

# Highly Convergent Synthesis of Dendrimerized Chitosan–Sialic Acid Hybrid<sup>1</sup>

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**ABSTRACT:** Surface bound chitosan–sialodendrimers with a high degree of substitution were successfully prepared using a doubly convergent approach. Poly(amidoamine) (PAMAM) dendrimers ( $G = 1-5$ ) having a 1,4-diaminobutane core were amidated to *N*-carboxyethylchitosan methyl ester under conditions that prevented cross-linking. The extent of attachment (DS) to the polysaccharide backbone decreased from 0.53 to 0.11 with increasing dendrimer generation. Peracetylated *p*-formylphenyl  $\alpha$ -sialoside was then successfully attached to the primary amine end groups of the dendrimerized chitosan hybrid with degree of substitution (DS) ranging from 0.7 to 1.4 using reductive amination. Water-soluble chitosan–sialodendrimer hybrids were finally obtained after protecting group hydrolysis and chitosan *N*-succinylation.

## Introduction

Chitosan is a polysaccharide formed primarily of repeating residues of D-glucosamine. Since chitosan itself is nontoxic and biodegradable<sup>2</sup> and shows widespread biological activities,<sup>3–6</sup> it is an appealing bioactive polymer for further development. Dendrimers, on the other hand, are monodispersed and chemically well-defined macromolecules.<sup>7</sup> Over the past 10 years, a lot of scientific effort have gone into the design and synthesis of dendrimers. Dendrimers are also attractive molecules owing to their multifunctional properties and are potentially useful for medical applications, for host–guest chemistry, and as dendritic catalysts.<sup>8</sup> The preparation of chitosan–dendrimer hybrid for the purpose of generating novel dendrimerized chitosan is described herein. Despite existing strategies toward dendrimerized/dendronized polymers (path A or B, Scheme 1),<sup>9</sup> there is limited reports toward dendrimerized polysaccharide, especially for chitin and chitosan. Recently, we prepared chitosan–sialodendrimer with tetra(ethylene glycol) spacer.<sup>1c</sup> The synthesis of this type of hybrid (path A, Scheme 1) was however limited due to low dendrimer attachment, particularly at high generation because of the steric hindrance inherent to preformed sialodendrimers. The stepwise preparation of surface bound functional groups is expected to avoid such problem (path C, Scheme 1). Moreover, this type of hybrid is a novel form of dendrimerized polymer between polymer chain and dendrimer. In this study, the successful preparation of surface bound type of chitosan–sialodendrimer hybrid using a doubly convergent approach (path C) is reported.

## Experimental Section

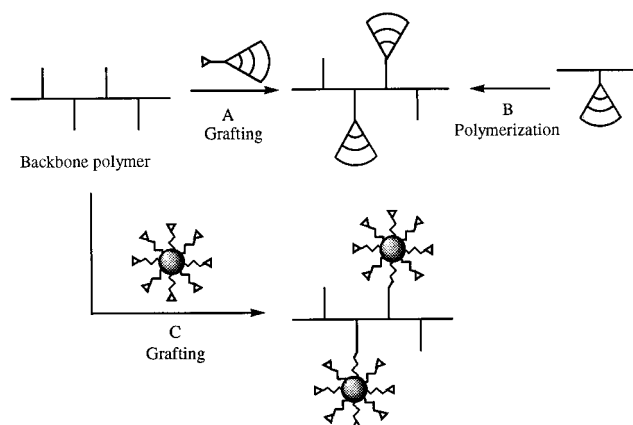
**Materials.** Chitosan (Flonac C, NHAc = 0.2, DP = 140, FW of unit = 169) was purchased from Kyowa Tecnos Co, Japan. Dialysis membrane (MW 12 000 cutoff) was purchased from Sigma Co. 1,4-Diaminobutane and other reagents were from Aldrich Co. and used without further purification.

**General Methods.** The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker 500 MHz AMX NMR spectrometer.

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Scheme 1



Proton chemical shifts ( $\delta$ ) are given relative to internal  $\text{CHCl}_3$  for  $\text{CDCl}_3$  or 3-(trimethylsilyl)propionic-2,2,3,3-*d*<sub>4</sub> acid sodium salt (TMSP) for  $\text{D}_2\text{O}$  or 0.5 M DCl in  $\text{D}_2\text{O}$  solution. Carbon chemical shifts are also given relative to  $\text{CDCl}_3$  or water-soluble TMSP. The degree of polymerization (DP) of chitosan was determined by GPC using pullulan as standard. The primary amino groups in the hybrids were quantified by colorimetric determination using ninhydrin at 570 nm.

