Novel Dendritic α-Sialosides: Synthesis of Glycodendrimers Based on a 3,3′-Iminobis(propylamine) Core

Diana Zanini and René Roy*

Department of Chemistry, University of Ottawa, Ottawa, Ontario K1N 6N5 Canada

Received June 3, 1996[⊗]

The cluster or multivalent effect has been recognized as an effective means by which to increase binding interactions between carbohydrates and proteins. In fact, it has been demonstrated that sialylated multibranched L-lysine dendrimers were potent inhibitors of hemagglutination of human erythrocytes by *Influenza* viruses. In a continuation of these studies, the synthesis of novel glycodendrimers with even valencies from 2 to 16 and ending with equidistant thiosialoside residues is described. These symmetrical dendrimers were more readily characterized by standard NMR spectral techniques than previously reported nonsymmetrical dendrimers of this general type. The synthesis of the dendritic core was based on the regioselective protection of the primary amines of 3,3′-iminobis(propylamine) (**4**) using benzyl cyanoformate. The resulting secondary amine **5** was alkylated with *tert*-butyl bromoacetate to provide divalent core structure **6** with Cbz protected amines and acid protected *tert*-butyl ester. Sequential deprotection by trifluoroacetolysis or hydrogenation afforded acid **7** or diamine **8** as key precursors, respectively. The two fragments were coupled using HOBt/DIC strategy to provide Cbz-protected dendrimers with valencies of 2, 4, 8, and 16 in the first, second, third, and fourth generations, respectively, in reasonable to good yields (42−82%). Cbz-protected precursors were efficiently transformed into N-chloroacetylated dendrimers by hydrogenolysis and treatment of the resulting amines with chloroacetic anhydride (82−91%). N-Chloroacetylated dendrimers were then treated with an excess of 2-thiosialic acid derivative **3** to give fully protected sialodendrimers in 76−96% yields. Deprotection of sialodendrimers under Zemplén conditions followed by methyl ester saponification and purification by gel permeation chromatography afforded symmetrical dendritic α-thiosialosides **21**, **23**, **25**, and **27** in fair yields (47−58%). These novel sialodendrimers, in keeping with their design, are currently being evaluated as inhibitors of human erythrocyte hemagglutination by *Influenza* viruses.

Introduction

Carbohydrate−protein interactions are responsible for a wide range of biological phenomena including cancer cell metastasis, inflammation, and infections by bacteria and viruses.[1] For example, binding of the viral membrane glycoprotein hemagglutinin (HA) to host cell sialyloligosaccharides present on glycolipids and glycoproteins mediates the infections of mammalian host tissues.[2] *N*-Acetylneuraminic acid (sialic acid, NeuAc) represents the most ubiquitous member of the sialic acid family of derivatives present on cell surface glycolipids and glycoproteins and constitutes the key epitope recognized as being essential for virus binding. The critical role played by α-sialosides has been confirmed by inhibition experiments with synthetic α-sialosides,[3] X-ray data,[4] and proton NMR spectroscopic studies.[5]

Unfortunately, carbohydrate−protein interactions, as opposed to protein−protein interactions, usually have low dissociation constants (K_D's) and, therefore, their overall attractive forces are generally weak.[6] NeuAc and viral HA interactions appear to be no exception ($K_D \approx 3$ mM).[5] In order to compensate for the low K_D's of natural oligosaccharides in the design of potent adhesion inhibitors, a strategy based on the cluster effect was successfully proposed. Clusters of α-sialosides have been prepared.[7] In addition, neoglycoproteins and glycopolymers have also been designed to improve the inhibitory potential of glycoconjugates.[8] These sialylated neoglycoproteins and glycopolymers, by virtue of their multivalent epitopes, possess the fundamental features necessary to compensate for the low affinity of individual α-sialosides. Sialylated polymers, because of their high valencies, have contributed to increased inhibitory activity by a factor of a few thousand (mM to μM range).[9] Still, neoglycoproteins and glycopolymers have ill defined chemical structures and are immunogenic. Consequently, they may not constitute ideal candidates for therapeutic uses.

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1996.

(1) (a) Varki, A. *Glycobiology* **1993**, *3*, 97. (b) Dwek, R. A. *Chem Rev.* **1996**, *96*, 683 and references cited therein.

(2) Corfield, A. P.; Schauer, R. In *Sialic Acids, Chemistry, Metabolism, and Functions*; Schauer, R., Ed.; Cell Biology Monograph, Vol. 10; Springer-Verlag: New York, 1982, p 5.

(3) Pritchett, T. J.; Brossner, R.; Rose, U.; Paulson, J. C. *Virology* **1987**, *160*, 502.

(4) Weiss, W.; Brown, J. H.; Cusack, S.; Paulson, J. C.; Skechel, J. J.; Wiley, D. C. *Nature* **1988**, *333*, 426.

(5) Sauter, N. K.; Bednarski, M. D.; Wurzburg, B. A.; Hanson, J. E.; Whitesides, G. M.; Skechel, J. J.; Wiley, D. C. *Biochemistry* **1989**, *28*, 8388.

(6) Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719.

(7) (a) Unverzagt, C.; Kelm, S.; Paulson, J. C. *Carbohydr. Res.* **1994**, *251*, 285. (b) Sabesan, S.; Duus, J. Ø.; Neira, S.; Domaille, P.; Kelm, S.; Paulson, J. C.; Bock, K. *J. Am. Chem. Soc.* **1992**, *114*, 8363. (c) Glick, G. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1991**, *113*, 4701.

(8) (a) Lee, R. T.; Lee, Y. C. Enhanced Biochemical Affinities of Multivalent Neoglycoconjugates in *Neoglycoconjugates: Preparation and Applications*; Lee, Y. C., Lee, R. T., Eds.; Academic Press, San Diego, CA, 1994; p 23. (b) Roy, R. Design and Syntheses of Glycoconjugates. In *Modern Methods in Carbohydrate Synthesis*; Khan, S.-H., O'Neil, R., Eds.; Harwood Academic: Amsterdam, 1995; p 378. (c) Magnusson, G.; Chernyak, A. Ya.; Kihlberg, J.; Kononov, L. O. Enhanced Biochemical Affinities of Multivalent Neoglycoconjugates. In *Neoglycoconjugates*: *Preparation and Applications*; Lee, Y. C., Lee, R. T., Eds.; Academic Press: San Diego, CA, 1994; p 53.

(9) (a) Sigal, G. B.; Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 3787. (b) Roy, R.; Andersson, F. O.; Harms, G.; Kelm, S.; Schauer, R. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1478. (c) Byramova, N. E.; Mochalova, L. V.; Belyanchikov, J. M.; Matrosovich, M. N.; Bovin, N. V. *J. Carbohydr. Chem.* **1991**, *10*, 691. (d) Roy, R.; Laferrière, C. A. *Carbohydr. Res.* **1988**, *177*, C1 and references cited therein.

(10) Roy, R. *Polym. News* **1996**, *21*, 226.

S0022-3263(96)01047-X CCC: $12.00

On the other hand, dendrimers with covalently attached carbohydrates represent chemically well defined polymers with known densities.[10] In general, they may be prepared by divergent or convergent approaches and may also be synthesized as polydispersed structures.[11] Bidirectional dendrimers can be used advantageously as models for cell surface multiantennary glycoproteins as they share unique structural parities. It therefore seems a natural extension to consider bidirectional dendritic α-sialosides as potent inhibitors of viral hemagglutination.

