), 131.73 (=CH=), 125.01 (=CH₂), 72.34 (OCH₂), 69.81 (OCH₂), 69.78 (OCH₂), 69.71 (OCH₂), 69.59 (OCH₂), 69.04 (OCH₂), 60.21 (OCH₂).

2.2.5. 28-Oxo-3,6,9,12,15,18,21,24-octaoxa-27-azatriacont-29-en-1-yl [Methyl(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate]-(2→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**7**)

Powdered molecular sieves 4 A (2.26 g) was added to a solution of imidate **6** (2.16 g, 1.79 mmol) and acrylamide alcohol **5** (1.02 g, 2.29 mmol) in DCM (20 mL) under an Ar atmosphere, and the suspension was stirred at RT for 30 min under an Ar atmosphere. To the suspension was dropwise added BF₃•OEt (0.45 mL, 3.58 mmol) at −15 °C under an Ar atmosphere and the mixture was stirred at the same temperature for 30 min, followed by further stirring at RT for 1 h. When TLC showed consumption of the starting materials, the suspension was passed through a pad of Celite. The filtrate was diluted with CHCl₃, and the organic solution was successively washed with satd aq NaHCO₃ and brine, dried over anhyd Na₂SO₄, filtered, and evaporated in vacuo. The residue yielded the desired glycoside **7** (0.953 g, 35%) and the corresponding hemiacetal **8** (1.13 g, 59%) after chromatographic purification on silica gel with 5:1 (*v/v*) EtOAc—MeOH as the eluent; *R_f* 0.33 [4:1 EtOAc—MeOH (*v/v*)]; ¹H NMR (400 MHz, CDCl₃) δ 6.78 (br s, 1 H, NHCO), 6.29 [dd, 1 H, *J*_{trans} = 17.0 Hz & *J*_{gem} = 1.8 Hz, CH₂ (trans)], 6.17 (dd, 1 H, *J*_{cis} = 10.1 Hz, CH=), 5.61 [dd, 1 H, =CH₂ (cis)], 5.54 (ddd, 1 H, *J*_{7'',8''} = 9.2 Hz, *J*_{8'',9''a} = 5.1 Hz, & *J*_{8'',9''a} = 2.7 Hz, H-8''), 5.39 (dd, 1 H, *J*_{6'',7''} = 2.8 Hz, H-7''), 5.18 (t, 1 H, *J*_{2,3} = *J*_{3,4} = 9.3 Hz, H-3), 4.97–4.83 (m, 4 H, H-2, H-2', H-4', & H-4''), 4.67 (d, 1 H, *J*_{1',2'} = 8.0 Hz, H-1'), 4.55 (d, 1 H, *J*_{1,2} = 8.0 Hz, H-1), 4.52 (dd, 1 H, *J*_{2',3'} = 10.2 & *J*_{3',4'} = 3.3 Hz, H-3'), 4.48–4.38 (m, 2 H, H-6 & H-9a''), 4.27–3.86 (m, 8 H), 3.84 (s, 3 H, COOMe), 3.76–3.53 (m, 41 H, OCH₂CH₂), 2.58 (dd, 1 H, *J*_{3''ax,3''eq} = 12.7 Hz & *J*_{3''eq,4''} = 4.6 Hz, H-3''eq), 2.16, 2.09, 2.09, 2.08, 2.07, 2.06, 2.04, 2.03, 2.00, 1.85 (each s, 10 Ac), 1.67 (t, 1 H, *J*_{3''ax,4''} = 12.4 Hz, H-3''ax); ¹³C NMR (101 MHz, CDCl₃) δ 170.99 (C=O), 170.77 (C=O), 170.62 (C=O), 170.51 (C=O), 170.44 (C=O), 170.37 (C=O), 169.90 (C=O), 169.77 (C=O), 169.70 (C=O), 168.09 (C=O), 165.79 (C=O), 131.25 (CH=), 126.17 (CH₂=), 101.13 (C-1'), 100.78 (C-1), 96.92 (C-2''), 77.48, 77.16, 76.84, 76.41, 73.51, 72.83, 72.15, 71.91, 71.52, 70.81, 70.71, 70.68, 70.65 (OCH₂), 70.37, 70.32, 70.06, 69.97, 69.46, 69.21, 67.91, 67.45, 67.05, 62.44, 62.36 (C-6), 61.66 (C-6'), 60.52, 53.26 (OMe), 49.26 (C-5''), 39.45 (OCH₂), 37.54 (C-3''), 23.30 (CH₃), 21.65 (CH₃), 21.08 (CH₃), 20.96 (CH₃), 20.92 (CH₃), 20.86 (CH₃), 20.82 (CH₃), 20.74 (CH₃); IR (KBr) 3431 (ν_{N-H}), 3277 (ν_{N-H}), 2943 (ν_{C-H}), 2880 (ν_{C-H}), 1748 (ν_{C=O}, ester), 1665 (ν_{C=O}, amide I), 1545 (δ_{N-H}, amide II), 1371 (δ_{C-O}), 1043 (ν_{C-O-C}) cm⁻¹; [α]_D²⁵ = −4.96° (*c* 1.16, CHCl₃); MALDI-TOF MS calcd for [M+Na]⁺: 1539.584. Found: *m/z* 1539.488, calcd for [M+K]⁺: 1555.558. Found: *m/z* 1555.476.

