ELSEVIER

Carbohydrate Polymers 49 (2002) 195–205

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Chemical modification of chitosan 11: chitosan–dendrimer hybrid as a tree like molecule

Hitoshi Sashiwa[a,1,*], Yoshihiro Shigemasa[b], Rene Roy[a]

[a]*Department of Chemistry, University of Ottawa, Ottawa, Ont., Canada K1N 6N5*
[b]*Faculty of Engineering, Tottori University, Tottori, 680-8552, Japan*

Received 6 February 2001; revised 6 August 2001; accepted 7 August 2001

Abstract

Tetraethylene glycol was modified by two different approaches to synthesize the scaffold of dendrimer. Poly(amido amine) (PAMAM) dendrimers ($G = 1$–3) having tetraethylene glycol spacer were prepared and attached to chitosan by reductive *N*-alkylation. On chitosan molecules, the degree of substitution of dendrimers was 0.03–0.18. Sialic acid residue bound PAMAM dendrimers of each generation were successfully attached to chitosan.

Keywords: Chitosan; Poly(amido amine); Dendrimer; Sialic acid; Reductive *N*-alkylation

1. Introduction

Chitosan is a polysaccharide formed primarily by repeating D-glucosamine and shows some biological activities like immunological (Nishimura, Nishimura, Nishi, Saiki, Tokura & Azuma, 1984), antibacterial (Tanigawa, Tanaka, Sashiwa, Saimoto & Shigemasa, 1992), and wound healing activity (Minami, Okamoto, Matsuhashi, Sashiwa, Saimoto, Shigemasa et al., 1992). Chitosan itself is a non-toxic and biodegradable polymer in animal body (Sashiwa, Saimoto, Shigemasa, Ogawa & Tokura, 1990; Shigemasa, Saito, Sashiwa & Saimoto, 1994). On the other hand, dendrimers are monodisperse high molecular weight macromolecules. They represent chemically well-defined structure and are prepared by divergent or convergent methods (Frechet, 1994). Many scientific efforts have gone into the design and synthesis of dendrimers over the last 10 years. Dendrimers offer a lot of possibilities in molecular design owing to their multifunctional properties such as medical applications, host–guest chemistry, dendritic catalysts etc (Bosman, Janssen & Meijer, 1999). In our approach for the chemical modification of chitosan, dendrimer is one of the attractive molecules because of its multifunctionality. Moreover, chitosan–dendrimer hybrid is a novel molecule like 'tree type molecule', which is capable of leading a variety of functional molecules like sugar, peptide, lipid, drug etc at the surface of dendrimer. In this tree type molecule based on chitosan, we imagine the trunk to be chitosan, main-branch the spacer, sub-branch the dendrimer, and leaf or flower some functional molecule. For the biomedical use of hybrid, ideally, all components are desirable to be non-toxic, biocompatible and biodegradable. Until now, we have reported the preparation of several kinds of hyperbranched chitosan–dendrimer hybrids which included tetraethylene glycol spacer, as a rapid communication (Sashiwa, Shigemasa & Roy, 2000, 2001a–c).

In this study, we describe the preparation of non-sugar bound chitosan–dendrimer hybrids and sialic acid bound hybrids.

2. Experimental section

2.1. Materials

Chitosan (Flonac C, NHAc = 0.2, degree of polymerization (DP) = 140) was purchased from Kyowa Tecnos Co., Japan. Tetra(ethylene glycol) and other reagents were purchased from Aldrich Co., and used without further purification. Dialysis membrane (MW 12,000 cut off) was purchased from Sigma Co.

* Corresponding author. Address: Department of Organic Materials, Functional Polymer Section, Osaka National Research Institute, 1-8-31 Midorigaoka, Ikeda City, Osaka 563-8577, Japan.

E-mail address: h-sashiwa@aist.go.jp (H. Sashiwa).

[1] Present address: National Institute of Advanced Industrial Science and Technology, 41ST, 1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan.

0144-8617/02/$ - see front matter
PII: S0144-8617(01)00322-8

2.2. General methods

The ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz AMX NMR spectrometer. Proton chemical shifts (δ) are given relative to internal $CHCl_3$ for $CDCl_3$ or 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (water soluble TMS: 0 ppm) for D_2O or 0.5 M DCl in D_2O solution. Carbon chemical shifts are also given relative to $CDCl_3$ or water soluble TMS (0 ppm). The degree of substitution (DS) of the hybrid was determined by ^1H NMR. The DP of chitosan was determined by GPC with pullulan as standard.

2.3. Preparation of 11-hydroxy-3,6,9-trioxadecanal acetal (*5*)

Compound **5** was prepared from tetraethylene glycol according to Bencomo, Valdes, Albernas, Santana, Rensoli and Martinez (1991). A typical procedure is as follows. To a solution of tetraethylene glycol (28.3 g: 146 mmol) in CH_2Cl_2 (40 ml) and triethylamine (18.18 g: 180 mmol), benzoyl chloride (20.53 g: 146 mmol: 1.0 equiv.) was added at 0°C. The mixture was stirred at 0°C for 30 min and rt for 1 day, washed with water (100 ml), dried and evaporated. The residue was suspended in toluene (50 ml) and hexane (100 ml), and extracted with EtOH/H_2O (1/2:150 ml). The aqueous layer was evaporated to ca. 100 ml and extracted with $CHCl_3$ to yield monobenzoate (**2**: yield, 38%). Data for **2**: ^1H NMR ($CDCl_3$) $\delta = 2.88$ (brs, 1 H, O*H*), 3.52 (t, $J = 4.84$ Hz, 2 H, CH_2CH$_2$OH), 3.57–3.66 (m, 10 H, OCH_2CH_2), 3.78 (t, $J = 4.84$ Hz, 2 H, CH_2CH$_2$OBz), 4.42 (t, $J = 4.84$ Hz, 2 H, CH_2OBz), 7.38 (t, $J = 7.40$, 2 H, H-3,5 of Ph), 7.50 (t, $J = 7.40$ Hz, 1 H, H-4 of Ph), 8.00 (m, 2 H, H-2,6 of Ph); ^{13}C NMR $\delta =$ 61.6 (*C*H$_2$OH), 64.0 (*C*H$_2$OBz), 69.2 (*C*H$_2$CH$_2$OBz), 70.3–70.7 (OCH_2CH_2O), 72.4 (*C*H$_2$CH$_2$OH), 128.3 (C-3,5 of Ph), 129.6 (C-2,6 of Ph), 130.1 (C-1 of Ph), 132.9 (C-4 of Ph), 166.5 (C=O); FAB-MS calcd for $C_{15}H_{22}O_6$ 298, found 299.1 ($M^+ + 1$, 27.5% base peak).

The oxidation of monobenzoate (**2**) was performed partly according to Taber, Amedio and Jung (1987). To a solution of **2** (15.6 g: 52 mmol) in CH_2Cl_2 (100 ml), dimethyl sulfoxide (100 mmol: 1.9 equiv.) and phosphorus pentaoxide (100 mmol: 1.9 equiv.) were added at 0°C. The mixture was stirred at 0°C for 30 min and at rt for 30 min. The flask was immersed in the ice-water bath; then, triethylamine (400 mmol: 7.7 equiv.) was added and stirred at 0°C for 30 min. The reaction was quenched with 10% aq. HCl and extracted with CH_2Cl_2. The organic extracts were washed with brine, evaporated to dryness, added to 10 ml of water and lyophilized to remove dimethyl sulfoxide. The aldehyde **3** was obtained in 90% yield. Data for **3**: ^1H NMR ($CDCl_3$) $\delta = 3.6$–3.7 (m, 8 H, OCH_2CH_2O), 3.79 (t, $J = 4.90$ Hz, 2 H, CH_2CH$_2$OBz), 4.08 (s, 2 H, CH_2CHO), 4.44 (t, $J = 4.90$ Hz, CH_2OBz), 7.39 (t, $J = 7.40$, 2 H, H-3,5 of Ph), 7.50 (t, $J = 7.40$ Hz, 1 H, H-4 of Ph), 8.00 (d, 2 H, H-2,6 of Ph), 9.65 (s, 1 H, C*H*O); ^{13}C NMR ($CDCl_3$) $\delta =$ 64.0 (*C*H$_2$OBz), 69.1 (*C*H$_2$CH$_2$OBz), 70.2–71.1 (OCH_2CH_2O), 76.7 (*C*H$_2$CHO), 128.3 (C-3,5 of Ph), 129.6 (C-2,6 of Ph), 130.0 (C-1 of Ph), 132.9 (C-4 of Ph), 166.4 (C=O), 200.8 (*C*HO); FAB-MS (pos) calcd for $C_{15}H_{20}O_6$ 296, found 297.1 ($M^+ + 1$, 0.8% base peak).

The aldehyde (**3**: 8.8 g: 30 mmol) was treated with *p*-toluenesulfonic acid (15 mmol: 0.5 equiv.) and ethylene glycol (42 mmol: 1.4 equiv.) in benzene (50 ml) and refluxed for 1 h. The mixture was then washed twice with sat. $NaHCO_3$, dried and evaporated to obtain acetal **4** in 94% yield. Data for **4**: ^1H NMR ($CDCl_3$) $\delta = 3.53$ (d, $J =$ 4.1 Hz, 2 H, OCH_2CH), 3.6–3.7 (m, 8 H, OCH_2CH_2O), 3.81 (t, $J = 4.85$ Hz, 2 H, CH_2CH$_2$OBz), 3.84–3.94 (m, 4 H, C$H_2$of acetal), 4.45 (t, $J = 4.84$ Hz, 2 H, CH_2OBz), 5.01 (t, $J = 4.10$ Hz, 1 H, C*H* of acetal), 7.41 (t, $J = 7.60$, 2 H, H-3,5 of Ph), 7.51 (t, $J = 7.60$ Hz, 1 H, H-4 of Ph), 8.02 (d, 2 H, H-2,6 of Ph); ^{13}C NMR ($CDCl_3$) $\delta = 64.1$ (*C*H$_2$OBz), 65.0 (CH_2 of acetal), 69.2 (*C*H$_2$CH$_2$OBz), 70.3–71.1 (OCH_2CH_2O), 72.0 (OCH_2CH), 102.6 (*C*H of acetal), 128.3 (C-3,5 of Ph), 129.6 (C-2,6 of Ph), 130.1 (C-1 of Ph), 132.9 (C-4 of Ph), 166.5 (C=O); FAB-MS (pos) calcd for $C_{17}H_{24}O_7$ 340, found 341.2 ($M^+ + 1$, 4.2% base peak).