In fact, it has already been demonstrated that sialylated dendrimers scaffolded onto multibranched L-lysine afforded conjugates that were as potent as polymers in the inhibition of hemagglutination of human erythrocytes by *Influenza* viruses.[12] However, the lack of symmetry within the poly-L-lysine dendrimers previously reported have rendered high field proton NMR spectral characterization rather cumbersome and although the fractal topology of these L-lysine based-glycodendrimers is likely to better mimic cell surface multiantennary glycoproteins, novel symmetrical "sialodendrimers" having chemically equivalent thiosialoside residues represent compounds of interest with possible therapeutic advantages. In attempts to generate such compounds, and in efforts to meet difficult synthetic challenges, we present herein the design and synthesis of a new family of symmetrical dendrimers with even valencies between 2 and 16 based on a 3,3′-iminobis(propylamine) (**4**) core.

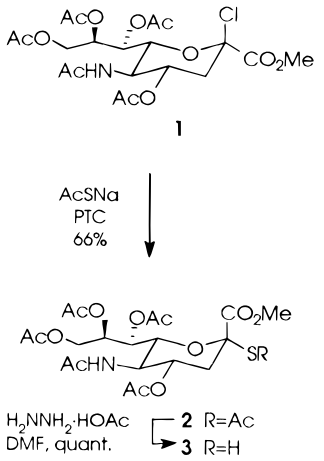
Although, the dendrimers presented here bear some resemblance to commercially available Starburst PAMAM dendrimers, they have the distinct advantage of not being susceptible to base-catalyzed retro-Michael degradations. In addition, this methodology eliminates the need for large excesses of reagents used to ensure complete conversion, a problem commonly associated with the Starburst PAMAM dendrimer synthesis.

Results and Discussion

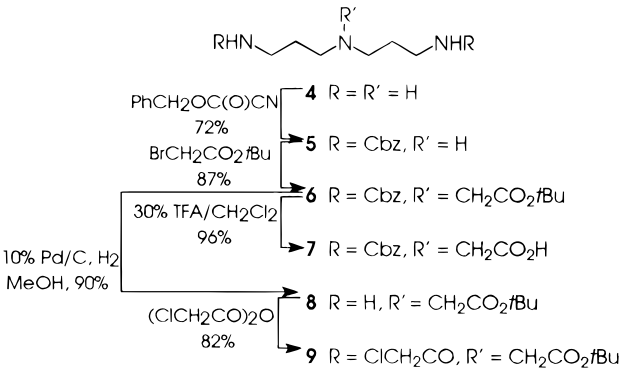
The approach described for the synthesis of these glycodendrimers was based on the convergent assembly of suitably multibranched dendrimers having *N*-chloroacetylated end groups to which thiolated carbohydrate derivatives could be added at a later stage.[10,12,13] This strategy allows the incorporation of different carbohydrate haptens onto prebuilt dendritic structures. Moreover, the choice of thiolated glycosides stemmed from the desire to generate glycodendrimers that would be resistant to glycohydrolase activity. The method is general, high-yielding and readily amenable to existing commercially available amine-terminated dendrimers.

The α-thiosialoside functionality was introduced under phase transfer catalysis (PTC) by treatment of acetochloroneuraminic acid (**1**) with thioacetic acid[12] to obtain methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosonate **2**. The thioacetate derivative **2** was chemoselectively converted to thiol **3** (H_2NNH_2·HOAc) using a

Scheme 1



Scheme 2



modification[14] of our previously established methodology (Scheme 1).[12]

First, second, third, and fourth generation N-chloroacetylated dendrimers **9**, **13**, **16**, and **19** with valencies of 2, 4, 8, and 16 were efficiently and conveniently synthesized in solution[15] using Cbz protecting group strategy and carbodiimide−hydroxybenzotriazole (HOBt) coupling chemistry. Key monomeric precursors **7** and **8** were prepared in the following manner.

The primary amines of 3,3′-iminobis(propylamine) (**4**) were regioselectively protected using the method of Murahashi *et al.*[16] to give diamine **5** (benzyl cyanoformate, CH_2Cl_2, 72% yield). Diamine **5** was then alkylated with *tert*-butyl bromoacetate in good yield (CH_3CN, 87%) to provide divalent core structure **6** with Cbz protected amines and *tert*-butyl protected acid functionalities. Trifluoroacetolysis of **6** gave acid **7** (96% yield) and hydrogenolysis of the Cbz groups of **6** afforded diamine **8** (90%) (Scheme 2). Acid **7** and diamine **8** represent key precursors in the synthesis of the glycodendrimers described here.

Compounds **7** and **8** were coupled using diisopropylcarbodiimide (DIC) and HOBt to provide tetravalent Cbz-protected dendrimer **10** in 82% yield. In order to avoid tedious silica gel chromatography, it was found that the crude mixture containing **10** could be treated with anion exchange resin (HO^-, Amberlite IRA-400) thereby eliminating HOBt and excess acid used in the coupling. The residue was then concentrated and diisopropylcarbodiimide urea and **10** were easily separated by chromatog-

(11) (a) Ardoin, N.; Astruc, D. *Bull. Soc. Chim. Fr.* **1995**, *132*, 875. (b) Issberner, J.; Moors, R.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2413. (c) Fréchet, J. M. J. *Science* **1994**, *263*, 1710 and references cited therein.

(12) (a) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1869. (b) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *ACS Symp. Ser.* **1994**, *560*, 104.

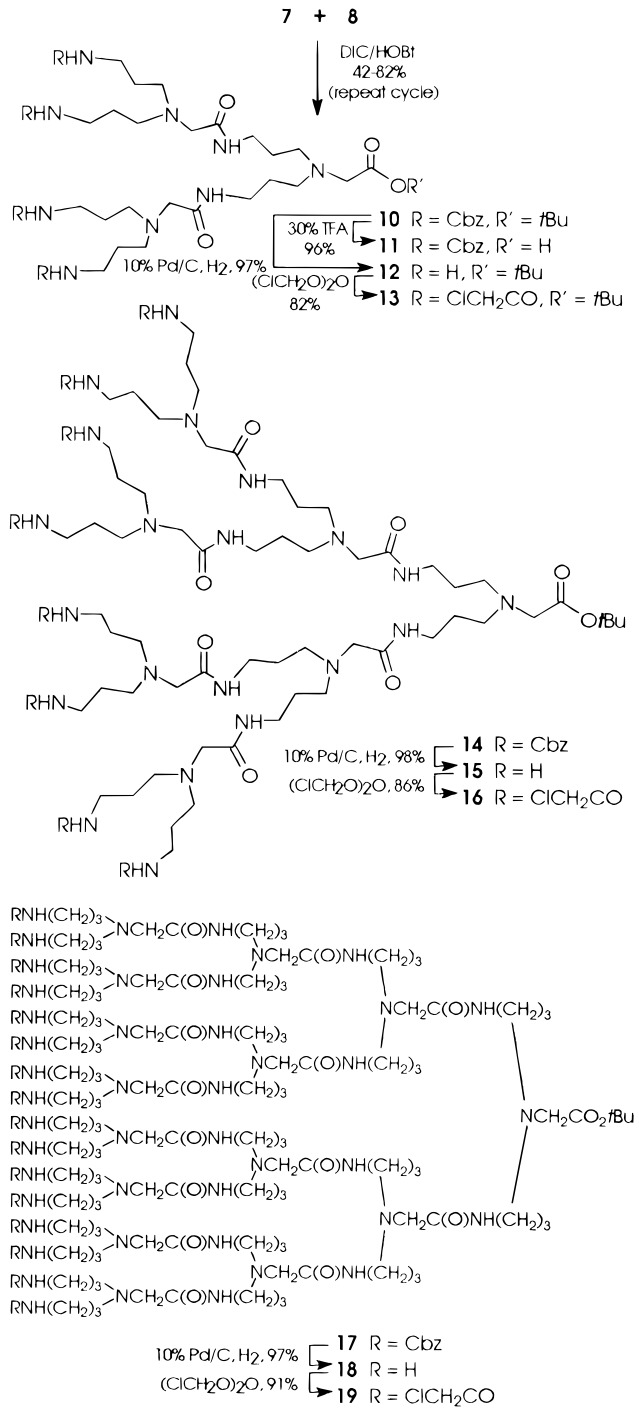
(13) Pagé, D.; Zanini, D.; Roy, R. *Bioorg. Med. Chem.* **1996**, *4*, in press.