Anal. Calcd for $C_{65}H_{100}N_2O_{38} \bullet 3 H_2O$: C, 49.68; H, 6.80; N, 1.78. Found: C, 49.64; H, 6.69; N, 1.57.

2.2.6. 28-Oxo-3,6,9,12,15,18,21,24-octaoxa-27-azatriacont-29-en-1-yl (5-Acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosyl)-(2 \rightarrow 3)-O-(β -D-galactopyranosyl)-(1 \rightarrow 4)- β -D-glucopyranoside (**9**)

Acetate **7** (0.902 g, 0.594 mmol) was treated with 0.1 M methanolic NaOMe (9 mL) at RT for 4 h under an Ar atmosphere. TLC indicated complete conversion of **7**, and Dowex 50W-x8 (H^+) was added to the reaction mixture until pH reached pH 7 on pH paper. The suspension was passed through a pad of cotton, and the filtrate was concentrated in vacuo. The residue was allowed to react with 0.05 M aq NaOH (9 mL) at RT for 3 h under an Ar atmosphere. To the reaction mixture was added Dowex 50W-x8 (H^+) until pH reached pH 7 on pH paper and the mixture was filtered. The filtrate was directly lyophilized and gave a white powdery mass **9** (0.586 g) in 91% yield: R_f 0.56 [5:4:1 $CHCl_3$ —MeOH— H_2O (v/v)]; 1H NMR (400 MHz, D_2O) δ 6.33 (dd, 1 H, $J_{trans} = 17.2$ Hz & $J_{cis} = 10.0$ Hz, CH=), 6.24 [dd, 1 H, $J_{gem} = 1.6$ Hz, =CH₂ (trans)], 5.81 [dd, 1 H, =CH₂ (cis)], 4.57 (d, 1 H, $J_{1'2'} = 8.0$ Hz, H-1'), 4.55 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 3.52 (t, 2 H, $J = 5.2$ Hz, CH₂N), 3.38 (t, 1 H, $J_{2,3} = 8.3$ Hz, H-2), 2.79 (dd, 1 H, $J_{3''ax,3''eq} = 12.6$ Hz & $J_{3''eq,4''} = 4.6$ Hz, H-3''eq), 2.07 (s, 3 H, NAc), 1.89 (t, 1 H, $J_{3''ax,4''} = 12.2$ Hz, H-3''ax); ^{13}C NMR (101 MHz, D_2O) δ 175.02 (C-1''), 172.77 [C=O(Ac)], 168.63 [C=O(acryloyl)], 129.91 (CH=), 127.40 (=CH₂), 102.66 (C-1'), 102.15 (C-1), 99.24 (C''-2), 78.30, 75.50 (C-3'), 75.09, 74.78 (C-2'), 74.30, 73.05, 72.83 (C-2), 71.38, 69.59, 69.45, 68.74, 68.15, 67.95, 67.56, 62.79, 60.95, 60.09, 51.68 (C-5''), 39.28 (C-3''), 39.03 (CH₂N), 22.07 (CH₃); IR (KBr) 3399 (ν_{O-H} , ν_{N-H}), 2926 (ν_{C-H}), 2887 (ν_{C-H}), 1647 ($\nu_{C=O}$ amide I), 1558 (δ_{N-H}), 1070 (ν_{C-O-C}) cm^{-1} ; $[\alpha]_D^{25} = -2.11^\circ$ (c 1.13, H_2O); MALDI-TOF MS calcd for $[M+Na]^+$: 1105.463. Found: m/z 1105.656.

Anal. Calcd for $C_{44}H_{78}N_2O_{28} \bullet 4 H_2O$: C, 45.75; H, 7.50; N, 2.43. Found: C, 45.91; H, 7.54; N, 2.21.

2.2.7. Radical Polymerization

A solution of appropriate amounts of carbohydrate monomer and acrylamide (**AAM**) in 1:1 (v/v) deionized water—DMSO was deaerated under reduced pressure for a few minutes, and then N,N,N',N' -tetramethylethylenediamine (TEMED) (0.2 molar equivalent for the corresponding carbohydrate monomer) and ammonium persulfate (APS) (0.1 molar equivalent for the corresponding carbohydrate monomer) were added under an Ar atmosphere at RT. The mixture was stirred at RT for an appropriate time and diluted with 0.1 M aq pyridine—acetic acid buffer (pH 5.2). The viscous solution was dialyzed against distilled water, followed by lyophilization to provide the corresponding white powdery glycopolymers **10a**~**10d**. The results of radical polymerization are summarized in Table 1.