**Preparation of PAMAM Dendrimers.** The preparation of PAMAM dendrimers of each generation was carried out according to published procedure.<sup>10</sup> Briefly, to a solution of 1,4-diaminobutane (20 mmol) in methanol (100 mL) was added methyl acrylate (3.0 equiv/ $\text{NH}_2$ ). The mixture was stirred at 45 °C. After 5 days, the mixture was evaporated to dryness. The crude product was purified by column chromatography using a gradient of  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  from 20/1 to 5/1 in addition to a small amount of triethylamine to give half-generation methyl ester. To a solution of methyl ester in methanol was added ethylenediamine (5–10 equiv/ $\text{CO}_2\text{Me}$ ), and the mixture was stirred at room temperature for 3 days. The mixture was evaporated and dried in a vacuum to provide dendrimers ( $G = 1-5$ ). Selected data for **1**: <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  1.35 (s, 4 H,  $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 2.37 (m, 2 H,  $\text{CH}_2(\text{b})\text{N}$ ), 2.41 (t,  $J = 7.1$  Hz, 8 H,  $\text{CH}_2(\text{d})\text{CO}_2\text{Me}$ ), 2.72 (t,  $J = 7.1$  Hz, 8 H,  $\text{NCH}_2(\text{c})$ ), 3.64 (s, 12 H,  $\text{CO}_2\text{Me}$ ). <sup>13</sup>C NMR ( $\text{CDCl}_3$ )  $\delta$  24.6 ( $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 32.3 ( $\text{CH}_2(\text{d})\text{CO}$ ), 49.1 ( $\text{NCH}_2(\text{c})$ ), 51.9 ( $\text{CO}_2\text{Me}$ ), 53.4 ( $\text{CH}_2(\text{b})\text{N}$ ), 172.7 ( $\text{CO}_2\text{Me}$ ). FAB-MS (pos) calcd for  $\text{C}_{20}\text{H}_{36}\text{N}_2\text{O}_8$  432, found 433.3 ( $\text{M}^+ + 1$ , 46% base peak); a–d:  $[-\text{CH}_2(\text{a})\text{CH}_2(\text{b})\text{N}(\text{CH}_2(\text{c})\text{CH}_2(\text{d})\text{CO}_2\text{Me})_2]_2$ .

Data for **2**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.24 (br, 4 H,  $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 1.56 (br, 8 H,  $\text{NH}_2$ ), 2.21 (t,  $J = 5.7$  Hz, 12 H,  $\text{CH}_2(\text{b})\text{N}$  (4 H) and  $\text{CH}_2(\text{d})\text{CO}$  (8 H)), 2.54 (d,  $J = 5.7$  Hz, 8 H,  $\text{NCH}_2(\text{c})$ ), 2.64 (d,  $J = 5.6$  Hz, 8 H,  $\text{CONHCH}_2(\text{e})$ ), 3.11 (t,  $J = 5.6$  Hz, 8 H,  $\text{CH}_2(\text{f})\text{NH}_2$ ), 7.73 (m, 2 H,  $\text{CONH}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  24.8 ( $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 34.1 ( $\text{CH}_2(\text{d})\text{CO}$ ), 41.3 ( $\text{CH}_2(\text{f})\text{NH}_2$ ), 42.1 ( $\text{CONHCH}_2(\text{e})$ ), 50.0 ( $\text{NCH}_2(\text{c})$ ), 53.4 ( $\text{CH}_2(\text{b})\text{N}$ ), 172.8 ( $\text{CONH}$ ). FAB-MS (pos) calcd for  $\text{C}_{24}\text{H}_{52}\text{N}_{10}\text{O}_4$  544, found 545.3 ( $\text{M}^+ + 1$ , 36% base peak); a–f:  $[-\text{CH}_2(\text{a})\text{CH}_2(\text{b})\text{N}(\text{CH}_2(\text{c})\text{CH}_2(\text{d})\text{CONHCH}_2(\text{e})\text{CH}_2(\text{f})\text{NH}_2)_2]_2$ .

Data for **3**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.32 (s, 4 H,  $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 2.25 (t,  $J = 6.7$  Hz, 8 H,  $\text{CH}_2(\text{d})\text{CO}$ ), 2.34 (t,  $J = 7.1$  Hz, 16 H,  $\text{CH}_2(\text{h})\text{CO}_2\text{Me}$ ), 2.39 (s, 4 H,  $\text{CH}_2(\text{b})\text{N}$ ), 2.44 (t,  $J = 6.0$  Hz, 8 H,  $\text{CONHCH}_2(\text{e})$ ), 2.67 (t,  $J = 7.1$  Hz, 16 H,  $\text{NCH}_2(\text{g})$ ), 3.17 (dd,  $J = 5.8$  Hz,  $\text{NCH}_2(\text{c})$ ), 3.57 (s, 24 H,  $\text{CO}_2\text{Me}$ ), 7.05 (t, 4 H,  $\text{CONH}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  24.5 ( $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 32.5 ( $\text{CH}_2(\text{h})\text{CO}_2\text{Me}$ ), 32.6 ( $\text{CH}_2(\text{d})\text{CO}$ ), 37.0 ( $\text{CONHCH}_2(\text{e})$ ), 49.1 ( $\text{NCH}_2(\text{c})$ ), 49.7 ( $\text{NCH}_2(\text{g})$ ), 51.3 ( $\text{CO}_2\text{Me}$ ), 52.1 ( $\text{CH}_2(\text{b}, \text{f})\text{N}$ ), 172.8 ( $\text{CONH}$  and  $\text{CO}_2\text{Me}$ ); e–h:  $[-\text{CH}_2(\text{e})\text{CH}_2(\text{f})\text{N}(\text{CH}_2(\text{g})\text{CH}_2(\text{h})\text{CO}_2\text{Me})_2]_2$ ; a–d, see **1**.

