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Article

Preparation of *N*-Linked-Type GlcNAc Monomers for Glycopolymers and Binding Specificity for Lectin

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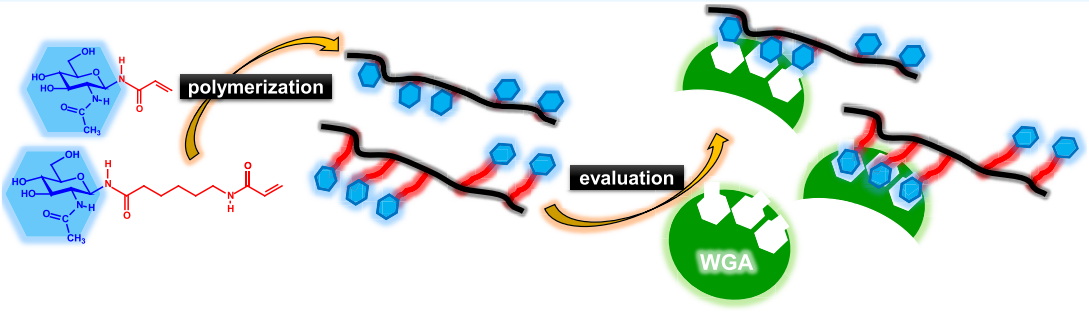

ABSTRACT: Glycomonomers having *N*-glycosidic linkages were prepared from a known glycosyl amine, *N*-acetyl-D-glucosamine (GlcNAc). Radical polymerization of the glycomonomers gave a series of glycopolymers displaying various sugar densities, which were models of the core structure of Asn-linked-type glycoproteins. In addition, fluorometric analyses of wheat germ agglutinin (WGA) against the glycopolymers were carried out, and the results showed unique binding specificities on the basis of flexibility of sugar moieties.

INTRODUCTION

Oligosaccharide portions on glycoconjugates play various roles in biological events, including as informative carriers for cell−cell interactions and markers of cellular differentiation, aging, and malignant alteration.[1] Interaction between a carbohydrate chain and a protein is one of major biological communications. A typical carbohydrate−protein interaction shows a millimolar-range affinity constant, while an antigen−antibody interaction shows a nanomolar-range affinity constant.[2] To enhance the weak affinities between carbohydrate chains and proteins, a wide variety of cluster-type compounds involving many carbohydrate residues have been used and have shown much higher affinities.[3] Dendrimers having carbohydrate residues at the terminal ends, so-called glycodendrimers, show remarkable enhancement of binding affinities for carbohydrate-recognizing proteins compared to those of the monomeric carbohydrate compounds.[4] Polymeric substances such as glycopolymers also show higher affinities than those of monomeric substances.[5] The use of multivalent-type substances is promising for achieving effective biological activities in biochemical uses and medicinal uses.[6] Various carbohydrate sequences including monosaccharides and oligosaccharides have been used for the preparation of sugar-cluster compounds.[7] Carbohydrate chains in glycoproteins in nature are roughly classified to *O*-glycosidic carbohydrate sequences and *N*-glycosidic ones.[8] The invariant "core" structure of *N*-linked-type glycoproteins is mannosyl-*β*-(1-4)-*N*-acetyl-D-glucosaminyl-*β*-(1−4)-*N*-acetyl-D-glucosaminyl-*β*-asparagine (Man-*β*-(1−4)-GlcNAc-*β*-(1−4)-GlcNAc-*β*-Asn). The pivotal structure of the reducing end position of the *N*-linked-type glycoproteins is GlcNAc-*β*-Asn as shown in Figure 1a. In our ongoing synthetic studies of glycopolymers,[9−11] *O*-glycosidic-type substances were investigated because of the easy chemical manipulation of the carbohydrate moieties. Figure 1c shows the core structure of an *O*-glycosidic-type compound, which was transformed into the corresponding glycodendrimers as well as the corresponding glycopolymers.[12] The sugar-cluster-type substances showed remarkable binding affinities against lectins depending on the basis of sugar densities on the multivalent compounds. However, preparation and investigation of multivalent compounds bearing *N*-glycosidic linkage have not been widely studied. The schematic structure of a mimic of *N*-linked-type glycoproteins is shown in Figure 1b. Glycopolymers bearing this type of *N*-glycosidic linkage between GlcNAc and the polymer backbone were prepared by Kallin et al.[13] Although biological responses of the polymer were not reported, glycopolymers having *N*-glycosidic linkages are of great

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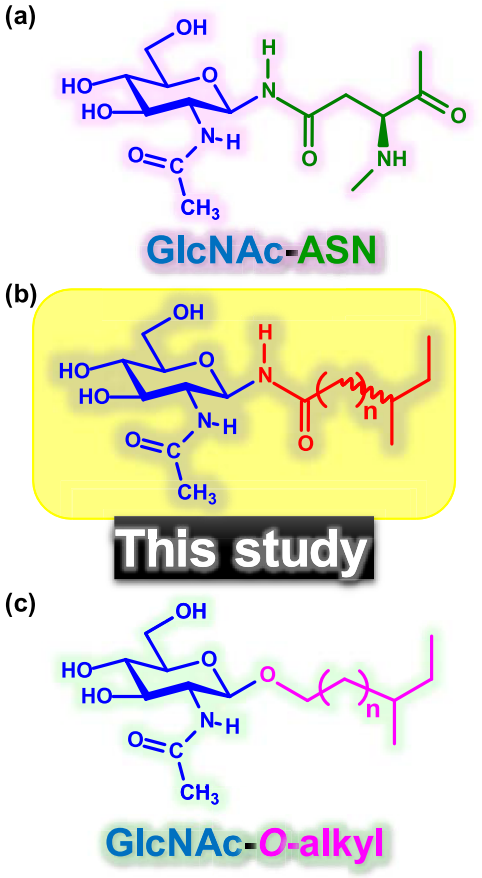

Figure 1. Schematic structures for glycosides related to GlcNAc. (a) The invariant "core" structure of *N*-linked type in nature, (b) a model structure of *N*-linked-type glycoside, and (c) a model structure of *O*-linked-type glycoside.

importance as *N*-glycoprotein models in view of biomimetic chemistry. In this paper, we report a simple and convenient method for the preparation of carbohydrate monomers based on GlcNAc closely related to the invariant "core" structure of *N*-linked-type glycoproteins and the glycopolymer. In addition to the preparation, the biological responses against wheat germ agglutinin (WGA), which is a lectin recognizing chitooligosaccharide, are also described.

EXPERIMENTAL SECTION

Materials and Methods. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Methanol (MeOH) was stored over molecular sieves (3 Å MS) before use. An ion-exchange resin (Amberlite IR120B) was regenerated to H^+ form before use. Acrylamide (**AAm**) was recrystallized from chloroform before use. IR spectra were observed using a Shimadzu IR Prestige-21 spectrometer (Shimadzu Corporation, Kyoto, Japan). NMR spectra were recorded at 500 MHz for ^{1}H and at 126 MHz for ^{13}C with a Bruker AVANCE 500 spectrometer (Bruker BioSpin MRI GmbH, Ettlingen, Germany) in chloroform-*d* ($CDCl_3$), dimethyl sulfoxide-d_6 (DMSO-d_6), methanol-d_4 (CD_3OD), or deuterium oxide (D_2O). 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 DMSO-d_6 (δ 2.50), and HDO in MeOD or D_2O (δ 4.78) for ^{1}H and $CHCl_3$ in $CDCl_3$ (δ 77.00) or CH_3 in DMSO-d_6 (δ 39.50) for ^{13}C. Ring-proton assignments in the ^{1}H NMR spectra were made by first-order analysis of the spectra and are supported by the results of homonuclear decoupling experiments and H−H or HMQC experiments. Elemental analyses were performed with a Fisons EA1108 (Thermo Fisher Scientific Inc., Waltham, MA) on samples that were extensively dried at 50−60 °C over phosphorus pentoxide for 4−5 h. Optical rotations were determined with a JASCO DIP-1000 digital polarimeter (JASCO Corporation, Tokyo, Japan). Fast atom bombardment mass (FAB MS) spectra were recorded with a JEOL JMS-HX110 spectrometer (JEOL Ltd., Tokyo, Japan). UV-vis spectra were uncorrected and were recorded with a Shimadzu UV-2550 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). For detection of the intermediates, TLC sheets were (a) dipped in a solution of 85:10:5 (v/v/v) MeOH−*p*-anisaldehyde−concd H_2SO_4 and