(14) Park, W. K. C.; Meunier, S. J.; Zanini, D.; Roy, R. *Carbohydr. Lett.* **1994**, *1*, 179.

(15) The approach described here is also amenable to solid-phase synthesis as described for L-lysine dendrimers (reference 12). Work is in progress to evaluate the efficiency of this approach.

(16) Murahashi, S.-I.; Naota, T.; Nakajima, N. *Chem. Lett.* **1987**, 879.

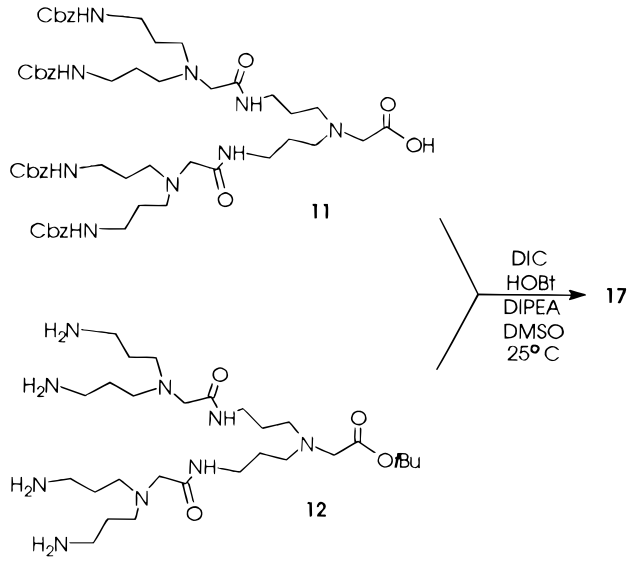
Scheme 3



raphy to afford **10**. Tetravalent dendrimer **10** was deprotected to give tetraamine **12** (10% Pd/C, H_2) which was coupled to acid **8** using the strategy described above to give Cbz-protected octamer **14** (66% yield). Repetition of deprotection procedures gave octaamine **15** which was again coupled to acid **8** providing hexadecavalent **17** (46%) (Scheme 3). All Cbz-protected dendrimers exhibited consistent NMR and mass spectral data which confirmed the purity of dendrimers **10**, **15**, and **17**. Dendrimer characterization was made easy by virtue of their symmetry. ^{1}H-NMR signals were superimposable for each generation, and characteristic ^{1}H NMR ($CDCl_3$) signals were observed at δ 2.50−2.55, 1.50−1.60, 3.00−3.20, and 5.05 ppm for α-, β-, γ-, and Cbz CH_2 residues, respectively.

Alternatively, hexadecavalent dendrimer **17** could also be prepared more efficiently by the convergent assembly of acid **11** and tetraamine **12**. Acid **11** was prepared by deprotection of tetravalent ester **10** with trifluoroacetic acid. Using the above established HOBt/DIC coupling techniques, **17** was isolated in 57% yield for the two step process (Scheme 4). Deprotection of the Cbz-protected

Scheme 4



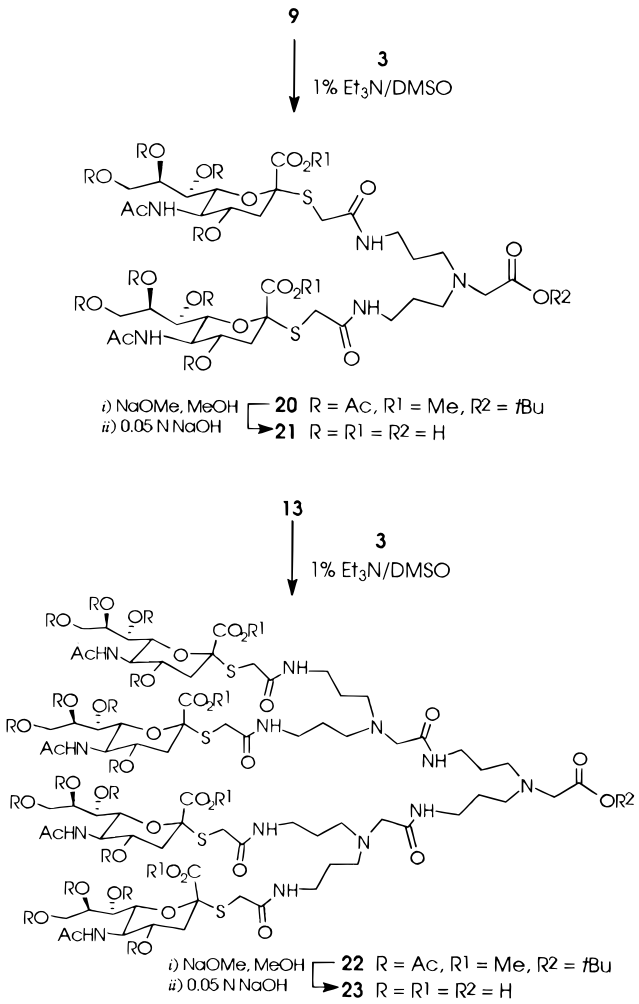
17 provided hexadecavalent amine **18** in excellent yield (97%).

N-Chloroacetylated di- (**9**), tetra- (**13**), octa- (**16**), and hexadecavalent (**19**) dendrimers were obtained by treating the corresponding amines (**8**, **12**, **15**, and **18**) with chloroacetic anhydride in nonprotic solvents (CH_3CN for **8**, DMF for **12**, and DMSO for **15** and **18**). Changes of solvent were necessary as the solubility properties of di- (**8**), tetra- (**12**), octa- (**15**), and hexadecavalent (**18**) amines were slightly different. Basic alumina chromatography using 20% H_2O/CH_3CN as eluent was used to remove the chloroacetate anions generated. Treatment of the crude mixtures with HO^- resin destroyed *N*-chloroacetyl functionalities. DIPEA and similar bases could be added to the reaction mixture to scavenge the chloroacetate anions. However, these bases were not easily removed during final purification as the multivalent chloroacetyl moiety was far too polar to allow easy isolation of desired compounds by standard chromatographic techniques.