Acetal **4** was debenzoated with 2 M NaOH in MeOH at rt, for 2 h. The mixture was extracted with $CHCl_3$, washed with brine and subjected to column with a gradient of CH_2Cl_2/MeOH (20/1–5/1) to give **5** in 95% yield. Data for **5**: ^1H NMR ($CDCl_3$) $\delta = 2.99$ (br, 1 H, O*H*), 3.45–3.50 (m, 4 H, OCH_2CH and CH_2CH$_2$OH), 3.52–3.63 (m, 10 H, OCH_2CH_2O), 3.75–3.90 (m, 4 H, C$H_2$of acetal), 4.95 (t, $J =$ 4.10 Hz, 1 H, C*H* of acetal); ^{13}C NMR ($CDCl_3$) $\delta = 61.5$ (*C*H$_2$OH), 64.9 (CH_2 of acetal), 70.2–71.0(OCH_2CH_2O), 71.8(OCH_2CH), 72.5 (*C*H$_2$CH$_2$OH), 102.5 (*C*H of acetal); FAB-MS (pos) calcd for $C_{10}H_{20}O_6$ 236, found 237.1 ($M^+ + 1$, 67.0% base peak).

2.4. Preparation of 11-amino-3,6,9-trioxadecanal acetal (*8*)

To a solution of **5** (1.4 g: 5.9 mmol) in CH_2Cl_2 (10 ml), triethylamine (12 mmol: 2.0 equiv.) and *p*-toluenesulfonyl chloride (TsCl: 9 mmol, 1.5 equiv.) were added. After stirring at rt for 1 day, the mixture was washed twice with aq. $NaHCO_3$. The organic layer was dried using anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was subjected to column chromatography and eluted with a gradient from hexane/EtOAc (1/1) to EtOAc to give tosylate **6** in 91% yield. Data for **6**: ^1H NMR ($CDCl_3$) $\delta = 2.40$ (s, 3 H, CH_3 of Ts, 3.51–3.65 (m, 12 H, OCH_2CH_2O), 3.82–3.96 (m, 4 H, CH_2 of acetal), 4.11 (t, $J = 4.80$ Hz, 2 H, CH_2OTs), 5.00 (t, $J = 4.06$ Hz, 1 H, C*H* of acetal), 7.30 (d, $J = 8.15$ Hz, H-3,5 of Ts), 7.76 (d, $J =$ 8.23 Hz, 2 H, H-2,6 of Ts); ^{13}C NMR ($CDCl_3$) $\delta = 21.5$ (*C*H$_3$ of Ts), 65.0 (C$H_2$of acetal), 68.6 (*C*H$_2$CH$_2$OTs), 69.2 (*C*H$_2$OTs), 70.5–71.1 (OCH_2CH_2O), 72.0 (OCH_2CH), 102.6 (*C*H of acetal), 127.9 (C-2,6 of Ts), 129.7 (C-3,5 of Ts),

133.0 (C-4 of Ts), 144.7 (C-1 of Ts); FAB-MS (pos) calcd for $C_{17}H_{26}O_8S_1$ 390, found 391.2 ($M^+ + 1$, 4.1% base peak).

To a solution of tosylate **6** (782 mg: 2 mmol) in 95% EtOH (20 ml), sodium azide (200 mg: 3 mmol: 1.5 equiv.) was added. The reaction was stirred under reflux for 5 h. After evaporation, the mixture was directly extracted with $CHCl_3$. The dried organic phase (Na_2SO_4) was concentrated and purified by column using a gradient from hexane/EtOAc (1/1) to EtOAc to give azide **7** in 90% yield. Data for **7**: ^{1}H NMR ($CDCl_3$) $\delta = 3.36$ (t, $J =$ 5.1 Hz, 2 H, CH_2N$_3$), 3.55 (d, $J = 4.10$ Hz, 2 H, CHCH_2), 3.61–3.69 (m, 10 H, OCH_2CH_2O), 3.84–3.97 (m, 4 H, C$H_2$of acetal), 5.03 (t, $J = 4.10$ Hz, 1 H, C*H* of acetal); ^{13}C NMR $\delta = 50.6$ (CH_2N$_3$), 65.0 (C$H_2$of acetal), 70.0–71.4 (OCH_2CH_2O), 72.0 (OCH_2CH), 102.6 (*C*H of acetal); FAB-MS (pos) calcd for $C_{10}H_{19}N_3O_5$ 261, found 261.8 ($M^+ + 1$, 17.3% base peak).

To a solution of azide **7** (480 mg: 1.84 mmol) in EtOH (20 ml), 450 mg of 10% palladium on carbon was added. Hydrogen was constantly bubbled through the solution at rt. After 2 h, the catalyst was removed by filtration and the filtrate was purified by column using a gradient from EtOAc to EtOAc/MeOH (10/1) to give amine (**8**) in 78% yield. Data for **8**: ^{1}H NMR ($CDCl_3$) $\delta = 2.12$ (brs, 2 H, NH_2), 2.85 (t, 2 H, $J = 5.00$ Hz, CH_2NH$_2$), 3.50 (t, $J =$ 5.00 Hz, 2 H, CH_2CH$_2$NH$_2$), 3.55 (d, $J = 4.10$ Hz, CHCH_2), 3.59–3.69 (m, 8 H, OCH_2CH_2O), 3.84–3.97 (m, 4 H, CH_2 of acetal), 5.02 (t, $J = 4.10$ Hz, 1 H, C*H* of acetal); ^{13}C NMR ($CDCl_3$) $\delta = 41.6$ (CH_2NH$_2$), 65.9 (CH_2 of acetal), 70.3–71.1 (OCH_2CH_2O), 72.0 (OCH_2CH), 73.0 (*C*H$_2$CH$_2$NH$_2$), 102.6 (*C*H of acetal); FAB-MS (pos) calcd for $C_{10}H_{21}N_1O_5$ 235, found 236.1 ($M^+ + 1$, 100% base peak).

2.5. Preparation of 11-(p-tolylsulfonyloxy)-3,6,9-trioxandecane-1-ol (**9**)

To a solution of tetraethylene glycol **1** (19.4 g:100 mmol) in $CHCl_3$ (30 ml) and triethylamine (120 mmol), TsCl (100 mmol: 1.0 equiv.) was added at 0°C The mixture was treated in a manner similar to the preparation of **2** and the crude product was purified by column using a gradient from hexane/EtOAc (1/1) to EtOAc to give monotosylate (**9**: $R_f = 0.3$ with ethyl acetate) in 40% yield. Ditosylate was also produced at the spot of $R_f = 0.7$ with EtOAc. Data for **9**: ^{1}H NMR ($CDCl_3$) $\delta = 2.40$ (s, 3 H, CH_3 of Ts), 2.47 (brs, 1 H, O*H*), 3.53–3.67 (m, 14 H, OCH_2CH_2O), 4.12 (t, $J =$ 4.90 Hz, 2 H, CH_2OTs), 7.29 (d, $J = 8.20$, 2 H, H-3,5 of Ts), 7.75 (d, $J = 8.20$ Hz, 2 H, H-2,6 of Ts); ^{13}C NMR ($CDCl_3$) $\delta = 21.5$ (CH_3 of Ts), 61.6 (*C*H$_2$OH), 68.6 (*C*H$_2$CH$_2$OTs), 69.2 (*C*H$_2$OTs), 70.3–70.6 (OCH_2CH_2O), 72.4 (*C*H$_2$CH$_2$OH), 127.9 (C-2,6 of Ts), 129.7 (C-3,5 of Ts), 133.0 (C-4 of Ts), 144.7 (C-1 of Ts); FAB-MS (pos) calcd for $C_{15}H_{24}O_7S_1$ 348, found 349.2 ($M^+ + 1$, 52.4% base peak).

2.6. Preparation of 11-(p-tolylsulfonyloxy)-3,6,9-trioxandecanal (**10**)

Oxidation of **9** was performed in a manner similar to that mentioned above. The crude product was extracted with diethyl ether to remove dimethyl sulfoxide. The compound **10** was obtained in quantitative yield. Data for **10**: ^{1}H NMR ($CDCl_3$) $\delta = 2.42$ (s, 3 H, CH_3 of Ts), 3.55–3.72 (m, 10 H, OCH_2CH_2O), 4.05–4.15 (m, 4 H, CH_2OTs and CH_2CHO), 7.31 (d, $J = 8.00$, 2 H, H-3,5 of Ts), 7.77 (d, $J = 8.00$ Hz, 2 H, H-2,6 of Ts), 9.69 (s, 1H, C*H*O); ^{13}C NMR ($CDCl_3$) $\delta =$ 21.6 (CH_3 of Ts), 68.7 (*C*H$_2$CH$_2$OTs), 69.2 (*C*H$_2$OTs), 70.3–70.7 (OCH_2CH_2O), 76.8 (*C*H$_2$CHO), 127.9 (C-2, 6 of Ts), 129.7 (C-3,5 of Ts), 133.0 (C-4 of Ts), 144.7 (C-1 of Ts), 200.8 (*C*HO). The compounds **6**, **7** and **8** were prepared from **10** according to the above mentioned procedure.