Table 1. Results of polymerizations using different ratios of carbohydrate monomers **9** and acrylamide (**AAM**).

Polymer	Monomer Ratio		Yield ¹ (%)	Polymer Composition ²			\overline{Mw} ³ (KDa)	$\overline{Mw}/\overline{Mn}$
	9	AAM		x	y	z		
10a	1	0	98	1	0	48	160	2.99
10b	1	4	99	1	4.6	175	239	1.86
10c	1	10	77	1	13	193	347	1.2
10d	1	25	74	1	26	112	321	1.19

¹ Total yields were calculated on the basis of quantities of monomers used. ² Polymer compositions of x:y were estimated on the basis of the results of 1H NMR. ³ The weight-average molecular weights (\overline{Mws}) were estimated by size-exclusion chromatography.

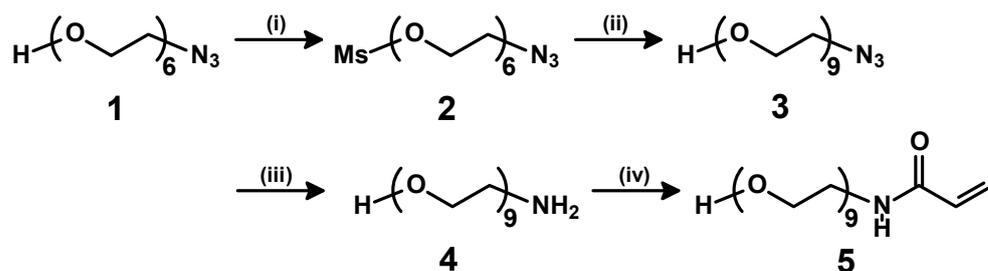
3. Results and Discussion

In our ongoing synthetic studies, glycomonomers having a shorter polymerizable linker than that in this study have been reported [16]. The radical polymerization reaction proceeded smoothly to afford the corresponding glycopolymers having various SLac-saccharide densities, and biological evaluations of the various glycopolymers for wheat germ agglutinin (WGA) were carried out. The synthetic assembly of SLac having a longer polymerizable linker is described.

3.1. Monomer Synthesis

3.1.1. Synthesis of PEG9 Linker

Our synthetic target as a polymerizable alcohol **5** is illustrated in Scheme 1. The elongation of oxyethylene repeating units was achieved by means of simple chemical reactions. Thus, a known azide alcohol **1** [16] was converted to the corresponding mesylate **2** in quantitative yield. An elongation of the triethylene glycol unit for **2** was carried out by using KO*t*-Bu as a nucleophilic reagent in order to activate the alcohol moiety generating the alkoxide in situ. The replacement of mesylate by triethylene glycol proceeded smoothly at refluxed temperature in *t*-BuOH to afford the corresponding azide alcohol **3** having nine polyethylene repeating units (PEG9) in 88% yield. The reduction of azide was achieved by means of typical hydrogenolysis conditions under a H₂ atmosphere giving the corresponding amine **4** in 78% yield.



Scheme 1. Reagents and conditions: (i) MsCl (1.2 molar equiv), Et₃N (1.2 molar equiv), DCM, 0 °C, 1 h; (ii) TEG (5.3 molar equiv), KO*t*-Bu (8.4 molar equiv), *t*-BuOH, reflux, 1 h, then **2**, reflux, 2.5 h; (iii) Pd(OH)₂/C, H₂, MeOH, RT, 6.5 h; (iv) HQ (0.02 molar equiv), DIEA (3.0 molar equiv), acryloyl chloride (2.0 molar equiv), MeOH, 0 °C, 1.5 h.

As the aminoalcohol **9** having a PEG9 linker had been obtained, our attention turned to the acryloylation of the amine of **9**. Consequently, acryloyl chloride was added to the solution of amine **4** in the presence of Et₃N in MeOH; however, none of the desired **5** was obtained, as shown in entry 1 in Table 2. The amide formation reaction proceeded on TLC, and subsequent polymerization of the acrylamide functional group would have occurred. In addition, a large amount of triethylamine-hydrochloride salt produced in the reaction hindered the isolation of pure **5** from the reaction mixture. The use of an ion exchange resin instead of Et₃N was suggested as a means of avoiding this situation. Therefore, IRA400J was used as the anion exchange resin for the reaction; however, the resin did not function as an acid scavenger, and no product at all was obtained, as shown in entry 2 in Table 2. Although amide formation would have proceeded in the reaction conditions, the polymerization of the acrylamide moiety also occurred simultaneously. It was found that protection of the free radical in the reaction mixture was needed to avoid this side reaction [17]. A free radical inhibitor was used for the preparation of acrylamide alcohol **5** [18]. Thus, a small amount of hydroquinone (HQ) was applied to the acryloylation, as shown in entry 3 in Table 2. The reaction proceeded, and the desired acrylamide derivative was obtained in 24% yield. We tried changing the base in addition to controlling the amounts of the base and the HQ (entry 3–6). When 3 molar equivalents of *N,N*-diisopropylethylamine (DIPEA)

