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for my parents Stefano and Vincenza Zanini

ABSTRACT

The syntheses and *in vitro* biological evaluation of three series of glycodendrimers is presented herein. This dissertation marks three "firsts" in the field of glycobiology. Chemically well defined hypervalent neoglycoconjugates, termed glycodendrimers, have been designed and prepared. These concepts were extended to include the enzymatic elongation of a glycoside on a dendritic core. Furthermore, the first direct correlation of the number of carbohydrate residues and the multivalent effect has been reported.

To further the understanding of multivalency and its role in carbohydrate-protein interactions, glycoconjugates with differing carbohydrate densities, conformations, and interglycosidic spacings must be prepared. The design and synthesis of glycodendrimers addresses these issues.

Solid phase synthesis on Wang resin was used to construct dendritic α-thiosialosides. The design of these new, hyperbranched clusters was based on the rational scaffolding of L-lysine core structures using established 9-fluorenylmethoxycarbonyl (Fmoc) protecting group and activated benzotriazolyl ester (HOBt) coupling procedures. Chain extension of the lysyl amino groups with chloroacetylglycylglycine active ester allowed introduction of the required functionality necessary for the coupling to an α-thiosialoside derivative prepared under phase transfer catalysed conditions. Well defined di-, tetra-, octa-, and hexadeca-valent dendritic α-thiosialosides were thus prepared.

The synthesis of structurally similar divergent and spherical dendrimers, with even valencies of between two and sixteen and ending with equidistant α-thiosialoside residues is also described. The synthesis of the dendritic core was based on the regioselective protection of the primary amines of 3,3'-iminobis(propylamine) using benzyl cyanoformate. The resulting secondary amine was monoalkylated with *tert*-butyl bromoacetate to provide a divalent core structure orthogonally protected with carbobenzyloxy (Cbz) protected amines and a *tert*-butyl ester. Selective deprotection *via* hydrogenation or trifluoroacetolysis afforded amine and acid key precursors, respectively. These were conjugated using standard HOBt/DIC strategy to give divergent, Cbz-

protected dendrimers with valencies between two and sixteen in the first through fourth generations. Tethering of the dimer and tetramer to both hexamethylenediamine and tris-(2-aminoethyl)amine provided spherical, hyperbranched dendritic structures with valencies between four and twelve. All Cbz-protected dendrimers were transformed into N-chloroacetylated dendrimers which were coupled to a thiolated sialic acid derivative.

Thiourelene *p*-phenyl α-thiosialoside containing dendrimers scaffolded on Starburst® PAMAM dendrimers were prepared using an isothiocyanate conjugation strategy. PAMAM cores, generation G0 to G3, were coupled to a sialic acid isothiocyanate derivative to give spherical, hypervalent thiourea derivatives containing four to thirty-two surface sialic acid residues.

The above concepts were easily extended to include the preparation of dendrimers with covalently attached N-acetylglucosamine, lactose, N-acetyllactosamine, mannose, Galβ-(1,3)-GalNAcα (T-antigen), and 3'-sulfo-Lewis^{x}-(Glc) residues. Furthermore, glycodendrimers were shown to be amenable to both chemical and enzymatic transformations *via* the 9-O-acetylation of octavalent α-thiosialodendrimers and the enzymatic galactosylation of dendritic N-acetylglucosamine.

The preparation of a custom designed heterobifunctional dendrimer containing biotin and four sialic acid residues is also presented. The synthesis was based on the tethering of a tetravalent, Cbz-protected, 3,3'-iminobis(propylamine)-based core to an amine functionalized biotin derivative using established HOBt/DIC coupling chemistry. Cbz deprotection *via* hydrogenation afforded the biotin-containing tetraamine which was conjugated to a sialic acid *p*-isothiocyanatophenyl derivative to provide the desired bi-directional glycodendrimer.

Binding studies *via* double immunodiffusion and/or turbidimetric analysis confirmed the ability of the glycodendrimers to cross-link and precipitate appropriate model lectins. In addition, when used in competitive enzyme linked lectin assays, all glycodendrimers showed improved inhibitory potentials.

ACKNOWLEDGMENTS

What follows is a short list of the people I would like to thank. Without their patience and support, this thesis would not be possible.

I would like to thank my supervisor, Professor René Roy. His enthusiasm for chemistry and its applications is boundless and makes working with him a pleasure. His knowledge of the field is extensive and makes one strive to learn more. His capacity for generating new ideas is phenomenal and makes one envious. I could not have asked for more from a supervisor and I am grateful.

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I am thankful for the fast and efficient technical support at the University of Ottawa. I would like to express my appreciation to Dr. Glenn Facey, Raj Capoor, and Dr. Clem Kazakoff. Their help and dedication to obtaining high quality, high resolution NMR and MS data allowed me to present these results.

Lastly, I would like to thank my parents Stefano and Vincenza Zanini and my sisters Giovanna, Lavinia, and Stefania. To be acknowledged in one's Ph. D. thesis seems like very small thanks for people who have done so much. I love you.

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LIST OF ABBREVIATIONS

ABTS	2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
Ac	acetyl
AcCl	acetyl chloride
AcOH	acetic acid
Ala	alanine
b	broad
BOC	*t*-butoxycarbonyl
bs	broad singlet
BSA	bovine serum albumin
t-Bu	*tert*-butyl
Cbz	carbobenzyloxy
CI	chemical ionization
COSY	correlation spectroscopy
d	doublet
Da	Dalton
DCC	dicyclohexylcarbodiimide
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhanced polarization transfer
DIC	diisopropylcarbodiimide
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMSO-d_6	hexadeuterated dimethylsulfoxide
ECA	*Erythrina Cristagalli* lectin
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

ELLA	enzyme linked lectin assay
eq.	equivalent(s)
EtOAc	ethyl acetate
EtOH	ethanol
Et_3N	triethylamine
FAB-MS	fast atom bombardment ionization mass spectrometry
Fmoc	9-fluorenylmethoxycarbonyl
Gal	galatose
Glc	glucose
GlcNAc	N-acetylglucosamine
GlcNAc β(1,4)GalT	N-acetylglucosamine β(1,4) galactosyltransferase
GPC	gel permeation chromatography
h	hour(s)
HMQC	heteronuclear multiple quantum coherence
HOBt	1-hydroxybenzotriazole
HRP	horseradish peroxidase
HSAc	thiol acetic acid
Hz	Hertz
IC_{50}	concentration required for 50% inhibition
kDa	kiloDaltons
Lac	lactose
LacNAc	N-acetyllactosamine
LFA	*Limax flavus* lectin
Lit.	literature
Lys	lysine
m	multiplet
M^+	parent molecular ion
MAP	multiple antigen peptide
Me	methyl

min	minute(s)
m.p.	melting point
MS	mass spectrometry
MW	molecular weight
m/z	mass to charge ratio
Neu(5)Ac	N-acetylneuraminic acid
NMR	nuclear magnetic resonance
Nu	nucleophile
O.D.	optical density
PAMAM	poly(amidoamine)
Ph	phenyl
pos.	positive
ppm	parts per million
PTC	phase transfer catalysis
R_f	retention factor
r.t.	room temperature
s	singlet
sLe^x	sialyl $Lewis^x$
SPPS	solid phase peptide synthesis
t	triplet
TBAHS	tetrabutylammonium hydrogen sulfate
TFA	trifluoroacetic acid
TLC	thin layer chromatography
TT	tetanus toxoid
WGA	wheat germ agglutinin

CHAPTER 1. Introduction

1.1. The Biological Significance of Carbohydrates

Glycobiology, a rapidly expanding field of late, deals with the nature and role of carbohydrates in biological events. Carbohydrates are among the most abundant organic constituents of plants and animals. They not only serve as an important source of chemical energy for living organisms but are involved in numerous biological functions such as cell growth, regulation, and differentiation, cellular trafficking, cancer metastasis, inflammation, and bacterial and viral infection processes.[1]

A cell surface is comprised mainly of glycolipids, glycoproteins, proteoglycans, and capsular polysaccharides.[2] This makes carbohydrates intimately involved in intracellular events. Figure 1.1.1 depicts ways in which this occurs. For example, N-acetyllactosamine (Galβ(1,4)GlcNAc, LacNAc) is well known as a biologically important disaccharide core structure of lactosaminoglycans and tumor-associated antigenic carbohydrates[3,4] and have been implicated in mouse colon cancers,[5] some thyroid disorders,[6] the sexual transmission of *Hemophilus Ducreyi* pathogen,[7] and corneal epithelial cell migration.[8]

---

1 Varki, A. *Glycobiology* **1993**, *3*, 97.

2 Sharon. N. (Ed.) in *Complex Carbohydrates: Their Chemistry, Biosynthesis, and Functions*, Addison-Wesley, Reading MA, **1975**.

3 Ando, K.; Kikugawa, K.; Beppu, M. *J. Biol. Chem.* **1994**, *269*, 19394.

4 Eggins, I.; Fenderson, B. A.; Toyokuni, T.; Dean, B.; Stroud, M. R.; Hakomori, S. *J. Biol. Chem.* **1989**, *264*, 9476.

5 Kawakani, H.; Ito, M.; Miura, Y.; Hirano, H. *J. Gastroenterology & Hepatology* **1994**, *9*, 567.

6 Fenouillet, E.; Thibault, V.; Miquelis, R. *Endocrinology* **1995**, *136*, 4204.

7 Melaugh, W.; Phillips, N. J.; Campagnari, A. A.; Tullius, M. V.; Gibson, B. W. *Biochemistry* **1994**, *33*, 13070.

8 Panjwani, N.; Zhao, A.; Ahmad, S.; Yang, Z. T.; Jungalwala, F.; Baum, J. *J. Biol. Chem.* **1995**, *270*, 14015.

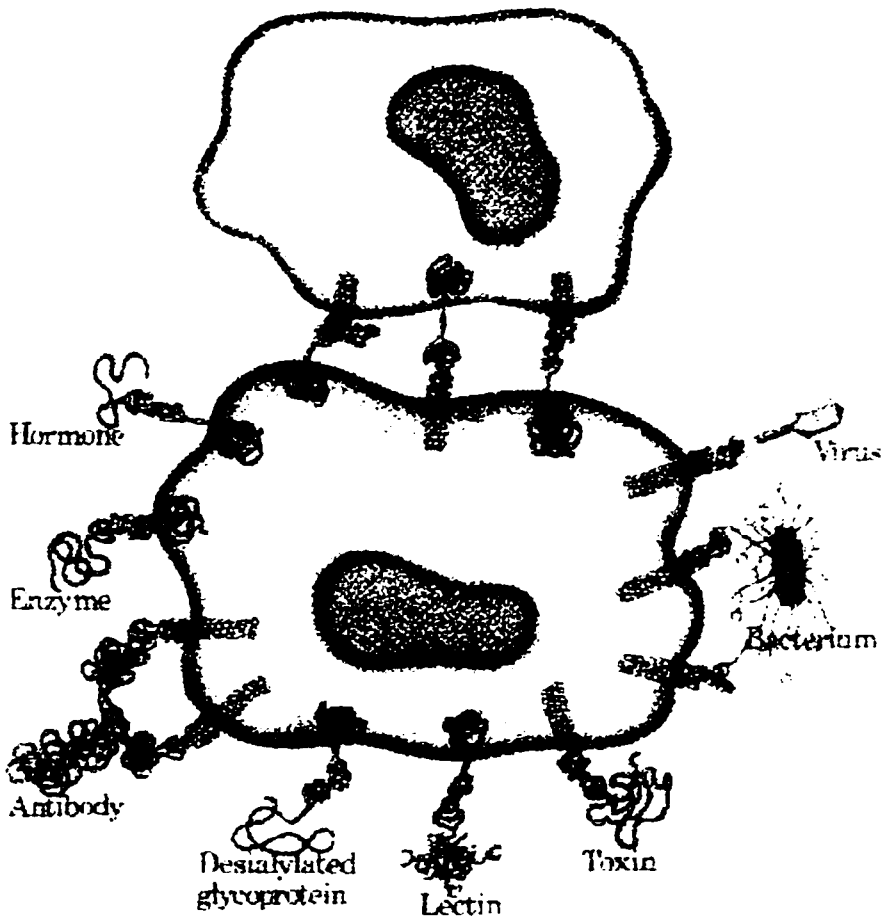

Figure 1.1.1.[9] Cell surface carbohydrate interactions.

Mannose (Man) protein binding interactions are involved in receptors found on macrophages,[10] hepatic sinusoidal cells,[11] fimbriated bacterial pathogens,[12,13] serum-type mannose binding proteins (MPB),[10] and placental receptors.[14]

---

[9] Figure taken from BioCarb Chemicals, **1990**.
[10] Drickamer, K.; Taylor, M. E. *Ann. Rev. Cell Biol.* **1993**, *9*, 237.
[11] Ashwell, G.; Harford, J. *Ann. Rev. Biochem.* **1982**, *51*, 531.
[12] Sharon, N.; Lis, H. *Science* **1989**, *246*, 227.
[13] Beachey, E. H. *J. Inf. Dis.* **1981**, *143*, 325.
[14] Curtis, B. M.; Scharnowske, S.; Watson, A. J. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 8356.

Sialic acid (NeuAc) terminated glycans (sialyloligosaccharides) present on cell surface glycoproteins and glycosphingolipids are involved in a large variety of biological events.[15] As boundary residues, sialic acids (Figure 1.1.2) are ideally positioned to participate in numerous carbohydrate-protein interactions mediating cell surface recognition phenomena. Sialic acids and sialyloligosaccharides have a variety of functions that span from trivial roles in structural, physical, stabilizing, and protective properties to very sophisticated roles in cell signal mediation[1] and as masking antigens where they disguise other carbohydrate antigens.[16]

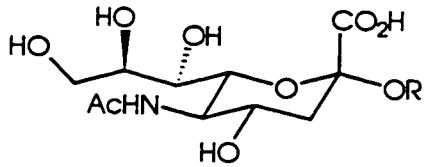

Figure 1.1.2. α-Sialosides as they appear on most natural cell surface gangliosides and sialylated glycoproteins.

More specifically, sialyl Lewis^x (NeuAc-α-(2,3)-Gal-β-(1,4)-[Fuc-α-(1,3)]-GlcNAc-β(1-OR), sLe^x, **1**, Figure 1.1.5) and related structures have been shown to be key players in the blockage of the coordinated sequence of events leading to inflammation.[17] SLe^x binds to selectins, sialoconjugate-binding molecules that mediate intercellular adhesions, at the onset of the migration of white blood cells to infection sites.[18,19]

Sialic acid itself has attracted interest by both academic and industrial scientists because of its involvement in *Influenza* virus infections. It plays two critical roles.

---

[15] Schauer, R. (Ed.) *Sialic Acids: Chemistry, Metabolism & Functions* (Cell Biology Monographs) Vol. 10, Springer-Verlag, Wien, **1992**.
[16] Schauer, R. in *Advances in Experimental Medicine and Biology* (Ed.: Wu, A. M.) Vol. 28, Plenum Press, New York, **1988**, pp. 147.
[17] Lasky, L. A. *Science* **1992**, *258*, 964.
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[19] Lasky, L. A. *Ann. Rev. Biochem.* **1995**, *64*, 113.

Firstly, it constitutes the key epitope recognized as being essential for virus binding. Secondly, sialic acid acts as a substrate for viral sialidase (neuraminidase) which is believed to be necessary for the release of viron particles from the infected cells. The critical role played by α-sialosides has been confirmed by inhibition experiments with synthetic α-sialosides,[20] X-ray data,[21] and proton NMR spectroscopic studies.[22]

Because viral and bacterial adhesion is so critical to infections, pharmaceutical industries are seriously considering the use of carbohydrates for prevention and treatment.

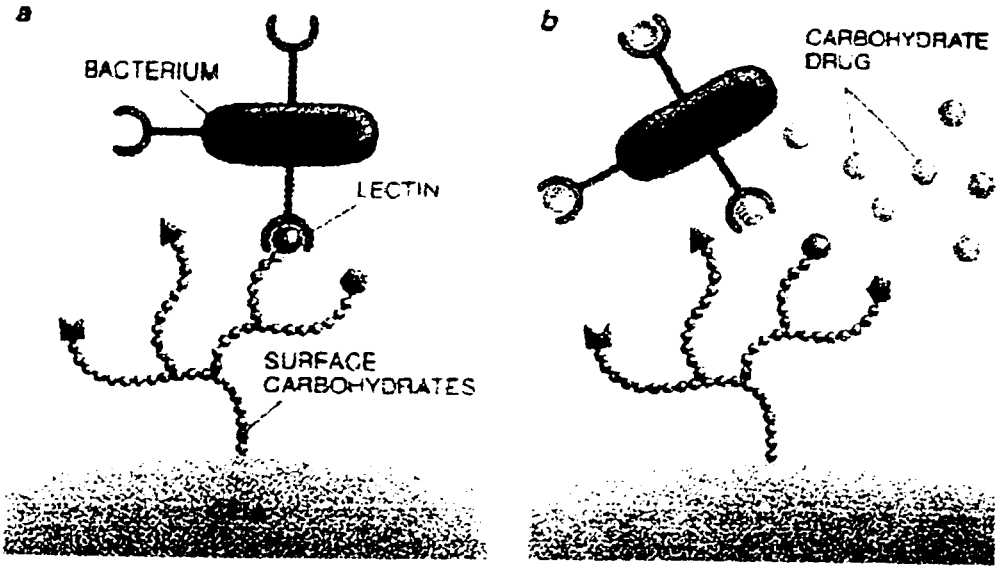


Figure 1.1.3.[23] Blocking bacterial attachment using carbohydrate drugs. (a) The onset of infection, when viral or bacterial surface proteins attach to host surface glycosides on susceptible host cells. (b) Similar carbohydrate-containing drugs could prevent attachment by binding to viral or bacterial surface proteins.

---

[20] Pritchett, T. J.; Brossner, R.; Rose, U.; Paulson, J. C. *Virology* **1987**, *160*, 502.

[21] Weiss, W.; Brown, J. H.; Cusack, S.; Paulson, J. C.; Skehel, J. J.; Wiley, D. C. *Nature* **1988**, *333*, 426.

[22] Sauter, N. K.; Bednarski, M. D.; Wurzburg, B. A.; Hanson, J. E.; Whitesides, G. M.; Skehel, J. J.; Wiley, D. C. *Biochemistry* **1989**, *28*, 8388.

[23] Figure taken from Sharon, N.; Lis, H. *Sci. Amer.* **1993**, 82.

Sugars that selectively inhibit adhesion could act as molecular decoys, intercepting viruses and/or pathogenic bacteria before they can attack cells. Figure 1.1.3 depicts this.

Part of the ability of carbohydrates to function as information molecules has been attributed to their structural diversity. Considering the smallest possible sugar chain: A—B in which A could be galactose and B mannose (Figure 1.1.4), galactose (A) could be linked to mannose (B) at four different positions: C-2, C-3, C-4, and C-6 forming a possibility of four different isomeric structures. Then too a galactose residue may take two anomeric functions, the number of possible isomers then doubles to eight. Furthermore, galactose may occur in the furanose form as well as in the pyranose form shown in Figure 1.1.4. This increases the number of possible isomeric forms to sixteen. When the number of carbohydrate residues increases to three, four, *etc.*, the number of isomeric sugar chains increases by geometrical progression.[24] This complexity has made research in carbohydrate chemistry relatively slow as compared with other scientific fields.

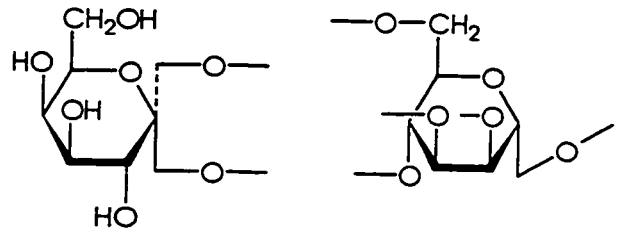


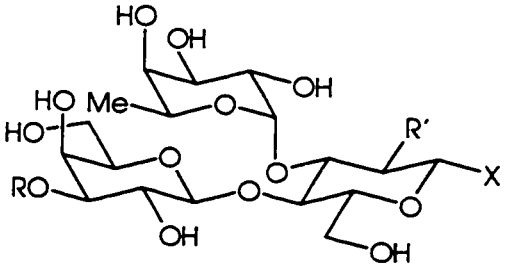
Figure 1.1.4. Possibilities for the construction of polymeric sugars.

Another difficulty to be overcome by carbohydrate chemists is that individual carbohydrate-protein interactions are often of low affinity with dissociation constants

[24] Kobata, A. *Acc. Chem. Res.* **1993**, *26*, 319.

(K_Ds) in the milliMolar range.[25] These drawbacks have led to the search for ligands of high specificity and high affinity.

Traditional methods of structure activity relationships (SAR) have led to the development of effective inhibitors for various carbohydrate-protein interactions. For example, SAR relationships towards L-, P-, and E-selectins have been performed to improve the poor binding properties of sialyl Lewis^X (**1**, Figure 1.1.5).[26] It was recognized that the N-acetylglucosamine residue (GlcNAc) could be replaced by a glucose (Glc) residue to provide a simpler sLe^X-(Glc) analog (**2**) which was more active than sLe^X (**1**) against all three selectins.[27,28] Furthermore, it was found that the sialic acid residue could be replaced by a negatively charged group (**3**, Figure 1.1.5) and that the corresponding sulfated analog **3** was a better ligand for E-selectins, but not for L- and P-selectins.[27] An analog containing both modifications afforded new derivative **4** (3'-sulfo-Le^X-(Glc)) which was as active as **1** towards E-selectins but had lower affinity towards L- and P-selectins.[28,29] Research is ongoing in the exploration of carbohydrate lead compounds *via* these methods.



1 R = α-NeuAc, R' = NHAc (sLeX)
2 R = α-NeuAc, R' = OH (sLeX-(Glc))
3 R = SO_3^-, R' = NHAc (3'-sulfo-LeX)
4 R = SO_3^-, R' = OH (3'-sulfo-LeX-(Glc))

Figure 1.1.5. Sialyl Lewis^X and related structures.

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[25] Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719.
[26] Roy, R. in *Carbohydrates: Targets for Drug Design* (Ed.: Witczak, Z. J.) Marcel Dekker, Inc., New York, **1997**, pp. 82.
[27] Dasgupta, F.; Rao, B. N. N. *Exp. Opin. Invest. Drugs* **1994**, *3*, 709.
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[29] Lubineau, A.; Le Gallic, J.; Lemoine, R. *Bioorg. Med. Chem.* **1994**, *2*, 1143.

1.2. The Cluster or Multivalent Effect

These strategies, while they constitute viable tools with which to investigate lead compounds, neglects another class of interactions. It is now recognized that multiple carbohydrate-protein interactions may cooperate in some recognition events to amplify affinity. This too can account for the interactions between extracellular proteins and carbohydrates.

These cluster or multivalent cooperative effects rely on the arrangement of multiple receptors in such a way as to bind multiple carbohydrates having well organized architectures. It is known that numerous receptors have clustered carbohydrate domains. For example, the biological forms of L-, P-, and E-selectins[19,30,31] and the natural ligands themselves, *i. e.* GLYCAM-1, CD34, MAdCAM-1 for L-selectin and PSGL-1 for P-selectin,[32,33] are likely to have clustered arrangements. Many mannose receptors found on serum-type binding proteins, macrophages, and hepatic sinusoidal cells too have carbohydrate recognition domains organized in a multivalent manner.[34,35]

Consequently, neoglycoproteins[36,37,38] and glycopolymers[37,39] have been successfully used to demonstrate that multivalency does indeed amplify carbohydrate protein interactions by factors as high as thousands. Figure 1.2.1 illustrates possible ways in which this could happen.[40,41,42]

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30 Feizi, T. *Curr. Opin. Struct. Biol.* **1993**, *3*, 701.
31 McEver, R.P.; Cummings, R. D.; Varki, A. *J. Biol. Chem.* **1993**, *268*, 12764.
32 Rosen, S. D.; Bertozzi, C. R. *Curr. Opin. Cell Biol.* **1994**, *6*, 663.
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35 Taylor, M. E.; Bezouška, K.; Drickamer, K. *J. Biol. Chem.* **1992**, *267*, 1719.
36 Stowell, C. P.; Lee, Y. C. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225.
37 Lee, Y. C.; Lee, R. T. (Eds.) *Neoglycoconjugates: Preparations and Applications*, Academic Press, San Diego, **1994**.
38 Lee, Y. C.; Lee, R. T. *Methods Enzymol.* **1994**, *242* and *247*.
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40 Drickamer, K.; Taylor, M. E. *Ann. Rev. Cell Biol.* **1993**, *9*, 237.
41 Barondes, S. H.; Cooper, D. N. W.; Gitt, M. A.; Leffler, H. *J. Biol. Chem.* **1994**, *269*, 20807.
42 Kiessling, L. L.; Pohl, N. L. *Chem. Biol.* **1996**, *3*, 71.

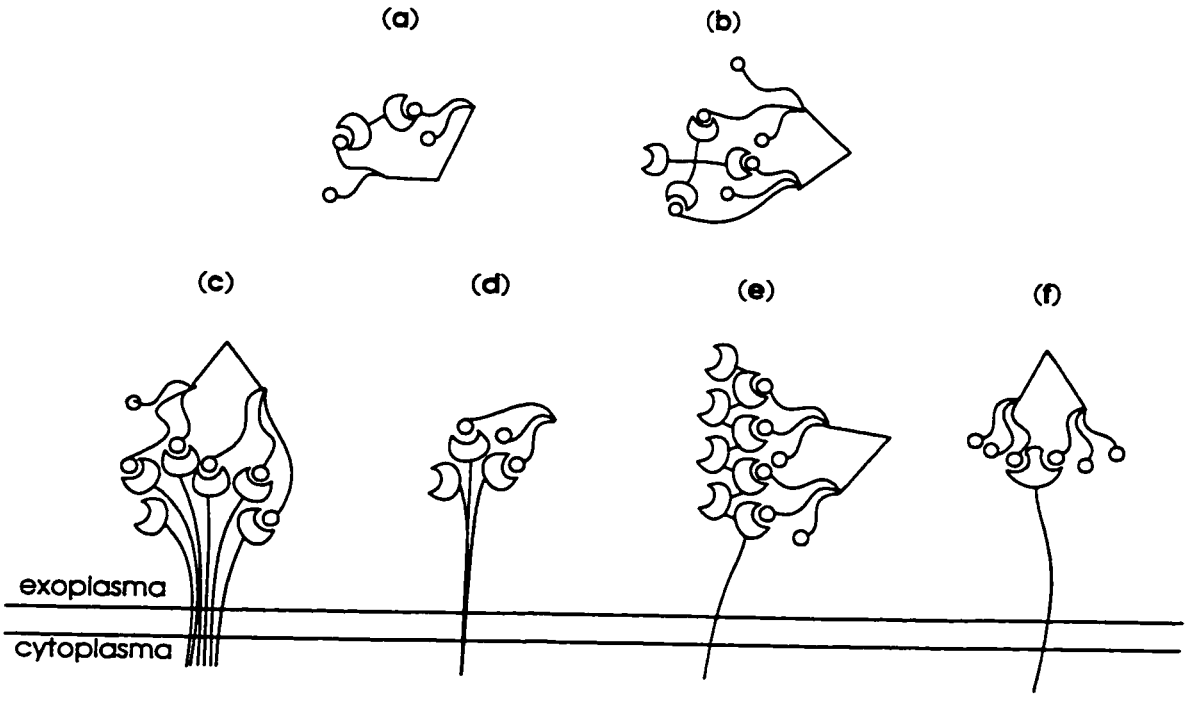

Figure 1.2.1. Amplification of specific carbohydrate-protein recognition processes *via* multivalent interactions: (a) and (b) soluble di- and tetra-valent receptors, *e.g.* galectins and phytohemagglutinins, (c) clustered monovalent receptors at the cell surface, *e.g.* hepatic asialoglycoprotein receptor, (d) and (e) oligomeric receptors, *e.g.* macrophage mannose receptor, and (f) receptors that bind more than one carbohydrate simultaneously.

However, neoglycoproteins and glycopolymers, by their very nature, have ill defined chemical structures. They are heterogenous in size and carbohydrate content. Thus, while they can demonstrate the role of multivalency in recognition processes they fail to allow precise biophysical analyses of the cluster effect. Chemically well defined glycoconjugates with systematically varied shapes and carbohydrate densities would constitute useful tools for a complete understanding of these important recognition processes. In addition, the use of chemically well defined glycoconjugates would offer better insights into the geometrical organization of the receptors under investigation.

Recently, much work has been devoted to the design and synthesis of such neoglycoconjugates. Compounds spanning from glycoclusters, with as little as two or three conjugated carbohydrate haptens, to spherical glycodendrimers, with numerous

surface carbohydrate residues, have been reported. These novel glycoconjugates, shown schematically in Figure 1.2.2, are being employed to probe these multiple interactions and the roles they play.

The remainder of this chapter will focus on current syntheses of neoglycoconjugates with varying shapes and carbohydrate valencies, excluding glycodendrimers. This thesis dissertation discusses glycodendrimers: their syntheses, *in vitro* biological testing, and possible applications.

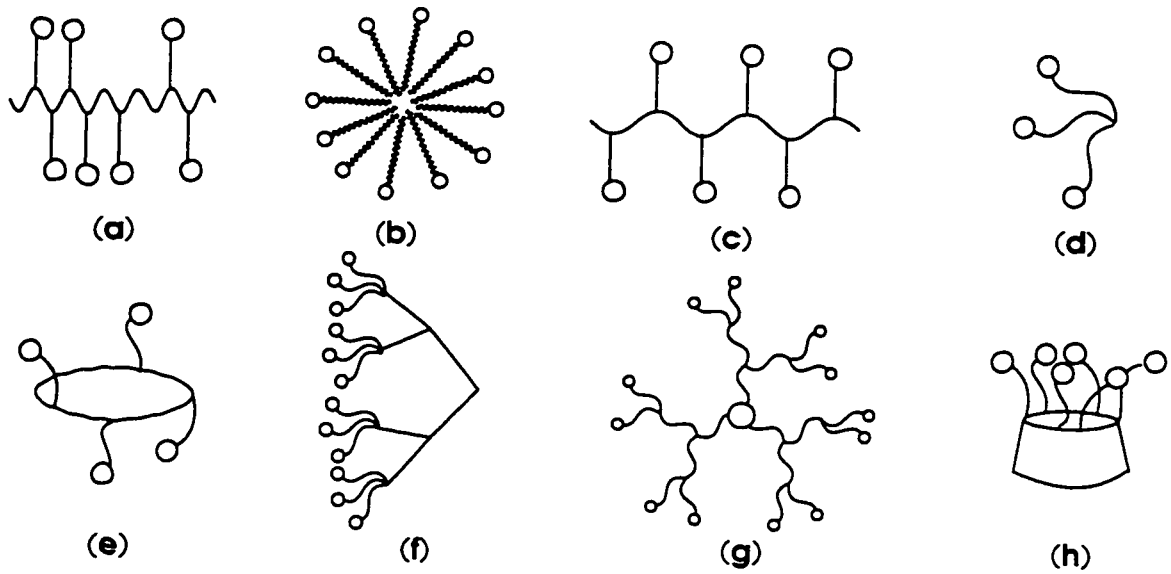

Figure 1.2.2. Multivalent carbohydrate ligands: possible shapes and valencies. (a) glycopolymers, (b) liposomes and micelles, (c) glycotelomers and glycopeptoids, (d) glycoclusters, (e) cyclic glycopeptides, (f) glycodendrimers (dendrons), (g) spherical glycodendrimers, and (h) cyclodextrins and glycocalixarenes.

1.3. Neoglycoproteins and Glycopolymers

Neoglycoproteins, neoglycolipids, and glycopolymers represent classical multivalent carbohydrate clusters. Many reviews currently exist on the subject.[36-39,43,44,45]

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[43] Roy, R. in *Modern Methods in Carbohydrate Synthesis* (Eds.: Khan, S. -H.; O'Neil, R.) Harwood Academic, Amsterdam, **1996**, pp. 378.

Increased binding avidity of these synthetic glycoconjugates have led to their use as models in a number of carbohydrate-protein interaction studies,[46,47] vaccines,[48] inhibitors of cell adhesions by viruses, bacteria, and toxins,[49] ligands in affinity chromatography,[50,51] and as drug carriers.[52,53,54]

Neoglycoproteins were first introduced as animal vaccines to prepare anti-carbohydrate antibodies.[48] These glycoconjugates were required because carbohydrates by themselves are poorly immunogenic since they are T cell independent antigens resulting in activation of only low affinity IgM immunoglobulins.[55] However, when conjugated to highly immunogenic protein carriers, carbohydrates become T cell dependent antigens capable of stimulating IgG antibodies with high affinities and specificities.

Stowell and Lee[38] have described the chemical methods available for the syntheses of neoglycoproteins. In early reports, allyl α-sialoside (**6**) was ozonolyzed and the resulting aldehyde conjugated to both bovine serum albumin (BSA) and tetanus toxoid (TT).[56] Similarly, *p*-formylphenyl sialoside **7** was coupled to both BSA and TT

---

[44] Bovin, N. V.; Korchagina, E. Yu; Zemlyanukhina, T. V.; Byramova, N. E.; Ivanov, A. E.; Zubov, U. P.; Mochalova, L. V. *Glycoconjugate J.* **1993**, *10*, 142.

[45] Magnusson, G.; Chernyak, A. Ya; Kihlberg, J. Kononov, O. in *Neoglycoconjugates: Preparations and Applications* (Ed.: Lee, Y. C.; Lee, R. T) Academic Press, San Diego, **1994**, pp. 53.

[46] Goldstein, I. J.; Poretz, R. O. in *The Lectins. Properties, Functions, and Applications in Biology and Medicine* (Eds.: Liener, I. E.; Sharon, N.; Goldstein, I. J.) Academic Press, Orlando, **1986**, pp. 35.

[47] Lemieux, R. U. *Acc. Chem. Res.* **1996**, *29*, 373.

[48] Dick, W. E.; Beurret, M. in *Contribution to Microbiology and Immunology* (Eds.: Cruse, J. M.; Lewis, Jr., R. E.) Vol. 10, Karger, Basel, **1989**, pp.48.

[49] Paulson, J. C. in *The Receptors* (Ed.: Coun, M.) Academic Press, New York, **1985**, pp. 131.

[50] Pazur, J. H. *Adv. Carbohydr. Chem. Biochem.* **1981**, *39*, 405.

[51] Schnaar, R. L. *Anal. Biochem.* **1984**, *143*, 1.

[52] Seymour, L. W. *Adv. Drug. Deliv. Rev.* **1994**, *14*, 89.

[53] Molema, G.; Meijer, D. K. F. *Adv. Drug. Deliv. Rev.* **1994**, *14*, 25.

[54] Monsigny, M.; Roche, A. -C.; Midoux, P.; Mayer, R. *Adv. Drug. Deliv. Rev.* **1994**, *14*, 1.

[55] Becker, R. S. *Springer Semin. Immunopathol.* **1993**, *15*, 217.

[56] Roy, R.; Laferrière, C. A.; Gamian, A.; Jennings, H. J. *J. Carbohydr. Chem.* **1987**, *6*, 161.

(Scheme 1.3.1).[57] It was concluded that threshold carbohydrate densities were required for efficient binding to model lectins, *i. e.* neoglycoproteins effectively conveyed the existence of cluster or multivalent effects. However, both conjugates were only mild inhibitors of *Influenza* virus[58] and conjugates with high NeuAc contents were highly immunogenic in rabbits, triggering IgG antibodies with high specificities against α-sialosides.[59] Sialoconjugate syntheses such as neoglycoproteins to be used as therapeutic inhibitors of the hemagglutination of *Influenza* viruses and other biological phenomena may have serious immunological consequences. The syntheses of potentially non-immunogenic, multivalent glycopolymers were then considered as an alternative.

In addition to their immunochemical properties, glycopolymers have many advantages. They may possess reasonably uniform and a wide range of molecular weights, carbohydrate densities and functionalities. Their purifications and characterizations are easier than their neoglycoprotein counterparts and they may be produced inexpensively and on large scale.[60]

Many strategies exist for the preparation of glycopolymers.[37,43-45,61,62] Carbohydrate monomers with alkenyl[63] or styryl[64] glycosides and N-[65] or O-acryloyl[66] residues have been prepared. These monomers can be copolymerized with other monomers such as acrylamide. Alternatively, carbohydrate precursors with amino or

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[57] Roy, R.; Tropper, F. D.; Romanowska, A.; Letellier, M.; Cousineau, L.; Meunier, S. J.; Boratyñski, J. *Glycoconjugate J.* **1991**, *8*, 75.

[58] Gamian, A.; Chomik, M.; Laferrière, C. A.; Roy, R. *Can. J. Microbiol.* **1991**, *37*, 233.

[59] Roy, R.; Laferrière, C. A.; Pon, R. A.; Gamian, A. *Methods Enzymol.* **1994**, *247*, 351.

[60] Roy, R. *Trends. Glycosci. Glycotechnol.* **1996**, *8*, 79.

[61] Bovin, N. V.; Gabius, H. J. *Chem. Soc. Rev.* **1995**, *24*, 413.

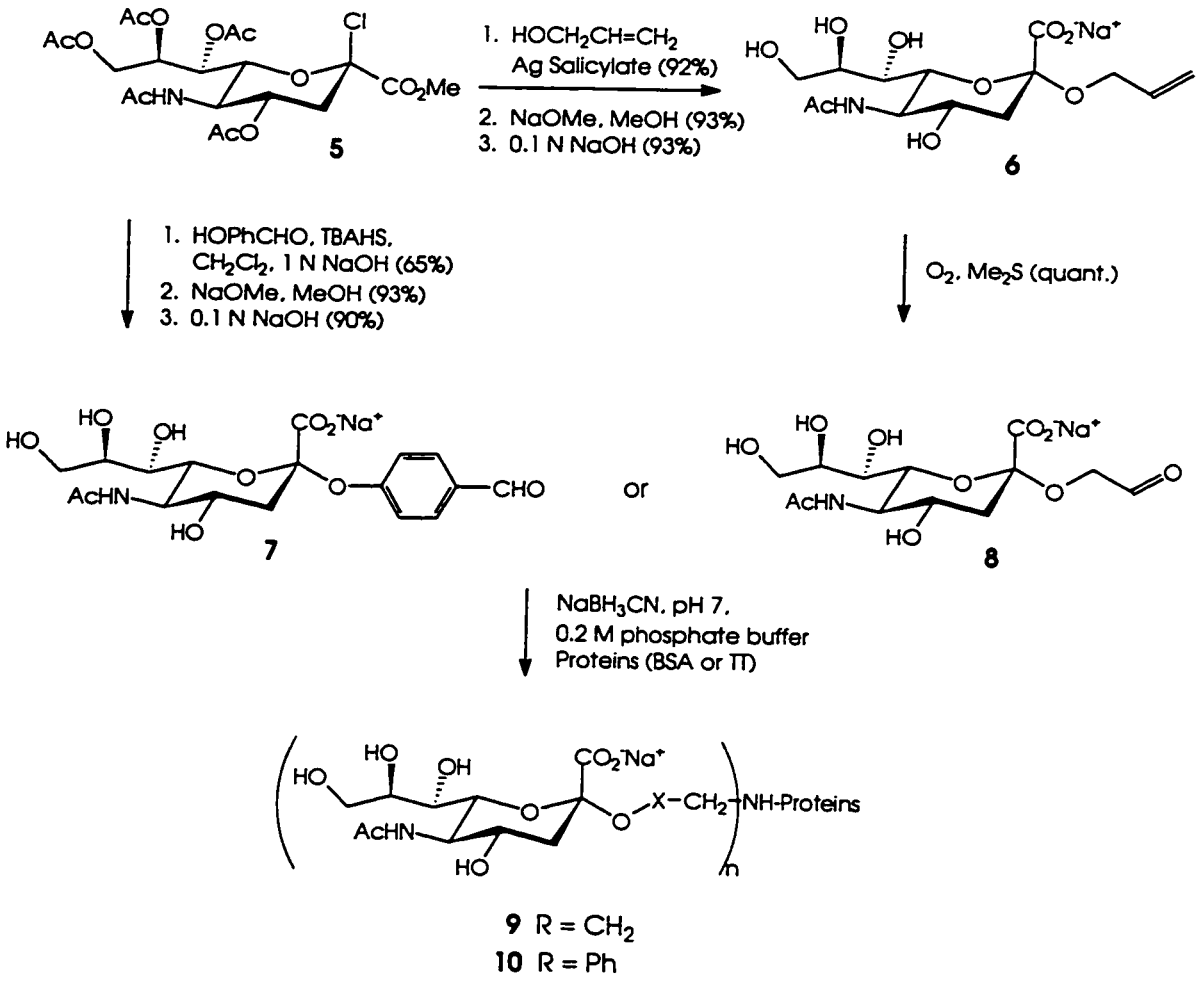
[62] Chernyak, A.Ya *ACS Symp. Ser.* **1994**, *560*, 133.

[63] Representative examples include: a) Horeskí, V.; Smolek, O.; Kocourek, J. *Biochim. Biophys. Acta* **1978**, *538*, 293; b) Kochetkov, N. K. *Pure Appl. Chem.* **1984**, *56*, 923; c) Kosma, P.; Waldstätten, P.; Daoud, L.; Schulz, G.; Unger, F. M. *Carbohydr. Res.* **1989**, *194*, 145; d) Nishimura, S. -I.; Matsuoka, K.; Furuike, T.; Nishi, N.; Tokura, S.; Nagani, K.; Murayama, S.; Kurita, K. *Macromolecules* **1994**, *27*, 157.

[64] Kobayashi, K.; Kobayashi, A.; Tobe, S.; Akaike, T. in *Neoglycoconjugates: Preparations and Applications* (Eds.: Lee, Y. C.; Lee, R. T.) Academic Press, San Diego, **19994**, pp. 262.

[65] Roy, R.; Tropper, F. D.; Romanowska, A. *Bionconjugate Chem.* **1992**, *3*, 256.

[66] Chytrý, V.; Driguez, H. *Makromol. Chem.; Rapid Commun.* **1992**, *13*, 499.



Scheme 1.3.1. Synthesis of Neoglycoproteins.

carboxyl functionalities can be synthesized and efficiently grafted to pre-formed polymers containing either active ester or amino groups.[67]

Polysialosides have been extensively studied as *Influenza* virus hemagglutination inhibitors. Polymers with O-,[56,67,68,69] S-,[70] and C-[71,72] α-sialosides have been made using

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[67] Byramova, N. E.; Mochalova, L. V.; Belyanchikov, I. M.; Matrosovich, M. N.; Bovin, N. V. *J. Carbohydr. Chem.* **1991**, *10*, 691.

[68] a) Roy, R.; Laferrère C. A. *Can J. Chem.* **1990**, *68*, 2045; b) Roy, R.; Laferrière, C. A. *Carbohydr. Res.* **1988**, *177*, C1; c) Roy, R.; Laferrière, C. A. *J. Chem. Soc., Chem. Commun.* **1990**, 1709.

the above techniques. These strategies too have been employed to generate polymers containing more complex oligosaccharides including sialyl Lewis^X,[73] sialyl Lewis^a,[74] 3'-sulfo-Lewis^X,[75] and GM_3 saccharide.[76] Scheme 1.3.2 depicts some representative examples of glycopolymers.[70-72,75]

Multivalent α-sialopolymers **14** and **15** were found to be potent inhibitors of the hemagglutination of human erythrocytes by *Influenza* A and C viruses.[58] Water soluble copolyacrylamide **17** combined glycomimetic and multivalent strategies to inhibit the binding of both L- and E-selectins in the μMolar range.[75] Glycopolymer **17** is the most potent L- and E-selectin inhibitor presently available and demonstrates that SAR and multivalent strategies may work cooperatively to give ligands with large binding affinities.

Still, while neoglycoproteins and glycopolymers can effectively demonstrate the cluster effect, their intrinsic heterogeneity precludes precise biophysical analyses of multivalency and the role it plays in carbohydrate-protein interactions. Chemically well-defined glycoconjugates with known glycosidic contents are necessary.

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[69] Lees, W. J.; Spaltenstein, A.; Kingery-Wood, J. E.; Whitesides, G. M. *J. Med. Chem.* **1994**, *37*, 3419.

[70] Roy, R.; Andersson, F.; Harms, G.; Kelm, S.; Schauer, R. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1478.

[71] a) Sigal, G. B.; Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 3789; b) Sparks, M. A.; Williams, K. W.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 4179.

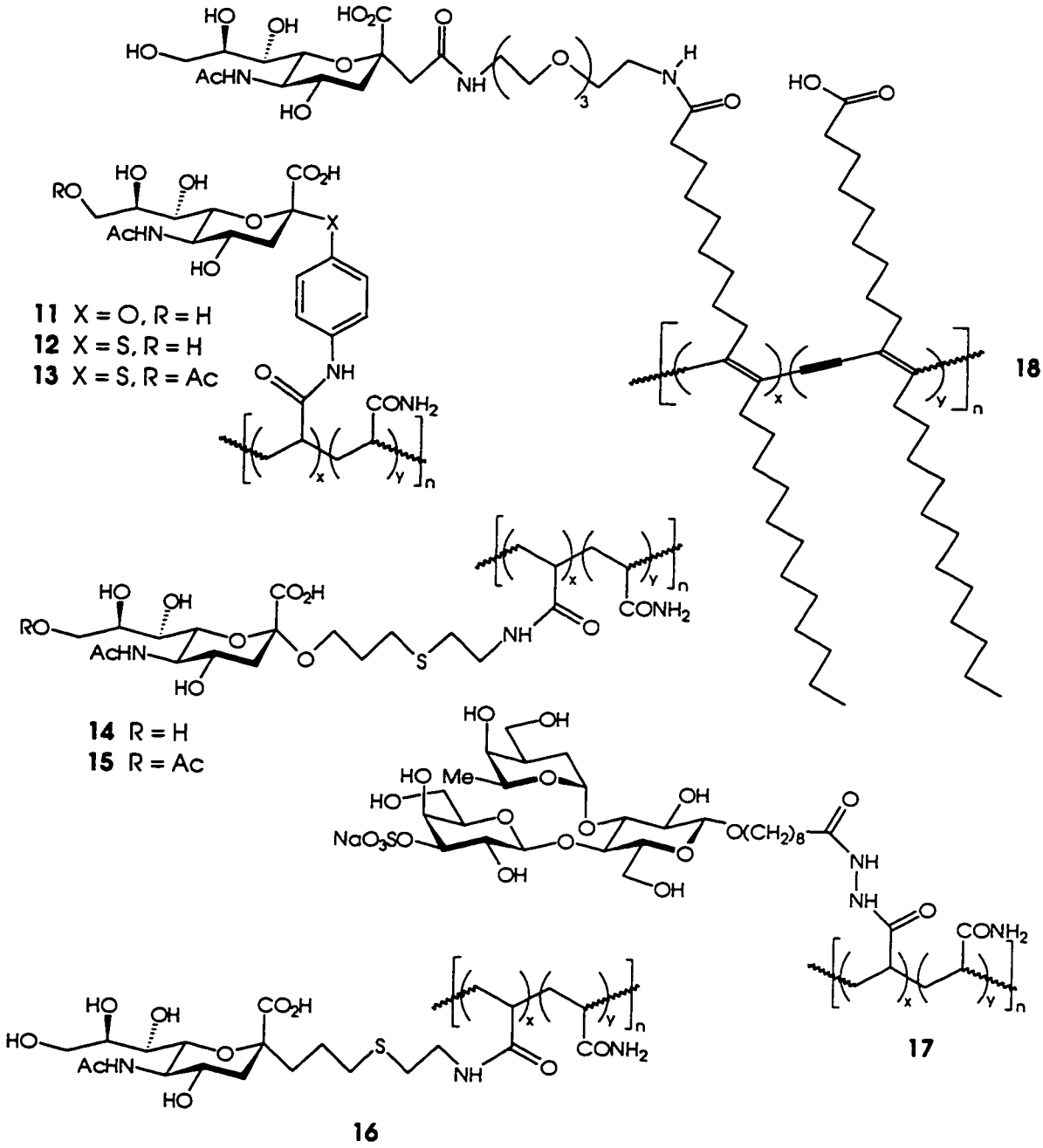
[72] a) Charych, D. H.; Nagy, J. O.; Spevak, W.; Bednarski, M. D. *Science* **1993**, *261*, 585; b) Spevak, W.; Nagy, J. O.; Charych, D. H.; Schaeffer, M. E.; Gilbert, J. H.; Bednarski, M. D. *J. Am. Chem. Soc.* **1993**, *115*, 1146; c) Nagy, J. O.; Wang, O.; Gilbert, J. H.; Schaeffer, M. E.; Hill, T. G.; Callstrom, M. R.; Bednarski, M. D. *J. Med. Chem.* **1992**, *35*, 4501.

[73] Nifant'ev, N. E.; Tsetkov, Yu E.; Shashkov, S.; Tuzikov, A. B.; Meslennikov, I. V.; Popova, I. S.; Bovin, N. V. *Bioorg. Khim.* **1994**, *20*, 552.

[74] Nifant'ev, N. E.; Shashkov, S.; Tsetkov, Yu E.; Tuzikov, A. B.; Abramenko, I. Gluzman, D. F.; Bovin, N. V. *ACS Symp. Ser.* **1994**, *560*, 267.

[75] Roy, R.; Park, W. K. C.; Srivastava, O. P.; Foxall, C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1399.

[76] Cao, S.; Roy, R. *Tetrahedron Lett.* **1996**, *37*, 3421.



Scheme 1.3.2. Examples of glycopolymers.

1.4. Glycoclusters

Initial attempts at fine-tuning geometry and valency requirements included the syntheses of glycoclusters. Glycoclusters fill the need for chemically well defined neoglycoconjugates but lack the requirement for high valency glycoderivatives.

Much work on the design and synthesis of galactosyl, mannosyl, and sialosyl clusters has been done.[26,77] Investigations by Lee and Lee[78,79,80] on rat and rabbit hepatocytes have resulted in the generation of potent trivalent N-acetylgalactosamine (GalNAc) inhibitors. Continued studies by Kichler and Schuber[81] on trivalent galactosyl (Gal) clusters further confirmed that trivalent oligosaccharides containing Gal/GalNAc moieties situated at the apexes of a triangle with sides 1.5, 2.2, and 2.5 nm constituted the optimum geometry for rat and rabbit hepatocyte receptors.[82]

By carefully adjusting the intra-sialosyl distance in a number of divalent clusters, Glick and Knowles[83,84] have obtained a sialosyl conjugate **19** (Scheme 1.4.1) in which the two sialic acids were 5.7 nm apart and this sialocluster was 100 fold more potent than methyl α-sialoside in *Influenza* virus X-31 inhibitions.

Other sialyl Lewis^X clusters have been synthesized, either enzymatically or chemically. 1,4-Butanediol (**20**),[85] 1,5-pentanediol (**21**),[85] galactoside (**22** and **23**),[85] and nitromethane-trispropionic acid (**24**)[86] have all been used successfully as scaffolding elements to demonstrate that multivalent interactions are involved in selectin binding

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77 Roy, R. *Top. Curr. Chem* **1997**, *187*, 241.

78 Lee, R. T.; Lee, Y. C. in *Neoglycoconjugates: Preparations and Applications* (Eds.: Lee, Y. C.; Lee, R. T.) Academic Press, San Diego, **1994**, pp.23.

79 Lee, Y. C. in *Carbohydrate Recognition in Cellular Function, CIBA Found. Symp. 145* (Eds.: Bock, G.; Harnette, S.) Wiley, Chichester, **1989**, pp.80.

80 Lee, R. T.; Lee, Y. C. *Glycoconjugate J.* **1987**, *4*, 317.

81 Kichler, A.; Schuber, F. *Glycoconjugate J.* **1995**, *12*, 275.

82 Lee, R. T.; Lin, P.; Lee, Y. C. *Biochemistry* **1984**, *23*, 4255.

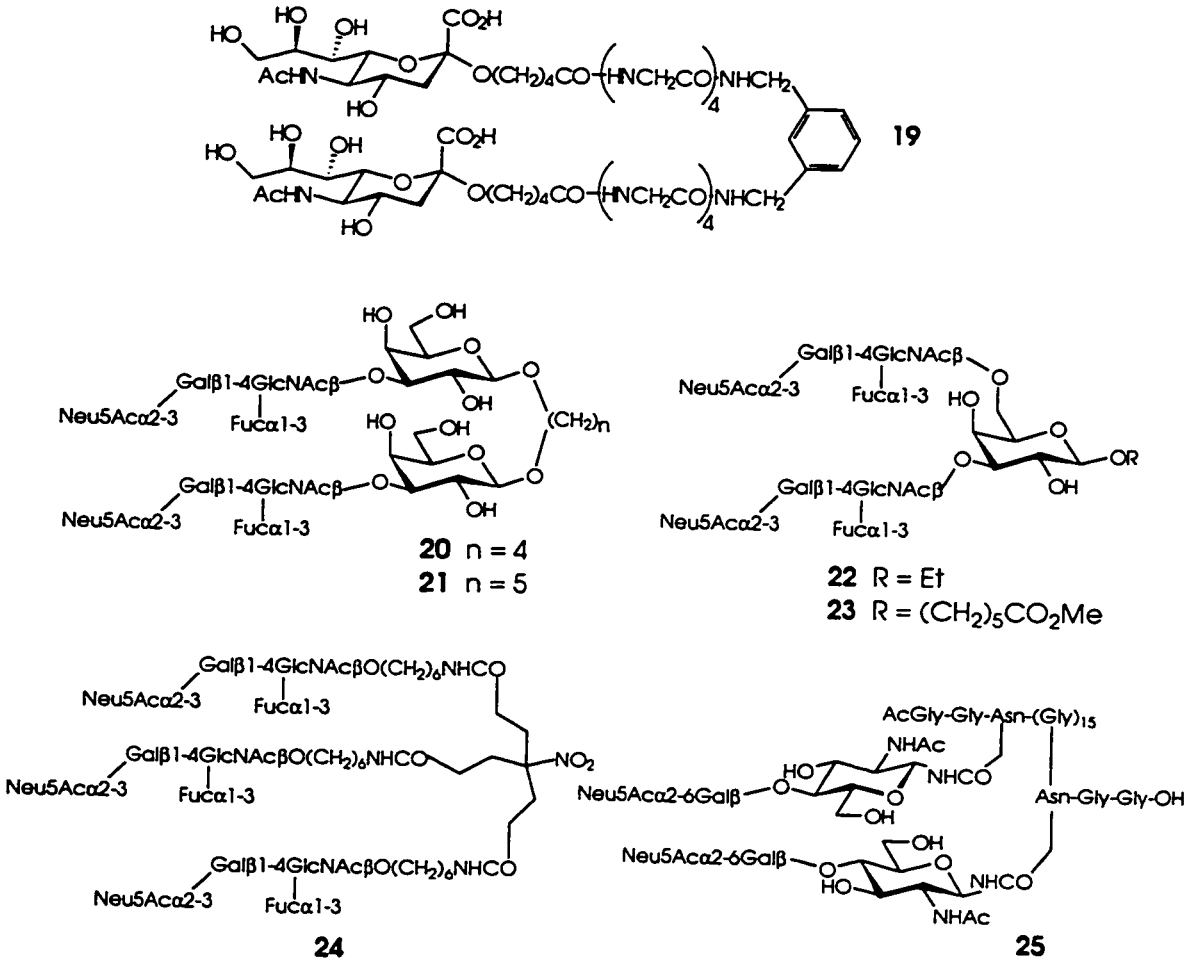
83 Glick, G. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1991**, *113*, 4701.

84 Glick, G. D.; Toogood, P. L.; Wiley, D. C.; Skehel, J. J.; Knowles, J. R. *J. Biol. Chem.* **1991**, *266*, 23660.

85 DeFrees, S. A.; Kosch, W.; Way, W.; Paulson, J. C.; Sabesan, S.; Halcomb, R. L.; Huang, D. -H.; Ichikawa, Y.; Wong, C. -H. *J. Am. Chem. Soc.* **1995**, *117*, 66.

86 Kretzschmar, G.; Sprengard, U.; Kunz, H.; Bartnik, E.; Schmidt, W.; Toepfer, A.; Hörsch, B.; Krause, M.; Seiffge, D. *Tetrahedron* **1995**, *51*, 13015.

processes. Unverzagt *et al.*[87] have branched sialyl-α-(2,6)-β-LacNAc dimers at different positions of synthetic peptides, including compact glycine-rich (**25**) and helical proline-rich (not shown) peptides, to give clusters which were eight and four fold more potent over the corresponding monovalent trisaccharide, respectively.