Data for **4**:  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.48 (brs,  $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 2.47 (t,  $J = 6.9$  Hz,  $\text{CH}_2(\text{b}, \text{f})\text{N}$  and  $\text{CH}_2(\text{d}, \text{h})\text{CO}$ ), 2.66 (t,  $J = 6.4$  Hz,  $\text{NCH}_2(\text{c}, \text{g})$ ), 2.77 (t,  $J = 6.6$  Hz,  $\text{CONHCH}_2(\text{e}, \text{i})$ ), 3.29 (t,  $J = 6.6$  Hz,  $\text{CH}_2(\text{j})\text{NH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  23.4 ( $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 32.1–32.4 ( $\text{CH}_2(\text{d}, \text{h})\text{CO}$ ), 39.4 ( $\text{CH}_2(\text{j})\text{NH}_2$ ), 41.0 ( $\text{CONHCH}_2(\text{e}, \text{i})$ ), 48.3–48.7 ( $\text{NCH}_2(\text{c}, \text{g})$ ), 50.8 ( $\text{CH}_2(\text{b}, \text{f})\text{N}$ ), 174.2 and 174.5 ( $\text{CONH}$ ); e–j:  $[-\text{CH}_2(\text{e})\text{CH}_2(\text{f})\text{N}(\text{CH}_2(\text{g})\text{CH}_2(\text{h})\text{CONHCH}_2(\text{i})\text{CH}_2(\text{j})\text{NH}_2)_2]_2$ .

Data for **6**:  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.47 (brs,  $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 2.47 (d,  $J = 6.9$  Hz,  $\text{CH}_2\text{N}$  and  $\text{CH}_2\text{CO}$ ), 2.66 (d,  $J = 6.4$  Hz,  $\text{NCH}_2$ ), 2.84 (d,  $J = 6.6$  Hz,  $\text{CONHCH}_2$ ), 3.33 (br,  $\text{CH}_2\text{NH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  23.3 ( $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 32.1–32.3 ( $\text{CH}_2\text{CO}$ ), 36.3 ( $\text{CH}_2\text{NH}_2$ ), 39.2–39.9 ( $\text{CONHCH}_2$ ), 48.6 ( $\text{NCH}_2$ ), 50.8 ( $\text{CH}_2\text{N}$ ), 174.1–174.7 ( $\text{CONH}$ ).

Data for **8**:  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.37 (d,  $J = 6.9$  Hz,  $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 2.47 (br,  $\text{CH}_2\text{N}$  and  $\text{CH}_2\text{CO}$ ), 2.66 (br,  $\text{NCH}_2$ ), 2.84 (br,  $\text{CONHCH}_2$ ), 3.35 (br,  $\text{CH}_2\text{NH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.7 ( $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 32.2–32.3 ( $\text{CH}_2\text{CO}$ ), 36.3 ( $\text{CH}_2\text{NH}_2$ ), 39.3–40.1 ( $\text{CONHCH}_2$ ), 48.6 ( $\text{NCH}_2$ ), 50.8 ( $\text{CH}_2\text{N}$ ), 174.1–174.7 ( $\text{CONH}$ ).

