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Globular Carbohydrate Macromolecule "Sugar Balls" 3. "Radial-Growth Polymerization" of Sugar-Substituted α-Amino Acid *N*-Carboxyanhydrides (GlycoNCAs) with a Dendritic Initiator [1]

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Abstract: "Radial-Growth Polymerization (RGP)", polymerization initiated with multivalent dendrimer to afford dendrimer-based star polymer, was proposed as a new class of polymerization systems. Oligoglycopeptide-type sugar balls, i.e., oligo[*O*-(β-D-glucopyranosyl)-L-serine]-persubstituted poly(amido amine) dendrimer (**4a**) and oligo[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-serine]-persubstituted poly(amido amine) dendrimer (**4b**), were obtained by ring-opening oligomerization of sugar-substituted α-amino acid *N*-carboxyanhydrides (glycoNCAs), i.e., *O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (**1a**) and *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (**1b**), respectively, with poly(amido amine) dendrimer (**2**, generation, 3.0-5.0) as a macroinitiator, followed by quantitative deacetylation with hydrazine monohydrate. Yields and M_w/M_n values of the products of the oligomerization were 97-99% and 1.0_3-1.1_1, respectively. Sugar balls **4a** and **4b** were soluble in water and dimethyl sulfoxide. Molecular recognition ability of **4a** and **4b** was examined by erythrocyte agglutination inhibition assays using wheat germ agglutinin (WGA).

INTRODUCTION

Precision synthesis of macromolecules by controlled organic reactions is important in order to produce useful polymeric compounds as functional materials. Living polymerization is a powerful methodology to synthesize linear polymers with narrow molecular weight distributions. We have already reported synthetic utilities of sugar-substituted α-amino acid *N*-carboxyanhydrides (glycoNCAs) to afford glycopeptides as a first example of living polymerization of sugar-bearing monomers.[2] A variety of glycopeptide-carrying polymers, e.g., graft-type[3] and block-type[2,4] synthetic

glycoconjugates, have been prepared on the basis of a strategy to synthesize carbohydrate polymers by using living polymerization.[5] Cell recognition ability of these saccharide-containing polymers has been evaluated with examining inhibition activities of erythrocyte agglutination by lectin.[3] Artificial glycoconjugates having sugar residues with molecular recognition ability and molecular information are indispensable as simplified model compounds of natural glycoconjugates as well as polymeric materials for biochemical and biomedical applications such as drug delivery systems (DDS).

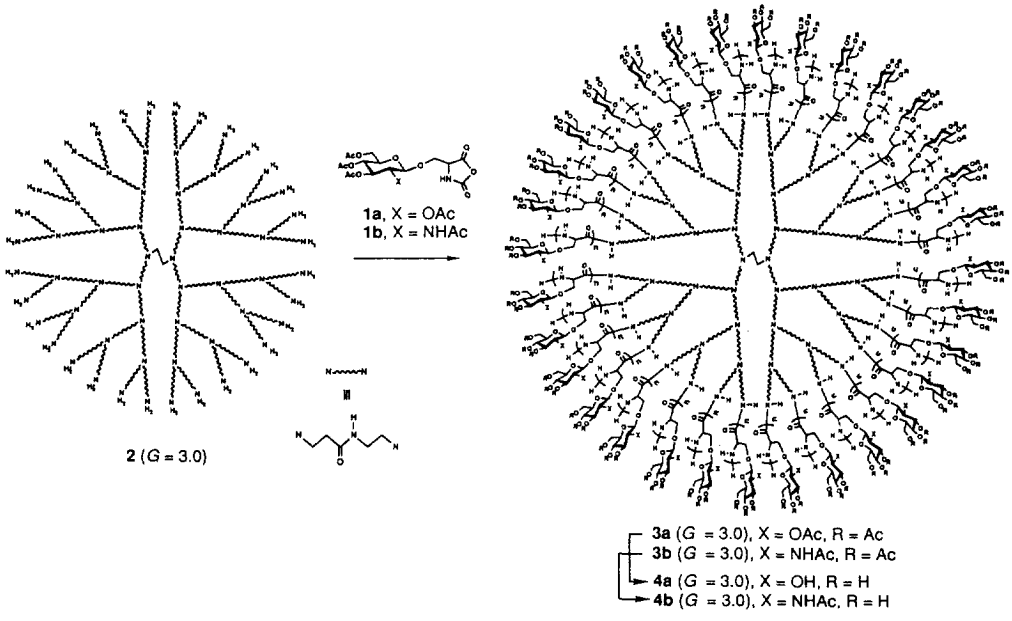
Under the concept of intersugar space regulated carbohydrate polymers by three-dimensional macromolecular architectures, we have presented synthesis and molecular recognition of "Sugar Balls", sugar-persubstituted dendrimers.[6] One of the most important features of dendrimers should be their ideally spherical shape of higher-order structure.[7] Sugar balls have a structure of an internal dendrimer skeleton covered with an external sugar layer. Concerning with recent progress of glycotechnology and glycobiology, sugar balls are a typical example for functionalization of dendrimer. Besides sugar-bearing dendrimers,[8] peptide dendrimers[9] and nucleic acid dendrimers[10] have been reported.