Table 1. Chemical Shifts and Multiplicities of GlcNAc Derivatives 1−11 in ^{1}H NMR Spectroscopic Analyses in D_2O with HDO, DMSO-d_6 with DMSO, $CDCl_3$ with TMS, or CD_3OD with TMS as Internal Standards

	chemical shifts (δ), multiplicity								
compounds	**1**[a]	**2**[b]	**5**[d]	**6**[e]	**7**[f]	**8**[e]	**9**[e]	**10**[f]	**11**[f]
H-6b	3.73, dd	3.40, dd	3.55, dd	4.11, dd	4.53, dt	4.09, dd	4.09, dd	3.39, dd	3.41, dd
H-6a	3.86, dd	3.63, dd	3.76, dd	4.32, dd	3.65, ddd	4.30, dd	4.31, dd	3.64, br d	3.65, dd
H-5	3.53, ddd	3.10, ddd	3.16, ddd	3.83, ddd	3.08, m[a]	3.76, ddd	3.76, ddd	3.08, ddd	3.08, ddd
H-4	3.84, t	3.05, dd	3.20, t	5.12, t	3.08, m[a]	5.13, t	5.13, t	3.30, dd	3.29, dd
H-3	3.61, dd	3.29, dd	3.32, t	5.15, t	3.30, m[a]	5.05, dd	5.05, t	3.05, t	3.05, t
H-2	3.47, dd	3.51, ND[c]	3.46, t	4.21, q	3.52, q	4.12, dt	4.12, ddd	3.52, q	3.52, q
H-1	5.13, d	4.78, d	3.91, d	5.22, t	4.79, t	5.07, dd	5.07, dd	4.79, t	4.78, t
NH				6.52, d	7.80,d	6.07, d	6.07, d	7.85, d	7.87, d
NAc	1.95, s	1.77, s	1.91, s	1.93, s	1.78, s	1.96, s	1.95, s	1.77, s	1.78, s
OAc				2.09, s		2.09, s	2.09, s		
				2.08,s		2.08, s	2.08, s		
				2.05, s		2.05, s	2.05, s		
NH (aglycon)				7.30, d	8.00, d	6.97, d	6.96, d	8.00, d	7.97, d

[a]Measured in D_2O. [b]Measured in DMSO-d_6 with D_2O. [c]Overlapped and could not be determined. [d]Measured in CD_3OD. [e]Measured in $CDCl_3$. [f]Measured in DMSO-d_6.

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Table 2. First-Order Coupling Constants of GlcNAc Derivatives 1−11 in ^{1}H NMR Spectroscopic Analyses in D_2O with HDO, DMSO-d_6 with DMSO, $CDCl_3$ with TMS, or CD_3OD with TMS as Internal Standards

	first-order coupling constants (Hz)								
compounds	**1**	**2**	**5**	**6**	**7**	**8**	**9**	**10**	11
$J_{6a,6b}$	12.5	11.9	11.8	12.5	11.9	12.5	12.5	12.0	12.0
$J_{5,6b}$	5.1	5.5	5.7	2.3	5.6	2.3	2.2	5.4	5.0
$J_{5,6a}$	2.2	1.9	2.3	4.4	1.8	4.3	4.3	1.8	2.0
$J_{4,5}$	10.0	9.7	9.8	9.8	ND[a]	9.9	9.9	9.7	10.0
$J_{3,4}$	10.2	8.4	9.8	9.5	ND[a]	9.4	9.4	9.6	8.0
$J_{2,3}$	8.7	10.1	9.9	10.0	9.8	10.3	10.5	9.5	9.5
$J_{1,2}$	9.7	9.8	9.2	9.3	9.5	9.9	9.9	9.6	9.4
$J_{2,NH}$				8.6	8.9	8.7	8.0	8.8	8.3
$J_{1,NH}$				8.6	9.2	8.4	8.4	9.1	9.2

[a]Overlapped and could not be determined.

Table 3. Chemical Shifts of GlcNAc Derivatives 1−11 in ^{13}C NMR Spectroscopic Analyses in D_2O, DMSO-d_6, or $CDCl_3$

	chemical shifts (δ)							
compounds	**1**[a]	**2**[b]	**6**[c]	**7**[b]	**8**[c]	**9**[c]	**10**[b]	**11**[b]
C-1	78.56	78.82	80.21	78.83	80.33	80.32	78.75	78.88
C-2	54.36	54.57	53.23	54.56	53.50	53.49	54.69	54.54
C-3	74.12	74.38	72.91	74.52	72.98	72.96	74.47	74.46
C-4	69.43	70.49	67.89	70.55	67.67	67.67	70.55	70.54
C-5	77.65	78.68	73.46	78.69	73.53	73.52	78.86	78.69
C-6	60.44	60.96	61.77	60.96	61.74	61.73	61.07	60.95
CH_3 (NAc)	21.88	22.86	23.01	22.85	23.08	23.08	22.94	22.78

[a]Measured in D_2O. [b]Measured in DMSO-d_6. [c]Measured in $CDCl_3$.

heated for a few minutes (for carbohydrate) or (b) dipped in an aqueous solution of 5 wt % $KMnO_4$ and heated similarly (for detection of C═C double bonds). Column chromatography was performed on silica gel (silica gel 60; 63−200 μm, E. Merck, Darmstadt, Germany). Flush column chromatography was also performed on silica gel (silica gel 60, spherical neutral; 40−100 μm, E. Merck, Darmstadt, Germany). All extractions were concentrated below 45 °C under diminished pressure. Dialysis was performed against distilled water using a dialysis tubing [molecular cutoff (MWCO): 12 k−16 kDa, Dow Chemical Co., Midland, MI]. Wheat germ agglutinin (WGA; a lectin from Triticum vulgaris) was purchased from SIGMA (Sigma-Aldrich, Inc., St. Louis, MO).

*2-Acetamido-2-deoxy-β-D-glucopyranosylamine (**5**).*[13] To a solution of *N*-acetyl-D-glucosamine (GlcNAc, 10.12 g, 45.8 mmol) in MeOH (100 mL) was added ammonium hydrogen carbonate (NH_4HCO_3) until saturation in MeOH. The suspension was stirred for 6 d at rt under a N_2 atmosphere while additional NH_4HCO_3 was added to the suspension in order to maintain NH_4HCO_3-saturated conditions. When TLC showed complete consumption of GlcNAc, the mixture was evaporated to afford a yellowish syrup, which was diluted with water (100 mL). The aqueous solution was lyophilized to provide powdery **5**, which was further diluted with water (100 mL), followed by lyophilization to give powdery **5** (10.2 g, quantitative yield): R_f 0.20 [65:30:4 $CHCl_3$−MeOH−H_2O (v/v/v)]; IR (NEAT) 3375 (ν_{O-H} & ν_{N-H}), 2914 (ν_{C-H}), 2884 (ν_{C-H}), 1651 ($\nu_{C=O}$, amide I), 1557 (δ_{N-H}, amide II), 1084 (ν_{C-O-C}), 1040 (ν_{C-N}); ^{1}H NMR (500 MHz, CD_3OD) signals are summarized in Tables 1 and 2.

*N-Acrylamido-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosylamine (**6**).* Amine **5** (1.47 g, 5.36 mmol) was dissolved in satd aq $NaHCO_3$−THF [20 mL, 1:1 (v/v)][14] at 0 °C under an Ar atmosphere. To the solution was dropwise added a solution of acryloyl chloride (0.65 mL, 8.04 mmol) in THF (10 mL) at 0 °C under an Ar atmosphere, and the stirring was continued for 4 h at the same temperature. The mixture was concentrated *in vacuo* to afford crude **1** (2.56 g). The crude acrylamide was acetylated with Ac_2O (10 mL) in pyr (30 mL) at 0 °C for 2.5 h under an Ar atmosphere. The whole mixture was evaporated and coevaporated with toluene to give the residue, which was poured into ice-cold water. The mixture was extracted with $CHCl_3$, washed successively with 1 M aq H_2SO_4, water, satd aq $NaHCO_3$, and brine, dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. The residual syrup was subjected to a column of silica gel with 1:1 (v/v) $CHCl_3$−MeOH as the eluent to yield pure **6** (0.244 g, 11.3%) as a white solid: R_f 0.46 [5:4:1 $CHCl_3$−EtOAc−MeOH (v/v/v)]; IR (KBr) 3308 (ν_{N-H}), 3292 (ν_{N-H}), 3073 (ν_{C-H}), 2941 (ν_{C-H}), 1749 ($\nu_{C=O}$, ester), 1668 ($\nu_{C=O}$, amide I), 1533 (δ_{N-H}, amide II), 1229 (ν_{C-O}), 1040 (ν_{C-O-C}); all ^{1}H NMR (500 MHz, $CDCl_3$) signals are summarized in Tables 1 and 2; ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.05 (C═O), 171.69 (C═O), 170.70 (C═O), 169.31 (C═O, aglycon), 130.30 (CH═), 128.19 (CH_2═), 20.70 (CH_3), 20.68 (CH_3), 20.56 (CH_3), and other signals are summarized in Table 3.

