# Chemical Modification of Chitosan. 3. Hyperbranched Chitosan−Sialic Acid Dendrimer Hybrid with Tetraethylene Glycol Spacer

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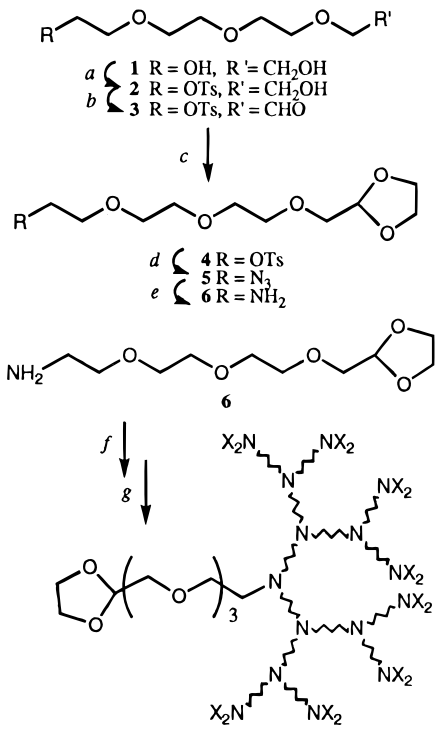
Chitosan is a polysaccharide composed mainly of *β*-(1−4)-2-amino-2-deoxy-D-glucopyranose repeating units. Chitosan shows interesting biological properties such as immunological,[1] antibacterial,[2,3] and wound healing activity.[4] Moreover, it is a nontoxic[3] and biodegradable polymer,[5] and the free amino groups of chitosan offer great potential for further derivatization. Alternatively, dendrimers also offer several possibilities in molecular design owing to their multifunctional attachment sites. They have been used to scaffold neoglycoconjugates,[6] probes, catalysts, and so on.[7] Herein we report the preparation of sialic acid bound dendronized[8] chitosan−dendrimer hybrids. These molecules may have the potential to inhibit the hemagglutination of human erythrocytes by *influenza* virus hemagglutinin as recently observed with straight polymers and hyperbranched polymers.[6] As polymer backbones used so far are highly toxic, it is expected that scaffolding poly(amidoamine) (PAMAM) dendrimer onto nontoxic chitosan core would present biopharmaceutical advantage.

To build up the desired long spacer necessary to allow multiple branch construction, commercial tetraethylene glycol **1** was modified into aminoacetal **6** in five steps (Scheme 1). Monotosylate **2** was prepared from **1** in only 40% yield (TsCl, 1.0 equiv, $Et_3N$, $CH_2Cl_2$) owing to the simultaneous formation of undesired ditosylate. According to a published procedure,[9,10] oxidation (DMSO, $P_2O_5$, $CH_2Cl_2$) and acetal formation ($HOCH_2CH_2OH$, *p*TsOH, PhH) were performed in good yields (**3**, 92%; **4**, 89%). After azide displacement and reduction [(i) $NaN_3$, EtOH; (ii) $H_2$, 10% Pd−C, EtOH],[11] amine **6** was obtained in 23% overall yield from **1**. Using **6** as an amine source, poly(amidoamine) (PAMAM) dendrimers were prepared according to Tomalia et al.[12] by repeating cycle of methyl acrylate and ethylenediamine treatment. The methyl esters of each dendrimers **7a**−**c** ($G = 0.5, 1.5, 2.5$) were purified by silica gel column chromatography ($CH_2Cl_2$/MeOH = 10/1) which were obtained in moderate to good yields (**7a**, $G = 0.5$, 70%; **7b**, $G = 1.5$, 73%; **7c**, $G = 2.5$, 57%). The terminal amines of each generation ($G = 1$, $G = 2$, and $G = 3$, **8a**−**c**) were obtained from each methyl esters quantitatively by treatment with excess ethylenediamine at room temperature for 3 days.