**Preparation of Chitosan–Dendrimer Hybrid.** A typical procedure is as follows: *N*-carboxyethylchitosan methyl ester<sup>1b</sup> (**11**; DS = 1.2; 100 mg; 0.54 mmol of  $\text{CO}_2\text{Me}$ ) was dispersed in MeOH (50 mL). To a suspension was added **2** (0.54 mmol; 1.0 equiv/ $\text{CO}_2\text{Me}$ ) in MeOH (50 mL). The mixture was stirred at room temperature. After 3 days, the mixture was evaporated to dryness, dispersed in 0.2 M NaOH at room temperature for 2 h, dialyzed, and lyophilized to give **12** (130 mg). Selected data for **12** (DS = 0.53):  $^1\text{H}$  NMR (0.5 M  $\text{DCl}/\text{D}_2\text{O}$ )  $\delta$  1.96 (s, 2.1 H,  $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 2.13 (s, 0.6 H,  $\text{NHAc}$ ), 2.94 (t,  $J = 6.6$  Hz, 4.2 H,  $\text{CH}_2(\text{d})\text{CO}$ ), 3.24 (t,  $J = 5.9$  Hz, 4.2 H,  $\text{CONHCH}_2(\text{e})$ ), 3.40 (br, H-2 of  $\text{GlcN-R}$  and  $\text{CH}_2(\text{a})\text{CH}_2(\text{b})\text{N}$ ), 3.59 (m,  $\text{CH}_2(\text{c})$  and  $\text{CH}_2\text{NH}_2(\text{f})$ ), 3.6–4.2 (brm, H-2 of  $\text{GlcNac}$ , H-3,4,5,6 of chitosan), 4.60 (br, H-1 of  $\text{GlcNac}$ ), 5.11 (br, H-1 of  $\text{GlcN-R}_2$ ), 5.30 (br, H-1 of  $\text{GlcN-R}$ ).  $^{13}\text{C}$  NMR (0.5 M  $\text{DCl}/\text{D}_2\text{O}$ )  $\delta$  23.3 ( $\text{CH}_2(\text{a})\text{CH}_2\text{N}$ ), 25.0 ( $\text{NHAc}$ ), 31.7 ( $\text{CH}_2(\text{d})\text{CO}$ ), 39.7 ( $\text{CH}_2(\text{f})\text{NH}_2$ ), 41.9 ( $\text{CONHCH}_2(\text{e})$ ), 52.2 ( $\text{NCH}_2(\text{c})$ ), 55.0 ( $\text{CH}_2(\text{b})\text{N}$ ), 62.6 (C-6 of sugar backbone), 63.4 (C-2), 73.7 (C-3), 77.6 (C-5), 81.7 (C-4), 99.2 (C-1), 175.5 ( $\text{CONH}$ ), 178.0 ( $\text{NHAc}$ ); for a–f assignment see **2**.

Data for **13** (DS = 0.40):  $^1\text{H}$  NMR (0.5 M  $\text{DCl}/\text{D}_2\text{O}$ )  $\delta$  1.92 (s, 1.6 H,  $\text{CH}_2(\text{a})$ ), 2.12 (s, 0.6 H,  $\text{NHAc}$ ), 2.91 (t,  $J = 6.7$  Hz, 6.4 H,  $\text{CH}_2\text{CO}$ ), 3.23 (t,  $J = 5.8$  Hz, 6.4 H,  $\text{CONHCH}_2$ ), 3.38–3.44 (m, H-2 of  $\text{GlcN-R}$  and  $\text{CH}_2(\text{b})\text{N}$ ), 3.57 (m,  $\text{CH}_2(\text{c})$  and  $\text{CH}_2\text{NH}_2(\text{f})$ ), 3.6–4.2 (brm, H-2 of  $\text{GlcNac}$ , H-3,4,5,6 of chitosan), 5.11 (br, H-1 of  $\text{GlcN-R}_2$ ), 5.30 (br, H-1 of  $\text{GlcN-R}$ ).  $^{13}\text{C}$  NMR (0.5 M  $\text{DCl}/\text{D}_2\text{O}$ )  $\delta$  23.4 ( $\text{CH}_2(\text{a})$ ), 25.0 ( $\text{NHAc}$ ), 31.9–32.1 ( $\text{CH}_2\text{CO}$ ), 39.7 ( $\text{CH}_2\text{NH}_2$ ), 41.9 ( $\text{CONHCH}_2$ ), 51.3–52.5 ( $\text{NCH}_2$ ), 54.7 ( $\text{CH}_2(\text{b})\text{N}$ ), 62.6 (C-6 of chitosan), 63.4 (C-2), 73.7 (C-3), 77.6 (C-5), 81.7 (C-4), 99.2 (C-1), 174.9–181.3 ( $\text{CONH}$  and  $\text{NHAc}$ ).

For hybrids **14**, **15**, and **16**, characteristic signals were found at  $\delta$  1.92 ( $\text{CH}_2(\text{a})$ ), 2.12 ( $\text{NHAc}$ ), 2.91 ( $\text{CH}_2\text{CO}$ ), 3.23 ( $\text{CONHCH}_2$ ), 3.38–3.44 ( $\text{CH}_2(\text{b})\text{N}$ ), 3.57 ( $\text{NCH}_2$  and  $\text{CH}_2\text{NH}_2$ ) in  $^1\text{H}$  NMR (0.5 M  $\text{DCl}/\text{D}_2\text{O}$ ) and at  $\delta$  23.4 ( $\text{CH}_2(\text{a})$ ), 25.0 ( $\text{NHAc}$ ), 31.5–32.0 ( $\text{CH}_2\text{CO}$ ), 39.7 ( $\text{CH}_2\text{NH}_2$ ), 41.9 ( $\text{CONHCH}_2$ ), 51.0–

52.5 ( $\text{NCH}_2$ ), 175–180 ( $\text{CONH}$ ) in  $^{13}\text{C}$  NMR in addition with the chitosan backbone signals of **11**. The DS of dendrimer was estimated by  $^1\text{H}$  NMR from the peak ratio at  $\delta$  1.92 ( $\text{CH}_2(\text{a})$ ) and 2.12 ( $\text{NHAc}$ : 0.6 H).