The present article describes synthesis of dendrimer-based star polymers, oligoglycopeptide-type sugar balls, by the new macromolecular design of living polymer-persubstituted dendrimers. The oligoglycopeptide-type sugar balls were synthesized by living oligomerization of glycoNCAs with poly(amido amine) (PAMAM) dendrimer as a multi-functional initiator. To our best knowledge, dendrimer-initiated chain polymerization has not been published until now.[1] This polymerization system is coined as "Radial-Growth Polymerization (RGP)", since dendrimer-based living polymerizations offer highly ordered star-type macromolecules with a number of arms, which should be different from conventional star polymers. The resulting sugar balls in this study are dendritic nanocapsules surrounded radially by oligoglycopeptide chains. The oligoglycopeptide-type sugar balls are regarded as block copolymers between globular dendrimer and linear living polymer. Considering widespread utilities of common linear block copolymers, the linear polymer-persubstituted dendrimers have fundamentally significant potential to future functional materials. Synthetic routes to the dendrimer-based star-shaped polymers are classified into two major approaches, i.e., (a) living polymerization initiated by a dendritic initiator (this work) and (b) living polymerization terminated by a dendritic terminator. In order to construct a well-defined dendrimer-based star polymer, the former method would be preferable to the latter, because steric hindrance between the dendrimer and the living polymer is easy to be expected. In case of the former dendritic initiator system, strictly clean chain reaction with rapid initiation and slow propagation is required. In this respect, the living polymerization system of glycoNCAs is suitable to synthesize dendrimer-based star polymers.

RESULTS AND DISCUSSION

Synthesis of novel oligoglycopeptide-type sugar balls by ring-opening polymerization of glycoNCAs initiated with poly(amido amine) dendrimer.

Dendrimer-initiated ring-opening oligomerization of sugar-substituted NCAs (glycoNCAs), i.e., *O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (**1a**) and *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (**1b**), was carried out as an example of radial-growth polymerization (RGP). Poly(amido amine) (PAMAM) dendrimer (generation 3.0-5.0, **2** (G = 3.0-5.0)) with an ethylenediamine core was used as a multi-functional initiator. As shown in Scheme 1, glycoNCAs **1a** and **1b** were polymerized with **2** in chloroform at 25-27 °C for 24-80 hours under nitrogen. The reaction conditions and results of the polymerization are listed in Table 1.



Scheme 1 Synthesis of oligoglycopeptide-type sugar balls **4** by ring-opening oligomerization of glycoNCAs **1** initiated with dendrimer **2**, followed by deacetylation. Ideal structures of **2**, **3**, and **4** (generation, 3.0) are illustrated.

The short chain length of the glycopeptide segment was set up, because the number of glycopeptide units is appropriate for characterization of the resulting sugar balls and for evaluation of their recognition ability. White powdery products were obtained in quantitative yields by reprecipitations from chloroform to diethyl ether. The M_w/M_n values determined by size exclusion chromatography (SEC) were reasonably narrow, while those of **2** (G = 3.0, 4.0, and 5.0) were 1.0_2, 1.0_3, and 1.0_3, respectively. In the living oligomerization of the glycoNCAs, side reaction to form hydantoic acid derivatives has never been observed,[2-4,11] although common ring-opening polymerization of NCAs involves termination by the hydantoic acid production.[12] The peculiarly selective reactivity of glycoNCA

Table 1. Ring-opening oligomerization of *O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine NCA (**1a**) and *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine NCA (**1b**) with PAMAM dendrimers **2** [ethylenediamine core; $G = 3.0\text{-}5.0$] [a]

Run no.	Monomer	Initiator	$[M]_0$ [b] / $[\text{-NH}_2]_0$	$[M]_0$, mmol/L	Temp., °C	Time, h	Product	Yield, %	M_w/M_n [c]
1	**1a**	**2** (*G*=3.0)	3.64	19.5	27	24	**3a** (*G*=3.0, DP=3.8)	97.9	1.1_1
2	**1b**	**2** (*G*=3.0)	3.51	24.0	27	36	**3b** (*G*=3.0, DP=3.5)	96.5	1.0_8
3	**1a**	**2** (*G*=4.0)	3.17	18.4	27	36	**3a** (*G*=4.0, DP=3.2)	97.0	1.0_7
4	**1b**	**2** (*G*=4.0)	3.66	19.8	25	42	**3b** (*G*=4.0, DP=3.7)	98.6	1.1_0
5	**1b**	**2** (*G*=5.0)	3.50	26.0	27	80	**3b** (*G*=5.0, DP=3.5)	99.3	1.0_3
6	**1a**	**2** (*G*=3.0)	1.18	10.1	- 30	0.25	**3a** (*G*=3.0, DP=1.0)	95.7[de]	1.0_3

[a] Solvent; $CHCl_3$, under nitrogen.
[b] $[\text{Monomer}]_0/[\text{terminal primary amino groups of } \mathbf{2}]_0$.
[c] By SEC in Me_2SO at 27 °C (poly(8-oxa-6-azabicyclo[3.2.1]octan-7-one) standard).
[d] M_n, 1.92×10^4 (by VPO in $CHCl_3$ at 35 °C); M_n, 1.92×10^4 (calc.).
[e] Calculated from assumption of DP(peptide) = 1.0.

is reasonably explained by the intramolecular hydrogen bonding between 2- or 6-acetyl carbonyl groups of the sugar residue and N-H of the NCA ring of **1**.[4]

Figure 1 shows SEC profiles of initiator **2** ($G = 4.0$) and product polymer **3a** ($G = 4.0$) in run no. 3. The sharp unimodal peak ($M_w/M_n = 1.0_7$) of **3a** ($G = 4.0$) indicates the absence of the tertiary amine-catalyzed polymerization which should bring homopolymer of **1** with broad molecular weight distributions. In other words, the polymerization proceeds exclusively from the terminal amino groups on the spherical surface via "nucleophilic addition mechanism".

