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Globular Carbohydrate Macromolecule "Sugar Balls" IV. Synthesis of Dendritic Nanocapsules with Molecular Recognition Sites on Periphery

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ABSTRACT: 8-Anilino-1-naphthalenesulfonic acid sodium salt (ANS)-encapsulated [*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-L-serine]-persubstituted poly(amido amine) (PAMAM) dendrimers ($G=6.0$, 7.0, and 8.0) and ANS-encapsulated [*O*-(*β*-D-galactopyranosyl)-L-serine]-persubstituted PAMAM dendrimers ($G=7.0$ and 8.0) were synthesized by polymer reaction between PAMAM dendrimers and glycoNCAs (*i.e.*, *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (NCA) and *O*-(tetra-*O*-acetyl-*β*-D-galactopyranosyl)-L-serine NCA) in the presence of ANS in chloroform, followed by deacetylation with hydrazine monohydrate in methanol. Number of ANS molecules per the mono(glycopeptide)-type sugar ball capsule was determined by quantitative UV analysis of the hydrolysis product of the capsule under a basic condition. The results suggested that the encapsulation efficiency depended on the surface density of sugar residues. UV and fluorescent analyses and gel filtration chromatography of the capsule indicated the existence of ANS in a relatively hollow internal cavity of the mono(glycopeptide)-type sugar ball capsule. An inhibition test of hemagglutinating activity of wheat germ agglutinin (WGA) lectin demonstrated that the capsule has a highly specific molecular recognition ability owing to the sugar residues.

KEY WORDS Nanocapsule / Dendrimer / Carbohydrate Polymer / Molecular Recognition / Supramolecule / α-Amino Acid *N*-Carboxyanhydride (NCA) /

The architectural features of dendrimers include their precise constitutions with high overall symmetries, their well-defined internal cavities, and their nanometer dimensions. Their numerous applications have been conceivable, and particularly creation of unimolecular-nanocapusules is one of the most attractive research subjects. As early as in 1982, Maciejewski proposed the possibility of constructing a dendritic "core-shell molecule" to "topologically" entrap small molecules.[1] Although some papers concerning dendritic capsule[2–8] have been published since the first article of dendrimer in *Polymer Journal*,[9] nanocapsule with molecular recognition sites on its periphery has not been reported yet.

Molecular recognition in dendrimer chemistry is important from viewpoints of both molecular science and materials science.[10] For instance, fully sugar-substituted poly(amido amine) dendrimer "Sugar Balls" have specific binding property with lectins and are regarded as significant model compounds of naturally occurring glycoconjugates as well as functional materials for biochemical and biomedical applications such as drug delivery systems (DDS).[11–14] In spite of some papers about carbohydrate-containing dendrimers aimed for sophisticated recognition ability originating from sugar residue function,[15–19] encapsulation into sugar-based dendrimer has not been reported at all. Therefore, we investigated the synthesis and characterization of fluorescent probe-encapsulated mono(glycopeptide)-persubstituted dendrimers in this study.

Hitherto we have reported molecular architecture of carbohydrate polymer by living ring-opening polymerization of sugar-substituted α-amino acid *N*-carboxyanhydrides (glycoNCAs), *i.e.*, block-type,[20–22] graft-type,[23] and star-type by radial-growth polymerization (RGP) of glycoNCAs with poly(amido amine) (PAMAM) dendrimer as a multi-functional initiator.[12] The anionic ring-opening polymerization of glycoNCAs initiated by primary amines has two important features. First, the polymerization proceeds without side reactions to yield polypeptides with controlled chain length. Secondly, the initiation is much faster than the propagation. Synthesis of mono(glycopeptide)-persubstituted PAMAM dendrimers reflects a clean and a rapid initiation/slow propagation system.[14] The present article describes the synthesis of nanocapsules with molecular recognition sites on the periphery by polymer reaction of PAMAM dendrimer with galactose and *N*-acetyl-D-glucosamine derivative-substituted NCAs in the presence of a fluorescent probe (Scheme 1). The mono(glycopeptide)-type sugar ball capsule should be important not only for the investigation of the biological function of sugar balls but also for the development of a novel active-targeting drug delivery system employing sugar balls.