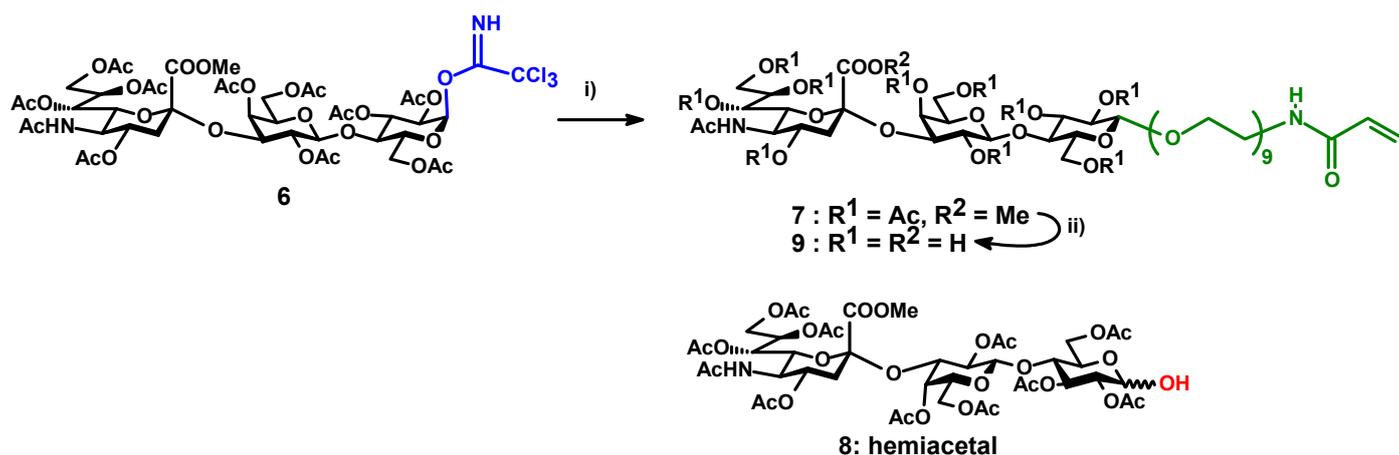
and 0.02 molar equivalent of HQ were used for the reaction, the best yield of acrylamide 5 was obtained.

Table 2. Results of acryloylation for aminoalcohol 4.

Entry	CH ₂ =CHCOCl (Molar Eq)	Base (Molar Eq)	HQ (Molar Eq)	Solvent	Yield (%)
1	2	Et ₃ N, 3	-	MeOH	-
2	2	IRA400J	-	MeOH	-
3	2	Et ₃ N, 3	0.02	MeOH	24
4	1.2	Et ₃ N, 1.5	0.02	DCM	8
5	2	DIPEA, 3	0.02	MeOH	31
6	2	DIPEA, 3	0.04	MeOH	30

3.1.2. Synthesis of SLac-PEG9 Monomer

Given the success in the preparation of acrylamide alcohol, our next area of interest was the incorporation of the alcohol into SLac. Because of the efficiency and easy handling associated with the glycosidation reaction between a carbohydrate donor and an acceptor, the use of the imidate strategy presented by Schmidt [19] was selected in this case (Scheme 2). The trisaccharide moiety of SLac was prepared using the method previously reported [20,21]. Thus, a glycosidation reaction between a freshly prepared imidate 6 as a glycosyl donor and acrylamide alcohol 5 as a glycosyl acceptor was carried out in the presence of BF₃•OEt as a Lewis acid catalyst to afford the desired SLac derivative 7 in 35% yield. Since we were not able to remove moisture from the reaction mixture prior to the condensation reaction, the use of two molar equivalents of BF₃•OEt was needed. A previous report [22] indicated that PEG typically binds 2–3 water molecules per ethylene oxide unit, and a low yield of the glycosidation was therefore encountered. In addition to the glycoside 7, the corresponding hemiacetal 8 was also obtained in 59% yield. The hemiacetal 8 was produced by the attack of water molecules on the oxocarbenium ion as an intermediate. Zemplén's transesterification [23] followed by subsequent saponification for fully protected SLac glycoside 7 furnished the desired glycomonomer 9 in 95% yield in two steps.