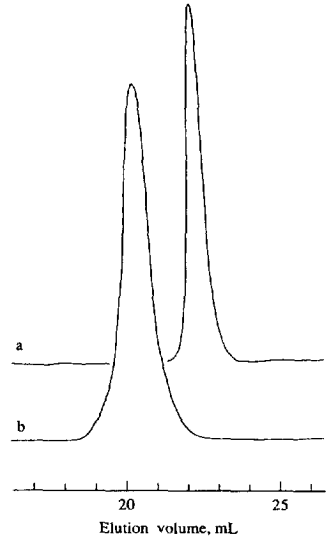


Figure 1. SEC charts of a) poly(amido amine) dendrimer **2** (ethylenediamine core; $G = 4.0$) and b) product polymer of ring-opening oligomerization of *O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (**1a**) initiated with dendrimer **2** ($G = 4.0$) (run no. 3) (refractive index detector; eluent, Me_2SO; temp., 27 °C).

As shown in the experimental section, IR, ^1H and ^{13}C NMR analyses supported the structure of oligoglycopeptide-type sugar ball precursors **3a** and **3b**. Especially, ^1H and ^{13}C NMR spectral patterns of the acetylated oligoglycopeptide segment of **3a** and **3b** quite resembled those of oligo[*O* - (tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine] and oligo[*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine] prepared by the living oligomerization of **1a** and **1b** with *n*-

hexylamine as an initiator, respectively. Figure 2 shows ^{13}C NMR spectrum of **3a** (G = 4.0). Peaks due to the PAMAM dendrimer skeleton and the acetylated oligoglycopeptide segment were observed as indicated in the figure. The following spectral data suggest that **3a** has a desired structure of a dendrimer having oligopeptide chains at all the dendritic terminal groups, instead of a dendrimer with a small number of long polypeptide chains. Multiplets (at δ 99.7, 99.3, and 99.1 ppm) of the anomeric carbons are caused by slightly different electron density of the α- and ω-ends and internal repeating units of the oligoglycopeptide segment. In the case of glyco-mono-peptide-substituted sugar ball (DP = 1.0, *vide infra*) and poly[*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine] with higher DPs, clear singlets of the anomeric protons appeared at δ 99.6 and 99.4 ppm, respectively. ^{13}C NMR peak pattern of peptide α-carbons of **3a** (G = 4.0) at 54.3 and 53.0-52.5 ppm was similar to that of living oligo[*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine] initiated with *n*-hexylamine. DPs of oligopeptide of **3a** (G = 3.0 and 4.0), which were estimated by peak intensities of the α-carbons of the ^{13}C NMR spectra, were 3.8 and 3.2, respectively. These values are in good agreement with the feed molar ratios of the monomer to the terminal primary amino groups. Furthermore, in the spectra of **3**, no signals ascribed to the α- and β-carbons of unreacted terminal amino groups of PAMAM dendrimer was detected, whereas those of **2** were observed at δ 41.0 and 41.7 ppm, respectively.

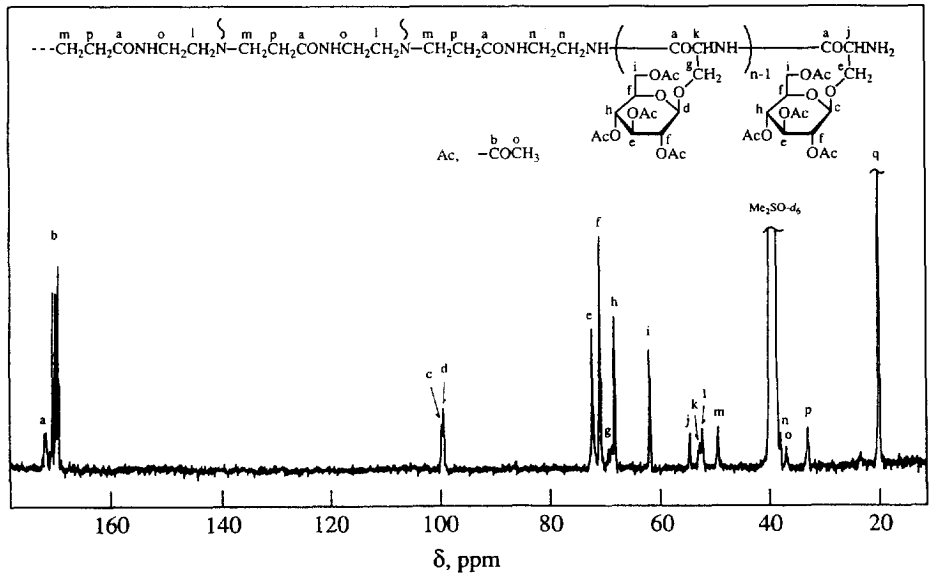


Figure 2. ^{13}C NMR spectrum of acetylated oligoglycopeptide-type sugar ball, oligo[*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine]-persubstituted poly(amido amine) dendrimer (**3a** (G = 4.0, DP of peptide = 3.2)) (5.0% Me_2SO-d_6 solution; TMS; 27 °C; 100 MHz).