EXPERIMENTAL

Measurements

^{1}H and ^{13}C NMR spectra were recorded with a Brucker ARX-400 NMR spectrometer (400 MHz and 100 MHz, respectively). IR measurements were performed by a JASCO FT/IR-610 spectrophotometer. Size exclusion chromatography (SEC) was taken with a JASCO PU-980 with an RI detector of JASCO RI-930 (column, superdex 200 HR 10/30 (Pharmacia Biotech); eluent, 50 mmol L^{-1} K_2HPO_4 aq; temp, 27°C; flow rate, 0.50 mL min^{-1}; standard, globular proteins (Pharmacia Biotech)). Gel filtration chromatography was performed by a JASCO PU-980 with a UV detector of JASCO UV-970 and a fluorescence detector of JASCO FP-920S

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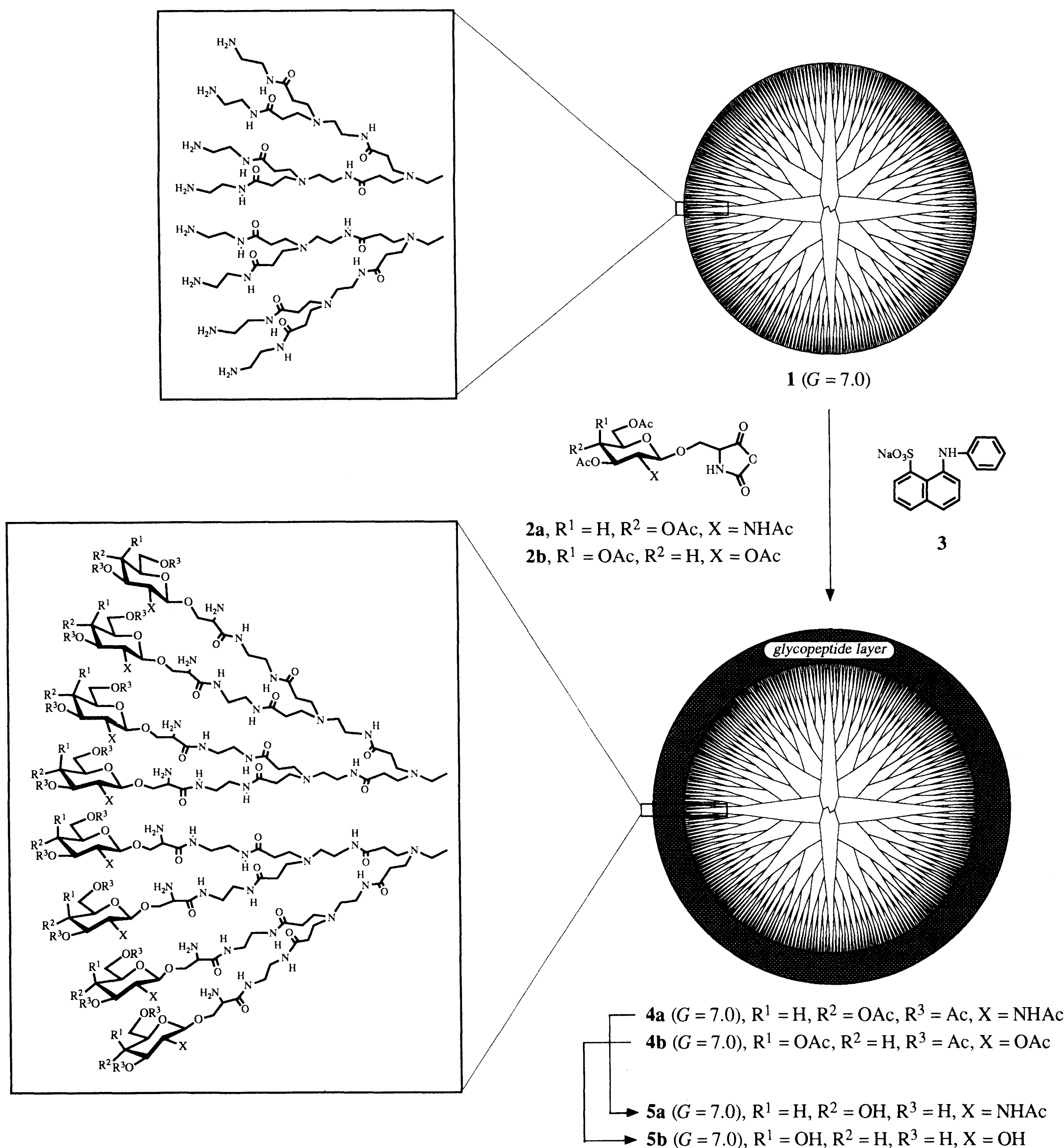


Scheme 1. Synthesis of glycopeptide-type sugar ball capsules.

(column, sephadex G-50 (ϕ 16 mm × 12 cm) (Pharmacia Biotech); eluent, 75 mmol L^{-1} $(NH_4)_2CO_3$ aq + CH_3CN (5 : 1, v/v); temp, 27°C; flow rate, 1.0 mL min^{-1}). UV measurement for quantification of guest molecules was carried out by using a microplate spectrophotometer system SPECTRAmax 250 from Molecular Devices Co. UV and fluorescent spectra were recorded by a JASCO Ubest-30 UV/Vis spectrophotometer and a Hitachi F-4500 fluorescence spectrophotometer, respectively.