*N-Acrylamido-2-acetamido-2-deoxy-β-D-glucopyranosylamine (**1**).*[13] Acetate **6** (0.230 g, 0.574 mmol) was dissolved in MeOH (9.0 mL) at rt under an Ar atmosphere, and NaOMe (0.015 g, 0.172 mmol) was added to the solution under an Ar atmosphere. The solution was allowed to react at rt for 1.5 h under an Ar atmosphere. When TLC showed complete conversion of acetate into the corresponding alcohol, IR120B (H^+) was added until pH 7 on wet pH paper. The suspension was filtered through a pad of cotton and concentrated *in vacuo* to give the desired acrylamide **1** in 81.0% yield as a white solid: R_f 0.71 [1:1 $CHCl_3$−MeOH (v/v)]; IR (KBr) 3306 (ν_{O-H} & ν_{N-H}), 3260 (ν_{N-H}), 3092 (ν_{C-H}), 2955 (ν_{C-H}), 2932 (ν_{C-H}),

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2911 (ν_{C-H}), 2849 (ν_{C-H}), 1670 ($\nu_{C=O}$, amide I), 1653 ($\nu_{C=C}$), 1537 (δ_{N-H}, amide II), 1223 (ν_{C-O}), 1090 (ν_{C-O-C}), 1040 (ν_{C-N}); ^{1}H NMR (500 MHz, D_2O) δ 6.25 [dd, 1 H, J_{gem} = 2.7 Hz, J_{trans} = 17.2 Hz, =CH_2(trans)], 6.21 [dd, 1 H, J_{cis} = 8.8 Hz, CH=], 5.82 [dd, 1 H, =CH_2(cis)], and other signals are summarized in Tables 1 and 2; ^{13}C NMR (126 MHz, D_2O) δ 174.78 (C=O, NAc), 168.89 (C=O, aglycon), 129.32 (CH_2=), 129.26 (CH=), and other signals are summarized in Table 3.

*2-Acetamido-N^1-(6-bromohexanoyl)-2-deoxy-β-D-glucopyranosylamine (**7**).* To a solution of amine **5** (10.13 g, 46.0 mmol) in MeOH (100 mL) was added Na_2CO_3 (4.90 g, 46.2 mmol) at rt, and the suspension was cooled to 0 °C under a N_2 atmosphere. Dropwise addition of a solution of 6-bromohexanoyl chloride (12.8 mL, 83.6 mmol) in THF (70 mL) was performed at 0 °C under a N_2 atmosphere, and the mixture was allowed to react for a further 4 h with stirring. After consuming the amine **5** on a TLC plate, the reaction mixture was filtered through a pad of celite, and the filtrate was evaporated *in vacuo* to afford the corresponding residue, which was purified by crystallization from MeOH to give desired hexanamide with bromine at ω-position **7** (9.21 g, 50.4%) as white needles: R_f 0.75 [65:30:4 $CHCl_3$−MeOH−H_2O (v/v/v)]; $[\alpha]_D^{25}$ +26.9° (*c*. 1.0, MeOH); mp. 174 °C; IR (KBr) 3306 (ν_{O-H} & ν_{N-H}), 3262 (ν_{N-H}), 2934 (ν_{C-H}), 2851 (ν_{C-H}), 1661 ($\nu_{C=O}$, amide I), 1541 (δ_{N-H}, amide II), 1437 (δ_{C-O}), 1375 (δ_{C-O}), 1323 (δ_{C-O}), 1111 (δ_{C-O}), 1088 (ν_{C-O}), 1038 (ν_{C-O-C}); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.53 (t, 2 H, J_{vic} = 6.7 Hz, $BrCH_2$), 2.05 (m, 2 H, $COCH_2$), 1.77 (m, 2 H, $BrCH_2CH_2$), 1.46 (m, 2 H, $COCH_2CH_2$), 1.33 (m, 2 H, $BrCH_2CH_2CH_2$), and other signals are summarized in Tables 1 and 2; ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.31 (C=O, NAc), 169.84 (C=O, aglycon), 35.21 ($COCH_2$), 34.93 ($BrCH_2$), 31.96 ($BrCH_2CH_2$), 27.00 ($BrCH_2CH_2CH_2$), 24.14 ($COCH_2CH_2$), and other signals are summarized in Table 3.

Anal. Calcd for $C_{14}H_{25}N_2O_6Br$·0.2 H_2O: C, 41.95; H, 6.39; N, 6.99. Found: C, 41.95; H, 6.40; N, 6.82.

*2-Acetamido-3,4,6-tri-O-acetyl-N^1-(6-bromohexanoyl)-2-deoxy-β-D-glucopyranosylamine (**8**).* An alcohol **7** (3.01 g, 7.58 mmol) was treated with Ac_2O (6.50 mL, 68.8 mmol) and pyr (30 mL) at rt for 3.5 h under a N_2 atmosphere. The mixture was diluted with toluene, and the mixture was concentrated *in vacuo*, and the residue was poured into ice-cold water. The mixture was extracted with $CHCl_3$, and the organic solution was successively washed with 1 M aq H_2SO_4, water, satd aq $NaHCO_3$, and brine, dried over anhyd $MgSO_4$, filtered and evaporated *in vacuo*. The residual syrup was crystallized from abs EtOH to yield white needle-like crystalline **8** (2.47 g, 62.3%): R_f 0.44 [9:1 $CHCl_3$−MeOH (v/v)]; $[\alpha]_D^{25}$ −4.05° (*c*. 1.0, $CHCl_3$); mp. 201 °C; IR (KBr) 3325 (ν_{N-H}), 2951 (ν_{C-H}), 2870 (ν_{C-H}), 1742 ($\nu_{C=O}$, ester), 1670 ($\nu_{C=O}$, amide I), 1659 ($\nu_{C=O}$, amide I), 1545 (δ_{N-H}, amide II), 1526 (δ_{N-H}, amide II), 1458 (δ_{C-O}), 1373 (δ_{C-O}), 1302 (δ_{C-O}), 1233 (δ_{C-O}), 1190 (ν_{C-O}), 1049 (ν_{C-O-C}); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.40 (t, 2 H, J_{vic} = 6.7 Hz, $BrCH_2$), 2.20 (m, 2 H, $COCH_2$), 1.86 (m, 2 H, $BrCH_2CH_2$), 1.62 (m, 2 H, $COCH_2CH_2$), 1.44 (m, 2 H, $BrCH_2CH_2CH_2$), and other signals are summarized in Tables 1 and 2; ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.39 (C=O), 171.99 (C=O), 171.91 (C=O), 170.66 (C=O), 169.23 (C=O, aglycon), 51.18 ($BrCH_2$), 36.28 ($COCH_2$), 28.53 ($BrCH_2CH_2$), 26.20 ($COCH_2CH_2$), 24.52 ($BrCH_2CH_2CH_2$), 20.72 ($COCH_3$), 20.67 ($COCH_3$), 20.56 ($COCH_3$), and other signals are summarized in Table 3.

Anal. Calcd for $C_{20}H_{31}N_2O_9Br$: C, 45.90; H, 5.97; N, 5.35. Found: C, 46.11; H, 6.04; N, 5.29.