To clarify the rapid initiation / slow propagation system, formation of a glycopeptide-type sugar ball precursor **3a** (G = 3.0, DP = 1.0) was attempted by terminating the reaction at -30 °C for 15 min (run no. 6). The results are listed in Table 1. The yield was nearly quantitative on the basis of the structure of **3a** (G = 3.0, DP = 1.0). The M_w/M_n value was close to that of the corresponding dendritic initiator **2** (G = 3.0, M_w/M_n = 1.0_2). The structure of **3a** (G = 3.0, DP = 1.0) was confirmed by IR, ^1H and ^{13}C NMR measurements. Molecular weight of **3a** (G = 3.0, DP = 1.0) measured by vapor pressure osmometry (VPO) agreed well with the calculated value (M_n (VPO), 1.92×10^4; M_n (calc), 1.92×10^4). These results indicate that all amino groups of **2** (G = 3.0) react with **1a** to afford glyco-mono-peptide-type sugar ball precursor **3a** (G = 3.0, DP = 1.0), and that the initiation is definitely faster than the propagation. Consequently, it was clearly demonstrated that initiation of ring-opening polymerization of the glycoNCA occurred exclusively with primary amino groups of PAMAM dendrimer.

Table 2. Deacetylation of oligoglycopeptide-type sugar ball precursors **3a** and **3b**[a]

Peracetylated Sugar Ball[b]		$H_2NNH_2{\cdot}H_2O$[c],	Temp.,	Time,	Oligoglycopeptide-type Sugar Ball	
	mg	mL (mmol)	°C	h		Yield, mg (%)
3a (G=3.0, DP=3.8)	33	0.50 (10)	19	4.0	**4a** (G=3.0, DP=3.8)	21 (99)
3b (G=3.0, DP=3.5)	53	0.76 (16)	26	4.0	**4b** (G=3.0, DP=3.5)	37 (96)
3a (G=4.0, DP=3.2)	34	0.50 (10)	27	10	**4a** (G=4.0, DP=3.2)	22 (98)
3b (G=4.0, DP=3.7)	52	0.73 (15)	23	24	**4b** (G=4.0, DP=3.7)	38 (98)
3b (G=5.0, DP=3.5)	49	0.73 (15)	25	72	**4b** (G=5.0, DP=3.5)	35 (98)

[a] Solvent, methanol.
[b] Samples prepared in run nos. 1-5 of Table 1 were used.
[c] Added as a methanol solution of 3.0 mol/L.

Deacetylation of the sugar moieties of **3** was undertaken by using hydrazine monohydrate in methanol at 19-27 °C. The results are summarized in Table 2. During the deprotection procedure, neither isomerization of the β-glycoside bond nor racemization of the peptide was observed. Oligoglycopeptide-type sugar balls **4a** and **4b** were obtained from the sugar ball precursors **3a** and **3b** in 98-99% yield and in 96-98% yield, respectively. The structure of **4a** and **4b** was identified by IR, ^1H and ^{13}C NMR spectroscopies. Theoretical molecular weights of obtained **4b** (G = 3.0, 4.0, and 5.0) were 3.73×10^4, 7.98×10^4, and 1.44×10^5, respectively. Calculated numbers of sugar residues per sugar ball of **4b** (G = 3.0 and 5.0) were 105 and 389, respectively, on the basis of DPs (peptide) of 3.5. The weight fractions of the oligoglycopeptide parts to the whole sugar balls of **4b** (G = 3.0 and 5.0) are both 82%. Although numbers of sugar residues per sugar ball molecule increase depending on the

generation, the weight fractions of the sugar moiety to the sugar ball do not vary so much. Diameters of **4b** (G = 3.0 and 5.0, DP = 3.5) were estimated by using a Corey-Pauling-Koltum (CPK) model. Diameters of three-dimensional contracted forms of sugar ball **4b** (G = 3.0 and 5.0, DP = 3.5) are 48 Å and 62 Å, while those of their extended models are 84 Å and 114 Å, respectively.[13] Intersugar distances of the terminal *N*-acetyl-D-glucosamine units of sugar ball **4b** (G = 3.0 and 5.0, DP = 3.5) are 17 Å and 11 Å (for the contracted models), 30 Å and 21 Å (for the extended models), respectively.[14] Although interior saccharides of oligopeptide chains should also participate in interaction with receptors, the present dendrimer-based star polymer architecture is preferable to design larger sugar balls having appropriate intersugar distances, in comparison with the previous sugar balls derived from disaccharide lactose and maltose.[6]