Materials

PAMAM dendrimers [ethylenediamine core; generation 6.0 (**1** (G = 6.0)), generation 7.0 (**1** (G = 7.0)), and generation 8.0 (**1** (G = 8.0))] were prepared by the stepwise method of Tomalia *et al.*[9] from PAMAM dendrimers [ethylenediamine core; generation 4.0 (**1** (G = 4.0))]. Theoretical molecular weights of PAMAM dendrimers were calculated from branching ratio (the ratio of tertiary amine structure in all branching points) obtained by ^{13}C NMR measurements in D_2O at 27°C. *O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (NCA) (**2a**) and *O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-L-serine NCA (**2b**) were prepared by phosgenation of the corresponding α-amino acids according to the previous papers.[20,24] 8-Anilino-1-naphthalenesulfonic acid sodium salt (ANS) (**3**) was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Chloroform was dried over calcium hydride, followed by distillation under nitrogen and stored over molecular sieves 3A. Hydrazine monohydrate was used without purification.

Typical Procedure for the Synthesis of Fluorescent Probe-Encapsulated Mono(glycopeptide)-Type Sugar Balls

In a flask with a three-way stopcock was placed 0.488 g of a methanol solution (7.64 wt%) of **1** ($G=8.0$) [**1** ($G=8.0$), 37.3 mg, (1.87×10^{-7} mol)], followed by addition of 0.660 g of a methanol solution (0.256 wt%) of **3** [**3**, 1.69 mg, (5.26×10^{-6} mol)] under nitrogen. After evaporation of the mixture, the remaining solid was dissolved in 9.57 mL of chloroform at $-30°C$. To the stirred mixture was added 9.57 mL of a chloroform solution (20 mmol L^{-1}) of **2a** [**2a**, 88.2 mg (1.91×10^{-4} mol)]. After the polymer reaction for 30 min at $-30°C$, 19.1 mL of methanol was poured for termination of the polymer reaction, followed by evaporation of the solvent. Powdery product was obtained by repeated reprecipitations from chloroform to diethyl ether for three times. The yield was 0.103 g (97.4%).

Deacetylation of the products was carried out as follows. In a test tube was placed 87.3 mg of the product, followed by addition of 8.73 mL of a methanol solution (1.0 mol L^{-1}) of hydrazine monohydrate [hydrazine monohydrate, 0.437 g, (8.73×10^{-3} mol)] at 0°C. After the reaction mixture was stirred at 27°C for 3.0 h, 0.886 g (1.77×10^{-2} mol) of acetone was added dropwise at 0°C to quench hydrazine. The mixture was evaporated, and then the sugar ball capsule **3**@**5a**, where A@B indicates that A is encapsulated into B, was isolated from free **3** and by-product by gel filtration chromatography (column, sephadex G-50 (ϕ 26 mm × 26 cm) (Pharmacia Biotech); eluent, 75 mmol L^{-1} $(NH_4)_2CO_3$ aq + CH_3CN (100 : 15, v/v); temp, 20°C; flow rate, 2.0 mL min^{-1}) After lyophilization for three times to remove ammonium carbonate, powdery **3**@**5a** ($G=8.0$) was obtained in 88.5% yield (61.4 mg).

3@**5a** ($G=8.0$): ^1H NMR (D_2O, 27°C, 400 MHz) δ 2.04 ($NHCOCH_3$), 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), 3.43 (H-4 and H-5 of the pyranose ring), 3.54 (C*H* of L-serine and H-3 of the pyranose ring), 3.70 (C*H*HOH, C*H*H of L-serine, and H-2 of the pyranose ring), 3.91 (CH*H*OH), 3.98 (CH*H* of L-serine), 4.49 (H-1 of the pyranose ring). ^{13}C NMR (D_2O, 27°C, 100 MHz) δ 25.0 (methyl carbons of *N*-acetyl groups), 35.3 (NCH_2CH_2CO of PAMAM), 39.4 ($NHCH_2CH_2N$ of PAMAM), 41.3 ($NHCH_2CH_2NH$ of PAMAM), 51.7 (NCH_2CH_2CO of PAMAM), 54.0 ($NHCH_2CH_2N$ of PAMAM), 57.0 (α-carbon of L-serine), 58.2 (C-2 of the pyranose ring), 63.4 (CH_2OH), 72.6 (C-4 of the pyranose ring), 74.3 (CH_2 of L-serine), 76.4 (C-3 of the pyranose ring), 78.6 (C-5 of the pyranose ring), 104.1 (C-1 of the pyranose ring), 176.9, 177.4 (carbonyl carbons). IR (KBr disk, cm^{-1}) 3294 (ν_{O-H}, ν_{N-H}), 2951, 2883 (ν_{C-H}), 1647 ($\nu_{C=O}$ (amide)), 1559 (δ_{N-H}(amide)), 1077 (ν_{C-O-C}).