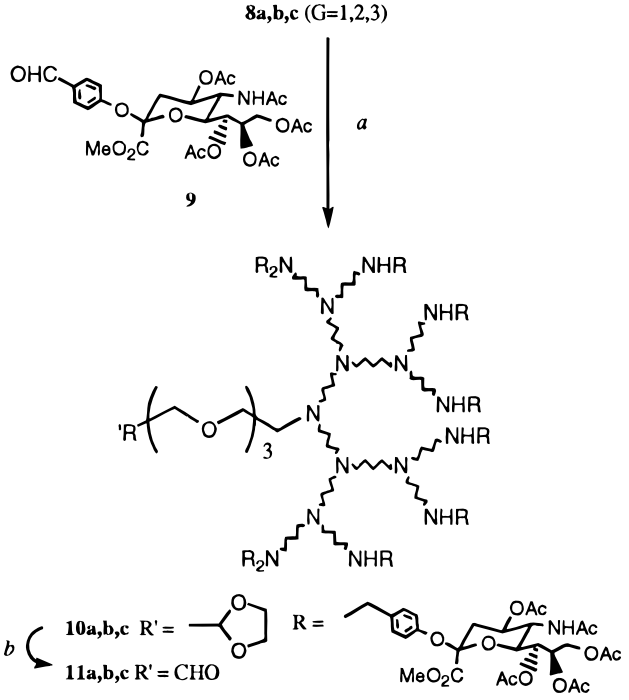
The sialic acid residues were initially attached onto each dendrimer by reductive N-alkylation with known *p*-formylphenyl α-sialoside **9**[13] using $NaCNBH_3$ in methanol (Scheme 2).[14] Since 3 equiv of aldehyde **9** was used

**Scheme 1**[a]




[a] Reagents: (a) TsCl, $Et_3N$, $CH_2Cl_2$, 0 °C r.t., 16 h, 40%; (b) DMSO, $P_2O_5$, $CH_2Cl_2$, 0°C r.t., 2 h, 92%; (c) $HO(CH_2)_2OH$, *p*TsOH, PhH, Reflux, 4 h, 89%; (d) $NaN_3$, EtOH, Reflux, 4 h, 90%; (e) 10% Pd/C, EtOH, $H_2$, r.t., 6 h, 78%; (f) $H_2C{=}CHCO_2Me$, MeOH, 45 °C, 5 days; (g) $H_2N(CH_2)_2NH_2$, MeOH, r.t., 3 days.

**Scheme 2**[a]




[a] Reagents: (a) $NaBH_3CN$, r.t., 16 h; (b) 80% aqueous TFA, r.t., 16 h.