2.7. Preparation of methyl ester (**11**) ($G = 0.5$)

A typical procedure is as follows. To a solution of amine (**8**: 3 mmol) in MeOH (30 ml) was added methyl acrylate (9 mmol: 3.0 equiv.). The mixture was stirred at 40°C for 5 days. After 5 days, the mixture was evaporated and purified by column using a gradient of CH_2Cl_2/MeOH from 20/1 to 5/1 in the presence of triethylamine to give methyl ester **11** in 70% yield. Data for **11**: ^{1}H NMR ($CDCl_3$) $\delta = 2.43$ (t, $J = 7.24$ Hz, 4 H, CH_2COOMe), 2.65 (t, $J = 6.20$ Hz, 2 H, OCH$_2$CH_2N), 2.80 (t, $J = 7.24$ Hz, 4 H, NCH_2), 3.50 (t, $J =$ 6.20 Hz, 2 H, OCH_2CH$_2$N), 3.55 (d, $J = 4.10$ Hz, 2 H, CHCH_2), 3.64 (s, 6 H, COO*Me*), 3.56–3.70 (m, 8 H, OCH_2), 3.87–3.96 (m, 4 H, C$H_2$of acetal), 5.03 (t, $J =$ 4.10 Hz, 1 H, C*H* of acetal); ^{13}C NMR ($CDCl_3$) $\delta = 32.6$ (*C*H$_2$CO), 49.9 (NCH_2), 51.5 (COO*Me*), 53.2 (OCH$_2$CH_2N), 65.0 (C$H_2$of acetal), 69.6 (OCH_2CH$_2$N), 70.3–71.9 (OCH_2CH_2O), 72.0 (OCH_2CH), 102.7 (*C*H of acetal), 172.9 (*C*OOMe); FAB-MS (pos) calcd for $C_{18}H_{33}N_1O_9$ 407, found 408.1 ($M^+ + 1$, 81% base peak).

2.8. Preparation of diamine (**12**)

To a solution of methyl ester (**11**:1.15 mmol) in MeOH, ethylenediamine (23 mmol: 20 equiv.) was added and the mixture was stirred at rt for 3 days. The mixture was evaporated and then dried in vacuo to obtain diamine **12** ($G = 1$). Data for **12**: ^{1}H NMR ($CDCl_3$) $\delta = 2.00$ (brs, 4 H, NH_2), 2.32 (t, $J = 6.20$ Hz, 4 H, CH_2(b)CONH), 2.59 (t, $J =$ 5.14 Hz, 2 H, OCH$_2$CH_2N), 2.71 (t, $J = 6.20$ Hz, 4 H, NCH_2(a)), 2.76 (t, $J = 5.9$ Hz, 4 H, CONHCH_2(c)), 3.23 (t, $J = 5.9$ Hz, 4 H, CH_2(d)NH$_2$), 3.45–3.65 (m, 12 H, OCH_2CH_2O), 3.83–3.92 (m, 4 H, CH_2 of acetal), 4.97 (t, $J = 4.10$ Hz, 1 H, C*H* of acetal), 7.57 (br, 2 H, CON*H*); ^{13}C NMR ($CDCl_3$) $\delta = 34.3$ (*C*H$_2$(b)CONH), 40.8 (*C*H$_2$(d)NH), 41.8 (CONH*C*H$_2$(c)), 51.0 (NCH_2(a)), 53.8 (OCH$_2$CH_2N), 65.0 (CH_2CH_2), 69.2 (OCH_2CH$_2$N), 70.1–71.0 (OCH_2CH_2O), 71.9 (OCH_2CH), 102.4 (*C*H of acetal), 172.9 (*C*ONH); FAB-MS (pos) calcd for $C_{20}H_{41}N_5O_7$ 463,

found 463.5 ($M^+ + 1$, 21.4% base peak). (a)–(d): $OCH_2CH_2N(CH_2(a)CH_2(b)CONHCH_2(c)CH_2(d)NH_2)_2$.

2.9. Preparation of dendrimers

The preparation of dendrimers of methyl esters **13**, **15** and amines **14**, **16** were prepared as mentioned above. The yield of **13** and **15** was 73 and 57%, respectively. The amines **14** and **16** were obtained in quantitative yields. Data for **13**: ^{1}H NMR ($CDCl_3$) $\delta = 2.43$ (t, $J = 7.24$ Hz, 4 H, NCH_2), 2.65 (t, $J = 6.20$ Hz, 2 H, OCH_2CH_2N), 2.23 (br, 4 H, NCH_2(a)), 2.30 (br, 8 H, NCH_2(e)), 2.38 (br, 4 H, CH_2(d)N), 2.62 (br, 10 H, OCH_2CH_2N (2 H), and CH_2(f)COOMe (8 H)), 2.70 (br, 4 H, CH_2(b)CONH), 3.14 (br, 4 H, $CONHCH_2$(c)), 3.4–3.6 (m, 20 H, OCH_2CH_2O (8 H) and COO*Me* (12 H)), 3.74–3.84 (m, 4 H, $CH_2CH_2$of acetal), 4.88 (t, $J = 4.10$ Hz, 1 H, C*H* of acetal), 7.10 (br, 2 H, CON*H*); ^{13}C NMR $\delta = 32.4$ (CH_2(f)COOMe), 33.3 (CH_2(b)CONH), 36.8 ($CONHCH_2$(c)), 49.0 (NCH_2(e)), 49.9 (NCH_2(a)), 51.3 (COO*Me*), 52.4 (OCH_2CH_2N), 52.7 (CH_2(d)N), 64.7 (CH_2CH_2), 68.8 (OCH_2CH_2N), 70.0–70.8 (OCH_2CH_2O), 71.7 (OCH_2CH), 102.4 (*C*H of acetal), 171.9 (*C*ONH), 172.7 (COOMe); FAB-MS (pos) calcd for C36H65N5O15 807, found 807.7 ($M^+ + 1$, 57.4% base peak).

(a)–(f): $OCH_2CH_2N(CH_2(a)CH_2(b)CONHCH_2(c)CH_2(d)N(CH_2(e)CH_2(f)CO_2Me)_2)_2$

Data for **14**. ^{1}H NMR ($CDCl_3$) $\delta = 2.33$ (br, 12 H, NCH_2 (a)(4 H) and (e) (8 H)), 2.49 (br, 2 H, OCH_2CH_2N), 2.73 (br, 12 H, CH_2CONH (b)(4 H) and (f)(8 H)), 2.81 (br, 12 H, $CONHCH_2$ (c)(4 H) and (g)(8 H)), 3.04 (br, 8 H, CH_2(h)NH_2), 3.18 (br, 4 H, CH_2N(d)), 3.45–3.65 (m, 12 H, OCH_2CH_2O), 3.83–3.92 (m, 4 H, $CH_2CH_2$of acetal), 4.98 (t, $J = 4.10$ Hz, 1 H, C*H* of acetal), 7.93 (br, 4 H, CON*H(2)*), 7.98 (br, 2 H, CON*H(1)*); ^{13}C NMR $\delta = 33.7$ (CH_2(b)CO), 34.2 (CH_2(f)CO), 40.9 ($CONHCH_2$(c)), 41.2 (CH_2(h)NH_2), 44.2 ($CONHCH_2$(g)), 46.3 (CH_2N (d)), 50.2(NCH_2(e)), 50.4(NCH_2(a)), 52.7 (OCH_2CH_2N), 65.0 (CH_2CH_2), 68.8 (OCH_2CH_2N), 70.2–71.0 (OCH_2CH_2O), 71.9 (OCH_2CH), 102.5 (*C*H of acetal), 172.8 (*C*ONH(1)), 173.2 (*C*ONH(2)); FAB-MS (pos) calcd for $C_{40}H_{81}N_{13}O_{11}$ 919, found 920.6 ($M^+ + 1$, 4.1% base peak).

(e)–(h) and 1, 2: $N(CH_2(e)CH_2(f)CONH(2)\ CH_2(g)CH_2(h)NH_2)_2)_2$

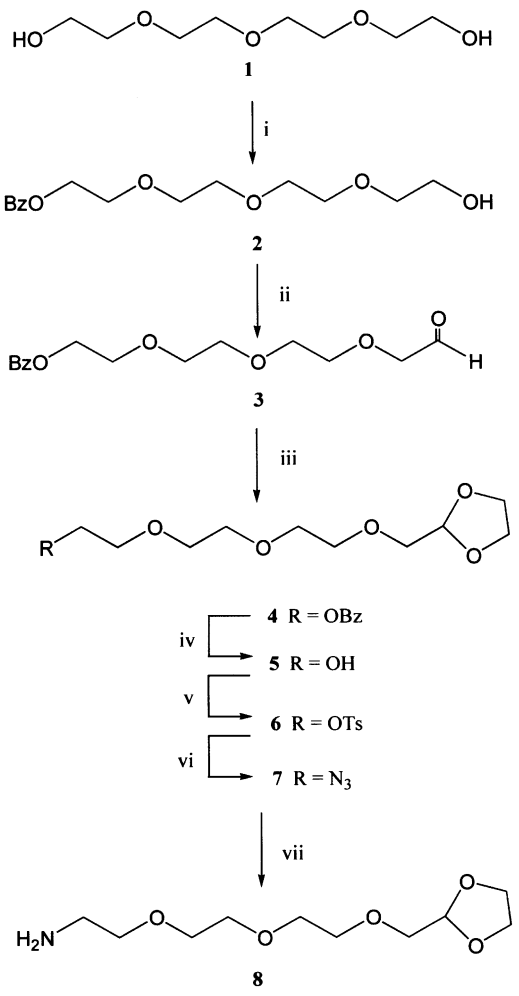
Data for **15**. ^{1}H NMR ($CDCl_3$) $\delta = 2.41$ (t, $J = 6.7$ Hz, 28 H, NCH_2(a, e, i)), 2.53 (t, $J = 6.0$ Hz, 12 H, CH_2N(d, h)), 2.61 (br, 2 H, OCH_2CH_2N), 2.74 (t, $J = 6.7$ Hz, 28 H, CH_2CONH(b, f) and CH_2(j)COOMe), 3.25 (m, 12 H, $CONHCH_2$(c, g)), 3.53 (d, $J = 4.0$ Hz, 2 H, $CHCH_2O$), 3.55–3.63 (m, 10 H, OCH_2CH_2O), 3.65 (s, 24 H, COO*Me*), 3.85 and 3.95 (m, 4 H, $CH_2CH_2$of acetal), 5.01 (br, 1 H, C*H* of acetal), 7.00 (br, 4 H, CON*H*(2)), 7.70 (br, 2 H, CON*H*(1)); ^{13}C NMR $\delta = 32.6$ (CH_2(j)COOMe), 33.6 (CH_2(b,f)CONH), 37.2 ($CONHCH_2$(c, g)), 49.2 (CH_2N(d, h)), 49.9 (NCH_2(a, e)), 50.2 (NCH_2(i)), 51.6 (COO*Me*), 52.6 (OCH_2CH_2N), 65.0 (CH_2CH_2), 70.3 (OCH_2CH_2N), 70.4–71.1 (OCH_2CH_2O), 72.0 (OCH_2CH), 102.6 (*C*H of acetal), 172.1 (*C*ONH), 173.0 (*C*OOMe); FAB-MS (pos) calcd for $C_{72}H_{129}N_{13}O_{27}$ 1607, found 1607.1 (M^+, 9.0% base peak).