*2-Acetamido-3,4,6-tri-O-acetyl-N^1-(6-azidohexanoyl)-2-deoxy-β-D-glucopyranosylamine (**9**).* To a solution of an alkylbromide **8** (1.97 g, 3.76 mmol) in DMF (20 mL) was added NaN_3 (0.490 g, 7.54 mmol) at rt under a N_2 atmosphere, and the mixture was allowed to warm at 80 °C. When the reaction mixture was stirred for 4 h at 80 °C under a N_2 atmosphere, TLC showed completion of the reaction. The mixture was filtered through a pad of celite, and the filtrate was concentrated *in vacuo* to afford the corresponding residue, which was diluted with $CHCl_3$ and washed with water. The mixture was partitioned, and the organic layer was washed with water and brine, dried over anhyd $MgSO_4$, filtered, and evaporated *in vacuo*. Crystallization of the residue from abs EtOH gave the desired azide **9** (1.60 g, 87.8%) as white needles: R_f 0.44 [9:1 $CHCl_3$−MeOH (v/v)]; $[\alpha]_D^{25}$ −3.07° (*c*. 1.0, $CHCl_3$); mp. 176 °C; IR (KBr) 3308 (ν_{N-H}), 3275 (ν_{N-H}), 2941 (ν_{C-H}), 2874 (ν_{C-H}), 2104 ($\nu_{N=N=N}$), 1742 ($\nu_{C=O}$, ester), 1661 ($\nu_{C=O}$, amide I), 1539 (δ_{N-H}, amide II), 1234 (ν_{C-O}), 1045 (ν_{C-O-C}); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.27 (t, 2 H, J_{vic} = 6.7 Hz, N_3CH_2), 2.19 (m, 2 H, $COCH_2$), 1.63 (m, 2 H, $N_3CH_2CH_2$), 1.60 (m, 2 H, $COCH_2CH_2$), 1.37 (m, 2 H, $N_3CH_2CH_2CH_2$), and other signals are summarized in Tables 1 and 2; ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.36 (C=O), 171.98 (C=O), 171.89 (C=O), 170.65 (C=O), 169.22 (C=O, aglycon), 51.18 (N_3CH_2), 36.28 ($COCH_2$), 28.53 ($N_3CH_2CH_2$), 26.19 ($COCH_2CH_2$), 24.52 ($N_3CH_2CH_2CH_2$), 20.72 ($COCH_3$), 20.67 ($COCH_3$), 20.56 ($COCH_3$), and other signals are summarized in Table 3.

Anal. Calcd for $C_{20}H_{31}N_5O_9$: C, 49.48; H, 6.44; N, 14.42. Found: C, 49.54; H, 6.39; N, 14.31.

*2-Acetamido-N^1-(6-azidohexanoyl)-2-deoxy-β-D-glucopyranosylamine (**10**).* Trans-esterification of an acetate **9** (1.47 g, 3.03 mmol) in MeOH (15 mL) was carried out in the presence of NaOMe (0.052 g, 0.963 mmol) at rt under a N_2 atmosphere, and the mixture was stirred at rt for 4 h under a N_2 atmosphere. The mixture was treated with IR120B (H^+) until pH 7 on wet pH paper, and the suspension was passed through a cotton pad. The filtrate was concentrated *in vacuo*, followed by crystallization from abs EtOH to give white needle-like crystalline **10** (0.670 g) in 61.5% yield: R_f 0.09 [9:1 $CHCl_3$−MeOH (v/v)]; $[\alpha]_D^{25}$ +30.3° (*c*. 1.0, MeOH); mp. 221 °C; IR (KBr) 3300 ($\nu_{O-H,\ N-H}$), 3261 (ν_{N-H}), 3090 (ν_{C-H}), 2934 (ν_{C-H}), 2864 (ν_{C-H}), 2097 ($\nu_{N=N=N}$), 1665 ($\nu_{C=O}$, amide I), 1545 (δ_{N-H}, amide II), 1443 (δ_{C-H}), 1373 (δ_{C-H}), 1321 (δ_{C-H}), 1267 (δ_{C-H}), 1111 (ν_{C-O}), 1088 (ν_{C-O}), 1038 (ν_{C-O-C}); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.39 (m, 2 H, N_3CH_2), 2.04 (m, 2 H, $COCH_2$), 1.51 (m, 2 H, $N_3CH_2CH_2$), 1.45 (m, 2 H, $COCH_2CH_2$), 1.25 (m, 2 H, $N_3CH_2CH_2CH_2$), and other signals are summarized in Tables 1 and 2; ^{13}C NMR (126 MHz, DMSO-d_6) δ 50.78 (N_3CH_2), 35.52 ($COCH_2$), 28.18 ($N_3CH_2CH_2$), 25.82 ($COCH_2CH_2$), 24.80 ($N_3CH_2CH_2CH_2$), and other signals are summarized in Table 3.

Anal. Calcd for $C_{14}H_{25}N_5O_6$·0.7 H_2O: C, 45.20; H, 7.15; N, 18.83. Found: C, 45.45; H, 7.01; N, 18.51.

*2-Acetamido-N^1-(6-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine (**11**).* Hydrogenolysis of an azide **10** (0.550 g, 1.53 mmol) in MeOH (5.5 mL) was performed in the presence of $Pd(OH)_2$/C (0.294 g) at rt for 2 h under a H_2

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Table 4. Results of Polymerizations Using Different Ratios of Carbohydrate Monomers 1 and 2 and Acrylamide (AAm)

polymer	monomer ratio		time	total yield[a]	polymer composition			sugar content	$\overline{Mw}$[d]	$\overline{Mw}/\overline{Mn}$
	sugar	AAm	h	%	x[b]	y[b]	n[c]	wt %	kDa	
3a		0		87	1	0	471	100	129	2.4
3b		1		81	1	1	472	79	169	2.2
3c	1	5	3	87	1	6	320	39	226	1.7
3d		10		98	1	11	234	26	254	1.8
3e		15		81	1	17	168	18	246	1.8
3f		20		93	1	22	146	15	274	1.7
4a		0		43	1	0	698	100	270	2.0
4b		1		87	1	1	633	84	295	2.3
4c	1	5	2	57	1	5	423	52	308	2.1
4d		10		97	1	11	263	33	310	2.0
4e		15		79	1	15	245	25	371	1.4
4f		20		72	1	21	183	21	345	1.5

[a]Total yields were calculated on the basis of quantities of monomers used. [b]Polymer compositions of x:y were estimated on the basis of the results of ^{1}H NMR, and the structures of the glycopolymers are shown in Figure 2. [c]The degrees of polymerizations were estimated on the basis of the results of weight-average molecular weights ($\overline{Mw}$s). [d]The $\overline{Mw}$s were estimated by size-exclusion chromatography in a water solution using a Shodex GF-510HQ column. Calibration curves were obtained using pullulan standards (5.9, 11.8, 22.8, 47.3, 112, 212, and 404 kDa; Shodex P-82).

atmosphere. TLC indicated complete conversion of **10** into **11** judged by a ninhydrin test, and the $Pd(OH)_2$/C was removed by filtration with a pad of celite. The filtrate was concentrated *in vacuo*, and the residue was purified by means of column chromatography on IR120B (H^+). The adsorbed amine **11** was eluted with 2 M aq NH_3, and the eluant was evaporated *in vacuo*, followed by lyophilization to afford light-yellowish powdery **11** (0.533, quant): R_f 0.02 [65:30:4 $CHCl_3$−MeOH−H_2O (v/v/v)]; $[\alpha]_D^{25}$ +24.9° (*c*. 1.0, MeOH); IR (KBr) 3397 ($\nu_{O-H,\ N-H}$), 3294 (ν_{N-H}), 3090 (ν_{C-H}), 2934 (ν_{C-H}), 2866 (ν_{C-H}), 1655 ($\nu_{C=O}$, amide I), 1545 (δ_{N-H}, amide II), 1375 (δ_{C-H}), 1317 (δ_{C-H}), 1080 (ν_{C-O}), 1047 (ν_{C-O-C}); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.48 (t, 1 H, J = 6.9 Hz, NH_2CH_a), 2.42 (t, 1 H, J = 7.0 Hz, NH_2CH_a), 2.04 (m, 2 H, $COCH_2$), 1.43 (m, 2 H, $NH_2CH_2CH_2$), 1.32 (m, 2 H, $COCH_2CH_2$), 1.20 (m, 2 H, $NH_2CH_2CH_2CH_2$), and other signals are summarized in Tables 1 and 2; ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.49 (C═O, NAc), 169.87 (C═O, aglycon), 49.40 (NH_2CH_2), 35.54 ($COCH_2$), 29.42 ($NH_2CH_2CH_2$), 26.42 ($COCH_2CH_2$), 25.09 ($NH_2CH_2CH_2CH_2$), and other signals are summarized in Table 3.