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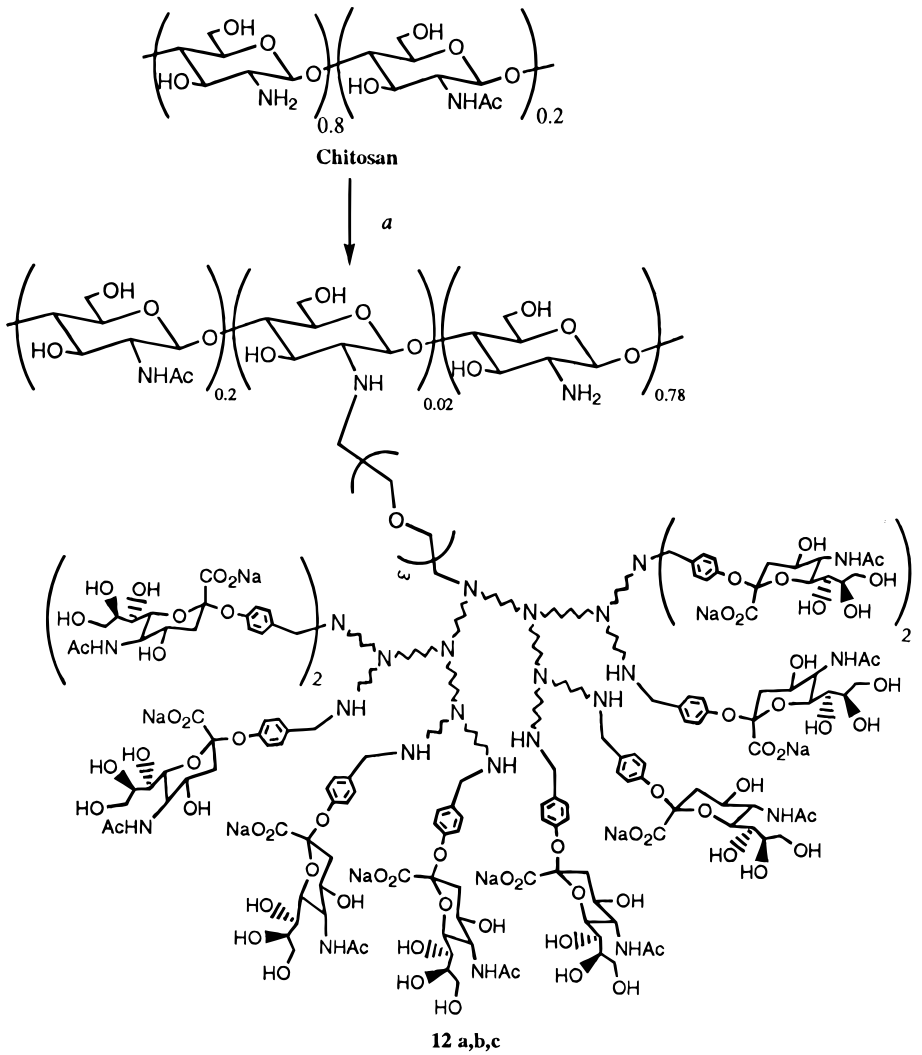
**Scheme 3**[a]




[a] Reagents: (a) (i) **11a**,**b**,**c**, $NaBH_3CN$, MeOH, r.t., 16 h; (ii) 0.1 M NaOH, r.t., 2 h.

to ensure complete amine substitutions, partial N,N-disubstitution of **10a**−**c** inevitably occurred. (On average, 10 mol of sialosides was bound to 8 mol of amine in dendrimer **10c**, 3 sialosides in G1 **10a**, and 5 in G2 **10b**.) The deprotection of dendritic sialoacetal **10a**−**c** was carried out with 80% aqueous trifluoroacetic acid ($CF_3CO_2H$) at room temperature for 16 h to provide aldehydes **11a**−**c**. The aldehyde-ending sialodendrimers were obtained by evaporation to dryness, followed by drying in vacuo for a day.[15] In the high field $^1H$ NMR (500 MHz) spectra of **11a**−**c**, the typical hydrated acetal proton ($\delta$ 5.10 ppm) completely disappeared. Compounds **11a**−**c** were then directly used in the reductive amination of chitosan without any further purification (Scheme 3).[16] The deprotection of O-acetyl groups and methyl ester of the α-sialoside moiety was performed with 0.1 M NaOH at room temperature for 2 h, and the resulting hyperbranched hybrids **12a**−**c** (220 mg) were obtained from 200 mg of chitosan. The purification of hybrids **12a**−**c** was performed by exhaustive dialysis ($H_2O$; MW = 12 000 cutoff) which could be separated from aldehyde **11a**−**c** (MW ≤ 7567) and its deprotected form (MW ≤ 5967). From the $^1H$ and $^{13}C$ NMR spectra of **12a**−**c**, the corresponding signals of the chitosan backbone ($\delta$ 3.4−4.2 and 4.6 ppm), dendrimer backbone ($\delta$ 2.80 ppm), and the phenyl α-sialoside residue ($\delta$ 2.28, 6.95, and 7.35 ppm; Nac, Ar) were clearly observed. The degree of substitutions (DS) were determined to be 0.08 (**12a**, 47%), 0.04 (**12b,** 25%), and 0.02 (**12c**, 25%), which suggests that only a small proportion of aldehyde **11a**−**c** reacted with chitosan. The low reactivity in the third generation could be ascribed to the steric hindrance of the high molecular weight of **11c** (MW = 7567). From the DS values (0.02−0.08) of the products and the DP (140) in the original chitosan, it was assumed that an average of 2.8−11.2 molecules of sialodendrimers were attached per molecule of the polysaccharide (approximately 28−33.6 sialoside residues/polysaccharide chain). In conclusion, we successfully prepared dendronized chitosan−sialic acid hybrids via tetraethylene glycol spacer. The low degree of substitution observed in the higher generation was in line with previous reports where polymer backbones are conjugated to preformed dendrons.[8] Work is now in progress to scaffold poly(amidoamine) dendrimers onto chitosan prior to sialic acid attachment. These compounds are currently being evaluated as inhibitors of hemagglutination of influenza viruses.