Scheme 1.4.1. Examples of sialoclusters.

[87] Unverzagt, C.; Kelm, S.; Paulson, J. C. *Carbohydr. Res.* **1994**, *251*, 285.

Still, many of these investigations are dependent on trial and error which necessitates much time and effort. Large numbers of clusters with varying shapes and valencies are needed to define the topography of any given receptor. The preparation of glycodendrimers that allow for carbohydrate attachment in the later stages is an attractive approach in the generation of multivalent glycoconjugates combining valency, conformation, and geometry requirements.

1.5. Dendrimers

Dendrimers (cascade molecules, arborols) are molecules with tree-like shapes. They are considered monodisperse, high molecular weight macromolecules. Classical polymers are related to these assemblies but are not so well defined. Dendrimers represent chemically well defined polymers and may be prepared by divergent or convergent methods.[88] Much scientific effort has gone into the design and synthesis of dendrimers over the last ten years. The reasons for this are numerous. Potential applications for dendrimers include those in medicinal engineering, photocopier toners, imaging, radiation and gene therapy, catalysis, charge separation, and as carriers of bioactive molecules with both agrochemical and pharmaceutical applications.[88]

In the divergent strategy for the syntheses of dendrimers, each branch bearing a terminal function is divided into several branches. The same functionality is introduced at the end of the new branches in order to continue the division process in the next generation.

The pioneering "cascade" molecules of Vögtle in 1978 were the first reported dendritic molecules.[89] These involved the conjugate addition of amines to acrylonitrile to give fifth generation dendrimers like **29** (Scheme 1.5.1).

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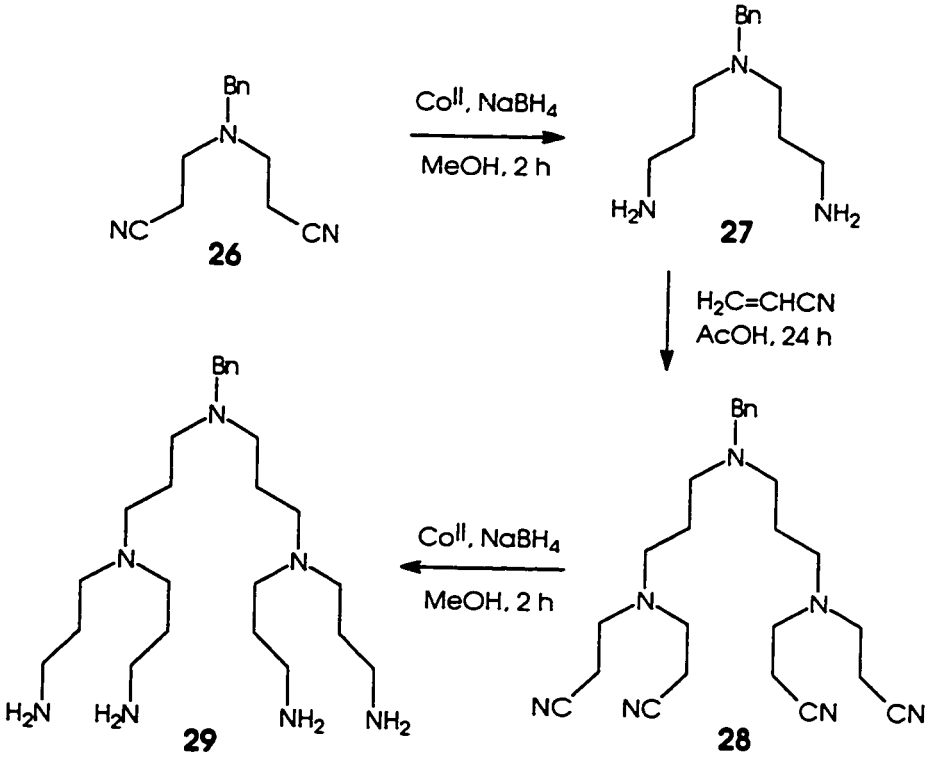
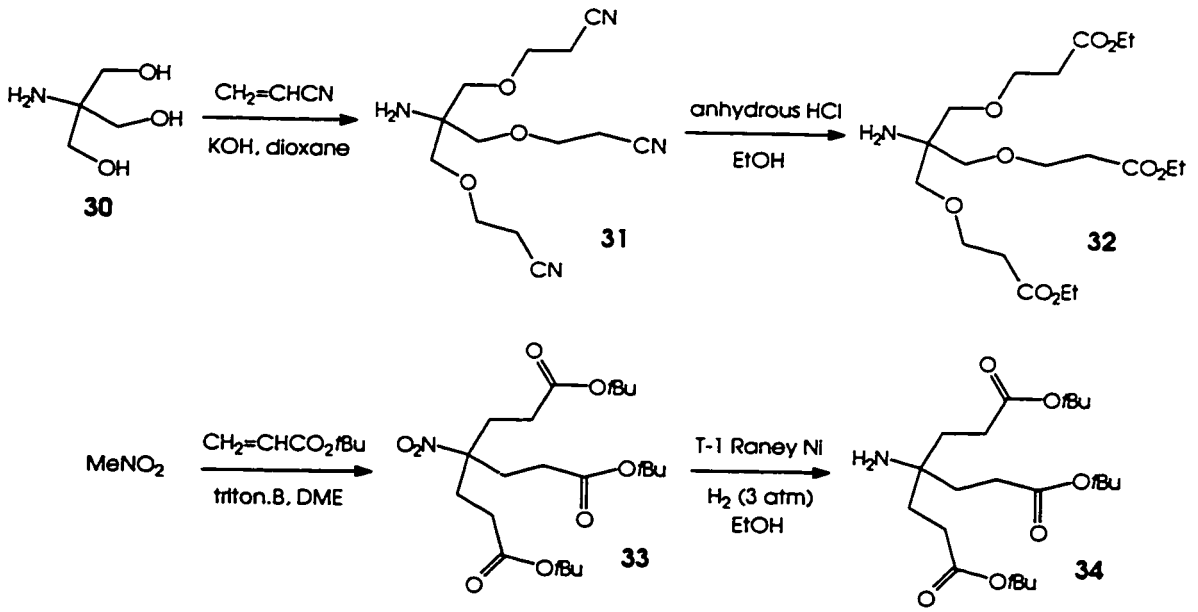
[88] a) Dvornic, P. R.; Tomalia, D. A. *Curr. Opin. Colloid Interface Sci.* **1996**, *1*, 221; b) Ardoin, N.; Astruc, D. *Bull. Soc. Chim. Fr.* **1995**, *132*, 875; c) Issberner, J.; Moors, R.; Vöglte, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2413; d) Fréchet, J. M. J. *Science* **1994**, *263*, 1710 and references cited therein.

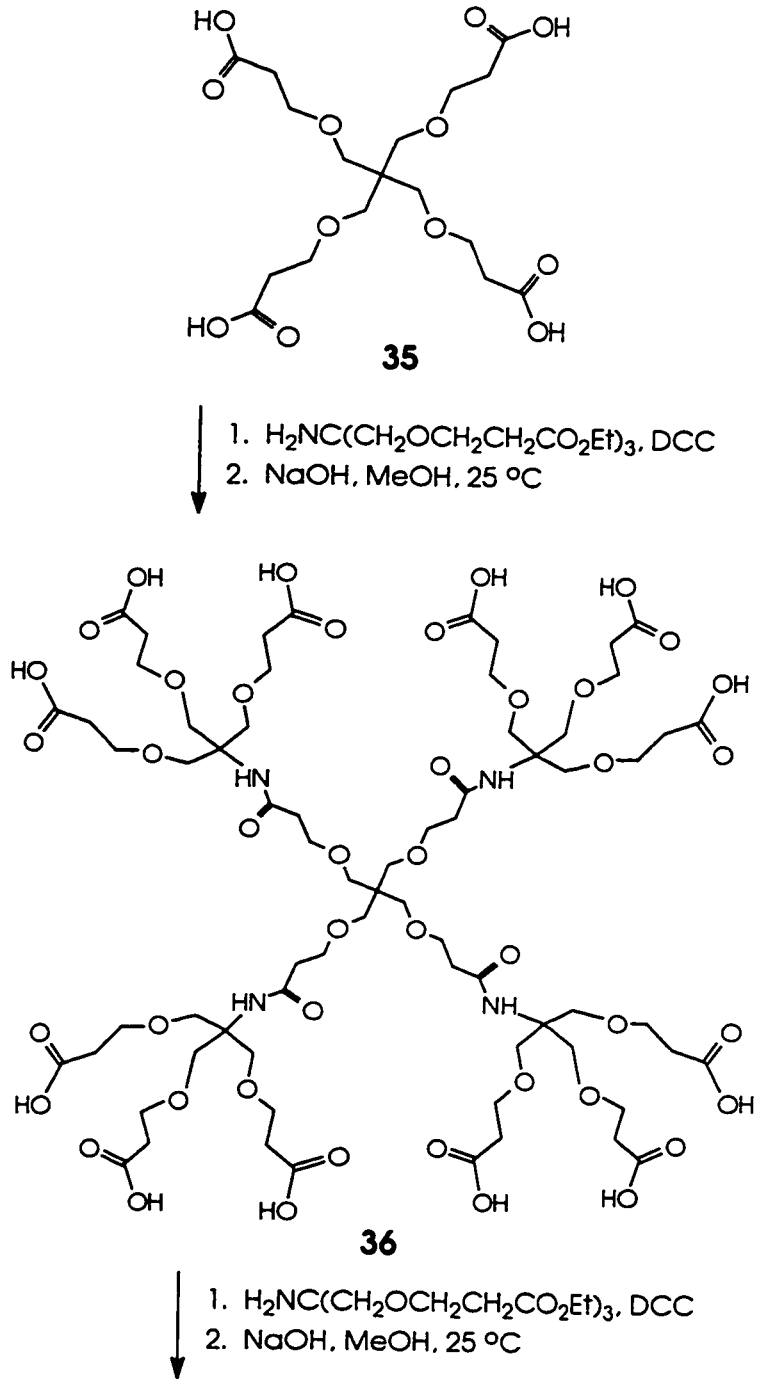
[89] a) Buhlein, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 155; b) Vögtle, F.; Weber, E. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 753.



Scheme 1.5.1. Vögtle's pioneering cascade molecules.



Scheme 1.5.2. Newkome's synthesis of dendritic cores.



Scheme 1.5.3. Newkome's synthesis of dendritic cores.

Newkome, nearly ten years later, reported a variety of synthetic schemes, which also used the conjugate addition to acrylonitrile or α,β-unsaturated esters such as CH_2=$CHCO_2tBu$ by nitromethane anion or polyols to build dendritic cores such as **32** and **34**(Scheme 1.5.2).[90,91,92]

In addition, polyacyl chloride or polycarboxylic acid cores were allowed to react with aminotriester cores for iterative synthesis (Scheme 1.5.3).[93] Similarly, 1,3,5-benzenetricarbonyl trichloride was reacted with aminoesters such as H_2NC—$(CH_2OCH_2CH_2CO_2Et)_3$, obtained by reaction of $H_2NC(CH_2OH)_3$ with acrylonitrile and KOH in dioxane, giving the tris-nitrile followed by reaction with anhydrous HCl in refluxing EtOH.[91,94]

The dendrimers introduced by Denkewalter *et al.*[95] represent the first examples of well-defined, monodisperse tree-like molecules until the tenth generation. The core was benzylhydrylamine and the branching molecule was an activated amino ester of amine-protected L-lysine. This concept was extended to solid phase on a Merrifield resin by Tam *et al.*[96,97] L-Lysine based dendrimers are discussed in detail in Chapter 3.

Tomalia achieved the first successful synthesis of highly branched poly(amidoamine) (PAMAM) dendrimers to generation ten with a defined number of surface groups.[98,99] These too are discussed later in this dissertation (Chapter 5).

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[90] Newkome, G. R.; Yao, Z. -Q.; Baker, G. R.; Gupta, V. K.; Russo, P. S.; Saunders, M. J. *J. Am. Chem. Soc.* **1986**, *108*, 849.

[91] Newkome, G. R.; Moorefield, C. N.; Theroilt, K. J. *J. Org. Chem.* **1988**, *53*, 5552.

[92] Newkome, G. R.; Nayak, A.; Behera, R. K.; Moorefield, C. N.; Baker, G. R. *J. Org. Chem.* **1992**, *57*, 358.

[93] Newkome, G. R.; Lin, X.; Weis, C. D. *Tetrahedron Assymetry* **1991**, *2*, 957.

[94] Newkome, G. R.; Lin, X.; Young, Y. K. *Synlett.* **1992**, 53.

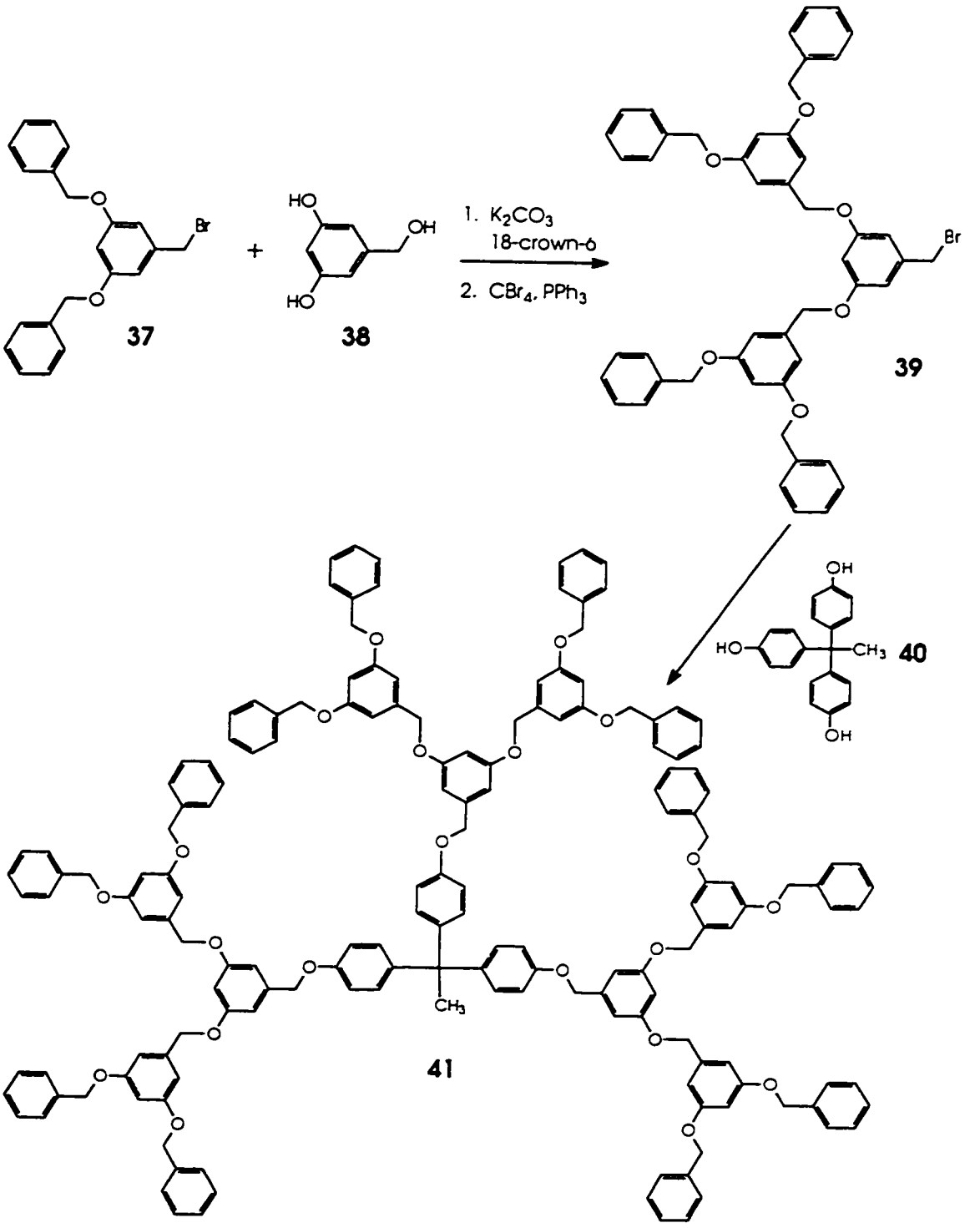
[95] Denkewalter, R. G.; Kolc, J. F.; Lokasavage, W.J. *US Pat. 4* 410688 (**1983**); *Chem. Abstr.* **1984**, *100*, 103907.

[96] Tam, J. P. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4929 and 5409.

[97] Posnett, D. N.; McGrath, H.; Tam, J. P. *J. Biol. Chem.* **1988**, *264*, 1719.

[98] Tomalia, D. A.; Naylor, A. N.; Goddard III, W. A. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 617.

[99] Tomalia, D. A.; Dvornic, P. R. *Nature* **1994**, 617.



Scheme 1.5.4. Fréchet's convergent synthesis of dendrimers.

The convergent method was reported independently by Fréchet[100,101,102] and Miller.[103] It consist of using a heterobifunctional core in such a way that some functional groups selectively react. Scheme 1.5.4 depicts Fréchet's method as an example.

Research in dendritic technology has given chemists the tools with which to design chemically well defined multivalent arrays. The next step was to apply this knowledge to the field of glycobiology.

1.6. Glycodendrimers

The term glycodendrimers has come to refer to chemically well defined, tree-like molecules with covalently attached carbohydrate residues. Glycodendrimers represent novel, monodisperse biopolymers with known glycosidic densities. They bridge the gap between small clusters and large polymers, generally having valencies between these two classes of neoglycoconjugates. The lengths of the branches, as well as the choice of the carbohydrate hapten, allows for control of intra-glycosidic bond distances.[104]

The first glycodendrimer synthesis in which up to 16 sialic acid residues were attached to the periphery of a multi-branched L-lysine core appeared in 1993.[105,106] The work presented in that paper stemmed directly from this thesis dissertation and is discussed fully in Chapter 3.

Few examples of glycodendrimers exist at present and most of these examples have originated here. These are the topics of later chapters. Still, it would be unjust to ignore the work reported outside of this laboratory, after 1993.

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[100] Hawker, C. J.; Fréchet, J. M. J. *J. Chem. Soc., Chem. Commun.* **1990**, 1010.
[101] Hawker, C, J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638.
[102] Fréchet, J. M. J.; Hawker, C. J.; Wooley, K. L. *J. Macromol. Sci.* **1994**, *A31(11)*, 1627.
[103] Miller, T. M.; Neenan, T. X. *Chem. Mat.* **1990**, *2*, 346.
[104] Roy, R. *Polymer News* **1996**, *21*, 226.
[105] Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1869.
[106] Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *ACS Symp. Ser.* **1994**, *560*, 104.

Spherical Starburst® (PAMAM) dendrimers[88a,98,99,107] made of poly(amidoamine) are commercially available and have been used to attach various carbohydrate derivatives. Disaccharide lactones of lactose and maltose have been directly conjugated to the amine terminated dendrimers and these spherical glycodendrimers (with 12, 24, and 48 glycan residues, **43** and **44**, Scheme 1.6.1) were shown to bind strongly and reversibly to Concanavalin A and pea lectins.[108] Toyokuni and Singhal[109] have attached T_N-antigen (α-D-GalNAc-O-Ser) peptide to fifth generation (48 amines) PAMAM dendrimers to give glycodendrimers **45** (Scheme 1.6.1) which were found to be non-immunogenic.

Lindhorst *et al.*[110] have used an isothiocyanate coupling reaction to attach various glycosides to dendrimers. Peracetyl glycosyl isothiocyanates of β-D-glucose, α-D-mannose, β-D-galactose, β-cellobiose, and β-lactose have been conjugated to poly(amidoamine) dendrimers in this fashion (**47** to **51**, Scheme 1.6.2). D-Mannopyranoside dendrimers **48** showed improved inhibitory potencies in the agglutination of a type 1-fimbriated *Escherichia coli* clone with yeast cells.[111]

Only two other groups have recently looked to the generation of glycodendrimers based on core structures other than poly(amidoamine) dendrimers. Ashton *et al.*[112] have condensed tri- (**52**) and hexa-glucosylated (**53**) derivatives onto a trifunctional 1,3,5-benzene tricarboxylic acid (**54**) core *via* amide bond formation to generate glycodendrimers with valencies of nine and eighteen (Scheme 1.6.3).

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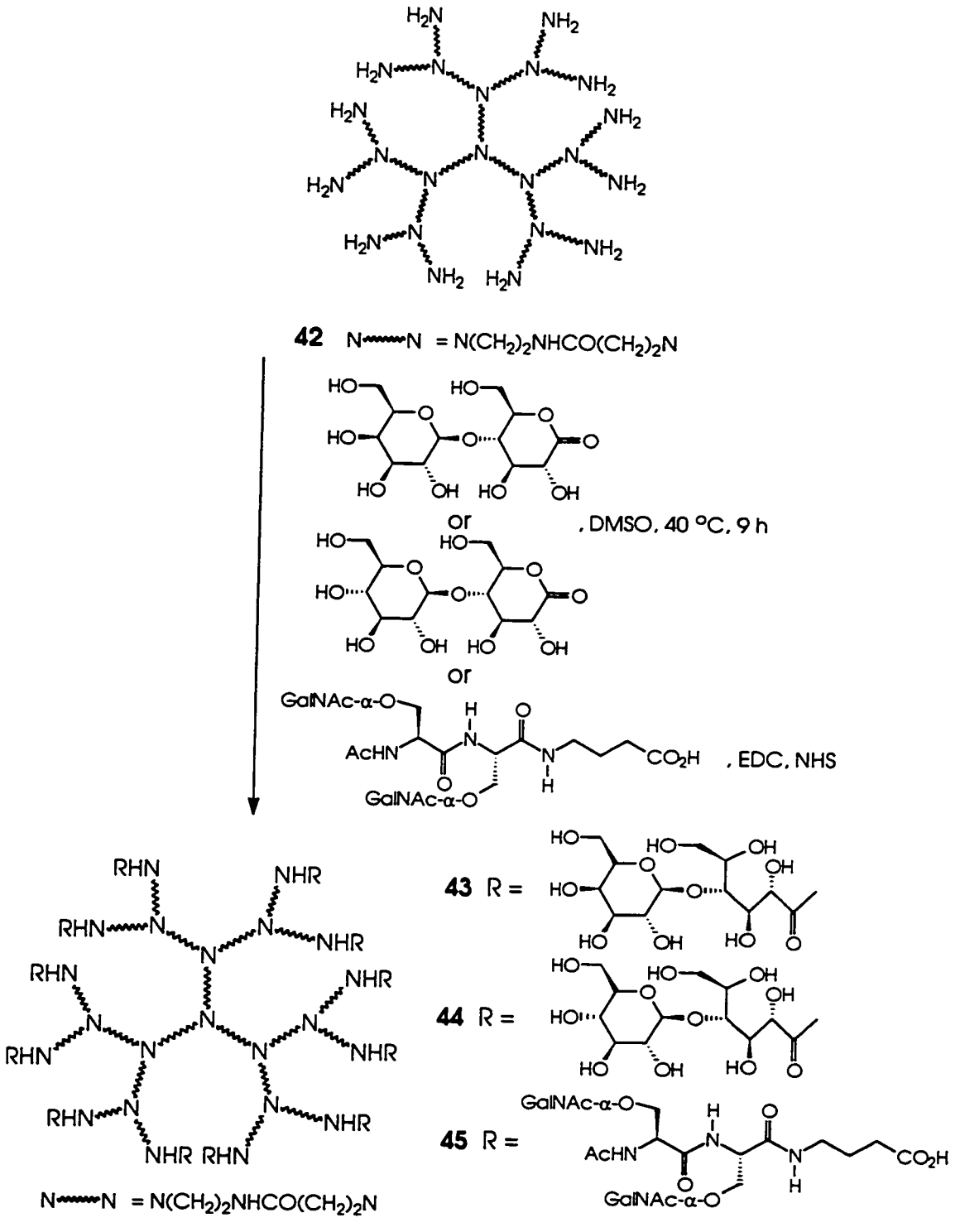
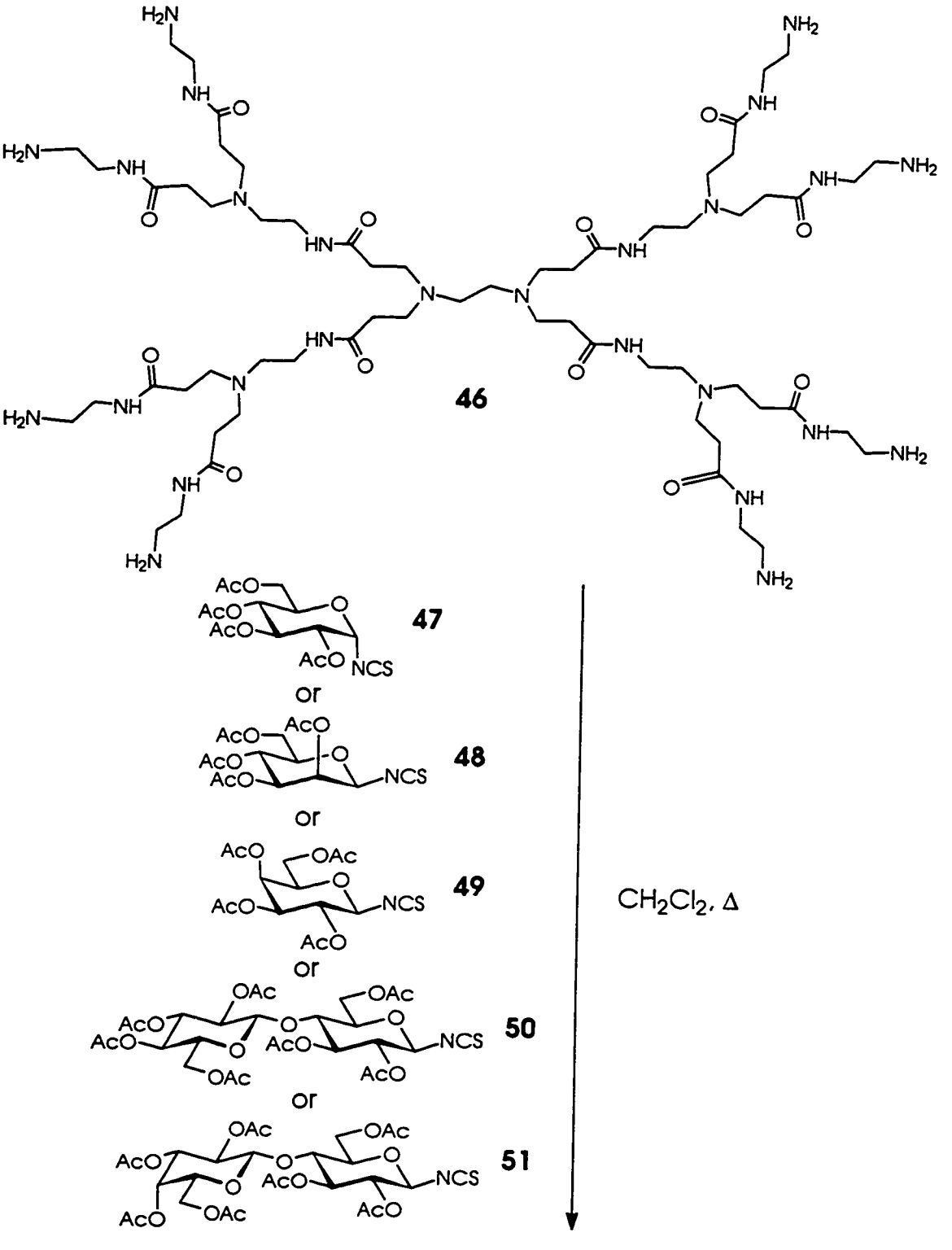
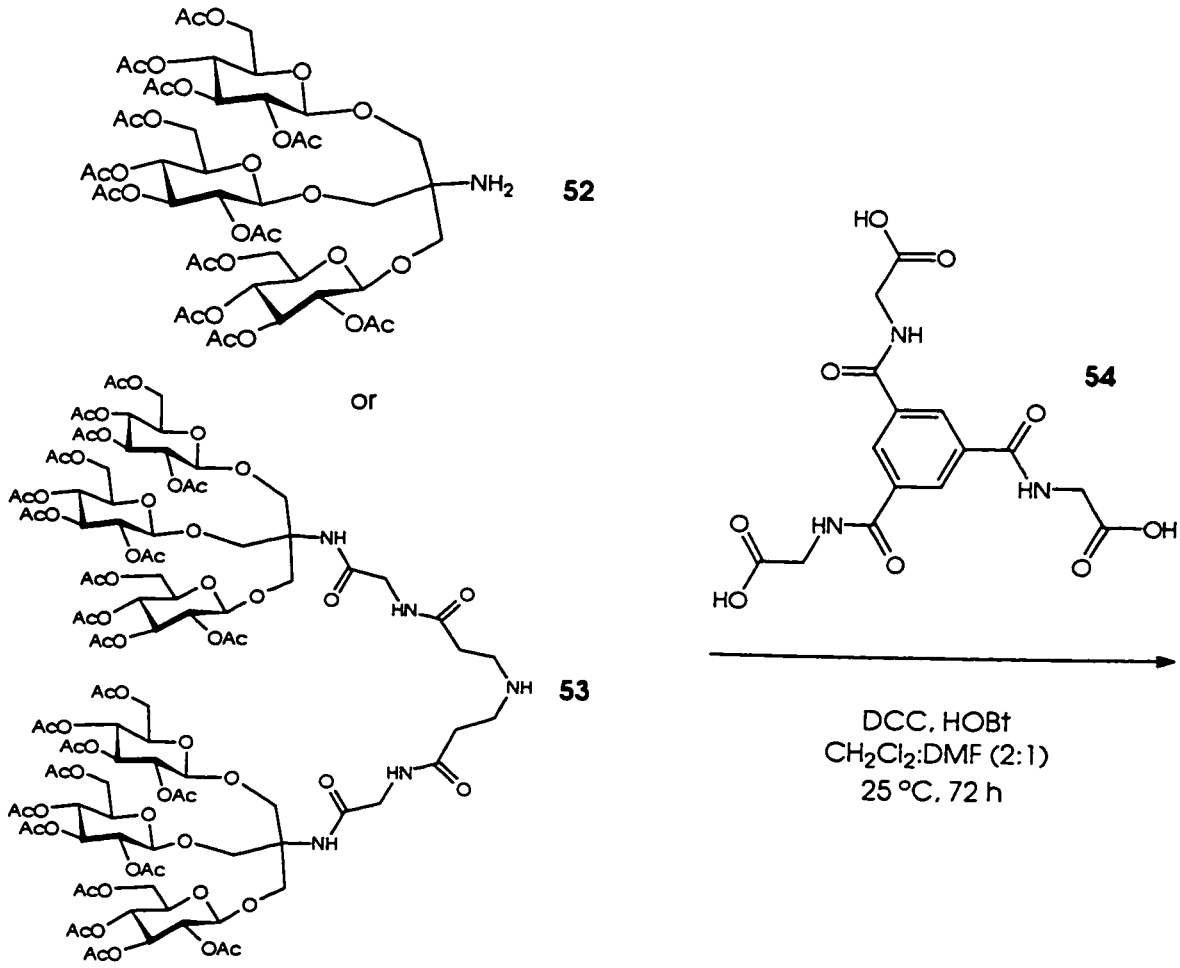
[107] Tomalia, D. A.; Durst, H. D. *Topics Curr. Chem.* **1993**, *165*, 193.
[108] Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391.
[109] Toyokuni, T.; Singhal, K. *Chem. Soc.Rev.* **1995**, 231.
[110] Lindhorst, T. K.; Kieburg, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1953.
[111] Krallmann, U.; Lindhorst, T. K. *Proc. of the XVIIIth Int. Carbohydr. Symp.* **1996**, Milan, July 21-26, p. 587.
[112] Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1996**, *2*, 1115.



Scheme 1.6.1. Glycofunctionalization of poly(amidoamine) dendrimers from references 108 and 109.



Scheme 1.6.2. Isothiocyanate coupling strategy in the generation of glycodendrimers.



Scheme 1.6.3. Synthesis of nona- and octadeca-valent glucosylated dendrimers.

In order to avoid base catalyzed retro-Michael degradations to which poly(amidoamine) dendrimers are susceptible, we have synthesized novel dendritic α-thiosialosides based on a 3,3'-iminobis(propylamine) core to give glycodendrimers of up to sixteen in valency.[113,114] Chapter 4 discusses this work.

[113] Zanini, D.; Roy, R. *J. Org. Chem.* **1996**, *61*, 7348.
[114] Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2088.

A better understanding of the role of multivalent carbohydrate-protein interactions in biological systems is necessary. Presently, novel neoglycoconjugates with varying degrees of valency, conformation, and geometry are being synthesized. Glycodendrimers represent the latest in this line of novel neoglycoconjugates and have made enormous impact in the glycobiological realm. All of the work that comes after the first reported glycodendrimer preparation (see above) attests to this. The first glycodendrimer synthesis and other glycodendrimer syntheses, their design and *in vitro* biological testing is presented here.

1.7. Immunochemical Techniques

1.7.1. Lectins

In order to assess the specific role of multivalency and its biological implications in carbohydrate-protein interactions, the synthetic neoglycoconjugates presented here and above must be evaluated both *in vitro* and *in vivo*. Commonly employed techniques include double immunodiffusion, turbidimetric analysis, and competitive enzyme linked lectin assays (ELLA) with either plant lectins or antibodies. These are discussed in this section.

Lectins are carbohydrate recognizing proteins of non-immune origin.[115] They are often arranged in clusters (Figure 1.2.1)[40-42] and, as such, may be used as models for other glycoside binding receptors where multivalent factors are important.

Lectins are relatively small proteins (usually 40 to 160 kDa) made up of several subunits. They usually exist as several isolectins (lectins with differing isoelectric points) and some have metal chelates. Lectins are carbohydrate specific.[116] That is, a lectin in its carbohydrate recognition domain (CRD) discriminates between glycosides. Three lectins,

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[115] Van den Eijnden, D. H.; Joziasse, D. H. *Carbohydrates in Europe* **1994**, 11.
[116] Liener, I. E.; Sharon, N.; Goldstein, I. J. (Eds.) *The Lectins. Properties, Functions, and Applications in Biology and Medicine*, Academic Press, Orlando, **1986**.

wheat germ agglutinin (WGA), *Limax flavus* (LFA) lectin, and *Erythrina Cristagalli* (ECA) lectin have been used extensively in this dissertation. These lectins will be discussed briefly here.

Wheat germ agglutinin (WGA) is a dimeric, carbohydrate-free protein composed of two subunits.[117] Subunit molecular weight ranges from 17.5 to 24 kDa. The carbohydrate binding specificity of WGA has been studied by a variety of techniques such as hapten inhibition of hemagglutination and of specific precipitation of glycoconjugates,[118,119] changes in lectin fluorescence (intrinsic) or in chromatogenic ligands (extrinsic),[120,121] equilibrium dialysis,[122,123] NMR, and X-ray diffraction.[124] Studies showed that each WGA monomeric subunit contains two identical and independent binding sites for N-acetylglucosamine (GlcNAc).[117] Carbohydrate-binding specificity of WGA is listed in Table 1.7.1.1.

WGA also binds to N-acetylneuraminic acid, but to a lesser extent than for GlcNAc. The specific binding of sialic acid to WGA is based on the similarity in configuration of this sugar to GlcNAc at positions C-2 (N-acetamido group) and C-3 (hydroxyl group) of the pyranose ring (Figure 1.7.1.1). These are the positions critical to binding between a glycoside and WGA.[117]

Limax flavus (LFA) is a sialic acid specific binding animal lectin. It consists of two equal sized, noncovalently associated subunits (M_r = 22,000).[125,126] It is specific for

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[117] Goldstein, I. J.; Poretz, R. D. in *The Lectins. Properties, Functions, and Applications in Biology and Medicine* (Eds.: Liener, I. E.; Sharon, N.; Goldstein, I. J.) Academic Press, Orlando, **1986**, pp. 103.

[118] Allen, A. K.; Neuberger, A.; Sharon, N. *Biochem. J.* **1973**, *131*, 155.

[119] Goldstein, I. J.; Hammarström, S.; Sundblad, G. *Biochim. Biophys. Acta* **1975**, *405*, 63.

[120] Privat, J. P.; Delmotte, F.; Monsigny, M. *FEBS Lett.* **1974**, *46*, 224.

[121] Van Landschoot, A.; Loontiens, F. G.; Clegg, R. M.; Sharon, N.; Bruyne, C. K. *Eur. J. Biochem.* **1977**, *79*, 275.

[122] Nagata, Y.; Burger, M. M. *J. Biol. Chem.* **1974**, *249*, 3116.

[123] Levine, D.; Kaplan, M J.; Greenaway, P. J. *Biochem. J.* **1972**, *129*, 847.

[124] Wright, C. S.; Gavilanes, F.; Peterson, D. L. *Biochemistry* **1984**, *23*, 280 and references cited therein.

[125] Goldstein, I. J.; Poretz, R. D. in *The Lectins. Properties, Functions, and Applications in Biology and Medicine* (Eds.: Liener, I. E.; Sharon, N.; Goldstein, I. J.) Academic Press, Orlando, **1986**, pp. 211.

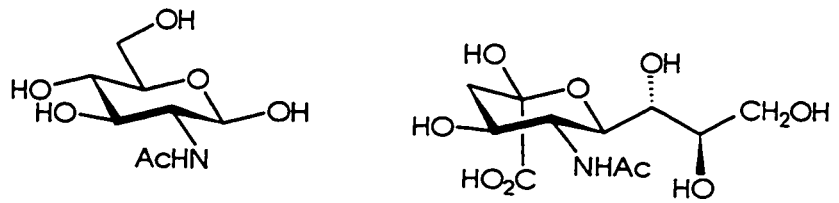
[126] Fischer, E.; Brossmer, R. *Glycoconjugate J.* **1995**, *12*, 707.

Table 1.7.1.1. Carbohydrate-binding specificity of WGA.[a]

Inhibitor	Relative Inhibitory Potency[b]
N-Acetylglucosamine	1.0
Sialic Acid	<0.25
Glucosamine	<0.1
N-Acetylgalactosamine	0.19

[a]Inhibition of wheat germ agglutinin-*p*-azophenyl N-acetyl-β-glucosaminide-bovine serum albumin precipitation by various saccharides. Data taken from reference 119.

[b]N-Acetylglucosamine is normalized to 1.0 (5.9 μM N-acetylglucosamine required for 50% inhibition).



β-D-GlcNAc α-D-Neu5Ac

Figure 1.7.1.1. Comparison of structural features shared by N-acetylglucosamine and sialic acid.

N-acetylneuraminic acid and N-glycolylneuraminic acid (Neu5Gc) residues with the binding affinity being much greater for Neu5Ac than for Neu5Gc, as found in hemagglutination inhibition experiments.[127]

Erythrina Cristagalli (ECA) lectins are composed of two non-covalently linked subunits. The subunits are identical, or nearly identical, in some cases differing only in molecular weight. ECA lectins have molecular weights in the range of 56 to 68 kDa.[128]

[127] Miller, R. L.; Collawn, Jr., J. F.; Fish, W. W. *J. Biol. Chem.* **1982**, *257*, 7574.

[128] Goldstein, I. J.; Poretz, R. D. in *The Lectins. Properties, Functions, and Applications in Biology and Medicine* (Eds.: Liener, I. E.; Sharon, N.; Goldstein, I. J.) Academic Press, Orlando, **1986**, pp. 160.

Binding studies have revealed that N-acetyllactosamine exhibits the highest affinity for ECA, where most of the binding energy is contributed by the non-reducing terminal galactose residue.[128,129] ECA-protein interactions are not significantly inhibited by GlcNAc residues.

1.7.2. Double Immunodiffusion

Double immunodiffusion[130] is one of the most technically simple forms of testing for agglutination. In this technique, a drop of antigen or glycoconjugate is placed into a well cut in an agar-coated glass plate. Antibody or lectin is placed in an adjacent well. The two are allowed to diffuse outwards over time and a precipitate forms between the two wells if there is molecular recognition (Figure 1.7.2.1). The precipitin line (precipitate) is directly visible and may be permanently stained and/or photographed. Double immunodiffusion is an easily performed, quick, qualitative method requiring no special equipment nor exhaustive reagent purifications.

1.7.3. Turbidimetric Analysis

Turbidimetric analysis too is a simple, qualitative test for agglutination resulting from the formation of an insoluble cross-linked lattice. The neoglycoconjugate or antigen being tested is placed in a microtiter well together with the lectin or antibody. Optical density (O.D.) is then measured as a function of time. If neither neoglycoconjugate nor lectin are in excess, then some of the reactions between these two will be with adjacent molecules and will result in the formation of a neoglycoconjugate/lectin polymer or lattice (Figure 1.7.3.1). This large complex becomes insoluble and precipitates out of solution, thereby increasing the observed O.D.

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[129] Bhattacharyna, L.; Haraldsson, M.; Sharon, N.; Lis, H.; Brewer, F. *Glycoconjugate J.* **1989**, *6*, 141.

[130] Kemeny, D. M. *a practical guide to ELISA* Pergamon Press, Oxford, **1991**.

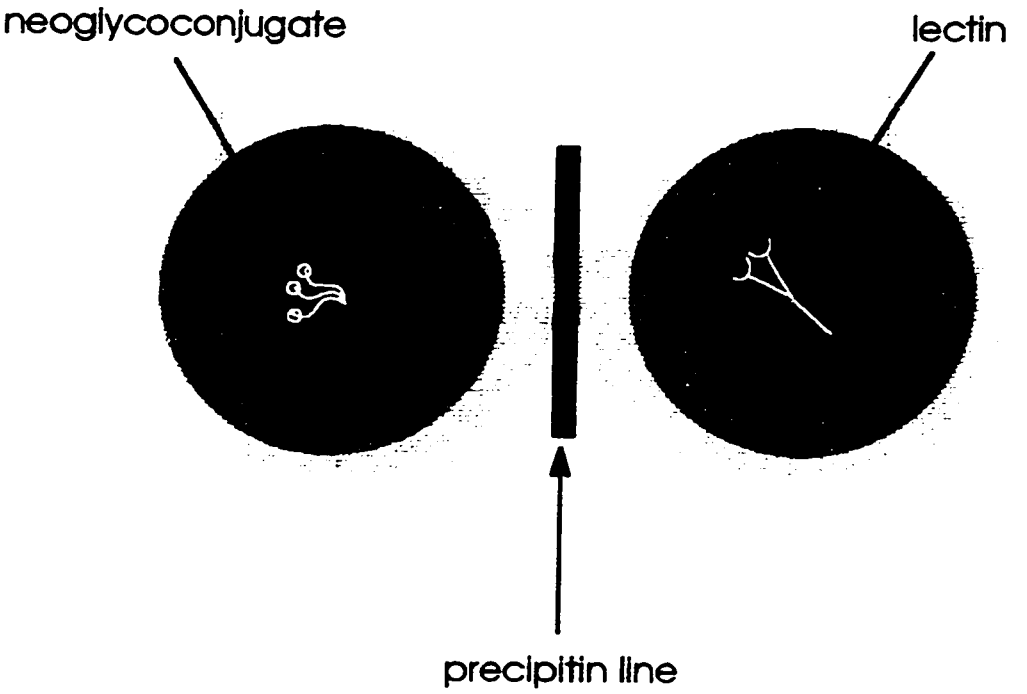

Figure 1.7.2.1. Schematic of the double immunodiffusion assay.

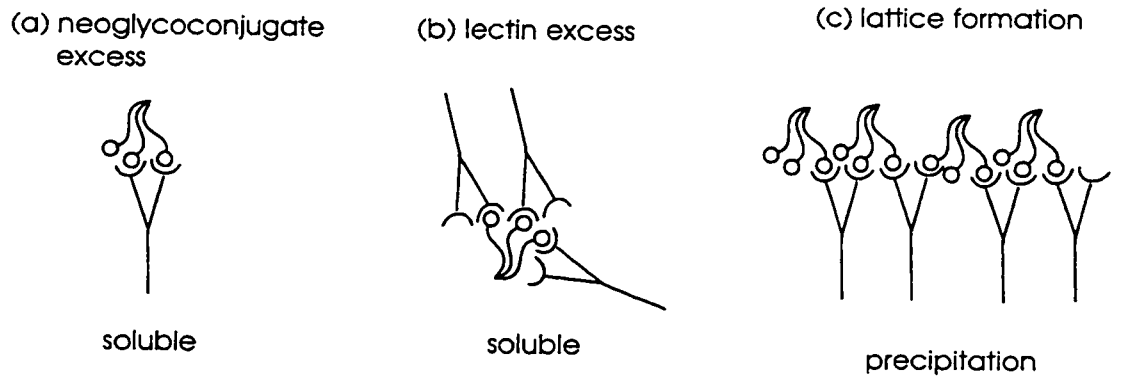

Figure 1.7.3.1. Schematic of turbidimetric analysis.

1.7.4. Enzyme Linked Lectin Assays (ELLA)

ELLA is a quantitative competitive inhibition assay that makes it possible to obtain an estimate (IC_{50}) of a particular hapten concentration necessary to inhibit a given interaction.[130] In competitive solid phase ELLA, a microtitre plate is coated with natural (*e.g.* glycoproteins, polysaccharides) or synthetic (*e.g.* glycopolymers) carbohydrate ligands. An enzyme linked probe/receptor (*e.g.* peroxidase or phosphatase linked lectin) is added to the plate together with the glycoconjugate inhibitor. If the glycoconjugate is an effective inhibitor, it will prevent the protein probes/receptors from binding to the carbohydrate ligand coated to the plate. Hence, the solid phase coated ligand competes with the free ligand in the binding to the enzyme-linked receptor (Figure 1.7.4.1). A multivalent neoglycoconjugate with improved inhibitory potential binds effectively to the receptor, and thus reduces receptor-plated ligand interactions. Once plotted as per cent inhibition versus inhibitor concentration, these data provide an estimate of the concentration of multivalent neoglycoconjugate necessary for 50% inhibition (IC_{50}).

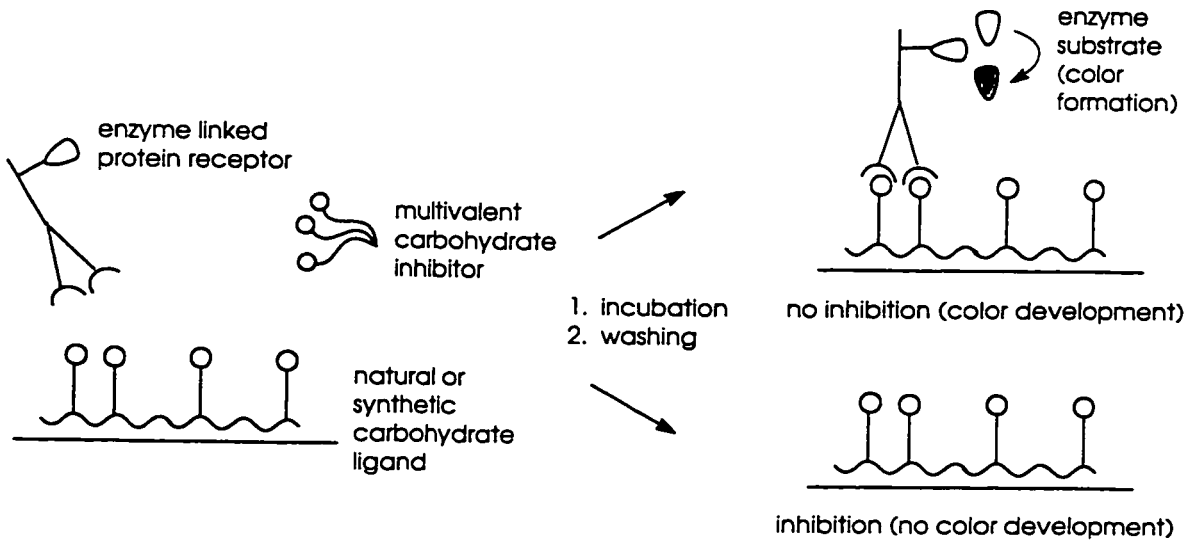

Figure 1.7.4.1. Schematic of competitive enzyme linked lectin assays.

Chapter 2. Carbohydrate Haptens

2.1. Introduction

As already stated, carbohydrates have key roles in a number of biological functions.[1] Table 2.1.1 lists some sugar specificities of bacterial and/or viral receptors in cell surface carbohydrate-protein interactions.[131] Any of these glycosides would constitute good targets in the design of glycodendrimers, as dendritic structures incorporating these sugars should inhibit the respective glycoside-receptor interaction.

Table 2.1.1. Sugar specificities of cell surface carbohydrate lectins.

Saccharide	Bacterial/Viral Receptor
D-GlcNAc	HIV Virus
Lactose	*Escherichia coli* *Actinomyces spp.* *E. coli*
Sialic Acid (NeuAc)	Lung tissues (Cancer Metastasis) Mycoplasma *Influenza* virus
Sialyloligosaccharides	E-, P-, L- Selectins (Inflammation) *H. pilori* (Gastric Ulcer)

Furthermore, in the design of viable inhibitors, consideration must be given to the action of glycohydrolase enzymes. Thioglycosides are an important family of carbohydrate derivatives and have been recognized as inhibitors of a number of glycohydrolases.[132] Thus, thioglycoside derivatives of the sugars listed in Table 2.1.1 represent stable and versatile carbohydrate haptens in the choice of model sugars in the preparation of glycodendrimers.

[131] Sharon, N. *FEBS Lett.* **1987**, *217*, 145.
[132] Steers, Jr., E.; Cuatrecasas, P.; Pollard, H. B. *J. Biol. Chem.* **1971**, *246*, 196.

Sialic acid was chosen as the first carbohydrate hapten since it is known to be the immunodominant structure recognized by *Influenza* virus hemagglutinins[15] and also because neosialoproteins[58,59] and sialopolymers[69,71] were already proven to be potent inhibitors of viral attachment to human erythrocytes. Still, the conjugation strategies, as outlined below, were easily extended to the syntheses of glycodendrimers containing lactose (Lac), N-acetylglucosamine (GlcNAc), N-acetyllactosamine (LacNAc), mannose (Man), and T-antigen (Galβ-(1,3)-GalNAcα).

2.2. Conjugation Strategies

In the syntheses of glycodendrimers, attractive strategies include those that allow for the incorporation of carbohydrate residues onto pre-formed dendritic cores at a late stage of the syntheses.[60,104] In this manner, a variety of glycodendrimers containing different sugar moieties with and without aglycon spacers may be prepared.

The first approach followed for the syntheses of glycodendrimers was based on the convergent syntheses of suitably branched dendrimers having N-chloroacetylated end groups to which thiolated carbohydrate derivatives could be added (Scheme 2.2.1).[105,106,133] S_N2 displacement of the chorine by thiolated glycosyl derivatives was shown to be general, high yielding, and easily amenable to commercially available dendrimers. In addition, a distinct advantage of the N-chloroacetyl strategy is that it enables the determination of the extent of glycoside incorporation by high field ^{1}H-NMR spectroscopy. ^{1}H-NMR N-chloroacetyl signals generally appear as well resolved singlets at 4.01 ppm ($CDCl_3$, DMSO-d_6) and can be precisely integrated (±3%) relative to other signals. Following carbohydrate attachment, the chloromethylene signal shifts upfield due to chlorine displacement. However, a disadvantage of this methodology resides in the fact that multivalent N-chloroacetyl groups make the desired derivatives extremely polar (Chapters 3 and 4). As a result, an additional approach to carbohydrate-dendrimer conjugation was investigated.

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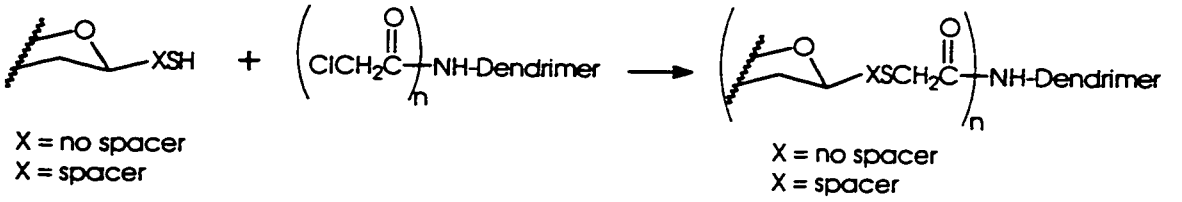
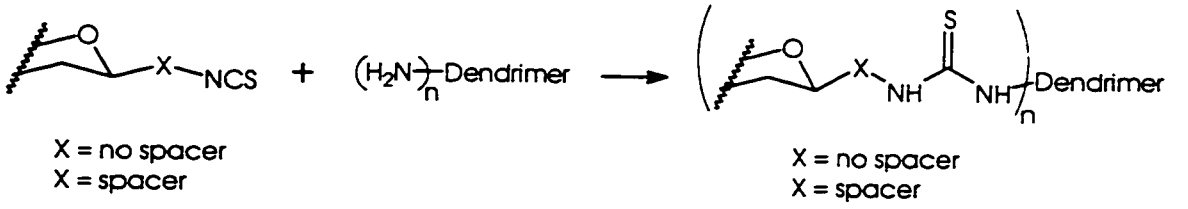
[133] Zanini, D.; Park, W. K. C.; Roy, R. *Tetrahedron Lett.* **1995**, *36*, 7383.



Scheme 2.2.1. N-Chloroacteylated conjugation strategy in the synthesis of glycodendrimers.

The second approach included the convergent syntheses of amine-terminated dendritic cores to which glycosyl isothiocyanate derivatives could be added at a later stage in the synthesis (Scheme 2.2.2). The transformation of isothiocyanates and amines to thiourea derivatives has been applied to the generation of multivalent glycoconjugates.[110,134,135] Sugar isothiocyanates may be synthesized *via* a variety of routes[136] and represent stable, useful precursors to glycodendrimer preparations, some of which are even commercially available.



Scheme 2.2.2. Isothiocyanate conjugation strategy in the synthesis of glycodendrimers.

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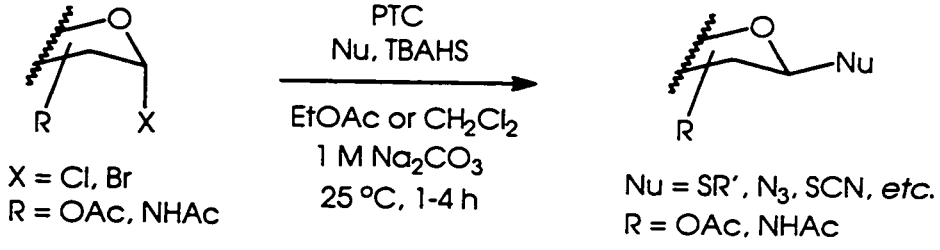
[134] Pagé, D.; Aravind, S.; Roy, R. *Chem. Commun.* **1996**, 1913.
[135] Pagé, D.; Roy, R. *Glyconjugate J.* **1997**, in press.
[136] Witczak, Z. J. *Adv. Carbohydr. Chem. Biochem.* **1986**, *44*, 91.

2.3. Glycosyl Derivatives

Liquid-liquid phase transfer catalysis (PTC) has been used for the syntheses of a wide range of anomeric glycosyl derivatives including C-, N-, O-, S-, and Se-glycosides.[137,138] These reactions have been successfully applied to mono- and di-saccharides, sialic acid, 2-deoxy-2-acetamido sugars, and to D-xylose.[43,138] PTC reactions are based on phase transfer catalyzed reactions between thiols and glycosyl halides. They are generally performed under mild conditions and are easily amenable to large scale syntheses. Anomeric nucleophilic substitutions occur stereospecifically; *i. e.* complete inversion occurs at the anomeric center. A general PTC reaction is outlined in Scheme 2.3.1.



Scheme 2.3.1. A general PTC reaction.

PTC reactions are well suited for the syntheses of carbohydrate derivatives providing the desired compounds in a mild, quick, and high yielding fashion. They were used in the preparation of glycosyl derivatives **58**, **59**, **64**, and **65** (Scheme 2.3.2).