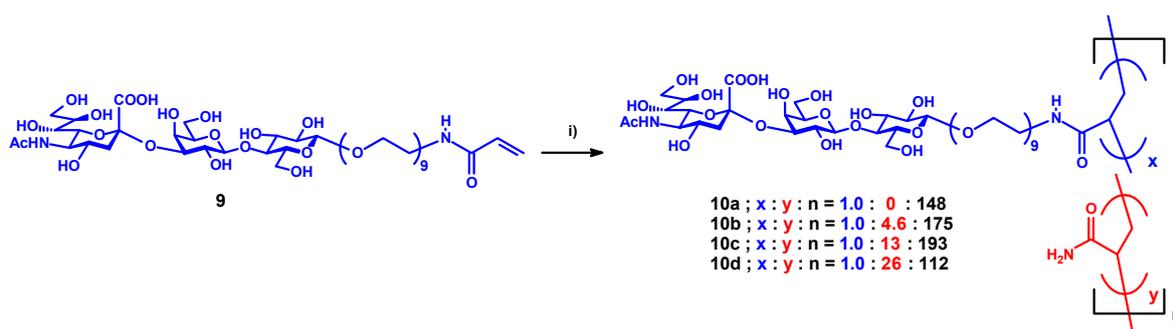


Scheme 2. Reagents and conditions: (i) 5 (1.2 molar equiv), MS4A, BF₃•OEt (2.0 molar equiv), DCM, −15 °C, 0.5 h, then RT, 1 h; (ii) 0.1 M MeONa in MeOH, RT, 4 h, then 0.05 M aq NaOH, RT, 3 h.

3.1.3. Polymerization of SLac-PEG9 Monomer

Working towards the completion of the preparation of SLac having polymerizable PEG9 aglycon, our next objective was the synthetic assembly of the SLac moieties by means of radical polymerization. We polymerized various glycomonomers in aqueous media using a convenient radical polymerization technique [11,24,25]. Thus, the glycomonomer 9

was allowed to polymerize with or without acryl amide as a mediator for controlling the distance between SLac residues in a 1:1 water—DMSO solvent system in the presence of APS and TEMED as shown in Scheme 3. The reaction proceeded smoothly to yield the corresponding water-soluble glycopolymers **10a**~**10d**, and the results are summarized in Table 1. It is interesting to note that, according to the results of the polymerization, when a higher ratio of **A**Am was used as comonomer in the polymerization reaction, the yields were worse. It was found that the glycomonomer **9** having a PEG9 moiety as the aglycon showed higher polymerization activity than that of **A**Am. We concluded that the reason for the effect of the PEG9 moiety on the yields was that PEG9 moiety displayed higher hydrophilic activity in this case. The polymer compositions for each polymerization were close to the charged ratio of the corresponding monomers.



Scheme 3. Reagents and conditions: (i) **A**Am, TEMED, APS, H₂O:DMSO = 1:1 (v/v), RT, 3 h.

¹H NMR spectra of the monomer **9**, homopolymer **10a**, and copolymer **10b** as an example of the copolymers are shown in Figure 3. Acrylamide moieties at approximately 5.8~6.5 ppm in monomer **9** in Figure 3a completely disappeared after polymerization, as shown in Figure 3b,c). These spectra supported the complete incorporation of the SLac moiety into the polymer. Characteristic protons due to the sialic acid moiety at approximately 2.8 ppm for H-3''_{eq} and at approximately 1.9 ppm for H-3''_{ax} were clearly observed in each spectrum. In addition, each anomeric proton signal of galactose and glucose residues of SLac trisaccharide appeared as two doublets at approximately 4.5 ppm.

3.1.4. Biological Response to WGA Determined by a Fluorometric Assay

We examined the biological activities of the series of glycopolymers that were prepared for carbohydrate-binding proteins prior to application as inhibitors for various pathogenic viruses. We recently carried out biological evaluation of lectins as carbohydrate-binding proteins [11], and the same procedure was used in this study. Wheat germ agglutinin (WGA) [26] is one of the well-known lectins that recognizes mainly *N*-acetyl-D-glucosamine and their oligomers [27,28]; however, *N*-acetyl-neuraminic acid (Neu5Ac) is also recognized [29,30]. In our previous studies, investigation with a series of glycopolymers as carbohydrate substrates was carried out by means of changes in fluorescence emission from tryptophane (Trp) residues of WGA on its own when aliquots of the glycopolymer solution were added to the analytical solution. Fluorometric analyses of a glycopolymer **10b** as an example in 0.65 μM of WGA solution in 50 mM Tris-HCl buffer (pH 7.5) at 4 °C is shown in Figure 4. The WGA solution was maintained at 4 °C ± 0.1 °C for all measurements in order to avoid nonspecific binding of the glycopolymer—WGA, and specific excitation of Trp on WGA was performed at λ₂₉₅ nm [31,32]. The fluorescence spectrum of the WGA solution upon addition of an aliquot of a polymer solution **10b** was monitored, and the spectra are shown in Figure 4a. The intensity of the spectrum gradually increased, and each value at 348 nm of the spectra was recorded. Figure 4b shows changes in the intensities of the emission spectra. A Hill plot analysis [33] was carried out by using the parameters shown as Figure 4c, and the results provided the corresponding association constant *K*_a of 11.3 × 10⁵ M⁻¹, which is 35 times higher than that of the monomeric SLac derivative