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## References and Notes


(1) Nishimura, K.; Nishimura, S.; Nishi, N.; Saiki, I.; Tokura, S.; Azuma, I. *Vaccine* **1984**, *2*, 93.

(2) Tanigawa, T.; Tanaka, Y.; Sashiwa, H.; Saimoto, H.; Shigemasa, Y. In *Advances in Chitin and Chitosan*; Brine, C. J., Sandford, P. A., Zikakis, J. P., Eds.; Elsevier: London, 1992; p 206.

(3) Uchida, Y. In *Chitin, Chitosan Handbook*; Japanese Society of Chitin and Chitosan, Ed.; Gihodo Co.: Japan, 1995; Chapter 10, p 302.

(4) Minami, S.; Okamoto, Y.; Matsuhashi, A.; Sashiwa, H.; Saimoto, H.; Shigemasa, Y.; Tanigawa, T.; Tanaka, Y.; Tokura, S. In *Advances in Chitin and Chitosan*; Brine, C. J., Sandford, P. A., Zikakis, J. P., Eds.; Elsevier: London, 1992; p 61.

(5) Sashiwa, H.; Saimoto, H.; Shigemasa, Y.; Ogawa, R.; Tokura, S. *Int. J. Biol. Macromol.* **1990**, *12*, 295. (b) Shigemasa, Y.; Saito, K.; Sashiwa, H.; Saimoto, H. *Int. J. Biol. Macromol.* **1994**, *16*, 43.

(6) Reuter, J. D.; Myc, A.; Hayes, M. M.; Gan, Z.; Roy, R.; Qin, D.; Yin, R.; Piehler, L. T.; Esfand, R.; Tomalia, D. A.; Baker, J. R., Jr. *Bioconjugate Chem.* **1999**, *10*, 271. (b) Zanini, D.; Roy, R. *J. Org. Chem.* **1996**, *61*, 7348. (c) Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2088. (d) Mammen, M.; Choi, S.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2754. (e) Roy, R. *Carbohydr. Eur.* **1999**, *27*, 34.

(7) Issberner, J.; Moors, R.; Vogtle, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2413. (b) Fréchet, J. M. J. *Science* **1994**, *263*, 1710 and references therein. (c) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665.

(8) Schlüter, A. D.; Rabe, J. P. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 864.

(9) Taber, D. F.; Amedio, J. C., Jr.; Jung, K. Y. *J. Org. Chem.* **1987**, *52*, 5621.

(10) Verez Bencomo, V.; Valdes, M. T. C.; Albernas, J. R. M.; Santana, V. F.; Rensoli, M. H.; Martinez, C. S. *Carbohydr. Res.* **1991**, *217*, 263.

(11) Meunier, M.; Wu, Q.; Wang, S. N.; Roy, R. *Can. J. Chem.* **1997**, *75*, 1472.

(12) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117.

(13) Roy, R.; Tropper, D. F.; Romanowska, A.; Letellier, M.; Cousineau, L.; Meunier, S. J.; Boratynski, J. *Glycoconjugate J.* **1991**, *8*, 75.