Glycosyl halides **57**, **60**, and **61** were prepared according to previously published procedures.[139,140,141] These were treated with tetra-n-butylammonium hydrogen sulfate

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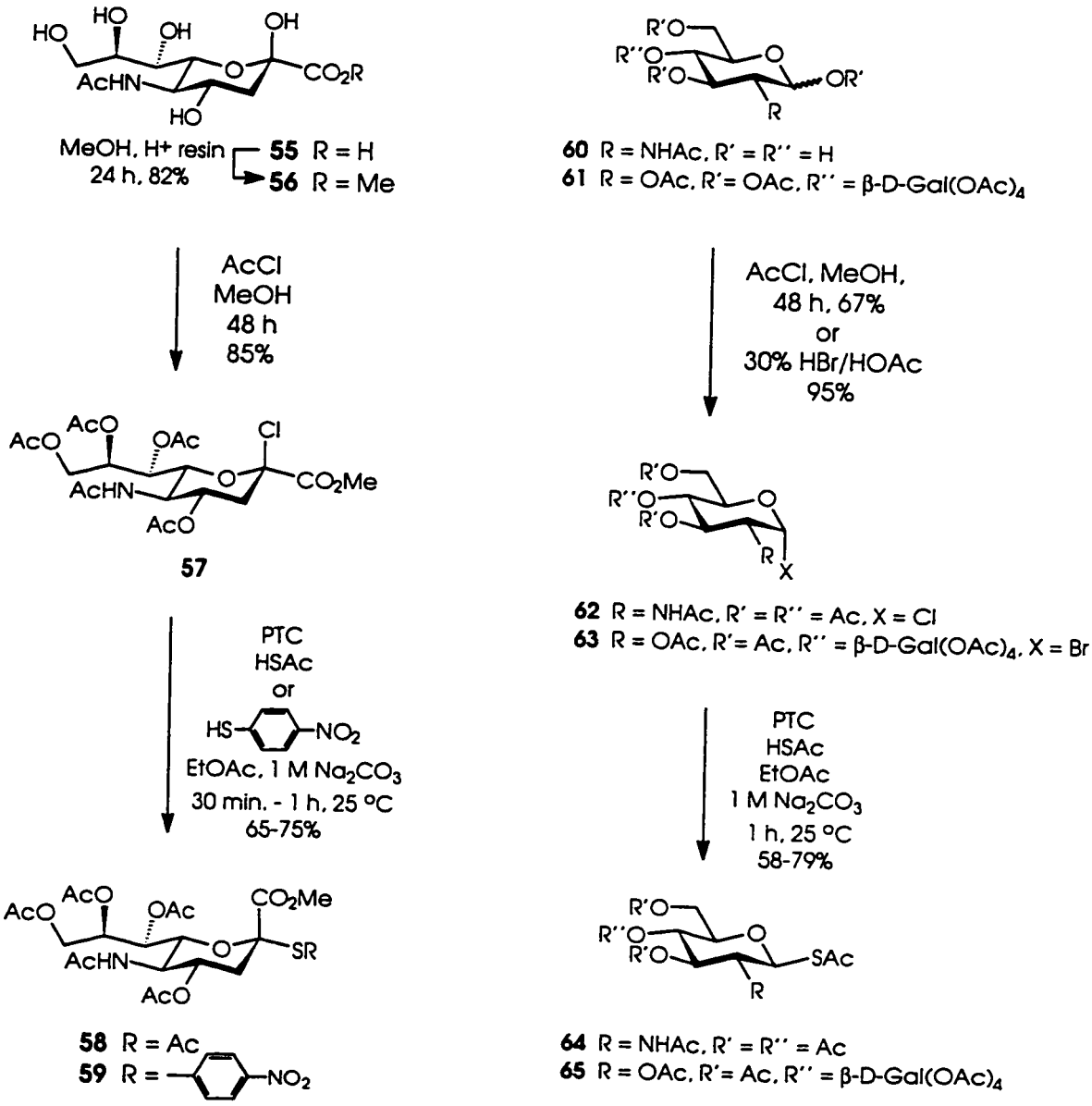
[137] Roy, R. in *Handbook of Phase Transfer Catalysis* (Eds.: Sasson, Y.; Neumann, R.) Chapman & Hall, Glasgow, **1997**, in press.
[138] Roy, R.; Tropper, F. D.; Cao, S.; Kim, J. -M. *ACS Symp. Ser.* **1997**, *659*, 163.
[139] Jeanloz, R. W.; Stoffyn, P. J. *Methods in Carbohydr. Chem.* **1962**, *1*, 221.
[140] Horton, D. *Methods in Carbohydr. Chem.* **1972**, *6*, 282.
[141] Kuhn, R.; Luts, P.; MacDonald, D. L. *Chem. Ber.* **1966**, *99*, 611.



Scheme 2.3.2. PTC synthesis of thioglycoside derivatives.

(TBAHS, 1 eq., 25 °C) as catalyst with either thiolacetic acid or *p*-nitrothiophenol (1.5 eq., 25 °C) in equal volumes of ethyl acetate and 1 M Na_2CO_3 to give thioglycosides **58**, **59**, **64**, and **65** in 58 to 79% isolated yields. Reactions occurred with complete stereocontrol to give the *trans* glycosides shown as judged by well documented data for compounds **58** and **59** (^{1}H-NMR δ 2.77 for H_{3eq})[142] and by ^{1}H- and ^{13}C-NMR spectral data for derivatives **64** and **65** ($J_{1,2} \approx 10$ Hz, C-1 δ 80-86 ppm).

In the first conjugation strategy, thiolated carbohydrate derivatives were coupled to pre-formed N-chloroacetylated dendrimers. Thiolated derivatives **66** to **68** were obtained by the chemoselective de-S-acetylation of thioacetate glycosides **58**, **64**, and **65**. Low temperate Zemplén conditions have been previously employed for the generation of anomeric thiols[143] and in this manner too was generated sialic acid thiol derivative **66** (Scheme 2.3.3). However, in extending this strategy to incorporate glycodendrimers containing sugars with longer spacer arms and non-anomeric thioacetates, it was found that mild, low temperature Zemplén conditions often caused partial de-O-acetylations when O-acetates and S-acetates were simultaneously present in carbohydrate molecules.

Hydrazinium acetate (H_2NNH_2•HOAc) has been used in regioselective anomeric de-O-acetylations,[144] but this reagent had not been previously explored in chemoselective de-S-acetylation reactions. It was found that de-S-acetylation could be achieved using hydrazinium acetate for both anomeric and primary thioacetates.[145] De-O-acetylation was not observed under these conditions and, in addition, chemoselective de-S-acetylation generally occurred in high yield, requiring minimal reaction times.[145] Thus, thioacetate derivative **66** was also generated by the treatment of **58** with hydrazinium acetate in DMF

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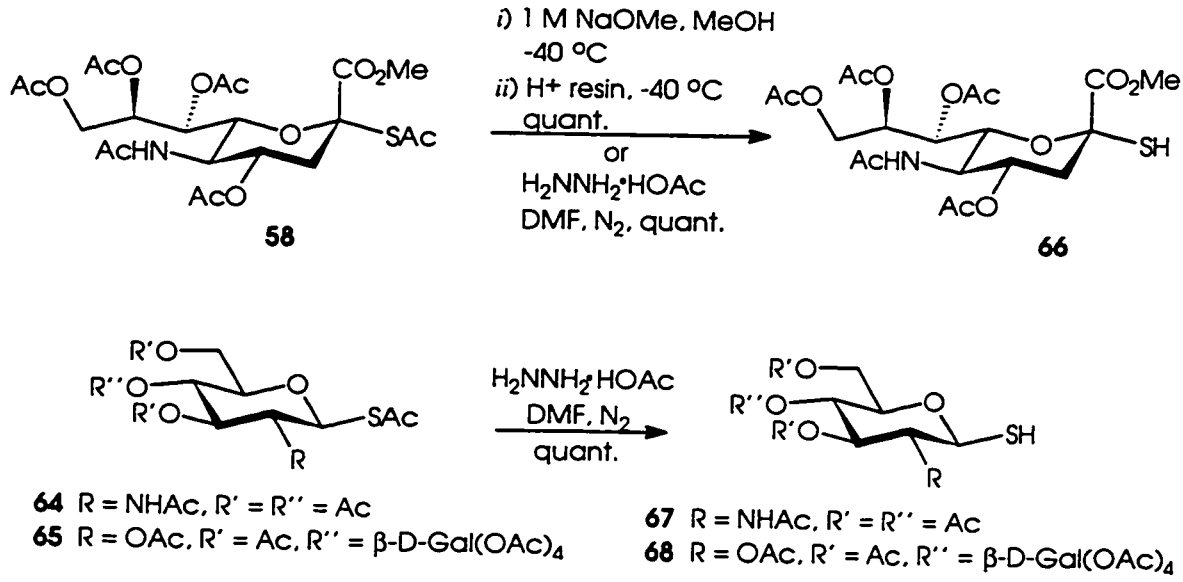
[142] a) Dabrowski, U.; Friebolin, H.; Brossman, R.; Supp, M. *Tetrahedron Lett.* **1979**, *20*, 4637; b) Paulsen, H.; Tiez, H. *Carbohydr. Res.* **1984**, *125*, 47; c) Okamoto, K.; Kodo, T.; Goto, T. *Tetrahedron Lett.* **1986**, *27*, 5229; d) Okamoto, K.; Kodo, T.; Goto, T. *Tetrahedron* **1987**, *43*, 5919; e) Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H.; Maguro, H. *Tetrahedron Lett.* **1988**, *29*, 6317.

[143] Hasegawa, A.; Nakamura, J.; Kiso, M. *J. Carbohydr. Chem.* **1986**, *5*, 11.

[144] Excoffier, G.; Gagnaire, D.; Utille, J. -P. *Carbohydr. Res.* **1975**, *39*, 368.

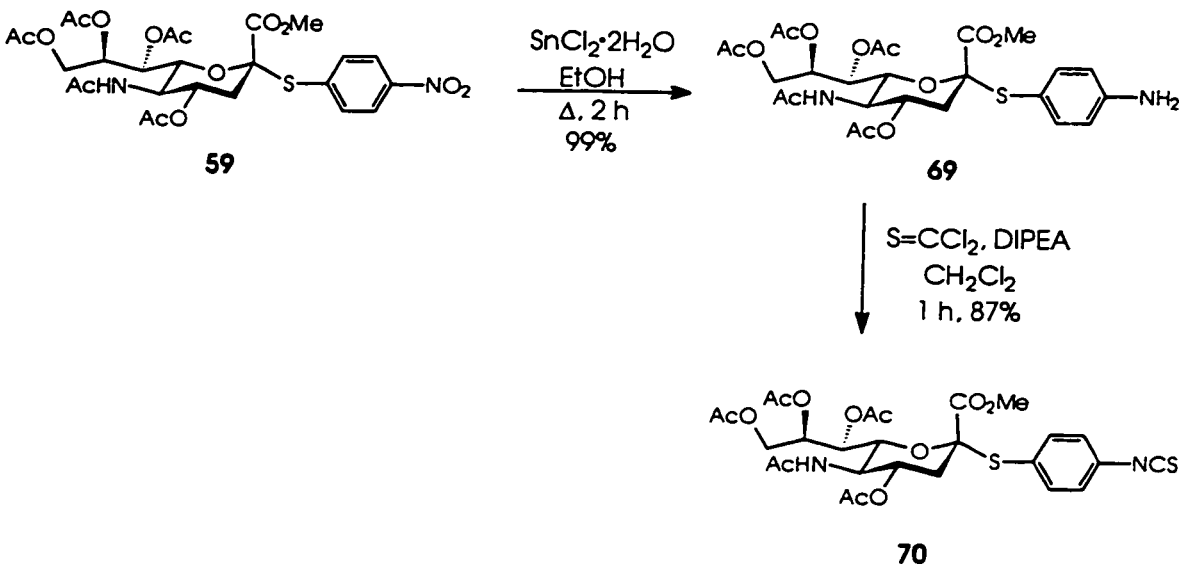
[145] Park, W. K. C.; Meunier, S. J.; Zanini, D.; Roy, R. *Carbohydr. Lett.* **1995**, *1*, 179.

(N_2, 25 °C, quantitative) and used directly for dendrimer conjugation. Thiols **67** and **68** were synthesized from thioacetates **64** and **65** in the same way (Scheme 2.3.3).



Scheme 2.3.3. Synthesis of thiolated glycosides.

The second conjugation strategy included the coupling of glycosl isothiocyanate derivatives to pre-built amine-terminated dendritic cores. The nitro group in sialic acid derivative **59** was efficiently reduced with tin (II) chloride in refluxing ethanol to give the corresponding *p*-aminophenylthioglycoside **69** (2 h, 99%). Compound **69** was treated with thiophosgene in dichloromethane (2 eq., DIPEA) to provide *p*-isothiocyanatophenyl glycoside **70** in 87% yield (Scheme 2.3.4). Isolated derivative **70** was used for dendrimer conjugation.



Scheme 2.3.4. Synthesis of glycoside isothiocyanate derivative.

2.4. Conclusions

Phase transfer catalyzed reactions provided a mild and stereospecific entry into anomeric thioglycoside derivatives. These stable derivatives were synthesized in an easy and high yielding manner and could be further transformed into useful glycoside derivatives such as *p*-isothiocyanatophenyl glycosyl compounds for direct conjugation to readily available amine-terminated dendrimers.

Two conjugation strategies have been proposed in the syntheses of glycodendrimers - the S_N2 displacement of the chlorine atom in pre-formed N-chloroacetylated dendritic structures by thiol glycosides and an isothiocyanate coupling strategy between isothiocyanate glycosides and pre-built amine containing dendrimers.

2.5. Experimental Methods

General Methods

^{1}H- and ^{13}C-NMR were recorded on a Bruker AMX500, Varian XL300, or Gemini 200 spectrometer at 500, 300, and 200 MHz for protons and 125.7, 75.4, and 50.3 MHz for carbons, respectively. Proton chemical shifts (δ) are given relative to internal chloroform (7.24 ppm) for $CDCl_3$ solutions, DMSO (2.49 ppm) for DMSO-d_6 solutions, and to HOD (4.76 ppm) for H_2O solutions unless indicated otherwise. Repeated exchange of protons for deuterium with D_2O and lyophilization for unprotected carbohydrates to simplify proton spectra was performed. Carbon chemical shifts are given relative to $CDCl_3$ (77.0 ppm) and DMSO-d_6 (39.5 ppm). Spectral analyses were performed as first order approximations and were based on shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and 1- and 2-dimensional distortionless enhancement by polarization transfer (DEPT) experiments.

Mass spectra were obtained using a VG 7070-E spectrometer (EI and CI) or Kratos IIH instrument (FAB-glycerol). Xenon was used as the neutral carrier atom in FAB-MS experiments.

Melting points were determined on a Gallenkamp apparatus and are uncorrected.

Optical rotations ($[\alpha]_D$) were measured on a Perkin Elmer 241 polarimeter and were run at room temperature.

Infrared spectra were recorded on Bomem Michelson series FT-IR apparatus. Anhydrous KBr discs were prepared as support for solid compounds. For solutions, NaCl sealed cells were used.

UV-VIS spectra were recorded on a Gilford Response I instrument using quartz cuvettes.

Optical densities (O.D.) for enzyme linked lectin assays (ELLA) and turbidimetric analysis were measured on a Dynatech MR600 Microplate Reader.

Elementary analyses were performed by Guelph Chemical Laboratories Ltd. (Ontario), by M-H-W Laboratories (Phoenix, AZ), or by this department.

The pH of aqueous solutions was measured using a Fischer Scientific Model 805 NP instrument fitted with a Fischer Scientific E-N5 pencil electrode. Qualitative pH measurements were routinely performed with Hydron test paper.

Thin layer chromatography (TLC) was performed using silica gel 60-F254 glass plates. Reagents used for developing plates include ceric sulfate (1% w/v) and ammonium molybdate (2.5% w/v) in 10% (v/v) aqueous sulfuric acid, isatin (0.2% w/v) in ethanol with sulfuric acid (5% v/v), iodine, dilute aqueous potassium permanganate, ninhydrin (2% w/v) in aqueous pyridine (4% v/v) and UV light. TLC plates were heated to ≈150 °C when necessary.

The ninhydrin (Kaiser)[146] color test was used for primary amine detection for both solutions and solid-phase resin samples. Reagents include: ninhydrin (10% w/v) in ethanol, phenol (80 % w/v) in ethanol, and potassium cyanide (1×10^{-4} w/v) in pyridine. Equal volumes of reagents (250 μL) and test solutions or a small resin sample were combined and heated to ≈120 °C for 5 minutes.

Purifications were performed by gravity or flash chromatography on silica gel 60 (230-400 Mesh, E. Merck No. 9385). Solvents were reagent grade and evaporated under reduced pressure using a Büchi rotary evaporator connected to a water aspirator.

Purifications were also performed *via* preparative scale size exclusion chromatography. Columns were connected to a Pharmacia Peristaltic Pump P3 and eluted with distilled H_2O. Waters Differential Refractometer R401 or R403 apparatus was used for detection and recorded on a Linear 1200 or 2000 chart recorder. Fractions were collected using LKB 2112 Redirac or Pharmacia Model 5051 fraction collectors.

Purifications by dialysis were performed using benzoylated cellulose tubing with 2000 Da molecular weight cut off from Sigma.

Lyophilization was carried out on a VIRTIS-24 freeze dryer.

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[146] Atherton, E.; Sheppard, R. C. in *Solid Phase Peptide Synthesis: A Practical Approach* (Eds.: Rickwood, D.; Hames, B. D.) IRL Press, New York, **1989**, p. 108.

All chemical and solvents used in experiments were of reagent grade. Further purifications were performed, when necessary, following published procedures.[147]

Amberlite IRA-400 anion exchange resin and Amberlite IR-120 cation exchange resin were used for synthetic purposes unless stated otherwise.

Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonyl chloride)onate (acetochloroneuraminic acid) (57).[141]

N-Acetylneuraminic acid **55** (1.00 g, 3.24 mmol) and Amberlite IR-120 H^+ resin (2.0 g) in methanol (50 mL) were stirred for 24 h at 25 °C. Completion of the reaction was monitored by TLC (R_f(isopropanol:H_2O, 7:3) 0.74). The clear mixture was filtered and evaporated to dryness. The residue was recrystallised from methanol/ether. Methyl ester **56** was isolated as white crystals in 82% yield (0.85 g, 2.64 mmol); m. p. 181.6-184.0 °C; $[\alpha]_D$ -27.2 ° (c = 1.2, H_2O). Lit.[148] m. p. 180.0-182.0 °C; $[\alpha]_D$ -28 ° (c = 1.0, H_2O).

Methyl ester **56** (obtained above) (0.65 g, 2.01 mmol) was combined with 100 μL MeOH in acetyl chloride (21 mL). The mixture was sealed and left stirring for 48 h at room temperature. Reaction progress was monitored by TLC (R_f(EtOAc) 0.45). The resultant clear solution was evaporated under reduced pressure and co-evaporated with toluene to remove residual HCl. Title compound **57** was dried under vacuum and used for subsequent reactions without further purification. It was isolated in 98% yield (1.00 g, 1.97 mmol). This product can be recrystallised from CH_2Cl_2/ether to give pure **57** as white crystals in 85% yield (0.85 g, 1.67 mmol); m. p. 105.5-108.0 °C; $[\alpha]_D$ -60.5 ° (c = 1.0, $CHCl_3$). Lit.[149] m. p. 105.0 °C (dec.); $[\alpha]_D$ -68.0 ° (c = 1.0, $CHCl_3$).

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147 Perrin, D. D.; Armarego, W. C.; Perrin, D. R. *Purification of Laboratory Compounds, 2nd Edition*, Pergamom Press, London, **1980**.

148 Ogura, H.; Furuhata, K. *Carbohydr. Res.* **1986**, *158*, 37.

149 Sharma, M. N.; Edy, R. *Carbohydr. Res.* **1984**, *127*, 201.

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 (58).

To a solution of freshly prepared acetochloroneuraminic acid **57** (1.00 g, 1.97 mmol) in EtOAc (20 mL) was added a solution of sodium thiolacetate (0.29 g, 2.96 mmol) and tetra-n-butylammonium hydrogen sulfate (0.66 g, 1.97 mmol) in 1 M sodium carbonate (20 mL). The two phase mixture was stirred vigorously for 30 min at room temperature and the reaction monitored by TLC (R_f(EtOAc) 0.39). The reaction mixture was then diluted with 75 mL each of EtOAc and saturated aqueous sodium bicarbonate. The organic phase was separated and washed with saturated $NaHCO_3$ (2 × 75 mL) followed by saturated sodium chloride (75 mL). After drying over Na_2SO_4, the organic phase was filtered and concentrated under reduced pressure. Title compound **58** was purified by recrystallisation from EtOAc/hexanes. Isolated yield was 65% (0.70 g, 1.28 mmol); m. p. 80.0-85.0 °C; $[\alpha]_D$ 49.0 ° (c = 1.1, $CHCl_3$). Lit.[150] m. p. 75.0-80.0 °C; $[\alpha]_D$ 46.7 ° (c = 1.0, MeOH).

Methyl (4-Nitrophenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5,-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (59).

To a solution of freshly prepared acetochloroneuraminic acid **57** (3.30 g, 6.47 mmol) in EtOAc (60 mL) was added a solution of *p*-nitrothiophenol (1.21 g, 7.73 mmol) and TBAHS (2.20 g, 6.47 mmol) in 1 M Na_2CO_3 (60 mL). The mixture was stirred vigorously for 1 h at room temperature and the reaction monitored by TLC (R_f(EtOAc) 0.51). The reaction mixture was next diluted with 125 mL each of EtOAc and saturated $NaHCO_3$. The organic phase was separated and washed with saturated $NaHCO_3$ (2 × 100 mL) followed by saturated NaCl (100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexanes to EtOAc to give compound **59** as an off-

[150] Rothermel, J.; Faillard, H. *Biol. Chem. Hoppe-Seyler* **1989**, *370*, 1077.

white foam in 75% yield (3.05 g, 4.85 mmol); m. p. 167.0-173.0 °C; $[\alpha]_D$ 28.5 ° (c = 1.2, $CHCl_3$). Lit.[150] m. p. 168.0-172.0 °C; $[\alpha]_D$ 27.6 ° (c = 1.0, MeOH).

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride (62).[139]

N-Acetylglucosamine **60** (2.00 g, 9.04 mmol) was combined with MeOH (200 μL) in acetyl chloride (50 mL). The mixture was sealed and left stirring for 48 h at room temperature. The reaction was monitored by TLC (R_f(EtOAc) 0.63). The resultant clear solution was evaporated under reduced pressure and co-evaporated with toluene. Glycosyl chloride **62** was dried under vacuum and purified by silica gel column chromatography using a gradient of hexanes to EtOAc as eluent. Title compound **62** was isolated as a beige solid in 67% yield (2.22 g, 6.07 mmol); m. p. 125.0-128.1 °C; $[\alpha]_D$ 105.0 ° (c = 1.0, $CHCl_3$). Lit.[140] m. p. 127.0-128.0 °C; $[\alpha]_D$ 110.0 ° (c = 1.0, $CHCl_3$).

2,2',3,3',4',6,6'-Hepta-O-acetyl-α-D-lactosyl bromide (63).

To a solution of peracetylated lactose **61**[138] (1.00 g, 1.48 mmol) dissolved in EtOAc (20 mL) was slowly added 30 wt. % HBr in glacial acetic acid (0.60 g, 2.22 mmol) while stirring. Reaction progress was monitored by TLC (R_f(EtOAc:hexanes, 2:1) 0.46) and judged complete in 1 h. The reaction mixture was then diluted with CH_2Cl_2 (100 mL) and washed with 75 mL saturated $NaHCO_3$ portions until neutral in pH. The organic phase was then washed with H_2O (2 × 50 mL) and saturated NaCl (50 mL) and dried over Na_2SO_4. The organic phase was concentrated under reduced pressure to give lactosyl bromide **63** as a white foam in 95% yield (0.98 g, 1.41 mmol). The product was used directly, without further purification. ^{1}H- and ^{13}C-NMR spectral data agree with previously reported values.[151,152] ^{1}H-NMR ($CDCl_3$) δ 1.78, 1.84, 2 × 1.88, 1.90, 1.93, 1.97 (7s, 21H, OAc's), 3.68 (m, 1H, H-5), 3.82 (dd, 1H, $J_{3,4}$ 9.1 Hz, $J_{4,5}$ 8.7 Hz, H-4), 3.85 (m, 1H, H-5'), 4.02-4.13 (m, 4H, H-6, H-6'), 4.45 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.92

[151] Roy, R.; Tropper, F. D.; Andersson, F.O.; Grand-Maître, C. *Synthesis* **1991**, *7*, 734.
[152] Roy, R.; Tropper, F. D.; Grand-Maître, C. *Can. J. Chem.* **1991**, *69*, 1462.

(dd, 1H, $J_{3',4'}$ 3.4 Hz, H-3'), 5.09 (m, 2H, H-2, H-2'), 5.22 (dd, 1H, H-3), 5.33 (dd, 1H, $J_{4',5'}$ 1Hz, H-4'), 5.64 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1); ^{13}C-NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 20.7, 20.8 (OAc's), 60.8 (C-5), 61.7 (C-6'), 66.5 (C-4'), 68.9, 70.4 (C-2, C-2'), 70.6 (C-3'), 70.9 (C-6), 72.5 (C-3), 73.4 (C-5'), 75.6 (C-4), 91.4 (C-1), 100.8 (C-1'), 168.8-170.3 (C=O's).

2-Acetamido-3,4,6-tri-O-acetyl-1-S-acetyl-2-deoxy-1-thio-α-D-glucopyranose (64).

To a solution of N-acetylglucosaminyl chloride **62** (2.00 g, 5.47 mmol) in EtOAc (20 mL) was added a solution of sodium thiolacetate (0.80 g, 5.47 mmol) and TBAHS (1.85 g, 5.47 mmol) in 1 M Na_2CO_3 (20 mL). The mixture was stirred vigorously for 1 h and the reaction monitored by TLC (R_f(EtOAc) 0.45). The mixture was next diluted with 75 mL of each EtOAc and saturated $NaHCO_3$. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ (2 × 75 mL), followed by saturated NaCl (75 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Title compound **64** was purified by silica gel column chromatography using a gradient of hexanes to EtOAc as eluent and isolated as an off-white powder in 58% yield (1.29 g, 3.17 mmol); m. p. 178.5-180.1 °C, $[\alpha]_D$ -2.62 ° (c = 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.89 (s, 3H, NAc), 2 × 2.01, 2.05 (3s, 9H, OAc's), 2.34 (s, 3H, SAc), 3.77 (m, 1H, H-5), 4.07 (dd, 1H, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 4.21 (dd, 1H, $J_{5,6'}$ 4.6 Hz, H-6'), 4.33 (m, 1H, H-2), 5.08-5.15 (m, 3H, H-1, H-3, H-4), 5.75 (d, 1H, $J_{2,NH}$ 9.8 Hz, NH); ^{13}C-NMR ($CDCl_3$) δ 20.5, 20.6, 20.7 (OAc's), 23.1 (NAc), 30.8 (SAc), 52.2 (C-2), 61.8 (C-6), 67.8 (C-3), 74.1 (C-4), 76.6 (C-5), 81.6 (C-1), 169.2, 170.0, 170.7, 171.3 (C=O's), 193.6 (SC=O); FAB-MS (pos.) calcd. for $C_{16}H_{21}NO_9S$: 405.1; found: 406.1 (M^+ + 1, 31.4%). Anal. calcd. for $C_{16}H_{21}NO_9S$: C, 47.40; H, 5.72; N, 3.45; S, 7.91; found: C, 47.28; H, 5.82; N 3.40; S, 7.73.

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1-4)-O-2,3,6-tri-O-acetyl-1-S-acetyl-1-thio-α-D-glucopyranose (65).

To a solution of freshly prepared lactosyl bromide **63** (6.15 g, 8.80 mmol) in EtOAc (40 mL) was added a solution of sodium thiolacetate (1.30 g, 13.2 mmol) and TBAHS (3.00 g, 8.80 mmol) in 1 M Na_2CO_3 (40 mL). The mixture was stirred vigorously for 1 h and the reaction monitored by TLC (R_f(EtOAc:hexanes, 2:1) 0.38). The mixture was next diluted with 100 mL of each EtOAc and saturated $NaHCO_3$. The organic layer was separated and washed successively with saturated aqueous $NaHCO_3$ (2 × 100 mL) and saturated NaCl (100 mL). After drying over Na_2SO_4, the organic layer was concentrated under reduced pressure and purified *via* silica gel column chromatography using a gradient of hexanes to EtOAc as eluent. Title compound **65** was isolated as an off-white foam in 79% yield (4.80 g, 6.95 mmol); m. p. 67.3-74.0 °C; $[\alpha]_D$ -1.20 (c = 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.78, 1.84, 2 × 1.87, 1.90, 1.93, 1.97 (7s, 21H, OAc's), 2.20 (s, 3H, SAc), 3.61-3.64 (m, 2H, H-4, H-5'), 3.77 (dd, 1H, $J_{5,6a}$ 6.6 Hz, $J_{6a,6b}$ 1.5 Hz, H-6a), 3.93-3.97 (m, 3H, H-5, H-6'), 4.27 (dd, 1H, $J_{5,6b}$ 12.0 Hz), H-6b, 4.35 (d, 1H, $J_{1',2'}$ 7.88 Hz, H-1'), 4.81 (dd, 1H, $J_{2',3'}$ 3.5 Hz, $J_{3',4'}$ 10.4 Hz, H-3'), 4.86-4.91 (m, 2H, H-2, H-2'), 5.06 (d, 1H, $J_{1,2}$ 10.5 Hz, H-1), 5.09 (dd, 1H, $J_{2,3}$ 9.02 Hz, $J_{3,4}$ 9.02 Hz, $J_{4',5'}$ 0.8 Hz, H-4'); ^{13}C-NMR ($CDCl_3$) δ 20.3, 2 × 20.4, 20.5, 20.6, 20.8 (OAc's), 30.6 (SAc), 61.0 (C-5), 62.0 (C-6'), 66.7 (C-4'), 68.9, 69.2 (C-2, C-2'), 70.6 (C-3'), 70.8 (C-6), 73.5 (C-5'), 77.0 (C-4), 79.9 (C-1), 100.6 (C-1'), 168.8-170.1 (C=O's), 191.7 (SC=O); FAB-MS (pos.) calcd. for $C_{28}H_{38}O_{18}S$: 694.18; found: 695.3 (M^+ + 1, 0.9%). Anal. calcd. for $C_{28}H_{38}O_{18}S$: C, 48.45; H, 5.55; found: C, 49.12; H, 5.68.

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (66).

Procedure 1. To a solution of **58** (143.5 mg, 0.261 mmol) in dry methanol (5 mL), cooled to -40 °C, was added a 1 M solution of sodium methoxide (250 μL, 0.249 mmol). The mixture was stirred at -40 °C for 10 min and monitored by TLC (R_f(EtOAc)

0.35). The solution was then treated with Amberlite IR-120 H^+ exchange resin at -40 °C for 15 min. The solution was filtered and evaporated at room temperature under reduced pressure to give title compound **66** as a yellow resin in quantitative yield (132.5 mg, 0.261 mmol). Derivative **66** was used without further purification.

Procedure 2. To a degassed solution of **58** (100 mg, 0.182 mmol) in DMF (100 μL) was added hydrazinium acetate (182 μL of a degassed 2.5M solution of H_2NNH_2•HOAc in DMF) under N_2 at room temperature. The reaction was monitored by TLC (R_f(EtOAc) 0.35). After 20 min, the mixture was diluted with degassed EtOAc (10 mL) and washed successively with degassed H_2O (2 × 10 mL) and saturated NaCl (10 mL). The organic phase was then dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Compound **66** was isolated in quantitative yield (92.4 mg, 0.182 mmol) and used immediately without any further purification.

^{1}H-NMR ($CDCl_3$) δ 1.88 (s, 3H, NAc), 2.03, 2.05, 2.13, 2.14 (4s, 12H, OAc's), 2.04 (dd, 1H, $J_{3ax,3eq}$ 12.8 Hz, $J_{3ax,4}$ 11.7 Hz, H-3ax), 2.80 (dd, 1H, $J_{3e,4}$ 4.8 Hz, H-3eq), 3.17 (s, 1H, SH), 3.73 (dd, 1H, $J_{5,6}$ 10.8 Hz, $J_{6,7}$ 1.9 Hz, H-6), 3.83 (s, 3H, OCH_3), 4.06 (m, 1H, H-5), 4.10 (dd, 1H, $J_{8,9}$ 5.5 Hz, $J_{9,9'}$ 12.4 Hz, H-9), 4.49 (dd, 1H, $J_{8,9'}$ 2.1 Hz, H-9'), 4.91 (ddd, 1H, $J_{4,5}$ 10.3 Hz, H-4), 5.17 (d, 1H, $J_{NH,5}$ 10.5 Hz, NH), 5.28-5.33 (m, 2H, H-7, H-8); ^{13}C-NMR ($CDCl_3$) δ 20.8, 20.8, 21.1 (OAc's), 23.1 (NAc), 38.9 (C-3), 49.1 (C-5), 53.4 (OCH_3), 62.2 (C-9), 67.7 (C-7), 69.4 (C-4), 70.1 (C-8), 75.1 (C-6), 81.6 (C-2), 170.0-170.9 (C=O's).

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-α-D-glucopyranose (67).

To a degassed solution of **64** (18.7 mg, 0.046 mmol) in DMF (100 μL) was added hydrazinium acetate (46 μL of a degassed 2.5M solution in DMF) under N_2 at room temperature. After 20 min, the reaction was judged complete by TLC (R_f(EtOAc) 0.40) and diluted with degassed EtOAc (10 mL). The solution was washed successively with degassed H_2O (2 × 10 mL) and saturated NaCl (10 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give title compound **67** as a

yellow resin in quantitative yield (16.7 mg, 0.046 mmol). Derivative **67** used immediately without any further purification; ^{1}H-NMR ($CDCl_3$) δ 1.89 (s, 3H, NAc), 2 × 2.01, 2.05 (3s, 9H, OAc's), 3.17 (s, 1H, SH), 3.77 (m, 1H, H-5), 4.07 (dd, 1H, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 4.21 (dd, 1H, $J_{5,6'}$ 4.6 Hz, H-6'), 4.33 (m, 1H, H-2), 5.08-5.15 (m, 3H, H-1, H-3, H-4), 5.75 (d, 1H, $J_{2,NH}$ 9.8 Hz, NH); ^{13}C-NMR ($CDCl_3$) δ 20.5, 20.6, 20.7 (OAc's), 23.1 (NAc), 52.2 (C-2), 61.8 (C-6), 67.8 (C-3), 74.1 (C-4), 76.6 (C-5), 81.6 (C-1), 169.2, 170.0, 170.7, 171.3 (C=O's).

2,3,4,5-Tetra-O-acetyl-β-D-galactopyranosyl-(1-4)-O-2,3,6-tri-O-acetyl-1-thio-α-D-glucopyranose (68).

To a degassed solution of **65** (100 mg, 0.144 mmol) in DMF (100 μL) was added H_2NNH_2•HOAc (144 μL of a degassed 2.5M solution of in DMF) under N_2 at room temperature. The reaction was monitored by TLC (R_f(EtOAc:hexanes, 2:1) 0.34). After 20 min, the mixture was diluted with degassed EtOAc (10 mL) and washed successively with degassed H_2O (2 × 10 mL) and saturated NaCl (10 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give title compound **68** as a yellow resin in quantitative yield (93.9 mg, 0.144 mmol). Compound **68** was used immediately without any further purification; ^{1}H-NMR ($CDCl_3$) δ 1.78, 1.84, 2 × 1.87, 1.90, 1.93, 1.97 (7s, 21H, OAc's), 3.18 (s, 1H, SH), 3.61-3.64 (m, 2H, H-4, H-5'), 3.77 (dd, 1H, $J_{5,6a}$ 6.6 Hz, $J_{6a,6b}$ 1.5 Hz, H-6a), 3.93-3.97 (m, 3H, H-5, H-6'), 4.27 (dd, 1H, $J_{5,6b}$ 12.0 Hz), H-6b, 4.35 (d, 1H, $J_{1',2'}$ 7.88 Hz, H-1'), 4.81 (dd, 1H, $J_{2',3'}$ 3.5 Hz, $J_{3',4'}$ 10.4 Hz, H-3'), 4.86-4.91 (m, 2H, H-2, H-2'), 5.06 (d, 1H, $J_{1,2}$ 10.5 Hz, H-1), 5.09 (dd, 1H, $J_{2,3}$ 9.02 Hz, $J_{3,4}$ 9.02 Hz, $J_{4',5'}$ 0.8 Hz, H-4'); ^{13}C-NMR ($CDCl_3$) δ 20.3, 2 × 20.4, 20.5, 20.6, 20.8 (OAc's), 61.0 (C-5), 62.0 (C-6'), 66.7 (C-4'), 68.9, 69.2 (C-2, C-2'), 70.6 (C-3'), 70.8 (C-6), 73.5 (C-5'), 77.0 (C-4), 79.9 (C-1), 100.6 (C-1'), 168.8-170.1 (C=O's).

Methyl (4-Aminophenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (69).

p-Nitrophenyl derivative **59** (40.0 mg, 0.064 mmol) was suspended in absolute ethanol (10 mL) to which was added tin (II) chloride dihydrate ($SnCl_2 \bullet 2H_2O$, 75.0 mg, 0.402 mmol). The reaction mixture was stirred at 70 °C for 2 h and monitored by TLC (R_f(EtOAc) 0.41). The reaction mixture was then cooled and poured onto ice-water and the final pH adjusted to ≈8 with $NaHCO_3$. The resulting mixture was filtered and the clear filtrate extracted with EtOAc (3 × 20 mL). The organic layers were combined, washed successively with saturated $NaHCO_3$ (20 mL), H_2O (20 mL), and saturated NaCl (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Title compound **69** was obtained as an off-white powder in 99% yield (37.7 mg, 0.063 mmol) and used directly in subsequent reactions without further purification; m. p. 96.0-101.0 °C, $[\alpha]_D$ 26.0 ° (c = 1.0, $CHCl_3$); Lit.[150] m. p. 98.0-102.0 °C, $[\alpha]_D$ 26.1 ° (c = 1.270, MeOH).

Methyl (4-Isothiocyanatophenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (70).

p-Aminophenyl derivative **69** (35.0 mg, 0.058 mmol) was dissolved in CH_2Cl_2 (20 mL) containing diisopropylethylamine (DIPEA, 18.7 mg, 0.145 mmol). Thiophosgene (16.7 mg, 0.145 mmol) was added and the solution stirred at 25 °C for 1 h. The solvent was evaporated under reduced pressure and the residue purified by silica gel column chromatography using a gradient of hexanes to EtOAc as eluent to afford **70** in 87% yield (32.6 mg, 0.051 mmol); m. p. 125.0-130.1 °C; $[\alpha]_D$ 26.3 ° (c = 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.85 (s, 3H, NAc), 2.00, 2.03, 2.04, 2.13 (4s, 12H, OAc's), 2.02 (m, 1H, H-3ax), 2.80 (dd, 1H, $J_{3ax,3eq}$ 12.8 Hz, $J_{3eq,4}$ 4.7 Hz), 3.57 (s, 3H, OCH_3), 3.78-3.90 (m, 2H, H-5, H-6), 3.96-4.11 (m, 1H, H-9), 4.31 (m, 1H, H-9'), 4.83 (ddd, 1H, $J_{3ax,4}$ 11.7 Hz, $J_{4,5}$ 10.3 Hz, H-4), 5.10 (d, 1H, $J_{NH,5}$ 9.9 Hz, NH), 5.23-5.27 (m, 2H, H-7, H-8), 7.16 (d, 2H, $J_{o,m}$ 8.6 Hz, H-ortho), 7.45 (d, I H, H-meta); ^{13}C-NMR ($CDCl_3$) δ 20.7, 20.9 (OAc's), 23.1 (NAc), 38.1 (C-3), 49.3 (C-5), 52.8 (OCH_3), 60.3 (C-9), 62.0 (C-7), 67.5

(C-7), 69.2 (C-4), 69.4 (C-8), 74.5 (C-6), 126.0 (C-ortho), 127.9 (N=C=S), 132.9 (C-para), 137.1 (C-ipso), 137.2 (C-meta), 167.5-170.8 (C=O's); FAB-MS (pos.) calcd. for $C_{27}H_{32}N_2O_{12}S$: 640.14; found: 641.2 ($M^+ + 1$, 18.0%). Anal. calcd. for $C_{27}H_{32}N_2O_{12}S$: C, 50.62; H, 5.03; N, 4.37; found: C, 50.23; H, 5.10; N 4.52.

Chapter 3. Synthesis of L-Lysine-Based Glycodendrimers

3.1. Introduction

The dendrimers introduced by Denkewalter *et al.*[95] represent the first examples of well-defined, monodisperse tree-like molecules until generation 10. The core used was based on benzylhydrylamide **71** and the construction proceeded with N^{α},N^{ε}-BOC-L-lysine *p*-nitrophenyl activated ester (**72**) as the branching residue (Scheme 3.1.1).[153] Generation 10 has 2048 terminal amino groups and its diameter reaches approximately 100 Å according to measurements made by intrinsic viscosity or small angle X-ray scattering.[154,155]

Tam *et al.*[96,97] have fixed these L-lysine-based trees on a Merrifield resin. "Fixing" peptides on a resin, or solid phase peptide synthesis (SPPS), is based on the sequential addition of N-α-protected amino acid residues to an insoluble polymeric support resulting in a peptide with its C-terminus attached to the linker of the support. The N-α-protection is generally the acid-labile *t*-butoxycarbonyl (BOC) group or the base-labile 9-fluorenylmethoxycarbonyl (Fmoc) group. Its removal allows for the coupling of the next protected amino acid carboxylic group to the newly generated α-amino group of the resin supported amino acid residue, after its deprotection. The coupling process is carried out by an *in situ* coupling reagent such as dicyclohexylcarbodiimide (DCC) or diisopropylcarbodiimide (DIC) and a pre-activated amino acid such as pre-formed activated esters or symmetrical anhydrides of the amino acid to be coupled.[156,157]

SPPS has several advantages. Every coupling may be effectively monitored for the disappearance of reacting amine *via* the ninhydrin (Kaiser) color test.[146,158] In this

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[154] Aharoni, S. M.; Crosby III, C. R.; Walsh, E. K. *Macromolecules* **1982**, *15*, 1093.
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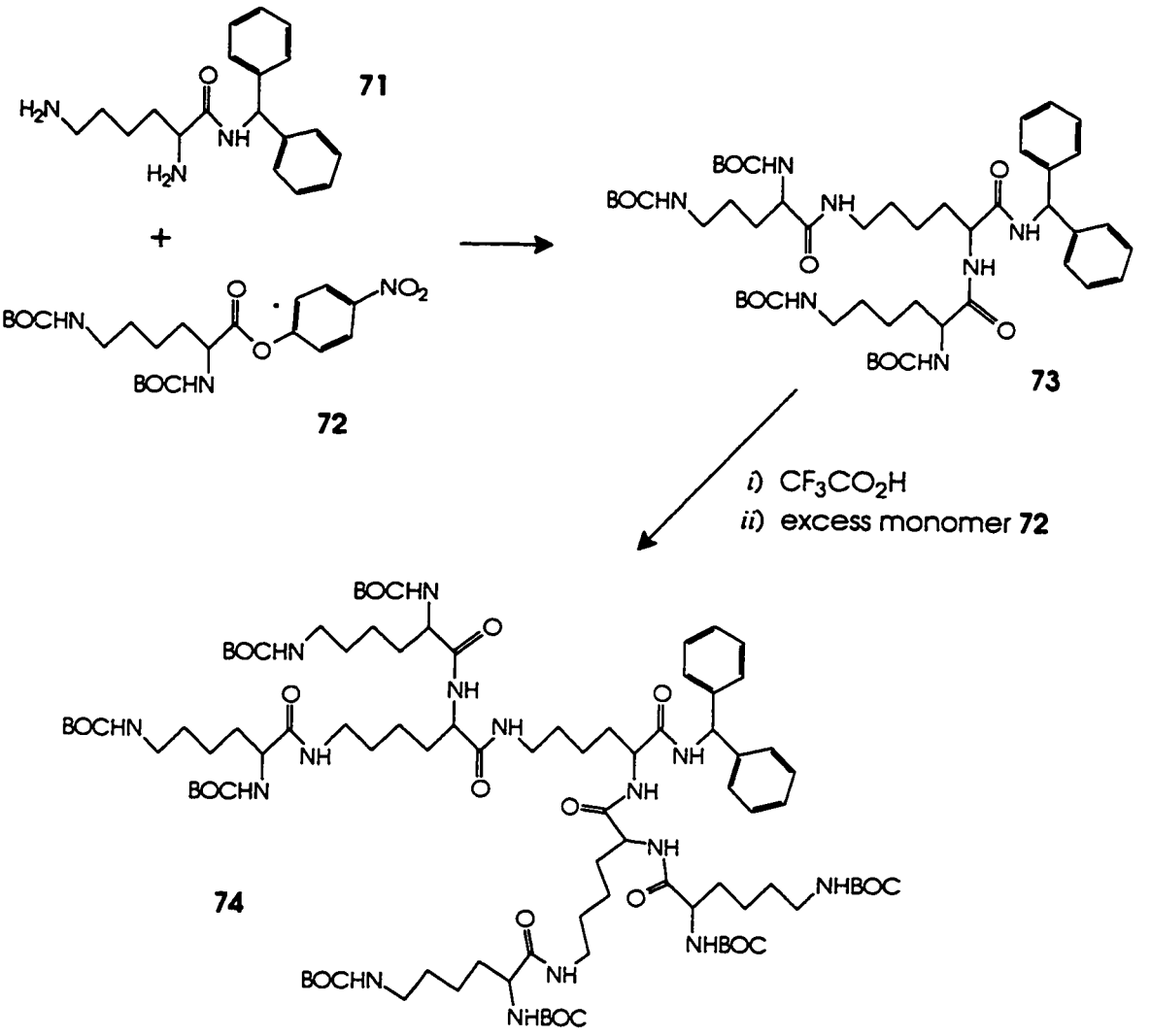
[158] Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

way, coupling reactions may be made to go to completion. Furthermore, any excess or unreacted, soluble reagents can be removed from the desired resin-bound peptide by simple filtration and washing. Subsequently, high purity peptides may be generated.



Scheme 3.1.1. Synthesis of L-lysine-based dendrimers by Denkewalter *et al.*[95]

The solid phase synthesis of Tam *et al.*[96,97,159] was first introduced as a novel approach to anti-peptide antibodies. A number of peptide antigens were anchored to the relatively small, immunogenically inert, resin-bound, branched L-lysine core and the macromolecular, multivalent peptide antigen conjugate was cleaved from the support using standard techniques. It was found that these conjugates did not require the use of a carrier protein to elicit an antibody response.[96,97,159]

Tam's technique has come to be known as the multiple antigen peptide (MAP) system and is pictured in Figure 3.1.1. Using this MAP system as a dendritic support, the first glycodendrimers were synthesized.[105,106]

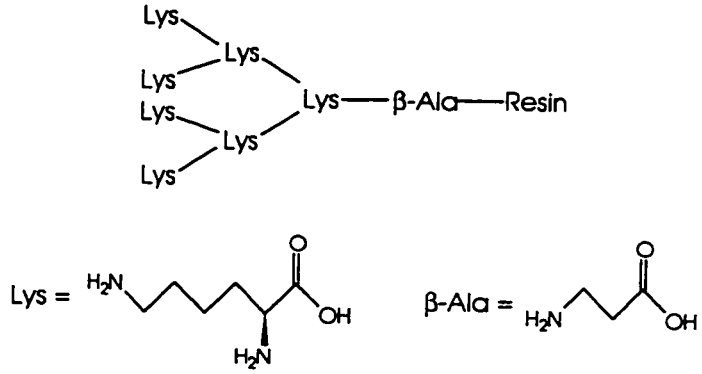


Figure 3.1.1. The multiple antigen peptide (MAP) system.

3.2. L-Lysine Core

Glycodendrimers were first prepared based on L-lysine as a core unit upon which the scaffolding of subsequent generations was constructed.[105,106] The synthesis of each dendritic structure was based on well established and high yielding solid phase peptide chemistry using 9-fluorenylmethoxycarbonyl (Fmoc) amino-protecting groups and benzotriazolyl esters (HOBt) with diisopropylcarbodiimide (DIC) as coupling reagents.

[159] Tam, J. P. (Ed.) *Synthetic Peptides: Approaches to Biological Problems*, **1988**, pp.3.

Multivalent L-lysine structures were capped with a chloroacetylglycylglycine spacer still using the Fmoc/HOBt strategy.

Specifically, dendritic L-lysine cores were elaborated on *p*-benzyloxybenzyl alcohol (Wang) resin (0.58 mmol/g substitution). A β-alanyl spacer was originally anchored to the Wang resin using an Fmoc/HOBt ester strategy (Fmoc-β-Ala-OBt, 2 eq., 0.5 eq. DMAP, 1 eq. DIC, DMF, 2.5 h). The extent of coupling was established by the spectrophotometric quantitation of the released dibenzofulvene chromophore at 300 nm following piperidine treatment.[160,161]

N^{α},N^{ε}-Di-Fmoc-L-lysine was synthesized in 68% yield using a well established procedure with 9-fluorenylmethylchloroformate in 10% sodium bicarbonate.[162] The corresponding benzotriazolyl ester **75** was freshly prepared in N,N-dimethylformamide (DMF) with one equivalent each of HOBt and DIC (0 °C for 15 min, then 25 °C for 1 h). Derivative **75** was coupled to the N-α-protected resin-bound β-alanine (2 eq. **75**, DIC, DMF, 2.5 h). Deprotection followed by coupling with benzotriazolyl ester **75** was the usual cycle for the synthesis of higher generation L-lysine cores (Scheme 3.2.1). In each cycle, the Fmoc protecting group was removed by the usual β-elimination process using 20% piperidine in DMF (1 × 3 min, 1 × 7 min).

The products resulting from each sequential generation were directly treated with pre-formed chloroacetylglycylglycine benzotriazolyl ester **76** prepared by the above procedure (Scheme 3.2.1). The required chloroacteylglycylglycine is commercially available and did not necessitate individual glycine residue coupling followed by N-capping with chloroacetic anhydride as is commonly done.

The ninhydrin (Kaiser) color test[146] was used to monitor each coupling and, when necessary, couplings were repeated.

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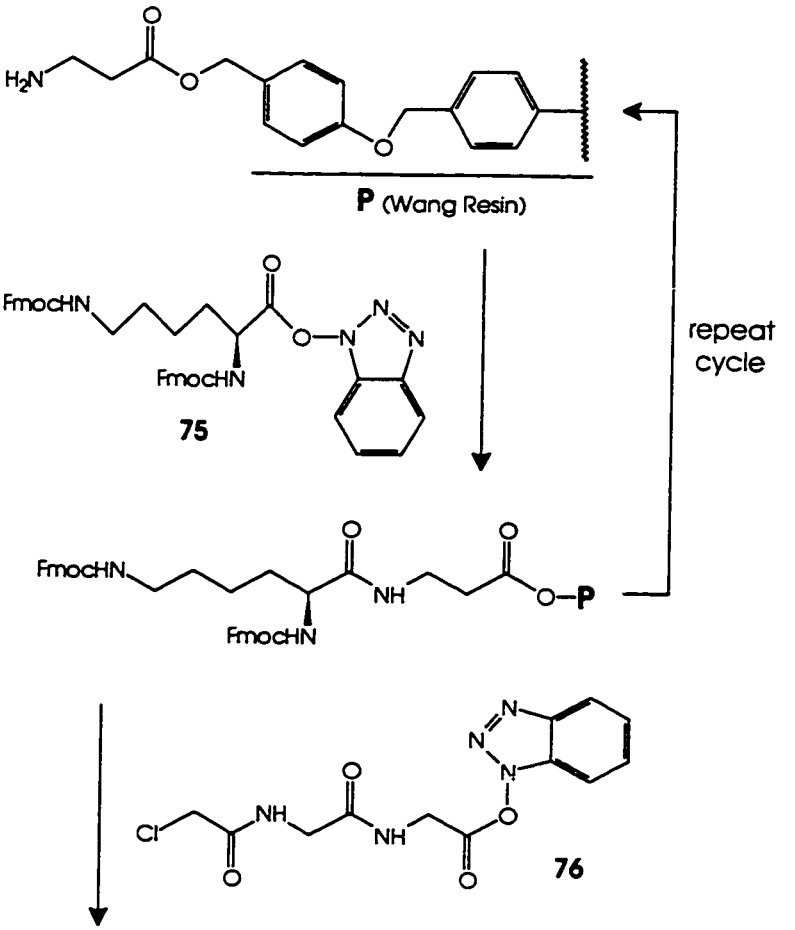
[160] Sheppard, R. C. *Chem. Britain* **1983**, *19*, 402.
[161] Meienhofer, J.; Waki, M.; Heimer, E. P.; Lambros, T. J.; Makofske, R. C.; Chang, C. D. *Int. J. Peptide Protein Res.* **1979**, *13*, 35.
[162] Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.



Nα,Nε-(ClCH$_2$CO-Gly-Gly-)$_2$-Lys-β-Ala-O-R

95% TFA, 1.5 h, 93% → **77** R = **P**; **78** R = H

Nα,Nε-(ClCH$_2$CO-Gly-Gly-)$_4$-Lys$_2$-Lys-β-Ala-O-R

95% TFA, 1.5 h, 90% → **79** R = **P**; **80** R = H

Nα,Nε-(ClCH$_2$CO-Gly-Gly-)$_8$-Lys$_4$-Lys$_2$-Lys-β-Ala-O-R

95% TFA, 1.5 h, 99% → **81** R = **P**; **82** R = H

Nα,Nε-(ClCH$_2$CO-Gly-Gly-)$_{16}$-Lys$_8$-Lys$_4$-Lys$_2$-Lys-β-Ala-O-R

95% TFA, 1.5 h, 90% → **83** R = **P**; **84** R = H

Scheme 3.2.1. Synthetic sequence for N-chloroacetylated dendrimers.

Using this solid phase approach, di- (**77**), tetra- (**79**), octa- (**81**), and hexadeca- (**83**) valent dendrimers were obtained in the first, second, third, and fourth generations respectively. For quality and structural determination purposes, the corresponding unbound N-chloroacetylated acid derivatives **78**, **80**, **82**, and **84** were released from the polymer support by treatment with aqueous trifluoroacetic acid (95% TFA, 1.5 h). N-Chloroacetylated dendrimers **78**, **80**, **82**, and **84** were generally obtained in >90% yields with 90-95% purity. Dendrimers **78**, **80**, **82**, and **84** exhibited characteristic ^{1}H-NMR signals. From the ratio of the β-alanyl α-CH_2 residues at 2.36 ppm to those of both the L-lysyl ε-CH_2 and chloroacetyl methylene groups (≈3.00 and 4.12 ppm, respectively), the integrity of the dendritic core could be evaluated. Figure 3.2.1 shows these characteristic signals.

3.3. Conjugation of Sialic Acid to L-Lysine Core

Using the S_N2 chloride displacement by a thiol conjugation strategy (as outlined in Chapter 2, Section 2.2), each dendrimer generation was treated with a slight excess of 2-thiosialic acid derivative **66** (1.2 eq. per N-chloroacetyl functionality, 1% triethylamine/DMF, 25 °C, 16 h). Before the bulk of the dendrimers were released from the polymeric support, aliquots were withdrawn and hydrolyzed as above (95% TFA, 1.5 h). The completeness of the couplings was estimated from the ^{1}H-NMR spectra of the sialylated dendrimers which showed characteristic signals for any residual N-chloroacetyl methylene groups at 4.12 ppm (DMSO-d_6). Where required, couplings were repeated (Schemes 3.3.1 to 3.3.4).