9. Other glycopolymers that were prepared were analyzed in the same manner as that described for **10b**, and all of the profiles of the fluorescence spectra of the glycopolymer—WGA interaction are shown in the supporting information. Table 3 summarizes the results of biological evaluations based on fluorometric assays. Changes in fluorescence intensities of the glycopolymers as represented by $\Delta F'/F_0$ were 17% to 75%, and the K_a values were $0.32 \times 10^5 \text{ M}^{-1}$ to $11.3 \times 10^5 \text{ M}^{-1}$. The K_a values of the glycopolymers were higher than the K_a value of a monomeric compound **9**, and this phenomenon strongly supported a positive sugar-clustering effect [3]. Glycopolymer **10b** used as the substrate showed the highest K_a value. In addition, Gibbs free energies ΔG_a^0 were calculated from the relationship $\Delta G_a^0 = -RT \ln K_a$, and the values of $-\Delta G_a^0$ of glycopolymers were 24~32 kJ/mol on the basis of the sugar unit.

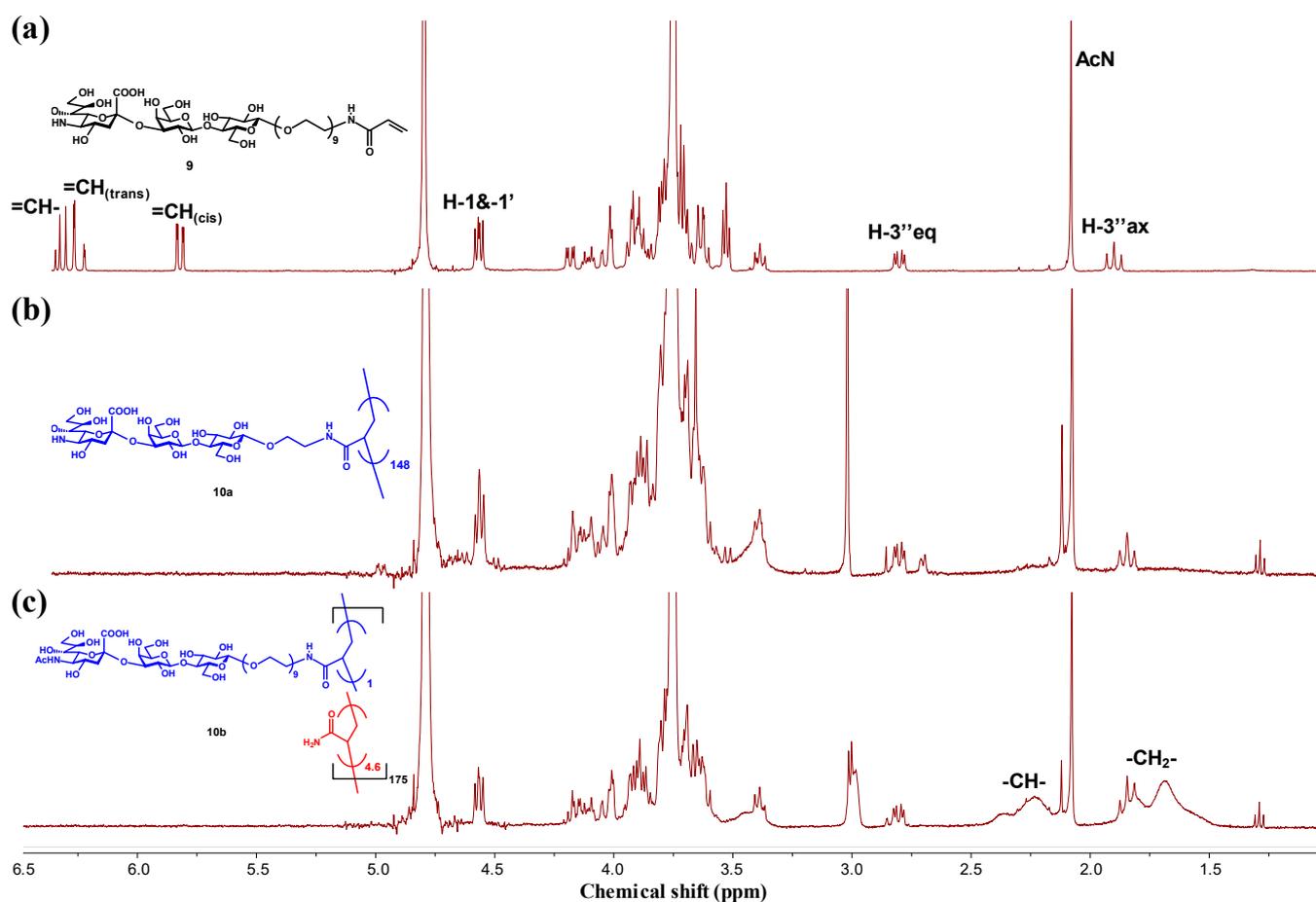


Figure 3. ^1H NMR spectra of (a) glycomonomer **9**, (b) homopolymer **10a**, and (c) copolymer **10b** in D_2O .