(e)–(j) and 1, 2: $N(CH_2(e)\ CH_2(f)CONH(2)CH_2(g)CH_2(h)N(CH_2(i)CH_2(j)COOMe)_2)_2)_2$

Data for **16**. ^{1}H NMR (D_2O) $\delta = 2.50$ (br, 30 H, NCH_2 a (4 H), e (8 H), i (16 H), and OCH_2CH_2N (2 H)), 2.78 (br, 28 H, CH_2CONH b (4 H), f (8 H), and j (16 H), 2.89 (br, 28 H, $CONHCH_2$ c (4 H), g (8 H), and k (16 H)), 3.30 (br, 16 H, CH_2(l)NH_2), 3.36 (br, 12 H, CH_2N d (4 H) and h (8 H)), 3.70–3.80 (m, 12 H, OCH_2CH_2O), 4.02–4.10 (m, 4 H, $CH_2$of acetal), 5.14 (br, 1 H, C*H* of acetal); ^{13}C NMR $\delta = 31.9$ (CH_2(b)CONH), 32.1 (CH_2(f)CONH), 32.4 (CH_2(j)CONH), 35.1 ($CONHCH_2$(g)), 36.4 ($CONHCH_2$(k)), 39.4 ($CONHCH_2$(c)), 41.2 (CH_2(h)NH_2), 41.8 (CH_2N(d)), 44.0 (CH_2N(h)), 48.7 (NCH_2(a)), 49.6 (NCH_2(e)), 50.9 (NCH_2(i)), 52.9 (OCH_2CH_2N), 64.5 (CH_2CH_2), 69.1 (OCH_2CH_2N), 70.2 (OCH_2CH), 72.3 (OCH_2CH_2O), 101.1 (*C*H of acetal), 174.2 (*C*ONH(1 and 2)), 174.6 (*C*ONH(3))). (i)–(l) and 3: $-((N(CH_2(i)CH_2(j)CONH(3)CH_2(k)CH_2(l)NH_2)_2)_2)_2$.

2.10. Removal of acetal and the preparation of chitosan–dendrimer hybrid

The removal of acetal from compound **11** was performed as follows. Compound **11** (149 mg: 0.37 mmol) was dissolved in trifluoroacetic acid (2.0 g) and water (1.0 g). After stirring at rt for 1 day, the mixture was evaporated and dried in vacuum to give aldehyde. Chitosan (110 mg: 0.52 mmol of NH_2) was dissolved in water (10 ml) containing AcOH (30 mg). To the solution was added **17** (0.37 mmol: 0.7 equiv./NH_2), which dissolved in a mixed solvent of water (2 ml) and MeOH (8 ml). The mixture was diluted with MeOH (30 ml) and stirred. After 1 h, $NaCNBH_3$ (1.6 mmol: 4.3 equiv./**17**) was added to the mixture and stirred continuously for 1 day. The reaction was stopped by precipitation with sat. Na_2CO_3 (5 ml) and acetone (100 ml). The precipitate was collected by filtration, dispersed with water containing sat. Na_2CO_3 (5 ml), dialyzed for 2 days, and lyophilized to give chitosan–dendrimer hybrid **20** (110 mg: 100% of recovery). Hybrid **21** and **22** were also obtained in the same manner and ca. 100% of recovery. Data for **20** ($G = 1$, DS = 0.14): ^{1}H NMR (0.5 M DCl/D_2O) $\delta = 2.06$ (s, 0.6 H, NHAc), 2.87 (br, 0.28 H, NCH_2(1)), 2.98 (t, $J = 6.0$ Hz, 0.56 H, NCH_2(a)), 3.19 (br, H-2 of GlcN), 3.34 (br, H-2 of GlcN-R), 3.39 (br, CH_2(2)), 3.51 (br, 0.28 H, CH_2 (8)), 3.58 (t, $J = 6.0$ Hz, 0.56 H, CH_2(b)COOH), 3.6–4.0 (m, H-2 of GlcNAc, H-3,4,5,6 of chitosan, OCH_2 (3,4,5,6,7)), 4.59 (br, H-1 of GlcNAc), 4.89 (br, 0.66 H, H-1 of GlcN), 5.07 (br, H-1 of GlcN-R); ^{13}C NMR $\delta = 25.0$ (NHAc), 31.1 (CH_2(b)COOH), 50.0 (CH_2(2)), 53.0 (NCH_2(1, a)), 56.8 (CH_2N(8)), 58.7 (C-2 of chitosan), 63.2 (C-6), 66.7 (CH_2(7)), 72.0–73.0 (C-3, OCH_2 (3,4,5,6,)), 77.6 (C-5), 79.3 (C-4 of GlcN and GlcN–R)), 81.4 (C-4 of NHAc),



Scheme 1. Reagents and conditions: i, BzCl, Et_3N, CH_2Cl_2, 38%; ii, P_2O_5, DMSO CH_2Cl_2, 90%; iii, ethylene glycol, *p*TsOH, C_6H_6, 94%; iv, 2 M NaOH/MeOH, 95% v, TsCl, Et_3N, CH_2Cl_2, 91%; vi, NaN_3, EtOH, 90%; vii, H_2, 10% Pd/C, EtOH, 78%.

99.4 (C-1 of GlcN-R), 100.4 (C-1 of GlcN), 104.1 (C-1 of GlcNAc), 177.0 (*C*OOH), 177.5 (NHAc). 1-8 and a,b: GlcN–CH_2(1)CH_2(2)OCH_2(3)CH_2(4)OCH_2(5)CH_2(6)–OCH_2(7)CH_2(8)N(CH_2(a)CH_2(b)CO_2H)$_2$

Data for **21** ($G = 2$, DS = 0.10). ^{1}H NMR (0.5 M DCl/D_2O) $\delta = 2.06$ (s, 0.6 H, NHAc), 2.87 (br, 0.6 H, N*C*H_2(1, a)), 2.98 (t, $J = 6.0$ Hz, 0.8 H, N*C*H_2(e)), 3.19 (br, 0.7 H, H-2 of GlcN), 3.34 (br, H-2 of GlcN–R), 3.57 (t, $J = 6.0$ Hz, 0.8 H, C*H*$_2$(f)COOH), 3.6–4.0 (m, H-2 of GlcNAc, H-3,4,5,6 of chitosan, OC*H*$_2$ (3,4,5,6,7)), 4.59 (br, 0.2 H, H-1 of GlcNAc), 4.89 (br, 0.7 H, H-1 of GlcN); ^{13}C NMR $\delta =$ 25.0 (NHAc), 31.0 (*C*H_2(f)COOH), 31.5(*C*H_2(b)CONH), 37.5 (CONH*C*H_2(c)), 52.6 (N*C*H_2(a, e)), 55.9 (*C*H_2N(8, d)), 58.7 (C-2 of chitosan), 62.9 (C-6), 72.5–72.9 (C-3, OC*H*$_2$ (3,4,5,6,)), 77.6 (C-5), 79.3 (C-4 of GlcN, GlcN–R)), 81.4 (C-4 of NHAc), 100.0 (C-1 of GlcN), 104.1 (C-1 of GlcNAc), 175.9 (NH*C*O), 176.9 (*C*OOH), 177.5 (NHAc).

(a)–(f): N(CH_2(a)CH_2(b)CONHCH_2(c)CH_2(d)N(CH_2(e)CH_2(f)CO_2H)$_2$)$_2$, 1–8 see compound **20**.