**Preparation of Sialodendrimer–Chitosan Hybrids 18–22.** Peracetylated *p*-formylphenyl  $\alpha$ -sialoside (**17**) was prepared according to Roy et al.<sup>11</sup> A typical procedure is as follows: To a suspension of dendrimer **12** ( $G = 1$ , 50 mg) in water (10 mL) and MeOH (30 mL) containing AcOH (30 mg) was added **17** (0.83 mmol, ca. 3.0 equiv/ $\text{NH}_2$ ) in MeOH (10 mL). After stirring at room temperature for 1 h,  $\text{NaCNBH}_3$  (2.4 mmol) was added and the solution continuously stirred at 50 °C for 2 h and then at room temperature for 1 day. The mixture was evaporated to dryness and then treated with 0.1 M NaOH at room temperature for 2 h, then dialyzed, and finally lyophilized to give compound **18** (140 mg). Compounds **19–22** were also prepared in a similar manner. Selected data for **18** (DS (Neu5Ac) = 0.70):  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.60 (br, 2.12 H ( $\text{CH}_2(\text{a})$ ), 1.98 (dd,  $J = 12.1$  Hz, 0.70 H, H-3ax of Neu5Ac), 2.11 and 2.12 (m, 2.7 H,  $\text{NHAc}$  of chitosan and Neu5Ac), 2.51–2.61 (br, 8.48 H,  $\text{CH}_2\text{CO}$  and  $\text{NCH}_2$ ), 2.80 (br, 4.24 H,  $\text{CH}_2\text{NH}$ ), 2.95 (dd,  $J = 12.1$  Hz, 0.70 H, H-3eq of Neu5Ac), 3.0–4.2 (m,  $\text{NCH}_2$  of dendrimer, H-2 of  $\text{GlcNac}$ , H-3,4,5,6 of chitosan and H-4,7,8,9 of Neu5Ac), 4.15 (d,  $J = 10.2$  Hz, 0.70 H, H-5 of Neu5Ac), 7.20 (d,  $J = 8.5$  Hz, 1.40 H, H-ortho of  $\text{C}_6\text{H}_4\text{-O}$ ), 7.41 (d,  $J = 8.5$  Hz, 1.40 H, H-meta of  $\text{C}_6\text{H}_4\text{-O}$ ).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  22.9 ( $\text{CH}_2(\text{a})$ ), 24.9–25.3 ( $\text{NHAc}$  of chitosan and Neu5Ac), 33.6 ( $\text{NHCOCH}_2$ ), 39.0 ( $\text{CONHCH}_2$ ), 43.6 (C-3 of Neu5Ac), 51.5 ( $\text{NCH}_2$ ), 54.7 ( $\text{CH}_2\text{C}_6\text{H}_4$ ), 55.1 ( $\text{CH}_2\text{N}$  and C-5 of Neu5Ac), 63.2 (C-6 of chitosan), 65.2 (C-6 of chitosan), 65.6 (C-9 of Neu5Ac), 71.0–71.3 (C-7, C-4, and C-8 of Neu5Ac), 74.5 (C-6 of Neu5Ac), 77.7 (C-5 of chitosan), 80.4 (C-4 of chitosan), 105.5 (C-2 of Neu5Ac), 124.4 (C-ortho), 133.8 (C-para, C-meta), 156.4 (C-*ipso*), 175.4–183.2 ( $\text{NHCO}$ ,  $\text{CO}_2\text{Na}$  of Neu5Ac).