Table 3. Results of binding assays for WGA on the basis of fluorescence measurements.

Substrate	$\Delta F'/F_0$ (%)	$-\Delta G_a^0$ ¹ (kJ/mol)	K_a ($\times 10^5 \text{ M}^{-1}$)	Relative Potency ²
9	75	24	0.32	1
10a	46	30	4.5	14
10b	29	32	11.3	35
10c	20	31	8.0	25
10d	17	29	2.8	9

¹ ΔG_a^0 was calculated from the relationship $\Delta G = -RT \ln K_a$. ² Relative potencies were calculated on the basis of K_a at 348 nm of monomeric SLac glycoside **9**.

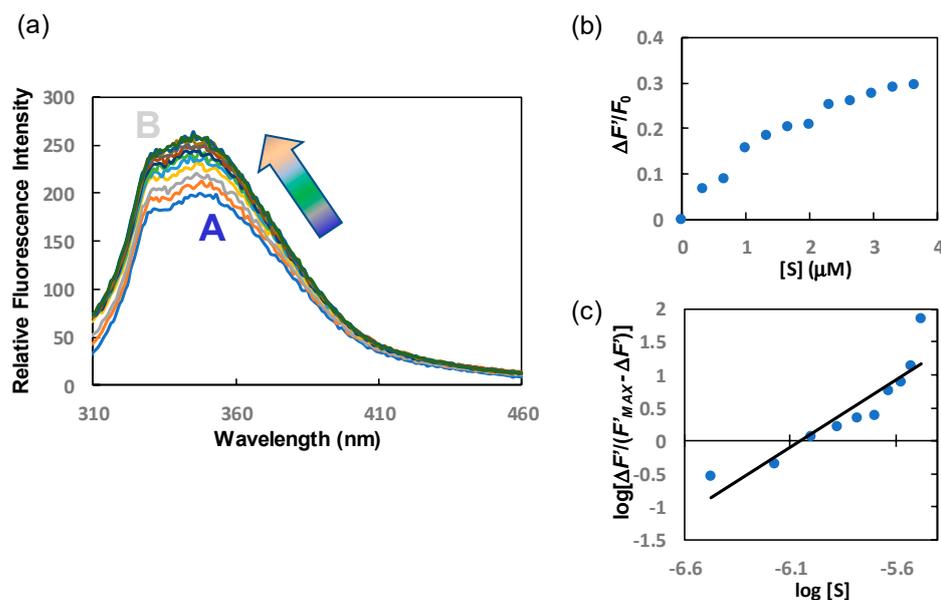


Figure 4. Biological evaluations of the WGA—carbohydrate interaction using glycopolymer **10b**. (a) Changes in fluorescence emission spectra of WGA (0.65 μM , 3.0 mL, 50 mM Tris-HCl buffer containing 1.25 M NaCl and 25 mM CaCl_2 , pH 7.5, 4 ± 0.1 $^\circ\text{C}$) from **A** to **B** upon addition of aliquots of polymer **10b** (138.0 μM). (b) Plots of $\Delta F'/F_0$ versus $[S]$, where $\Delta F'$ is change in the intensity at 348 nm of WGA with various concentrations of **10b**, F_0 is the intensity of WGA alone, and $[S]$ is the total ligand concentration based on the sugar residue concentration. (c) Hill plots of $\log[\Delta F'/(F'_{\text{MAX}}-\Delta F')]$ versus $\log [S]$.

In the current study, a series of SLac polymers having shorter aglycon moieties was prepared, and preliminary biological evaluations for WGA using fluorometric assays were carried out. The results suggested that there is a relationship between a larger degree of freedom of the sugar moiety in SLac polymers having longer aglycon than that of our current study [16] and the sugar densities on the corresponding glycopolymers. Further chemical manipulations and control of the sugar densities of the SLac derivatives are now being carried out, and the results will be reported elsewhere in the near future.