Quantification of Guest Molecules in Mono(glycopeptide)-Type Sugar Ball Capsules

The experimental procedure in quantification of **3** in **3**@**5a** ($G=8.0$) was as follows. First a calibration line was made by using **3** and a reference sample **5a** ($G=8.0$). In each of polypropylene test tubes (4 pieces) was placed 20.0 μL of an aqueous solution (15.0 mg mL^{-1}) of **5a** ($G=8.0$) [**5a** ($G=8.0$), 0.300 mg, (6.70×10^{-10} mol)], followed by addition of 0, 10.0, 20.0, and 30.0 μL of an aqueous solution (6.45 mg mL^{-1}) of **3** [**3**, 0, 0.646, 1.29, and 1.94 μg, (0, 2.01×10^{-9}, 4.02×10^{-9}, and 6.03×10^{-9} mol)], respectively. Distilled water was added to a total volume of 50.0 μL in each tube. After addition of 50.0 μL of 10.0 mol L^{-1} NaOH aq solution, the mixture was kept at 80°C for 8.0 h. Then 90.0 μL of the hydrolyzed solution was moved to a 96 well microplate and the absorbance was measured at 350 nm. From collected data by repeating this procedure for 3 times, linear calibration plots were obtained for the relationship between the absorbance and the molar ratio of **3** to **5a** ($G=8.0$). Secondly hydrolysis of **3**@**5a** ($G=8.0$) was carried out. In each of polypropylene test tubes (3 pieces) was placed 20.0 μL of an aqueous solution (15.0 mg mL^{-1}) of **3**@**5a** ($G=8.0$) [**3**@**5a** ($G=8.0$), 0.300 mg], followed by addition of 30 μL of distilled water. After addition of 50.0 μL of 10.0 mol L^{-1} NaOH aq solution, **3**@**5a** ($G=8.0$) was hydrolyzed at 80°C for 8.0 h. The absorbance was measured as stated above and the number of **3** in **3**@**5a** ($G=8.0$) was determined from the average absorbance with the calibration plots (relative uncertainty $\pm 6\%$).

Agglutination Inhibition Assay

The agglutination inhibition assays were carried out according to the literature.[25] The minimum inhibition concentrations of sugar residues of **5a**, **5b**, **3**@**5a**, **3**@**5b**, *N*-acetyl-D-glucosamine, and D-galactose were determined within 2-fold serial dilutions. Wheat germ agglutinin (WGA) (Sigma Chemical Co.) was used at a concentration of 5.0×10^{-4} g L^{-1} in a phosphate buffer solution. Substrates were incubated for 6 h at 27°C prior to the addition of an actinase E-treated human erythrocyte suspension (blood type B). The mixture was kept for 1 h at 27°C and then examined for hemagglutination.

RESULTS AND DISCUSSION

Synthesis of Fluorescent Probe-Encapsulated Mono-(glycopeptide)-Type Sugar Balls

The results of the synthesis of 8-anilino-1-naphthalenesulfonic acid sodium salt (**3**)-encapsulated mono(glycopeptide)-type sugar balls **3**@**5a** and **3**@**5b** are summarized in Table I. The polymer reaction of PAMAM dendrimer **1** ($G=6.0$, 7.0, and 8.0) with *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine NCA (**2a**) and *O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-L-serine NCA (**2b**) in the presence of **3** proceeded quantitatively without interruption by **3** under conditions as shown in Table I, although a large excess of **3** ($[\mathbf{3}]_0/[\mathbf{1}]_0=1.10\times10^3$) disturbed the reaction. In each polymer reaction, **3** was hardly detected in the supernatant of the reprecipitation by means of UV analysis. Fluorescence spectra of the product, a mixture of **3** and **4a**, and **3** in chloroform are shown in Figure 1. Both spectra of the product and the mixture of **3** and **4a** indicate the increase in intensity and λ_{max}. Perhaps dipole–dipole interaction between **3** and peracetylated sugar ball **4** in the non-polar solvent led most of **3** into the complexation with **4** irrespective of the internal or

Table I. Synthesis of mono(glycopeptide)-type sugar ball capsules

Polymer reaction[a]				Deacetylation[d]					
Dendrimer[b] G	GlycoNCA	$[3]_3/[1]_0$	Yield[c] %	Product	Yield[e] %	N[f]	$M_n\times10^{-5}$ Calcd.[e]	$M_n\times10^{-5}$ SEC[g]	M_w/M_n[g]
8.0	**2a**	28.2	97.4	**3@5a**	88.5	2.6	4.49	3.69	1.0_2
8.0	**2a**	6.1	97.7	**3@5a**	92.9	1.2	4.48	3.69	1.0_2
7.0	**2a**	10.3	97.7	**3@5a**	93.0	0.8	2.31	2.58	1.0_2
6.0	**2a**	10.2	96.9	**3@5a**	85.2	0.4	1.18	1.60	1.0_2
8.0	**2b**	9.0	94.0	**3@5b**	89.2	0.8	4.13	3.67	1.0_3
7.0	**2b**	10.0	95.3	**3@5b**	97.7	0.2	2.13	2.26	1.0_2