The polymer bound peracetylated dendrimers **85**, **89**, **92**, and **93** were released from the polymer support as above for N-chloroacetylated dendrimers **78**, **80**, **82**, and **84** and obtained in 66-99% yields after solvent removal under reduced pressure (Schemes 3.3.1 to 3.3.4). The ^{1}H- and ^{13}C-NMR spectra (Figures 3.3.1 and 3.3.2) revealed the integrity of the α-sialoside linkages as well as the ratio of the β-alanyl residues relative to

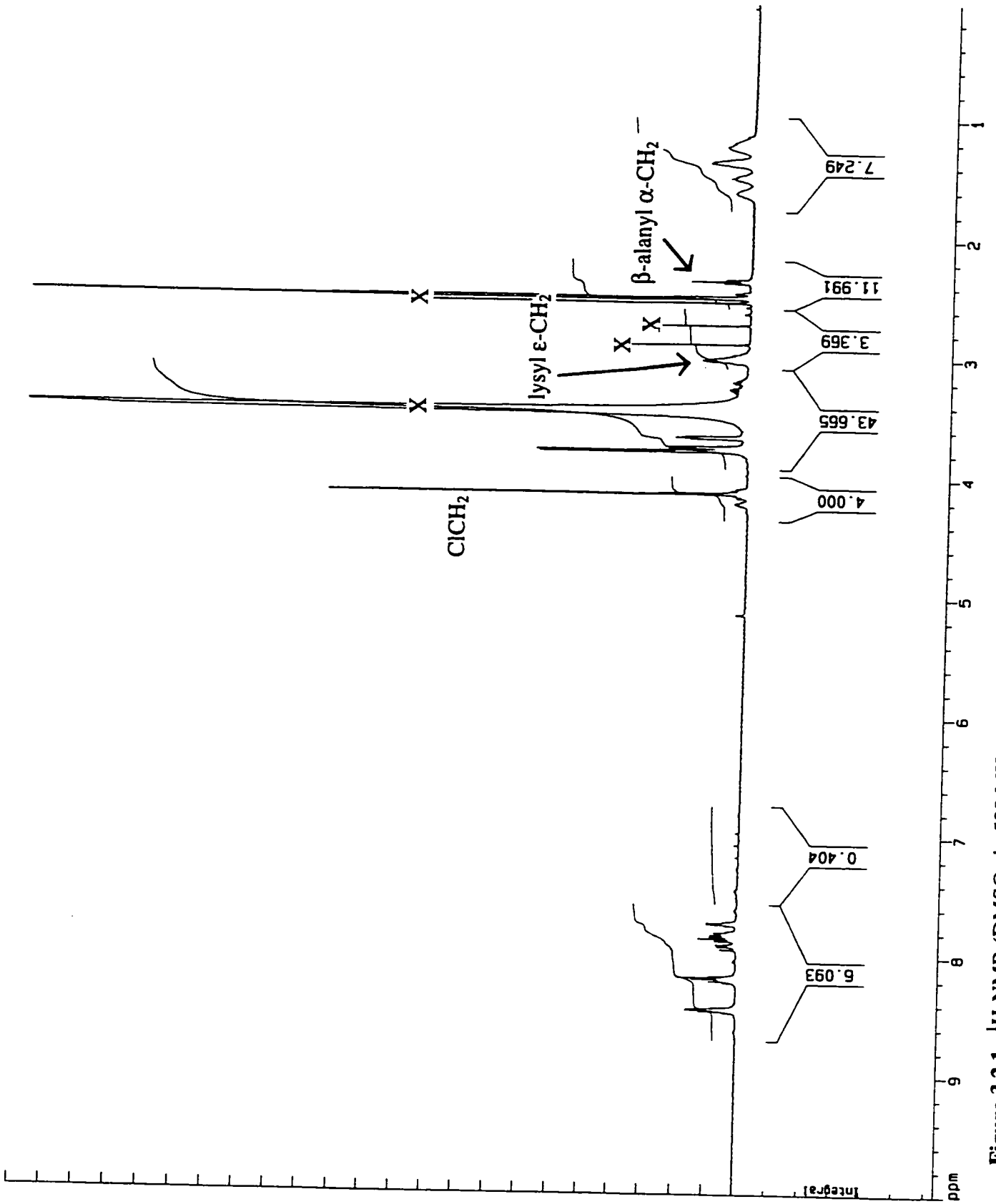
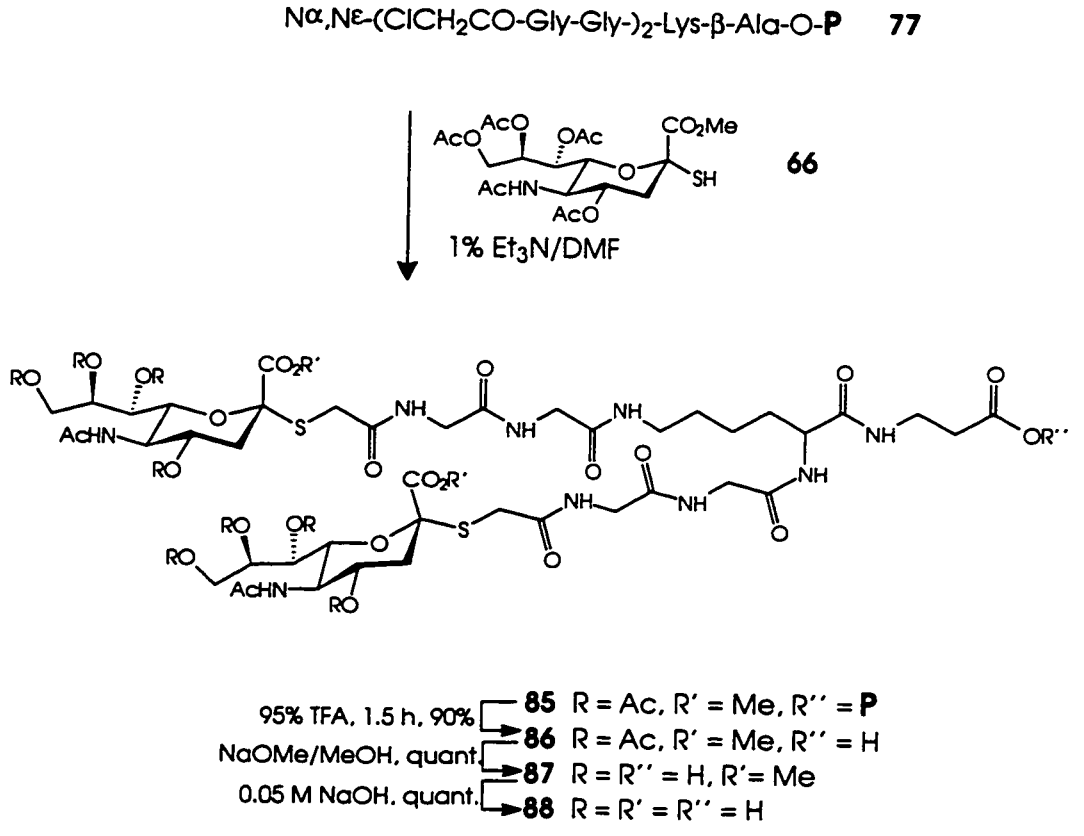


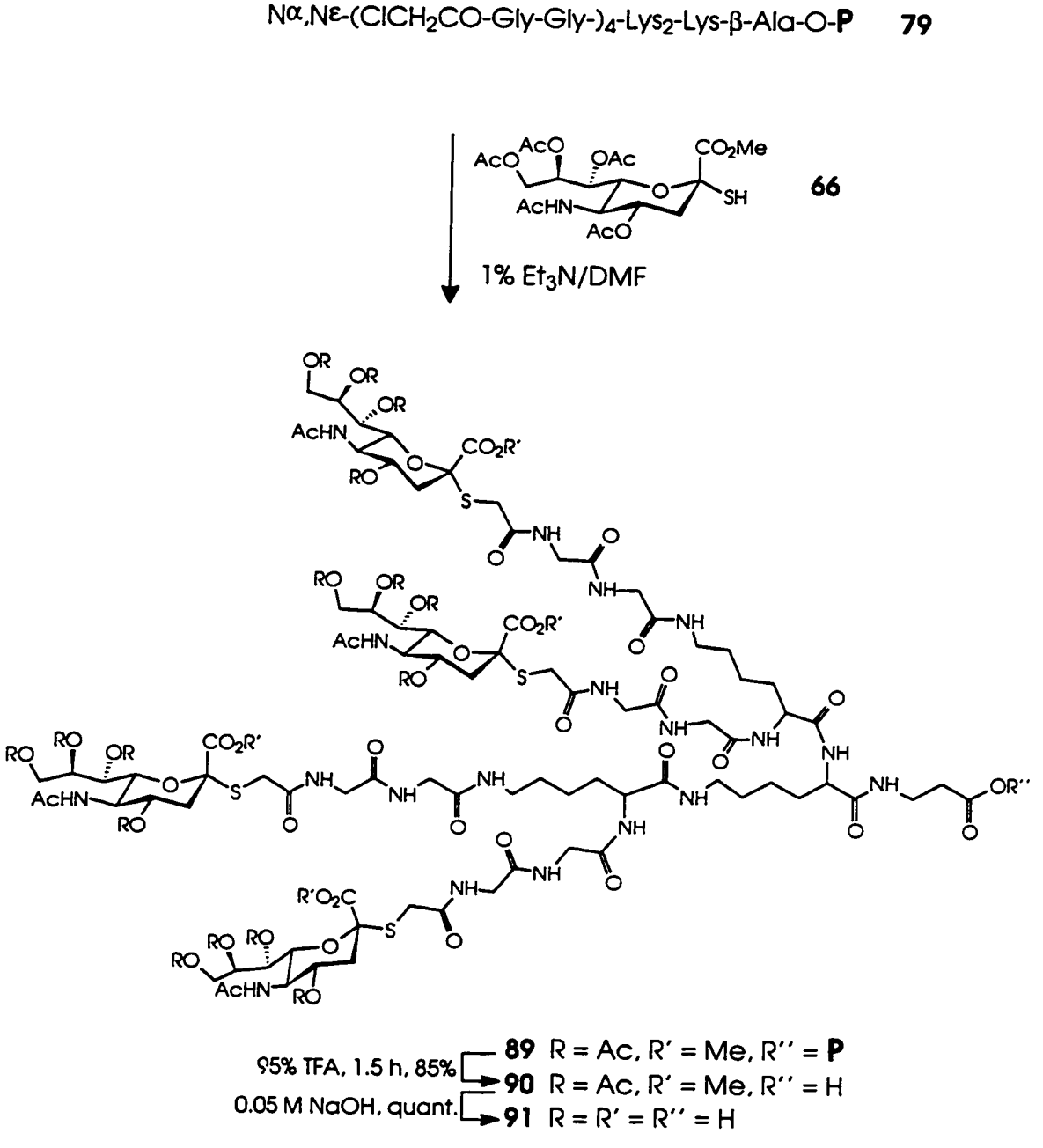
Figure 3.2.1. ^{1}H-NMR (DMSO-d_6, 500 MHz) spectrum of N-chloroacetylated tetravalent L-lysine-based dendrimer **80**.

those of both L-lysyl and sialyl signals (β-alanyl α-CH_2, lysyl ε-CH_2, NAc, H-3ax, H-3eq, H-4 residues, and C-2 signal at 2.36, 2.99, 1.65, 1.78, 2.64, 4.70, and 81.3 ppm, respectively).

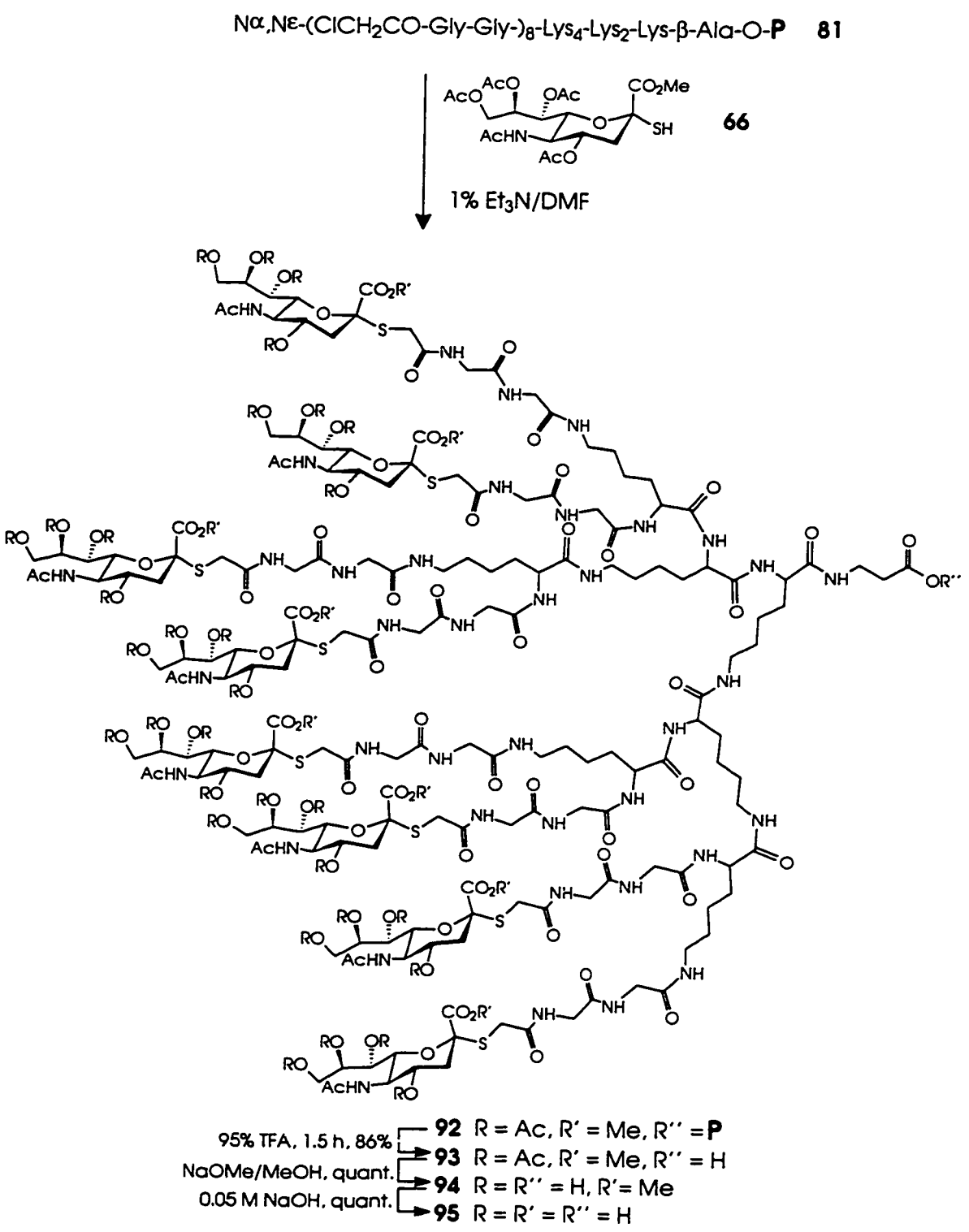
Each of the protected dendrimers (**86**, **90**, **93**, and **97**) was de-esterified with NaOMe/MeOH (25 °C, 1 h) followed by 0.05 M NaOH (25 °C, 2 h) or directly with 0.05 M NaOH (25 °C, 2 h) with Amberlite IR-120 H^+ resin treatment after each step to afford fully deprotected α-thiosialodendrimers **88**, **91**, **95**, and **98** in essentially quantitative yields (Schemes 3.3.1 to 3.3.4). All dendrimers were fully water soluble and could be purified by gel permeation chromatography on Biogel P-2 columns using H_2O as eluent. Furthermore, the larger octa- (**95**) and hexadeca- (**98**) meric dendrimers could be dialyzed using benzoylated dialysis tubing (MW cutoff 2 kDa).



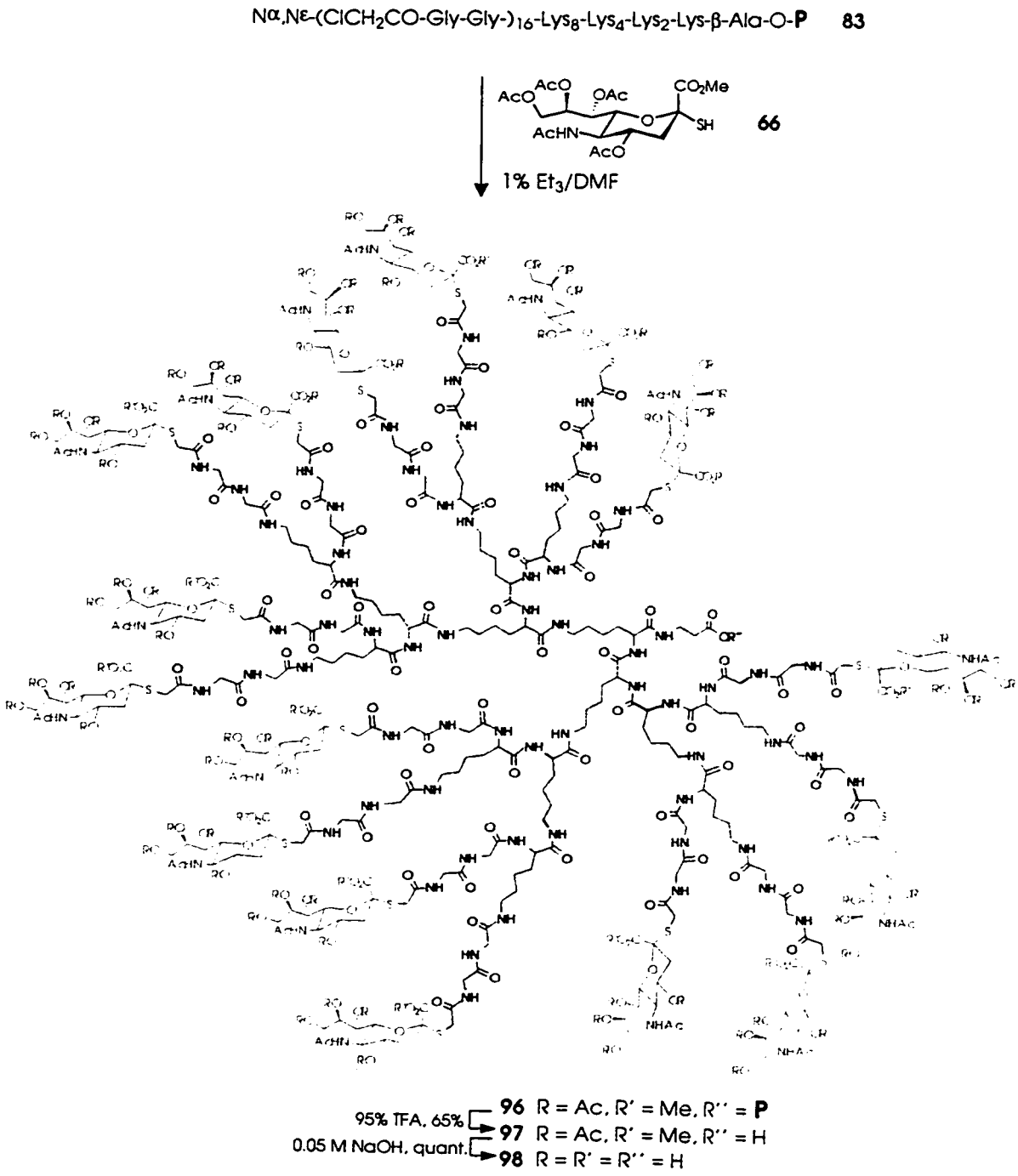
Scheme 3.3.1. Synthesis of divalent L-lysine-based sialodendrimer.



Scheme 3.3.2. Synthesis of tetravalent L-lysine-based sialodendrimer.



Scheme 3.3.3. Synthesis of octavalent L-lysine-based sialodendrimer.



Scheme 3.3.4. Synthesis of hexadecavalent L-lysine-based sialodendrimer.

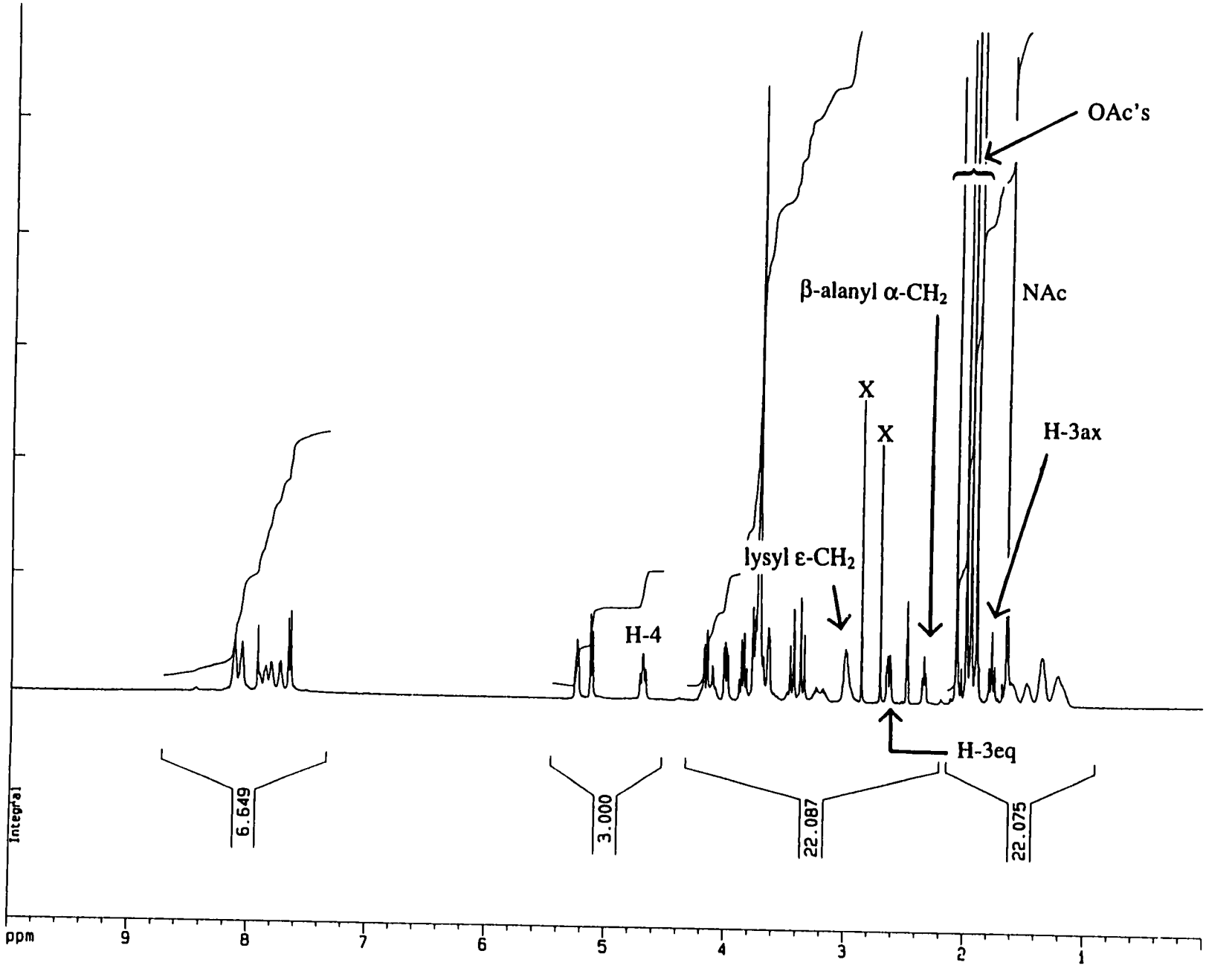


Figure 3.3.1. ^{1}H-NMR (DMSO-d_6, 500 MHz) spectrum of tetravalent L-lysine-based α-thiosialodendrimer **90**.

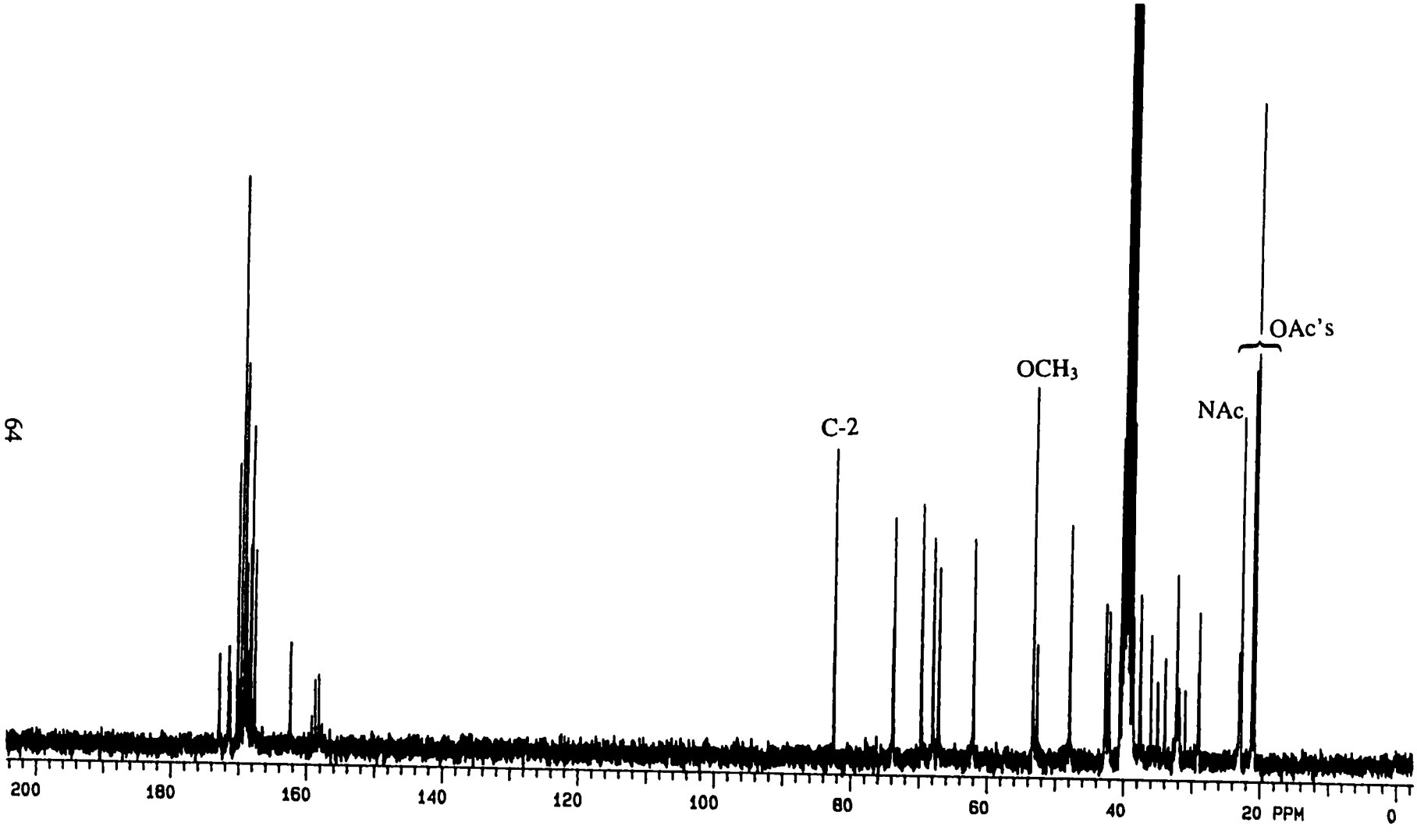
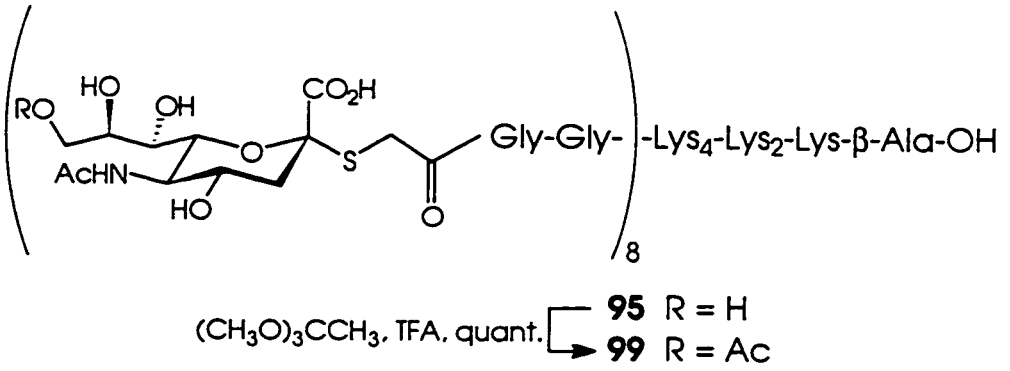


Figure 3.3.2. ^{13}C-NMR (DMSO-d_6, 300 MHz) spectrum of tetravalent L-lysine-based α-thiosialodendrimer **90**.

3.4. 9-O-Acetylated Sialic Acid Residues on L-Lysine Core

As already stated, α-sialosides play a critical role in *Influenza* virus infections.[15] Still, different virus isolates have shown slight variations in their receptor specificities including O-acyl substitutions.[163] For *Influenza* A and B viruses, the receptor determinants have been identified as N-acetylneuraminic acid. The *Influenza* C virus receptors require 9-O-acetyl sialosides for recognition processes to lead to infection.[163]

To also gain access to *Influenza* C virus hemagglutinin (HA) inhibitors, octavalent sialic acid-based dendrimer **95** was treated with an excess of trimethylorthoacetate in dimethyl sulfoxide containing a catalytic amount of trifluoroacetic acid. After stirring overnight at room temperature, the reaction mixture was exhaustively dialyzed against H_2O. Following lyophilization, the 9-O-acetylated dendrimer **99** was obtained in quantitative yield (Scheme 3.4.1). The high field proton NMR spectrum (500 MHz, D_2O, Figure 3.4.1) revealed only a single positional isomer as judged by the unique O-acetyl signal appearing at 2.24 ppm and integrating for 8 × 3 protons. The intensity of the above O-acetyl signal was found to be identical to that of the N-acetyl signal at 2.13 ppm and demonstrated the regioselectivity of the process. The chemical shift of one of the corresponding H-9 protons (4.18 ppm) was accordingly shifted downfield to 4.38 ppm in **99** (Figure 3.4.1).



Scheme 3.4.1. Selective 9-O-acetylation of octameric sialodendrimer.

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[163] Herrler, G.; Rott, R.; Klenk, H. -D.; Muller, H. -P.; Shukla, A. K.; Schauer, R. *EMBO J.* **1985**, *4*, 1503.

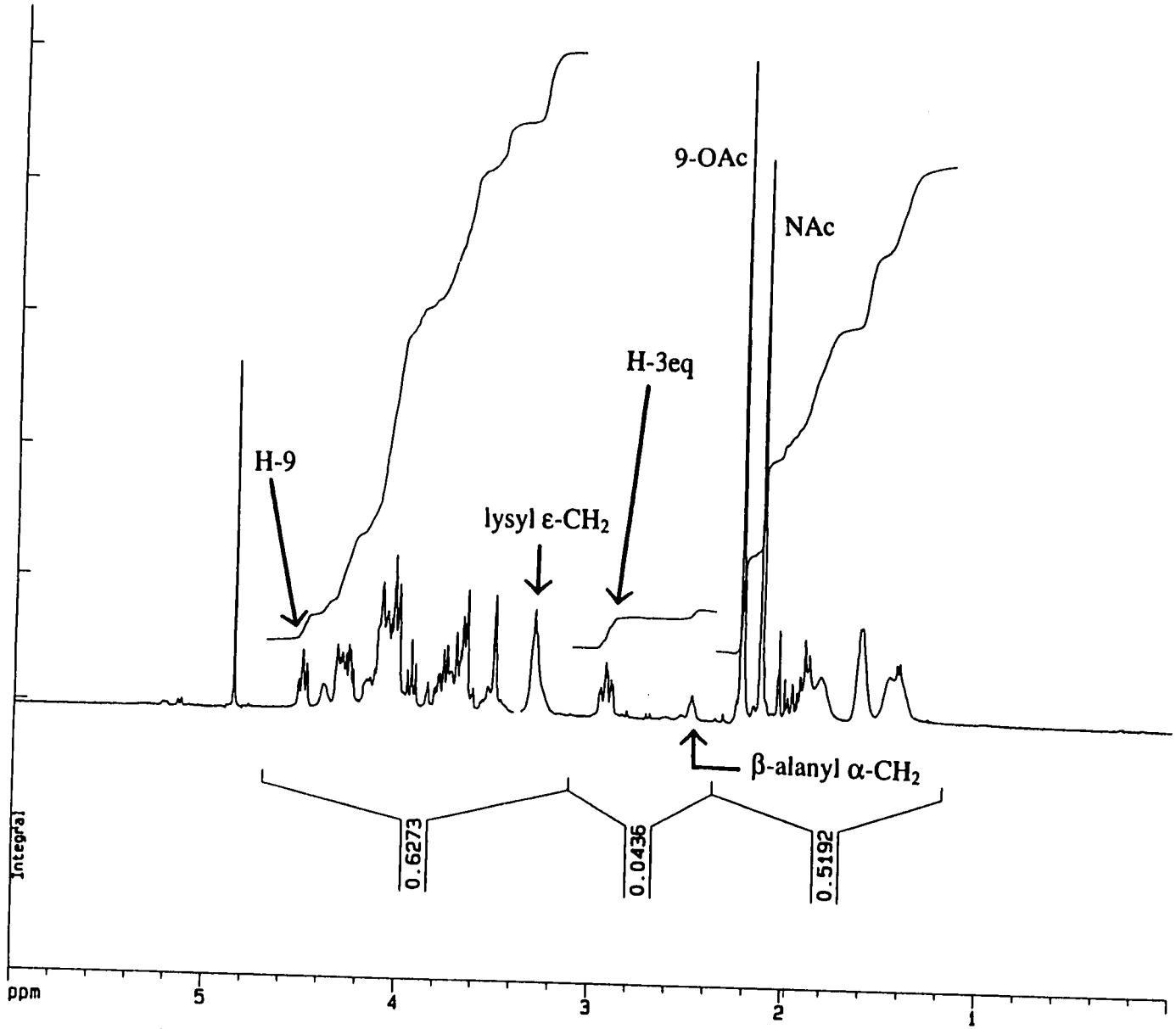


Figure 3.4.1. ^{1}H-NMR (D_2O, 500 MHz) spectrum of 9-O-acetylated octameric α-thiosialodendrimer **99**.

3.5. Immunochemical Assays

The antigenic properties of dendritic α-thiosialosides **88**, **91**, **95**, and **98** were first established in two model assays. In the first, double immunodiffusion, the plant lectin wheat germ agglutinin (WGA) (20 μL of a 2 mg/mL solution in phosphate buffer saline, pH 7.3) was used to fill the center well of an agarose coated plate. Glycodendrimer solutions (20 μL of 2 mg/mL in PBS of **88**, **91**, **95**, and **98**) and a negative control (20 μL of 2 mg/mL solution in PBS of copolyacrylamide MW ≈100,000) were used to fill adjacent wells. Diffuse precipitin lines were visible with octa- (**95**) and hexadeca- (**98**) valent α-thiosialodendrimers (Figure 3.5.1). The double immunodiffusion assay served to confirm the capacity of the synthesized α-thiosialodendrimers to bind to WGA.

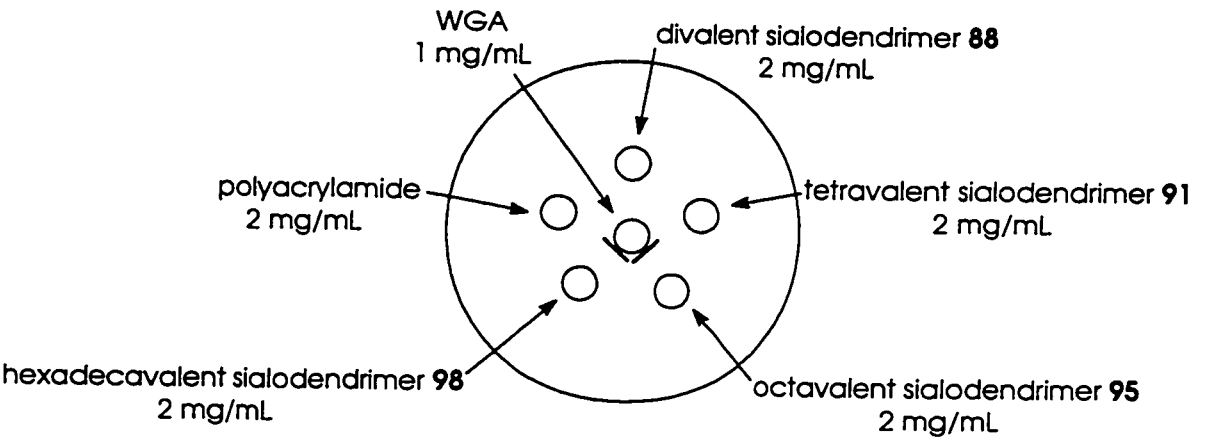


Figure 3.5.1. Double immunodiffusion assay between WGA (center well) and sialodendrimers **88**, **91**, **95**, and **98**.

In the second, more sensitive test, model studies using WGA in solid phase immunoassay format established the antigenic properties of the glycodendrimers. The dendrimers' capacity to be used as coating haptens in microtiter plates was evaluated by a direct enzyme linked lectin assay (ELLA). Thus, di- (**88**), tetra- (**91**), octa- (**95**), and hexadeca- (**98**) valent sialylated dendrimers together with poly(acrylamide-co-*p*-

acrylamidophenylthio-α-sialoside) **12**[70] employed as a positive reference were used to coat the wells of microtiter plates. After the usual incubation time, blockings, and washings, the presence of adsorbed sialic acid residues was confirmed by treating the plates with horseradish peroxidase labeled WGA (WGA/HRP) using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and hydrogen peroxide as enzyme substrates. The results of these control experiments are illustrated in Figure 3.5.2. The octa- (**95**) and hexadeca- (**98**) valent dendrimers were almost as efficient coating antigens as the phenylthio-α-sialoside copolymer **12**. The poor coating properties of the di- (**88**) and tetra- (**91**) valent dendritic structures was attributed to their lack of lipophilic components.

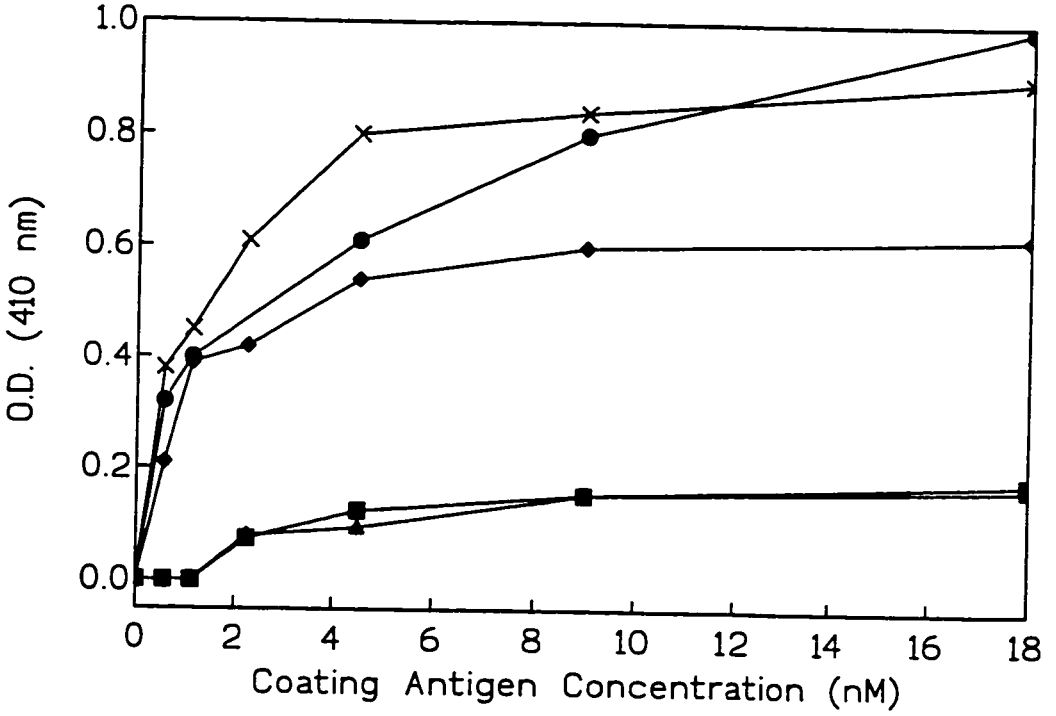


Figure 3.5.2. ELLA using α-thiosialodendrimers **88** (■), **91** (▲), **95** (◆), and **98** (●) and copolymer **12** (×) as coating antigens in microtiter plates with WGA/HRP and ABTS-H_2O_2 as peroxidase substrates.

In another set of experiments, the dendrimers were evaluated for their ability to inhibit the strong binding interactions between WGA and glycopolymer **12**. ELLA was used in competitive inhibition assays. Glycopolymer **12** was first taken as a coating antigen *via* adsorption in the microtiter plate wells. Pre-incubated mixtures of phenylthio-α-sialoside or sialodendrimers at various concentrations with WGA/HRP were then added to each well for the competitive inhibition experiments. Residual enzymatic activity was then measured with ABTS-H_2O_2 as peroxidase substrates and the results plotted as a function of per cent inhibition (Figure 3.5.3). Results clearly showed that the synthesized α-thiosialodendrimers were far superior to the monosialoside in inhibition experiments, thereby demonstrating the effect of multivalency. The

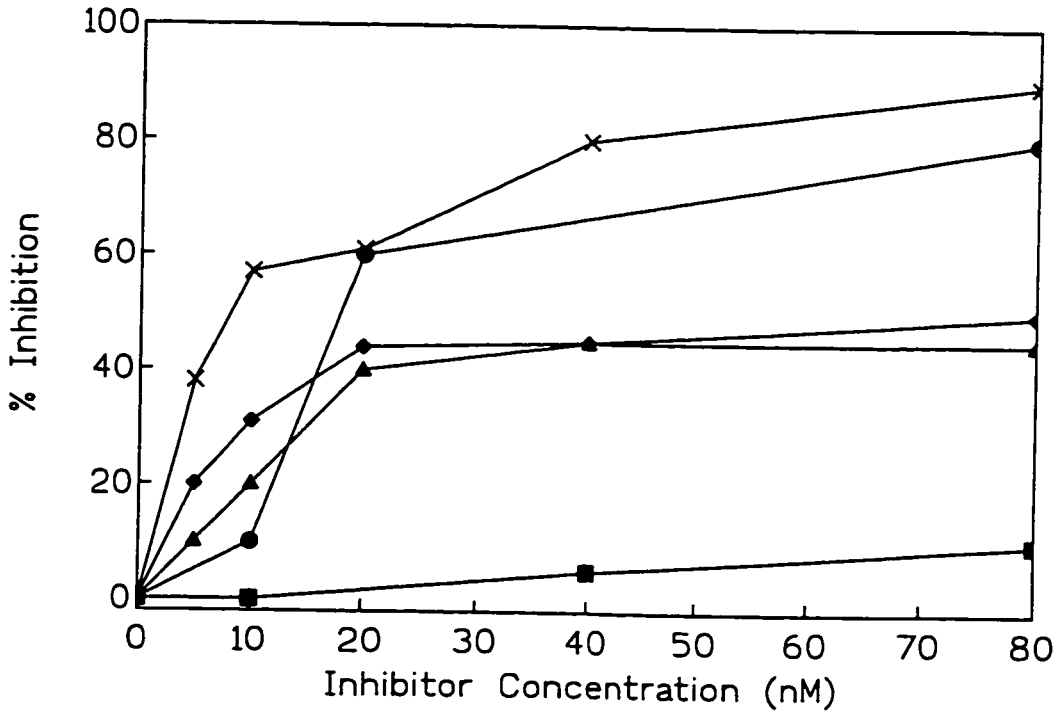


Figure 3.5.3. Inhibition of binding of sialopolymer **12** to WGA/HRP in ELLA. Inhibitors used include: phenyl α-thiosialoside (■), and sialodendrimers **88** (▲), **91** (◆), **95** (●), and **98** (×).

concentrations required for 50% inhibition (IC_{50}) were >236, 40, 30, 18, and 8 nM for monomer phenyl-α-thiosialoside[70], di- (**88**), tetra- (**91**), octa- (**95**), and hexadeca- (**98**) valent dendrimers, respectively (>236, 80, 120, 144, and 128 nM, respectively on a per sialoside residue basis).

IC_{50}'s as a function of dendrimer valency are plotted in Figure 3.5.4. A drop in IC_{50} is concurrent with an increase in valency.

Preliminary experiments with *Influenza* A virus (strain X-31) showed that the dendrimers were potent inhibitors of the hemagglutination of human erythrocytes. Dendrimers **88**, **91**, **95**, and **98** exhibited inhibitions of 625, 312.5, 156, and 19 μM, respectively. Even the divalent dendrimer **88** was at least five times more efficient than monosialosides (≈3 mM) and the hexadecavalent dendrimer **98** was as potent as previously described sialopolymers.

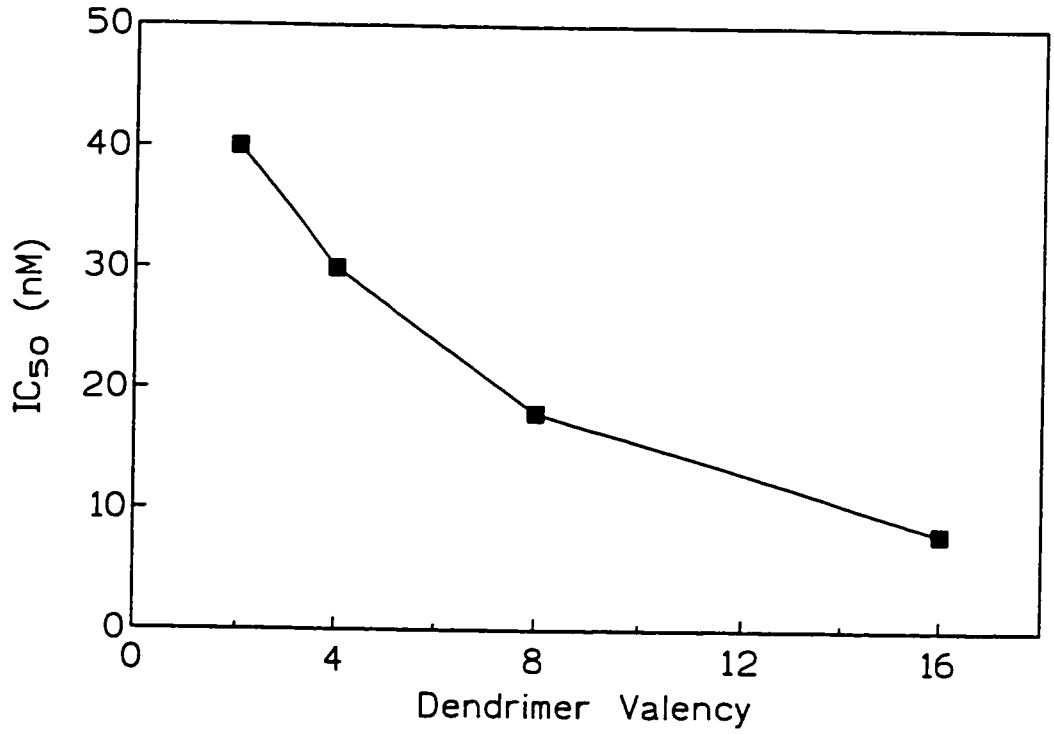


Figure 3.5.4. Effect of dendrimer valency on the inhibition of sialopolymer **12** and WGA/HRP. Data taken from Figure 3.5.3.

3.6. Conjugation of Other Glycosides to L-Lysine Core

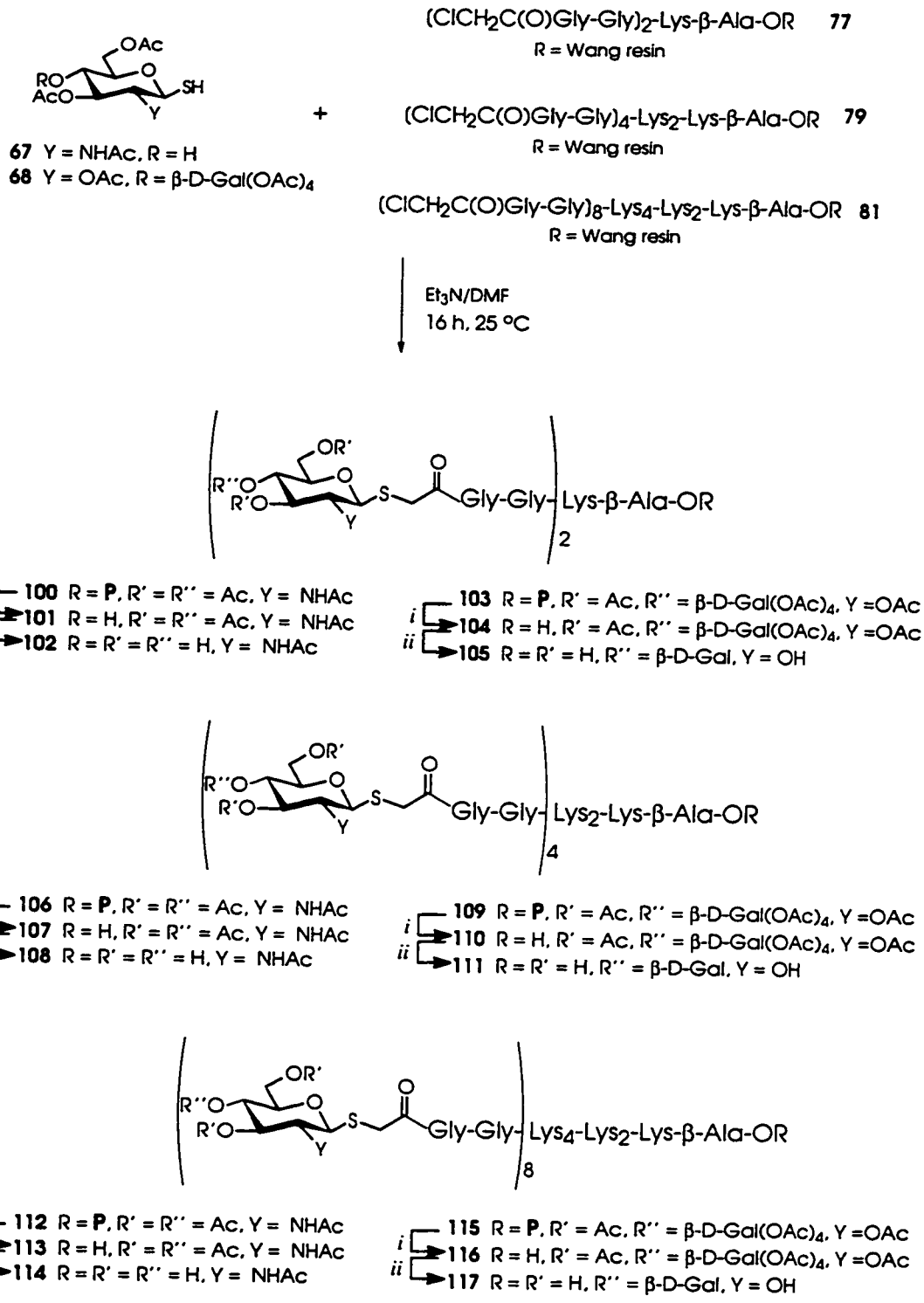
As already stated, proof is growing that multivalency amplifies individual carbohydrate protein interactions. The synthesis and biological evaluation of the L-lysine-based α-thiosialodendrimers attests to this. To further demonstrate the multivalency effect and to extend this approach to other carbohydrate-protein interactions, glycodendrimers bearing sugar residues other than sialic acid were generated.[164]

N-Chloroacetylated dendrimer backbones **77**, **79**, and **81** (Scheme 3.6.1) were synthesized as described in the previous section. Coupling of 1-thio-N-acetylglucosamine derivative **67** and of 1-thio-lactose derivative **68** was done on solid phase. Polymer supported (Wang resin, 0.58 mmol/g substitution) dendrimers **77**, **79**, and **81** were placed in a 1% Et_3N/DMF solution containing peracetylated thioglycosides **67** or **68** (1.2 eq. per N-chloroacetyl functionality, 16 h). Before the bulk of the dendrimers were released from the polymeric support, aliquots were withdrawn and hydrolyzed (95% aq. TFA, 1.5 h). Completeness of the reaction was monitored by the ^{1}H-NMR spectra of the dendritic glycosides which showed characteristic signals for any residual N-chloroacetyl groups at 4.12 ppm (DMSO-d_6, Figure 3.6.1). Where required, couplings were repeated (Scheme 3.6.1).

Resin-bound glycodendrimers **100**, **103**, **106**, **109**, **112**, and **115** were released from the polymeric support (95% TFA, 1.5 h) and obtained in 65-99% yields after dissolution in the minimum amount of TFA and precipitation in ether (Scheme 3.6.1).

Each of the protected dendrimers **101**, **104**, **107**, **110**, **113**, and **116** were de-esterified with NaOMe/MeOH (25 °C, 1 h) followed by treatment with Amberlite IR-120 cation exchange resin to afford fully deprotected glycodendrimers **102**, **105**, **108**, **111**, **114**, and **117** in essentially quantitative yields. ^{1}H-NMR spectra confirmed the integrity of the peptide backbone (β-alanyl α-CH_2 and lysyl ε-CH_2 at 2.36 and 3.00 ppm,

[164] Zanini, D.; Park, W. K. C.; Roy, R. *Tetrahedron Lett.* **1995**, *36*, 7383.



Scheme 3.6.1. Synthesis of N-acetylglucosamine-based and lactose-based dendrimers: *i*) 95% aq. TFA, 1.5 h, 65-99%; *ii*) NaOMe/MeOH, then H^+ resin, 95%-quant.

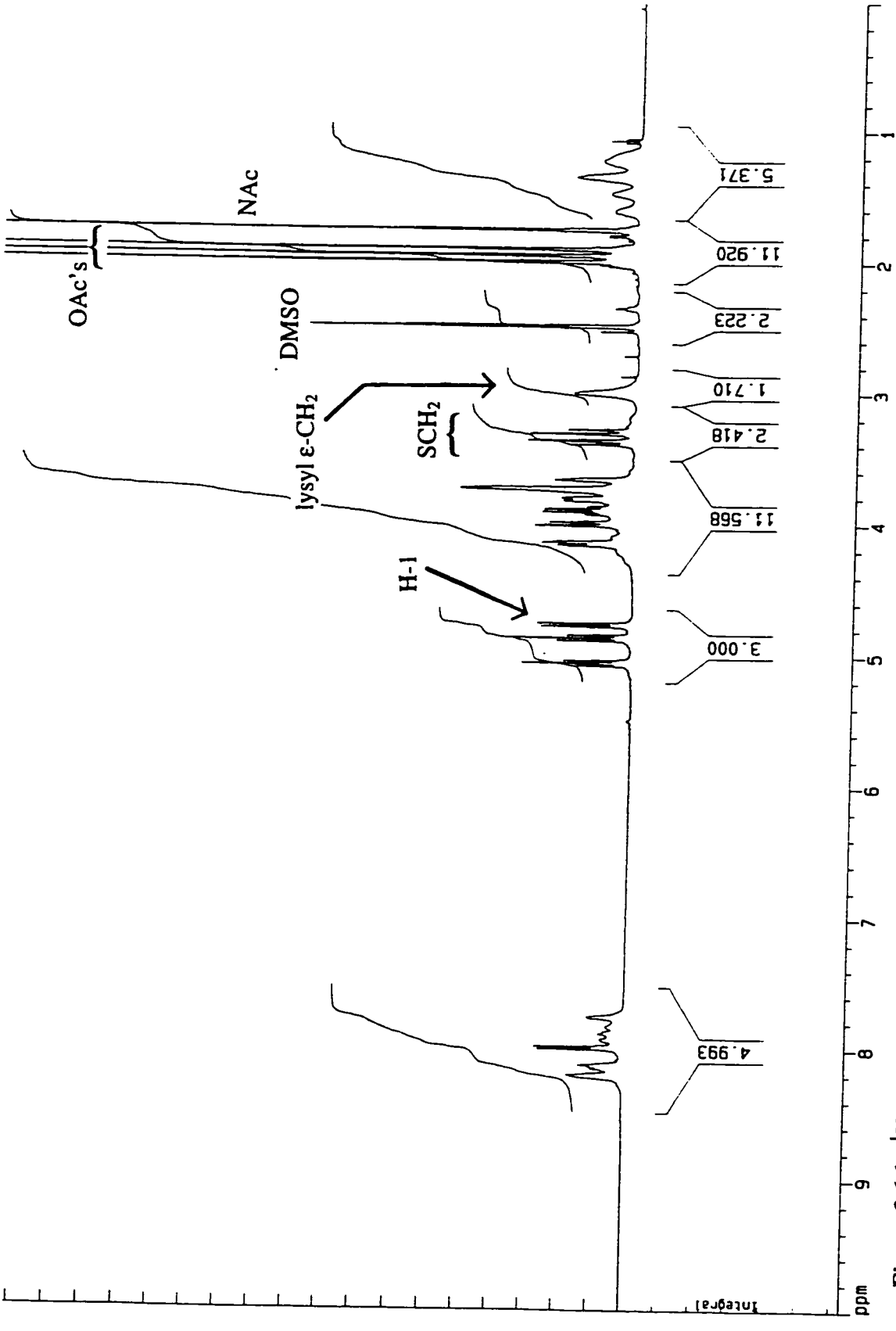


Figure 3.6.1. ^{1}H-NMR (DMSO-d_6, 500 MHz) spectrum of peracetylated octavalent N-acetylglucosaminylated dendrimer **113**.