Data for **22** ($G = 3$, DS = 0.13). ^{1}H NMR (0.5 M DCl/D_2O) $\delta = 2.05$ (s, 0.6 H, NHAc), 2.87 (t, $J = 6.4$ Hz, 1.82 H, N*C*H_2(1, a, e)), 2.96 (t, $J = 6.4$ Hz, 2.08 H, N*C*H_2(i)), 3.18 (br,, 0.67 H, H-2 of GlcN), 3.34 (br, H-2 of GlcN–R), 3.46 (m, 1.56 H, C*H*$_2$N(d, h)), 3.56 (t, $J = 6.4$ Hz, 2.08 H, C*H*$_2$COOH), 3.6–4.0 (m, H-2 of GlcNAc, H-3,4,5,6 of chitosan, OC*H*$_2$ (3,4,5,6,7)), 4.68 (br, 0.2 H, H-1 of GlcNAc), 4.89 (br, 0.67 H, H-1 of GlcN); ^{13}C NMR $\delta = 25.2$ (NHAc), 31.1 (*C*H_2(j)COOH), 37.5 (*C*H_2CONH (c, g)), 52.6 (N*C*H_2(1,a,e,i)), 55.9 (*C*H_2N(8,d,h)), 58.7 (C-2 of chitosan), 62.9 (C-6), 72.6–72.9 (C-3, OC*H*$_2$ (3,4,5,6,)), 77.6 (C-5), 79.3 (C-4 of GlcN, GlcN–R)), 100.4 (C-1 of GlcN), 104.1 (C-1 of GlcNAc), 175.9 (NH*C*O), 177.0 (*C*OOH). g–j: N((–CONHCH_2(g)CH_2(h)N (CH_2(i)CH_2(j)CO_2H)$_2$)$_2$)$_2$, 1–8, (a)–(f) see compounds **20**, **21**.

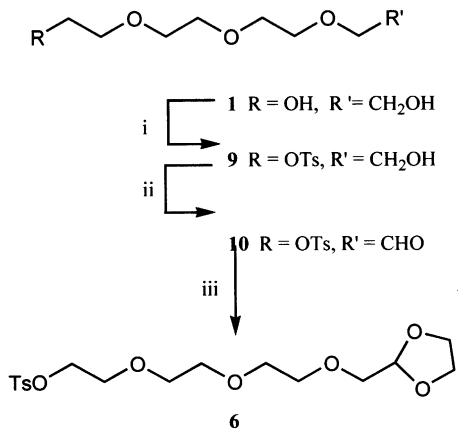
2.11. Preparation of sialic acid bound dendrimer (G = 1 and 3)

To a solution of dendrimer 16 ($G = 3$, 0.1 mmol, 0.8 mmol of NH_2) in MeOH (20 ml), *p*-formylphenyl-α-sialoside **23** (2.4 mmol, 3.0 equiv./NH_2) in MeOH (10 ml) was added. After stirring at rt for 1 h, $NaCNBH_3$ (2.4 mmol) was added and continuously stirred at rt for 1 day. The mixture was evaporated and crude **26** was obtained. Acetal was removed from compound **26** and attached to chitosan (200 mg) as described above. The mixture was continuously treated with 0.5 M NaOH, dialyzed, lyophilized and compound **32** was obtained ($G = 3$ in a good yield (210 mg). Succinylation of compounds **30**–**32** was performed according to the previous literature (Sashiwa et al., 2001a).

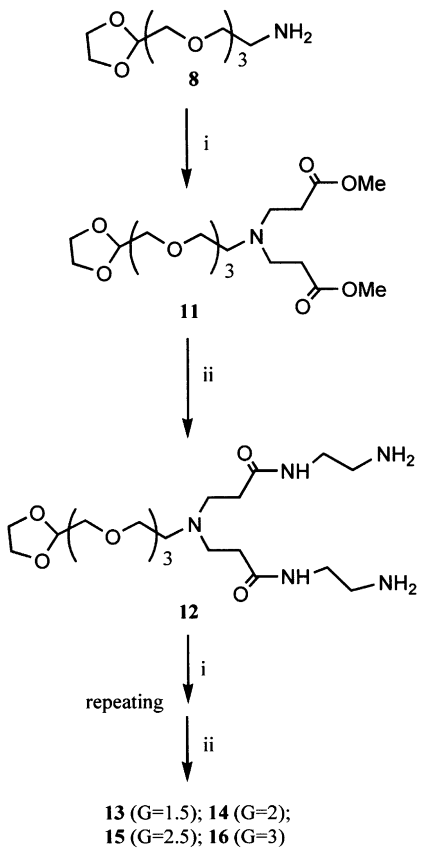
Data for **24**. ^{1}H NMR ($CDCl_3$) $\delta = 1.88$ (t, 3 H, $J =$ 12.2 Hz, H-3ax of Neu5Ac), 2.00–2.10 (m, NHAc and OAc), 2.75–3.20 (br, C*H*$_2$ of dendrimer), 3.70–3.80 (m, OC*H*$_2$C*H*$_2$O of spacer), 4.02–4.10 (m, 4 H, C*H*$_2$ of acetal), 5.15 (br, 1 H, C*H* of acetal), 6.95 and 7.35 (br, 12 H, Ph); ^{13}C NMR $\delta = 25.2$ (NHAc), 42.0 (C-3 of Neu5Ac), 55.0 (*C*H_2 of dendrimer), 62.6 (O*C*H_2*C*H_2O of spacer), 64.5 (*C*H_2 of acetal), 101.0 (*C*H of acetal), 118.5 and 134.5 (Ph), 174.4 (*C*ONH).

Data for **25**. ^{1}H NMR ($CDCl_3$) $\delta = 1.86$ (t, 5 H, $J =$ 12.2 Hz, H-3ax of Neu5Ac), 2.00–2.13 (m, NHAc and OAc), 2.75–3.20 (br, C*H*$_2$ of dendrimer), 3.70–3.80 (m, OC*H*$_2$C*H*$_2$O of spacer), 4.00–4.10 (m, 4 H, C*H*$_2$ of acetal), 5.16 (br, 1 H, C*H* of acetal), 6.96 and 7.33 (br, 20 H, Ph); ^{13}C NMR $\delta = 25.1$ (NHAc), 42.1 (C-3 of Neu5Ac), 55.3 (*C*H_2 of dendrimer), 62.4 (O*C*H_2*C*H_2O of spacer), 64.5 (*C*H_2 of acetal), 101.2 (*C*H of acetal), 118.5 and 134.5 (Ph), 174.5 (*C*ONH).

Data for **26**. ^{1}H NMR ($CDCl_3$) $\delta = 1.89$ (t, 10 H, $J =$ 12.2 Hz, H-3ax of Neu5Ac), 2.01–2.12 (m, NHAc and OAc), 2.75–3.20 (br, C*H*$_2$ of dendrimer), 3.70–3.80 (m, OC*H*$_2$C*H*$_2$O of spacer), 4.02–4.10 (m, 4 H, C*H*$_2$ of acetal), 5.14 (br, 1 H, C*H* of acetal), 6.95 and 7.35 (br, 40 H, Ph); ^{13}C NMR $\delta = 25.0$ (NHAc), 42.2 (C-3 of Neu5Ac), 55.0 (*C*H_2 of dendrimer), 62.8 (O*C*H_2*C*H_2O of spacer), 64.6 (*C*H_2



Scheme 2. Reagents and conditions: i, TsCl, Et_3N, CH_2Cl_2, 40%; ii, P_2O_5, DMSO, CH_2Cl_2, 92%, iii, ethylene glycol, *p*TsOH, C_6H_6, 89%.



Scheme 3. Reagents and conditions: i, $H_2C{=}CHCO_2MeOH$, 45°C, 5 days; ii, ethylenediamine, MeOH, rt, 3 days.

of acetal), 101.1 (*C*H of acetal), 118.8 and 134.6 (Ph), 174.2 (*C*ONH).

Data for **30** ($G = 1$, DS = 0.08). ^{1}H NMR (0.5 M DCl/D_2O) $\delta = 1.91$ (t, $J = 12.4$ Hz, 0.24 H, H-3ax of Neu5Ac), 2.09 and 2.10 (d, NHAc of chitosan and Neu5Ac), 2.35 (dd, $J = 5.0$ Hz, 0.24 H, H-3eq of Neu5Ac), 2.90 (br, N*C*H_2), 3.22 (br, H-2 of GlcN and CH_2CO), 3.46 (br, CH_2), 3.6–4.0 (m, H-2 of GlcNAc, H-3,4,5,6 of chitosan and H-4,7,8,9 of Neu5Ac), 4.09 (d, $J = 10.2$ Hz, H-5 of Neu5Ac), 4.60 (br, H-1 of GlcNAc), 4.91 (br, H-1 of GlcN), 7.00 (d, $J =$ 8.5 Hz, 0.48 H, H-*ortho* of C_6H_4–O), 7.41 (d, $J = 8.5$ Hz, 0.48 H, H-*meta* of C_6H_4–O); ^{13}C NMR $\delta = 25.1$ (NHAc), 31.7 (NHCO*C*H_2), 37.2 (CONH*C*H_2), 41.7 (C-3 of Neu5Ac), 52.7 (N*C*H_2), 53.5 (*C*H_2Ph), 55.0 (*C*H_2N and C-5 of Neu5Ac), 58.6 (C-2 of chitosan), 63.0 (C-6 of chitosan and C-9 of Neu5Ac), 66.1 (C-7 of Neu5Ac), 69.5 (C-4 of Neu5Ac), 71.2 (C-8 of Neu5Ac), 73.1 (C-3 of chitosan and C-6 of Neu5Ac), 77.7 (C-5 of chitosan), 79.4 (C-4 of GlcN), 81.5 (C-4 of NHAc), 100.1 (C-2 of Neu5Ac),100.4 (C-1 of GlcN), 104.1 (C-1 of GlcNAc), 118.9 (C-*ortho*), 134.8 (C-*para*, C-*meta*), 153.6 (C-*ipso*), 177.6 (NHCO).