Each dendrimer generation (**9**, **13**, **16**, and **19**) was then treated with a slight excess of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosonate (**3**) to provide completely protected glycodendrimers **20**, **22**, **24**, and **26**. DMSO was removed by lyophilization and the residue redissolved in the minimum amount of DMSO and precipitated from ethyl acetate (Schemes 5−7) to give **20**, **22**, **24**, and **26** in good to excellent yields (76 to 96%). The polarity of the NeuAc end groups dismisses the possibility of purification by silica gel chromatography for glycodendrimers of higher generation. Dendrimers **22**, **24**, and **26** are soluble only in polar solvents, and precipitation from these solvents must be accomplished with ethyl acetate. Using other solvents such as ether or hexanes also precipitated α-thiosialoside **3** and some disulfide byproduct formed during the reaction. This phenomenon was not envisioned for glycosides less polar than NeuAc.

Complete coupling was evident upon analysis of NMR spectral data. The disappearance of the *N*-chloroacetyl signal at 4.01 ppm ($CDCl_3$ for the di- (**9**), tetra- (**13**), and, octavalent (**16**) dendrimers, and DMSO-d_6 for the hexa-

Scheme 5



decamer (**19**)) and the emergence of new signals corresponding to the incorporated NeuAc residues (H-4 at 4.83 ppm, H-3 equatorial at 2.70 ppm, and *N*-Ac at 1.83 ppm, $CDCl_3$ for di- (**20**) and tetravalent (**22**) α-sialosides and DMSO-d_6 for the octa- (**24**) and hexadecameric (**26**) protected glycodendrimers) clearly established the extent of sialoside incorporation (Figure 1).

Deprotection of glycodendrimers **20**, **22**, **24**, and **26** by sequential ester hydrolysis ((i) NaOMe/MeOH; (ii) 0.05 N NaOH) followed by gel permeation chromatography (GPC, Biogel-P2, H_2O as eluent) afforded fully deprotected glycodendrimers **21**, **23**, **25**, and **27** with two, four, eight, and sixteen NeuAc residues in moderate yields (47−58%). Surprisingly, a two-step deprotection strategy was necessary to ensure complete de-O-acetylation. No deprotection was observed with shorter reaction times nor when Zemplén deprotection was avoided and NaOH treatment performed directly.

The strategy presented here is a convenient and efficient way of synthesizing dendritic glycosides. However, higher generation N-chloroacetylated dendrimers and protected glycodendrimers are very polar compounds and not readily isolable by standard chromatography techniques (silica gel chromatography, reverse phase chromatography). Alternative methods of carbohydrate conjugation are currently under investigation. Still, the distinct advantage of the N-chloroacetylated strategy resides in the ability to determine the extent of glycoside coupling by high field ^{1}H-NMR spectroscopy. As already stated, the well resolved singlet corresponding to the chloromethylene groups at 4.01 ppm ($CDCl_3$, DMSO-d_6) can be precisely integrated (±3%) relative to other signals. Following carbohydrate attachment, the signal was shifted upfield (3.41 and 3.81 ppm) due to the sulfur atom and is further transformed into two doublets by virtue of the newly created diastereotopic protons.

In conclusion, this methodology enabled the synthesis of symmetrical sialodendrimers with sialic acid densities corresponding to 2^n, where *n* represents the *n*'th generation of the 3,3′-iminobis(propylamine) core. All yields were fair to excellent, and the biological testing of glycodendrimers **21**, **23**, **25**, and **27**, including enzyme linked lectin assays (ELLA) with *Limax flavus* lectin to various mucins and the inhibition of hemagglutination of human erythrocytes by *Influenza* viruses, is in progress and will be reported in due course.

Experimental Methods

General Methods. Proton chemical shifts (δ) are given relative to internal $CHCl_3$ (7.24 ppm) for $CDCl_3$ solutions, to internal dimethyl sulfoxide (2.49 ppm) for DMSO-d_6 solutions, and to internal HOD (4.76 ppm) for D_2O solutions. Carbon chemical shifts are given relative to deuterochloroform (77.0 ppm) and DMSO-d_6 (39.4 ppm). Assignments were based on COSY, HMQC, and DEPT experiments. Thiosialic acid derivatives **2** and **3** were synthesized as previously described.[12,14]

Preparation of 3,3′-Bis(carbobenzyloxy)-3,3′-iminobis(propylamine) (5). To a solution of 3,3′-iminobis(propylamine) (**4**) (2.00 g, 0.015 mol) in dry CH_2Cl_2 (50 mL) was added dropwise a solution of benzyl cyanoformate (4.91 g, 0.030 mol) in dry CH_2Cl_2 (25 mL) over a period of 2 h at 25 °C. Hydrogen cyanide generated during the reaction was carefully introduced into a solution of sodium hydroxide in water. After removal of the organic solvent, the residue was subjected to column chromatography and eluted with $CHCl_3$ and MeOH (2:1) to give compound **5** as a white solid in 72% yield (4.36 g, 0.11 mol).

5: ^{1}H-NMR ($CDCl_3$) δ 1.31 (bs, 1H, NH amine), 1.63 (t, 4H, β-CH_2), 2.61 (t, 4H, $J_{\alpha,\beta}$ 7.4 Hz, α-CH_2), 3.24 (m, 4H, γ-CH_2), 5.06 (s, 4H, Cbz-CH_2), 5.49 (s, 2H, Cbz-NH), 7.24−7.31 (m, 10H, Cbz-Ph); ^{13}C-NMR ($CDCl_3$) δ 29.6 (β-C), 39.5 (γ-C), 47.4 (α-C), 66.5 (Cbz-CH_2), 128.0 (2×), 128.3, and 128.5 (Cbz-Ph, ortho, meta, para), 136.7 (Cbz-Ph, C-1), 156.5 (C═O); FAB-MS (pos) calcd for $C_{22}H_{29}N_3O_4$ 399.22, found 400.3 ($M^+ + 1$, 83.7% base peak).

Preparation of Monomer 6. To a solution of **5** (4.00 g, 0.010 mol) in acetonitrile (25 mL) was added *tert*-butyl bromacetate (1.94 g, 0.010 mol). One equivalent of diisopropylethylamine (DIPEA, 1.28 g, 0.010 mol) was added and the solution stirred for 30 min at 25 °C. After solvent evaporation, the residue was subjected to column chromatography and eluted with a gradient of hexane/ethyl acetate to give yellow resin **6** in 87% yield (4.42 g, 0.009 mol).