Solubility of the sugar balls

The results of a solubility test of oligoglycopeptide-type sugar balls **4a** (G = 3.0) and **4b** (G = 3.0) and their peracetylated derivatives **3a** (G = 3.0) and **3b** (G = 3.0) are shown in Table 3. In order to compare the property, solubilities of PAMAM dendrimer **2** (G = 3.0), oligo[*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine], and oligo[*O*-(β-D-glucopyranosyl)-L-serine] were also listed in the table. Good solvents for the sugar ball precursors **3a** (G = 3.0) and **3b** (G = 3.0) were different from

Table 3. Solubilities of oligoglycopeptide-type sugar ball precursors **3a** and **3b** (G = 3.0) and oligoglycopeptide-type sugar balls **4a** and **4b** (G = 3.0)[a]

Solvent	Dendrimer[b]	Peracetylated Sugar Ball		Peracetylated Glycopeptide[c]		Sugar Ball		Glycopeptide[d]
	2	**3a** DP:3.8	**3b** DP:3.5	DP:31	DP:5.0	**4a** DP:3.8	**4b** DP:3.5	DP:5.3
hexane	−	−	−	−	−	−	−	−
diethyl ether	−	−	−	−	−	−	−	−
benzene	−	−	−	−	+	−	−	−
dioxane	−	+	−	−	+	−	−	−
chloroform	−	+	+	+	+	−	−	−
pyridine	−	+	+	−	+	−	−	−
acetone	−	+	−	−	+	−	−	−
ethanol	+	−	+	−	+	−	−	−
methanol	+	−	+	−	+	−	−	−
dimethyl sulfoxide	+	+	+	−	+	+	+	−
water	+	−	+	−	−	+	+	+

[a] (+), Soluble; (−), insoluble. Temp., room temp.; conc., 1.0 mg/mL.
[b] Poly(amido amine) dendrimer (generation 3.0).
[c] Poly[(*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine].
[d] Poly[(*O*-(β-D-glucopyranosyl)-L-serine].

PAMAM dendrimer **2**. Solubility of the peracetylated sugar ball **3a** ($G = 3.0$) was intermediate between those of linear oligo[*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine] having DPs of 5.0 and 31. It was also found that the solubility of **3** was affected by the functional groups at the 2-position of the sugar moiety. This implies that sugar-derivatives of glycopeptide influence effectively the characteristics. Oligoglycopeptide-type sugar balls **4a** and **4b** were soluble in water and dimethyl sulfoxide, although the other solvents examined did not dissolve the sugar balls. This is easily understandable, because solubility of polyglycopeptide is generally limited as shown in the results of oligo[*O*-(β-D-glucopyranosyl)-L-serine].

Evaluation of recognition ability of oligoglycopeptide-type sugar balls 4a and 4b by an inhibition test of hemagglutinating activity of wheat germ agglutinin (WGA) lectin.

In order to elucidate molecular recognition function of **4a** and **4b**, their inhibition ability was investigated against erythrocyte association with wheat germ agglutinin (WGA) lectin, which shows a specific interaction with *N*-acetyl-D-glucosamine (GlcNAc) and *N*-acetyl-D-neuraminic acid.[15] The lectin and extracellular saccharides of erythrocyte link to generate agglutination. The inhibition test using sugar balls **4a** ($G = 3.0$) and **4b** ($G = 3.0$), and monosaccharides (D-glucose (Glc) and GlcNAc) as inhibitors was performed. As shown in Table 4, only **4b** ($G = 3.0$) inhibited the hemagglutination under the sugar concentration of 2×10^{-4} mol/L in this experiment. This result indicates the specific interaction between GlcNAc-bearing **4b** ($G = 3.0$) and WGA. The difference between **4b** ($G = 3.0$) and monosaccharide GlcNAc seems to be due to a cluster effect with a high local concentration of sugar residues.

Table 4. Inhibition of hemagglutinating activity of lectin by oligoglycopeptide-type sugar balls **4a** (D-glucose-type, $G = 3.0$) and **4b** (*N*-acetyl-D-glucosamine-type, $G = 3.0$) and monosaccharide D-glucose and *N*-acetyl-D-glucosamine [a]

Inhibitor	Minimum inhibitory concentration, mol/L[b]
Glc-type sugar ball **4a** ($G = 3.0$, DP of peptide=3.8)[c]	N.I.[d]
GlcNAc-type sugar ball **4b** ($G = 3.0$, DP of peptide=3.5)[c]	2×10^{-4}
D-glucose	N.I.[d]
N-acetyl-D-glucosamine	N.I.[d]

[a] Lectin, wheat germ agglutinin (WGA); [lectin] = 2.5×10^{-3} g/L.
[b] Based on sugar units.
[c] See the text and Tables 1 and 2.
[d] Not inhibited by 10 mg/mL.

In the present paper, synthesis of novel globular sugar-polypeptide conjugates, oligoglycopeptide-type sugar balls, was described as an example of Radial-Growth Polymerization (RGP). The new class of polymerization systems is based on a clean living polymerization, e.g., ring-opening polymerization of glycoNCAs in this study. RGP will offer numerous novel dendrimer-based

star polymers of AB_n-type block copolymers between a dendrimer and linear polymers. RGP and the resulting new polymers are believed to open new material sciences and applications.

EXPERIMENTAL SECTION

Materials.

