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Globular Carbohydrate Macromolecule "Sugar Balls". 1. Synthesis of Novel Sugar-Persubstituted Poly(amido amine) Dendrimers

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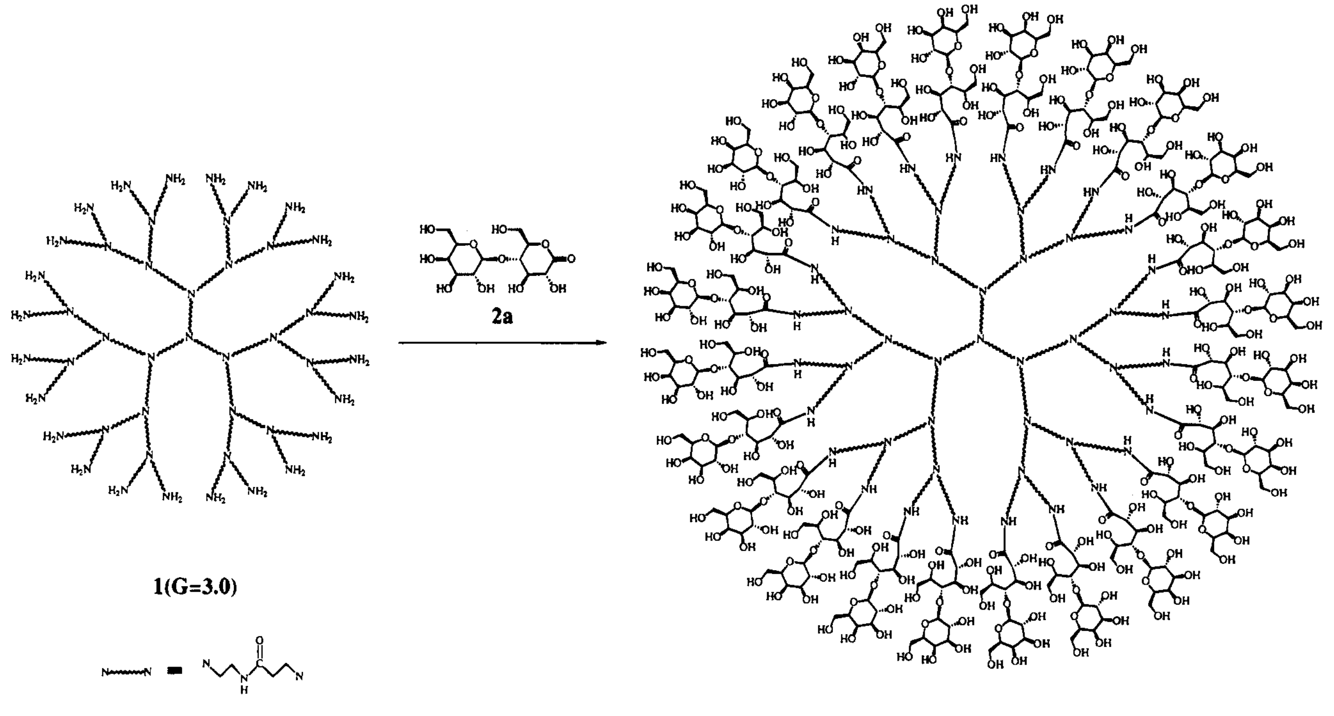
This paper describes new three-dimensional architectures of artificial glycoconjugates, fully sugar-substituted globular dendrimers "sugar balls", as mimics of naturally occurring multiantennary oligosaccharides. Sugar balls have a highly ordered structure with arranged saccharides on the peripheries of dendrimers. The great feature of sugar balls is a controlled geometric arrangement of their sugar moieties, which possess molecular information and function as a molecular recognition marker.