(14) To a solution of dendrimer **8c** ($G = 3$, 0.1 mmol, 0.8 mmol of $NH_2$) in MeOH (40 mL) was added **9** (2.4 mmol, 3 equiv/$NH_2$). After stirring at room temperature for 1 h, $NaCNBH_3$ (4.8 mmol) and AcOH (4.8 mmol) were added. After a day, the mixture was treated with saturated $Na_2CO_3$ and extracted with $CH_2Cl_2$, followed by drying to give sialodendrimer acetal **10c**. Data for **10c:** $^1$H NMR ($D_2O$) $\delta$ 1.89 (t, $J = 12.2$ Hz, H-3 ax of Neu5Ac), 2.12 (NHAc), 2.75−3.20 (br, C$H_2$ of dendrimer), 3.70−3.80 (m, C$H_2$C$H_2$O of spacer), 4.02−4.10 (m, 4 H, C$H_2$C$H_2$ of acetal), 5.14 (br, 1 H, C*H* of acetal), 6.95 and 7.35 (Ph); $^{13}$C NMR $\delta$ 25.0 (NHAc), 42.2 (C-3 of Neu5Ac), 55.0 (*C*H$_2$ of dendrimer), 62.8 (C$H_2$C$H_2$O of spacer), 64.6 (C$H_2$C$H_2$ of acetal), 101.1 (*C*H of acetal), 118.8 and 134.6 (Ph), 174.2 (*C*ONH).

(15) Acetal **10c** (0.1 mmol) was dissolved in $CF_3CO_2H$ (1.4 mL) and $H_2O$ (0.5 mL). After stirring at room temperature for a day, the mixture was evaporated and dried in vacuo to give aldehyde **11c**.

(16) Chitosan (NHAc = 0.2, DP = 140) was used in this study. Chitosan (200 mg: 0.94 mmol of $NH_2$) was dissolved in $H_2O$ (10 mL) and AcOH (1.0 mmol). To a solution was added aldehyde **11c** (0.1 mmol: 0.11 equiv/$NH_2$) and MeOH (30 mL). After stirring for 1 h, $NaCNBH_3$ (1.6 mmol) was added to the mixture and stirred for a day. The reaction was quenched by precipitation with saturated $Na_2CO_3$ (5 mL) and acetone (100 mL). The precipitate was collected by filtration, dispersed with 0.1 M NaOH (20 mL) at room temperature for 2 h, dialyzed, and lyophilized to give hybrid **12c** (220 mg). The degree of substitution (DS) was estimated from the ratio of phenyl proton at $\delta$ 6.95−7.35 ppm and H-1 proton of chitosan ($\delta$ 4.6−5.1 ppm). All compounds showed satisfactory NMR (Bruker AMX 500 MHz). Selected NMR data for **12c** (DS = 0.02): $^1$H NMR (0.2 M DCl/$D_2O$) $\delta$ 2.04 and 2.06 (NHAc of chitosan and Neu5Ac), 2.28 (H-3*eq* of Neu5Ac), 2.80 (−C$H_2$CONH− of dendrimer), 3.18 (H-2 of GlcN and NC$H_2$), 3.4−4.2 (ring proton of chitosan, Neu5Ac, and −C$H_2$O− of spacer), 4.60 (H-1 of GlcNAc), 5.1 (H-1 of GlcN and *N*-alkylated GlcN), 6.95 (H-*ortho* of Ph), 7.35 (H-*meta* of Ph); $^{13}$C NMR $\delta$ 25.0 (NHAc of chitosan and Neu5Ac), 42.1 (C-3 of Neu5Ac), 55.1 ($CH_2$ of dendrimer), 58.7 (C-2 of chitosan), 62.9 (C-6 of chitosan and $CH_2$O of spacer), 72.9 (C-3 of chitosan), 77.6 (C-5 of chitosan), 79.2 (C-4 of chitosan), 100.4 (C-1 of GlcN), 104.1 (C-1 of GlcNAc), 118.8 (Ph), 134.6 (Ph), 177.5 (NHCO).


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