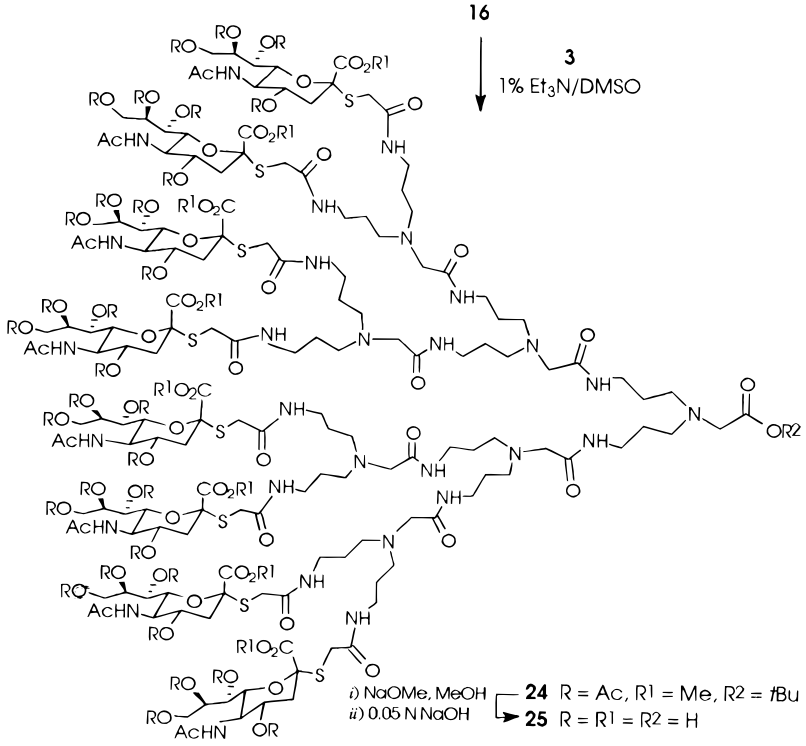
6: ^{1}H-NMR ($CDCl_3$) δ 1.40 (s, 9H, *t*Bu), 1.62 (t, 4H, β-CH_2), 2.51 (t, 4H, $J_{\alpha,\beta}$ 6.3 Hz, α-CH_2), 3.10 (s, 2H, $NCH_2C(O)$), 3.24 (m, 4H, γ-CH_2), 5.05 (s, 4H, Cbz-CH_2), 5.72 (bs, 2H, Cbz-NH), 7.24−7.31 (m, 10H, Cbz-Ph); ^{13}C-NMR ($CDCl_3$) δ 27.0 (β-C), 28.1 (CH_3's), 39.4 (γ-C), 52.2 (α-C), 56.1 ($NCH_2C(O)$), 66.3 (Cbz-CH_2), 81.4 ($OCMe_3$), 2 × 127.9 and 128.4 (Cbz-Ph, ortho, meta, para), 136.9 (Cbz-Ph, C-1), 156.6 (C═O); FAB-MS (pos) calcd for $C_{28}H_{39}N_3O_6$ 513.28, found 514.3 ($M^+ + 1$, 29.1% base peak).

Synthesis of Acids 7 and 11. Typical Procedure: A solution of **6** (2.00 g, 3.90 mmol) in 50 mL of 30% trifluoroacetic acid in CH_2Cl_2 was stirred vigorously for 3 h at 25 °C. The solution was concentrated and dried under vacuum overnight. The trifluoroacetate salt of **7** was recovered as a yellow resin in 96% yield (2.14 g, 3.75 mmol) and used without further purification.

The trifluoroacetate salt of **11** was isolated as above to provide **11** as a yellow resin in quantitative yield.

7: ^{1}H-NMR ($CDCl_3$) δ 1.78 (m, 4H, β-CH_2), 3.11 (m, 8H, α-CH_2, γ-CH_2), 3.79 (s, 2H, $NCH_2C(O)$), 4.99 (s, 4H, Cbz-CH_2), 5.81 (bs, 2H, Cbz-NH), 7.24−7.28 (m, 10H, Cbz-Ph), 10.6−11.2 (bs, CO_2H); ^{13}C-NMR ($CDCl_3$) δ 27.8 (β-C), 37.6 (γ-C), 53.0 (α-C), 53.4 ($NCH_2C(O)$), 66.9 (Cbz-CH_2), 114.9 and 117.2 (tri-

Scheme 6



fluoroacetate salt), 127.9, 128.2, and 128.4 (Cbz-Ph, ortho, meta, para), 136.3 (Cbz-Ph, C-1), 157.4 (acid C═O), 161.0 and 161.3 (Cbz C═O's), 167.9 (trifluoracetate salt C═O); FAB-MS (pos) calcd for $C_{24}H_{31}N_3O_6$ 457.22, found 458.2 ($M^+ + 1$, 17.2% base peak).

Preparation of Di- (8), Tetra- (12), Octa- (15), and Hexadeca- (18) Amines. Typical Procedure: To compound **6** (400 mg, 0.78 mmol) was added MeOH (10 mL) containing 10% Pd/C (40 mg). H_2 was bubbled through the solution and the mixture stirred for 30 min at 25 °C. The mixture was then filtered and the Pd/C washed twice with MeOH. All filtrates were combined and dried down giving compound **8** in 90% yield (264 mg, 0.70 mmol). Yellow resin **8** was used as is.

Amines **12**, **15**, and **18** were obtained in a similar manner, but with longer reaction times (4 h for **12**, 20 h for both **15** and **18**), as white solids in yields of 97, 98, and 97%, respectively.

8: ^{1}H-NMR ($CDCl_3$) δ 1.34 (s, 9H, *t*Bu), 1.49 (m, 4H, β-CH_2), 1.75 (bs, 4H, NH_2), 2.49 (t, 4H, $J_{\alpha,\beta}$ 7.0 Hz, α-CH_2), 2.64 (t, 4H, $J_{\beta,\gamma}$ 6.7 Hz, γ-CH_2), 3.07 (s, 2H, $NCH_2C(O)$); ^{13}C-NMR ($CDCl_3$) δ 28.1 (CH_3's), 30.9 (β-C), 40.3 (γ-C), 52.0 (α-C), 56.0 ($NCH_2C(O)$), 170.9 (C═O); mass spectrum (CI) (rel intensity) *m*/*z* 246.1 (M^+, 51.6%).

12: ^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H, *t*Bu), 2.44−2.78 (m, 28H, α-CH_2's, γ-CH_2NH_2's, NH_2's); FAB-MS (pos) calcd for $C_{28}H_{61}N_9O_4$ 587.48, found 588.5 ($M^+ + 1$, 7.3% base peak).

15: ^{1}H-NMR ($CDCl_3$) δ 1.42 (s, 9H, *t*Bu), 1.45−1.72 (m, 44H, β-CH_2's, NH_2's).

Synthesis of Cbz-Protected Dendrimers 10, 14, 17. Typical Procedure: To a solution of **8** (170 mg, 0.45 mmol) in CH_3CN (15 mL) were added acid **7** (1.2 equiv. per amine group, 617 mg, 1.08 mmol) already dissolved in CH_3CN (2 mL) and neutralized with DIPEA. To the stirred solution were added diisopropylcarbodiimide (DIC, 136 mg, 1.08 mmol) and hydroxybenzotriazole (HOBt, 146 mg, 1.08 mmol), and the mixture was stirred at 25 °C. The pH was kept at 9 by the addition of DIPEA. Completion of the reaction was monitored by ninhydrin test. After 3 h, the solution was treated with HO^- resin for 15 min in order to remove excess acid and HOBt. The solution was concentrated and the residue subjected to column chromatography using a slow gradient of CH_3CN to 20% water in CH_3CN. Tetravalent **10** was isolated as a yellow resin in 82% yield (448 mg, 0.40 mmol).

Compounds **14** and **17** were prepared in a similar fashion from amines **12** and **15**, respectively. Differences include solvent used (DMF and DMSO for compounds **14** and **17**) and reaction times required for complete coupling as measured via ninhydrin testing (20 h and 48 h, respectively). After chromatography, isolated yields were 66% for **14** and 46% for **17**.

Using the same coupling procedure with tetraamine **12** and acid **11** also gave compound **17** in a higher yield of 57%.