The synthesis of sugar-containing polymeric materials is currently of considerable interest in a wide variety of fields such as glycotechnological, biochemical, and medical applications,[1] because sugar moieties of complex carbohydrates play essential roles in various life processes, e.g., cellular recognition,[2] transport,[3] and adhesion.[4] It is known that multiantennary oligosaccharides show higher affinity to the multisubunit receptors than simple monosaccharide-receptor binding.[5] A geometric arrangement of terminal sugar residues of natural multiantennary oligosaccharides should be important for their recognition. We have already attained well-defined biofunctional glycopolymers, e.g., monodisperse glycopeptides[6] and sugar-substituted poly(2-oxazolines),[7] by utilizing living polymerizations. An intersugar distance controllable graft-type glycoconjugate has also been prepared.[7] Recently Roy et al.[8] have reported the preparation of sugar-carrying multibranching glycopolymers. However, shape-controlled carbohydrate-based polymers with a definite geometrical pattern have never been synthesized until now.

We present herein the new concept of intersugar space regulated carbohydrate macromolecular design exploiting a globular dendrimer skeleton. Structural peculiarity of dendrimers is their fractal symmetry in constitution and shape with a central core.[9] Tomalia and co-workers[10] have synthesized poly(amido amine) (PAMAM) dendrimers by the divergent-growth procedure. Fréchet et al.[11] have developed a convergent-growth approach to polyether and polyester macromolecules. Synthesis of nucleic acid dendrimers[12] and peptide dendrimers[13] has also appeared. Recently PAMAM dendrimers have been used as building blocks for multilayered assemblies.[14] Delocalized π-stacks have been formed on PAMAM dendrimers modified with naphthalenediimide groups.[15]

Lactose and maltose derivative persubstituted PAMAM dendrimers (generation 2.0−4.0) (**3a** (*G* = 2.0−4.0) and **3b** (*G* = 2.0−4.0)) were synthesized by the reaction of the amine-terminated PAMAM dendrimers (generation 2.0−4.0) (**1** (*G* = 2.0−4.0)) with an excess amount of *O*-β-D-galactopyranosyl-(1→4)-D-glucono-1,5-lactone (**2a**) and *O*-α-D-glucopyranosyl-(1→4)-D-glucono-1,5-lactone (**2b**),[16] respectively (Scheme 1, Chart 1). PAMAM dendrimers were prepared with ammonia as an initiator core according to the previous paper.[10] Reaction of **1** and **2** was carried out in dimethyl sulfoxide (DMSO) at 27−40 °C under a nitrogen atmosphere.[17] White powdery compound **3** was obtained, after remaining **2** was removed by dialysis. The structure of **3** was determined by IR and ^{13}C NMR measurements.[18] Size exclusion chromatography (SEC) analysis suggested that **3** consists of a single component.[19] The unreacted amine-terminated branch of the PAMAM dendrimer was not detected in the ^{13}C NMR spectra. In the ^{13}C NMR

Scheme 1



1(G=3.0)

2a

3a(G=3.0)

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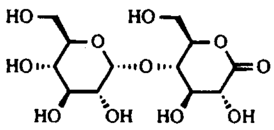
Chart 1



2b

Table 1. Diameter and Intersugar Distance of Sugar Balls 3(G=2.0−4.0)

		diameter[b] (Å)		intersugar distance[b] (Å)	
sugar ball	sugar[a]	contracted	extended	contracted	extended
3(G=2.0)	12	30	62	18	37
3(G=3.0)	24	38	71	16	29
3(G=4.0)	48	47	86	13	24

[a] Theoretical number of terminal sugar residues. [b] Estimated by the three-dimensionally contracted or extended CPK models according to ref 9a. The inter sugar distance is calculated between C-4 carbons of the terminal sugar residues.