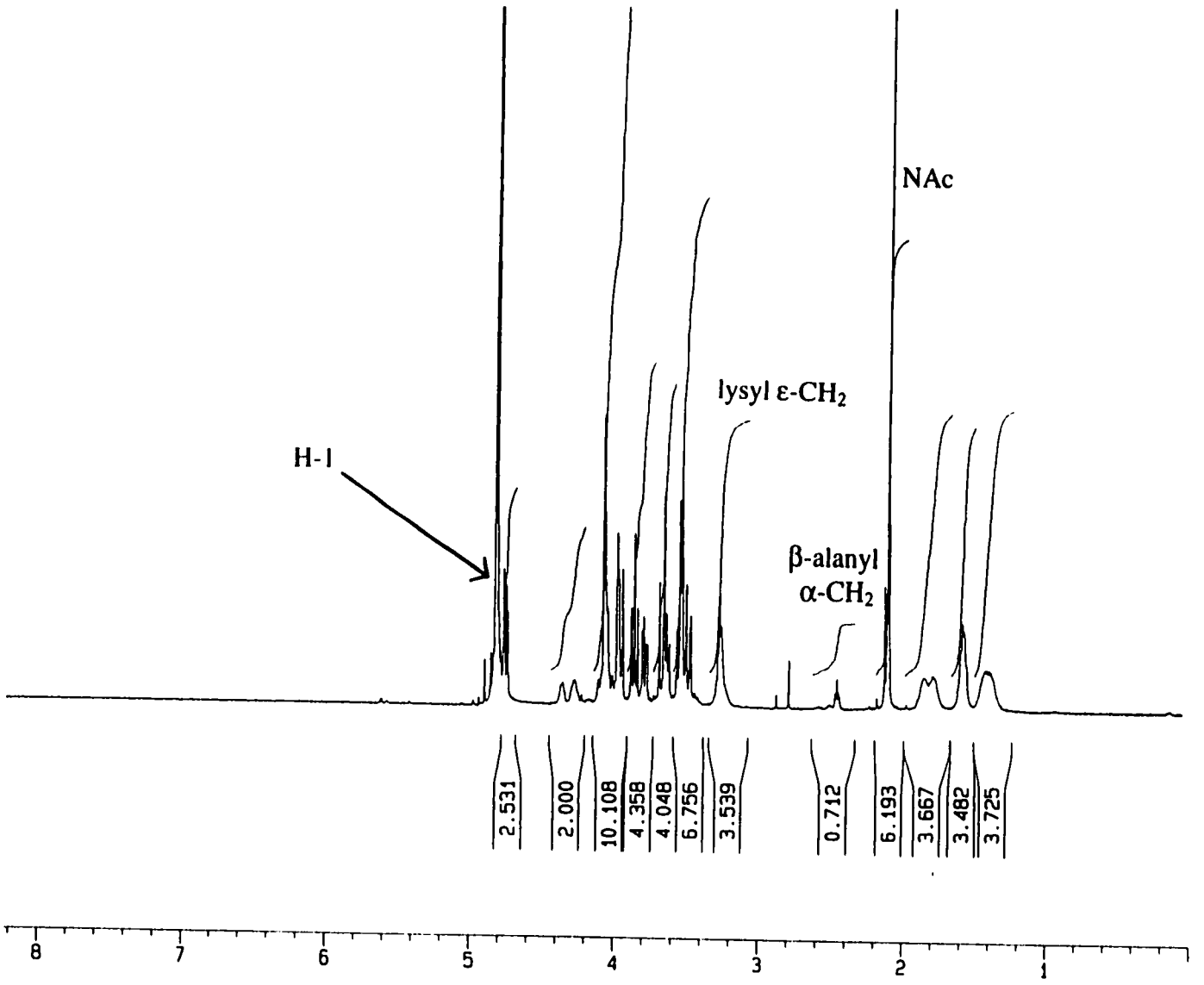
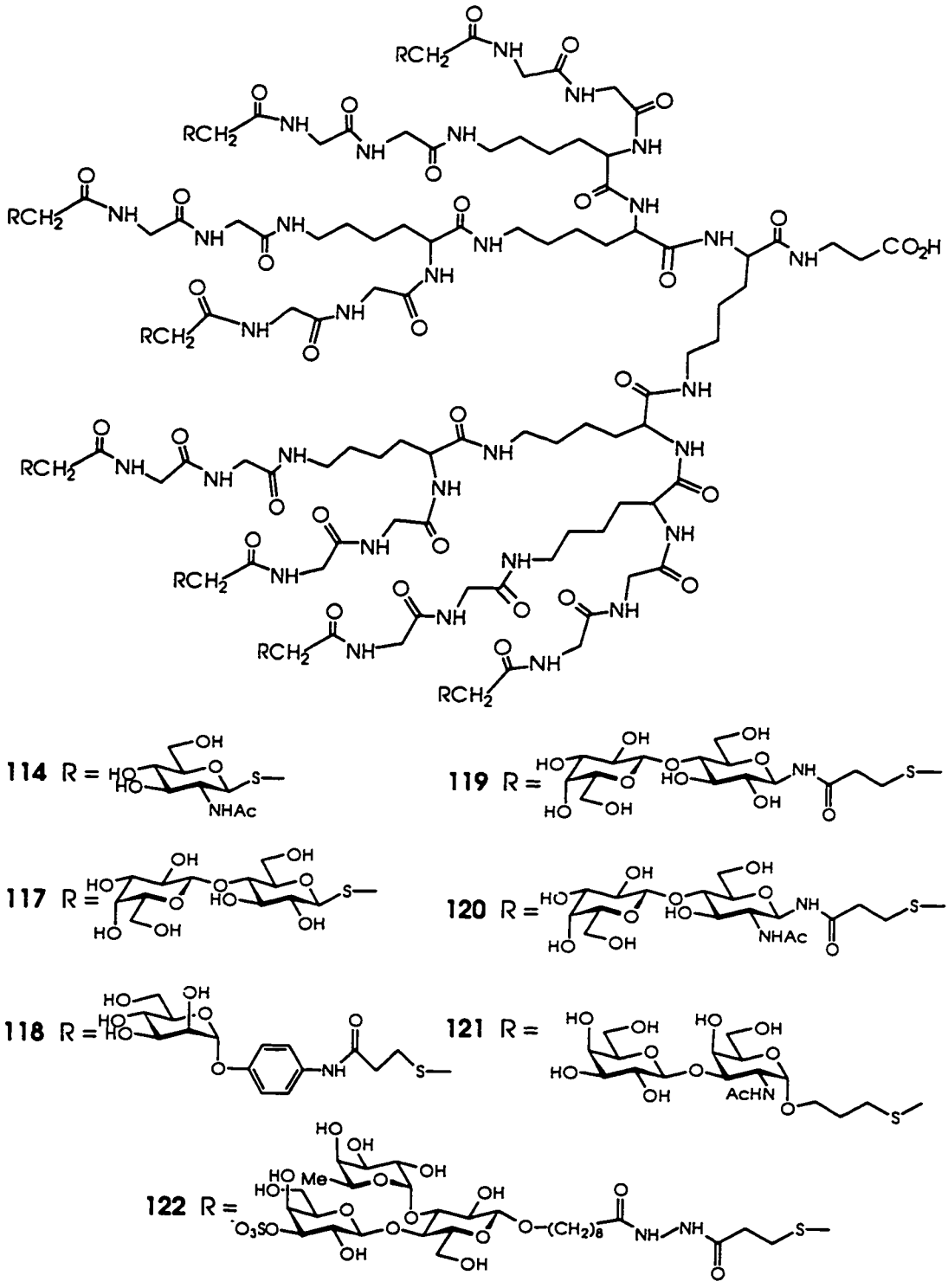


Figure 3.6.2. ^{1}H-NMR (D_2O, 500 MHz) spectrum of fully deprotected octavalent N-acetylglucosaminylated dendrimer **114**.



Scheme 3.6.2. Typical structures of glycodendrimers based on the L-lysine core.

respectively) and the incorporation of glycosidic residues. Figures 3.6.2 and 3.6.3 are included as examples.

In addition, using this solid phase, convergent approach, glycodendrimers containing β-D-lactosides with spacers **119**,[164] N-acetyllactosaminides **120**,[164] α-D-mannosides **118**,[165] T-antigen **121**,[166] and 3'-sulfo-Lewis^{X}-(Glc) analog **122**[167] were prepared (Scheme 3.6.2).

The variety of carbohydrate residues that may be conjugated to the dendritic backbone demonstrates the universality of this approach and allows an entry into the syntheses of even more complex dendritic oligosaccharide compounds (Chapter 6).

3.7. Immunochemical Assays

Preliminary biochemical testing of dendrimers GlcNAc-based **102**, **108**, **114** and Lac-containing **105**, **111**, **117** included double immunodiffusion assays using WGA for **102**, **108**, and **114** and *Arachis hypogaea* lectin (peanut lectin) for dendrimers **105**, **111**, and **117** (Figures 3.7.1 and 3.7.2). All dendrimers exhibited precipitin bands. Precipitin bands for the divalent dendrimers were transient and as valency increased, precipitation lines became noticeably stronger and less diffuse. These experiments served to confirm the ability of the dendrimers to bind to their respective lectins.

Octavalent GlcNAc-based dendrimer **114** was further tested for its binding properties to WGA *via* turbidimetric analysis. The time course formation of insoluble precipitin complexes between WGA and dendrimer **114** is illustrated in Figure 3.7.3. Maximum turbidity was reached after only 45 minutes. These micro-quantitative precipitation experiments confirmed the direct binding and cross-linking properties of N-acetylglucosamine dendrimers with WGA.

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[165] Pagé, D.; Zanini, D.; Roy, R. *Bioorg. Med. Chem.* **1996**, *4*, 1949.
[166] Roy, R.; Zanini, D.; Baek, M. -G., unpublished data.
[167] Roy, R.; Park, W. K. C.; Zanini, D.; Foxall, C.; Srivastava, O. P. *Carbohydr. Lett.* **1997**, *2*, 259.

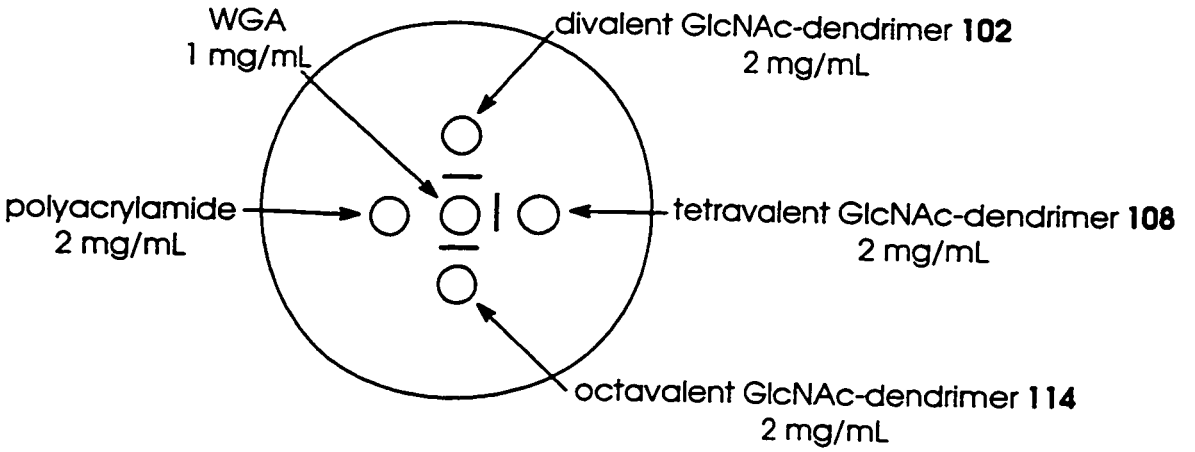

Figure 3.7.1. Double immunodiffusion between WGA and GlcNAc-based dendrimers **102**, **108**, and **114**.

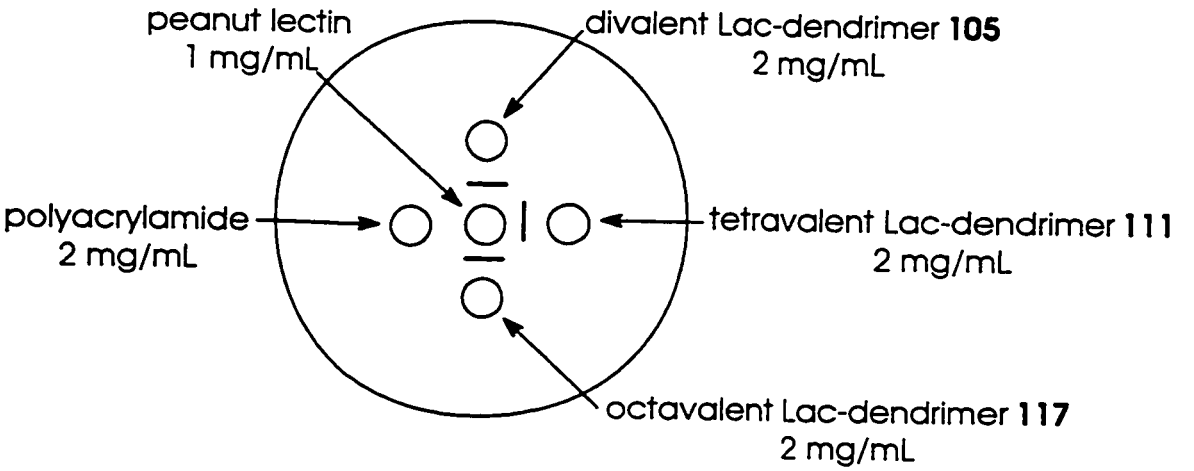

Figure 3.7.2. Double immunodiffusion between *Arachis hypogaea* lectin (peanut lectin) and Lac-based dendrimers **105**, **111**, and **117**.

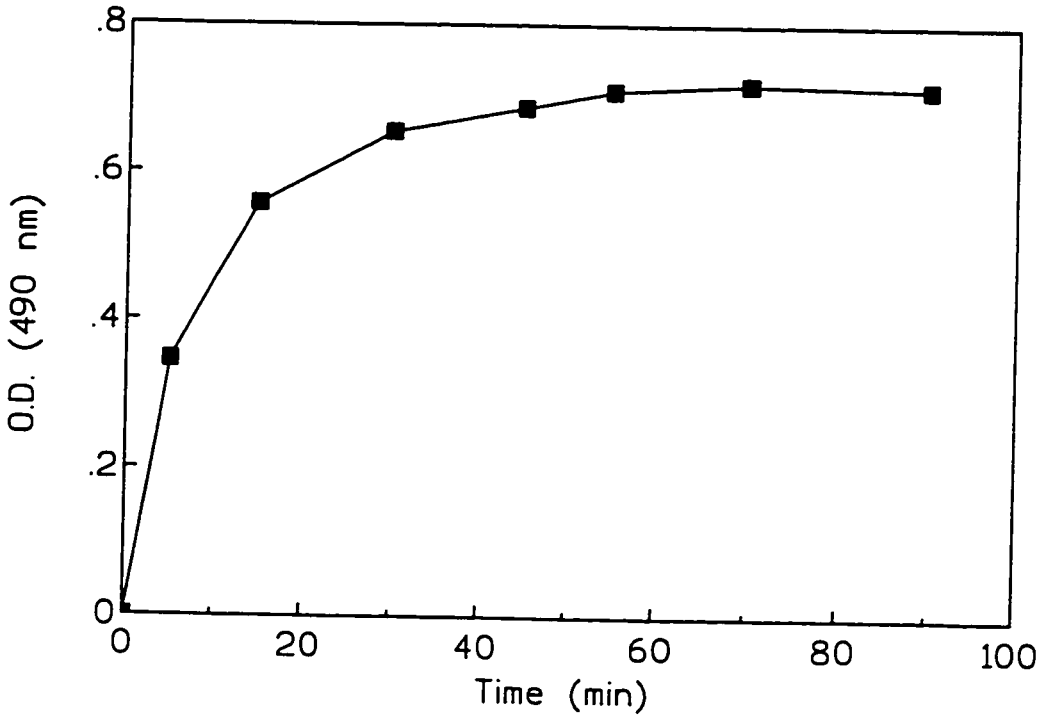

Figure 3.7.3. Turbidimetric analysis for binding between WGA and octameric N-acetylgucosaminide **114**.

When tested in ELLA, N-acetylglucosaminyl dendrimers **102**, **108**, and **114** showed enhanced binding affinities as compared to their monomeric precursor. Using porcine stomach mucin type III as coating antigen, GlcNAc dendrimers **102**, **108**, and **114** were tested for their ability to inhibit WGA/porcine stomach mucin type III binding. Figure 3.7.4 plots per cent inhibition as a function of inhibitor concentration.

IC_{50}'s for GlcNAc dendrimers **102**, **108**, and **114** were 3100, 509, and 88 μM, respectively (6200, 2040, and 703 μM on a per glycosidic residue basis). When plotting IC_{50}'s as a function of dendrimer valency (Figure 3.7.5), the curve generated for this set of data clearly indicates that multivalency enhances the inhibitory potencies for the WGA/porcine stomach mucin type III interaction. The inhibitory potential of dendritic structure **114** represents more than a 20 fold increase over the analogous monomer.

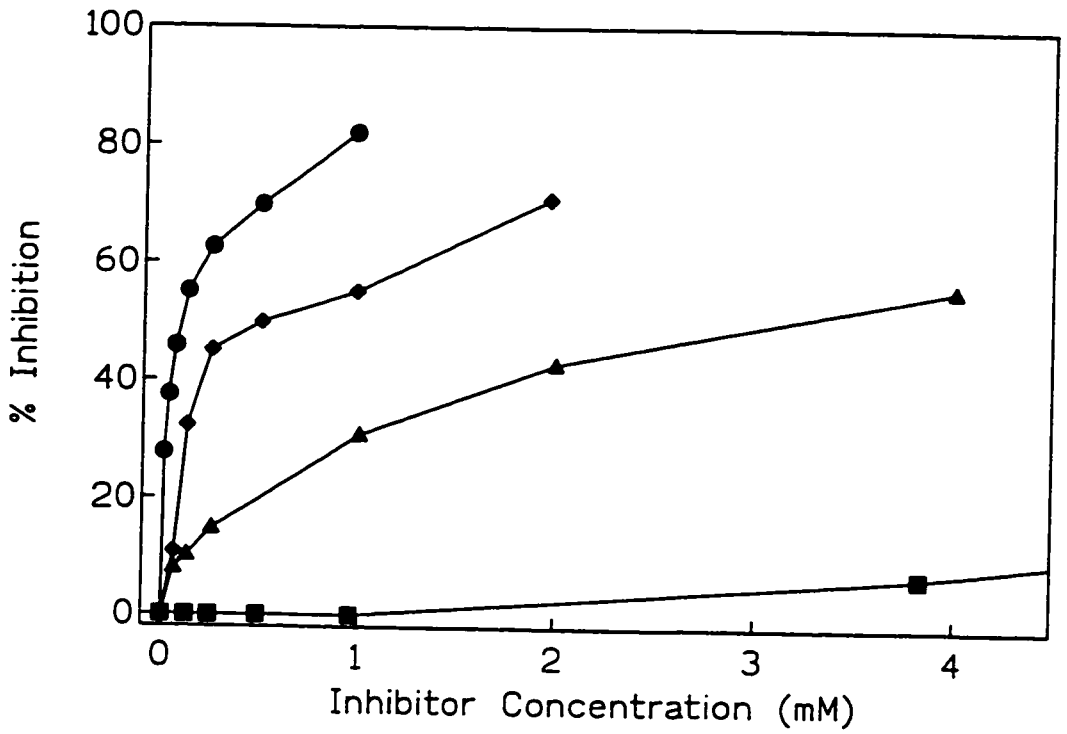

Figure 3.7.4. Inhibition of binding of porcine stomach mucin type III and WGA/HRP in ELLA. Inhibitors used include 2-acetamido-2-deoxy-α-D-glucopyranose[168] (■), and divalent (▲), tetravalent (◆), and octavalent (●) GlcNAc-based dendrimers **102**, **108**, and **114**, respectively.

These findings are not unique. L-Lysine-based dendrimers bearing mannose residues (**118**) showed enhanced binding affinity as compared with their monomeric precursors[165] and, in addition, L-lysine-based dendrimers containing 3'-sulfo-Lewis^x-(Glc) (**122**) are presently the most effective E- and L-selectin antagonists known.[167]

[168] Lee, Y. C.; Lee, R. T. *Carbohydr. Res.* **1974**, *37*, 193.

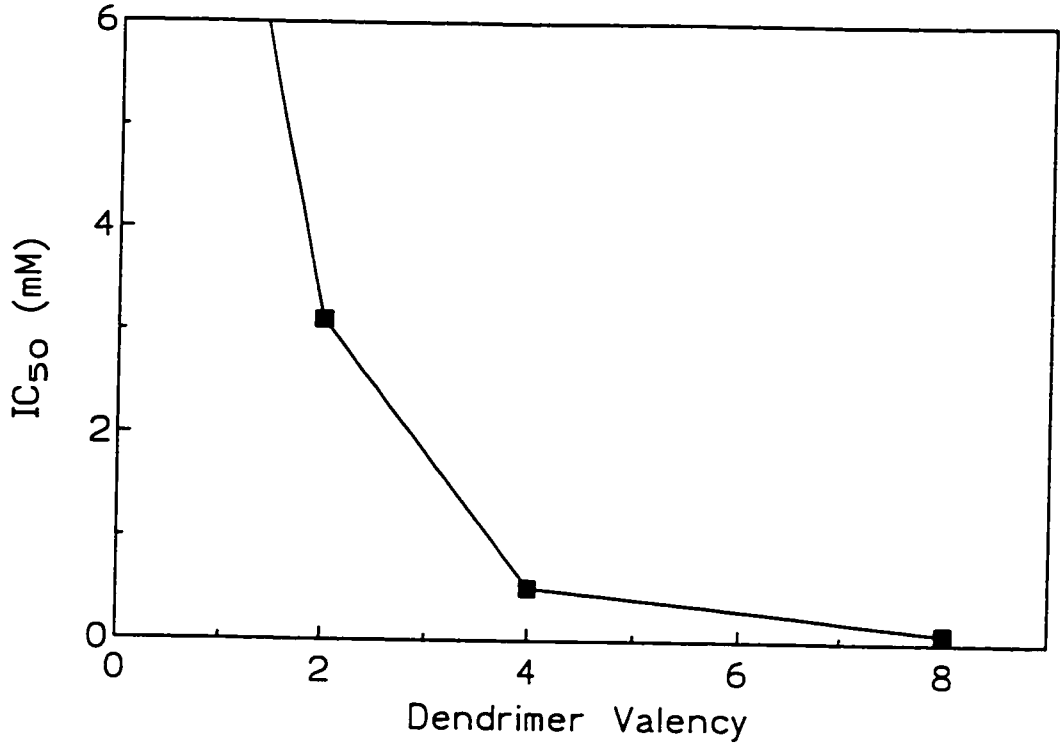

Figure 3.7.5. Effect of dendrimer valency on the inhibition of porcine stomach mucin type III and WGA/HRP. Data taken from Figure 3.7.4.

3.8. Conclusions

Solid phase synthesis on Wang resin, along with HOBt/DIC chemistry, was used to generated hyperbranched L-lysine cores which were functionalized with an N-chloroacetylglycylglycine spacer. Peracetylated 1-thioglycopyranoses were added to the solid phase for nucleophilic substitution of the N-chloroacetyl groups. In this manner, glycodendrimers containing α-thiosialosides, β-D-lactosides, N-acetyllactosaminides, α-D-mannosides, T-antigen, and 3'-sulfo-Lewis^x-(Glc) analog were prepared. This solid phase, convergent strategy has been shown to proceed effectively, generating the first reported glycodendrimers in moderate to good yields and with high purity.

In immunochemical assays, all glycodendrimers exhibited binding with appropriate model lectins. Furthermore, when tested in ELLA, the glycodendrimers showed increases in inhibitory potential with a concurrent increase in valency.

The multivalency effect has been demonstrated using novel neoglycoconjugates - well-defined, monodisperse, multibranched structures with covalently attached carbohydrate residues or glycodendrimers.

3.9. Experimental Methods

Fmoc-β-alanine-OBt active ester.

Fmoc-β-Ala (Novabiochem, Ca) (0.99 g, 3.19 mmol) was dissolved in the minimum amount of DMF (3 mL). N-Hydroxybenzotriazol (HOBt, 0.43 g, 3.19 mmol) also dissolved in DMF (8 mL) was added to the Fmoc-amino acid solution that was cooled to 4 °C (ice bath). Diisopropylcarbodiimide (DIC, 497 μL, 3.19 mmol) was added dropwise to the cooled solution and the mixture stirred at 4 °C for 15 min. The solution was then left at room temperature with stirring for 1 h and added directly to the Wang resin. Isolation of the Fmoc-β-ala-OBt active ester was not performed. The benzotriazolyl ester formation was monitored by TLC (R_f($CHCl_3$:MeOH:HOAc, 85:10:5) 0.74).

N^{α}, N^{ε}-Di-Fmoc-L-lysine-OBt active ester (75).

To an ice cold solution of L-lysine hydrochloride (0.692 g, 3.8 mmol) in 10% aqueous $NaHCO_3$ (10 mL) was added 9-fluorenylmethyl chloroformate (1.96 g, 7.6 mmol) dissolved in the minimum amount of dioxane. The solution was stirred at 0 °C for 2.5 h during which time a precipitate had formed. Water was then added to the heterogeneous mixture and the resulting solid compound was washed with a limited volume of ether to remove small quantities of 9-fluorenylmethanol and the high melting

polymer of dibenzofulvene.[162] The white precipitate was collected and successively washed with 1 M HCl and water until the filtrate was found neutral. N^{α},N^{ε}-di-Fmoc-L-lysine was dried in a dessicator over P_2O_5. It was obtained in 68% yield (1.52 g, 2.58 mmol); m. p. 120.0-124.0 °C, $[\alpha]_D$ -6.5 ° (c = 1, DMF); R_f($CHCl_3$:MeOH:HOAc, 85:10:5) 0.78; ^{1}H-NMR (DMSO-d_6) δ 1.12-1.80 (m, 6H, β-CH_2, γ-CH_2, δ-CH_2), 2.97 (m, 2H, ε-CH_2) 3.91 (m, 1H, α-CH), 4.12-4.38 (m, 6H, Fmoc-$CHCH_2$), 7.21-7.95 (m, aryl, NH); ^{13}C-NMR (DMSO-d_6) δ 21.6 (γ-C), 27.6 (δ-C), 29.1 (β-C), 45.3 (ε-C), 52.4 (α-C), 63.8 (Fmoc-CH_2), 65.0 (Fmoc-*C*H), 118.8, 123.9, 125.7, 126.2, 139.3, 142.4 (Fmoc C's), 154.8 (Fmoc C=O), 172.6 (CO_2H).

N^{α},N^{ε}-Di-Fmoc-L-lysine (1.38 g, 2.33 mmol) was dissolved in the minimum amount of DMF (8 mL). N-Hydroxybenzotriazol (HOBt, 3.19 g, 2.36 mmol) also dissolved in DMF (8 mL) was added to the Fmoc-amino acid solution that was cooled to 4 °C (ice bath). Diisopropylcarbodiimide (DIC, 365 μL, 2.33 mmol) was added dropwise to the cooled solution and the mixture stirred at 4 °C for 15 min. The solution was then left at room temperature with stirring for 1 h and added directly to the Wang resin. Isolation of the N^{α},N^{ε}-di-Fmoc-L-lysine-OBt active ester was not performed. The benzotriazolyl ester formation was monitored by TLC (R_f($CHCl_3$:MeOH:HOAc, 85:10:5) 0.89).

Chloroacetylglycylglycine-OBt active ester (76).

Chloroacetylglycylglycine (0.10 g, 0.47 mmol) was dissolved in the minimum amount of DMF (3 mL). N-Hydroxybenzotriazol (HOBt, 0.077 g, 0.57 mmol) also dissolved in DMF (8 mL) was added to the Fmoc-amino acid solution that was cooled to 4 °C (ice bath). Diisopropylcarbodiimide (DIC, 74 μL, 0.47 mmol) was added dropwise to the cooled solution and the mixture stirred at 4 °C for 15 min. The solution was then left at room temperature with stirring for 1 h and added directly to the Wang resin. Isolation of the chloroacetylglycylglycine-OBt active ester was not performed. The

benzotriazolyl ester formation was monitored by TLC (R_f($CHCl_3$:MeOH:HOAc, 90:8:2) 0.32).

Divalent N-chloroacetylated poly-L-lysine dendrimer (78).

The solid phase synthesis was accomplished manually using a glass funnel equipped with a coarse sintered glass filter and a bottom stopcock T-valve. Wang resin (2.51 g) having 0.58 mmol/g of hydroxyl group substitution (100-200 mesh, Advance Chemtech, LO, KY) was suspended in DMF. The resin was allowed to swell for 1 h at room temperature by bubbling nitrogen through the funnel. This process was repeated three times. After draining the DMF, pre-formed Fmoc-β-Ala-OBt (2.92 mmol, see above) and DMAP (0.09 g, 0.73 mmol) in DMF were added to the resin which was agitated by bubbling N_2 for 2.5 h. The excess reagents and DMF were evacuated and the resin washed with fresh DMF (5 × 1 min). The Fmoc-β-Ala-OBt coupling was repeated until aliquots treated with 20% piperidine showed greater than 85% substitution as judged by the quantitative spectrophotometric analysis of the dibenzofulvene chromophore.[160,161] The acyl capping of unsubstituted hydroxyl groups was achieved using acetic anhydride (276 μL, 2.92 mmol) in DMF (6 mL) containing DMAP (0.18 g, 1.46 mmol). After acetylation, the resin was washed with DMF (5 × 1 min). The Fmoc-protecting groups were removed by treatment with 20% piperidine in DMF (1 × 3 min, 2 × 5 min). Pre-formed N^{α},N^{ε}-di-Fmoc-L-lysine-OBt active ester (**75**) (2.33 mmol, see above) in DMF (12 mL) was added to the resin and the mixture agitated by bubbling N_2 at room temperature for 2.5 h. The resin was washed with DMF (5 × 1 min). Again, the Fmoc-protecting groups were removed by treatment with 20% piperidine in DMF (1 × 3 min, 2 × 7 min). Pre-formed chloroacetylglycylglycine-OBt active ester (**76**, 4.66 mmol, see above) in DMF (10 mL) was next added to the resin and the reaction allowed to proceed at room temperature for 2.5 h, after which time the resin was washed with DMF (5 × 1 min). A ninhydrin (Kaiser) color test[146] was performed after each deprotection step and after each coupling step in order to ensure complete substitution. When necessary,

couplings were repeated. The resin was dried under vacuum overnight. The resin bound divalent N-chloroacetylated dendrimer **77** was treated with 95% aqueous trifluoroacetic acid (2 mL for 33.4 mg, 0.014 mmol) and the mixture was stirred at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **78** was obtained as an off-white solid in 93% yield (7.8 mg, 0.013 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 2H, lysyl γ-CH_2), 1.33 (m, 2H, lysyl δ-CH_2), 1.46 and 1.60 (2m, 2H, lysyl β-CH_2, unequiv.), 2.36 (t, 2H, J 7.0 Hz, β-alanyl α-CH_2), 2.99 (m, 2H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.65 and 3.74 (2d, 4H, J 5.8 Hz, glycyl CH_2's), 3.77 (d, 4 H, J 5.8 Hz, glycyl CH_2's), 4.12 (s, 4H, $ClCH_2$'s), 4.13 (m, 1H, lysyl α-CH), 7.72 (t, 1H, J 5.8 Hz, lysyl ε-NH), 7.92 (m, 2 H, β-alanyl NH, lysyl α-NH), 8.18 (m, 2H, glycyl NH), 8.45 (m, 2H, glycyl NH), 10.80 (bs, 1H, CO_2H); ^{13}C-NMR (by HMQC, DMSO-d_6) δ 22.7 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 33.8 (β-alanyl α-C), 34.3 (β-alanyl β-C), 38.7 (lysyl ε-C), 41.7 (4 × glycyl C), 42.4 (ClC), 52.5 (lysyl α-C); FAB-MS (pos.) calcd. for $C_{21}H_{33}Cl_2N_7O_9$ 598.4; found 598.3 (7.9% base peak).

Tetravalent N-chloroacetylated poly-L-lysine dendrimer (80).

The Fmoc-protecting groups of resin bound dipeptide $(Fmoc)_2$-Lys-β-Ala (1.05 mmol, see above) were removed by treatment with 20% piperidine in DMF (1 × 3 min, 2 × 7 min). Pre-formed N^{α},N^{ε}-di-Fmoc-L-lysine-OBt active ester (**75**, 4.20 mmol, see above) in DMF (15 mL) was next added to the resin and the reaction allowed to proceed at room temperature for 2.5 h, after which time the resin was washed with DMF (5 × 1 min). Again, the Fmoc-protecting groups were removed by treatment with 20% piperidine/DMF (1 × 3 min, 2 × 7 min). Pre-formed chloroacetylglycylglycine-OBt active ester (**76**, 8.40 mmol, see above) in DMF (15 mL) was added to the resin and the

mixture agitated with bubbling N_2 for 2.5 h. The resin was washed with DMF (5 × 1 min). A ninhydrin (Kaiser) color test[146] was performed after each deprotection step and after each coupling step in order to ensure complete substitution. When necessary, couplings were repeated. The resin was dried under vacuum overnight. The resin bound tetravalent N-chloroacetylated dendrimer **79** was treated with 95% aqueous trifluoroacetic acid (4 mL for 73.0 mg, 0.026 mmol) and the mixture was stirred at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **80** was obtained as an off-white solid in 90% yield (28.9 mg, 0.023 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.19 (m, 6H, lysyl γ-CH_2), 1.35 (m, 6H, lysyl δ-CH_2), 1.49 and 1.60 (2m, 6H, lysyl β-CH_2, unequiv.), 2.35 (t, 2H, J 7.0 Hz, β-alanyl α-CH_2), 3.01 (m, 6H, lysyl ε-CH_2), 3.25 and 3.27 (2m, 2H, β-alanyl β-CH_2), 3.65, 3.75, 3.77 (3,, 16H, glycyl CH_2's), 3.77 (d, 4 H, J 5.8 Hz, glycyl CH_2's), 4.12 (s, 8H, $ClCH_2$'s), 4.13 (m, 3H, lysyl α-CH), 7.95-8.80 (4 m, 19H, NH's); ^{13}C-NMR (by HMQC, DMSO-d_6) δ 22.7 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 33.8 (β-alanyl α-C), 34.3 (β-alanyl β-C), 38.7 (lysyl ε-C), 42.0 (glycyl C), 42.4 (ClC), 52.5 (lysyl α-C); FAB-MS (pos.) calcd. for $C_{45}H_{71}Cl_4N_{15}O_{17}$ 1236.0; found 1236.9 (M^+ + 1).

Octavalent N-chloroacetylated poly-L-lysine dendrimer (82).

The Fmoc-protecting groups of resin bound $(Fmoc)_4$-Lys_2-Lys-β-Ala (0.70 mmol, see above) were removed by treatment with 20% piperidine in DMF (1 × 3 min, 2 × 7 min). N^{α},N^{ε}-Di-Fmoc-L-lysine-OBt active ester (**75**, 5.60 mmol, see above) in DMF (15 mL) was added to the resin and the mixture agitated by bubbling N_2 for 2.5 h. The resin was washed with DMF (5 × 1 min). Again, the Fmoc-protecting groups were removed by treatment with 20% piperidine/DMF (1 × 3 min, 2 × 7 min). Pre-formed chloroacetylglycylglycine-OBt active ester (**76**, 11.21 mmol, see above) in DMF (15 mL)

was next added to the resin and the reaction allowed to proceed at room temperature for 2.5 h, after which time the resin was washed with DMF (5 × 1 min). A ninhydrin (Kaiser) color test[146] was performed after each deprotection step and after each coupling step in order to ensure complete substitution. When necessary, couplings were repeated. The resin was dried under vacuum overnight. The resin bound octavalent N-chloroacetylated dendrimer **81** was treated with 95% aqueous trifluoroacetic acid (10 mL for 331 mg, 0.078 mmol) and the mixture was stirred at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **82** was obtained as an off-white solid in 99% yield (195 mg, 0.077); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 14H, lysyl γ-CH_2), 1.33 (m, 14H, lysyl δ-CH_2), 1.70 (m, 14H, lysyl β-CH_2), 2.38 (m, 2H, β-alanyl α-CH_2), 3.03 (m, 14H, lysyl ε-CH_2), 3.32 (m, 2H, β-alanyl β-CH_2), 3.65 and 3.74 (2m, 32H, glycyl CH_2's), 4.13 (s, 8H, $ClCH_2$'s), 4.14 (m, 7H, lysyl α-CH), 7.70-8.57 (m, 31H, NH's); ^{13}C-NMR (by HMQC, DMSO-d_6) δ 22.7 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 33.8 (β-alanyl α-C), 34.3 (β-alanyl β-C), 38.7 (lysyl ε-C), 41.7 (glycyl C), 42.4 (ClC), 52.5 (lysyl α-C).

Hexadecavalent N-chloroacetylated poly-L-lysine dendrimer (84).

The Fmoc-protecting groups of resin bound dipeptide $(Fmoc)_8$-Lys_4-Lys_2-Lys-β-Ala (0.068 mmol, see above) were removed by treatment with 20% piperidine in DMF (1 × 3 min, 2 × 10 min). Pre-formed N^{α},N^{ε}-di-Fmoc-L-lysine-OBt active ester (**75**, 1.08 mmol, see above) in DMF (15 mL) was next added to the resin and the reaction allowed to proceed at room temperature for 2.5 h, after which time the resin was washed with DMF (5 × 1 min). Again, the Fmoc-protecting groups were removed by treatment with 20% piperidine/DMF (1 × 3 min, 2 × 10 min). Pre-formed chloroacetylglycylglycine-OBt active ester (**76**, 2.16 mmol, see above) in DMF (15 mL) was added to the resin and the

mixture agitated with bubbling N_2 for 2.5 h. The resin was washed with DMF (5 × 1 min). A ninhydrin (Kaiser) color test[146] was performed after each deprotection step and after each coupling step in order to ensure complete substitution. When necessary, couplings were repeated. The resin was dried under vacuum overnight. The resin bound hexadecavalent N-chloroacetylated dendrimer **83** was treated with 95% aqueous trifluoroacetic acid (1 mL for 4.0 mg, 0.00055 mmol) and the mixture was stirred at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **84** was obtained as an off-white solid in 90% yield (2.6 mg, 0.00050 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 30H, lysyl γ-CH_2), 1.33 (m, 30H, lysyl δ-CH_2), 1.55 (m, 30H, lysyl β-CH_2, unequiv.), 2.36 (m, 2H, β-alanyl α-CH_2), 3.01 (m, 30H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.65 and 3.74 (2m, 64H, glycyl CH_2's), 4.12 (s, 32H, $ClCH_2$'s), 4.13 (m, 15H, lysyl α-CH), 7.72-8.30 (m, NH's); ^{13}C-NMR (by HMQC, DMSO-d_6) δ 22.7 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 33.8 (β-alanyl α-C), 34.3 (β-alanyl β-C), 38.7 (lysyl ε-C), 41.7 (glycyl C), 42.4 (ClC), 52.5 (lysyl α-C).

Divalent dendritic α-thiosialoside (86).

Polymer-bound divalent N-chloroacetylated dendrimer **77** (0.022 mmol, see above) was washed with DMF (5 × 1 min). The α-thiosialoside **66** (27.4 mg, 0.054 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic α-thiosialoside **85** was dried under vacuum overnight and then treated with 95% TFA (3 mL for 31.5 mg, 0.0058 mmol) at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether.

The precipitate was collected and dried under vacuum. Title compound **86** was obtained as an off-white solid in 90% yield (11.3 mg, 0.0052 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 2H, lysyl γ-CH_2), 1.33 (m, 2H, lysyl δ-CH_2), 1.46 and 1.60 (2m, 2H, lysyl β-CH_2, unequiv.), 1.78 (dd, 2H, 2 × J ≡ 12.1 Hz, H-3ax), 1.65 (s, 6H, NAc), 1.91, 1.96, 1.99, 2.06 (4s, 24H, OAc's), 2.36 (t, 2H, J 7 Hz, β-alanyl α-CH_2), 2.64 (dd, 2H, $J_{3ax,3eq}$ 12.5 Hz, $J_{3eq,4}$ 4.6 Hz, H-3eq), 2.99 (m, 2H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.37 and 3.47 (2d, 2 × 2H, J 7.1 Hz, SCH_2's), 3.65 and 3.74 (2d, 4H, J 5.8 Hz, glycyl CH_2's), 3.72 (s, 6H, CO_2CH_3), 3.77 (m, 6 H, H-6, glycyl CH_2's), 3.86 (m, 2H, H-5), 4.02 (dd, 2H, $J_{8,9}$ 5.8 Hz, $J_{9,9'}$ 12.2 Hz, H-9), 4.13 (m, 1H, lysyl α-CH), 4.16 (dd, 2H, $J_{8,9'}$ 3.1 Hz, H-9'), 4.70 (ddd, 2H, $J_{4,5}$ 10.8 Hz, H-4), 5.12 (dd, 2H, J 2.0 and 8.3 Hz, H-7), 5.25 (m, 2H, H-8), 7.62-8.16 (m, 9H, NH's); ^{13}C-NMR (DMSO-d_6) δ 20.6 (OAc's), 22.6 (NAc), 22.8 (lysyl γ-C), 28.8 (lysyl δ-C), 31.7 (lysyl β-C), 32.1 (2 × SC), 33.8 (β-alanyl α-C), 34.9 (β-alanyl β-C), 37.4 (C-3), 38.4 (lysyl ε-C), 42.0, 42.5, 42.6 (glycyl C's), 47.8 (C-5), 52.5 (lysyl α-C), 53.1 (OCH_3), 61.9 (C-9), 67.1 (C-7), 67.9 (C-4), 69.5 (C-8), 73.7 (C-6), 82.4 (C-2), 167.7-172.8 (C=O's).

De-O-acetylated divalent dendritic α-thiosialoside (87).

Dendritic α-thiosialoside **86** (11.3 mg, 0.0052 mmol) was dissolved in MeOH (2 mL) and 10% 1 M NaOMe/MeOH was added. The solution was stirred at room temperature for 1 h. The solution was then treated with Amberlite IR-120 H^+ resin for 15 min, and the solution taken to dryness under reduced pressure. The residue was then freeze dried. Divalent **87** was obtained as a white powder in quantitative yield (6.3 mg, 0.0052 mmol); ^{1}H-NMR (D_2O) δ 1.42 (m, 2H, lysyl γ-CH_2), 1.64 (m, 2H, lysyl δ-CH_2), 1.78, 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 1.94, 1.96 (2dd, 2H, J 12.1 H-3ax), 2.10 (s, 6H, NAc), 2.91, 2.96 (2dd, 2H, $J_{3ax,3eq}$ 12.6 Hz, $J_{3eq,4}$ 4.7 Hz, H-3eq), 2.69 (m, 2H, β-alanyl α-CH_2), 3.34 (m, 2H, lysyl ε-CH_2), 3.50-4.33 (m, 35H, peptide backbone and NeuAc residues excluding above, 3.83 (s, 6H, CO_2CH_3), NH's, CO_2H); ^{13}C-NMR (by

HMQC, D_2O) δ 18.8 (NAc), 19.2 (lysyl γ-C), 24.6 (lysyl δ-C), 27.4 (lysyl β-C), 29.5 (2 × SC), 30.8 (β-alanyl α-C), 32.4 (β-alanyl β-C), 35.8 (lysyl ε-C), 36.7, 37.3 (C-3, C-3'), 39.4 (4 × glycyl C's), 48.2 (C-5), 50.7 (lysyl α-C), 53.1 (OCH_3) 59.9 (C-9), 65.0 (C-7), 69.1 (C-4), 72.1 (C-8), 72.9 (C-6).

Fully deprotected divalent α-thiosialodendrimer (88).

A solution of the divalent dendritic methyl ester **87** (6.3 mg, 0.0052 mmol) dissolved in 0.05 M NaOH (2 mL) was stirred at room temperature for 2 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **88** was obtained as a white, lyophilized powder in quantitative yield (6.1 mg, 0.0052 mmol); ^{1}H-NMR (D_2O) δ 1.42 (m, 2H, lysyl γ-CH_2), 1.64 (m, 2H, lysyl δ-CH_2), 1.78, 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 1.90, 1.96 (2dd, 2H, J 12.1 H-3a), 2.12, 2.13 (2s, 6H, NAc), 2.69 (m, 2H, β-alanyl α-CH_2), 2.91, 2.96 (2dd, 2H, $J_{3ax,3eq}$ 12.6 Hz, $J_{3eq,4}$ 4.7 Hz, H-3eq), 3.34 (m, 2H, lysyl ε-CH_2), 3.50-4.33 (m, 29H, peptide backbone and NeuAc residues excluding above, NH's, CO_2H); ^{13}C-NMR (by HMQC, D_2O) δ 18.8 (NAc), 19.2 (lysyl γ-C), 24.6 (lysyl δ-C), 27.4 (lysyl β-C), 29.5 (2 × SC), 30.8 (β-alanyl α-C), 32.4 (β-alanyl β-C), 35.8 (lysyl ε-C), 36.7, 37.3 (C-3, C-3'), 39.4 (4 × glycyl C's), 48.2 (C-5), 50.7 (lysyl α-C), 59.9 (C-9), 65.0 (C-7), 69.1 (C-4), 72.1 (C-8), 72.9 (C-6); FAB-MS (pos.) calcd. for $C_{43}H_{69}N_9O_{25}S_2$ 1176.2; found 1176.4 (0.8% base peak).

Tetravalent dendritic α-thiosialoside (90).

Polymer-bound tetravalent N-chloroacetylated dendrimer **79** (0.014 mmol, see above) was washed with DMF (5 × 1 min). The α-thiosialoside **66** (34.0 mg, 0.067 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic α-thiosialoside **89** was dried under vacuum overnight and then treated with 95% TFA (6 mL for 65.7 mg, 0.015 mmol) at room temperature for

1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **90** was obtained as an off-white solid in 85% yield (39.9 mg, 0.013 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 6H, lysyl γ-CH_2), 1.33 (m, 6H, lysyl δ-CH_2), 1.46 and 1.60 (2m, 6H, lysyl β-CH_2, unequiv.), 1.78 (dd, 4H, 2 × J ≅ 12.1 Hz, H-3ax), 1.65 (s, 12H, NAc), 1.91, 1.96, 1.99, 2.06 (4s, 48H OAc's), 2.36 (t, 2H, J 7.0 Hz, β-alanyl α-CH_2), 2.64 (dd, 4H, $J_{3ax,3eq}$ 12.5 Hz, $J_{3eq,4}$ 4.6 Hz, H-3eq), 2.99 (m, 6H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.37 and 3.47 (2d, 8H, J 7.1 Hz, SCH_2's), 3.60-3.75 (m, 16H, glycyl CH_2's), 3.72 (s, 12H, CO_2CH_3), 3.86 (m, 4H, H-5), 4.02 (dd, 4H, H-9), 4.13-4.16 (m, 7H, lysyl α-CH, H-9'), 4.70 (ddd, 4H, $J_{4,5}$ 10.8 Hz, H-4), 5.12 (dd, 4H, J 2.0 and 8.3 Hz, H-7), 5.25 (m, 4H, H-8), 7.62-8.16 (m, 19H, NH's); ^{13}C-NMR (DMSO-d_6) δ 20.8 (OAc's), 21.0 (NAc), 22.4 (lysyl γ-C), 28.8 (lysyl δ-C), 31.7 (lysyl β-C), 32.1 (2 × SC), 33.8 (β-alanyl α-C), 34.9 (β-alanyl β-C), 35.9 (C-3), 38.4 (lysyl ε-C), 42.2, 42.6 (glycyl C's), 47.9 (C-5), 52.5 (lysyl α-C), 53.6 (OCH_3), 62.0 (C-9), 67.1 (C-7), 68.0 (C-4), 69.8 (C-8), 74.0 (C-6), 81.3 (C-2), 162.4-172.4 (C=O's).

Fully deprotected tetravalent α-thiosialodendrimer (91).

A solution of the tetravalent α-thiosialodendrimer **90** (9.7 mg, 0.0031 mmol) dissolved in 0.05 M NaOH (2 mL) was stirred at room temperature for 2 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **91** was obtained as a white, lyophilized powder in quantitative yield (7.4 mg, 0.0031 mmol); ^{1}H-NMR (D_2O) δ 1.42 (m, 6H, lysyl γ-CH_2), 1.64 (m, 6H, lysyl δ-CH_2), 1.80-1.98 (m, 10H, lysyl β-CH_2, H-3ax), 2.12, 2.13 (2s, 12H, NAc), 2.91, 2.96 (2dd, 4H, $J_{3ax,3eq}$ 12.6 Hz, $J_{3eq,4}$ 4.7 Hz, H-3eq), 2.69 (t, 2H, J 7.0 Hz, β-alanyl α-CH_2), 3.34 (m, 6H, lysyl ε-CH_2), 3.50-4.40 (m, 53H, peptide backbone and NeuAc

residues excluding above, NH's, CO_2H); ^{13}C-NMR (by HMQC, D_2O) δ 18.8 (NAc), 19.2 (lysyl γ-C), 24.6 (lysyl δ-C), 27.4 (lysyl β-C), 29.5 (2 × SC), 30.8 (β-alanyl α-C), 32.4 (β-alanyl β-C), 35.8 (lysyl ε-C), 36.7, 37.3 (C-3, C-3'), 39.4 (glycyl C's), 48.2 (C-5), 50.7 (lysyl α-C), 59.9 (C-9), 65.0 (C-7), 69.1 (C-4), 72.1 (C-8), 72.9 (C-6).

Octavalent dendritic α-thiosialoside (93).

Polymer-bound octavalent N-chloroacetylated dendrimer **81** (0.0070 mmol, see above) was washed with DMF (5 × 1 min). The α-thiosialoside **66** (34.0 mg, 0.067 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic α-thiosialoside **92** was dried under vacuum overnight and then treated with 95% TFA (3 mL for 30.4 mg, 0.0031 mmol) at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **93** was obtained as an off-white solid in 86% yield (17.0 mg, 0.0027 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 14H, lysyl γ-CH_2), 1.33 (m, 14H, lysyl δ-CH_2), 1.46 and 1.60 (2m, 14H, lysyl β-CH_2, unequiv.), 1.78 (m, 8H, H-3ax), 1.65 (s, 24H, NAc), 1.91, 1.96, 1.99, 2.06 (4s, 96H OAc's), 2.36 (m, 2H, β-alanyl α-CH_2), 2.64 (dd, 8H, $J_{3ax,3eq}$ 12.5 Hz, $J_{3eq,4}$ 4.6 Hz, H-3eq), 3.01 (m, 14H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.37 and 3.47 (2d, 16H, J 7.1 Hz, SCH_2's), 3.65-3.75 (m, 32H, glycyl CH_2's), 3.72 (s, 24H, CO_2CH_3), 3.86 (m, 8H, H-5), 4.02-4.13 (m, 15H, lysyl α-CH, H-9), 4.16 (m, 8H, H-9'), 4.70 (ddd, 8H, $J_{4,5}$ 10.8 Hz, H-4), 5.12 (m, 8H, H-7), 5.25 (m, 8H, H-8), 7.62-8.16 (m, 39H, NH's); ^{13}C-NMR (DMSO-d_6) δ 20.6 (OAc's), 22.6 (NAc), 22.8 (lysyl γ-C), 28.8 (lysyl δ-C), 31.7 (lysyl β-C), 32.1 (2 × SC), 37.4 (C-3), 38.4 (lysyl ε-C), 42.0, 42.5, 42.6 (glycyl C's), 47.8

(C-5), 52.5 (lysyl α-C), 53.1 (OCH_3), 61.9 (C-9), 67.1 (C-7), 67.9 (C-4), 69.5 (C-8), 73.7 (C-6), 82.4 (C-2), 167.7-172.8 (C=O's).

De-O-acetylated octavalent dendritic α-thiosialoside (94).

Dendritic α-thiosialoside **93** (15.1 mg, 0.0024 mmol) was dissolved in MeOH (2 mL) and 10% 1 M NaOMe/MeOH was added. The solution was stirred at room temperature for 1 h. The solution was then treated with Amberlite IR-120 H^+ resin for 15 min, and the solution taken to dryness under reduced pressure. The residue was then freeze dried. Octavalent **94** was obtained as a white powder in quantitative yield (11.8 mg, 0.0024 mmol); ^{1}H-NMR (D_2O) δ 1.42 (m, 14H, lysyl γ-CH_2), 1.64 (m, 14H, lysyl δ-CH_2), 1.78-2.05 (m, 22H, lysyl β-CH_2, H-3ax), 2.08 (s, 24H, NAc), 2.63 (m, 2H, β-alanyl α-CH_2), 2.90 (m, 8H, H-3eq), 3.25 (m, 14H, lysyl ε-CH_2), 3.52-4.39 (m, 137H, peptide backbone and NeuAc residues excluding above, 3.84 (s, 24H, CO_2CH_3), NH's, CO_2H); ^{13}C-NMR (by HMQC, D_2O) δ 18.8 (NAc), 19.2 (lysyl γ-C), 24.6 (lysyl δ-C), 27.4 (lysyl β-C), 29.5 (2 × SC), 35.8 (lysyl ε-C), 36.7, 37.3 (C-3, C-3'), 39.4 (glycyl C's), 48.2 (C-5), 50.7 (lysyl α-C), 53.1 (OCH_3) 59.9 (C-9), 65.0 (C-7), 69.1 (C-4), 72.1 (C-8), 72.9 (C-6).

Fully deprotected octavalent α-thiosialodendrimer (95).

A solution of the octavalent dendritic methyl ester **94** (11.8 mg, 0.0024 mmol) dissolved in 0.05 M NaOH (2 mL) was stirred at room temperature for 2 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **95** was obtained as a white, lyophilized powder in quantitative yield (11.6 mg, 0.0024 mmol); ^{1}H-NMR (D_2O) δ 1.42 (m, 14H, lysyl γ-CH_2), 1.64 (m, 14H, lysyl δ-CH_2), 1.79-1.96 (m, 22H, lysyl β-CH_2, H-3ax), 2.10 (s, 24H, NAc), 2.48 (m, 2H, β-alanyl α-CH_2), 2.90 (m, 8H, H-3eq), 3.26 (m, 14H, lysyl ε-CH_2), 3.39-4.42 (m, 113H, peptide backbone and NeuAc residues excluding above, NH's, CO_2H); ^{13}C-NMR (by HMQC, D_2O) δ 18.8 (NAc), 19.2 (lysyl γ-C), 24.6 (lysyl δ-C), 27.4 (lysyl β-C), 29.5

(2 × SC), 35.8 (lysyl ε-C), 36.7, 37.3 (C-3, C-3'), 39.4 (glycyl C's), 48.2 (C-5), 50.7 (lysyl α-C), 59.9 (C-9), 65.0 (C-7), 69.1 (C-4), 72.1 (C-8), 72.9 (C-6).

Hexadecavalent dendritic α-thiosialoside (97).