*2-Acetamido-N^1-(6-acrylamidohexanoyl)-2-deoxy-β-D-glucopyranosylamine (**2**).* A solution of acryloyl chloride (0.156 mL, 1.91 mmol) in THF (10 mL) was dropwise added to a solution of an amine **11** (0.533 g, 1.60 mmol) in saturated aq $NaHCO_3$−THF [20 mL (v/v)] at 0 °C under an Ar atmosphere. The reaction was stirred at 0 °C for 3 h under an Ar atmosphere. The progress of the reaction was monitored by means of TLC, and complete consumption of **11** was confirmed. The alkaline mixture was adjusted with 1 M aq HCl until pH 7, and the neutral mixture was concentrated *in vacuo*. Chromatographic purification of the residue on silica gel with 3:2 (v/v) $CHCl_3$−MeOH as the eluent gave crude **2**, which was further purified by passing through a column of Sephadex LH-20 with MeOH to provide white powdery **2** (0.204 g) in 32.9% yield after vacuum-drying: R_f 0.48 [3:2 $CHCl_3$−MeOH (v/v)]; mp. 222 °C; IR (KBr) 3310 (ν_{O-H} & ν_{N-H}), 3287 (ν_{O-H} & ν_{N-H}), 3258 (ν_{N-H}), 2938 (ν_{C-H}), 2864 (ν_{C-H}), 1676 ($\nu_{C=O}$, amide I), 1653 ($\nu_{C=C}$), 1541 (δ_{N-H}, amide II), 1088 (ν_{C-O}), 1036 (ν_{C-O-C}); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.07 (m, 2 H, $NHCH_2$), 2.03 (m, 2 H, $COCH_2$), 1.44 (m, 2 H, $COCH_2CH_2$), 1.40 (m, 2 H, $NH_2CH_2CH_2$), 1.20 (m, 2 H, $NH_2CH_2CH_2CH_2$), and other signals are summarized in Tables 1 and 2; ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.78 (C═O, NAc), 170.34 (C═O, aglycon), 164.71 (C═O, acryloyl), 131.83 (CH═), 125.13 (CH_2═), 38.46 ($NHCH_2$), 35.52 ($COCH_2$), 28.86 ($NHCH_2CH_2$), 26.08 ($COCH_2CH_2$), 24.89 ($NHCH_2CH_2CH_2$), and other signals are summarized in Table 3; FAB MS calcd for $[M + H]^+$: 388.2. Found: m/z 388.2.

Radical Polymerization. Deaeration of a solution of appropriate amounts of monomer **1** (or **2**) and acrylamide (**AAm**) in water was performed under diminished pressure for 30 min. *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED, 0.2 equiv of monomer) and ammonium persulfate (APS, 0.1 equivalents of monomer) were added to the reaction mixture at rt under an Ar atmosphere. The reaction mixture was allowed to stir at rt for 2 h. A 0.1 M aq pyridine-acetic acid buffer (pH 5.0) was added. Dialysis of the aqueous solution against deionized water, followed by lyophilization gave a white cotton-like polymer. The results of polymerization are summarized in Table 4.

Average Molecular Weight Estimation by Means of Size-Exclusion Chromatography (SEC). Weight-average molecular weights ($\overline{Mw}$) were determined by using a CLASS-VP system (Shimadzu Corp., Tokyo, Japan) equipped with a Shodex Asahipak GF-510HQ column (0.46 mm × 250 mm, Shoko, Co., Ltd., Tokyo, Japan) at 40 °C. A 0.3 M NaCl aqueous solution was used as the eluent, and the column was equilibrated before loading sample solution. A flow rate of 0.300 mL/min was used for the SEC system. A differential refractometer (Waters Corp., MA) was used for detection of the sample. Calibration curves were obtained using pullulan standards (5.9, 11.8, 22.8, 47.3, 112, 212, 404, and 788 kDa; Shodex P-82). The results of SEC measurements are summarized in Table 4.

Biological Evaluations of Glycopolymers for WGA. Fluorometric measurements were performed in a Teflon-stoppered cuvette (12.5 mm in width × 45 mm height) containing 3.0 mL of a sample. Emission spectra of WGA induced by excitation at 295 nm were uncorrected and were recorded with a Shimadzu RF-5300PC fluorescence spectrophotometer. The slit width used for the excitation and emission was 5.0 nm, and the interval of sampling was 1.0

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nm. The scan speed and the sensitivity were high. The cuvette was mounted in a thermostated holder, and measurement was carried out at 4 °C in order to eliminate the effect of nonspecific binding on the spectra. The concentration of WGA was estimated to be 0.65 μM by using the absorption coefficient at 280 nm ($E_{280}^{1\%}$ = 15.0 in 50 mM Tris-HCl buffer, 1.25 M NaCl, 0.25 mM $CaCl_2$, pH 7.4).[15] The results of biological evaluation by means of fluorometric measurements are summarized in Table 5.

Table 5. Results of Binding Assays for WGA on the Basis of Fluorescence Measurements

compounds	$\Delta\lambda$ (nm)	$\Delta F'/F_0$ (%)	$-\Delta G_a^{0a}$ (kJ/mol)	K_a (M^{-1})	relative potency[b]
GlcNAc	−4	8	19	4.6×10^3	1
3a				ND	
3b				ND	
3c				ND	
3d				ND	
3e				ND	
3f				ND	
4a	−10	22	29	2.6×10^5	56
4b	−5	33	30	3.7×10^5	80
4c	−6	39	30	4.7×10^5	102
4d	−5	46	32	12.8×10^5	278
4e	−4	26	32	10.5×10^5	228
4f	−3	23	32	8.9×10^5	194

[a]ΔG_a^0 was calculated from the relationship $\Delta G = -RT \ln K_a$. [b]Relative potencies were calculated on the basis of K_a at 348 nm of monomeric glycoside.[19]

RESULTS AND DISCUSSION

Our synthetic plan for the conversion of *N*-linked-type GlcNAc monomers **1** and **2** into the glycopolymers **3** and **4** is summarized in Figure 2. Glycomonomers were allowed to react with or without acrylamide (**AAm**), which plays the role of a controller of the distance between GlcNAc residues. Each glycomonomer was prepared from a known common precursor amine,[13] and the monomers underwent simple radical polymerization to yield the desired glycopolymers.

A β-glycosyl amine **5** was prepared by the method previously reported,[13,16] and the aminoalcohol **5** was converted to the corresponding glycosyl monomers **1** and **2** as shown in Scheme 1. Thus, the aminoalcohol was directly acryloylated with acryloyl chloride in the presence of a base to afford crude β-*N*-glycoside **1**, which was further acetylated in the usual manner since **1** could not be crystallized for isolation. Trans-esterification by Zemplén's method[17] of acetate **6** afforded the desired **1**. Structural elucidation of **1** was done by ^{1}H NMR spectroscopic analysis in DMSO-d_6 as shown in Figure 3a. Three kinds of specific proton signals according to an alkenyl group appeared around 6.3 to 5.5 ppm, and two kinds of characteristic proton signals according to amides, which are attributed to the corresponding acetamide at the C-2 position and the acrylamide at the aglycon were observed at 7.8 ppm and at 8.4 ppm, respectively.

Since the β-*N*-glycoside-type glycomonomer **1** having the shortest aglycon was obtained, our attention was turned toward an elongation of aglycon in order to examine the degree of freedom of the sugar moiety after conversion into the corresponding glycopolymer. Thus, β-glycosyl amine **5** was allowed to react with an acid chloride having appropriate methylene chains and a bromine atom at the ω-position. The amide formation reaction of amine **5** with commercially available 6-bromohexanoyl chloride proceeded in MeOH−THF in the presence of a base to afford the corresponding amide **7**, which was further acetylated to yield pure acetate **8** having mp 201 °C in 61% yield in 2 steps. The bromine atom in **8** was the converted into an azide, which was a precursor functional group of an amine. S_N2 replacement of **8** with NaN_3 in DMF proceeded smoothly to provide the corresponding crystalline azide **9** in 88% yield. The ester function in the azide **9** was removed by trans-esterification to yield the desired glycosyl monomer **10** in 62% yield. Quantitative reduction by