Preparation of *O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (**1a**) and *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (**1b**) was carried out by phosgenation of the corresponding α-amino acids according to the previous papers.[2,16] PAMAM dendrimers [ethylenediamine core, generation 3.0 (**2** (G = 3.0)) and generation 4.0 (**2** (G = 4.0))] were purchased from Aldrich Chemical Co. PAMAM dendrimer [ethylenediamine core, generation 5.0 (**2** (G = 5.0))] was prepared from **2** (G = 4.0) according to the stepwise method of Tomalia and coworkers.[13] Numbers of terminal amino groups of **3** (G = 3.0, 4.0, and 5.0) were estimated to be 30, 62, and 111, respectively, by NMR analysis.[13] All data in the text were calculated on the basis of the molecular structure with some defects of branches. Chloroform was dried by the conventional method, followed by distillation under nitrogen and stored over molecular sieves 3A. Hydrazine monohydrate was used without purification. Other reagents and solvents were purified by the conventional methods.

Measurements.

^1H and ^{13}C NMR measurements were performed by using a Bruker ARX-400 NMR spectrometer. IR spectra were recorded with a Jasco FT/IR-5MP spectrophotometer. Molecular weight was measured by vapor pressure osmometry (VPO) (Corona Model 117) in chloroform at 35 °C. Size exclusion chromatography (SEC) was taken with a Jasco Model DIP-1 high performance liquid-chromatograph apparatus (column, Shodex KF803→804, 8 ϕ x 600 mm; solvent, dimethyl sulfoxide; temp., 27 °C).

Typical procedure for ring-opening polymerization of sugar-substituted NCAs 1 with dendritic initiators 2.

The experimental procedure in run no. 1 was as follows. In a flask with a three-way stopcock was suspended 0.245 g (0.531 mmol) of **1a** in 20.4 mL of chloroform, followed by addition of 9.98 g of a chloroform solution (0.325 wt%) of **2** (G = 3.0) [**2** (G = 3.0), 32.4 mg (4.86×10^{-3} mmol)] at -30 °C under nitrogen. After stirring at 27 °C for 24 h, the reaction mixture was poured into 500 mL of diethyl ether and then purified twice by reprecipitation from chloroform to diethyl ether. After drying in vacuo, **3a** (G = 3.0) was isolated in 97.9% yield (0.248 g).

*Oligo[O-(tetra-O-acetyl-β-D-glucopyranosyl)-L-serine]-persubstituted poly(amido amine) dendrimer (**3a** (G = 3.0)):* IR (KBr disk); 3586 (ν_{N-H}), 3368 (ν_{N-H}), 2965 (ν_{C-H}), 2895 (ν_{C-H}), 1755 ($\nu_{C=O}$(ester)), 1663 ($\nu_{C=O}$(amide)), 1532 (δ_{N-H}(amide)), 1231 ($\nu_{C-C(=O)-O}$), 1042 (ν_{C-O-C}) cm^{-1}.

^1H NMR (Me_2SO-d_6, 22 °C, 400 MHz); δ 1.93-2.02 ($COCH_3$), 2.18 (NCH_2CH_2CO of PAMAM), 2.43 ($NHCH_2CH_2N$ of PAMAM), 2.66 (NCH_2CH_2CO of PAMAM), 3.08 ($NHCH_2CH_2N$ and $NHCH_2CH_2NH$ of PAMAM), 3.36 (CH of poly(L-serine) and NH_2), 3.71 (CH_2 of poly(L-serine)), 4.02 (CHOAc and H-5 of the pyranose ring), 4.20 (CHOAc), 4.70-4.92 (H-1, H-2, and H-4 of the pyranose ring), 5.23 (H-3 of the pyranose ring), 7.72-8.29 (NH).

^{13}C NMR (Me_2SO-d_6, 22 °C, 100 MHz); δ 20.2 (methyl carbons of acetyl groups), 33.0 (NCH_2CH_2CO of PAMAM), 36.8 ($NHCH_2CH_2N$ of PAMAM), 37.8 ($NHCH_2CH_2NH$ of PAMAM), 49.4 (NCH_2CH_2CO of PAMAM), 52.1 ($NHCH_2CH_2N$ of PAMAM), 52.5-53.0 (α-carbon of poly(L-serine)), 54.3 (α-carbon of the terminal unit of poly(L-serine)), 61.6 (CH_2OAc), 68.0 (C-4 of the pyranose ring), 68.0-69.4 (CH_2 of poly(L-serine)), 70.6 (C-2 and C-5 of the pyranose ring), 71.9 (C-3 of the pyranose ring and CH_2 of the terminal unit of poly(L-serine)), 99.1-99.3 (C-1 of the pyranose ring), 99.7 (C-1 of the terminal unit of the pyranose ring), 168.1-170.0 (carbonyl carbons of poly(L-serine) and acetyl groups), 171.1 (carbonyl carbons of PAMAM).

*Oligo[O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine]-persubstituted poly-(amido amine) dendrimer (**3b** (G = 3.0)):* IR (KBr disk); 3414 (ν_{N-H}), 2944 (ν_{C-H}), 1748 ($\nu_{C=O}$(ester)), 1659 ($\nu_{C=O}$(amide)), 1553 (δ_{N-H}(amide)), 1246 ($\nu_{C-C(=O)-O}$), 1047 (ν_{C-O-C}) cm^{-1}.