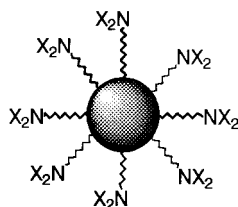
For sialylated hybrids **19**, **20**, **21**, and **22** in  $\text{D}_2\text{O}$  or 0.5 M  $\text{DCl}/\text{D}_2\text{O}$ , characteristic signals were found at  $\delta$  1.92 ( $\text{CH}_2(\text{a})$ ), 2.12 ( $\text{NHAc}$ ), 2.55 ( $\text{CH}_2\text{CO}$ ), 2.95 (H-3eq of Neu5Ac), 7.20 (H-ortho of  $\text{C}_6\text{H}_4\text{-O}$ ), 7.41 (H-meta of  $\text{C}_6\text{H}_4\text{-O}$ ) in  $^{13}\text{C}$  NMR and at  $\delta$  22.9 ( $\text{CH}_2(\text{a})$ ), 25.0 ( $\text{NHAc}$  of Neu5Ac), 33.6 ( $\text{NHCOCH}_2$ ), 39.0 ( $\text{CONHCH}_2$ ), 43.6 (C-3 of Neu5Ac), 54.7 ( $\text{CH}_2\text{C}_6\text{H}_4$ ), 65.6 (C-9 of Neu5Ac), 105.5 (C-2 of Neu5Ac), 124.4 (C-ortho), 133.8 (C-para, meta) in  $^{13}\text{C}$  NMR in addition with the sugar backbone signals of **11**. The DS of sialic acid moiety was estimated by  $^1\text{H}$  NMR from the peak ratio at  $\delta$  2.12 ( $\text{NHAc}$ : 0.6 H) and 2.95 (H-3eq of Neu5Ac). The DS estimated from the ratio at  $\delta$  2.12 ( $\text{NHAc}$ : 0.6 H) and 7.41 (H-meta of  $\text{C}_6\text{H}_4\text{-O}$ ) are also available.

**N-Succinylation of Sialodendrimer–Chitosan Hybrids 23–27.** Primary and secondary amino group N-succinylation in hybrids **18–22** was performed according to the literature.<sup>12</sup> A typical procedure is as follows. Hybrid **18** (50 mg) was dispersed in  $\text{H}_2\text{O}$  (10 mL) and MeOH (30 mL) containing AcOH (60 mg), and succinic anhydride (3.0 mmol/ $\text{NH}$  and  $\text{NH}_2$ ) was added in excess. The mixture was stirred at 60 °C for 4 h and cooled to room temperature. After 2 days, the mixture was concentrated to ca. 10 mL, NaOH (1.0 M, 10 mL) was added, and the solution was stirred at room temperature. After 2 h, the mixture was dialyzed against distilled water for 2 days and lyophilized to provide N-succinylated hybrids **23** (60 mg). For N-succinylated hybrids **23–27**, characteristic signals were found at  $\delta$  2.48 ppm ( $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ) in  $^1\text{H}$  NMR (0.5 M  $\text{DCl}/\text{D}_2\text{O}$ ) and at  $\delta$  37.0 ppm ( $\text{CH}_2\text{CO}_2\text{H}$ ) in  $^{13}\text{C}$  NMR in addition to the backbone signals of sialylated hybrids **18–22**.

## Results and Discussion

**Chitosan–Dendrimer Hybrid (12–16).** PAMAM dendrimers of  $G = 1–5$  were initially prepared from 1,4-diaminobutane according to published procedure (Scheme 2).<sup>10</sup> These methyl esters and amines (**1–10**) were structurally well-defined from  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. On the other hand, *N*-carboxyethylchitosan methyl ester (**11**) was prepared from chitosan according to our previous procedure.<sup>1b</sup> In this study, DS (degree of

Scheme 2



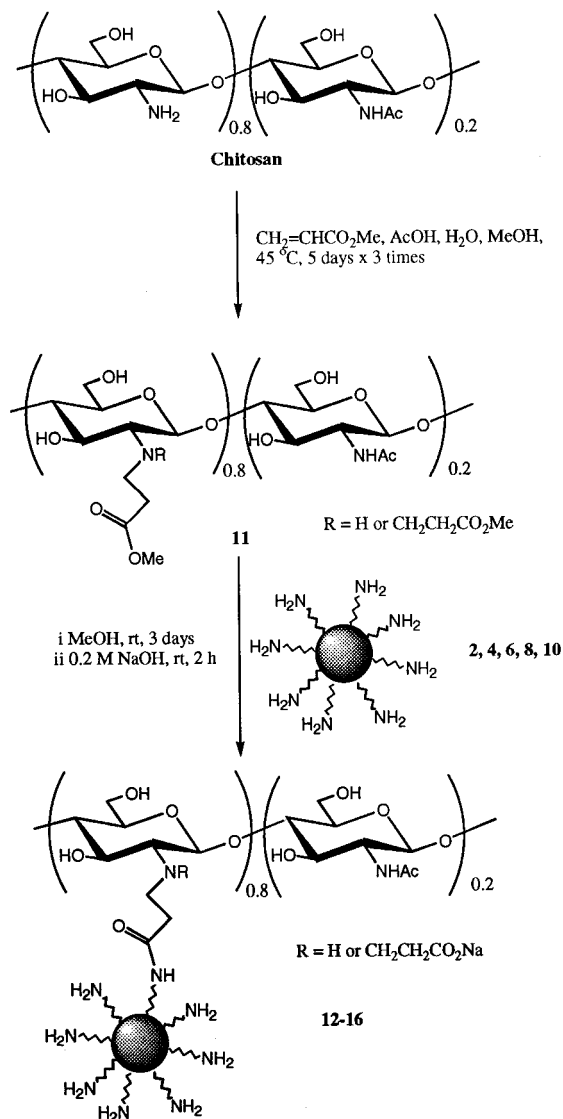
- 1** ( $G=0.5$ );  $X = \text{CH}_2\text{CH}_2\text{CO}_2\text{Me}$       **2** ( $G=1.0$ );  $X = (\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NH}_2$   
**3** ( $G=1.5$ )      **4** ( $G=2.0$ )  
**5** ( $G=2.5$ )      **6** ( $G=3.0$ )  
**7** ( $G=3.5$ )      **8** ( $G=4.0$ )  
**9** ( $G=4.5$ )      **10** ( $G=5.0$ )