10: ^{1}H-NMR ($CDCl_3$) δ 1.40 (s, 9H, *t*Bu), 1.52−1.59 (m, 12H, β-CH_2's), 2.47−2.57 (m, 12H, α-CH_2's), 3.02−3.27 (m, 18H, γ-CH_2's, $NCH_2C(O)$'s), 5.04 (s, 8H, Cbz-CH_2), 5.52 (bs, 4H, Cbz-NH), 7.24−7.36 (m, 20H, Cbz-Ph), 7.58 (bs, 2H, amide NH); ^{13}C-NMR ($CDCl_3$) δ 27.2 (β-C's), 28.1 (CH_3's), 37.3 and 38.9 (γ-C's), 52.0 and 52.4 (α-C's), 55.8 and 57.8 ($NCH_2C(O)$'s), 57.8 (Cbz-CH_2), 81.4 ($OCMe_3$), 128.0 and 128.5 (Cbz-Ph, ortho, meta, para), 136.7 (Cbz-Ph, C-1), 165.8−171.0 (C═O's); FAB-MS (pos) calcd for $C_{60}H_{85}N_9O_{12}$ 1123.63, found 1125.1 ($M^+ + 1$, 14.5% base peak).

14: ^{1}H-NMR ($CDCl_3$) δ 1.41 (s, 9H, *t*Bu), 5.03 (s, 16H, Cbz-CH_2), 7.24−7.32 (m, 40H, Cbz-Ph); ^{13}C-NMR ($CDCl_3$) δ 28.1 (CH_3's), 66.6 (Cbz-CH_2), 128.0, 128.1, and 128.5 (Cbz-Ph, ortho, meta, para), 136.6 (Cbz-Ph, C-1); FAB-MS (pos) calcd for $C_{124}H_{177}N_{21}O_{24}$ 2344.32, found 2346.6 ($M^+ + 1$, 0.4% base peak).

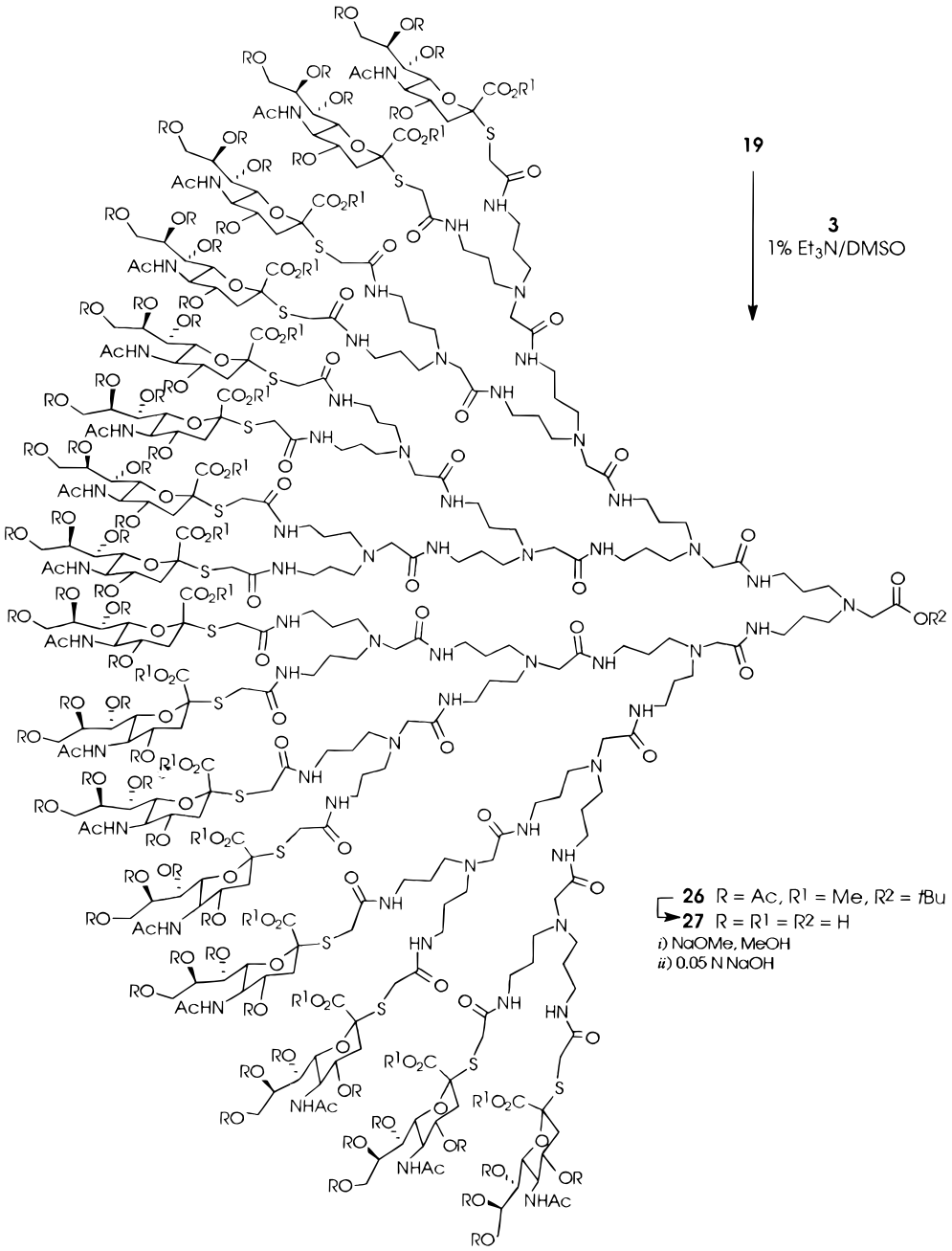
17: ^{1}H-NMR ($CDCl_3$) δ 1.41 (s, 9H, *t*Bu), 5.04 (s, 32H, Cbz-CH_2), 7.24−7.30 (m, 80H, Cbz-Ph); ^{13}C-NMR ($CDCl_3$) δ 28.1 (CH_3's), 66.5 (Cbz-CH_2), 128.0, 128.1, 128.3, and 128.5 (Cbz-Ph, ortho, meta, para), 136.7 (Cbz-Ph, C-1).

Preparation of N-Chloroacetylated Dendrimers 9, 13, 16, 19. Typical Procedure: To a solution of amine **8** (294 mg, 0.78 mmol) in CH_3CN (5 mL) was added chloroacetic anhydride (1.2 equiv per amine functionality, 310 mg, 1.86 mmol). The solution was stirred for 2 h at 25°C. The reaction was judged to be complete by ninhydrin test. The solvent was removed *in vacuo*, and the residue was subjected to basic alumina chromatography using 20% H_2O in CH_3CN as eluent in order to remove the chloroacetate anion of **9**. Compound **9** was isolated in 82% yield as a yellow resin (254 mg, 0.64 mmol).

N-Chloroacetylated dendrimers **13**, **16**, and **19** were isolated in 82, 86, and 91% yields, respectively. The only difference in their preparation included choice of solvent. Compound **12** was dissolved in DMF to give **13**; amines **15** and **18** were each dissolved in DMSO to prepare compounds **16** and **19,** respectively.