Data for **31** ($G = 2$, DS = 0.04). ^{1}H NMR (0.5 M DCl/D_2O) $\delta = 1.90$ (t, $J = 12.4$ Hz, 0.20 H, H-3ax of Neu5Ac), 2.09 and 2.11 (d, NHAc of chitosan and Neu5Ac), 2.33 (dd, $J = 5.0$ Hz, 0.20 H, H-3eq of Neu5Ac), 2.90 (br, N*C*H_2), 3.20 (br, H-2 of GlcN and CH_2CO), 3.48 (br, CH_2), 3.6–4.0 (m, H-2 of GlcNAc, H-3,4,5,6 of chitosan and H-4,7,8,9 of Neu5Ac), 4.10 (d, $J = 10.2$ Hz, H-5 of Neu5Ac), 4.60 (br, H-1 of GlcNAc), 4.90 (br, H-1 of GlcN), 7.02 (d, $J =$ 8.5 Hz, 0.48 H, H-*ortho* of C_6H_4–O), 7.41 (d, $J = 8.5$ Hz, 0.48 H, H-*meta* of C_6H_4–O); ^{13}C NMR $\delta = 25.0$ (NHAc), 31.5 (NHCO*C*H_2), 37.2 (CONH*C*H_2), 41.5 (C-3 of Neu5Ac), 52.7 (N*C*H_2), 53.5 (*C*H_2Ph), 55.0 (*C*H_2N and C-5 of Neu5Ac), 58.5 (C-2 of chitosan), 63.0 (C-6 of chitosan and C-9 of Neu5Ac), 66.0 (C-7 of Neu5Ac), 69.5 (C-4 of Neu5Ac), 71.0 (C-8 of Neu5Ac), 73.0 (C-3 of chitosan and C-6 of Neu5Ac), 77.7 (C-5 of chitosan), 79.5 (C-4 of GlcN), 81.5 (C-4 of NHAc), 100.0 (C-2 of Neu5Ac),100.5 (C-1 of GlcN), 104.0 (C-1 of GlcNAc), 118.8 (C-*ortho*), 134.8 (C-*para*, C-*meta*), 153.5 (C-*ipso*), 177.5 (NHCO).

Data for **32** ($G = 3$, DS = 0.02). ^{1}H NMR (0.5 M DCl/D_2O) $\delta = 1.91$ (t, $J = 12.4$ Hz, 0.2 H, H-3ax of Neu5Ac), 2.09 and 2.10 (d, NHAc of chitosan and Neu5Ac), 2.35 (dd, $J = 5.0$ Hz, 0.2 H, H-3eq of Neu5Ac), 2.91 (br, 0.44 H, N*C*H_2), 3.22 (br, 1.08 H, H-2 of GlcN and CH_2CO), 3.46 (br, 0.44 H, CH_2), 3.6–4.0 (m, H-2 of GlcNAc, H-3,4,5,6 of chitosan and H-4,7,8,9 of Neu5Ac), 4.09 (d, $J = 10.2$ Hz, H-5 of Neu5Ac), 4.41 (dd, $J = 7.0$ Hz, H-6 of Neu5Ac), 4.60 (br, 0.2 H, H-1 of GlcNAc), 4.91 (d, $J = 7.7$ Hz, 0.8 H, H-1 of GlcN), 7.00 (d, $J = 8.5$ Hz, 0.4 H, H-*ortho* of C_6H_4–O), 7.41 (d, $J = 8.5$ Hz, 0.4 H, H-*meta* of C_6H_4–O); ^{13}C NMR $\delta = 25.1$ (NHAc of chitosan and Neu5Ac), 31.7 (NHCO*C*H_2), 37.2 (CONH*C*H_2), 41.7 (C-3 of Neu5Ac), 52.7 (N*C*H_2), 53.5 (*C*$H_2C_6H_4$), 55.0 (*C*H_2N and C-5 of Neu5Ac), 58.6 (C-2 of chitosan), 63.0 (C-6 of chitosan and C-9 of Neu5Ac), 66.1 (C-7 of Neu5Ac), 69.5 (C-4 of Neu5Ac), 71.2 (C-8 of Neu5Ac), 73.1 (C-3 of chitosan and C-6 of Neu5Ac), 77.7 (C-5 of chitosan), 79.4 (C-4 of GlcN), 81.5 (C-4 of NHAc), 100.1 (C-2 of Neu5Ac), 100.4 (C-1 of GlcN), 104.1 (C-1 of GlcNAc), 118.9 (C-*ortho*), 134.8 (C-*para*, C-*meta*), 153.6 (C-*ipso*), 176.0 (NH*C*O), 177.6 (NHAc).

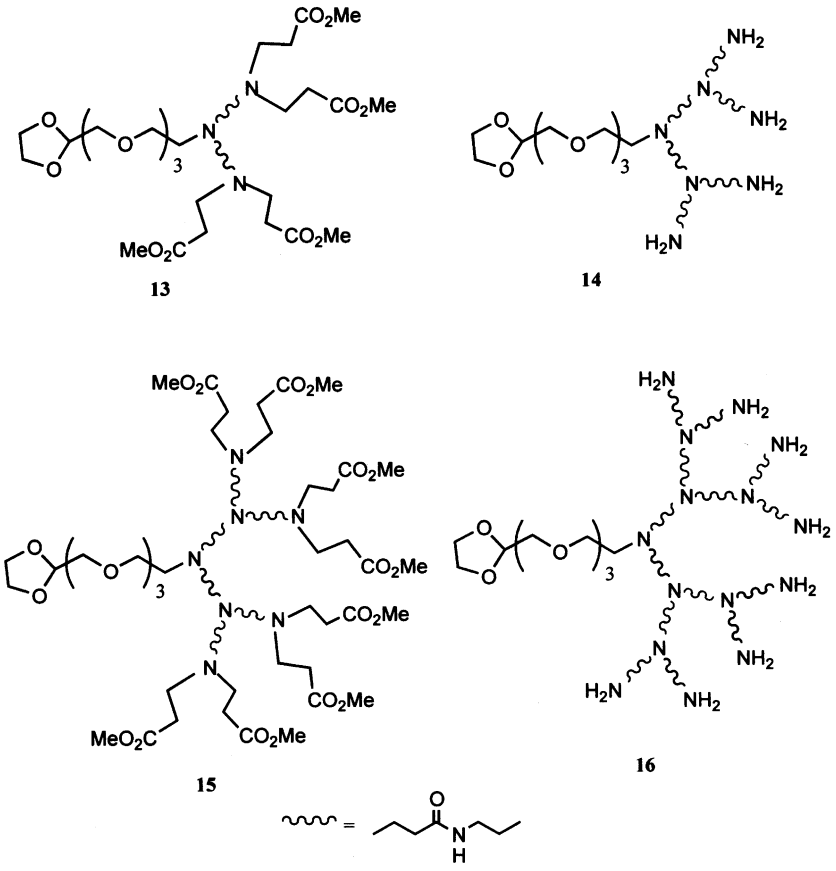


Chart 1.

3. Results and discussion

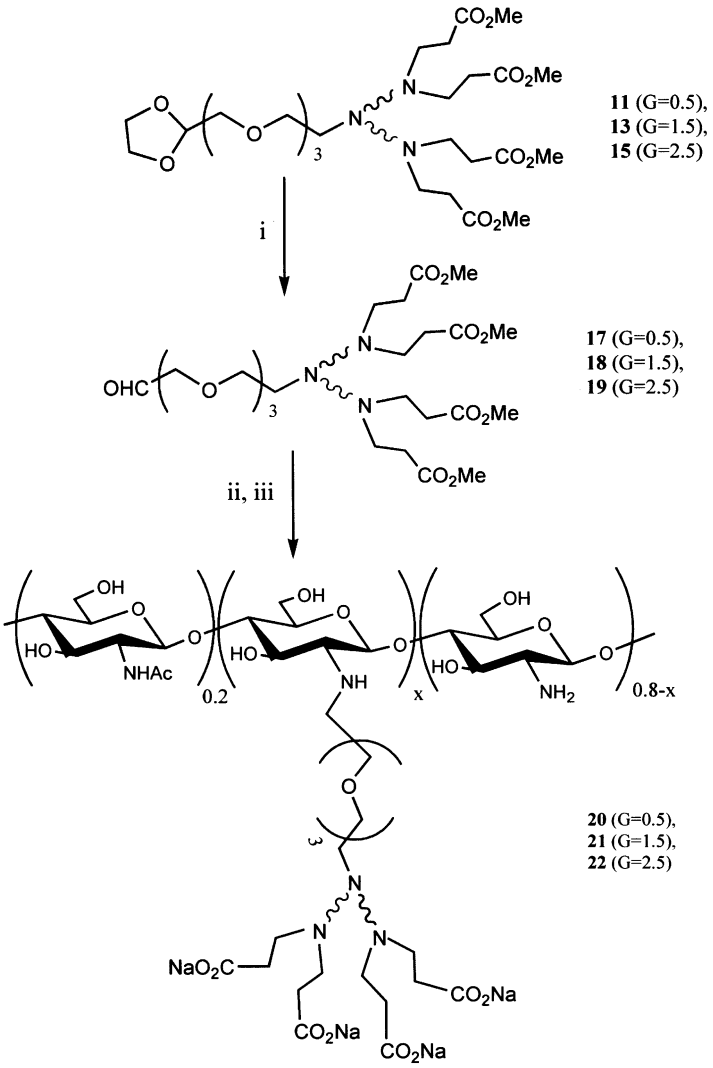
3.1. Modification of tetraethylene glycol

To build up the oligoethylene glycol spacer, we selected commercialized tetraethylene glycol **1** as the starting material (Scheme 1, Reagents and conditions: (i) BzC;, Et_3N, CH_2Cl_2, 38%; (ii) P_2O_5, DMSO, CH_2Cl_2, 90%; (iii) ethylene glycol, *p*TsOH, C_6H_6, 94%; (iv) 2 M NaOH/MeOH, 95%; (v) TsCl, Et_3N, CH_2Cl_2, 91%; (vi) NaN_3, EtOH, (0%; (vii) H_2, 10% Pd/C, EtOH, 78%). According to Bencome et al. (1991), monobenzoylation, oxidation, acetal formation and deprotection of benzoyl group were performed in four steps. Monobenzoation of tetraethylene glycol was performed with 1.0 equiv. of benzoyl chloride. The moderate yield (38%) of monobenzoate **2** is because of the simultaneous production of dibenzoate. The oxidation of **2** was performed according to Taber et al. (1987) and aldehyde **3** was obtained in excellent yield (90%). The aldehyde **3** was transformed to acetal with ethylene glycol to afford **4**, followed by deprotection of benzoyl group with 2 M NaOH/MeOH to give 11-hydroxy-3,6,9-trioxadecanal acetal **5**. To convert the hydroxyl group to amine, tosylation, azide formation and reduction were performed according to the authorized procedure (Maunier, Wu, Wang & Roy, 1997). After the tosylation of **5**, tosylate **6** was obtained in 28% overall yield in five steps from **1**. The azide **7** was also obtained in a excellent yield (90%), followed by reduction (H_2, 10% Pd–C) to give 11-amino-3,6,9-trioxadecanal acetal **8**, successfully.