substitution) means the number of functional group per hexosamine (GlcN and GlcNAc) residue in the chitosan backbone. The DS in ester **11** was 1.2, which includes 0.4 monoester and 0.4 diester ( $1.2 = 0.4 + 0.4 \times 2$ ). Tomalia et al. reported that the surface amines of PAMAM dendrimer successfully reacted with methyl esters of other PAMAM dendrimers to afford “core–shell *tecto*-(dendrimer) molecules”.<sup>13</sup> Furthermore, they also reported the synthesis of rod-shaped cylindrical dendronized polymers from poly(ethylene imine) cores without any cross-linking.<sup>14</sup> These reports lead us to propose a new approach toward hybridized dendrimers and polymers (path C, Scheme 1).

As shown in Scheme 3, PAMAM dendrimer (**2**;  $G = 1$ ) successfully attached to ester **11** to give chitosan–dendrimer hybrid **12** without gel formation. High-generation dendrimers (**4**, **6**, **8**, **10**;  $G = 2–5$ ) also successfully attached to **11** and gave hybrids **13–16**. The absence of gel formation strongly suggests that no intermolecular cross-linking had occurred. The slow reaction under our heterogeneous conditions may be responsible for the lack of intermolecular cross-linking. The results are summarized in Table 1. The DS of the hybrids gradually decreased with increasing dendrimer generation, thus indicating again that steric hindrance of the underivatized dendrimers would affect the reactivity, but to a much lesser extent than those of previously synthesized dendronized chitosan–sialodendrimers. All hybrids were soluble in acidic water (0.2 M HCl). Hybrids of low generation ( $G = 1$  and 2) were soluble even in neutral water. The chemical structures of hybrid **12–16** are shown in Table 2. From the ninhydrin analysis, the DS of amine groups gradually decreased (from C to D in Table 2), thus indicating that few amine groups formed amide linkages with other methyl esters in **11** (thus forming gel). From Table 2 analysis, 24–37% ( $1/3–1/4$ ) of the remaining amine groups were transformed into amides.

**Sialodendrimer–Chitosan Hybrid (18–22) and Their *N*-succinates (23–27).** Since several primary amino groups are available in these chitosan–dendrimer hybrids (**12–16**), these amines would be useful for further modifications. We tested the attachment of peracetylated *p*-formylphenyl  $\alpha$ -sialoside (**17**) to the various hybrids by reductive amination (Scheme 4). The results are summarized in Table 3. Although sialoside **17** was used in excess (3.0 equiv/  $\text{NH}_2$ ), only 22–23% could be covalently attached to dendrimers  $G = 1–3$ , and 64–70% of the primary amines were *N*-alkylated. The reactivity decreased with increasing generations, especially above  $G = 4$ . Some hybrids (**20–22**) were insoluble in neutral water and thus would be useless

Scheme 3



**Table 1. Reaction of *N*-Carboxyethylchitosan Methyl Ester (**11**) with Dendrimers<sup>a</sup>**

compd	dendrimer <sup>b</sup> NH <sub>2</sub> / CO <sub>2</sub> Me	product	yield <sup>c</sup> (%)	DS	solubility	
					H <sub>2</sub> O	0.2 M HCl
<b>2</b> ( $G = 1$ )	4	12	70	0.53	yes	yes
<b>4</b> ( $G = 2$ )	8	13	68	0.40	yes	yes
<b>6</b> ( $G = 3$ )	16	14	77	0.21	no	yes
<b>8</b> ( $G = 4$ )	32	15	70	0.17	no	yes
<b>10</b> ( $G = 5$ )	64	16	86	0.11	no	yes

<sup>a</sup> Solvent: MeOH, rt, 3 days. <sup>b</sup> Dendrimer (equiv/  $\text{CO}_2\text{Me}$ ) = 1.0. <sup>c</sup> Yield was estimated on the bases of the weight of **11**.

for biological evaluation. To this end, the remaining amino groups in the hybrids **18–22** were transformed by *N*-succinylation using succinic anhydride.<sup>12</sup> *N*-Succinylated hybrids **23–27** were obtained quantitatively. This process dramatically increased their water solubility. Although the actual DS values of succinylated hybrids **23–27** could not be directly estimated from their <sup>1</sup>H NMR spectra because of partial overlapping signals at  $\delta$  2.48 ( $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ) and at 2.55 ( $\text{CH}_2\text{CO}$ ), most primary amines in the PAMAM–dendrimer moiety and part of the secondary amines in both dendrimer moieties (sialic acid branches) and chitosan backbone were *N*-succinylated.