Polymer-bound hexadecavalent N-chloroacetylated dendrimer **83** (0.0068 mmol, see above) was washed with DMF (5 × 1 min). The α-thiosialoside **66** (66.0 mg, 0.130 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic α-thiosialoside **96** was dried under vacuum overnight and then treated with 95% TFA (8 mL for 103.0 mg, 0.0072 mmol) at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **97** was obtained as an off-white solid in 65% yield (58.8 mg, 0.0047 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 30H, lysyl γ-CH_2), 1.33 (m, 30H, lysyl δ-CH_2), 1.46 and 1.60 (2m, 30H, lysyl β-CH_2, unequiv.), 1.65 (s, 48H, NAc), 1.78 (dd, 16H, 2 × J ≅ 12.1 Hz, H-3ax), 1.91, 1.96, 1.99, 2.06 (4s, 192H, OAc's), 2.36 (m, 2H, β-alanyl α-CH_2), 2.64 (m, 16H, H-3eq), 3.01 (m, 30H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.37 and 3.47 (2d, 2 × 16H, J 7.1 Hz, SCH_2's), 3.65-3.75 (m, 64H, glycyl CH_2's), 3.72 (s, 48H, CO_2CH_3), 3.86 (m, 16H, H-5), 4.02-4.13 (m, 31H, lysyl α-CH, H-9), 4.16 (m, 16H, H-9'), 4.70 (ddd, 16H, $J_{4,5}$ 10.8 Hz, H-4), 5.12 (dd, 16H, J 2.0 and 8.3 Hz, H-7), 5.25 (m, 16H, H-8), 7.62-8.16 (m, NH's); ^{13}C-NMR (DMSO-d_6) δ 20.6 (OAc's), 22.6 (NAc), 22.8 (lysyl γ-C), 28.8 (lysyl δ-C), 31.7 (lysyl β-C), 32.1 (2 × SC), 37.4 (C-3), 38.4 (lysyl ε-C), 42.0, 42.5, 42.6 (glycyl C's), 47.8 (C-5), 52.5 (lysyl α-C), 53.1 (OCH_3), 61.9 (C-9), 67.1 (C-7), 67.9 (C-4), 69.5 (C-8), 73.7 (C-6), 82.4 (C-2), 167.7-172.8 (C=O's).

Fully deprotected hexadecavalent α-thiosialodendrimer (98).

A solution of the hexadecavalent dendritic methyl ester **97** (24.0 mg, 0.0019 mmol) dissolved in 0.05 M NaOH (2 mL) was stirred at room temperature for 2 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **98** was obtained as a white, lyophilized powder in quantitative yield (18.3 mg, 0.0019 mmol); ^{1}H-NMR (D_2O) δ 1.42 (m, 30H, lysyl γ-CH_2), 1.64 (m, 30H, lysyl δ-CH_2), 1.78-1.99 (m, 46H, lysyl β-CH_2, H-3ax), 2.12 (s, 48H, NAc), 2.46 (m, 2H, β-alanyl α-CH_2), 2.94 (m, 16H, H-3eq), 3.30 (m, 30H, lysyl ε-CH_2), 3.50-4.33 (m, peptide backbone and NeuAc residues excluding above, NH's, CO_2H); ^{13}C-NMR (by HMQC, D_2O) δ 18.8 (NAc), 19.2 (lysyl γ-C), 24.6 (lysyl δ-C), 27.4 (lysyl β-C), 29.5 (2 × SC), 35.8 (lysyl ε-C), 36.7, 37.3 (C-3, C-3'), 39.4 (4 × glycyl C's), 48.2 (C-5), 50.7 (lysyl α-C), 59.9 (C-9), 65.0 (C-7), 69.1 (C-4), 72.1 (C-8), 72.9 (C-6).

9-O-Acetylated octavalent dendritic α-thiosialoside (99).

Octavalent α-thiosialoside **95** (5.3 mg, 0.0012 mmol) was dissolved in DMSO (500 μL) and trimethylorthoacetate (22.4 μL, 20 equiv.) was added. Three drops of TFA were added and the solution stirred overnight at 25 °C. The solution was dialyzed (dialysis tubing MW cut off 2000) against H_2O. Title ester **99** was obtained as a lyophilized white powder (6.2 mg, 0.0012 mmol, quantitative); ^{1}H-NMR (D_2O) δ 1.42 (m, 14H, lysyl γ-CH_2), 1.64 (m, 14H, lysyl δ-CH_2), 1.79-1.96 (m, 22H, lysyl β-CH_2, H-3ax), 2.10 (s, 24H, NAc), 2.24 (s, 24H, 9-OAc), 2.48 (m, 2H, β-alanyl α-CH_2), 2.90 (m, 8H, H-3eq), 3.26 (m, 14H, lysyl ε-CH_2), 3.39-4.33 (m, 105H, peptide backbone and NeuAc residues excluding those listed, NH's, CO_2H), 4.38 (m, 8H, H-9); ^{13}C-NMR (by HMQC, D_2O) δ 18.8 (NAc), 19.2 (lysyl γ-C), 24.6 (lysyl δ-C), 27.4 (lysyl β-C), 29.5 (SC), 35.8 (lysyl ε-C), 36.7, 37.3 (C-3, C-3'), 39.4 (glycyl C's), 48.2 (C-5), 50.7 (lysyl α-C), 59.9 (C-9), 65.0 (C-7), 69.1 (C-4), 72.1 (C-8), 72.9 (C-6).

Double immunodiffusion assays between WGA and α-thiosialodendrimers 88, 91, 95, and 98.

Agar gel diffusion experiments were performed in 1% agarose containing 2% poly(ethyleneglycol) (PEG, MW 8,000) in PBS buffer. A glass plate was coated with 1% agarose containing 2% PEG in PBS. A central well and five surrounding wells were cut in the gel. Wheat germ agglutinin (WGA, 20 μL of a 1 mg/mL solution in PBS) was used to fill the center well. Outer wells were filled with α-thiosialodendrimers solutions (20 μL of 2 mg/mL of each of **88**, **91**, **95**, and **98**) and a negative control polyacrylamide (MW 100,000, 20 μL of a 2 mg/mL solution in PBS). Precipitin bands between WGA and neoglycoconjugates **88**, **91**, **95**, and **98** and polyacrylamide were allowed to form overnight at 4 °C.

Enzyme linked lectin assays (ELLA) using α-thiosialodendrimers 88, 91, 95, and 98 as coating antigens.

Linbro (Titertek) microtitration plates were coated with 10 μg/well of poly(acrylamide-co-*p*-N-acrylamidophenylthiosialoside) **12**[70] or with thiosialodendrimers **88**, **91**, **95**, and **98** (10 μg/well) at room temperature overnight. Wells were washed 5 times (4 times with PBS-Tween and once with PBS). The wells were then blocked with 200 μL/well of 1% bovine serum albumin/phosphate buffer (BSA/PBS) for 60 min at room temperature. After washing 5 times (4 times with PBS-Tween and once with PBS), the wells were then filled with 100 μL/well of serial dilution of WGA/horse radish peroxidase (WGA/HRP) from 10^{-2} to 10^{-5} mg/mL in PBS and incubated at room temperature for 3 h. The plates were washed with PBS-Tween (5×) and PBS (2×). Then 50 μL/well of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, 1 mg/4 mL) in citrate-phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. After 15 min, the optical density (O. D.) was measured at 410 nm relative to 570 nm. Blank wells did not contain conjugates. Each test was performed in triplicate.

Competitive inhibition ELLA using α-thiosialodendrimers 88, 91, 95, and 98 as inhibitors.

Linbro (Titertek) microtitration plates were coated overnight at room temperature with poly(acrylamide-co-*p*-N-acrylamidophenylthiosialoside) **12**[70] (10 μg/well). The wells were then washed 5 times (4 times with PBS-Tween and once with PBS) and then blocked with 200 μL/well of 1% BSA/PBS for 60 min at room temperature. Each of the following inhibitors were used as stock solutions of 1 mg/mL PBS: phenylthio-α-sialoside[70] as reference monovalent compound, di- (**88**), tetra- (**91**), octa- (**95**), and hexadeca- (**98**) valent dendrimers. Each inhibitor was added in serial two-fold dilutions (50 μL/well) in PBS with 50 μL of WGA/HRP (2.5 μg/mL) at 25 °C for 60 min. The above solutions were then transferred to the copolymer coated plates which were incubated for two hours at room temperature. The plates were washed as described above and the ABTS substrate was the added (50 μL/well). The O. D. was measured at 410 nm relative to 570 nm after 15 min. The per cent inhibitions were calculated as follows:

$$\% \text{ Inhibition} = (A_{(\text{without inhibitor})} - A_{(\text{with inhibitor})})/A_{(\text{without inhibitor})} \times 100$$

IC_{50}'s were reported as the concentration required for 50% inhibition of the coating antigen. Each test was performed in triplicate.

Divalent N-acetamidoglucosaminylated dendrimer (101).

Polymer-bound divalent N-chloroacetylated dendrimer **77** (0.082 mmol, see above) was washed with DMF (5 × 1 min). Thiolated N-acetylglucosamine derivative **67** (89.8 mg, 0.247 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic N-acetylglucosaminide **100** was dried under vacuum overnight and then treated with 95% TFA (10 mL for 244 mg, 0.082 mmol) at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL)

and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **101** was obtained as an off-white solid in 74% yield (76.2 mg, 0.061 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 2H, lysyl γ-CH_2), 1.33 (m, 2H, lysyl δ-CH_2), 1.46 and 1.61 (2 m, 2H, lysyl β-CH_2, unequiv.), 1.75 (s, 6H, NAc), 1.90, 1.95, 2.00 (3s, 18H OAc's), 2.36 (t, 2H, J 7.0 Hz, β-alanyl α-CH_2), 3.00 (m, 2H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.30 and 3.38 (2d, 2 × 2H, J 14.1 Hz, SCH_2's), 3.66 (d, 2H, J 5.8 Hz, glycyl CH_2), 3.73 (m, 6 H, glycyl CH_2's), 3.81 (m, 2H, H-5), 3.89 (dd, 2H, $J_{2,3}$ 9.9 Hz, H-2), 4.00 (d, 2H, $J_{5,6}$ 10.3 Hz, H-6), 4.14 (m, 3H, lysyl α-CH, H-6), 4.77 (d, 2H, $J_{1,2}$ 10.3 Hz, H-1), 4.86 (dd, 2H, J 9.7 Hz, H-4), 5.05 (dd, 2H, H-3). 7.73 (m, 1H, lysyl ε-NH), 7.90 (m, 2H, β-alanyl NH, lysyl α-NH), 7.98 (d, 2H, $J_{2,NH}$ 9.3 Hz, NHAc), 8.12, 8.20 (2m, 2 × 2H, glycyl NH's); ^{13}C-NMR (DMSO-d_6) δ 20.3, 20.4, 20.5 (OAc's), 22.6 (NAc), 22.7 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 32.6 (SC), 33.7 (β-alanyl α-C), 34.8 (β-alanyl β-C), 38.4 (lysyl ε-C), 42.0, 42.4 (glycyl C's), 52.0 (C-2), 52.5 (lysyl α-C), 61.9 (C-6), 68.4 (C-4), 73.6 (C-3), 74.7 (C-5), 82.8 (C-1), 168.4-172.8 (C=O's); FAB-MS (pos.) calcd. for $C_{49}H_{73}N_9O_{25}S_2$ 1252.3; found 1253.2 (M^+ + 1, 19.8%).

Fully deprotected divalent N-acetamidoglucosaminylated dendrimer (102).

A solution of the divalent dendritic N-acetylglucosaminide **101** (29.7 mg, 0.024 mmol) was dissolved in MeOH (5 mL) and 10% 1 M NaOMe/MeOH was added. The solution was stirred at room temperature for 1 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **102** was obtained as a white, lyophilized powder in quantitative yield (24.0 mg, 0.024 mmol); ^{1}H-NMR (D_2O) δ 1.40 (m, 2H, lysyl γ-CH_2), 1.57 (m, 2H, lysyl δ-CH_2), 1.77, 1.83 (2m, 2H, lysyl β-CH_2, unequiv.), 2.09 (s, 6H, NAc), 2.66 (t, 2H, J 6.5 Hz, β-alanyl α-CH_2), 3.26 (m, 2H, lysyl ε-CH_2), 3.46-3.97 (m, 26H, peptide backbone and GlcNAc residues excluding those listed, NH's, CO_2H), 4.27 (m, 1H, lysyl α-CH), 4.74 (dd, 2H, H-1); ^{13}C-NMR (D_2O) δ 21.7 (NAc), 21.8 (lysyl γ-C), 27.2 (lysyl δ-C), 30.1 (lysyl β-C), 32.9 (SC),

33.0 (β-alanyl α-C), 34.7 (β-alanyl β-C), 38.5 (lysyl ε-C), 41.8, 42.0, 42.4 (glycyl C's), 53.5 (lysyl α-C), 54.0, 60.4, 69.2, 74.5, 79.4 (C-2, C-3, C-4, C-5, C-6) 83.5 (C-1), 170.6-175.6 (C=O's); FAB-MS (pos.) calcd. for $C_{37}H_{61}N_9O_{19}S_2$ 999.4; found 1000.6 (M^+ + 1, <1%).

Divalent lactosylated dendrimer (104).

Polymer-bound divalent N-chloroacetylated dendrimer **77** (0.045 mmol, see above) was washed with DMF (5 × 1 min). Thiolated lactose derivative **68** (93.9 mg, 0.144 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic lactoside **103** was dried under vacuum overnight and then treated with 95% TFA (10 mL for 160.0 mg, 0.045 mmol) at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **104** was obtained as an off-white solid in 99% yield (81.6 mg, 0.044 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.20 (m, 2H, lysyl γ-CH_2), 1.36 (m, 2H, lysyl δ-CH_2), 1.47 and 1.60 (2 m, 2H, lysyl β-CH_2, unequiv.), 1.88, 1.96, 2 × 1.99 (6s, 42H, OAc's), 2.36 (t, 2H, J 6.9 Hz, β-alanyl α-CH_2), 2.99 (m, 2H, lysyl ε-CH_2), 3.22 (m, 2H, β-alanyl β-CH_2), 3.29 and 3.37 (2d, 2 × 2H, J 14.1 Hz, SCH_2's), 3.60-3.82 (m, 12H, glycyl CH_2's, H-4, H-5), 4.00 (m, 6H, H-6a, H-6'), 4.13 (m, 1H, lysyl α-CH), 4.21 (ddd, 2H, H-5'), 4.28 (dd, 2H, H-6b), 4.71-4.84 (m, 6H, H-1, H-2, H-2'), 4.89 (d, 2H, $J_{1,2}$ 10.1 Hz, H-1), 5.12-5.16 (m, 4H, H-3, H-3'), 5.21 (dd, 2H, H-4'), 7.74 (m, 1H, lysyl ε-NH), 7.90-7.93 (m, 2H, β-alanyl NH, lysyl α-NH), 8.12-8.21 (2m, 4H, glycyl NH's); ^{13}C-NMR (DMSO-d_6) δ 20.2, 20.3, 2 × 20.4, 20.6 (OAc's), 22.7 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 32.6 (SC), 33.7 (β-alanyl α-C), 34.8 (β-alanyl β-C), 38.4 (lysyl ε-C), 42.0, 42.4 (glycyl C's), 52.5 (lysyl

α-C), 60.9 (C-6'), 62.4 (C-6), 67.1 (C-4'), 68.9, 69.7 (C-2, C-2'), 70.0, 75.6 (C-3, C-3'), 73.2 (C-5'), 75.6, 76.0 (C-4, C-5), 81.2 (C-1), 99.9 (C-1'), 168.4-172.8 (C=O's); FAB-MS (pos.) calcd. for $C_{73}H_{103}N_7O_{43}S_2$ 1830.8; found 1853.7 (M^+ + Na, 7.4 %).

Fully deprotected divalent lactosylated dendrimer (105).

A solution of the divalent dendritic lactose **104** (22.3 mg, 0.012 mmol) was dissolved in MeOH (5 mL) and 10% 1 M NaOMe/MeOH was added. The solution was stirred at room temperature for 1 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **105** was obtained as a white, lyophilized powder in 95% yield (14.2 mg, 0.011 mmol); ^{1}H-NMR (D_2O) δ 1.39 (m, 2H, lysyl γ-CH_2), 1.57 (m, 2H, lysyl δ-CH_2), 1.78, 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 2.66 (t, 2H, J 6.5 Hz, β-alanyl α-CH_2), 3.27 (m, 2H, lysyl ε-CH_2), 3.50-4.20 (m, 56H, peptide backbone and Lac residues excluding those listed, NH's, CO_2H), 4.33 (m, 1H, lysyl α-CH), 4.51 (d, 2H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.67 (d, 2H, $J_{1,2}$ 9.9 Hz, H-1); ^{13}C-NMR (D_2O) δ 21.9 (lysyl γ-C), 27.3 (lysyl δ-C), 30.0 (lysyl β-C), 33.0 (2 × SC), 32.5 (β-alanyl α-C), 38.2 (β-alanyl β-C), 38.6 (lysyl ε-C), 41.9, 42.0, 42.4 (glycyl C's), 53.4 (lysyl α-C), 59.7, 60.6, 68.1, 70.6, 71.4, 72.1, 74.9, 75.2, 77.5, 78.2 (C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6, C-6'), 84.4 (C-1), 102.4 (C-1'), 170.5-175.5 (C=O's).

Tetravalent N-acetamidoglucosaminylated dendrimer (107).

Polymer-bound tetravalent N-chloroacetylated dendrimer **79** (0.041 mmol, see above) was washed with DMF (5 × 1 min). Thiolated N-acetylglucosamine derivative **67** (89.8 mg, 0.247 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic N-acetylglucosaminide **106** was dried under vacuum overnight and then treated with 95% TFA (10 mL for 173.5 mg, 0.041 mmol) at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced

pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **107** was obtained as an off-white solid in 99% yield (102.4 mg, 0.041 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 6H, lysyl γ-CH_2), 1.33 (m, 6H, lysyl δ-CH_2), 1.46 and 1.61 (2m, 6H, lysyl β-CH_2, unequiv.), 1.76 (s, 12H, NAc), 1.90, 1.95, 2.00 (3s, 36H OAc's), 2.36 (t, 2H, J 7.0 Hz, β-alanyl α-CH_2), 3.05 (m, 6H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.30 and 3.37 (2d, 8H, J 14.1 Hz, SCH_2's), 3.60-3.89 (m, 24H, glycyl H's, H-5, H-2), 4.00 (d, 4H, $J_{5,6}$ 10.3 Hz, H-6), 4.14-4.21 (m, 7H, lysyl α-CH, H-6), 4.77 (d, 4H, $J_{1,2}$ 10.3 Hz, H-1), 4.86 (dd, 4H, J 9.7 Hz, H-4), 5.05 (dd, 4H, H-3). 7.80-8.20 (m, NH's); ^{13}C-NMR (DMSO-d_6) δ 20.3, 20.4, 20.5 (OAc's), 22.6 (NAc), 22.8 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 32.6 (SC), 33.7 (β-alanyl α-C), 34.9 (β-alanyl β-C), 38.4 (lysyl ε-C), 42.0, 42.4 (glycyl C's), 52.0 (C-2), 52.5 (lysyl α-C), 61.9 (C-6), 68.5 (C-4), 73.5 (C-3), 74.7 (C-5), 82.8 (C-1), 168.9-170.1 (C=O's); FAB-MS (pos.) calcd. for $C_{101}H_{151}N_{19}O_{49}S_4$ 2543.6; found 2544.3 (M^+ + 1, 0.5%).

Fully deprotected tetravalent N-acetamidoglucosaminylated dendrimer (108).

A solution of the tetravalent dendritic N-acetylglucosaminide **107** (27.9 mg, 0.011 mmol) was dissolved in MeOH (5 mL) and 10% 1 M NaOMe/MeOH was added. The solution was stirred at room temperature for 1 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **108** was obtained as a white, lyophilized powder in 97% yield (20.0 mg, 0.011 mmol); ^{1}H-NMR (D_2O) δ 1.42 (m, 6H, lysyl γ-CH_2), 1.64 (m, 6H, lysyl δ-CH_2), 1.78, 1.84 (2m, 6H, lysyl β-CH_2, unequiv.), 2.09 (s, 12H, NAc), 2.69 (t, 2H, J 6.5 Hz, β-alanyl α-CH_2), 3.34 (m, 6H, lysyl ε-CH_2), 3.50-4.05 (m, 78H, peptide backbone and GlcNAc residues excluding those listed, NH's, CO_2H), 4.21, 4.25 (2m, 3H, lysyl α-CH), 4.74 (dd, 4H, H-1); ^{13}C-NMR (D_2O) δ 21.7 (NAc), 21.8 (lysyl γ-C), 27.4 (lysyl δ-C), 30.1, 30.2 (lysyl β-C), 32.9 (SC), 32.9 (β-alanyl α-C), 34.7 (β-alanyl β-C), 38.5, 38.6 (lysyl ε-C), 41.9, 42.1,

42.4 (glycyl C's), 53.3, 53.4 (lysyl α-C), 54.0, 60.4, 69.2, 74.5, 79.5 (C-2, C-3, C-4, C-5, C-6) 83.5 (C-1), 170.5-175.4 (C=O's).

Tetravalent lactosylated dendrimer (110).

Polymer-bound tetravalent N-chloroacetylated dendrimer **79** (0.014 mmol, see above) was washed with DMF (5 × 1 min). Thiolated lactose derivative **68** (43.2 mg, 0.066 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic lactoside **109** was dried under vacuum overnight and then treated with 95% TFA (10 mL for 74.8 mg, 0.014 mmol) at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **110** was obtained as an off-white solid in 84% yield (42.6 mg, 0.012 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.20 (m, 6H, lysyl γ-CH_2), 1.36 (m, 6H, lysyl δ-CH_2), 1.47 and 1.60 (2m, 6H, lysyl β-CH_2, unequiv.), 1.88, 1.96, 2 × 1.99 (6s, 84H, OAc's), 2.36 (t, 2H, J 6.9 Hz, β-alanyl α-CH_2), 2.99 (m, 6H, lysyl ε-CH_2), 3.22 (m, 2H, β-alanyl β-CH_2), 3.29 and 3.37 (2d, 8H, J 14.1 Hz, SCH_2's), 3.60-3.82 (m, 24H, glycyl CH_2's, H-4, H-5), 4.00 (m, 12H, H-6a, H-6'), 4.13 (m, 3H, lysyl α-CH), 4.21 (ddd, 4H, H-5'), 4.28 (dd, 4H, H-6b), 4.71-4.84 (m, 12H, H-1, H-2, H-2'), 4.89 (d, 4H, $J_{1,2}$ 10.1 Hz, H-1), 5.12-5.16 (m, 8H, H-3, H-3'), 5.21 (dd, 4H, H-4'), 7.74-8.20 (m, NH's); ^{13}C-NMR (DMSO-d_6) δ 20.2, 20.3, 2 × 20.4, 20.6 (OAc's), 22.7 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 32.6 (SC), 33.7 (β-alanyl α-C), 34.8 (β-alanyl β-C), 38.4 (lysyl ε-C), 42.0, 42.4 (glycyl C's), 52.5 (lysyl α-C), 60.9 (C-6'), 62.4 (C-6), 67.1 (C-4'), 68.9, 69.7 (C-2, C-2'), 70.0, 75.6 (C-3, C-3'), 73.2 (C-5'), 75.6, 76.0 (C-4, C-5), 81.2 (C-1), 99.9 (C-1'), 168.4-172.6 (C=O's).

Fully deprotected tetravalent lactosylated dendrimer (111).

A solution of the tetravalent dendritic lactose **110** (31.5 mg, 0.0089 mmol) was dissolved in MeOH (5 mL) and 10% 1 M NaOMe/MeOH was added. The solution was stirred at room temperature for 1 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **111** was obtained as a white, lyophilized powder in quantitative yield (22.6 mg, 0.0090 mmol); ^{1}H-NMR (D_2O) δ 1.39 (m, 6H, lysyl γ-CH_2), 1.57 (m, 6H, lysyl δ-CH_2), 1.78, 1.84 (2m, 6H, lysyl β-CH_2, unequiv.), 2.66 (t, 2H, J 6.5 Hz, β-alanyl α-CH_2), 3.27 (m, 6H, lysyl ε-CH_2), 3.50-4.20 (m, 86H, peptide backbone and Lac residues excluding those listed, NH's, CO_2H), 4.20 (2m, 3H, lysyl α-CH), 4.51 (d, 4H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.67 (d, 4H, $J_{1,2}$ 9.9 Hz, H-1); ^{13}C-NMR (D_2O) δ 21.9 (lysyl γ-C), 27.3 (lysyl δ-C), 30.0 (lysyl β-C), 33.0 (2 × SC), 32.5 (β-alanyl α-C), 38.2 (β-alanyl β-C), 38.6 (lysyl ε-C), 41.9, 42.0, 42.4 (glycyl C's), 53.4 (lysyl α-C), 59.7, 60.6, 68.1, 70.6, 71.4, 72.1, 74.9, 75.2, 77.5, 78.2 (C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6, C-6'), 84.4 (C-1), 102.4 (C-1'), 170.5-175.5 (C=O's).

Octavalent N-acetamidoglucosaminylated dendrimer (113).

Polymer-bound octavalent N-chloroacetylated dendrimer **81** (0.021 mmol, see above) was washed with DMF (5 × 1 min). Thiolated N-acetylglucosamine derivative **67** (89.8 mg, 0.247 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic N-acetylglucosaminide **112** was dried under vacuum overnight and then treated with 95% TFA (10 mL for 141.2 mg, 0.021 mmol) at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **113** was obtained as an off-white solid in 74% yield (78.0 mg, 0.015 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 14H, lysyl γ-CH_2), 1.33 (m, 14H, lysyl δ-CH_2), 1.46 and

1.61 (2m, 14H, lysyl β-CH_2, unequiv.), 1.76 (s, 24H, NAc), 1.90, 1.95, 2.00 (3s, 72H OAc's), 2.36 (m, 2H, β-alanyl α-CH_2), 3.00 (m, 14H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.30 and 3.37 (2d, 16H, J 14.1 Hz, SCH_2's), 3.60-3.89 (m, 48H, glycyl H's, H-5, H-2), 4.00 (d, 8H, $J_{5,6}$ 10.3 Hz, H-6), 4.14-4.21 (m, 25H, lysyl α-CH, H-6), 4.77 (d, 8H, $J_{1,2}$ 10.3 Hz, H-1), 4.86 (dd, 8H, J 9.7 Hz, H-4), 5.05 (dd, 8H, H-3). 7.80-8.20 (m, NH's); ^{13}C-NMR (DMSO-d_6) δ 2 × 20.3, 20.4 (OAc's), 22.6 (NAc), 22.8 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 32.5 (SC), 38.4 (lysyl ε-C), 41.9, 42.4 (glycyl C's), 52.0 (C-2), 52.5 (lysyl α-C), 61.9 (C-6), 68.4 (C-4), 73.5 (C-3), 74.6 (C-5), 82.7 (C-1), 168.3-170.0 (C=O's).

Fully deprotected octavalent N-acetamidoglucosaminylated dendrimer (114).

A solution of the octavalent dendritic N-acetylglucosaminide **113** (43.0 mg, 0.0084 mmol) was dissolved in MeOH (5 mL) and 10% 1 M NaOMe/MeOH was added. The solution was stirred at room temperature for 1 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **114** was obtained as a white, lyophilized powder in 99% yield (32.6 mg, 0.0083 mmol); ^{1}H-NMR (D_2O) δ 1.42 (m, 14H, lysyl γ-CH_2), 1.64 (m, 14H, lysyl δ-CH_2), 1.78, 1.84 (2m, 14H, lysyl β-CH_2, unequiv.), 2.11 (s, 24H, NAc), 2.69 (t, 2H, J 6.5 Hz, β-alanyl α-CH_2), 3.34 (m, 14H, lysyl ε-CH_2), 3.50-4.05 (m, 106H, peptide backbone and GlcNAc residues excluding those listed, NH's, CO_2H), 4.21, 4.25 (2m, 7H, lysyl α-CH), 4.74 (dd, 8H, H-1); ^{13}C-NMR (D_2O) δ 21.7 (NAc), 21.9 (lysyl γ-C), 27.4 (lysyl δ-C), 30.2 (lysyl β-C), 32.9 (SC), 38.5 (lysyl ε-C), 42.1, 42.4 (glycyl C's), 53.6 (lysyl α-C), 54.0, 60.4, 69.2, 74.5, 79.4 (C-2, C-3, C-4, C-5, C-6) 83.5 (C-1), 170.5-174.0 (C=O's).

Octavalent lactosylated dendrimer (116).

Polymer-bound octavalent N-chloroacetylated dendrimer **81** (0.015 mmol, see above) was washed with DMF (5 × 1 min). Thiolated lactose derivative **68** (93.9 mg, 0.144 mmol) dissolved in 1% Et_3N/DMF was added to the resin which was agitated by

bubbling N_2 at room temperature overnight (≈16 h). The resin was drained and washed with DMF (5 × 1 min). Resin-bound dendritic lactoside **115** was dried under vacuum overnight and then treated with 95% TFA (10 mL for 99.0 mg, 0.015 mmol) at room temperature for 1.5 h. The filtrate was collected and the resin beads rinsed three times with neat TFA. The combined filtrates were evaporated under reduced pressure. The residue was then dissolved in the minimum amount of neat TFA (<500 μL) and precipitated with ether. The precipitate was collected and dried under vacuum. Title compound **116** was obtained as an off-white solid in 65% yield (72.8 mg, 0.010 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.20 (m, 14H, lysyl γ-CH_2), 1.36 (m, 14H, lysyl δ-CH_2), 1.47 and 1.60 (2m, 14H, lysyl β-CH_2, unequiv.), 1.88, 1.96, 2 × 1.99 (6s, 168H, OAc's), 2.36 (m, 2H, β-alanyl α-CH_2), 3.01 (m, 14H, lysyl ε-CH_2), 3.22 (m, 2H, β-alanyl β-CH_2), 3.29 and 3.37 (2d, 16H, J 14.1 Hz, SCH_2's), 3.60-3.82 (m, 48H, glycyl CH_2's, H-4, H-5), 4.00 (m, 24H, H-6a, H-6'), 4.13 (m, 7H, lysyl α-CH), 4.21 (ddd, 8H, H-5'), 4.28 (dd, 8H, H-6b), 4.71-4.84 (m, 24H, H-1, H-2, H-2'), 4.89 (d, 8H, $J_{1,2}$ 10.1 Hz, H-1), 5.12-5.16 (m, 16H, H-3, H-3'), 5.21 (dd, 8H, H-4'), 7.70-8.20 (m, NH's); ^{13}C-NMR (DMSO-d_6) δ 20.2, 20.3, 2 × 20.4, 20.6 (OAc's), 22.7 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 32.6 (SC), 38.4 (lysyl ε-C), 42.0, 42.4 (glycyl C's), 52.5 (lysyl α-C), 60.9 (C-6'), 62.4 (C-6), 67.1 (C-4'), 68.9, 69.7 (C-2, C-2'), 70.0, 75.6 (C-3, C-3'), 73.2 (C-5'), 75.6, 76.0 (C-4, C-5), 81.2 (C-1), 99.9 (C-1'), 168.4-170.3 (C=O's).

Fully deprotected octavalent lactosylated dendrimer (117).

A solution of the octavalent dendritic lactose **116** (81.1 mg, 0.011 mmol) was dissolved in MeOH (5 mL) and 10% 1 M NaOMe/MeOH was added. The solution was stirred at room temperature for 1 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **105** was obtained as a white, lyophilized powder in 95% yield (52.8 mg, 0.010 mmol); ^{1}H-NMR (D_2O) δ 1.39 (m, 14H, lysyl γ-CH_2), 1.57 (m, 14H, lysyl δ-CH_2), 1.78, 1.84 (2m, 14H, lysyl β-CH_2, unequiv.), 2.66 (m, 2H, β-alanyl α-CH_2), 3.27 (m, 14H, lysyl ε-CH_2), 3.50-4.20 (m,

186H, peptide backbone and Lac residues excluding those listed, NH's, CO_2H), 4.33 (m, 7H, lysyl α-CH), 4.51 (d, 8H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.67 (d, 8H, $J_{1,2}$ 9.9 Hz, H-1); ^{13}C-NMR (D_2O) δ 21.9 (lysyl γ-C), 27.3 (lysyl δ-C), 30.0 (lysyl β-C), 33.0 (2 × SC), 38.6 (lysyl ε-C), 41.9, 42.0, 42.4 (glycyl C's), 53.4 (lysyl α-C), 59.7, 60.6, 68.1, 70.6, 71.4, 72.1, 74.9, 75.2, 77.5, 78.2 (C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6, C-6'), 84.4 (C-1), 102.4 (C-1'), 170.5-175.5 (C=O's).

Double immunodiffusion assay between WGA and N-acetylglucosaminylated dendrimers 102, 108, and 114.

Agar gel diffusion experiments were performed in 1% agarose containing 2% poly(ethyleneglycol) (PEG, MW 8,000) in PBS buffer. A glass plate was coated with 1% agarose containing 2% PEG in PBS. A central well and four surrounding wells were cut in the gel. Wheat germ agglutinin (WGA, 20 μL of a 1 mg/mL solution in PBS) was used to fill the center well. Outer wells were filled with GlcNAc-based dendrimers (20 μL of 2 mg/mL solutions of each of **102**, **108**, and **114**) and a negative control polyacrylamide (MW 100,000, 20 μL of a 2 mg/mL solution in PBS). Precipitin bands between WGA and neoglycoconjugates **102**, **108**, and **114** and polyacrylamide were allowed to form overnight at 4 °C.

Double immunodiffusion assay between *Arachis hypogaea* lectin (peanut lectin) and lactosylated dendrimers 105, 111, and 117.

Agar gel diffusion experiments were performed in 1% agarose containing 2% poly(ethyleneglycol) (PEG, MW 8,000) in PBS buffer. A glass plate was coated with 1% agarose containing 2% PEG in PBS. A central well and four surrounding wells were cut in the gel. Peanut lectin (20 μL of a 1 mg/mL solution in PBS) was used to fill the center well. Outer wells were filled with Lac-based dendrimers (20 μL of 2 mg/mL solutions of each of **105**, **111**, and **117**) and a negative control polyacrylamide (MW 100,000, 20 μL

of a 2 mg/mL solution in PBS). Precipitin bands between WGA and neoglycoconjugates **105**, **111**, and **117** and polyacrylamide were allowed to form overnight at 4 °C.

Turbidimetric analysis between WGA and octavalent N-acetylglucosaminylated dendrimer 114.

Tubidimetry experiments were performed in Linbro (Titertek) microtitration plates where 50 μL/well of stock lectin solutions prepared from WGA (1 mg/mL in PBS) were mixed with 50 μL of a stock solution of glycodendrimer **114** (0.5 mg/mL in PBS) and incubated at room temperature for 3 h. Turbidity of the solutions was monitored by reading the optical density (O. D.) at 490 nm at regular time intervals until no noticeable changes could be observed. Each test was performed in triplicate.

Competitive inhibition ELLA using porcine stomach mucin type III and N-acetylglucosaminylated dendrimers 102, 108, and 114 as inhibitors.

Linbro (Titertek) microtitration plates were coated with porcine stomach mucin type III at 100 μL/well of a stock solution of 5 μg/mL in 0.01 M phosphate buffer (pH 7.3). The wells were then washed three times with 300 μL/well of 0.01 M phosphate buffer (pH 7.3) containing 0.05% (v/v) Tween 20 (PBST). Washing with PBST was repeated after each incubation period. Wells were then blocked with 150 μL/well of 1% BSA/PBS for one hour at 37 °C. After washing, wells were filled with 100 μL/well of inhibitor solutions and incubated again at 37 °C for one hour. Inhibitors used include allyl 2-acetamido-2-deoxy-α-D-glucopyranoside[168] as reference monovalent compound, di- (**102**), tetra- (**108**), and octa- (**114**) valent GlcNAc dendrimers. Each inhibitor was added in serial two-fold dilutions (60 μL/well) in PBS with the appropriate lectin-enzyme conjugate concentration (1000-fold dilution of a 1 mg/mL stock WGA solution in PBS) on Nunclon (Delta) microtiter plates and incubated at 37 °C for one hour. These inhibitor solutions (100 μL) were transferred to the antigen-coated plates and incubated for a second hour at 37 °C. The plates were washed and 50 μL/well of 2,2'-azinobis(3-

ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg/4 mL) in citrate-phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 minutes by adding 50 μL/well of 1 M H_2SO_4 and the optical density was measured at 410 nm relative to 570 nm. Per cent inhibition was calculated as follows:

$$\% \text{ inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})})/A_{(\text{no inhibitor})} \times 100$$

IC_{50}'s were reported as the concentration required for 50% inhibition of the coating antigen. All tests were performed in triplicate.

Chapter 4. Synthesis of 3,3'-Iminobis(propylamine)-Based Glycodendrimers

4.1. Introduction

Chapter 3 demonstrated that sialylated dendrimers scaffolded onto multi-branched L-lysine afforded conjugates that were as potent as polymers in the inhibition of the hemagglutination of human erythrocytes by *Influenza* viruses.[105,106] However, the lack of symmetry within the poly-L-lysine dendrimers previously reported have rendered high field proton NMR spectral characterization rather cumbersome. Symmetrical sialodendrimers having chemically equivalent thiosialoside residues represent novel neoglycoconjugates that may be used to systematically explore multivalency, in addition to facilitating NMR spectral characterization.

In addition, a systematic study of the cluster effect and its role in carbohydrate-protein interactions must consider structural organization of carbohydrate residues. It is therefore logical to compare structurally similar divergent and spherical glycodendrimers. Herein is presented a new family of symmetrical dendrimers, both divergent and spherical in structure, with even valencies of between 2 and 16, based on a 3,3'-iminobis(propylamine) core.[113,114]

4.2. 3,3'-Iminobis(propylamine) Core

First, second, third, and fourth generation N-chloroacetylated dendrimers **128**, **132**, **135**, and **138** with valencies of two, four, eight, and sixteen were efficiently and conveniently synthesized in solution[169] using Cbz protecting group strategy and carbodiimide-hydroxybenzotriazolyl (HOBt) coupling chemistry. Key monomeric precursors **126** and **127** were prepared in the following manner.

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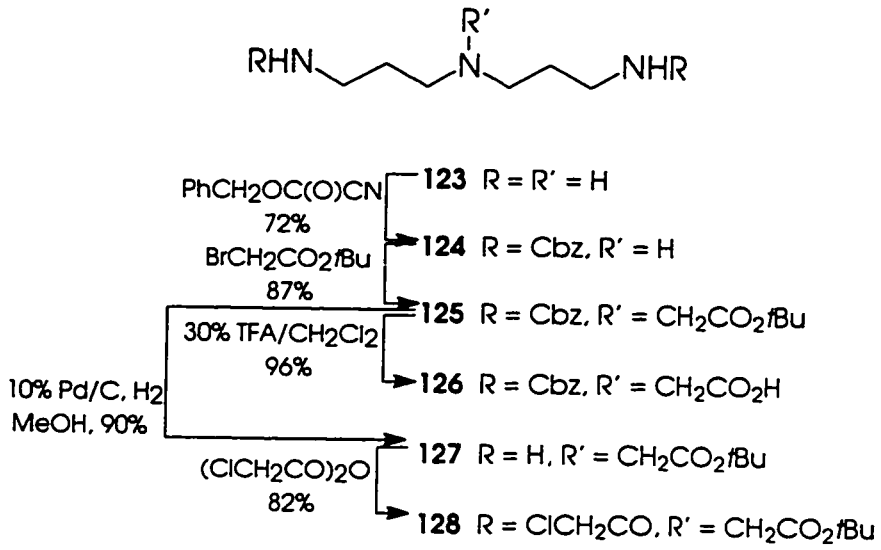
[169] The approach described here is amenable to solid-phase synthesis as described for L-lysine-based glycodendrimers (Chapter 3). Work is in progress to evaluate the efficiency of this approach.

The primary amines of 3,3'-iminobis(propylamine) **123** were regioselectively protected using the method of Murahashi *et al.*[170] to give diamine **124** (benzyl cyanoformate, 2 h, 25 °C, 72% yield). Diamine **124** was then alkylated with *t*-butyl bromoacetate in good yield to provide divalent core structure **125** with Cbz protected amines and *t*-butyl protected acid functionalities (CH_3CN, DIPEA, 30 min., 25 °C, 87% yield). Trifluoroacetolysis of **125** gave acid **126** (30% TFA/CH_2Cl_2, 3h, 25 °C, 96% yield) and hydrogenolysis of the Cbz groups of **125** afforded diamine **127** (10% Pd/C, H_2, MeOH, 30 min., 25 °C, 90% yield) (Scheme 4.2.1). Acid **126** and diamine **127** represent key precursors in the synthesis of the glycodendrimers described here.



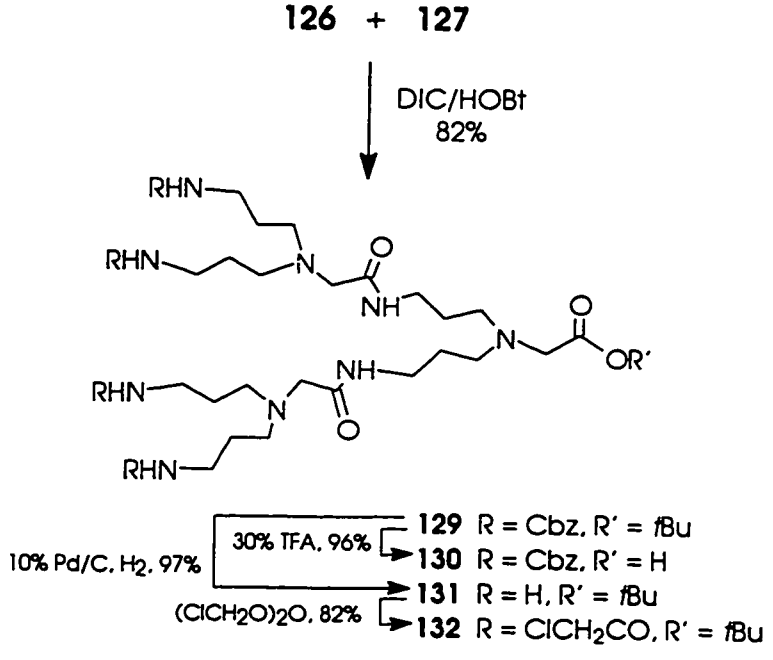
Scheme 4.2.1. Synthesis of key monomeric precursors for 3,3'-iminobis(propylamine) dendritic core.

Compounds **126** and **127** were coupled using diisopropylcarbodiimide (DIC) and HOBt to provide tetravalent Cbz-protected dendrimer **129** (DIC, HOBt, DIPEA, CH_3CN, 3 h, 25 °C). In order to avoid tedious silica gel chromatography, it was found that the

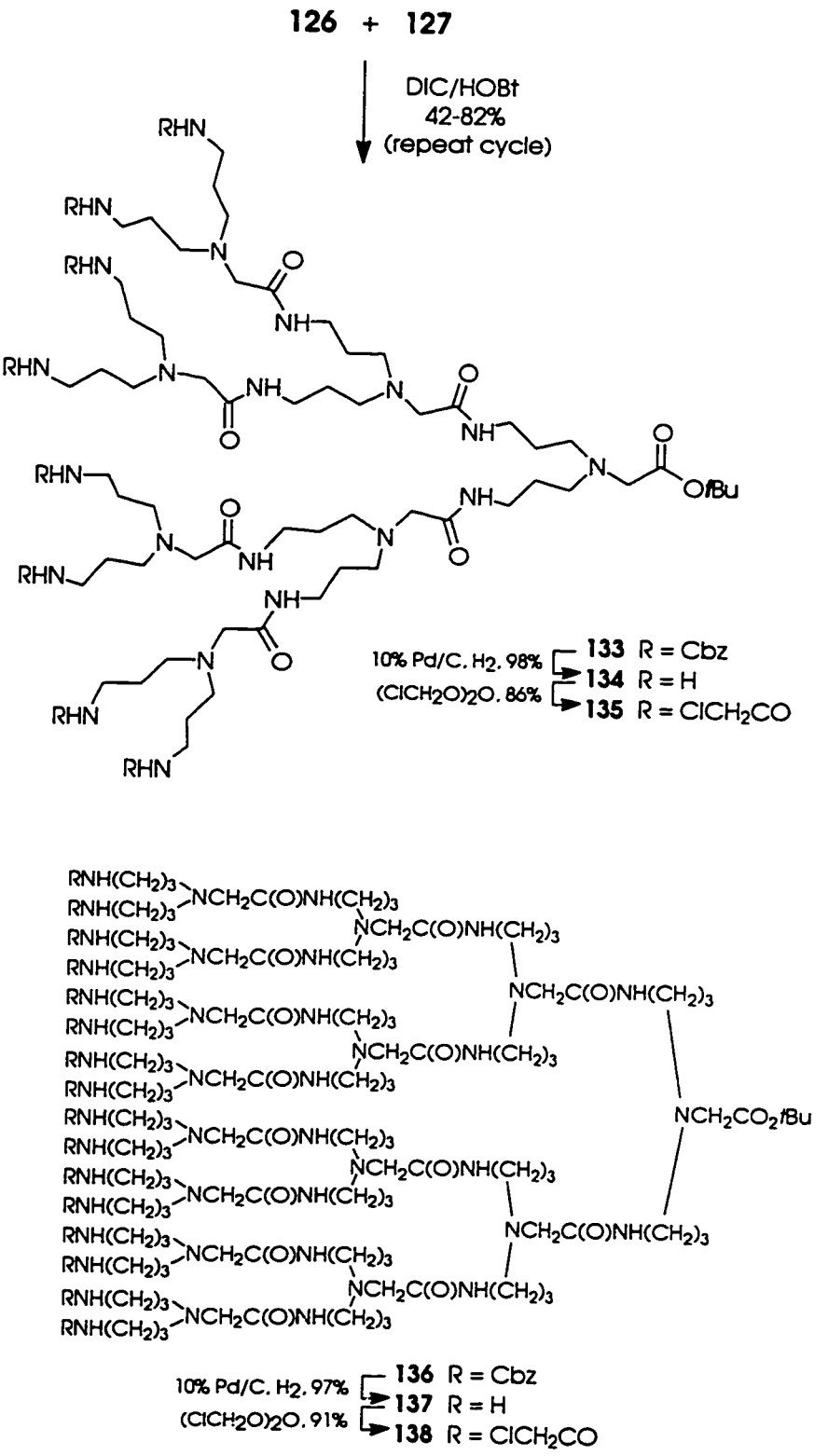
[170] Murahashi, S. -I.; Naota, T.; Nakajima, N. *Chem. Lett.* **1987**, 879.

crude mixture containing **129** could be treated with anionic resin (HO^-, Amberlite IRA-400) thereby eliminating HOBt and excess acid used in the coupling. The residue was then concentrated and diisopropylcarbodiimide urea and **129** were easily separated by silica gel chromatography to afford **129** in 82% yield. Tetravalent dendrimer **129** was deprotected to give tetraamine **131** (10% Pd/C, H_2, MeOH, 4h, 97% yield) which was coupled to acid **126** using the strategy described above to give Cbz-protected octamer **133** in 66% yield ((*i*) DIC, HOBt, DIPEA, DMF, 20 h, 25 °C; (*ii*) HO^- resin treatment, 15 min., 25 °C). Repetition of deprotection procedures gave octaamine **134** (10% Pd/C, H_2, MeOH, 20 h, 98% yield) which was again coupled to acid **126** providing hexadecavalent **136** in a yield of 46% ((*i*) DIC, HOBt, DIPEA, DMSO, 48 h, 25 °C; (*ii*) HO^- treatment, 15 min., 25 °C) (Schemes 4.2.2 and 4.2.3).

All Cbz-protected dendrimers exhibited consistent NMR and mass spectral data which confirmed the purity of dendrimers **129**, **134**, and **136**. Dendrimer characterization was made easy by virtue of their symmetry. ^{1}H-NMR signals were superimposable for



Scheme 4.2.2. Synthesis of N-chloroacetylated 3,3'-iminobis(propylamine) tetramer.



Scheme 4.2.3. Synthesis of N-chloroacetylated 3,3'-iminobis(propylamine) dendrimers.

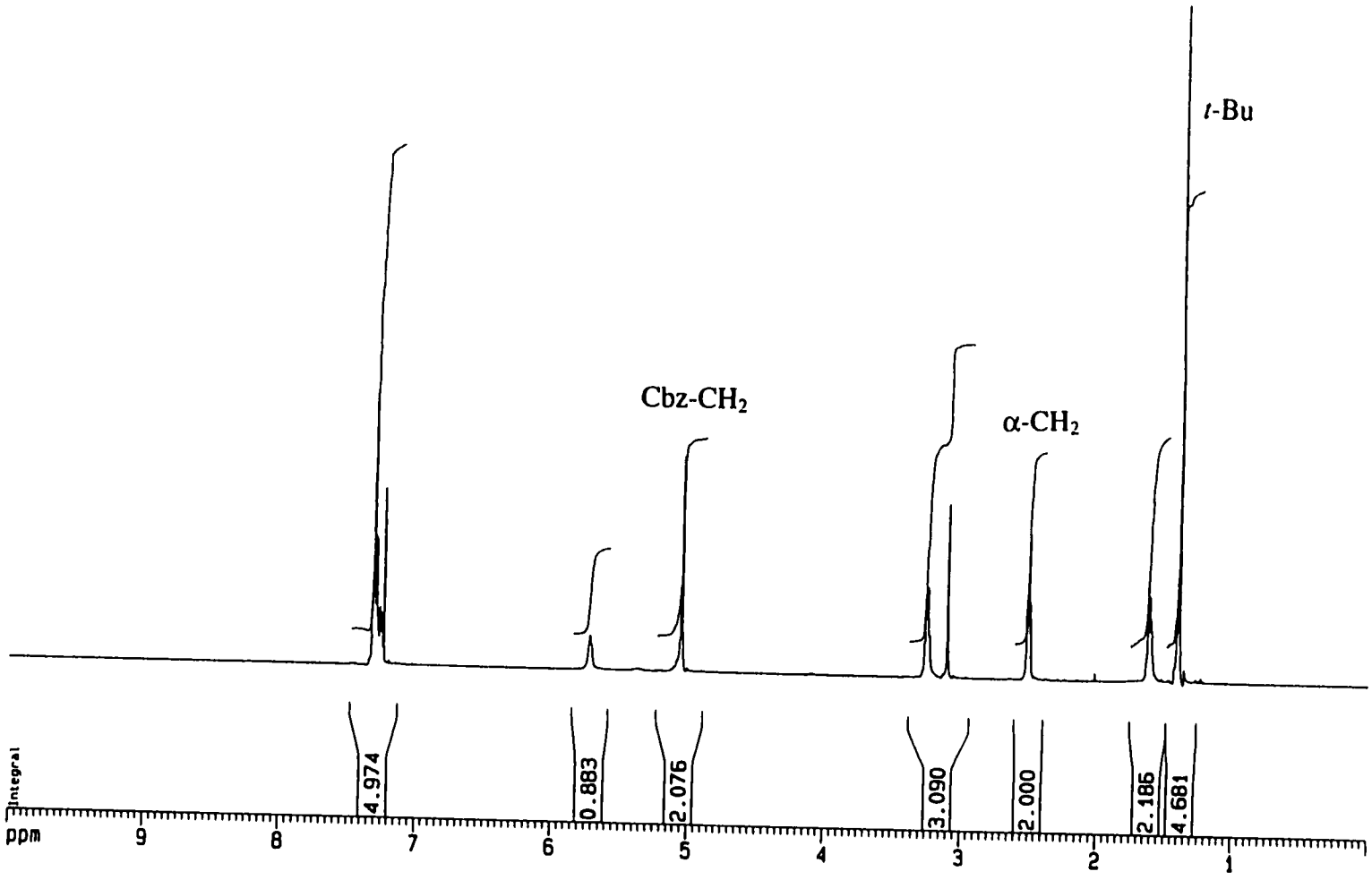
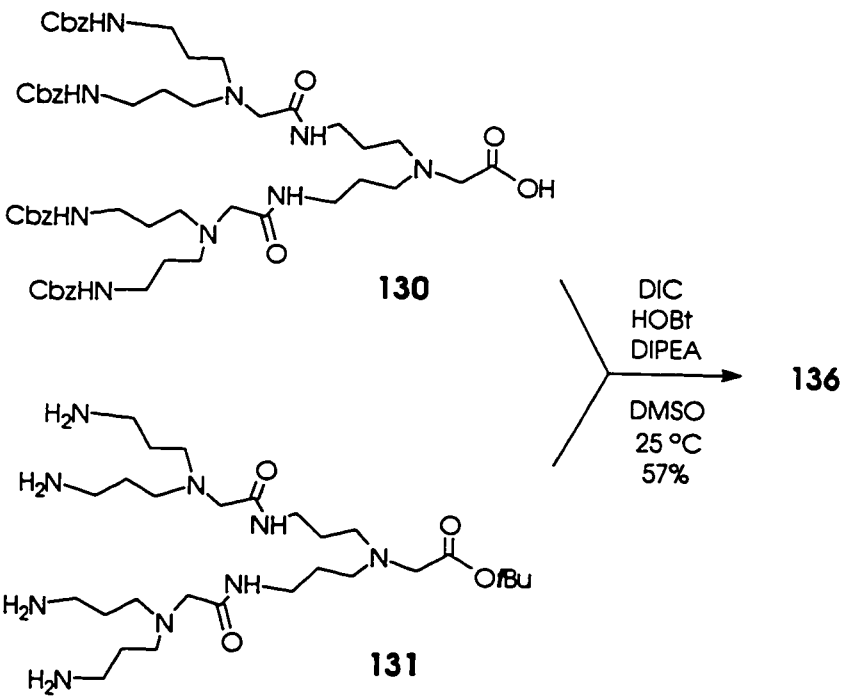


Figure 4.2.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of Cbz-protected divalent 3,3'-iminobis(propylamine)-based dendrimer **125**.

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's, respectively (Figure 4.2.1) Alternatively, octavalent dendrimer **136** could also be prepared more efficiently by the convergent assembly of acid **130** and tetraamine **131** (Scheme 4.2.4). Acid **130** was prepared by deprotection of tetravalent ester **129** with trifluoroacetic acid (30% TFA/CH_2Cl_2, 4 h, 25 °C, quantitative) (Scheme 4.2.2). Using the above coupling techniques ((*i*) DIC, HOBt, DIPEA, DMF, 20 h, 25 °C; (*ii*) HO^- resin treatment, 15 min, 25 °C) **136** was isolated in 57% yield for the two step process . Deprotection (10% Pd/C, MeOH, catalytic AcOH, 20 h, 25 °C) of the Cbz-protected **136** provided hexadecavalent amine **137** in excellent yield (97%).



Scheme 4.2.4. Convergent synthesis of hexadecavalent Cbz-protected 3,3'-iminobis(propylamine) dendrimer **136**.

N-Chloroacetylated di- (**128**), tetra- (**132**), octa- (**135**), and hexadeca- (**138**) valent dendrimers were obtained by treating the corresponding amines (**127**, **131**, **134**, and **137**) with chloroacetic anhydride in non-protic solvents (CH_3CN for **127**, DMF for **131**, and DMSO for **134** and **137**, 2 h, 25 °C). Changes of solvent were necessary as the solubility properties of di- (**127**), tetra- (**131**), octa- (**134**), and hexadeca- (**137**) valent amines were slightly different. Basic alumina chromatography using 20% H_2O/CH_3CN as eluent was used to remove the chloroacetate anions generated. N-Chloroacetylated di- (**128**), tetra- (**132**), octa- (**135**), and hexadeca- (**138**) valent dendrimers were obtained in 82, 82, 86, and 91% yields, respectively. Treating 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.

All NMR spectral data gave consistent results with characteristic ^{1}H-NMR signals at 4.01 ppm ($CDCl_3$ for **128**, DMSO-d_6 for **132**, **135**, and **138**) for the N-chloroacetyl methylene functionality (Figure 4.2.2).

4.3. Tethered 3,3'-Iminobis(propylamine) Core

The approach followed for the syntheses of the spherical dendrimers was based on the tethering of Cbz-protected dimer **126** and tetramer **130** with both hexamethylenediamine **139** and tris-(2-aminoethyl)amine **143** to give spherical dendrimers with valencies of between four and twelve. First and second generation divergent carbobenzyloxy protected dendrimers **126** and **130** with valencies of two and four were efficiently and conveniently synthesized, as described in Section 4.2.