[a] $[2]_0$, 10.0 mmol^{-1} L; $[2]_0/[\text{-NH}_2$ of **1**], 1.20; solv., $CHCl_3$; temp, −30°C; time, 15 min. [b] PAMAM dendrimer (ethylenediamine core). [c] Calculated on the assumption that the products contained all of **3**. [d] $[3@4]_0$, 10.0 mg mL^{-1}; $H_2NNH_2\cdot H_2O$, 1.00 mol L^{-1}; solv., MeOH; temp, 27°C; time, 3.0 h. [e] Calculated in consideration of the content of **3**. [f] Number of **3** molecules per mono(glycopeptide)-type sugar ball capsule. [g] Estimated by SEC in 50 mmol L^{-1} L K_2HPO_4 aq solution at 27°C (globular protein standard).

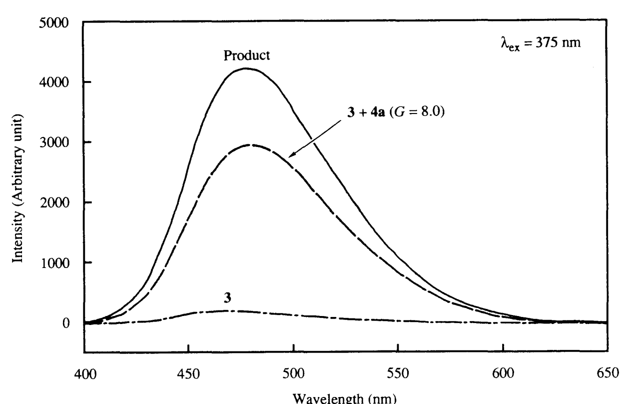


Figure 1. Fluorescence spectra of chloroform solutions (24 h after preparation) of the product of polymer reaction of **1** (G=8.0) and **2a** in the presence of **3** (1.0 mg mL^{-1}) (content; [**3**], 16 μg mL^{-1}; [**4a** (G=8.0)], 0.98 mg mL^{-1}, calculated on the assumption that the product contained all of the added **3**), the mixture of **3** (16 μg mL^{-1}) and acetylated glycopeptide-type sugar ball **4a** (G=8.0) (0.98 mg mL^{-1}), and **3** (16 μg mL^{-1}) at 20°C. Product of polymer reaction (——), **3**+**4a** (G=8.0) (— —), and **3** (- - -).

external area.[26]

After the deacetylation of the products of the polymer reaction, the isolation of **3@5** was performed by gel filtration chromatography. Because aromatic compounds adhere to sephadex gel, purified **3@5** is expected to contain no **3** bound on the surface (*vide infra*). The quantification of **3** in **3@5** was carried out by the absorbance at 350 nm of the reaction mixture after hydrolysis of **3@5** to avoid misleading by direct spectroscopic analysis of **3@5**. Number of **3** molecules per **3@5** was indicated as N in Table I. Two tendencies between encapsulation efficiency and the structure were recognized from the number of **3** per sugar ball capsule in the table. One is that an N-acetyl-D-glucosamine-type capsule tended to hold **3** more efficiently than a D-galactose-type capsule. The other is that the contents of **3** became larger with growing the generation of the dendrimer skeleton of each sugar-type capsule. The intersugar distances of **5** (G=6.0, 7.0, and 8.0) are estimated to be 14 Å, 11 Å, and 8 Å, respectively, for three dimensionally extended models by using a Corey–Pauling–Koltum (CPK) molecular model. Steric hindrance of N-acetyl-D-glucosamine residue is higher than that of D-galactose residue due to the N-acetyl group. The difference of the bulkiness of the sugar moieties is probably effective in aforementioned small intersugar space. Therefore, the increase of the encapsulation efficiency would be caused by enhancement of surface density of sugar residues.

The M_n and M_w/M_n values of **3@5** were estimated by SEC calibrated with globular protein standards in 50 mmol L^{-1} K_2HPO_4 aq at 27°C, signifying that relatively large macromolecules have been synthesized precisely. The measured number average molecular weights of **3@5** (G=7.0) were relatively close to the calculated values. This is considered that **3@5** (G=7.0) with internal cavities has nearly the same exclusion volumes as globular proteins having a compactly folded structure.[27] On the other hand, the measured number average molecular weights of **3@5** (G=8.0) were lower than the theoretical values and that of **3@5a** (G=6.0) was higher. Probably these reflect the increase in the surface density with changing the generation from 6.0 to 8.0.

The chemical structure of **3@5** was determined by ^{1}H and ^{13}C NMR and IR measurements as shown in the experimental section. In ^{13}C NMR spectra in D_2O quantitative substitution of the terminal primary groups of **1** with glycoNCA **2** was suggested by the fact that no signals ascribed to the α- and β-carbons of the unreacted primary amino group were observed at 42.4 and 44.2 ppm, respectively. However, in ^{1}H NMR spectra the peaks due to **3** in **3@5** were not observed owing to the low concentration.