**Table 2. Chemical Structure of Hybrids 12–16<sup>a</sup>**

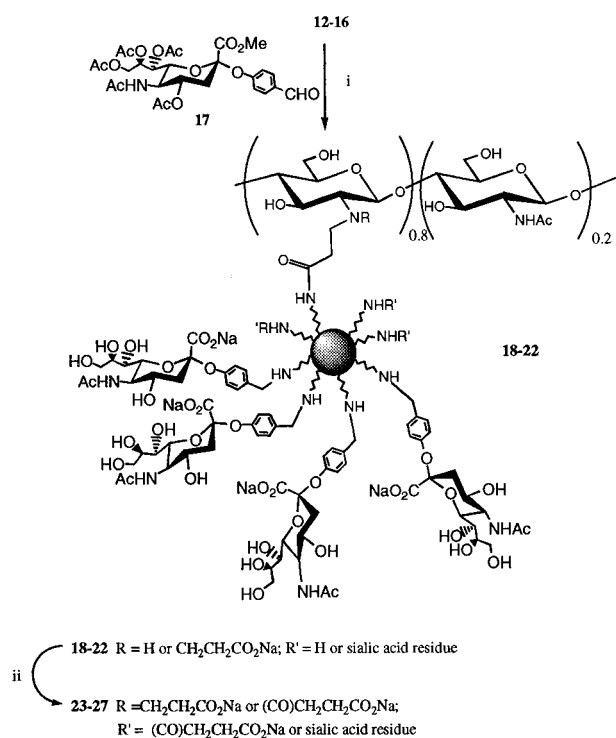
	A	B	DS (NH <sub>2</sub> )		CONH formed	
			C (calcd)	D (found)	E	%
<b>12</b> ( <i>G</i> = 1)	0.53	3	1.59	1.00	0.59	37
<b>13</b> ( <i>G</i> = 2)	0.40	7	2.80	2.00	0.80	29
<b>14</b> ( <i>G</i> = 3)	0.21	15	3.15	2.10	1.05	33
<b>15</b> ( <i>G</i> = 4)	0.17	31	5.27	4.00	1.27	24
<b>16</b> ( <i>G</i> = 5)	0.11	63	6.93	5.00	1.93	28

<sup>a</sup> A, DS of dendrimer; B, number of NH<sub>2</sub> in dendrimer moiety; C, calcd DS of NH<sub>2</sub> as  $C = A \times B$ ; D, found DA of NH<sub>2</sub> which was determined by ninhydrin analysis;  $E = C - D$ ; % =  $(E/C) \times 100$ .

**Table 3. Preparation of Sialodendrimer–Chitosan Hybrids 18–22<sup>a</sup>**

compd	DS (NH <sub>2</sub> )	sialoside	DS (Neu5Ac)	reactivity (%) <sup>b</sup>
<b>12</b> ( <i>G</i> = 1)	1.0	18	0.70	23
<b>13</b> ( <i>G</i> = 2)	2.0	19	1.40	23
<b>14</b> ( <i>G</i> = 3)	2.1	20	1.35	22
<b>15</b> ( <i>G</i> = 4)	4.0	21	1.27	11
<b>16</b> ( <i>G</i> = 5)	5.0	22	1.21	8

<sup>a</sup> Aldehyde **17**, 3.0 equiv/NH<sub>2</sub>. <sup>b</sup> % =  $(\text{DS}(\text{Neu5Ac})/\text{DS}(\text{NH}_2) \times 3) \times 100$ .

**Scheme 4**

i (a) **17**, NaCNBH<sub>3</sub>, AcOH, H<sub>2</sub>O, MeOH, rt 2 days (b) 0.5 M NaOH, rt, 2 h

ii (a) succinic anhydride, AcOH, H<sub>2</sub>O, MeOH, 60 °C-rt 2 days (b) 0.5 M NaOH, rt, 2 h

In conclusion, surface-bound chitosan–sialodendrimer hybrids with high degree of substitutions were successfully prepared. Given the fact that flu virus hemagglutinins exist as several clusters of trimers (200–300/virion),<sup>15</sup> it is likely that the novel dendrimerized chitosan–sialic acid hybrids described herein would

present added beneficial architectures not present in previous sialopolymers.<sup>16</sup> Preliminary biological evaluation of analogous hyperbranched sialodendrimers has already shown increased inhibitory properties.<sup>17</sup>

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## References and Notes

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