A slight excess of divalent acid **126** was coupled to hexamethylenediamine **139** to give Cbz protected tetravalent spherical dendrimer **140** (DIC, HOBt, DIPEA, 86%) which upon hydrogenolysis afforded amine **141** (10% Pd/C, H_2, 95%). N-Chloroacetylated dendrimer **142** was obtained by treatment of amine **141** with chloroacetic anhydride in

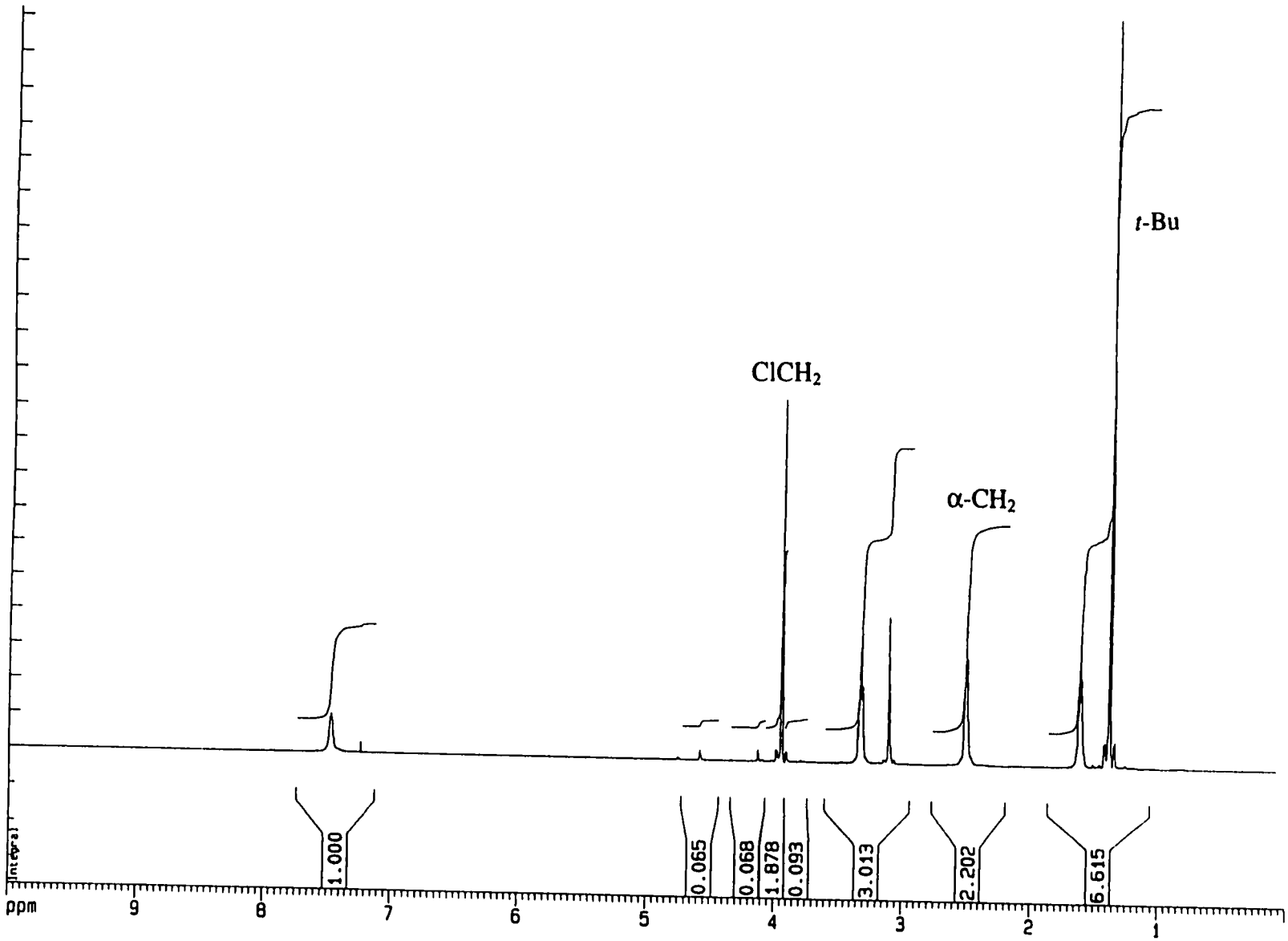
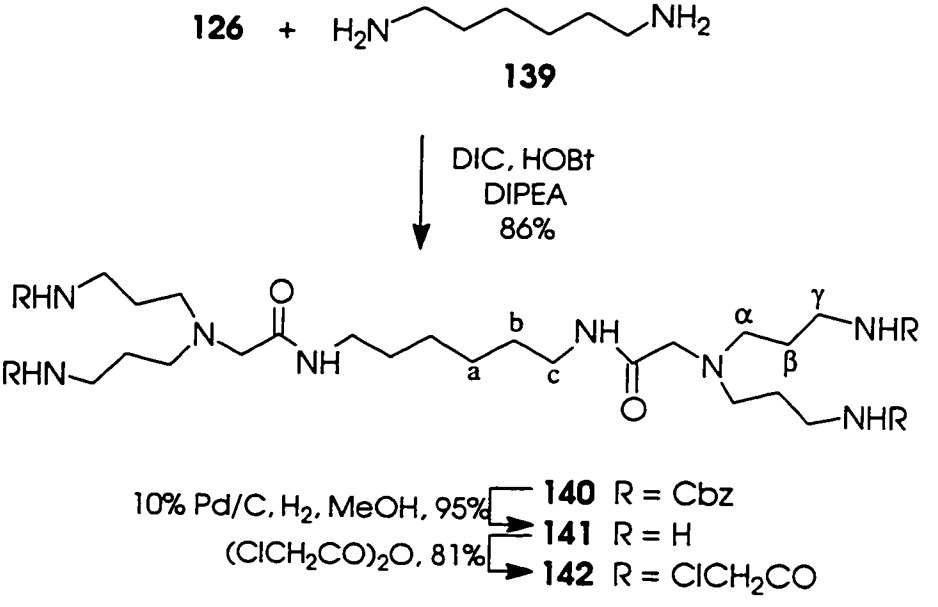
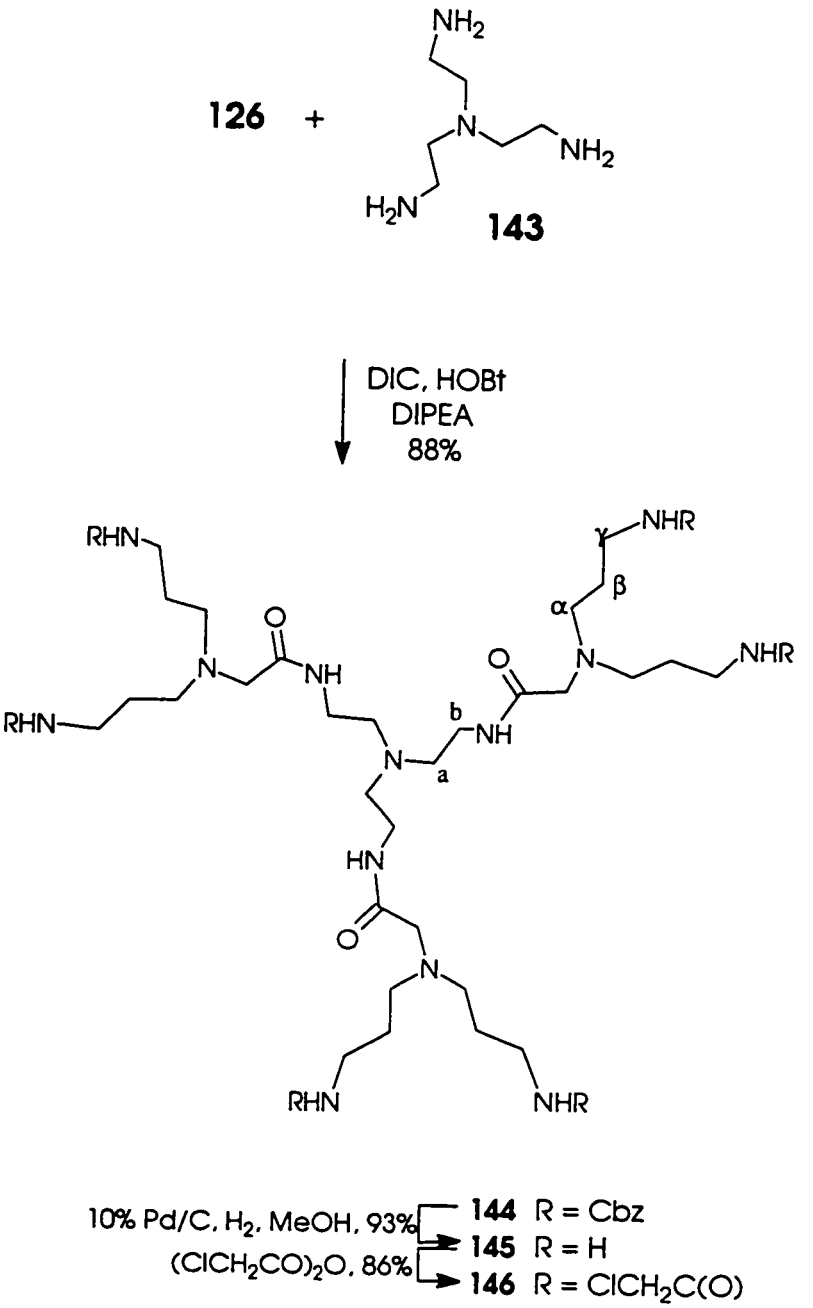


Figure 4.2.2. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of N-chloroacetylated divalent 3,3'-iminobis(propylamine)-based dendrimer **128**.

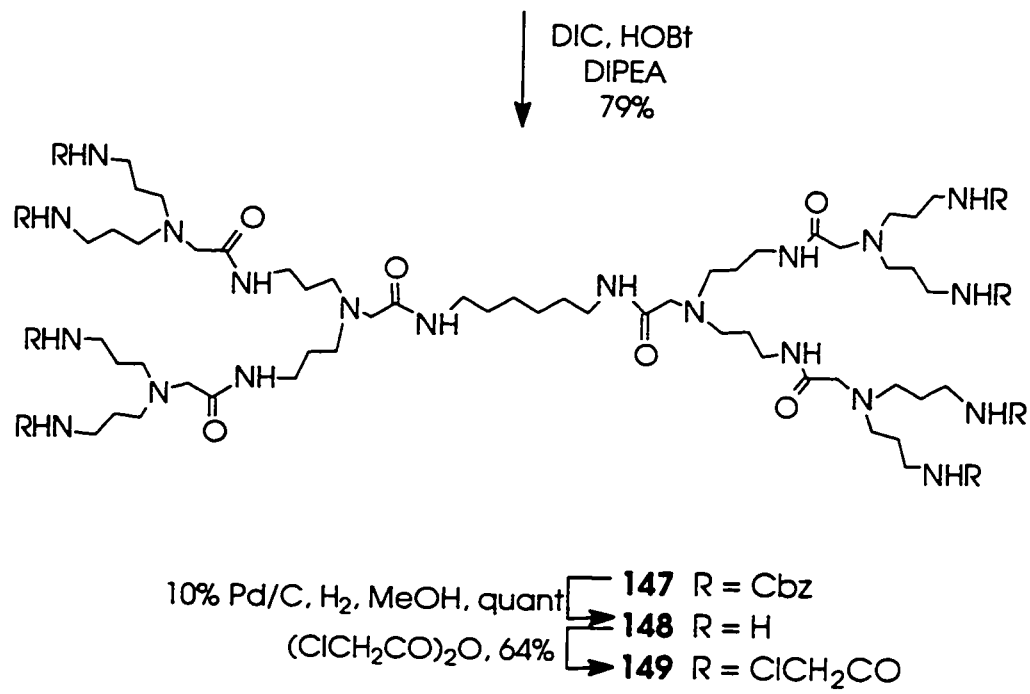
DMSO (81%). Multivalent N-chloroacetylated derivative **142** was too polar to allow easy isolation *via* standard silica gel chromatographic techniques. Instead, the crude mixture containing **142** was subjected to basic alumina column chromatography (20% H_2O/CH_3CN) to remove the chloroacetate anions generated (Scheme 4.3.1).



Scheme 4.3.1. Synthesis of tethered tetravalent 3,3'-iminobis(propylamine) dendrimer.

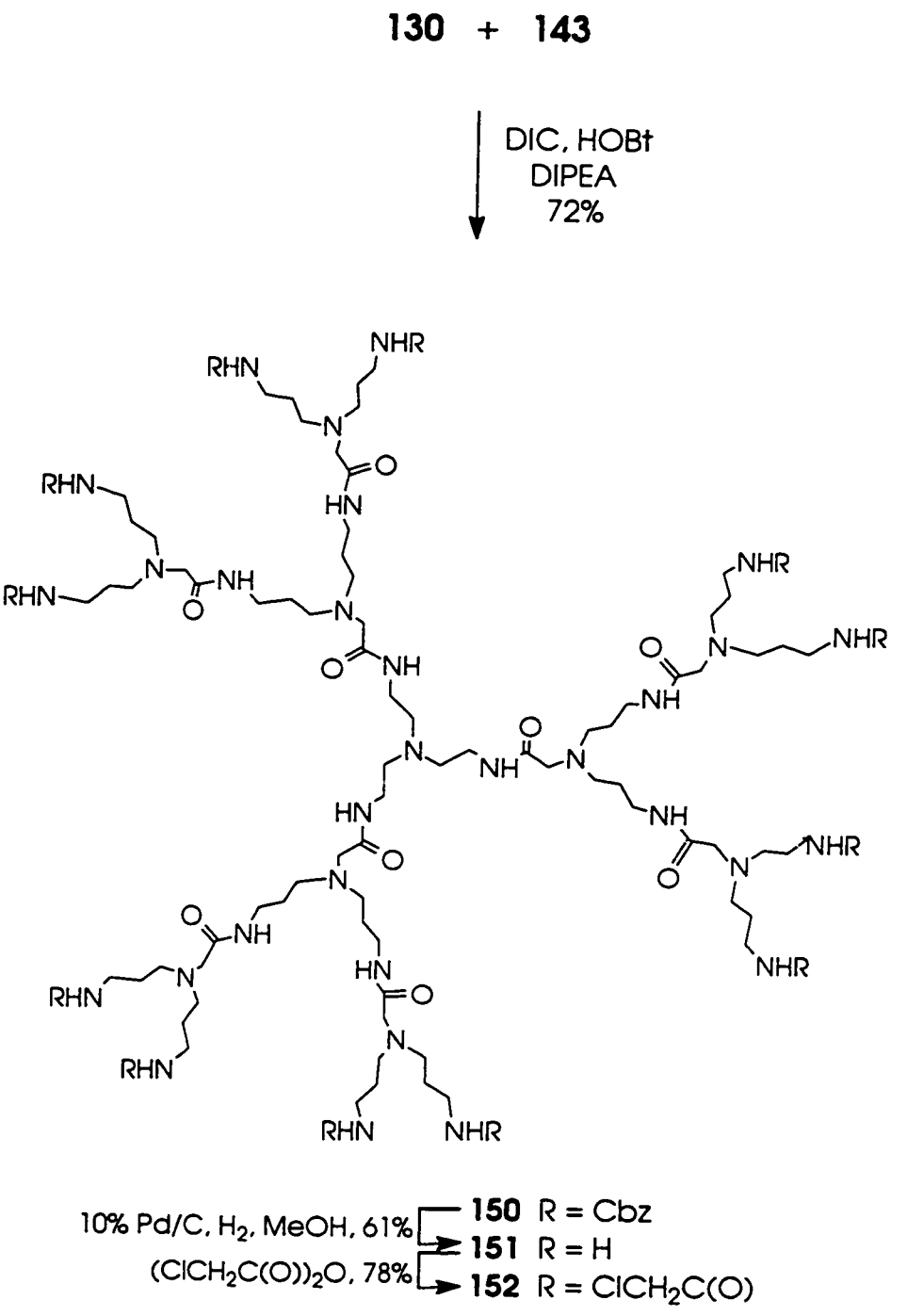


Scheme 4.3.2. Synthesis of tethered hexavalent 3,3'-iminobis(propylamine) dendrimer.



Scheme 4.3.3. Synthesis of tethered octavalent 3,3'-iminobis(propylamine) dendrimer.

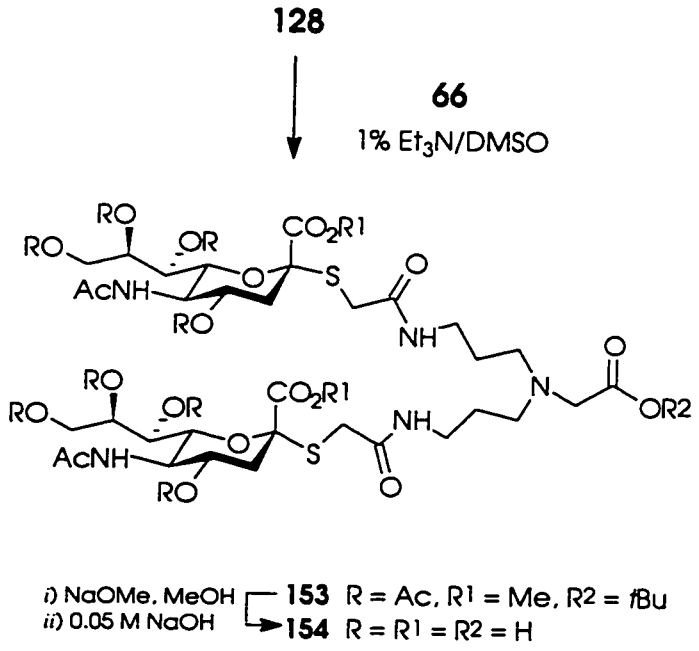
The corresponding N-chloroacetylated tethered hexa- (**146**), octa- (**149**), and dodeca- (**152**) valent dendrimers were generated in a similar manner. Tetravalent acid **130** was coupled to both hexamethylenediamine **139** and tris-(2-aminoethyl)amine **143** and divalent acid **146** was conjugated to tris-(2-aminoethyl)amine **143** to give Cbz-protected octa- (**149**), dodeca- (**152**), and hexa- (**146**) valent dendrimers, respectively (72-88%, Schemes 4.3.2 to 4.3.4). Hydrogenolysis of **144**, **147**, and **150** afforded hexa- (**145**), octa- (**148**), and dodeca-(**151**) amines (Pd/C, H_2, 61-99%). Amines **145**, **148**, and **151** were treated with chloroacetic anhydride in DMSO to generate N-chloroacetylated dendrimers **146**, **149**, and **152** (64-86%) (Schemes 4.3.2 to 4.3.4).



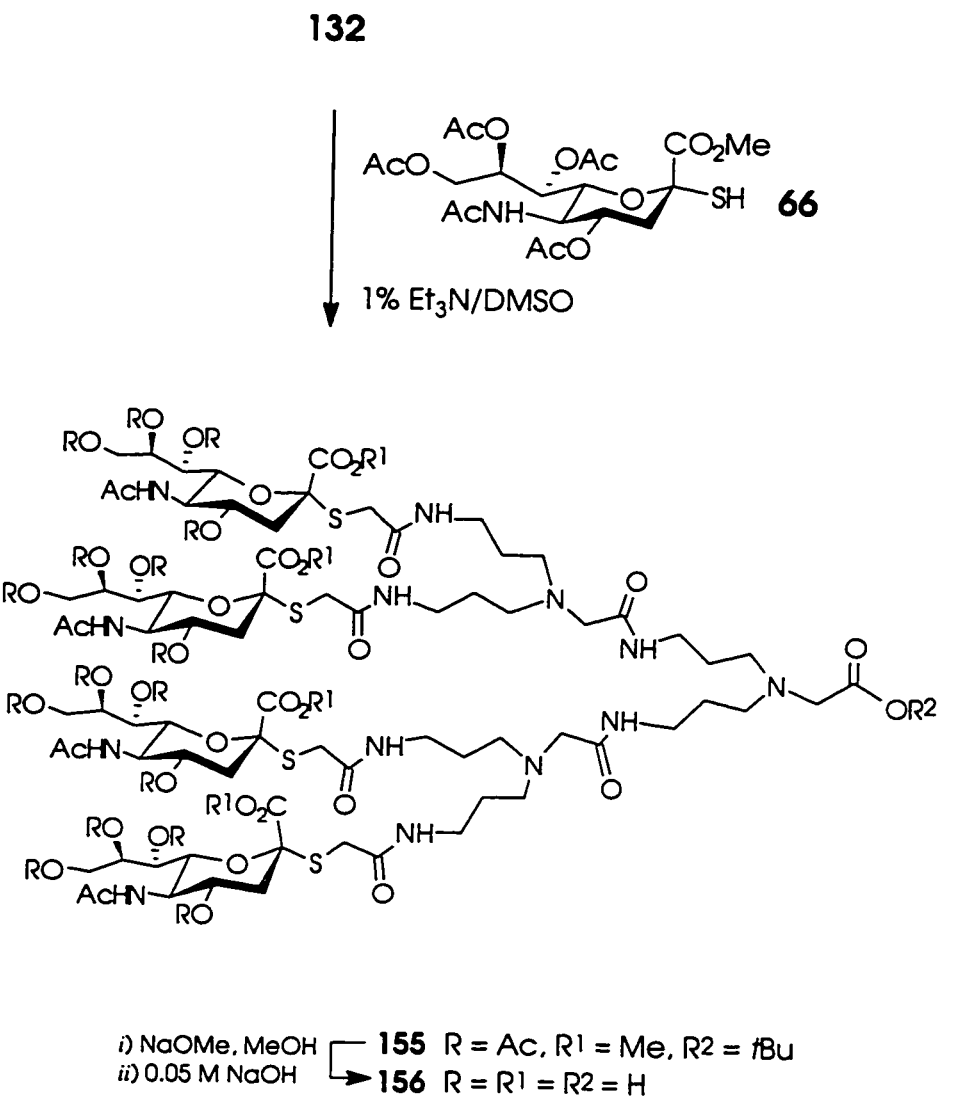
Scheme 4.3.4. Synthesis of tethered dodecavalent 3,3'-iminobis(propylamine) dendrimer.

4.4. Conjugation of Sialic Acid to 3,3'-Iminobis(propylamine) Core

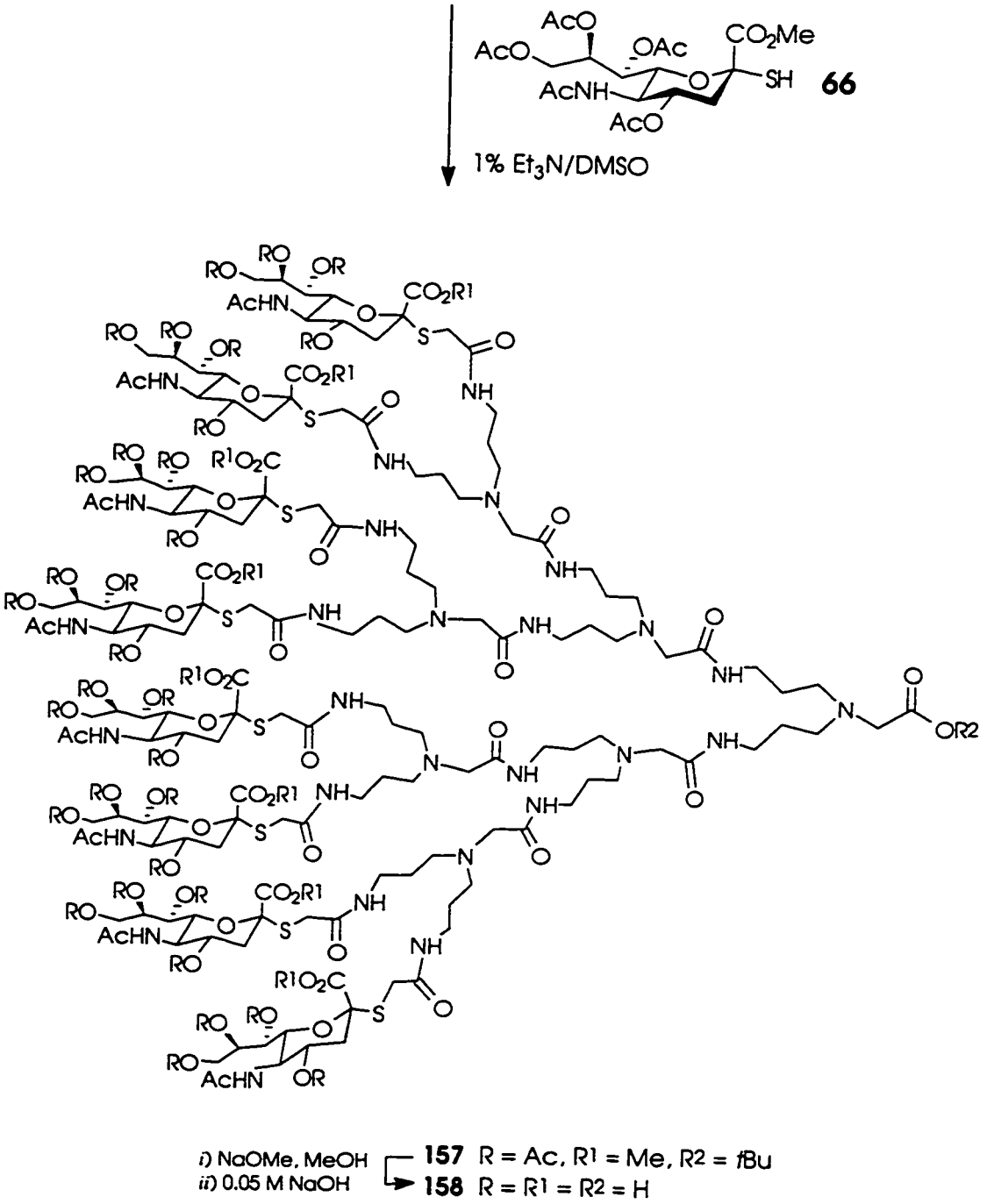
Each dendrimer generation (**128**, **132**, **135**, and **138**) was treated with an excess of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-*D-glycero*-α-*D-galacto*-2-nonulopyranosonate **66** (1.1 equivalents per N-chloroacetyl functionality, 1% Et_3N/DMSO, N_2, 16 h, 25 °C) to provide completely protected glycodendrimers **153**, **155**, **157**, and **159**. DMSO was removed by lyophilization and the residues redissolved in the minimum amount of DMSO and precipitated from ethyl acetate (Schemes 4.4.1 to 4.4.4) to give **153**, **155**, **157**, and **159** in good to excellent yields (96, 93, 84, and 76% respectively). The polarity of the NeuAc end groups dismisses the possibility of purification by silica gel chromatography for glycodendrimers of higher generation. Dendrimers **155**, **157**, and **159** 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 **66** and some disulfide by-product formed during the reaction.



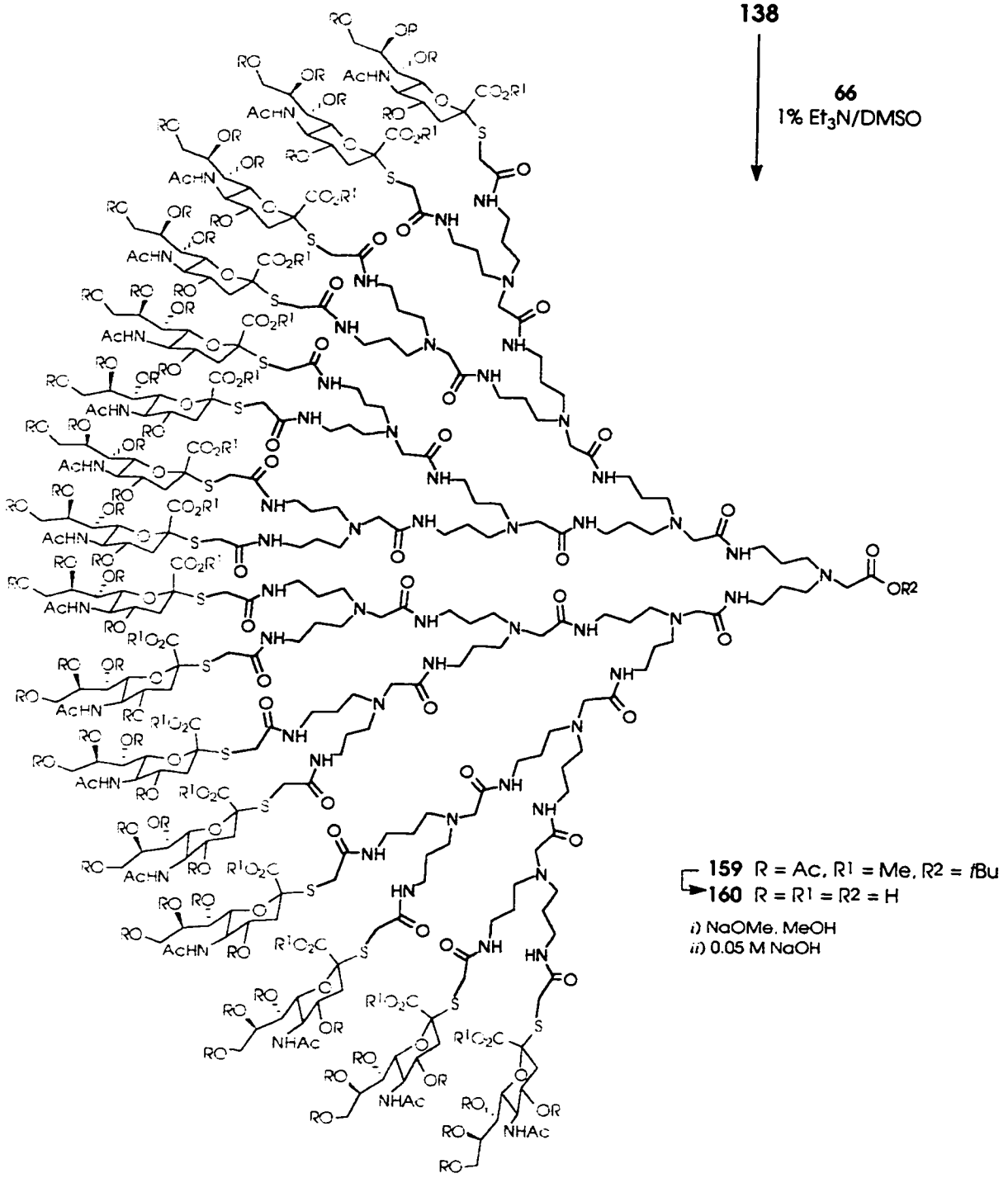
Scheme 4.4.1. Synthesis of divalent 3,3'-iminobis(propylamine) α-thiosialodendrimer.



Scheme 4.4.2. Synthesis of tetravalent 3,3'-iminobis(propylamine) α-thiosialodendrimer.



Scheme 4.4.3. Synthesis of octavalent 3,3'-iminobis(propylamine) α-thiosialodendrimer.



Scheme 4.4.4. Synthesis of hexadecavalent 3,3'-iminobis(propylamine) α-thiosialodendrimer.

Complete coupling was evident upon analysis of ^{1}H-NMR spectral data. The disappearance of the chloroacetyl signal at δ 4.01 ppm ($CDCl_3$ for the di- (**128**), tetra- (**132**), and octa- (**135**) valent dendrimers and DMSO-d_6 for the hexadecamer (**138**)) and the emergence of new signals corresponding to the incorporated NeuAc residues (H-4 at δ 4.83, H-3eq at 2.70, N-Ac at 1.83, and C-2 at 82.3 ppm, $CDCl_3$ for di- (**153**) and tetra- (**155**) valent α-sialosides and DMSO-d_6 for the octa- (**157**) and hexadeca- (**159**) meric protected glycodendrimers) clearly established the extent of sialoside incorporation and revealed the integrity of the α-sialoside linkages (Figure 4.4.1 and 4.4.2).

Deprotection of glycodendrimers **153**, **155**, **157**, and **159** by sequential ester hydrolysis ((*i*) NaOMe/MeOH, 8 h, 25 °C; (*ii*) 0.05 M NaOH, 8 h, 25 °C) followed by gel permeation chromatography (GPC, Biogel-P2, H_2O as eluent) afforded fully deprotected glycodendrimers **154**, **156**, **158**, and **160** with two, four, eight, and sixteen NeuAc residues in moderate yields (47 to 58%). Surprisingly, this two step deprotection strategy was necessary to ensure complete de-O-acetylation. No deprotection was observed under milder conditions of less time nor when Zemplén deprotection was avoided and NaOH treatment performed directly. The ^{1}H-NMR (D_2O) spectrum of neoglycoconjugate **154** is shown in Figure 4.4.3.

4.5. Conjugation of Sialic Acid to Tethered 3,3'-Iminobis(propylamine) Core

Each tethered N-chloroacetylated dendrimer (**142**, **146**, **149**, and **152**) 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*-nonulopyranosonate **66** to provide completely protected spherical α-sialodendrimers **161**, **163**, **165**, and **167** in good yields (83-87%). Dendrimers **161**, **163**, **165**, and **167** were isolated by precipitation from ethyl acetate (Schemes 4.5.1 to 4.5.4).

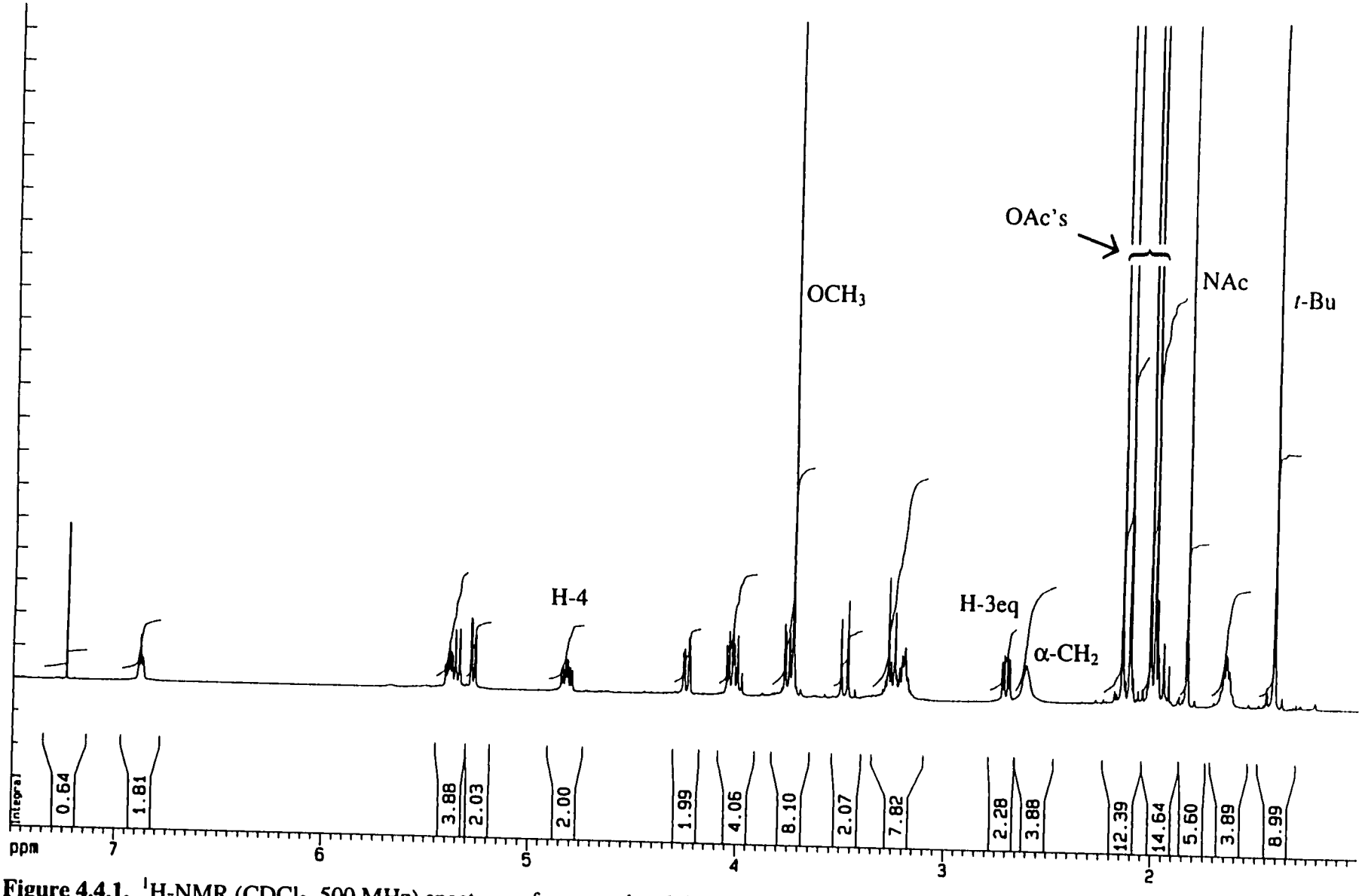


Figure 4.4.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of peracetylated divalent 3,3'-iminobis(propylamine)-based α-thiosialodendrimer **153**.

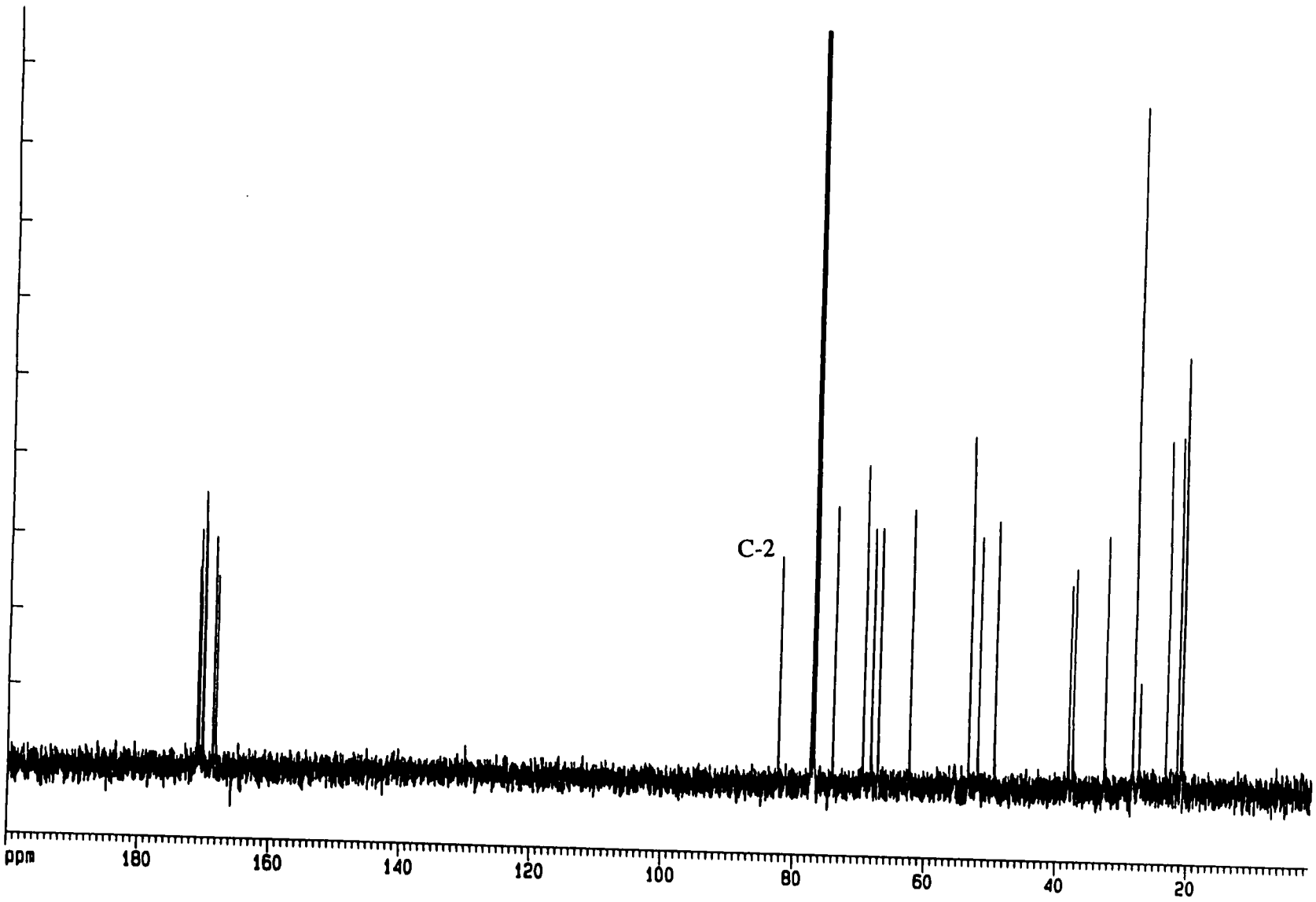


Figure 4.4.2. ^{13}C-NMR ($CDCl_3$, 500 MHz) spectrum of peracetylated divalent 3,3'-iminobis(propylamine)-based α-thiosialodendrimer **153**.

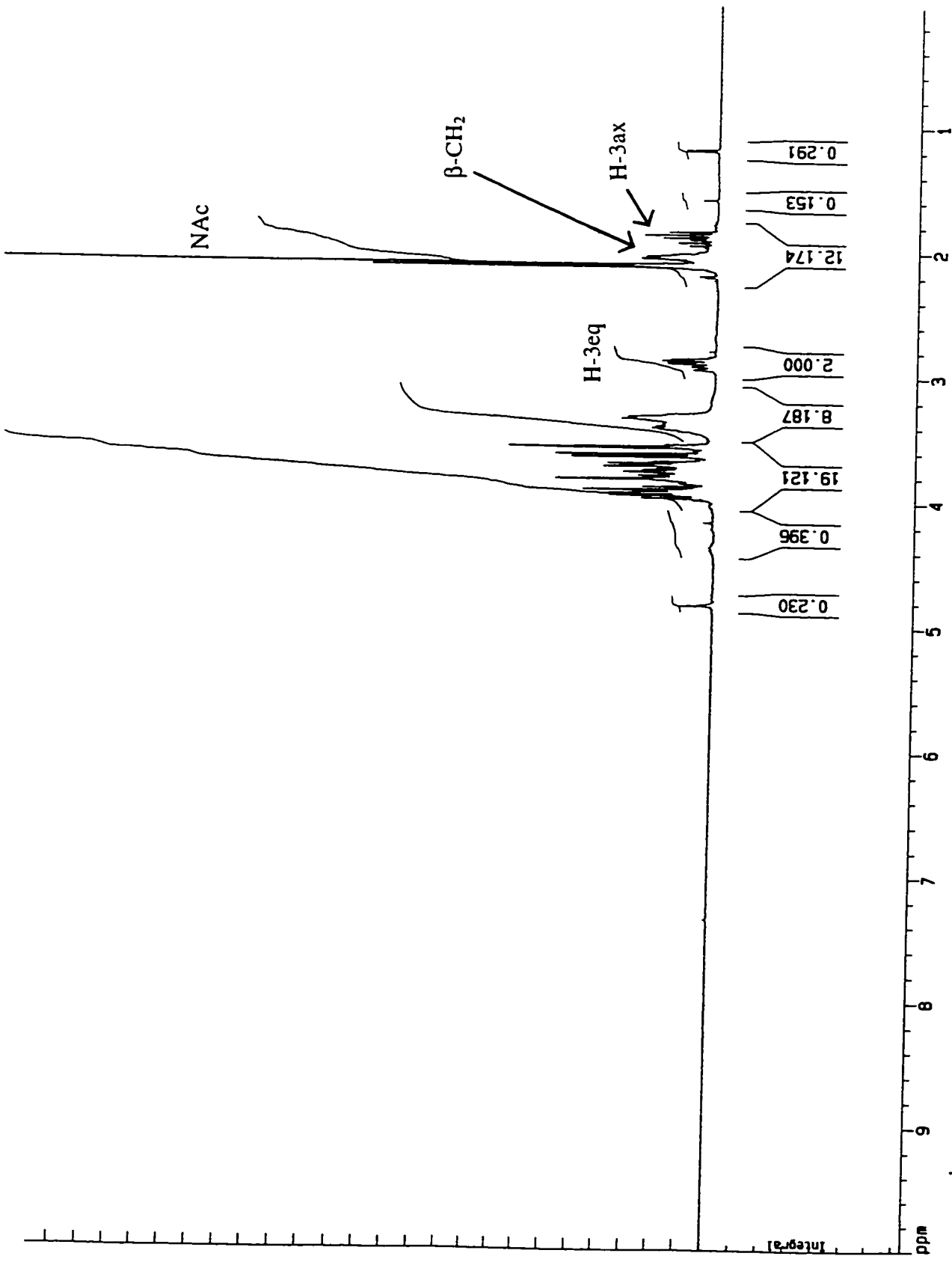
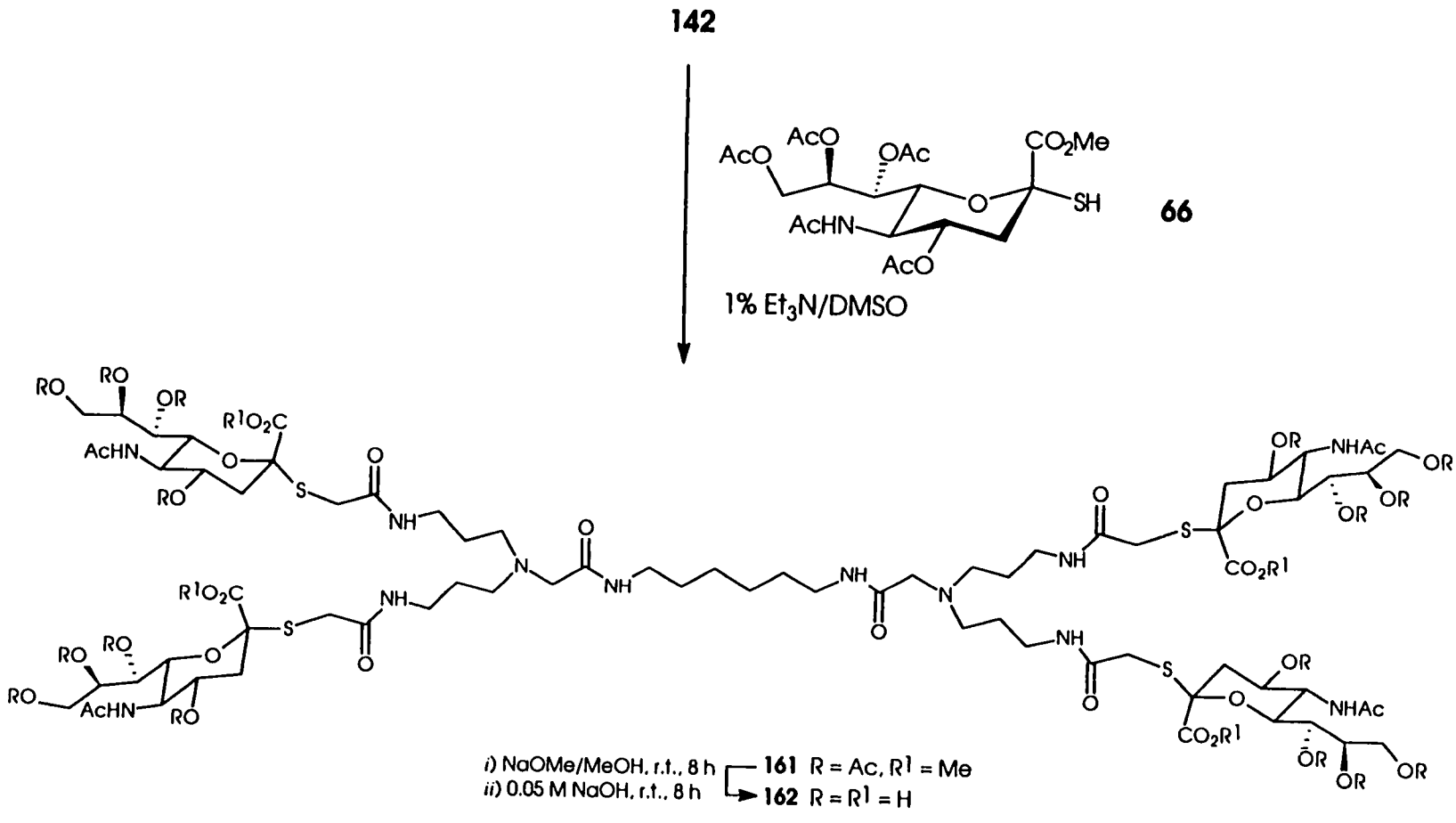
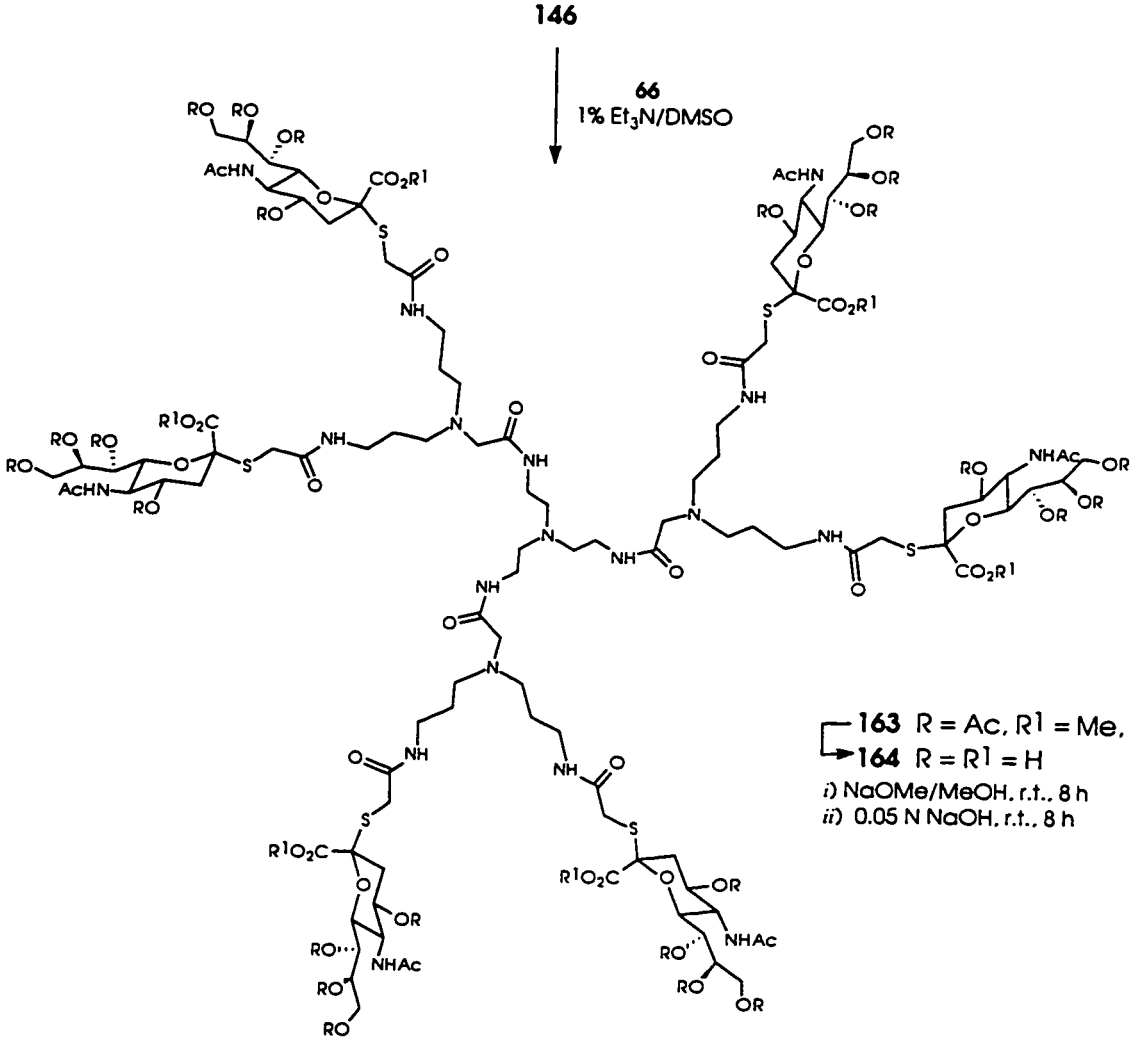


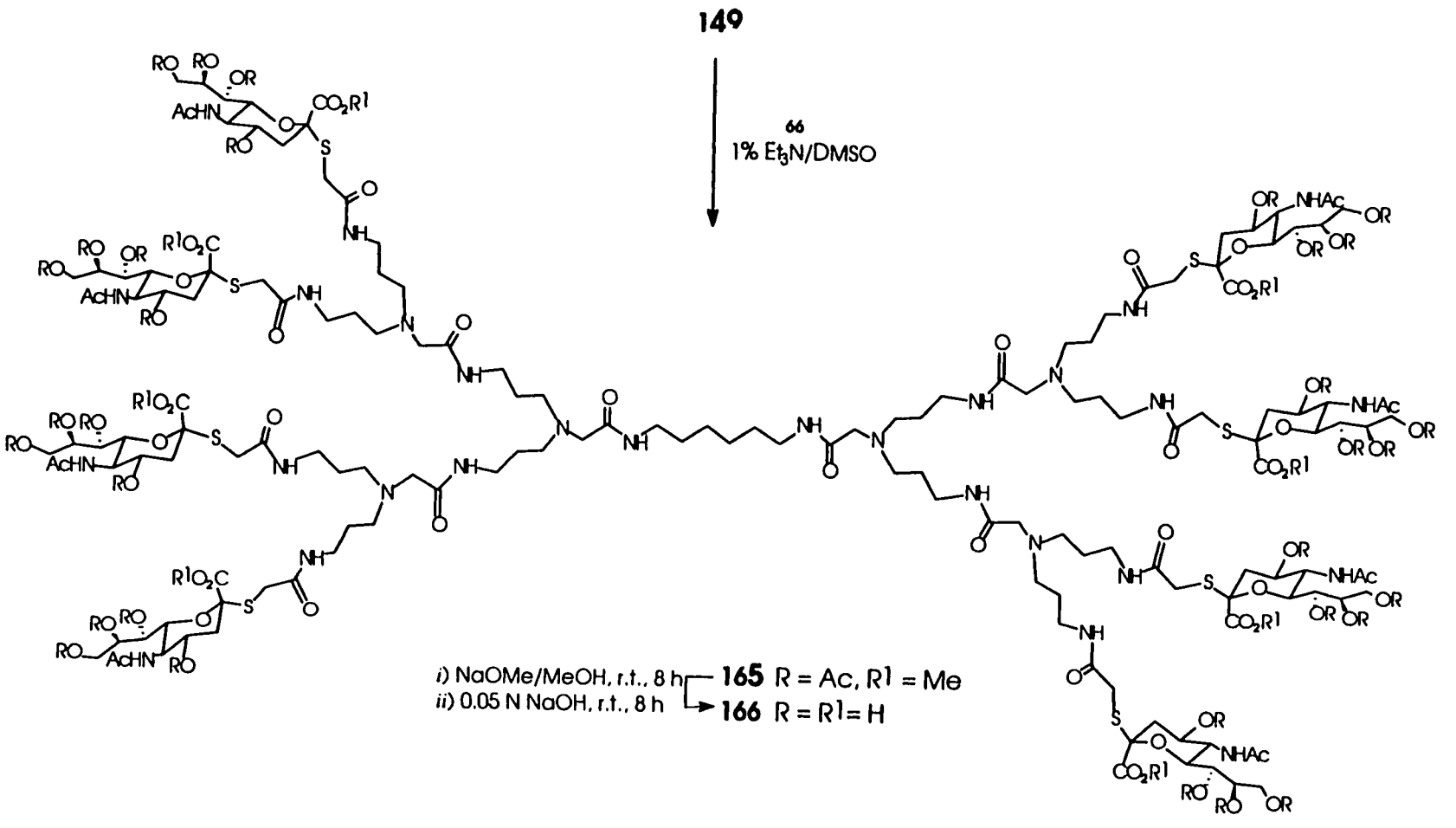
Figure 4.4.3. ^{1}H-NMR (D_2O, 500 MHz) spectrum of fully deprotected divalent 3,3'-iminobis(propylamine)-based α-thiosialodendrimer **154**.