9: ^{1}H-NMR ($CDCl_3$) δ 1.39 (s, 9H, *t*Bu), 1.60 (m, 4H, β-CH_2), 2.51 (t, 4H, $J_{\alpha,\beta}$ 6.2 Hz, α-CH_2), 3.11 (s, 2H, $NCH_2C(O)$), 3.33 (m, 4H, γ-CH_2), 3.95 (s, 4H, $ClCH_2$), 7.47 (bs, 2H, amide NH); ^{13}C-NMR ($CDCl_3$) δ 26.4 (β-C), 28.1 (CH_3's), 38.2 (γ-C), 42.7

Scheme 7



($ClCH_2$), 52.2 (α-C), 55.9 ($NCH_2C(O)$), 81.5 ($OCMe_3$), 166.2 and 171.0 (C=O's); FAB-MS (pos) calcd for $C_{16}H_{29}N_3O_4Cl_2$ 398.11, found 398.2 ($M^+ + 1$, 5.8% base peak).

13: ^{1}H-NMR ($CDCl_3$) δ 1.42 (s, 9H, *t*Bu), 4.01 (s, 8H, $ClCH_2$); ^{13}C-NMR ($CDCl_3$) δ 28.2 (CH_3's), 42.8 ($ClCH_2$); FAB-MS (pos) calcd for $C_{36}H_{69}N_9O_8Cl_4$ 893.29, found 894.4 ($M^+ + 1$, 1.3% base peak).

16: ^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H, *t*Bu), 4.02 and 4.03 (2s, 16H, $ClCH_2$'s); ^{13}C-NMR ($CDCl_3$) δ 28.2 (CH_3's), 41.0 and 42.8 ($ClCH_2$'s).

19: ^{1}H-NMR (DMSO-d_6) δ 1.38 and 1.39 (2s, 9H, *t*Bu), 4.03 (s, 32H, $ClCH_2$); ^{13}C-NMR (DMSO-d_6) δ 27.8 (CH_3's), 42.7 ($ClCH_2$).

Synthesis of Peracetylated Dendritic α-Thiosialosides 20, 22, 24, 26. Typical Procedure: N-Chloroacetylated dendrimer **9** (45 mg, 0.11 mmol) was dissolved in 1% Et_3N/DMSO (5 mL) and N_2 bubbled through the solution. To this was added thiol **3** (1.1 equiv per *N*-chloroacetyl functionality, 137 mg, 0.25 mmol) and the solution left stirring, under N_2, overnight at 25 °C. The solution was concentrated by lyophilization and subjected to column chromatography using a gradient of CH_3CN to 20% H_2O/CH_3CN giving off-white solid **20** in 96% yield (145 mg, 0.11 mmol).

Peracetylated NeuAc dendrimers **22**, **24**, and **26** were isolated in a similar manner. Of note, these protected glycodendrimers were too polar to be subjected to standard chromatographic techniques. Instead, they were isolated by redissolution in the minimum amount of DMSO (typically 300 μL) and precipitated with ethyl acetate. NMR spectral data shows the incorporation of ethyl acetate in the dendrimer core.

20: ^{1}H-NMR ($CDCl_3$) δ 1.41 (s, 9H, *t*Bu), 1.64 (t, 4H, *J* 6.7 Hz, β-CH_2), 1.83 (s, 6H, NAc), 1.99, 2.01, 2.11, and 2.14 (4s, 24H, OAc's), 2.02−2.10 (m, 2H, H-3ax), 2.61 (m, 4H, α-CH_2), 2.70 (dd, 2H, $J_{3eq,4}$ 12.7 Hz, $J_{3ax,3eq}$ 4.7 Hz, H-3eq), 3.81 (m, 8H, γ-CH_2, $NCH_2C(O)$, SCH_2), 3.49 (d, 2H, *J* 15.9 Hz, SCH_2), 3.73 (s, 6H, CO_2CH_3), 3.75−3.77 (m, 2H, H-6), 3.98−4.05 (m, 4H, H-5, H-9), 4.25 (dd, 2H, $J_{8,9}$, 12.4 Hz, $J_{9,9}$, 2.7 Hz, H-9′), 4.83 (ddd, 2H, H-4), 5.27 (dd, 2H, *J* 2.2 Hz, *J* 8.9 Hz, H-7), 5.35 (d, 2H, $J_{5,NHAc}$ 10.0 Hz, N*H*Ac), 5.37−5.39 (m, 2H, H-8), 6.88 (t, $J_{\alpha,NH}$ 5.6 Hz, amide NH); ^{13}C-NMR ($CDCl_3$) δ 3 × 20.8, 21.4 (OAc's), 23.1 (NAc), 27.2 (β-C), 28.2 (CH_3's), 32.5 (SCH_2), 37.4 (C-3), 38.0 (γ-C), 49.3 (C-5), 51.8 (α-C), 53.2 (MeO), 55.2 ($NCH_2C(O)$), 62.3 (C-9), 67.1 (C-7), 68.2 (C-8), 69.4 (C-4), 74.0