spectrum of **1** (G = 4.0) in D_2O, peaks due to α- and β-methylene carbons of terminal amino groups appeared at δ 41.9 and 41.2 ppm, respectively. No peak was observed at the region in the spectra of **3**. Lactobionamide formation was shown by the newly appeared carbonyl carbon signal of **3** at around 179 ppm. By the ^{1}H NMR spectroscopy and by vapor pressure osmometry (VPO),[20] it was found that sugar residues were introduced almost quantitatively to terminal groups of the PAMAM dendrimer. As several reaction examples of **2** with primary amines have already been reported,[16,21] it is assumed that selective and complete reaction between an ester linkage of **2** and an amino group of **1** proceeds without protection of hydroxyl groups of **2** under the condition. The resulting sugar balls showed hydrophilic properties. **3a** (G = 3.0) was soluble in water and DMSO, whereas it was insoluble in ethanol and chloroform, which dissolved **1** (G = 3.0). This result implies that the outer surface of the dendrimer is covered with a layer of hydrophilic saccharide.

Molecular structure of **3**(G=2.0 and 3.0) is predicted to be an oblate spheroid, while **3**(G=4.0) has the shape of a nearly symmetrical spheroid.[22] Diameters and intersugar distances of sugar balls were estimated by using a Corey−Pauling−Koltun (CPK) model.[9a] The values to three-dimensional contracted and extended forms are listed in Table 1. Geometric arrangement of multisubunit receptors has been recently investigated. For example, the sugar-combining sites of galactose/*N*-acetylgalactosamine-specific mammalian hepatic lectin have been ascertained to be situated in space at the vertexes of a triangle whose sides are 15, 22, and 25 Å.[23] The average intersugar distances of sugar balls **3** (G = 3.0−4.0) were within a range from 13 to 29 Å depending on their conformation. Thus, sugar balls, whose terminal sugar residues mainly contribute to the molecular recognition, are regarded as a new class of model compounds for multiantennary oligosaccharides.

The recognition ability of sugar balls was investigated by the quantitative precipitation method in a solution containing **3a** or **3b** and lectin, concanavalin A (Con A).[24] Con A specifically binds α-D-glucosyl or α-D-mannosyl residues.[16b,25] As shown in Figure 1, the turbidity of the D-glucose-bearing sugar ball **3b** (G = 3.0)/Con A solution increased and a precipitate was observed after about 1 h. Precipitation is reasonably explained by the formation of cross-linking between the tetrameric Con A molecule[26] and the sugar ball having a number of glucose residues. In order to estimate the binding strength, monosaccharide D-glucose was added to the mixture containing the aggregate of Con A and the sugar ball. When a 24-fold molar amount of D-glucose to **3b** was added, only a slight decrease of turbidity was observed (Figure 1A). The mixture became a clear homogeneous solution by the addition of a 1200-fold molar excess of D-glucose (Figure 1C). These results suggest strong interaction between Con A and **3b**. On the other hand, the D-galactose-bearing sugar ball **3a** (G = 3.0) did not precipitate Con A, since Con A carries no binding site for galactose. In this case, a decrease in absorption was not seen with the addition of a large amount of D-glucose.

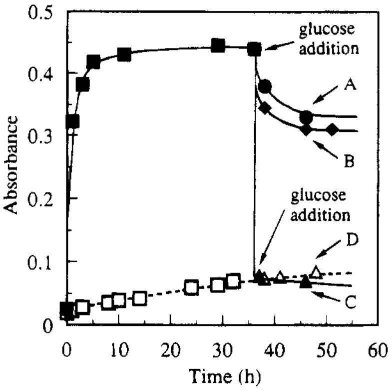


Figure 1. Interaction of Con A with sugar balls **3a** (G = 3.0) (□ and dashed line) and **3b** (G = 3.0) (■ and solid line). The absorbance at 500 nm was measured at 27 °C by a UV/vis spectrophotometer. Sugar ball **3**, 1.0 mg; Con A, 6.4 mg; buffer solution, 8.0 mL (pH 7.2); glucose addition after 36 h: A, 0.32 mg (●): B, 1.6 mg (◆): C, 16 mg (▲); D, 16 mg (△). A control buffer solution containing an equimolar amount of Con A was used.