To reduce the reaction step for the preparation of amine **8**, we tested the monotosylation of **1** under the same conditions (Scheme 2, Reagents and conditions: (i) TsCl, Et_3N, CH_2Cl_2, 40%; (ii) P_2O_5, DMSO, CH_2Cl_2, 92%; (iii) ethylene glycol, *p*TsOH, C_6H_6, 89%). The monotosylate **9** was successfully obtained with 1.0 equiv. of tosyl chloride, although the yield (40%) was not refined even in altering the molar ratio of the reagent. Simultaneous production of ditosylate was also observed. After oxidation and acetal formation, compound **6** was also obtained in 3 steps. The overall yield from **1** to **6** was slightly refined (Scheme 1, 28%; Scheme 2, 33%) and the reaction step could be reduced through the monotosylation. The azide formation and reduction were also performed and obtained amine **8**.

3.2. Preparation of PAMAM dendrimer

Using compound **8** as an amine source, the generation of poly(amido amine) (PAMAM) dendrimer was performed



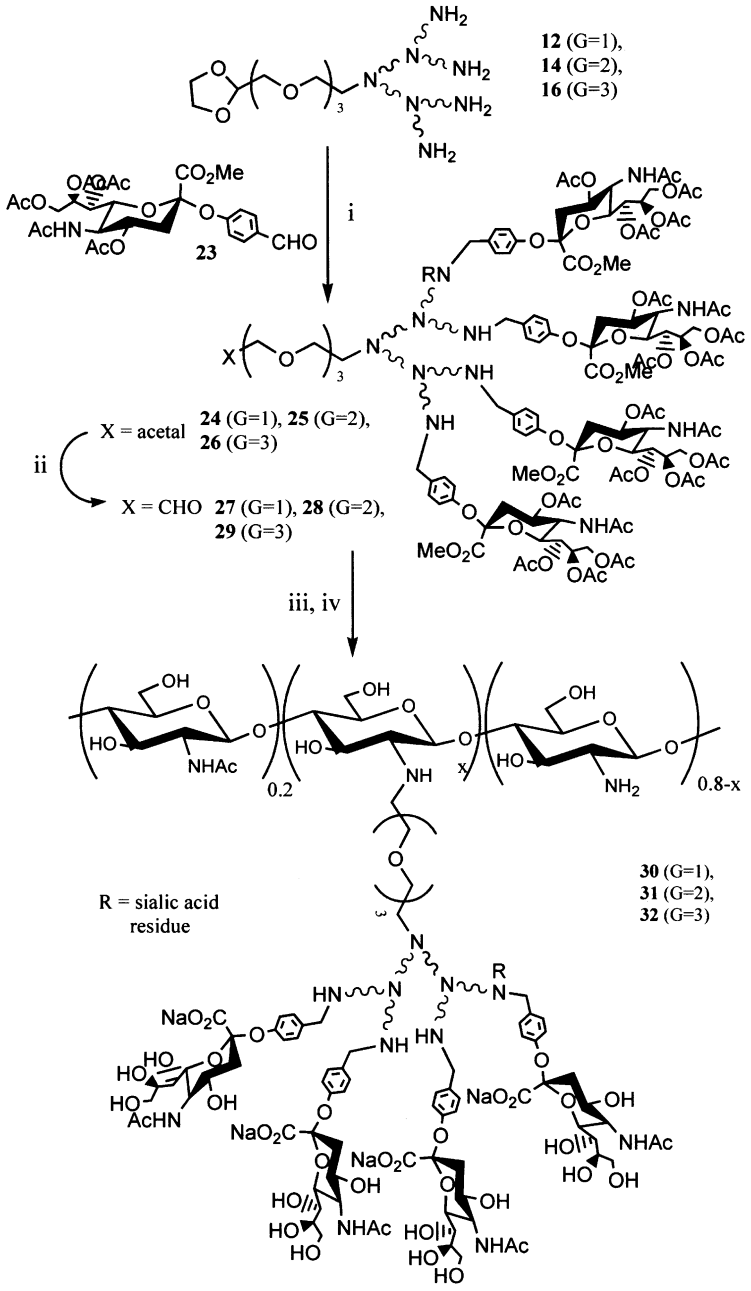
Scheme 4. Reagents and conditions: i, CF_3CO_2H/H_2O (4/1), rt, 1 day; ii, chitosan, $NaCNBH_3$, H_2O, AcOH, MeOH, rt, 1 day; iii, 0.5 M NaOH, rt, 2 h. Structure shows example for G = 1.5 (13, 18 and 21).

according to the reported procedure (Tomaria, Baker, Dewald, Hall, Kallos, Martin et al., 1985; Tomaria, Naylor & Goddard, 1990; Scheme 3, Reagents and conditions: (i) $H_2C{=}CHCO_2Me$, MeOH, 45°, 5 days; (ii) ethylenediamine, MeOH, rt, 3 days and Chart 1). Although the methyl esters were purified with column gave moderate or good yield (**11** = 70%, **13** = 73%, **15** = 57%), it was difficult to purify amines (**12**, **14**, **16**) with column owing to their high polarity. Since the excess amount of ethylenediamine (bp = 118°C) could be removed with evaporation and dried in vacuo, these amines (**12**, **14**) were used in the next generation without further purification. From the ^1H and ^{13}C NMR

Table 1
Molar ratio and DS of chitosan–dendrimer hybrid

Acetal	Molar ratio (equiv./NH_2)	Solvent[a]	Time (day)	DS	Reactivity[b] (%)
11	0.7	A	1	0.14	25
11	0.3	B	3	0.18	60
12	0.7	A	1	0.10	18
13	0.3	B	3	0.14	58
15	0.3	A	1	0.03	13
15	0.3	B	3	0.13	54

[a] Solvent using the deprotection of acetals: A, CF_3CO_2H/H_2O (4/1); B, CF_3CO_2H/?M HCl (4/1).
[b] Reactivity(%) = DS/(molar ratio × 0.8)100.



Scheme 5. Reagents and conditions: i, 23, $NaCNBH_3$, MeOH, rt, 1 day; ii, CF_3CO_2H/H_2O (4/1), rt, 1 day; iii, chitosan, $NaCNBH_3$, H_2O, AcOH, MeOH, rt, 1 day; iv 0.5 M NaOH, rt, 2 h. Structure shows example for G = 2 (14, 25, 28 and 31).

spectra, there were no peaks of ethylenediamine in each spectrum, thus demonstrating that it was not included in each amine.

3.3. Deprotection of acetal and attachment of dendrimer to chitosan

The deprotection of acetal was carried out with 80% CF_3COOH in water at rt for 1 day, then evaporated to dryness, followed by drying in vacuum for 1 day (Scheme 4, Reagents and conditions: (i) CF_3CO_2H/H_2O(4/1), rt, 1 day; (ii) chitosan, $NaCNBH_3$, H_2O, AcOH, MeOH, rt, 1 day; (iii) 0.5 M NaOH, rt, 2 h. Structure shows example for $G = 1.5$ (**13**, **18** and **21**)). However, the aldehydes **17**–**19**, were hard to purify with column because of the salt formation at tertiary amines with acid. Therefore, we tested the following reductive *N*-alkylation of chitosan (Hall & Yalpani, 1980; Yalpani & Hall, 1984) without any purification. From the ^1H NMR spectra of **17**–**19**, the typical CH proton of acetal (5.1 ppm) disappeared, although the signal of CHO proton could not be observed clearly. The reductive *N*-alkylation of chitosan was performed according to our previous report (Sashiwa & Shigemasa, 1999) and purified by dialysis from the mixture in the presence of Na_2CO_3.