Figure 2 shows UV spectra of aqueous solutions of **3@5a** (G=8.0, N=2.6), **3**, **5a** (G=8.0), and the mixture of **3** and **5a** (G=8.0). In spite of equal concentration in each of **3** and **5a** (G=8.0), the spectrum of **3@5a** (G=8.0, N=2.6) is different from that of the mixture of **3** and **5a** (G=8.0). The former is nearly the same as that of **5a** (G=8.0), while the latter looks like the combined spectrum of **3** and **5a** (G=8.0). The UV analysis suggests that the environment of **3** in **3@5a** (G=8.0) is different from that in the mixture.

To investigate the microscopic environment around **3** in **3@5a** (G=8.0), fluorescence analysis was performed. Fluorescent spectra of **3@5a** (G=8.0, N=2.6), **3**, **5a** (G=8.0), and the mixture of **3** and **5a** (G=8.0) are presented in Figure 3. In contrast to UV analysis, the spectrum of **3@5a** (G=8.0, N=2.6) looks like the com-

bined spectrum of **3** and **5a** ($G=8.0$), while that of the mixture of **3** and **5a** ($G=8.0$) shows a very strong peak intensity. Considering the fact of water penetration in PAMAM dendrimer,[28] **3** is expected to be in a relatively hollow core in the dendrimer skeleton of **3@5a** ($G=8.0$).[29] On the other hand, in the mixture of **3** and **5a** ($G=8.0$), **3** seems to be adsorbed on the dense surface of **5a** due to electrostatic interaction as reported by S. Jockusch *et al.*[30,31]

Figure 4 shows gel filtration chromatography profiles of aqueous solutions (24 h after preparation) of **3@5a** ($G=8.0$, $N=2.6$), **3**, **5a** ($G=8.0$), and the mixture of **3** and **5a** ($G=8.0$) with UV and fluorescence detectors. The chromatogram of **3@5a** ($G=8.0$, $N=2.6$) indicated only a single peak at the same elution volume as **5a** ($G=8.0$), which showed a strong fluorescence intensity in comparison with **5a** ($G=8.0$). Although the mixture of **3** and **5a** ($G=8.0$) is expected to form the complex on the periphery of **5a** ($G=8.0$) by fluorescent analysis, the elution profile showed both free **3** and sugar ball with the same fluorescent intensity as **5a** ($G=8.0$). This is ascribable to the adherence of aromatic compounds to sephadex gel as stated above. These chromatograms

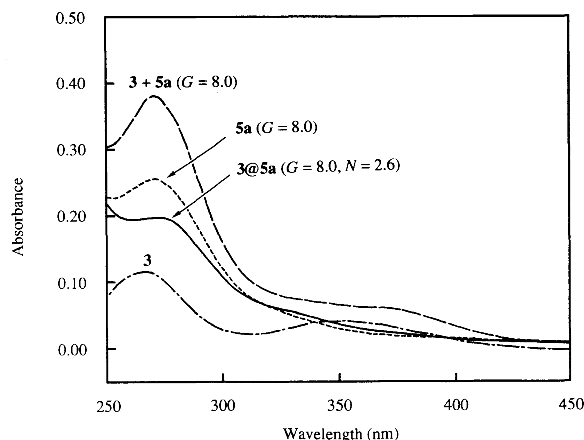
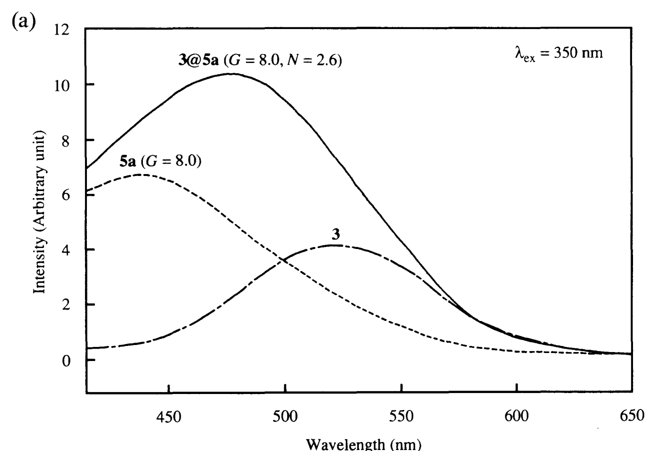


Figure 2. UV spectra of aqueous solutions (27 h after preparation) of glycopeptide-type sugar ball capsule **3@5a** ($G=8.0$, $N=2.6$) (1.0 mg mL^{-1}) (content; [**3**], 1.9 $\mu g\,mL^{-1}$; [**5a** ($G=8.0$)], 1.0 mg mL^{-1}), the mixture of **3** (1.9 $\mu g\,mL^{-1}$) and glycopeptide-type sugar ball **5a** ($G=8.0$) (1.0 mg mL^{-1}), **3** (1.9 $\mu g\,mL^{-1}$), and **5a** ($G=8.0$) (1.0 mg mL^{-1}) at 27°C. **3@5a** ($G=8.0$, $N=2.6$) (——), **3**+**5a** ($G=8.0$) (— —), **3** (- - -), and **5a** ($G=8.0$) (------).