Moreover, interaction of **3** (G = 3.0) with peanut agglutinin (PNA, *Arachis hypogaea*) was examined by UV/vis spectroscopy. PNA has a high specificity for terminal D-galactosyl residues.[27] In this instance, apparent aggregation between PNA and D-galactose-bearing sugar ball **3a** (G = 3.0) was confirmed, while **3b** (G = 3.0) showed no interaction with PNA. These phenomena are due to selective recognition between the lectin and the terminal carbohydrate residues of the sugar balls.

Variation of intersugar space will also be feasible in denser clustered sugar balls with an ethylenediamine initiator core.[10] Versatile supramolecular assemblies and covalent architecture of sugar-carrying biofunctional molecules should be achieved by this facile synthesis of globular sugar balls instead of polysaccharide dendrimers.

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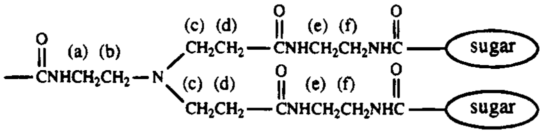
(17) A typical run was as follows. In a two-necked, round-bottomed flask equipped with a thermometer, a three-way stopcock, and a magnetic stir bar was introduced 270 mg (0.025 mmol) of **1** ($G = 4.0$) in 2.0 mL of dry DMSO under nitrogen. A 300-fold molar excess amount (2.6 g) of **2a** in 3.0 mL of DMSO was added by a gas-tight syringe to the solution with stirring. The mixture was stirred at 40 °C for 9 h. The solution was poured into a large amount of methanol. After the reprecipitation procedure, the precipitate was further purified by dialysis against water using a cellulose tube (MW cutoff 3500) and lyophilized to afford white powder **3a** ($G = 4.0$); yield 290 mg (43%).

(18) Lactobionamido-persubstituted poly(amido amine) (PAMAM) dendrimer (generation 4.0) (**3a** ($G = 4.0$)): IR (KBr) 3390 (ν_{O-H}), 2934 (ν_{C-H}), 1645 ($\nu_{C=O}$), 1551 (δ_{N-H}), 1370, 1262, 1080, 1047 (ν_{C-O}) cm^{-1}; ^{1}H NMR (D$_2$O, 400 MHz) δ 4.40 (d, $J = 7.7$ Hz), 4.02–3.35 (m), 3.16 (m), 3.00 (m), 2.69 (m), 2.48 (m), 2.28 (m); ^{13}C NMR (D$_2$O, 100 MHz) δ 178.5 (carbonyl carbon of lactobionamide), 175.6 and 174.6 (carbonyl carbons of the PAMAM dendrimer), 103.6 (C-1 of galactosyl residue), 81.8, 75.4, 72.7, 72.5, 71.9, 71.7, 71.3, 68.8, 62.1, and 61.2 (other carbons derived from **2a**), 51.4 (b), 48.8 (c), 39.2 (f), 37.1 (e), 36.7 (a), 32.4(d). (See the following chart).



(19) The M_w/M_n values of **1** ($G = 3.0$), **3a** ($G = 3.0$), and **3b** ($G = 3.0$) were all 1.02 (by SEC using Shodex KF 803 and 804 columns in dimethyl sulfoxide at 27 °C with poly(2-methyl-2-oxazoline) standards).

(20) The molecular weight of **3a** ($G = 2.0$) by VPO (in water at 60 °C) was 6.5×10^3, which agreed with the theoretical value, 6491.

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(24) Precipitation studies were undertaken in 8.0 mL of buffer solutions (0.01 M phosphate (pH 7.2), 0.5 M NaCl, 0.1 mM MnCl$_2$, and 0.1 mM CaCl$_2$) containing 6.4 mg of Con A (Sigma Chemical Co.) and 1.0 mg of **3** ($G = 3.0$). The solutions were incubated at 27 °C with monitoring by a UV/vis spectrophotometer. After 36 h, 0.32, 1.6, or 16 mg of D-glucose was added. Those correspond to a 1.0-, 5.0-, or 50-fold molar amount to the terminal sugar residues.

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