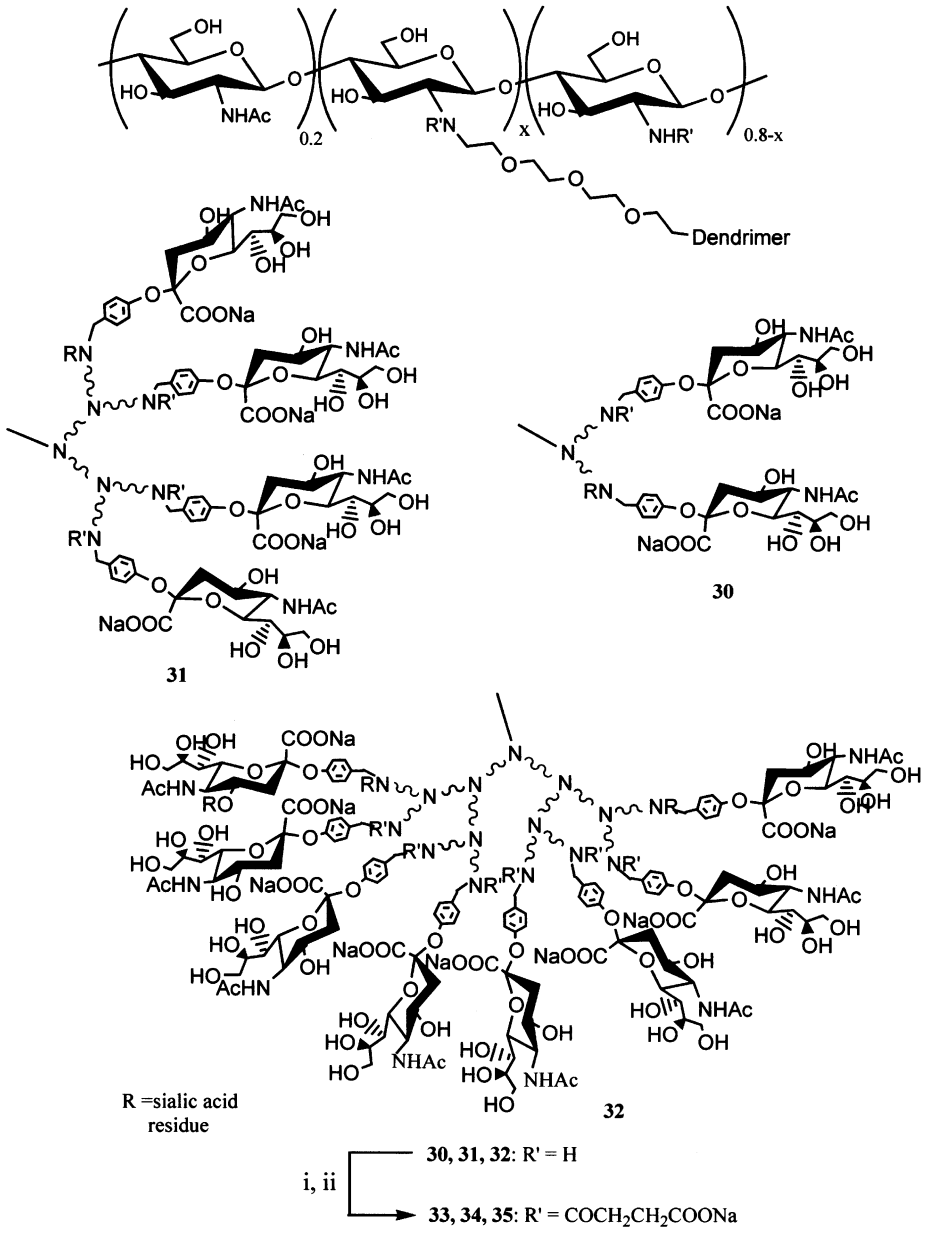


Chart 2. Reagents and conditions: (i) succinic anhydride, H_2O, AcOH, MeOH, rt, 1 day; (ii) 0.5 M NaOH, rt, 2 h.

From the ^1H and ^{13}C NMR spectra, the corresponding methylene signals of dendrimer and spacer were observed. The DS was estimated from the ratio of $COCH_2$ signals at 2.87–2.96 ppm against H-1 proton of chitosan (4.6–5.1 ppm). Table 1 shows the molar ratio of acetal and DS of products. The reactivity of aldehydes was gradually decreased with increasing the generation of dendrimer. However, the reactivity was extremely low (less than 25%) in 80% CF_3COOH in water (solvent A), which could have been caused by incomplete deprotection of acetal. To increase the reactivity, 2 M HCl (solvent B) was used instead of water and elongated the reaction time. As a result, a remarkable increase in reactivity was observed.

Table 2
Reactivity of sialoside bound dendrimers to chitosan (solvent for the deprotection of acetal: CF_3CO_2H/H_2O (4/1); reactivity (%), see Table 1)

Aldehyde			Product	DS	Reactivity (%)
Compd.	MW	Equiv.			
27	2157	0.2	**30**	0.08	47
28	3775	0.2	**31**	0.04	25
29	7587	0.1	**32**	0.02	25

To attach sialic acid to the dendrimer, we selected reported *p*-formylphenyl-α-sialoside **23** (Roy, Tropper, Romanowsky, Letellier, Cousineau, Meunier et al., 1991) by reductive *N*-alkylation with $NaCNBH_3$ (Scheme 5,

Reagents and conditions: (i) CF_3CO_2H/H_2O (4/1), rt, 1 day; (ii) chitosan, $NaCNBH_3$, H_2O, AcOH, MeOH, rt, 1 day; (iii) 0.5 M NaOH, rt, 2 h. Structure shows example for $G = 1.5$ (**13**, **18** and **21**) and Chart 2). Since 3 equiv. of **23** was used to eliminate remained primary amino groups in dendrimer, a part of *N*,*N*-disubstituted products of **30**, **31**, and **32** were obtained (**32**: 10 mol of sialoside bound to 8 mol of amine in dendrimer). The deprotection of acetal **24**–**26** was performed as above with 80% CF_3COOH in water to prevent the cleavage of glycoside linkage in sialoside. Since aldehydes **27**–**29** were also hard to purify, we consequently performed the reductive *N*-alkylation of chitosan without further purification. From the ^1H NMR spectra of **27**–**29**, typical CH proton of acetal (5.1 ppm) disappeared. The reductive *N*-alkylation of chitosan and the deprotection of acetyl groups and methyl ester in α-sialoside moiety were also performed with 0.5 M NaOH as above. From the ^1H and ^{13}C NMR spectra, the corresponding signals of dendrimer and phenyl-α-sialoside were observed in hybrid **30**–**32**. The reactivity of acetals **27**–**29** bound α-sialoside dendrimer are shown in Table 2. The DS of hybrids was 0.02–0.08, which reveals that 25–47% of aldehydes reacted towards chitosan. The low reactivity, especially for **28** and **29** would be caused by the steric hindrance because of high molecular weight of aldehydes. Hybrids **30**–**32** were insoluble in neutral water and these would not be useful towards biological evaluation. To improve the solubility, the remaining amino groups and secondary amines of the hybrids were transformed into **33**–**35** by succinylation with succinic anhydride in 90–100% yields. Complete succinylation was difficult due to the increasing steric hindrance of the polymer. All succinylated products **33**–**35** were soluble in water.

In conclusion, chitosan–dendrimer hybrids and chitosan–sialodendrimer hybrids with tetraethylene glycol spacer, were successfully prepared. We are now progressing further investigations like accurate molecular weight evaluation or conformational study in solution of these chitosan–dendrimer hybrids and will be publish in the near future. Finally, we expect that these chitosan–dendrimer hybrid as tree type molecule will be useful not only in the biomedical field, but also other field such as agriculture, electronics, life science etc.

Acknowledgement

We are indebted to Nippon Gaishi Co., Japan for a generous supply of *N*-acetylneuraminic acid.

References

Bencomo, V. V., Valdes, M. T. C., Albernas, J. R. M., Santana, V. F., Rensoli, M. H., & Martinez, C. S. (1991). *Carbohydrate Research*, *217*, 263–267.

Bosman, A. W., Janssen, H. M., & Meijer, E. W. (1999). *Chemical Reviews*, *991*, 665–688.

Frechet, J. M. J. (1994). *Science*, *263*, 1710–1715 and references cited therein.

Hall, L. D., & Yalpani, M. (1980). *Chemical Communications*, 1153–1154.

Maunier, M., Wu, Q., Wang, S. N., & Roy, R. (1997). *Canadian Journal of Chemistry*, *75*, 1472–1481.

Minami, S., Okamoto, Y., Matsuhashi, A., Sashiwa, H., Saimoto, H., Shigemasa, Y., Tanigawa, T., Tanaka, Y., & Tokura, S. (1992). In C. J. Brine, P. A. Sandford & J. P. Zikakis, *Advances in chitin and chitosan* (pp. 61–69). London: Elsevier.

Nishimura, K., Nishimura, S., Nishi, N., Saiki, I., Tokura, S., & Azuma, I. (1984). *Vaccine*, *2*, 93–99.

Roy, R., Tropper, D. F., Romanowsky, A., Letellier, M., Cousineau, L., Meunier, S. J., & Bratynski, J. (1991). *Glycoconjugate Journal*, *8*, 75–81.

Sashiwa, H., & Shigemasa, Y. (1999). *Carbohydrate Polymers*, *39*, 127–138.

Sashiwa, H., Saimoto, H., Shigemasa, Y., Ogawa, R., & Tokura, S. (1990). *International Journal of Biological Macromolecules*, *12*, 295–296.

Sashiwa, H., Shigemasa, Y., & Roy, R. (2000). *Macromolecules*, *33*, 6913–6915.

Sashiwa, H., Shigemasa, Y., & Roy, R. (2001a). *Macromolecules*, *34*, 3211–3214.

Sashiwa, H., Shigemasa, Y., & Roy, R. (2001b). *Macromolecules*, *34*, 3905–3909.

Sashiwa, H., Shigemasa, Y., & Roy, R. (2001c). *Carbohydrate Polymers*, in press.

Shigemasa, Y., Saito, K., Sashiwa, H., & Saimoto, H. (1994). *International Journal of Biological Macromolecules*, *16*, 43–49.

Taber, D. F., Amedio Jr, J. C., & Jung, K. Y. (1987). *Journal of Organic Chemistry*, *52*, 5621–5622.

Tanigawa, T., Tanaka, Y., Sashiwa, H., Saimoto, H., & Shigemasa, Y. (1992). In C. J. Brine, P. A. Sandford & J. P. Zikakis, *Advances in chitin and chitosan* (pp. 206–215). London: Elsevier.

Tomaria, D. A., Baker, H., Dewald, J., Hall, M., Kallos, G., Martin, S., Roeck, J., Ryder, J., & Smith, P. (1985). *Polymer Journal*, *17*, 117–132.

Tomaria, D. A., Naylor, A. M., & Goddard III, W. A. (1990). *Angewandte Chemistry, International Edition of England*, *29*, 138–175.

Yalpani, M., & Hall, L. D. (1984). *Macromolecules*, *17*, 272–281.