^1H NMR (Me_2SO-d_6, 27 °C, 400 MHz); δ 1.81 ($NHCOCH_3$), 1.91, 1.97, 2.03 ($OCOCH_3$), 2.31 (NCH_2CH_2CO of PAMAM), 2.58 ($NHCH_2CH_2N$ of PAMAM), 2.80 (NCH_2CH_2CO of PAMAM), 3.13 ($NHCH_2CH_2N$ and $NHCH_2CH_2NH$ of PAMAM), 3.62-3.97 (CH_2 and CH of poly(L-serine), H-2 and H-5 of the pyranose ring, and NH_2), 4.04 (CHOAc), 4.20 (CHOAc), 4.76 (H-1 of the pyranose ring), 4.85 (H-4 of the pyranose ring), 5.08 (H-3 of the pyranose ring), 8.00, 8.10 (NH).

^{13}C NMR (Me_2SO-d_6, 27 °C, 100 MHz); δ 20.3 (methyl carbons of *O*-acetyl groups), 22.7 (methyl carbons of *N*-acetyl groups), 32.4 (NCH_2CH_2CO of PAMAM), 36.2 ($NHCH_2CH_2N$ of PAMAM), 38.0 ($NHCH_2CH_2NH$ of PAMAM), 49.2 (NCH_2CH_2CO of PAMAM), 52.0 ($NHCH_2CH_2N$ of PAMAM), 53.0 (C-2 of the pyranose ring), 53.7 (α-carbon of poly(L-serine)), 61.7 (CH_2OAc), 67.9 (CH_2 of poly(L-serine)), 68.3 (C-4 of the pyranose ring), 70.9 (C-5 of the pyranose ring), 72.9 (C-3 of the pyranose ring), 99.6, 100.1 (C-1 of the pyranose ring), 169.3-170.1 (carbonyl carbons of poly(L-serine) and acetyl groups), 171.2 (carbonyl carbons of PAMAM).

Typical procedure for deprotection of O-acetyl groups of oligoglycopeptide-type sugar ball precursors 3. (Preparetion of oligoglycopeptide-type sugar ball 4.)

Deprotection of *O*-acetyl groups of **3** was performed as follows. In a test tube with a magnetic stirrer bar was placed 52.5 mg of **3b** (G = 3.0, DP = 3.5) (1.04×10^{-3} mmol), followed by adding 5.20 mL of a hydrazine monohydrate methanol solution (3.00 mol/L) at 0 °C. The reaction mixture was stirred at 26 °C. After 4.0 h, 1.98 g of acetone (34.1 mmol) was added dropwise at 0 °C to quench hydrazine. The mixture was evaporated, and then the product was purified by repeated reprecipitations from water to ethanol, and dried in vacuo. The yield was 37.2 mg (96.2%).

*Oligo[O-(β-D-glucopyranosyl)-L-serine]-persubstituted poly(amido amine) dendrimer (**4a** (G = 3.0))*: IR (KBr disk); 3306 (ν_{O-H}, ν_{N-H}), 2934 (ν_{C-H}), 1669 ($\nu_{C=O}$(amide)), 1534 (δ_{N-H}(amide)), 1080 (ν_{C-O-C}) cm^{-1}.

^1H NMR (D_2O, 27 °C, 400 MHz); δ 2.42 (NCH_2CH_2CO of PAMAM), 2.63 ($NHCH_2CH_2N$ of PAMAM), 2.82 (NCH_2CH_2CO of PAMAM), 3.31 ($NHCH_2CH_2N$ and $NHCH_2CH_2NH$ of PAMAM), 3.39-4.86 (CH_2 and CH of glycopeptide).

^{13}C NMR (D_2O, 27 °C, 100 MHz); δ 35.2 (NCH_2CH_2CO of PAMAM), 39.3 ($NHCH_2CH_2N$ of PAMAM), 41.2 ($NHCH_2CH_2NH$ of PAMAM), 51.5 (NCH_2CH_2CO of PAMAM), 54.0 ($NHCH_2CH_2N$ of PAMAM), 56.1, 56.5 (α-carbon of poly(L-serine)), 63.4 (CH_2OH), 71.2 (CH_2 of poly(L-serine)), 72.3 (C-4 of the pyranose ring), 75.7 (C-5 of the pyranose ring), 78.2 (C-2 of the pyranose ring), 78.6 (C-3 of the pyranose ring), 105.0, 105.2 (C-1 of the pyranose ring), 173.7 (carbonyl carbons of poly(L-serine)), 177.4 (carbonyl carbons of PAMAM).

*Oligo[O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-serine]-persubstituted poly(amido amine) dendrimer (**4b** (G = 3.0))*: IR (KBr disk); 3289 (ν_{O-H}, ν_{N-H}), 2940 (ν_{C-H}), 1651 ($\nu_{C=O}$(amide)), 1561 (δ_{N-H}(amide)), 1047 (ν_{C-O-C}) cm^{-1}.