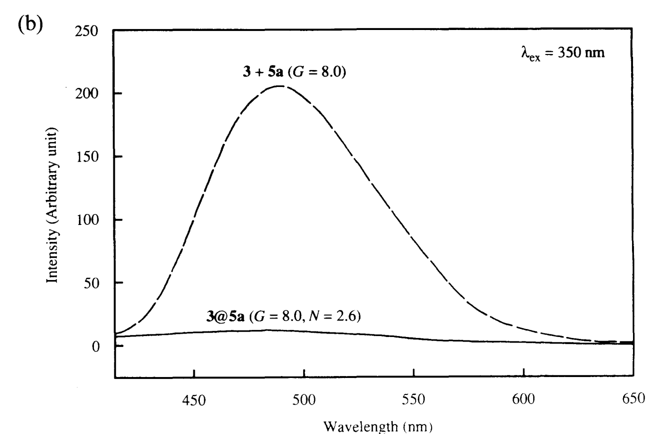


Figure 3. Fluorescence spectra of aqueous solutions (24 h after preparation) of glycopeptide-type sugar ball capsule **3@5a** ($G=8.0$, $N=2.6$) (1.0 mg mL^{-1}) (content; [**3**], 1.9 $\mu g\,mL^{-1}$; [**5a** ($G=8.0$)], 1.0 mg mL^{-1}), the mixture of **3** (1.9 $\mu g\,mL^{-1}$) and glycopeptide-type sugar ball **5a** ($G=8.0$) (1.0 mg mL^{-1}), **3** (1.9 $\mu g\,mL^{-1}$), and **5a** ($G=8.0$) (1.0 mg mL^{-1}) at 20°C. **3@5a** ($G=8.0$, $N=2.6$) (——), **3**+**5a** ($G=8.0$) (— —), **3** (- - -), and **5a** ($G=8.0$) (------).

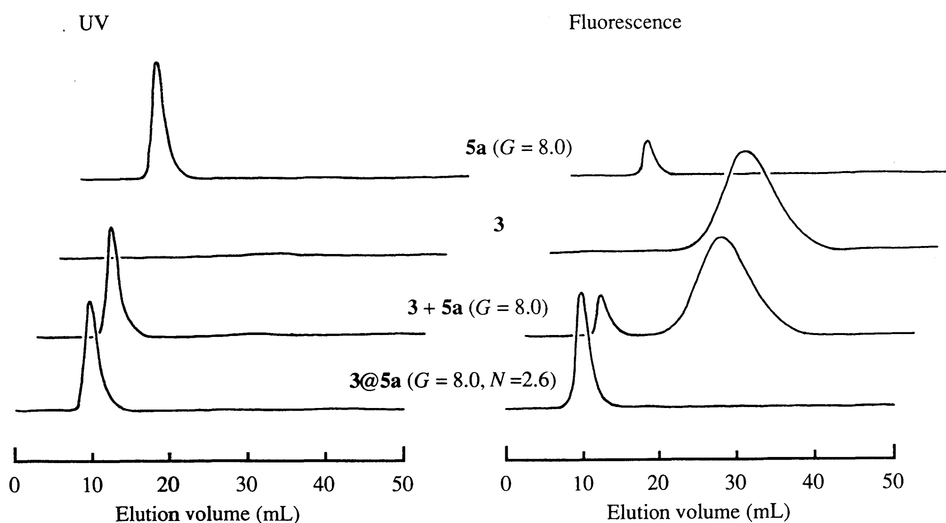


Figure 4. Gel filtration chromatogram of aqueous solutions (24 h after preparation) of glycopeptide-type sugar ball capsule **3@5a** ($G=8.0$, $N=2.6$) (1.0 mg mL^{-1}) (content; [**3**], 1.9 $\mu g\,mL^{-1}$; [**5a** ($G=8.0$)], 1.0 mg mL^{-1}), the mixture of **3** (1.9 $\mu g\,mL^{-1}$) and glycopeptide-type sugar ball **5a** ($G=8.0$) (1.0 mg mL^{-1}), **3** (1.9 $\mu g\,mL^{-1}$), and **5a** ($G=8.0$) (1.0 mg mL^{-1}). Column, sephadex G-50 (ϕ 16 mm × 12 cm); eluent, 75 mmol L^{-1} $(NH_4)_2CO_3$ aq $+CH_3CN$ (5 : 1, v/v); temp, 27°C; flow rate, 1.0 mL min^{-1}; detector, UV (220 nm) and fluorescence (*ex*, 350 nm; *em*, 515 nm).