4. Conclusions

Simple hydrophilic mimetics for the oligosaccharide moiety portion were achieved by means of PEG repeating units, and a polymerizable alcohol having 9 PEG repeats was prepared. Sialyl $\alpha(2 \rightarrow 3)$ lactose, which is known as a sugar moiety of GM3 ganglioside, was also synthesized and was coupled with the polymerizable alcohol to afford the desired glycomonomer, which was further polymerized with or without acrylamide to give the corresponding water-soluble glycopolymers. The glycopolymers had higher affinities than those of glycopolymers having sialyl lactose moieties with shorter aglycon moieties.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/polym15040998/s1>, Figure S1: ^1H NMR spectrum of 17-azido-3,6,9,12,15-pentaoxaheptadecyl methanesulfonate (**2**) in $\text{DMSO-}d_6$; Figure S2: ^{13}C NMR spectrum of 17-azido-3,6,9,12,15-pentaoxaheptadecyl methanesulfonate (**2**) in $\text{DMSO-}d_6$; Figure S3: IR spectrum of 17-azido-3,6,9,12,15-pentaoxaheptadecyl methanesulfonate (**2**); Figure S4: ^1H NMR spectrum of 26-azido-3,6,9,12,15,18,21,24-octaoxahexacosan-1-ol (**3**) in $\text{DMSO-}d_6$; Figure S5: ^{13}C NMR spectrum of 26-azido-3,6,9,12,15,18,21,24-octaoxahexacosan-1-ol (**3**) in $\text{DMSO-}d_6$; Figure S6: IR spectrum of 26-azido-3,6,9,12,15,18,21,24-octaoxahexacosan-1-ol (**3**); Figure S7: ^1H NMR spectrum of 26-amino-3,6,9,12,15,18,21,24-octaoxahexacosan-1-ol (**4**) in $\text{DMSO-}d_6$; Figure S8: ^{13}C NMR spectrum of 26-amino-3,6,9,12,15,18,21,24-octaoxahexacosan-1-ol (**4**) in $\text{DMSO-}d_6$; Figure S9: IR spectrum of 26-amino-3,6,9,12,15,18,21,24-octaoxahexacosan-1-ol (**4**); Figure S10: ^1H NMR spectrum of *N*-(26-hydroxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)acrylamide (**5**) in $\text{DMSO-}d_6$; Figure S11: ^{13}C NMR spectrum of

N-(26-hydroxy-3,6,9,12,15,18,21,24-octaohexacosyl)acrylamide (**5**) in DMSO-*d*₆; Figure S12: IR spectrum of *N*-(26-hydroxy-3,6,9,12,15,18,21,24-octaohexacosyl)acrylamide (**5**); Figure S13: ¹H NMR spectrum of 28-oxo-3,6,9,12,15,18,21,24-octaosa-27-azatriacont-29-en-1-yl [Methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosyl)onate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl- β -*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl- β -*D*-glucopyranoside (**7**) in CDCl₃; Figure S14: ¹³C NMR spectrum of 28-oxo-3,6,9,12,15,18,21,24-octaosa-27-azatriacont-29-en-1-yl [Methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosyl)onate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl- β -*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl- β -*D*-glucopyranoside (**7**) in CDCl₃; Figure S15: IR spectrum of 28-oxo-3,6,9,12,15,18,21,24-octaosa-27-azatriacont-29-en-1-yl [Methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosyl)onate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl- β -*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl- β -*D*-glucopyranoside (**7**); Figure S16: MALDI-TOF MS spectrum of 28-oxo-3,6,9,12,15,18,21,24-octaosa-27-azatriacont-29-en-1-yl [Methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosyl)onate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl- β -*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl- β -*D*-glucopyranoside (**7**); Figure S17: ¹H NMR spectrum of 28-oxo-3,6,9,12,15,18,21,24-octaosa-27-azatriacont-29-en-1-yl (5-acetamido-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosyl)-(2→3)-*O*-(β -*D*-galactopyranosyl)-(1→4)- β -*D*-glucopyranoside (**7**) in D₂O; Figure S18: ¹³C NMR spectrum of 28-oxo-3,6,9,12,15,18,21,24-octaosa-27-azatriacont-29-en-1-yl (5-acetamido-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosyl)-(2→3)-*O*-(β -*D*-galactopyranosyl)-(1→4)- β -*D*-glucopyranoside (**7**) in D₂O; Figure S19: IR spectrum of 28-oxo-3,6,9,12,15,18,21,24-octaosa-27-azatriacont-29-en-1-yl (5-acetamido-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosyl)-(2→3)-*O*-(β -*D*-galactopyranosyl)-(1→4)- β -*D*-glucopyranoside (**7**); Figure S20: MALDI-TOF MS spectrum of 28-oxo-3,6,9,12,15,18,21,24-octaosa-27-azatriacont-29-en-1-yl (5-acetamido-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosyl)-(2→3)-*O*-(β -*D*-galactopyranosyl)-(1→4)- β -*D*-glucopyranoside (**7**); Figure S21: Biological evaluations of the WGA—carbohydrate interaction using glycomonomer **9**; Figure S22: Biological evaluations of the WGA—carbohydrate interaction using homopolymer **10a**; Figure S23: Biological evaluations of the WGA—carbohydrate interaction using copolymer **10b**; Figure S24: Biological evaluations of the WGA—carbohydrate interaction using copolymer **10c**; Figure S25: Biological evaluations of the WGA—carbohydrate interaction using copolymer **10d**.

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