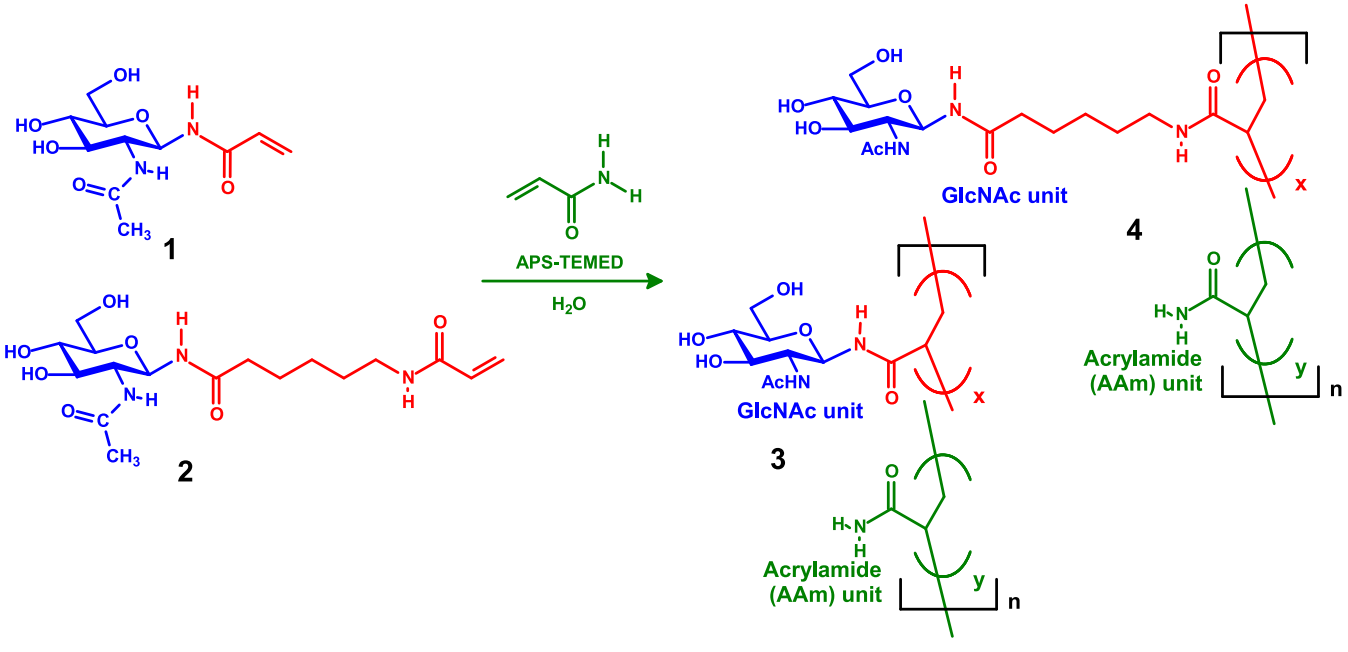

Figure 2. Synthetic plan for construction of glycopolymers.

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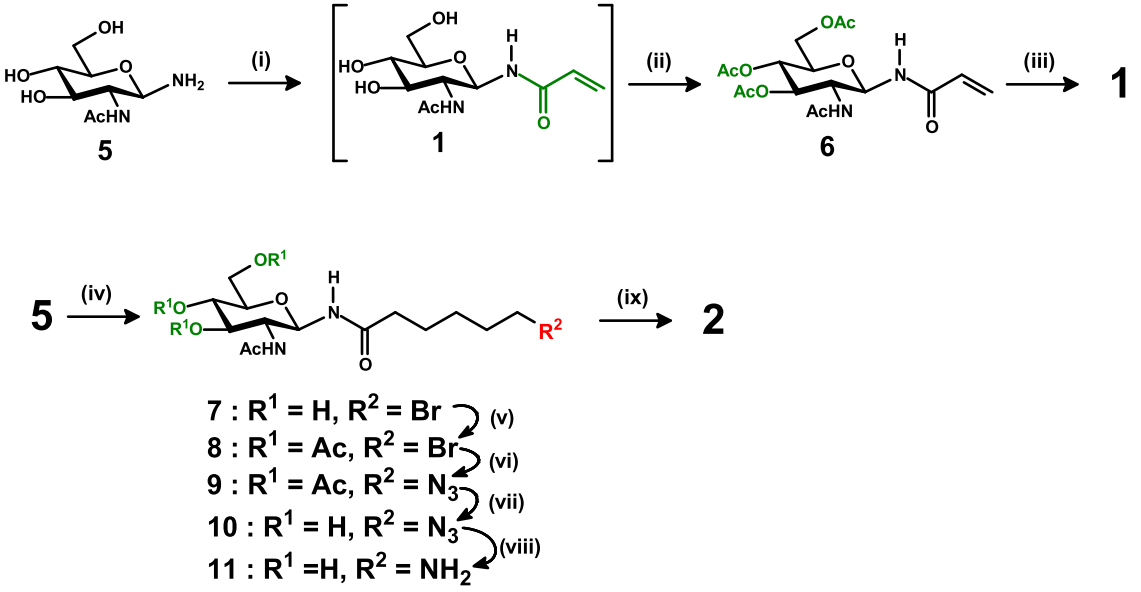
Scheme 1. Synthesis of Glycosyl Monomers Involving an *N*-Glycosidic Linkage Reagents and Conditions: (i) CH_2═CHCOCl, satd aq $NaHCO_3$−THF, 0 °C, Ar, 4 h, (ii) Ac_2O, pyr, 0 °C, Ar, 2.5 h, (iii) NaOMe, MeOH, rt, Ar, 1.5 h, (iv) 6-Bromohexanoyl chloride, $NaCO_3$, MeOH−THF, 0 °C, 4 h, (v) Ac_2O, pyr, 0 °C, N_2, 3.5 h, (vi) NaN_3, DMF, 80 °C, N_2, 4 h, (vii) NaOMe, MeOH, rt, N_2, 4 h, (viii) $Pd(OH)_2/C$, MeOH, rt, H_2, 2 h, (ix) CH_2═CHCOCl, satd aq $NaHCO_3$−THF, 0 °C, Ar, 3 h



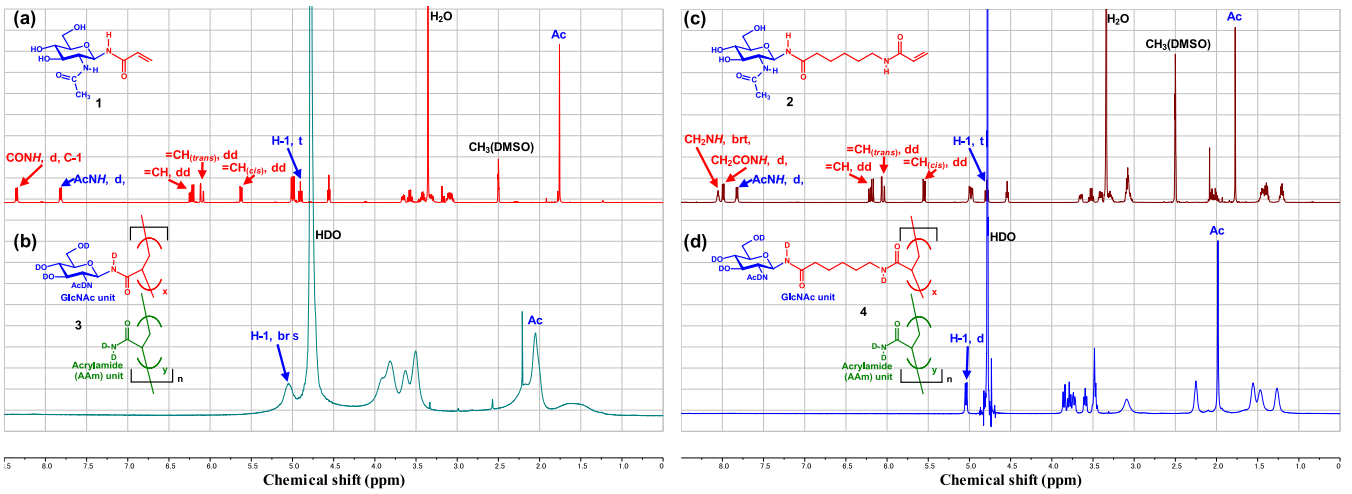


Figure 3. ^{1}H NMR spectra of (a) glycomonomer **1** measured in DMSO-d_6, (b) homopolymer **3a** derived from the corresponding monomer **1** measured in D_2O, (c) glycomonomer **2** measured in DMSO-d_6, and (d) homopolymer **4a** derived from the corresponding monomer **2** measured in D_2O.

means of $Pd(OH)_2/C$ under H_2 gave the corresponding amine **11** that was positive in a ninhydrin test, and purification was performed on a cation-exchange resin eluting with 2 M aqueous ammonium hydroxide to afford pure **11** after lyophilization. Base-mediated acryloylation of the amine **11** was carried out, and the reaction mixture was purified by a combination of silica gel chromatography and gel filtration using Sephadex LH-20 to remove residual silica mass to give white powdery **2** in 33% yield. The ^{1}H NMR in DMSO-d_6 is shown in Figure 3c. Three kinds of similar proton signals according to an alkenyl group compared with **1** as shown in Figure 3a appeared around 6.3 to 5.5 ppm. In addition to two kinds of characteristic proton signals according to amides for compound **1**, a further amide proton attributed to the terminal acrylamide appeared at 8.1 ppm as a broad triplet signal.