Table II. Inhibition of hemagglutinating activity of lectin by mono(glycopeptide)-type sugar ball capsule **3@5a** ($G=8.0$, $N=2.6$), mono(glycopeptide)-type sugar balls **5a** ($G=8.0$) and **5b** ($G=8.0$), N, N', N''-triacetylchitotriose, N-acetyl-D-glucosamine, and D-galactose[a]

Inhibitor		Minimum inhibitory concentration
		$\mathrm{mol\,L^{-1}}$ [b]
GlcNAc-type sugar ball capsule	**3@5a** ($G=8.0$, $N=2.6$)[c]	5×10^{-7}
GlcNAc-type sugar ball	**5a** ($G=8.0$)[c]	6×10^{-7}
Gal-type sugar ball	**5b** ($G=8.0$)[c]	N.I.[d]
N,N',N''-Triacetylchitotriose		4×10^{-4}
N-Acetyl-D-glucosamine		N.I.[e]
D-Galactose		N.I.[e]

[a] Lectin, wheat germ agglutinin (WGA); [lectin] $=5\times10^{-4}\,\mathrm{g\,L^{-1}}$. [b] Based on sugar units. [c] See the text and Table I.[14] [d] Not inhibited by $0.33\,\mathrm{g\,L^{-1}}$. [e] Not inhibited by $3.3\,\mathrm{g\,L^{-1}}$.

indicate clearly that **3** was entrapped into the sugar ball capsule and never leaked out. Additionally, aqueous solutions ($1.0\,\mathrm{mg\,mL^{-1}}$) of **3@5a** ($G=8.0$, $N=2.6$) gave the same charts both at 0 h and at 120 h later. Consequently, it was considered that encapsulation of **3** into **5a** ($G=8.0$) in water is not caused by equilibrium.

Evaluation of Recognition Ability of Mono(glycopeptide)-Type Sugar Ball Capsule **3@5a** *($G=8.0$) by an Inhibition Test of Hemagglutinating Activity of Wheat Germ Agglutinin (WGA) Lectin*

In order to confirm the molecular recognition ability of **3@5a** ($G=8.0$), the inhibition activity of erythrocyte agglutination was examined with WGA lectin which shows a specific interaction with N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-D-neuraminic acid.[32] The lectin interacts with extracellular saccharides of erythrocyte to cause agglutination. It means a highly efficient interaction, namely a high recognition ability, that an inhibitor shows a low minimum concentration to inhibit the hemagglutination. The results of the inhibition test using **3@5a** ($G=8.0$, $N=2.6$), **5a** ($G=8.0$), **5b** ($G=8.0$), and monosaccharides (GlcNAc and D-galactose) as inhibitors are summarized in Table II.

GlcNAc-bearing mono(glycopeptide)-type sugar ball (*i.e.*, **3@5a** ($G=8.0$, $N=2.6$) and **5a** ($G=8.0$)) gave relatively low minimum concentrations of 5×10^{-7} and $6\times10^{-7}\,\mathrm{mol\ L^{-1}}$, respectively. This finding indicates that mono(glycopeptide)-type sugar ball capsule **3@5a** ($G=8.0$) has a molecular recognition ability as high as **5a** ($G=8.0$) due to a cluster effect with a high local concentration of sugar residues and that the encapsulation of **3** into an internal cavity of **5a** ($G=8.0$) does not prevent the specific interaction.

CONCLUSION

The encapsulation of ANS into mono(glycopeptide)-type sugar ball was achieved by polymer reaction of PAMAM dendrimer and glycoNCA in the presence of ANS in chloroform, followed by deacetylation with hydrazine monohydrate in methanol. Number of ANS molecules per mono(glycopeptide)-type sugar ball capsule isolated by gel filtration chromatography was determined by quantitative UV analysis of the hydrolysis mixture of the capsule under a basic condition. The results suggested that the encapsulation efficiency depends on the surface density. The environment of ANS in mono(glycopeptide)-type sugar ball capsule was characterized by UV and fluorescent analyses, which showed the existence of ANS in a relatively hollow internal cavity in the dendrimer skeleton. Additionally, gel filtration chromatography showed not only that ANS molecules were encapsulated into mono(glycopeptide)-type sugar ball but also that the encapsulation is not an equilibrium process in water. Furthermore, an inhibition test of hemagglutinating activity of WGA lectin demonstrated that ANS-encapsulated mono(glycopeptide)-type sugar ball had a high molecular recognition ability. Therefore we conclude that the first synthesis of nanocapsules with molecular recognition sites on the periphery was achieved in this study. Further sophisticated molecular design of sugar ball capsules will be required for biochemical and biomedical applications such as drug delivery systems.

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