^1H NMR (D_2O, 27 °C, 400 MHz); δ 2.05 ($NHCOCH_3$), 2.66 (NCH_2CH_2CO of PAMAM), 2.99 ($NHCH_2CH_2N$ of PAMAM), 3.15 (NCH_2CH_2CO of PAMAM), 3.34 ($NHCH_2CH_2N$ and $NHCH_2CH_2NH$ of PAMAM), 3.43-4.61 (CH_2 and CH of glycopeptide).

^{13}C NMR (D_2O, 27 °C, 100 MHz); δ 24.9 (methyl carbons of *N*-acetyl groups), 33.7 (NCH_2CH_2CO of PAMAM), 38.1 ($NHCH_2CH_2N$ of PAMAM), 41.4 ($NHCH_2CH_2NH$ of PAMAM), 52.2 (NCH_2CH_2CO of PAMAM), 54.5 ($NHCH_2CH_2N$ of PAMAM), 57.4 (α-carbon of poly(L-serine)), 58.0 (C-2 of the pyranose ring), 63.3 (CH_2OH), 70.5 (CH_2 of poly(L-serine)), 72.5 (C-4 of

the pyranose ring), 76.4 (C-3 of the pyranose ring), 78.6 (C-5 of the pyranose ring), 103.5, 103.8, 104.0 (C-1 of the pyranose ring), 174.0 (carbonyl carbons of poly(L-serine) and *N*-acetyl groups), 177.6 (carbonyl carbons of PAMAM).

Agglutination inhibition assay.

The agglutination inhibition assays were performed as reported in the literature.[17] The minimum inhibition concentrations of sugar residues of **4**, D-glucose, and *N*-acetyl-D-glucosamine were determined within 2-fold serial dilutions. Wheat germ agglutinin (WGA) (Sigma Chemical Co.) was used at a final concentration of 2.5×10^{-3} g/L in a phosphate buffer solution. Substrates were incubated for 1 h at 25 °C prior to the addition of an actinase E treated human erythrocyte suspension (blood type A). The mixture was kept for 12 h at 25 °C and then examined for hemagglutination.

REFERENCES AND NOTES

1. Aoi, K.; Tsutsumiuchi, K.; Okada, M. *Polym. Prepr., Jpn.* **1996**, *45*, E73. This polymerization system has been presented at 45th annual meeting of the society of polymer science, Japan on May 27, 1996.
2. Aoi, K.; Tsutsumiuchi, K.; Okada, M. *Macromolecules* **1994**, *27*, 875.
3. Aoi, K.; Tsutsumiuchi, K.; Aoki, E.; Okada, M. *Macromolecules* **1996**, *29*, 4456.
4. Tsutsumiuchi, K.; Aoi, K.; Okada, M. *Macromolecules*, submitted.
5. Aoi, K.; Suzuki, H.; Okada, M. *Macromolecules* **1992**, *25*, 7073.
6. Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391.
7. For references of dendrimers, see: (a) Tomalia, D. A.; Durst, H. D. *Top. Curr. Chem.* **1993**, *165*, 193-313. (b) Fréchet, J. M. J.; Hawker, C. J. In *Comprehensive Polymer Science*, Second Supplement, Allen, G. Ed; Pergamon, Elsevier Science, Oxford, 1996; pp 71-132. (c) Newkome, G. R.; Moorefield, C. N. In *Advances in Dendritic Macromolecules*, Vol. 1;. Newkome, G. R. Ed; JAI Press: Greenwich, Conn., 1994; pp 1-68.
8. (a) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1869. (b) Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2088. (c) Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1996**, *2*, 1115. (d) Lindhorst, T. K.; Kieburg, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1953.
9. (a) Shao, J.; Tam, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 3893. (b) Zhang, L.; Tam, J. P. *J. Am. Chem. Soc.* **1997**, *119*, 2363.
10. Hudson, R. H. E.; Damha, M. J. *J. Am. Chem. Soc.* **1993**, *115*, 2119.
11. Okada, M. In *Polymeric Materials Encyclopedia*; Salamone, J. C., Ed.; CRC Press: Boca Raton, 1996; Vol. 4, pp. 2834-2840.
12. (a) Imanishi, Y. In *Ring-Opening Polymerization*; Ivin, K., Saegusa, T., Eds.; Elsevier Applied Science Publishers: New York, 1985; Vol. 2, Chapter 8. (b) Kricheldorf, H. R. In *Models of*

Biopolymers by Ring-Opening Polymerization; Penczek, S., Ed.; CRC Press: Florida, 1990; Chapter 1.

13. Tomalia, D. A.; Naylor, A. M.; Goddard III, W. A. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 138.
14. Average intersugar distance was estimated between C-4 carbons of terminal sugar residues.
15. Wright, C. S. *J. Biol. Chem.* **1992**, *267*, 14345.
16. (a) Rüde, E.; Westphal, O.; Hurwitz, E.; Fuchs, S.; Sela, M. *Immunochemistry* **1966**, *3*, 137. (b) Rüde, E.; Meyer-Delius, M. *Carbohydr. Res.* **1968**, *8*, 219. (c) Garg, H. G.; Jeanloz, R. W. *Carbohydr. Res.* **1976**, *49*, 482.
17. Osawa, T.; Matsumoto, I. *Methods Enzymol.* **1972**, *28*, 323.

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