Given the success of the preparation of *N*-linked-type GlcNAc monomers having appropriate linker lengths, the monomers **1** and **2** were converted into the corresponding glycopolymer **3** and **4**. A schematic image of the polymerization is shown in Figure 2. A typical polymerization protocol was applied to the glycomonomers, and polymerization with ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) as a radical initiator system proceeded in aqueous media at rt to give the desired glycopolymers after general workup procedures. The results of polymerizations are summarized in Table 4. The ^{1}H NMR spectrum of each homopolymer **3a** and **4a** are shown in Figure 3b,d, respectively. Characteristic broadening of proton signals in both spectra is observed, and there are no alkenyl signals. In comparison with polymers **3a** and **4a**, homopolymer **3a** showed a highly broadening NMR profile in the whole field,

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while homopolymer **4a** showed broadening signals around the polymer backbone and typical proton signals according to the pyranose ring protons. The results of ^{1}H NMR according to the glycopolymers **3a** and **4a** suggested that there are considerable differences in a degree of freedom of the carbohydrate moiety in each glycopolymer. The yields of radical polymerization were 43−98% based on the quantities of the corresponding monomers, and the polymer compositions were determined as sugar/**AAm** = 1.0:0−22 based on the results of ^{1}H NMR spectra. Since no differences between the charged ratio and the polymer composition were observed, randomness in the polymer composition for the sugar unit and the acrylamide unit is high. Although controlled average polymer composition was accomplished, simple radical polymerization has a limitation on the sequence of each monomer unit on the corresponding polymer. The sugar contents of the glycopolymers are also shown in Figure 4.

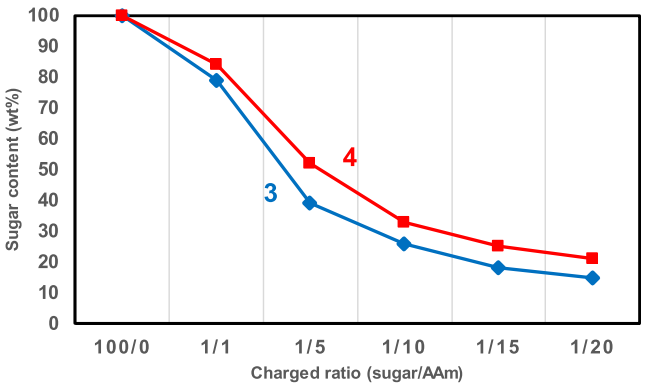

Figure 4. Relationship between charged ratio in polymerization and results of sugar content in **3** and **4**.

Weight-average molecular weights ($\overline{Mw}$s) were estimated by means of size-exclusion chromatography (SEC), and each glycopolymer had an appropriate molecular weight. The degrees of dispersion ($\overline{Mw}/\overline{Mn}$) were calculated according to the results of SEC, and the values were 1.4−2.4. The relationship between the charged ratio in polymerization and the results of sugar content is shown in Figure 4. The sugar contents in both glycopolymers strongly depended on the charged ratio of the monomers and could be controlled. In addition, because of the bulkiness of the carbohydrate portion close to the polymer backbone, incorporation of glycomonomer **1** having a shorter spacer arm into the corresponding polymer backbone was slightly low than that of glycomonomer **2** having a longer spacer arm.

Since the series of β-*N*-glycoside-type glycopolymers having appropriate spacers between the GlcNAc residue and the polymer backbone were systematically prepared, the biological responses of the polymers were investigated using wheat germ agglutinin (WGA), which is a lectin that has been shown to selectively recognize and bind to GlcNAc and GlcNAc-β(1,4)-linked oligosaccharide chains.[18] We previously reported a series of β-*O*-glycoside-type glycopolymers having various spacer arms between the GlcNAc residue and the polymer backbone by means of fluorometric measurements using WGA.[19] WGA has tryptophan residues, and it is known that changes in the environment of a tryptophan residue in a protein are highly sensitive in both a UV spectrum and fluorescence spectrum.[20] The uses of spectral changes of tryptophan residues in a protein for biological evaluations of carbohydrate−protein interactions have been reported.[15,21] The results of our ongoing synthetic studies using simple and convenient preparations of glycopolymers involving GlcNAc have been reported in order to apply a fluorometric assay for WGA because the tryptophan residues in WGA are highly sensitive for binding with GlcNAc.[10,22] The results of fluorometric analysis of a glycopolymer **3a** as a first trial in 0.65 μM of WGA solution in 50 mM Tris-HCl buffer (pH 7.4) at 4 °C are shown in Figure 5a. The WGA solution was maintained at 4 ± 0.1 °C for all measurements, and the excitation wavelength was used at λ 295 nm for specific excitation of Trp on WGA.[23] A fluorescence spectrum of the WGA solution was monitored every time upon addition of an aliquot of a polymer solution **3a** to the WGA solution, and the spectra are shown in Figure 5a. The fluorescence intensity of the spectra did not show any differences, and the results suggested that there is no interaction between the sugar moieties and the binding sites of WGA. All glycopolymers **3a**−**3f** having the shortest spacer arm between GlcNAc, and the corresponding polymer backbone were measured by the same protocol and showed no binding abilities on the basis of the corresponding fluorospectroscopic analyses. In addition to the spectra of **3a**, the spectra of **3d** having a polymer composition of sugar/**AAm** = 1:11 are shown in Figure 5b as a further example and other spectra according to the glycopolymers **3** are shown in the Supporting Information (Figures S27−S29). Further trials were performed by addition of an aliquot of a polymer solution **4a** to the WGA solution by using fluorometric analyses, and the results of the spectrum are shown in Figure 5c. In this case, the intensity of the spectrum gradually increased, and the results indicated effective binding between the sugar moieties in the glycopolymer **4a**, and the binding sites of WGA attributed to the change in the environmental behavior of tryptophane near the binding site of the WGA. Similarly, copolymer **4d** having a polymer composition of sugar/**AAm** = 1:11 was also demonstrated in comparison with copolymer **3d**, and the results of fluorescence profiles by addition of an aliquot of the polymer solution **4d** to the WGA solution are shown in Figure 5d. Since the investigation between copolymer **4d** and the WGA clearly showed considerable differences from the spectrum of WGA alone indicated as "A" to the spectrum according to final addition of an aliquot of the polymer solution **4d** to the WGA solution indicated as "B", efficient binding between copolymer **4d** and WGA was confirmed. Further spectra according to the glycopolymers **4** are shown in the Supporting Information (Figures S30−S35).

Since successful interactions of glycopolymer **4** and WGA were obtained, our attention was focused on the affinity of the polymer **4** against the WGA, and the analytical platform of **4b** as an example is shown in Figure 6. The fluorescence spectrum of WGA by addition of an aliquot of a polymer solution **4b** to the WGA solution was recorded, and the results are shown in Figure 6a. In this case, the intensity of the spectrum also gradually increased, and each value at 348 nm of the spectra was read, and the values of the intensities of emission spectra are plotted in Figure 6b. Figure 6c shows the results of Hill plot analysis[24] by using the parameters, and the results provided the corresponding association constant K_a of 3.7 × 10^5 M^{-1}, which is 80 times stronger than that of the monomeric GlcNAc derivative.[19] Other glycopolymers according to **4** were analyzed in the same manner as that described

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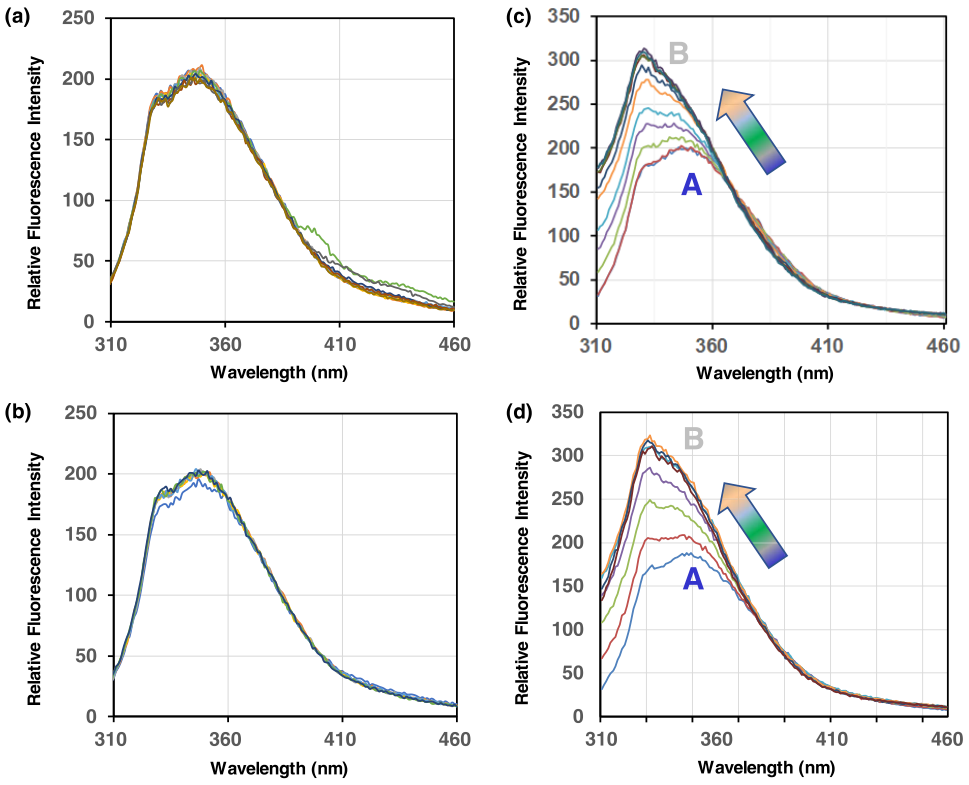