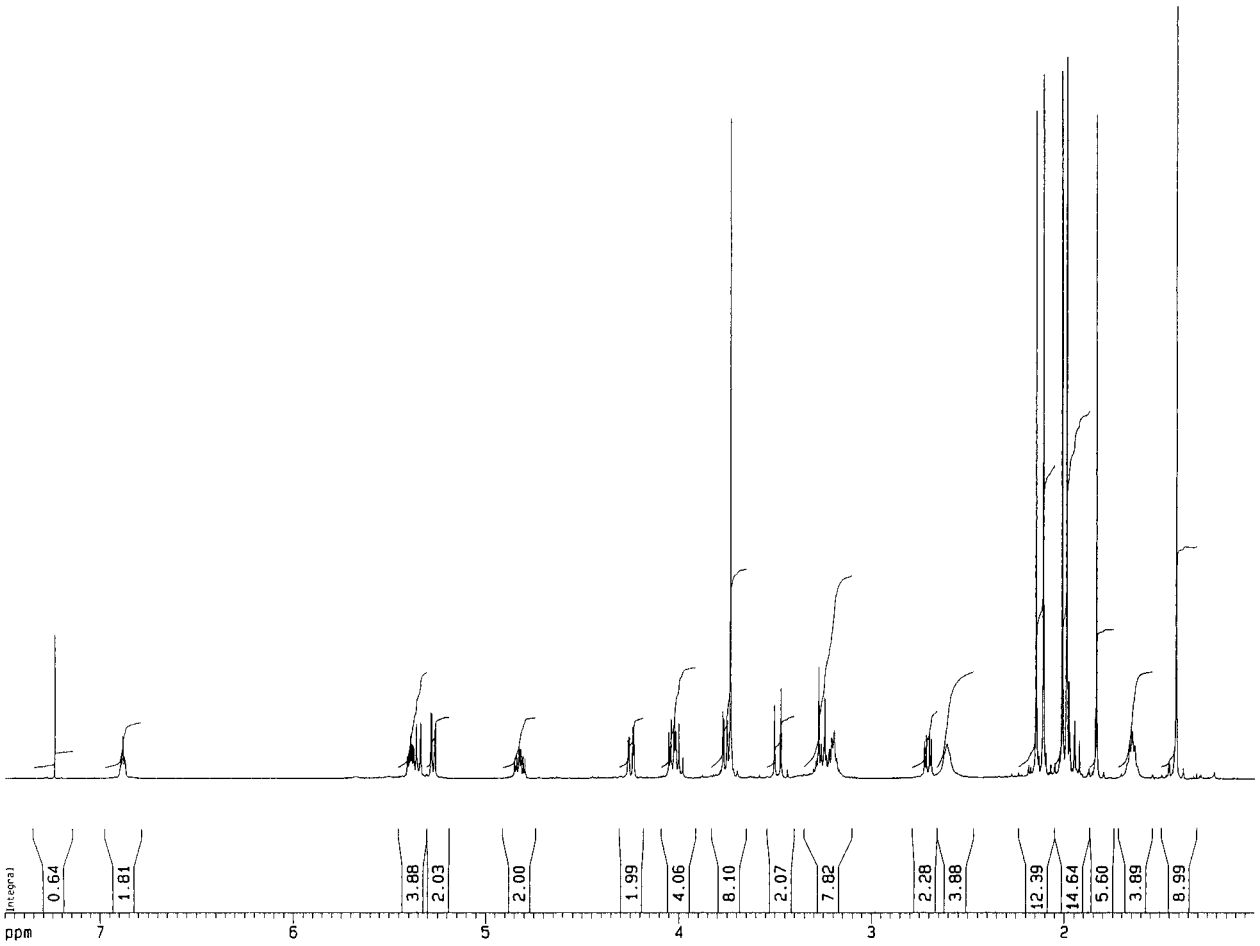


Figure 1. ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound **20**.

(C-6), 82.3 (C-2), 168.2−171.0 (C═O's); FAB-MS (pos) calcd for $C_{56}H_{85}N_5O_{29}S_2$ 1339.48, found 1340.8 ($M^+ + 1$, 28.0% base peak).

22: ^{1}H-NMR ($CDCl_3$) δ 1.44 (bs, 9H, *t*Bu), 1.84 (s, 12H, NAc), 1.99, 2.01, 2.02, and 2.11 (4s, 48H, OAc's), 3.76 (s, 12H, CO_2CH_3), 3.76−3.83 (m, 4H, H-6); ^{13}C-NMR ($CDCl_3$) δ 20.9, 21.0, and 21.5 (OAc's), 23.1 (NAc), 28.1 (CH_3's), 32.5 (SCH_2), 82.2 (C-2).

24: ^{1}H-NMR (DMSO-d_6) δ 1.40 (s, 9H, *t*Bu), 1.65 (s, 24H, NAc), 1.92, 1.98, 2.00, and 2.08 (4s, 96H, OAc's), 3.75 (s, 24H, CO_2CH_3), 3.76−3.95 (m, 24H, H-5, H-6, H-9); ^{13}C-NMR (DMSO-d_6) δ 2 × 20.6, 20.8, and 21.0 (OAc's), 22.6 (NAc), 27.8 (CH_3's), 32.5 (SCH_2), 82.3 (C-2).

26: ^{1}H-NMR (DMSO-d_6) δ 1.40 (bs, 9H, *t*Bu), 1.66 (s, 48H, NAc), 1.92, 1.97, 2.02, and 2.08 (4s, 192H, OAc's), 3.74 (s, 48H, CO_2CH_3), 3.74−3.82 (m, 48H, H-5, H-6, H-9); ^{13}C-NMR (DMSO-d_6) δ 2 × 20.6, 20.8, and 21.0 (OAc's), 22.6 (NAc), 28.1 (CH_3's by HMQC only), 32.2 (SCH_2), 82.3 (C-2).

Preparation of Fully Deprotected Dendritic α-Thiosialosides 21, 23, 25, 27. Typical Procedure: To peracetylated glycodendrimer **20** (50 mg, 0.04 mmol) dissolved in DMSO (0.5 mL) was added 1 N NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 8 h, MeOH was removed *in vacuo* and 0.05 N NaOH added (5 mL). Again the mixture was stirred for 8 h at 25 °C. Solvent was removed by lyophilization, and the residue was purified by gel permeation chromatography on Biogel-P2 column. Title compound **21** was isolated, after freeze-drying, as a white, spongy solid in 58% yield (21 mg, 0.02 mmol).

Glycodendrimers **23**, **25**, and **27** were synthesized as above in 58, 47, and 53% yields after GPC from compounds **22**, **24**, and **26**, respectively.

21: ^{1}H-NMR (D_2O) δ 1.86 and 1.93 (2dd, 2H, *J* 12.3 Hz, *J* 12.3 Hz, H-3ax), 1.97−2.08 (m, 4H, β-CH_2), 2.10 and 2.11 (2s, 6H, NAc), 2.84−2.95 (m, 2H, H-3eq), 3.30−3.49 (m, 8H, α-CH_2, γ-CH_2), 3.54−3.99 (m, 20H, $NCH_2C(O)$, SCH_2, and NeuAc residues excluding above); ^{13}C-NMR (D_2O) δ 21.6 and 21.7 (NAc), 23.1 (β-C), 32.6 (SCH_2), 36.2 (γ-C), 38.6 and 40.1 (C-3), 41.0 and 51.2 (C-5), 51.8, 52.3, and 52.5 (α-C's), 55.2 (NCH_2C-(O)), 62.2 and 62.8 (C-9), 66.8 and 67.4 (C-7), 67.7, 68.0, and 68.1 (C-8), 68.2, 71.2, and 71.4 (C-4), 74.1, 74.4, and 74.6 (C-6), 84.5 and 85.1 (C-2), 169.7-174.6 (C═O's); FAB-MS (pos) calcd for $C_{34}H_{57}N_5O_{20}S_2$ 919.30, found 920.3 ($M^+ + 1$, 0.6% base peak).

23: ^{1}H-NMR (D_2O) δ 1.76−2.18 (m, 28H, β-CH_2's, H-3ax, NAc), 2.80−2.94 (m, 4H, H-3eq), 2.55−2.68 and 3.20−3.95 (2m, 76H, α-CH_2's, γ-CH_2's, $NCH_2C(O)$, SCH_2, and NeuAc residues excluding above); ^{13}C-NMR (D_2O) δ 21.6 (NAc), 38.7 (SCH_2), 84.4 (C-2).

25: ^{1}H-NMR (D_2O) δ 1.84−2.15 (m, 60H, β-CH_2's, H-3ax, NAc), 2.86 (dd, 8H, $J_{3eq,4}$ 12.7 Hz, $J_{3ax,3eq}$ 4.8 Hz, H-3eq), 3.20−3.99 (m, 164H, α-CH_2's, γ-CH_2's, $NCH_2C(O)$, SCH_2, and NeuAc residues excluding above); ^{13}C-NMR (D_2O) (from HMQC) δ 21.5 (NAc), 32.5 (SCH_2).

27: ^{1}H-NMR (D_2O) δ 1.80−2.11 (m, 124H, β-CH_2's, H-3ax, NAc), 2.80−2.92 (m, 16H, H-3eq), 3.14−4.00 (m, 340H, α-CH_2's, γ-CH_2's, $NCH_2C(O)$, SCH_2, and NeuAc residues excluding above); ^{13}C-NMR (D_2O) δ 21.5 (NAc), 32.5 (SCH_2), 85.2 and 86.9 (C-2).

Acknowledgment. We thank Dr. Glenn Facey and Dr. Raj Capoor for running NMR spectral data. We are grateful to the National Sciences and Engineering Council of Canada (NSERC) for financial support and for a postgraduate scholarship to D.Z.

Supporting Information Available: ^{1}H and ^{13}C NMR data of chromatographically isolated compounds **5**, **6**, **8**−**10**, **14**, **17**, **20**, **21**, **23**, **25**, and **27** (24 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO961047Z