Figure 5. Results of measurement of the interaction of glycopolymers. (a) Changes in fluorescence emission spectra of WGA (0.65 μM, 3.0 mL, 50 mM Tris-HCl buffer containing 1.25 M NaCl, 25 mM $CaCl_2$, pH 7.4, 4°C) homopolymer **3a** upon addition of aliquots of polymer **3a** (0.50 mM). (b) Changes in fluorescence emission spectra of WGA (as that for **3a**) copolymer **3d** upon addition of aliquots of polymer **3d** (1.0 mM). (c) Changes in fluorescence emission spectra of WGA (as that for **3a**) homopolymer **4a** from **A** to **B** upon addition of aliquots of polymer **4a** (0.50 mM). (d) Changes in fluorescence emission spectra of WGA (as that for **3a**) homopolymer **4d** from **A** to **B** upon addition of aliquots of polymer **4d** (0.50 mM).

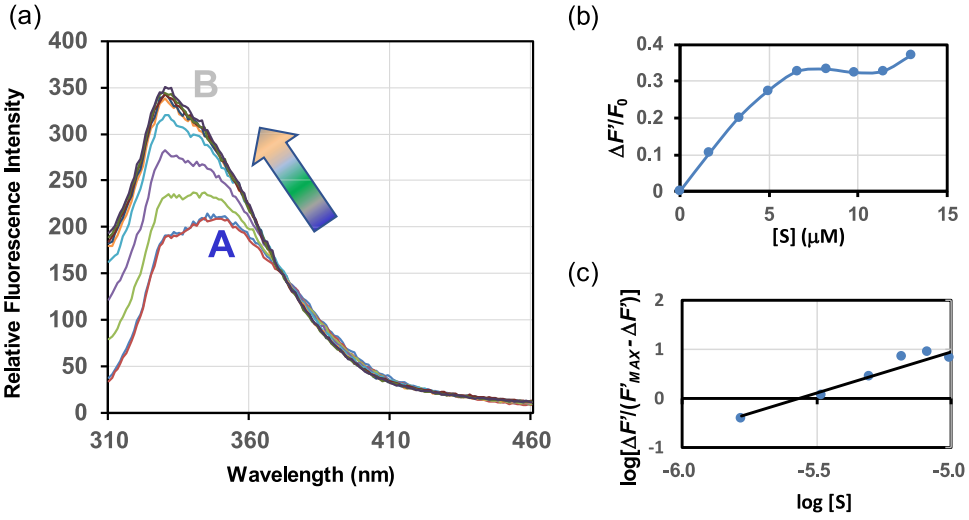

Figure 6. Results of measurement of the interaction of homopolymer **4b** with WGA. (a) Changes in fluorescence emission spectra of WGA (0.65 μM, 3.0 mL, 50 mM Tris-HCl buffer containing 1.25 M NaCl, 25 mM $CaCl_2$, pH 7.4, 4°C) from **A** to **B** upon addition of aliquots of polymer **4b** (0.50 mM). (b) Plots of $\Delta F'/F_0$ versus [S], where $\Delta F'$ is the change in the intensity at 348 nm of WGA with various concentrations of **4b**, 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 $[\Delta F'/(\Delta F'_{max} - \Delta F')]$ versus log [S].

for **4b**, and all of the profiles of fluorescence spectra of glycopolymers **4a**−**4f**−WGA interaction are shown in the Supporting Information. The results of biological evaluations by fluorometric assays are summarized in Table 5. Maximum wavelength shifts of each evaluation of the glycopolymer−WGA as shown by $\Delta\lambda$ were −3 to −10 nm and changes in fluorescence intensities of the glycopolymers as represented by $\Delta F'/F_0$ were 22−46%. In addition, the K_a values were 2.6×10^5 to 12.8×10^5 M^{-1} on the basis of sugar unit concentrations In comparison with these values for a monomeric compound;

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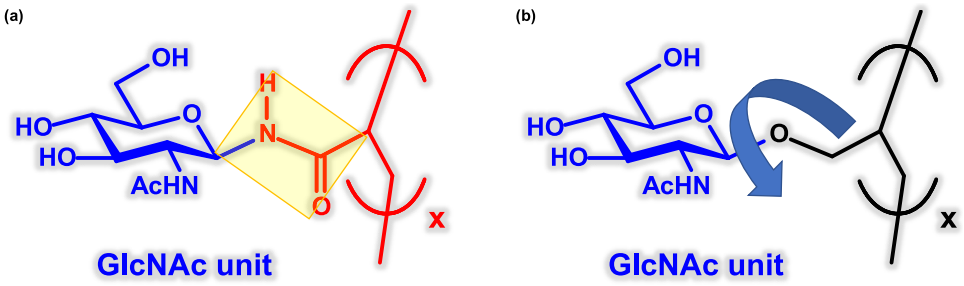

Figure 7. Schematic images of the (a) *N*-glycoside-type glycopolymer with no appropriate spacer and (b) *O*-glycoside-type glycopolymer with the shortest spacer.

much higher values were obtained and a positive glycoside cluster effect was therefore observed.[2] When glycopolymer **4d** was used as the substrate, the K_a value was the highest. 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 around 30 kJ/mol based on the sugar unit concentrations.

Although a series of glycopolymers **4** having an appropriate length of the spacer between the GlcNAc moiety and the polymer backbone had binding abilities for WGA, a series of glycopolymers **3** having an amide linkage alone between the GlcNAc moiety and the polymer backbone did not show any binding abilities for WGA. Schematic images of a glycopolymer having an *N*-glycosidic linkage between the GlcNAc moiety and the polymer backbone as shown in polymer **4** and a glycopolymer having an *O*-glycosidic linkage between the GlcNAc moiety and the polymer backbone as shown in our previous study[11] are shown in Figure 7. Since Figure 7b shows the *O*-glycoside-type glycopolymer having a high degree of freedom of the sugar moiety due to the connection using sp^3 hybrid orbitals, binding affinity of the polymer against WGA was around 10^5 M^{-1}. On the other hand, the *N*-glycoside-type polymer as shown in Figure 7a seems to show a low degree of freedom of the sugar moiety due to the connection using a rigid planar bond with a resonance effect, and hence no binding affinities of the polymer were observed. Since the difficulties in interpreting the binding/no binding results based on no structural data was reported by Unverzagt and Jiménez-Barbero,[25] a molecular resolution technique might be needed in order to speculate difference in the degree of freedom around the linkages. Further investigations related to the linkages and the lengths are now in progress, and the results will be reported elsewhere.

CONCLUSIONS

N-Glycoside-type glycomonomers having no methylene chains and appropriate methylene chains were prepared, and polymerizations of the monomers with or without **AAm** were carried out to produce a series of glycopolymers having various sugar densities. Biological evaluations of the polymers against WGA were performed on the basis of fluorometric analyses according to changes in the environment of the tryptophan residue in WGA. It was found that glycopolymers having direct linkage to the polymer backbone did not show any biological activities, while glycopolymers having an appropriate spacer length between the sugar and the polymer backbone showed binding activities with 10^5–10^6 M^{-1}. It was also found that there is a significant factor based on the degree of freedom of carbohydrate moieties in order to bind to the lectins. In addition, previous report[26] showed structure–activity relationships employing a GlcNAc carbamate motif with α-configuration and by systematic variation of the spacer length on divalent derivatives, and there was suitable length between carbohydrates at each terminal end bridging adjacent binding sites on WGA. Further investigation using the series of *N*-glycoside-type glycopolymers employing an *N*-glycosidic linkage and by systematic control of carbohydrate densities is underway, and the results will be reported elsewhere.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05151.

Spectral data including NMR, IR, fluorometric assays, and Hill plot's analyses for biological evaluations (PDF)

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Notes

The authors declare no competing financial interest.

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