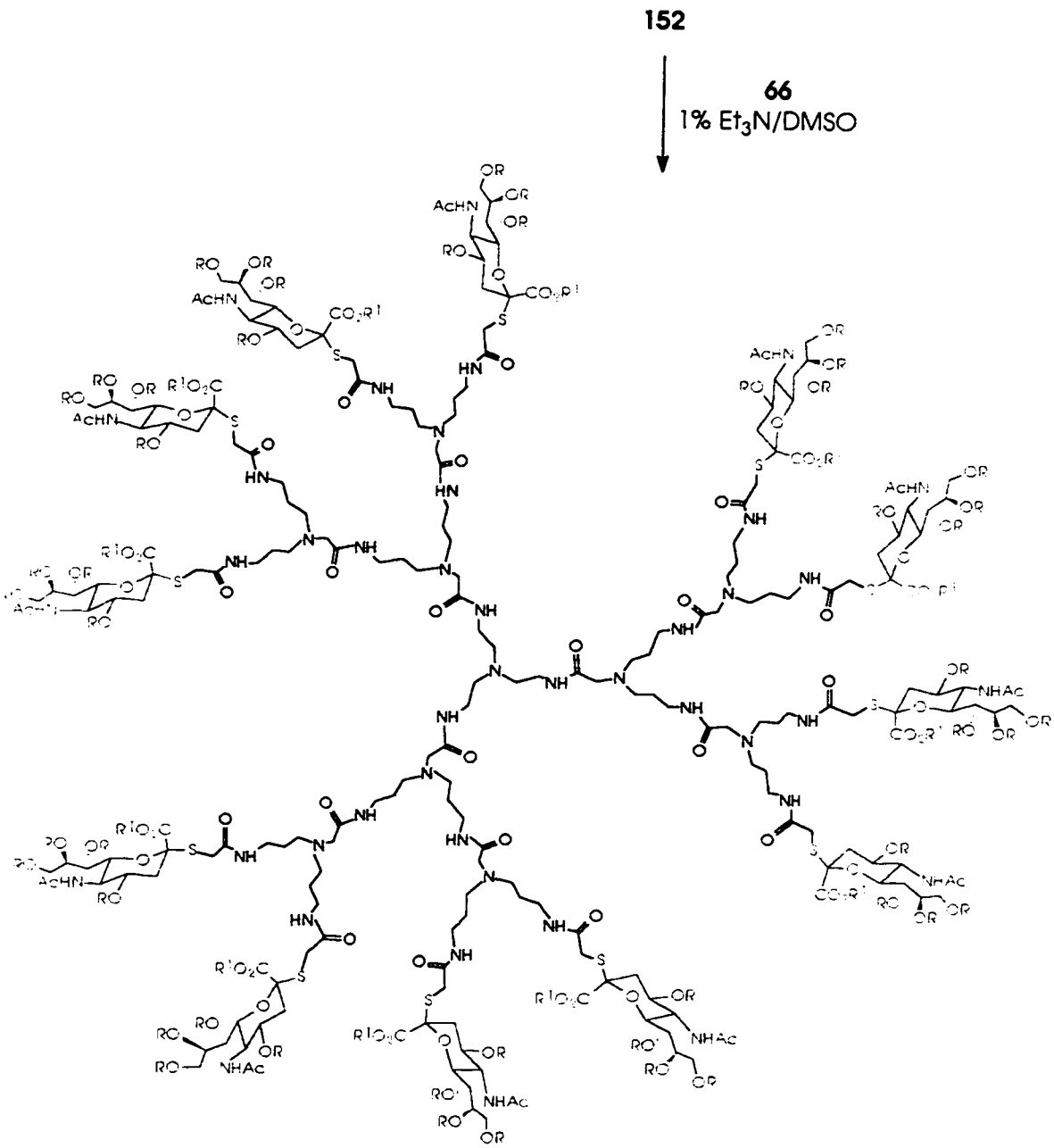
Scheme 4.5.1. Synthesis of tethered tetravalent 3,3'-iminobis(propylamine) α-thiosialodendrimer.



Scheme 4.5.2. Synthesis of tethered hexavalent 3,3'-iminobis(propylamine) α-thiosialodendrimer.



Scheme 4.5.3. Synthesis of tethered octavalent 3,3'-iminobis(propylamine) α-thiosialodendrimer.



Scheme 4.5.4. Synthesis of tethered dodecavalent 3,3'-iminobis(propylamine) α-thiosialodendrimer.

Complete coupling between the dendritic core and the α-thiosialoside was evident from the ^{1}H-NMR spectra of compounds **161**, **163**, **165**, and **167**. The disappearance of the N-chloroacetyl signals at 4.03 ppm (DMSO-d_6) and the emergence of new signals corresponding to the NeuAc residues (H-4 at 4.83 ppm, H-3eq at 2.70 ppm, and N-Ac at 1.83 ppm, DMSO-d_6) integrating for the desired valency clearly established the extent of sialoside incorporation.

Deprotection of sialodendrimers **161**, **163**, **165**, and **167** by sequential ester hydrolysis ((*i*) NaOMe/MeOH; (*ii*) 0.05 M NaOH) followed by gel permeation chromatography (GPC, Biogel-P2, H_2O as eluent) afforded fully deprotected tethered dendrimers **162**, **164**, **166**, and **168** with four, six, eight, and twelve NeuAc residues, respectively (56-73%) (Schemes 4.5.1 to 4.5.4).

4.6. Immunochemical Assays

To demonstrate the ability of these α-sialodendrimers to bind to the lectin from the slug *Limax flavus* (LFA), turbidimetric analysis was performed. The time course formation of insoluble precipitin complexes between LFA and dendrimer **156** is illustrated in Figure 4.6.1. Maximum turbidity was reached after only 30 minutes. These micro-quantitative precipitation experiments confirmed the direct binding and cross-linking properties of all α-sialodendrimers with LFA.

The efficiency of each dendrimer to inhibit the binding of horseradish peroxidase-labeled LFA to human α_1-acid glycoprotein (orosomucoid) was determined by enzyme linked lectin assays (ELLA). Human α_1-acid glycoprotein, which contains a large number of α-linked sialoside residues (10 to 12.5% by weight)[170] was used as a coating antigen and horseradish peroxidase-labeled LFA was used for quantitative detection. The results for the inhibition of binding of LFA to human α_1-acid glycoprotein are shown in

[170] Jeanloz, R. W. (Ed.) *Glycoproteins*, Elsevier Publishing Co., New York, **1972**, Vol. 5, pp. 565.

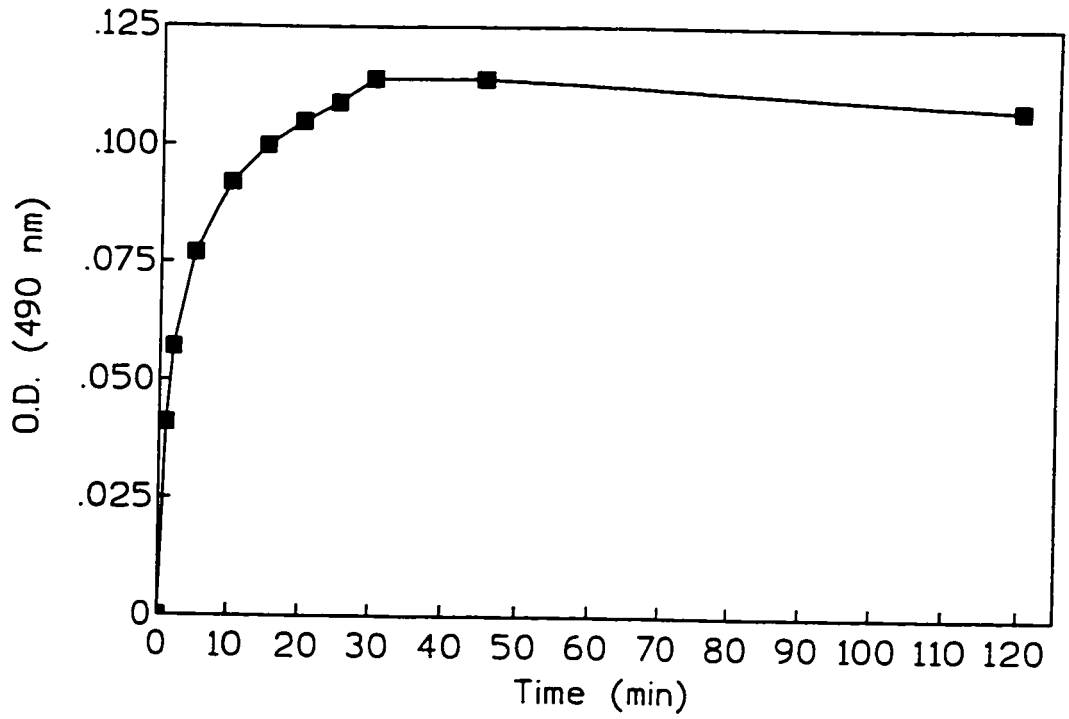


Figure 4.6.1. Turbidimetric analysis of LFA with tetrameric 3,3'-iminobis(propylamine) α-thiosialodendrimer **156**.

Figure 4.6.2. 5-Acetamido-5-deoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosonyl azide (NeuAcαN_3)[171] was used as a standard.

In the divergent α-sialodendrimer series, the best results were obtained with tetravalent dendrimer **156**. An IC_{50} of 11.8 nM was measured representing a 127 fold increase over that of NeuAcαN_3 (IC_{50} 1500 nM) used as a standard (Table 4.6.1). On a per sialoside basis, each residue was 32 times more potent than the corresponding monomer. Interestingly, no further increase in inhibitory potency was observed for third and fourth generations (**158** and **160**).

In the tethered α-sialodendrimer series, an increase in multivalency resulted in a steady increase in inhibitory potential. Tetra- (**162**), hexa- (**164**), octa- (**166**), and dodeca-

[171] Tropper, F.; Andersson, F. O.; Braun, S.; Roy, R. *Synthesis* **1992**, *7*, 618.

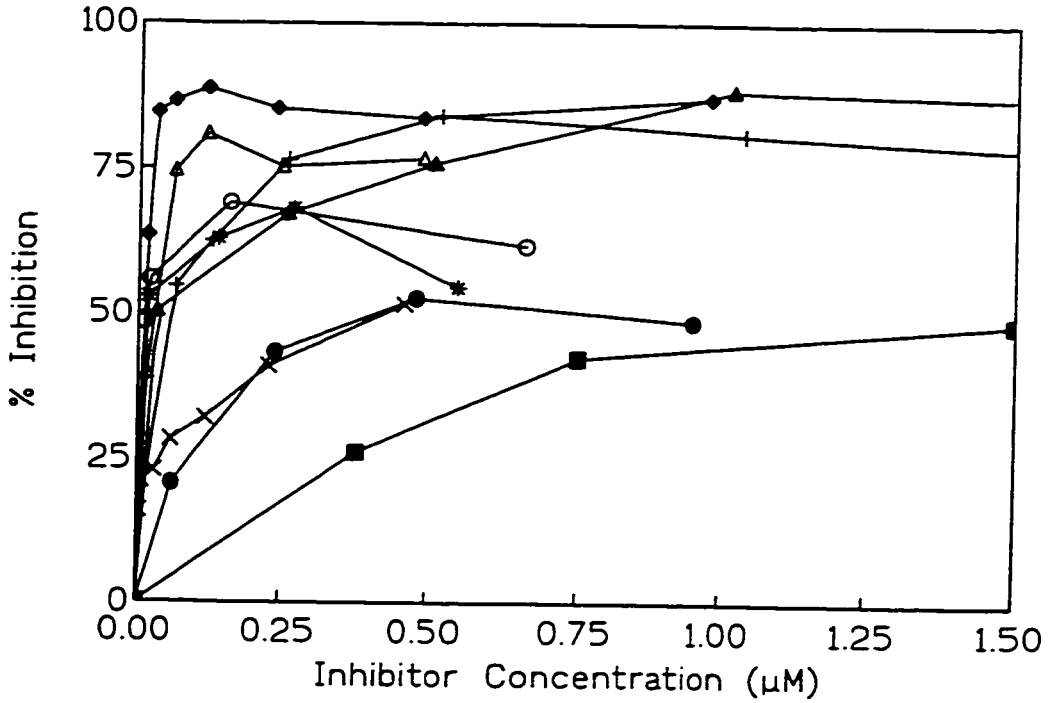


Figure 4.6.2. Inhibition of binding of LFA to human α_1-acid glycoprotein by NeuAcαN$_3$ (■), and glycodendrimers **154** (▲), **156** (◆), **158** (●), **160** (×), **162** (+), **164** (✱), **166** (Δ), and **168** (O).

Table 4.6.1. Inhibition of Binding of Human α_1-Acid Glycoprotein (Orosomucoid) to *Limax flavus* by Sialodendrimers.

Series	Compound	IC_{50}[a] (nM)	Relative Potency[a]
-	NeuAcαN$_3$	1500	1
Divergent	**154** (dimer)	176 (352)	8.5 (4.2)
	156 (tetramer)	11.8 (47.2)	127 (32)
	158 (octamer)	206 (1650)	7.3 (0.91)
	160 (hexadecamer)	425 (6800)	3.5 (0.22)
Tethered	**162** (tetramer)	58.7 (235)	26 (6.4)
	164 (hexamer)	16.9 (101)	89 (15)
	166 (octamer)	17.5 (140)	86 (11)
	168 (dodecamer)	8.22 (98.6)	182 (15)

[a]Values in parentheses are based on a per sialoside residue.

(**168**) valent dendrimers exhibited IC_{50}'s of 58.7, 16.9, 17.5, and 8.22 nM, respectively. This represents a 26 to 182 fold (6.4 to 15 fold/sialoside) jump in inhibitory potential. These data confirm previous findings that multivalency may be responsible for an increase in the binding of carbohydrate-protein interactions.

For the inhibition of the binding of LFA to human α_1-acid glycoprotein, the tethered dendrimers appear to be more effective than the divergent dendrimers. These results indicate that the increased inhibitory capacity cannot be solely attributed to an increase in multivalency. In fact, divergent hexadecamer **160** showed the poorest inhibition. It may be argued that glycoside conformation and position may be inappropriate in the higher homologues of the divergent dendrimer series to allow for effective inhibition.

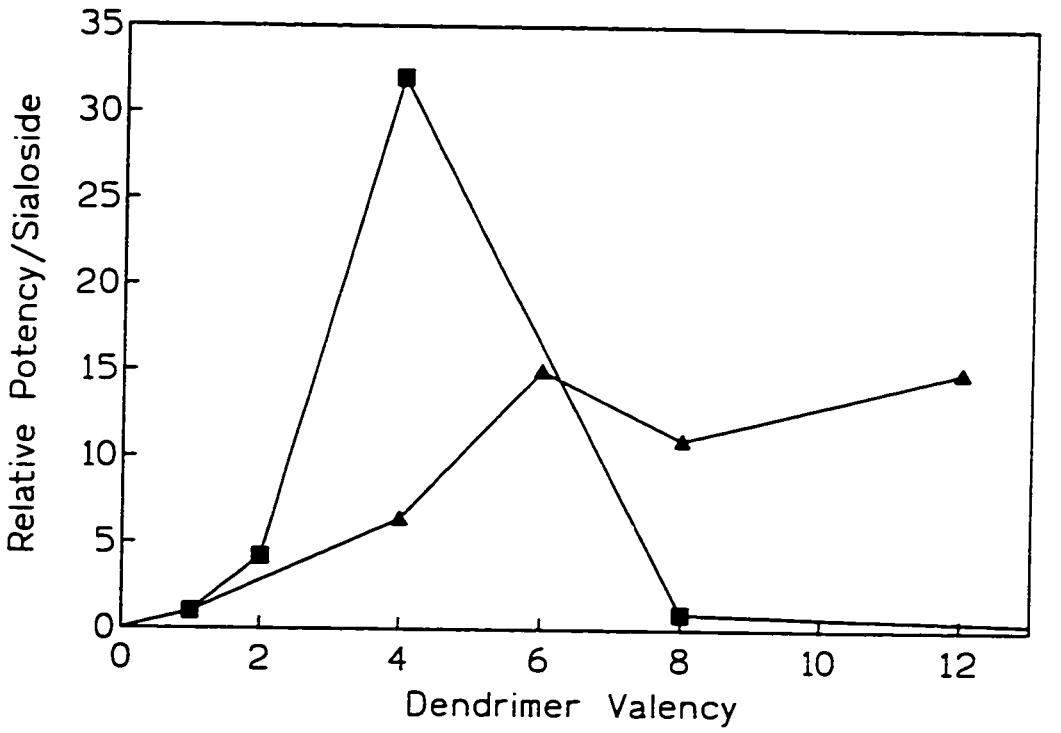


Figure 4.6.3. Relative potency per sialoside as a function of dendrimer valency for the divergent series **154**, **156**, **158**, and **160** (■) and for the tethered series **162**, **164**, **166**, and **168** (▲).

It is realistic to assume that not all sialic acid residues are involved in tight associations. In fact, when comparing relative potency on a per sialoside basis to dendrimer valency (Figure 4.6.3), values fell below one sialoside residue for divergent octamer **158** and hexadecamer **160**. This infers that some sialic acid residues are not involved in binding and negate the effect of some of the tight binding associations of NeuAc to human α_1-acid glycoprotein, thereby reducing some of the observed relative potency values. This phenomenon has been observed with some previously reported polymeric sialosides.[56,67,70,71]

4.7. Conclusions

α-Thiosialoside-containing dendrimers scaffolded on an orthogonally protected 3,3'-iminobis(propylamine) core were efficiently prepared *via* Cbz-protecting group and HOBt/DIC coupling strategies. Divergent and spherical dendrimers with even valencies of 2 to 16 were synthesized. The potential of these sialodendrimers to cross-link and precipitate *Limax flavus* lectin (LFA) was confirmed by turbidimetric analysis. When tested in ELLA using human α_1-acid glycoprotein (orosomucoid) as coating antigen and horseradish peroxidase-labeled LFA for detection, glycodendrimers exhibited increased inhibitory potential. The spherical α-sialodendrimers appeared to have structural organizations more suitable than the divergent dendrimers for the solid phase inhibition of the binding of human α_1-acid glycoprotein to LFA. These data confirm previous findings that multivalency may be responsible for an increase in the binding of carbohydrate-protein interactions.

4.8. Experimental Methods

3,3'-Bis(carbobenzyloxy)-3,3'-iminobis(propylamine) (124).

To a solution of 3,3'-iminobis(propylamine) **123** (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 **124** as a white solid in 72% yield (4.36 g, 0.11 mol); ^{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).

Cbz-protected divalent 3,3'-iminobis(propylamine)-based dendrimer (125).

To a solution of **124** (4.00 g, 0.010 mol) in acetonitrile (25 mL) was added *t*-butyl bromoacetate (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 **125** in 87% yield (4.42 g, 0.009 mol); ^{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 ($N\underline{C}H_2C(O)$), 66.3 (Cbz-CH_2), 81.4 ($O\underline{C}Me_3$), 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).

Cbz-protected divalent 3,3'-iminobis(propylamine)-based acid (126).

A solution of **125** (2.00 g, 3.90 mmol) in 50 mL 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 **126** was recovered as a yellow resin in 96% yield (2.14 g, 3.75 mmol) and used without further purification; ^{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 ($N\underline{C}H_2C(O)$), 66.9 (Cbz-CH_2), 114.9 and 117.2 (trifluoroacetate 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 (trifluoroacetate 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).

Divalent 3,3'-iminobis(propylamine)-based amine (127).

To compound **125** (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 **127** in 90% yield (264 mg, 0.70 mmol). Yellow resin **127** was used as is; ^{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 ($N\underline{C}H_2C(O)$), 170.9 (C=O); mass spectrum (CI) (rel intensity) *m/z* 246.1 (M^+, 51.6%).

N-Chloroacetylated divalent 3,3'-iminobis(propylamine)-based dendrimer (128).

To a solution of amine **127** (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 **128**. Compound **128** was isolated in 82% yield as a yellow resin (254 mg, 0.64 mmol); ^{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 ($ClCH_2$), 52.2 (α-C), 55.9 ($N\underline{C}H_2C(O)$), 81.5 ($O\underline{C}Me_3$), 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).

Cbz-protected tetravalent 3,3'-iminobis(propylamine)-based dendrimer (129).

To a solution of **127** (170 mg, 0.45 mmol) in CH_3CN (15 mL) was added acid **126** (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 was added diisopropylcarbodiimide (DIC, 136 mg, 1.08 mmol) and hydroxybenzotriazole (HOBt, 146 mg, 1.08 mmol) and the mixture 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 **129** was isolated as a yellow resin in 82% yield (448 mg, 0.40 mmol); ^{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 ($N\underline{C}H_2C(O)$'s), 57.8 (Cbz-CH_2), 81.4 ($O\underline{C}Me_3$), 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).

Cbz-protected tetravalent 3,3'-iminobis(propylamine)-based acid (130).

A solution of **129** (0.70 g, 0.62 mmol) in 50 mL 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 **130** was recovered as a yellow resin in quantitative yield (0.86 g, 0.62 mmol) and used without further purification; ^{1}H-NMR ($CDCl_3$) δ 1.83 (m, 12H, β-CH_2), 3.15 (m, 24H, α-CH_2, γ-CH_2), 3.92 (m, 6H, $NCH_2C(O)$), 5.15 (bs, 8H, Cbz-CH_2), 5.81 (bs, 4H, Cbz-NH), 7.21-7.40 (m, 20H, Cbz-Ph); ^{13}C-NMR ($CDCl_3$) δ 27.8 (β-C), 37.6 (γ-C), 53.0 (α-C), 53.4 ($N\underline{C}H_2C(O)$), 66.9 (Cbz-CH_2), 114.9 and 117.2 (trifluoroacetate 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 (trifluoroacetate salt C=O).

Tetravalent 3,3'-iminobis(propylamine)-based amine (131).

To compound **129** (150 mg, 0.13 mmol) was added MeOH (10 mL) containing 10% Pd/C (15 mg). H_2 was bubbled through the solution and the mixture stirred for 3 h 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 **131** in 97% yield (76.0 mg, 0.13 mmol). White solid **131** was used as is; ^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H, *t*Bu), 1.53-1.59 (m, 12H, β-CH_2's), 2.44-2.78 (m, 28H, α-CH_2's, γ-CH_2NH_2's, NH_2's), 3.04 (s, 4H, $NCH_2C(O)$'s), 3.16 (s, 2H, $NCH_2C(O)$), 3.23-3.31 (m, 4H, γ-CH_2's), 7.85 (bs, 2H, amide NH); ^{13}C-NMR ($CDCl_3$) δ 26.4, 26.7, 27.1, 27.4, and 28.9 (β-C's), 28.2 (CH_3's), 37.3, 37.4, 37.9, 38.7, 39.4, and 39.5 (γ-C's), 52.1, 52.5, and 52.7 (α-C's), 56.3 and 57.6 ($N\underline{C}H_2C(O)$'s), 172.0 and 172.6 (C=O's); FAB-MS (pos.) calcd. for $C_{28}H_{61}N_9O_4$ 587.48, found 588.5 ($M^+ + 1$, 7.3% base peak).

N-Chloroacetylated tetravalent 3,3'-iminobis(propylamine) dendrimer (132).

To a solution of amine **131** (52.1 mg, 0.089 mmol) in DMF (5 mL) was added chloroacetic anhydride (1.2 equiv. per amine functionality, 80 mg, 0.43 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 **132**. Compound **132** was isolated in 82% yield as a yellow resin (65.0 mg, 0.073 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.42 (s, 9H, *t*Bu), 1.60-1.10 (m, 12H, β-CH_2's), 2.47-2.51 (m, 12H, α-CH_2's), 3.06 (s, 4H, $NCH_2C(O)$'s), 3.14 (s, 2H, $NCH_2C(O)$), 3.30-3.36 (m, 12H, γ-CH_2's), 4.01 (s, 8H, $ClCH_2$), 7.35 (m, 4H, $ClCH_2C(O)N\underline{H}$), 7.65 (t, 2H, J 5.0 Hz, amide NH); ^{13}C-NMR ($CDCl_3$) δ 26.6 and 27.2 (β-C's), 28.2 (CH_3's), 37.5 and 37.9 (γ-C's), 42.8 ($ClCH_2$), 52.2 and 52.4 (α-C's), 56.0 and 57.7 ($N\underline{C}H_2C(O)$'s), 81.5 ($O\underline{C}Me_3$), 166.4 and 171.2 (C=O's); FAB-MS (pos.) calcd. for $C_{36}H_{69}N_9O_8Cl_4$ 893.29, found 894.4 ($M^+ + 1$, 1.3% base peak).

Cbz-protected octavalent 3,3'-iminobis(propylamine) dendrimer (133).

To a solution of **131** (151.4 mg, 0.26 mmol) in DMF (15 mL) was added acid **126** (1.2 equiv. per amine group, 713 mg, 1.25 mmol) already dissolved in CH_3CN (2 mL) and neutralized with DIPEA. To the stirred solution was added DIC (158 mg, 1.25 mmol) and HOBt (169 mg, 1.25 mmol) and the mixture 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 20 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. Octavalent **133** was isolated as a yellow resin in 66% yield (400.0 mg, 0.17 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.41 (s, 9H, *t*Bu), 1.58-1.65 (m, 28H, β-CH_2's), 2.42-2.56 (m, 28H, α-CH_2's), 2.96-3.33 (m, 42H, γ-CH_2's, $NCH_2C(O)$'s), 4.68 (s, 8H, Cbz-NH), 5.03 (s, 16H, Cbz-CH_2), 7.24-7.32 (m, 40H, Cbz-Ph); ^{13}C-NMR ($CDCl_3$) δ 27.0 and 27.4 (β-C's), 28.1 (CH_3's), 36.6, 37.2, 38.0, and 38.8 (γ-C's), 51.9, 52.2, and 52.4 (α-C's), 55.9 and 58.2 ($N\underline{C}H_2C(O)$'s), 66.6 (Cbz-CH_2), 128.0, 128.1, and 128.5 (Cbz-Ph, ortho, meta, para),

136.6 (Cbz-Ph, C-1), 156.7-171.6 (C=O's); FAB-MS (pos.) calcd. for $C_{124}H_{177}N_{21}O_{24}$ 2344.32, found 2346.6 (M^+ + 1, 0.4% base peak).

Octavalent 3,3'-iminobis(propylamine)-based amine (134).

To compound **133** (30.0 mg, 0.013 mmol) was added MeOH (10 mL) containing 10% Pd/C (3 mg). H_2 was bubbled through the solution and the mixture stirred for 20 h 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 **134** in 98% yield (16.0 mg, 0.012 mmol). White solid **134** was used as is; ^{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), 2.36-2.48 (m, 28H, α-CH_2's), 2.49 (t, 16H, J 6.8 Hz, γ-CH_2NH_2's), 3.00 (s, 14H, $NCH_2C(O)$'s), 3.15-3.31 (m, 12H, γ-CH_2's); ^{13}C-NMR ($CDCl_3$) δ 26.4, 26.7, 27.1, 27.4, and 28.9 (β-C's), 28.2 (CH_3's), 37.3, 37.4, 37.9, 38.7, 39.4, and 39.5 (γ-C's), 52.1, 52.5, and 52.7 (α-C's), 56.3 and 57.6 ($N\underline{C}H_2C(O)$'s), 172.0 and 172.6 (C=O's).

N-Chloroacetylated octavalent 3,3'-iminobis(propylamine)-based dendrimer (135).

To a solution of amine **134** (41.6 mg, 0.032 mmol) in DMSO (5 mL) was added chloroacetic anhydride (1.2 equiv. per amine functionality, 53.0 mg, 0.31 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 **135**. Compound **135** was isolated in 86% yield as a yellow resin (52.8 mg, 0.028 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H, *t*Bu), 1.46-1.69 (m, 28H, β-CH_2's), 2.48-2.60 (m, 28H, α-CH_2's), 3.00-3.42 (m, 42H, γ-CH_2's, $NCH_2C(O)$'s), 4.02 and 4.03 (2s, 16H, $ClCH_2$'s); ^{13}C-NMR ($CDCl_3$) δ 26.8 and 27.0 (β-C's), 28.2 (CH_3's), 37.1, 37.4, and 37.8 (γ-C's), 41.0 and 42.8 ($ClCH_2$'s), 52.4 (α-C's), 58.0 ($N\underline{C}H_2C(O)$'s), 81.5 ($O\underline{C}Me_3$), 166.4-171.4 (C=O's).

Cbz-protected hexadecavalent 3,3'-iminobis(propylamine) dendrimer (136).

Procedure 1. To a solution of **134** (22.1 mg, 0.017 mmol) in DMSO (15 mL) was added acid **126** (1.2 equiv. per amine group, 93.2 mg, 0.16 mmol) already dissolved in CH_3CN (2 mL) and neutralized with DIPEA. To the stirred solution was added DIC (21 mg, 0.16 mmol) and HOBt (22 mg, 0.16 mmol) and the mixture 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 48 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. Hexadecavalent **136** was isolated as a yellow resin in 46% yield (38.6 mg, 0.0081 mmol).

Procedure 2. To a solution of **131** (30.0 mg, 0.051 mmol) in DMF (5 mL) was added acid **130** (1.2 equiv. per amine group, 340 mg, 0.24 mmol) already dissolved in CH_3CN (5 mL) and neutralized with DIPEA. To the stirred solution was added DIC (31 mg, 0.24 mmol) and HOBt (33 mg, 0.24 mmol) and the mixture 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 20 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. Hexadecavalent **136** was isolated as a yellow resin in 57% yield (140.0 mg, 0.029 mmol).

^{1}H-NMR ($CDCl_3$) δ 1.41 (s, 9H, *t*Bu), 1.57-1.59 (m, 60H, β-CH_2's), 2.41-2.55 (m, 60H, α-CH_2's), 2.81, 2.91, 2.94, and 2.97 (4s, 30H, $NCH_2C(O)$'s), 3.12-3.38 (m, 60H, γ-CH_2's), 5.04 (s, 32H, Cbz-CH_2), 5.50-5.60 (2bs, 16H, Cbz-NH), 7.24-7.30 (m, 80H, Cbz-Ph); ^{13}C-NMR ($CDCl_3$) δ 26.0, 27.1, 27.2, 27.4, 27.5, and 27.6 (β-C's), 28.1 (CH_3's), 35.6, 36.6, 36.7, 36.8, 37.3, 38.1, 38.9, and 39.0 (γ-C's), 50.9, 51.9, 52.1, 52.3, and 52.4 (α-C's), 56.2, 58.2, and 58.4 ($N\underline{C}H_2C(O)$'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), 156.7-171.6 (C=O's).

Hexadecavalent 3,3'-iminobis(propylamine)-based amine (137).

To compound **136** (67.2 mg, 0.014 mmol) was added MeOH (10 mL) containing 10% Pd/C (7 mg). H_2 was bubbled through the solution and the mixture stirred for 20 h 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 **137** in 97% yield (38.7 mg, 0.014 mmol). White solid **137** was used as is; ^{1}H-NMR (DMSO-d_6) δ 1.42 (s, 9H, *t*Bu), 1.45-1.75 (m, 92H, β-CH_2, NH_2), 2.36-2.46 (m, 60H, α-CH_2), 2.50 (m, 32H, γ-CH_2NH_2), 3.00 (m, 30H, $NCH_2C(O)$), 3.15-3.30 (m, 28H, γ-CH_2); ^{13}C-NMR (DMSO-d_6) δ 26.4, 26.7, 27.1, 27.4, and 28.9 (β-C's), 28.2 (CH_3's), 37.3, 37.4, 37.9, 38.7, 39.4, and 39.5 (γ-C's), 52.1, 52.5, and 52.7 (α-C's), 56.3 and 57.6 ($N\underline{C}H_2C(O)$'s), 172.0 and 172.6 (C=O's).

N-Chloroacetylated hexadecavalent 3,3'-iminobis(propylamine)-based dendrimer (138).

To a solution of amine **137** (38.7 mg, 0.014 mmol) in DMSO (5 mL) was added chloroacetic anhydride (1.2 equiv. per amine functionality, 46.0 mg, 0.27 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 **138**. Compound **138** was isolated in 91% yield as a yellow resin (50.2 mg, 0.013 mmol); ^{1}H-NMR (DMSO-d6) δ 1.38 and 1.39 (2s, 9H, *t*Bu), 1.41-1.71 (m, 60H, β-CH_2's), 2.23-2.52 (m, 60H and DMSO, α-CH_2's), 2.97-3.40 (m, 90H and H_2O, γ-CH_2's, $NCH_2C(O)$'s), 4.03 (s, 32H, $ClCH_2$), 6.65, 7.27, 7.81, and 8.28 (4m, amide NH's); ^{13}C-NMR (DMSO-d6) δ 25.7, 26.2, 26.4, and 26.5 (β-C's), 27.8 (CH_3's), 36.4, 36.6, 37.1, and 38.7 (γ-C's), 42.7($ClCH_2$), 51.2 and 52.0 (α-C's), 56.2, 57.3, and 58.8 ($N\underline{C}H_2C(O)$'s), 164.7-171.5 (C=O's).

Cbz-protected tetravalent tethered 3,3'-iminobis(propylamine)-based dendrimer (140).

To a solution of hexamethylenediamine **139** (29.0 mg, 0.25 mmol) in CH_3CN (15 mL) was added acid **126** (1.2 equiv. per amine group, 343 mg, 0.60 mmol) already dissolved in CH_2Cl_2 (2 mL) and neutralized with DIPEA. To the stirred solution was added DIC (76 mg, 0.60 mmol) and HOBt (81 mg, 0.60 mmol) and the mixture 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 20 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 **140** was isolated as a yellow resin in 86% yield (214.0 mg, 0.22 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.24 (m, 4H, a-CH_2), 1.43 (m, 4H, b-CH_2), 1.55 (m, 8H, β-CH_2), 1.89 (m, 8H, α-CH_2), 2.94 (bs, 4H, $NCH_2C(O)$), 3.14 (m, 12H, γ-CH_2, c-CH_2), 5.02 (s, 8H, Cbz-CH_2), 5.52 (bs, 4H, Cbz-NH), 7.23-7.30 (m, 20H, Cbz-Ph), 7.58 (bs, 2H, NH amide); ^{13}C-NMR ($CDCl_3$) δ 26.1 (a-C), 27.4 (β-C), 29.3 (b-C), 38.6 (c-C), 38.9 (γ-C), 52.5 (α-C), 58.4 ($N\underline{C}H_2C(O)$), 66.5 (Cbz-CH_2), 127.8, 128.0, 128.3 (Cbz-Ph, ortho, meta, para), 136.7 (Cbz-Ph, C-1), 156.6-171.2 (C=O); FAB-MS (pos.) calcd. for $C_{54}H_{74}N_8O_{10}$ 994.55, found 995.5 (M^+ + 1, 75.6% base peak).

Tetravalent tethered 3,3'-iminobis(propylamine)-based amine (141).

To compound **140** (90.0 mg, 0.090 mmol) was added MeOH (10 mL) containing 10% Pd/C (9 mg). H_2 was bubbled through the solution and the mixture stirred for 20 h 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 **141** in 90% yield (264 mg, 0.70 mmol). White solid **141** was used as is; ^{1}H-NMR ($CDCl_3$) δ 1.23 (m, 4H, a-CH_2), 1.48 (m, 12H, b-CH_2, NH_2), 1.59 (m, 8H, β-CH_2), 2.49 (m, 8H, α-CH_2), 2.72 (m, 8H, γ-CH_2), 3.02 (s, 4H, $NCH_2C(O)$), 3.21 (m. 4H, c-CH_2), 7.56 (s, 2H, NH amide); ^{13}C-NMR

($CDCl_3$) δ 26.6 (a-C), 29.7 (b-C), 30.9 (β-C), 38.8 (c-C), 40.3 (γ-C), 51.9 (α-C), 58.7 ($N\underline{C}H_2C(O)$), 171.5 (C=O); mass spectrum (CI) (rel intensity) *m/z* 458.9 (M^+, base peak).

N-Chloroacetylated tetravalent tethered 3,3'-iminobis(propylamine)-based dendrimer (142).

To a solution of amine **141** (38.0 mg, 0.086 mmol) in DMSO (2 mL) was added chloroacetic anhydride (1.2 equiv. per amine functionality, 70 mg, 0.41 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 **142**. Compound **142** was isolated in 81% yield as a yellow resin (52 mg, 0.069 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.33 (m, 4H, a-CH_2), 1.51 (m, 4H, b-CH_2), 1.69 (m, 8H, β-CH_2), 2.50 (m, 8H, α-CH_2), 3.02 (bs, 4H, $NCH_2C(O)$), 3.24 (m, 4H, c-CH_2), 3.34 (m, 8H, γ-CH_2), 4.02 (s, 8H, $ClCH_2$); ^{13}C-NMR ($CDCl_3$) δ 26.1 (a-C), 26.9 (β-C), 29.3 (b-C), 38.1 (γ-C), 38.7 (c-C), 42.7 ($ClCH_2$), 52.5 (α-C), 58.4 ($N\underline{C}H_2C(O)$), 166.3, 171.1 (C=O); FAB-MS (pos.) calcd. for $C_{30}H_{54}N_8O_6Cl_4$ 762.29, found 763.3 (M^+ + 1, 61.9% base peak).

Cbz-protected hexavalent tethered 3,3'-iminobis(propylamine)-based dendrimer (144).

To a solution of 2-(aminoethyl)amine **143** (20.0 mg, 0.14 mmol) in CH_3CN (15 mL) was added acid **126** (1.2 equiv. per amine group, 288 mg, 0.50 mmol) already dissolved in CH_2Cl_2 (5 mL) and neutralized with DIPEA. To the stirred solution was added DIC (63 mg, 0.50 mmol) and HOBt (68 mg, 0.50 mmol) and the mixture 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 20 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. Hexavalent **144** was isolated as a yellow resin in 88% yield (182.0 mg, 0.12 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.55 (m, 12H, β-CH_2), 2.41 (m, 12H, α-CH_2), 2.53 (m, 6H, a-CH_2), 3.12-3.23 (m, 24H, γ-CH_2, b-CH_2, $NCH_2C(O)$), 5.02 (s, 12H, Cbz-CH_2), 5.65 (bs, 6H, Cbz-NH), 7.23-7.30 (m, 30H, Cbz-Ph), 7.54 (bs, 3H, NH amide); ^{13}C-NMR ($CDCl_3$) δ 27.3 (β-C), 37.4 (b-C), 38.9 (γ-C), 52.5 (α-C), 54.1 (a-C), 58.0 ($N\underline{C}H_2C(O)$), 66.5 (Cbz-CH_2), 127.8, 128.0, 128.5 (Cbz-Ph, ortho, meta, para), 136.7 (Cbz-Ph, C-1), 156.7-171.6 (C=O); FAB-MS (pos.) calcd. for $C_{78}H_{105}N_{13}O_{15}$ 1464.77, found 1466.4 (M^+ + 1, base peak).

Hexavalent tethered 3,3'-iminobis(propylamine)-based amine (145).

To compound **144** (80.0 mg, 0.055 mmol) was added MeOH (10 mL) containing 10% Pd/C (8 mg). H_2 was bubbled through the solution and the mixture stirred for 20 h 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 **145** in 93% yield (33.5 mg, 0.051 mmol). White solid **145** was used as is; ^{1}H-NMR ($CDCl_3$) δ 1.52-1.75 (m, 24H, β-CH_2, NH_2), 2.47-2.92 (m, 30H, α-CH_2, γ-CH_2, a-CH_2), 3.02 (s, 6H, $NCH_2C(O)$), 3.43 (m, 6H, b-CH_2); ^{13}C-NMR ($CDCl_3$) δ 30.5, 30.6 (β-C), 36.9 (b-C), 38.7 (γ-C), 52.7 (α-C), 53.7 (a-C), 58.8 ($N\underline{C}H_2C(O)$), 170.9 (C=O); FAB-MS (pos.) calcd. for $C_{30}H_{69}N_{13}O_3$ 659.56, found 660.5 (M^+ + 1, 3.1% base peak).

N-Chloroacetylated hexavalent tethered 3,3'-iminobis(propylamine)-based dendrimer (146).

To a solution of amine **145** (33.5 mg, 0.051 mmol) in DMSO (2 mL) was added chloroacetic anhydride (1.2 equiv. per amine functionality, 62.5 mg, 0.37 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 **146**. Compound **146** was isolated in 86% yield as a yellow resin

(49.0 mg, 0.044 mmol); ^{1}H-NMR (DMSO- d_6) δ 1.75 (m, 12H, β-CH_2), 2.47-2.50 (m, 12H, α-CH_2), 3.12 (m, 30H, γ-CH_2, a-CH_2, b-CH_2, $NCH_2C(O)$), 4.03 (s, 12H, $ClCH_2$); ^{13}C-NMR (DMSO- d_6) δ 26.1 (β-C), 36.7 (b-C), 37.0 (γ-C), 42.6 ($ClCH_2$), 51.9 (α-C), 53.1 (a-C), 60.2 ($N\underline{C}H_2C(O)$), 165.9-171.4 (C=O); FAB-MS (pos.) calcd. for $C_{42}H_{75}N_{13}O_9Cl_6$ 1115.39, found 1118.2 (M^+ + 3, 0.1% base peak).

Cbz-protected octavalent tethered 3,3'-iminobis(propylamine)-based dendrimer (147).

To a solution of hexamethylenediamine **139** (10.0 mg, 0.086 mmol) in CH_3CN (15 mL) was added tetravalent acid **130** (1.2 equiv. per amine group, 284 mg, 0.21 mmol) already dissolved in CH_2Cl_2 (2 mL) and neutralized with DIPEA. To the stirred solution was added DIC (26.5 mg, 0.21 mmol) and HOBt (28.0 mg, 0.21 mmol) and the mixture 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 20 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. Octavalent **147** was isolated as a yellow resin in 79% yield (150.2 mg, 0.068 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.21-1.25 (m, 4H, a-CH_2), 1.38-1.44 (m, 4H, b-CH_2), 1.54 (m, 24H, β-CH_2), 2.38 (m, 24H, α-CH_2), 2.90-3.34 (2m, 50H, γ-CH_2, c-CH_2, $NCH_2C(O)$), 5.01 (s, 16H, Cbz-CH_2), 5.64, 5.74 (2bs, 4H, NH amide), 5.84, 5.94 (2bs, 8H, Cbz-NH), 7.23-7.29 (m, 40H, Cbz-Ph), 7.39-7.41 (bs, 2H, NH amide); ^{13}C-NMR ($CDCl_3$) δ 26.4 (a-C), 27.3 (β-C), 29.3 (b-C), 36.6 (c-C), 38.8 (γ-C), 52.3 (α-C), 58.3 ($N\underline{C}H_2C(O)$), 66.4 (Cbz-CH_2), 127.9, 128.0, 128.4 (Cbz-Ph, ortho, meta, para), 136.6 (Cbz-Ph, C-1), 156.6-172.9 (C=O); FAB-MS (pos.) calcd. for $C_{118}H_{166}N_{20}O_{22}$ 2216.74, found 2218 (M^+ + 1, 3.2% base peak).

Octavalent tethered 3,3'-iminobis(propylamine)-based amine (148).

To compound **147** (150.2 mg, 0.068 mmol) was added MeOH (10 mL) containing 10% Pd/C (15 mg). H_2 was bubbled through the solution and the mixture stirred for 20 h 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 **148** in quantitative yield (77.0 mg, 0.068 mmol). White solid **148** was used as is; ^{1}H-NMR (DMSO-d_6) δ 1.22 (m, 4H, a-CH_2), 1.52 (m, 4H, b-CH_2), 1.65 (m, 24H, β-CH_2), 2.36-2.49 (2m, 24H, α-CH_2), 2.75 (m, 12H, $NCH_2C(O)$), 2.99-3.26 (m, 28H, γ-CH_2, c-CH_2), 4.91 (bs, NH_2); ^{13}C-NMR (DMSO-d_6) δ 25.5 (β-C), 26.6 (a-C), 29.1 (b-C), 37.8 (c-C), 38.4 (γ-C), 51.7, 52.0 (α-C), 57.0 ($N\underline{C}H_2C(O)$), 170.4-173.6 (C=O).

N-Chloroacetylated octavalent tethered 3,3'-iminobis(propylamine)-based dendrimer (149).

To a solution of amine **148** (77.0 mg, 0.068 mmol) in DMSO (2 mL) was added chloroacetic anhydride (1.2 equiv. per amine functionality, 111 mg, 0.65 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 **149**. Compound **149** was isolated in 81% yield as a yellow resin (76.0 mg, 0.043 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.22 (m, 4H, a-CH_2), 1.35 (m, 4H, b-CH_2), 1.54 (m, 24H, β-CH_2), 2.47-2.50 (m, 24H, α-CH_2), 2.85-3.30 (3m, 42H, γ-CH_2, c-CH_2, $NCH_2C(O)$), 4.03 (s, 16H, $ClCH_2$); ^{13}C-NMR (DMSO- d_6) δ 26.2 (a-C), 26.7 (β-C), 29.1 (b-C), 36.4 (c-C), 37.2 (γ-C), 42.7 ($ClCH_2$), 51.9 (α-C), 57.5 ($N\underline{C}H_2C(O)$), 165.8, 170.4 (C=O).

Cbz-protected dodecavalent tethered 3,3'-iminobis(propylamine)-based dendrimer (150).

To a solution of 2-(aminoethyl)amine **143** (10.0 mg, 0.068 mmol) in CH_3CN (15 mL) was added tetravalent acid **130** (1.2 equiv. per amine group, 340 mg, 0.25 mmol) already dissolved in CH_2Cl_2 (2 mL) and neutralized with DIPEA. To the stirred solution was added DIC (31.5 mg, 0.25 mmol) and HOBt (33.0 mg, 0.25 mmol) and the mixture 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 20 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. Dodecavalent **150** was isolated as a yellow resin in 72% yield (162.0 mg, 0.049 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.56 (m, 36H, β-CH_2), 2.39 (m, 42H, α-CH_2, a-CH_2), 2.89-3.35 (2m, 60H, γ-CH_2, b-CH_2, $NCH_2C(O)$), 5.01 (s, 24H, Cbz-CH_2), 5.68-5.82 (m, 12H, Cbz-NH), 7.24-7.30 (m, 60H, Cbz-Ph); ^{13}C-NMR ($CDCl_3$) δ 27.4 (β-C), 37.4 (b-C), 38.9 (γ-C), 52.4 (α-C), 58.3 ($N\underline{C}H_2C(O)$), 66.4 (Cbz-CH_2), 127.8, 128.0, 128.4 (Cbz-Ph, ortho, meta, para), 136.7 (Cbz-Ph, C-1), 156.7-172.9 (C=O).

Dodecavalent tethered 3,3'-iminobis(propylamine)-based amine (151).

To compound **150** (162.0 mg, 0.049 mmol) was added MeOH (10 mL) containing 10% Pd/C (16 mg). H_2 was bubbled through the solution and the mixture stirred for 20 h 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 **151** in 61% yield (51.0 mg, 0.030 mmol). White solid **151** was used as is; ^{1}H-NMR (DMSO-d_6) δ 1.53-1.65 (m, 24H, β-CH_2), 2.39-2.51 (m, 24H, α-CH_2), 2.74 (m, 12H, $NCH_2C(O)$), 2.93-3.27 (m, 36H, γ-CH_2, a-CH_2, b-CH_2), 5.00 (bs, NH_2); ^{13}C-NMR (DMSO-d_6) δ 25.3, 26.6 (β-C), 36.7 (b-C), 37.6, 37.8 (γ-C), 51.7, 52.0 (α-C), 57.0, 57.5 ($N\underline{C}H_2C(O)$), 170.8-173.3 (C=O).

N-Chloroacetylated dodecavalent tethered 3,3'-iminobis(propylamine)-based dendrimer (152).

To a solution of amine **151** (51.0 mg, 0.050 mmol) in DMSO (2 mL) was added chloroacetic anhydride (1.2 equiv. per amine functionality, 123 mg, 0.72 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 **152**. Compound **152** was isolated in 78% yield as a yellow resin (51.2 mg, 0.039 mmol); ^{1}H-NMR (DMSO- d_6) δ 1.54 (m, 36H, β-CH_2), 2.40 (m, 36H, α-CH_2), 2.48 (m, 6H, a-CH_2), 2.90-3.42 (3m, 60H, γ-CH_2, , b-CH_2, $NCH_2C(O)$), 4.03 (s, 24H, $ClCH_2$); ^{13}C-NMR (DMSO- d_6) δ 26.2, 26.5, 26.6 (β-C), 36.5 (b-C), 36.6, 36.8, 37.0 (γ-C), 42.7 ($ClCH_2$), 51.9 (α-C), 57.5 ($N\underline{C}H_2C(O)$), 165.8, 170.5 (C=O).

Peracetylated 3,3'-iminobis(propylamine) divalent α-thiosialodendrimer (153).

N-Chloroacetylated dendrimer **128** (45.0 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 **66** (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 **153** in 96% yield (145 mg, 0.11 mmol); ^{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$\underline{H}$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 ($N\underline{C}H_2C(O)$), 62.3 (C-9), 67.1 (C-7), 68.2 (C-8), 69.4 (C-4), 74.0 (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).

Fully deprotected 3,3'-iminobis(propylamine)-based divalent α-thiosialodendrimer (154).

To peracetylated glycodendrimer **153** (50.0 mg, 0.040 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 8 h, MeOH was removed *in vacuo* and 0.05 M 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 **154** was isolated, after freeze-drying, as a white, spongy solid in 58% yield (21.0 mg, 0.020 mmol); ^{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 ($N\underline{C}H_2C(O)$), 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).

Peracetylated 3,3'-iminobis(propylamine) tetravalent α-thiosialodendrimer (155).

N-Chloroacetylated dendrimer **132** (20.0 mg, 0.022 mmol) was dissolved in 1% Et_3N/DMSO (5 mL) and N_2 bubbled through the solution. To this was added thiol **66** (1.1 equiv. per N-chloroacetyl functionality, 54.5 mg, 0.11 mmol) and the solution left stirring, under N_2, overnight at 25 °C. The solution was concentrated by lyophilization. The residue was redissolved in the minimum amount of DMSO (≈300 μL) and

precipitated with ethyl acetate to give off-white solid **155** in 93% yield (58.0 mg, 0.020 mmol). NMR spectral data shows the incorporation of ethyl acetate in the dendrimer core; ^{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), 1.84-2.18 (m, 16H, β-CH_2's, H-3ax), 2.72 (dd, 4H, H-3eq), 3.05-3.55 (3m, 38H, α-CH_2's, γ-CH_2's, $NCH_2C(O)$'s, SCH_2's), 3.76 (s, 12H, CO_2CH_3), 3.76-3.83 (m, 4H, H-6), 3.90-4.03 (m, 8H, H-5, H-9), 4.28 (m, 4H, H-9'), 4.86 (m, 4H, H-4), 5.23-5.40 (2m, 12H, H-7, H-8, NHAc); ^{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), 37.0 (γ-C's), 37.4 (C-3), 45.8 (α-C's), 49.1 (C-5), 53.4 (MeO), 62.4 (C-9), 67.3 (C-7), 68.3 (C-8), 69.5 (C-4), 74.1 (C-6), 82.2 (C-2), 168.7-171.2 (C=O's).

Fully deprotected 3,3'-iminobis(propylamine)-based tetravalent α-thiosialodendrimer (156).

To peracetylated glycodendrimer **155** (58.0 mg, 0.020 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 8 h, MeOH was removed *in vacuo* and 0.05 M 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 **156** was isolated, after freeze-drying, as a white, spongy solid in 58% yield (25.0 mg, 0.012 mmol); ^{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 19.9, 21.7, 22.9, and 23.5 (β-C's), 21.6 (NAc), 34.0, 35.9, and 36.4 (γ-C's), 38.7 (SCH_2), 40.1 (C-3), 51.2 (C-5), 52.3, 54.2, and 55.2 (α-C's), 58.2, 58.8, and 59.8 ($NCH_2C(O)$), 62.2 and 62.7 (C-9), 66.7 and 67.3 (C-7), 68.0 and 68.2 (C-8), 69.0, 70.6, 71.2, and 71.4 (C-4), 74.0 and 74.5 (C-6), 84.4 (C-2), 163.8-175.6 (C=O's).

Peracetylated 3,3'-iminobis(propylamine) octavalent α-thiosialodendrimer (157).

N-Chloroacetylated dendrimer **135** (10.2 mg, 0.0054 mmol) was dissolved in 1% Et_3N/DMSO (5 mL) and N_2 bubbled through the solution. To this was added thiol **66** (1.1 equiv. per N-chloroacetyl functionality, 28.0 mg, 0.047 mmol) and the solution left stirring, under N_2, overnight at 25 °C. The solution was concentrated by lyophilization. The residue was then redissolved in ≈300 μL DMSO and precipitated with EtOAc to give off-white solid **157** in 84% yield (25.6 mg, 0.0045 mmol). NMR spectral data shows the incorporation of ethyl acetate in the dendrimer core; ^{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), 1.60-2.08 (m, 36H, β-CH_2's, H-3ax), 3.05 (dd, 8H, $J_{3eq,4}$ 7.1 Hz, $J_{3ax,3eq}$ 4.4 Hz, H-3eq), 2.98-3.40 (2m, 86H, α-CH_2's, γ-CH_2's, $NCH_2C(O)$'s, SCH_2's), 3.75 (s, 24H, CO_2CH_3), 3.76-3.95 (m, 24H, H-5, H-6, H-9), 4.17 (bd, 8H, H-9'), 4.71 (ddd, 8H, H-4), 5.15 (dd, 8H, H-7) 5.24 (m, 8H, H-8), 7.70 (m, 8H, N<u>H</u>Ac); ^{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), 37.2 (γ-C's), 37.4 (C-3), 45.5 (α-C's), 47.7 (C-5), 51.2 (N<u>C</u>H_2C(O)'s), 53.1 (MeO), 61.8 (C-9), 66.0 (C-7), 67.1 (C-8), 69.8 (C-4), 74.5 (C-6), 82.3 (C-2), 168.1-170.3 (C=O's).

Fully deprotected 3,3'-iminobis(propylamine)-based octavalent α-thiosialodendrimer (158).

To peracetylated glycodendrimer **157** (25.6 mg, 0.0045 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 8 h, MeOH was removed *in vacuo* and 0.05 M 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 **158** was isolated, after freeze-drying, as a white, spongy solid in 47% yield (9.0 mg, 0.0021 mmol); ^{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), 23.4 (β-C's), 32.5 (SCH_2), 40.1 (C-3), 51.2 (C-5), 53.0 (α-C's), 56.0 ($N\underline{C}H_2C(O)$), 62.3 (C-9), 67.8 (C-7), 68.2 (C-8), 71.3 (C-4), 74.5 (C-6).

Peracetylated 3,3'-iminobis(propylamine) hexadecavalent α-thiosialodendrimer (159).

N-Chloroacetylated dendrimer **138** (10.2 mg, 0.0025 mmol) was dissolved in 1% Et_3N/DMSO (5 mL) and N_2 bubbled through the solution. To this was added thiol **66** (1.1 equiv. per N-chloroacetyl functionality, 28.0 mg, 0.048 mmol) and the solution left stirring, under N_2, overnight at 25 °C. The solution was concentrated by lyophilization. The residue was then redissolved in ≈300 μL DMSO and precipitated with EtOAc to give off-white solid **159** in 76% yield (22.0 mg, 0.0019 mmol). NMR spectral data shows the incorporation of ethyl acetate in the dendrimer core; ^{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), 1.67-2.08 (m, 76H, β-CH_2's, H-3ax), 2.64 (dd, 16H, $J_{3eq,4}$ 12.6 Hz, $J_{3ax,3eq}$ 4.7 Hz, H-3eq), 3.00-3.46 (2m, 166H, α-CH_2's, γ-CH_2's, $NCH_2C(O)$'s, SCH_2's), 3.74 (s, 48H, CO_2CH_3), 3.74-3.82 (m, 48H, H-5, H-6, H-9), 4.18 (dd, 16H, $J_{8,9'}$ 11.8 Hz, $J_{9,9'}$ 2.5 Hz, H-9'), 4.70 (ddd, 16H, H-4), 5.15 (dd, 16H, H-7) 5.23 (m, 16H, H-8), 7.67 (m, 16H, $N\underline{H}Ac$); ^{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), 36.5 (γ-C's), 37.4 (C-3), 47.7 (C-5), 53.1 (MeO), 61.8 (C-9), 67.0 (C-7), 67.8 (C-8), 69.5 (C-4), 73.7 (C-6), 82.3 (C-2), 168.1-170.1 (C=O's).

Fully deprotected 3,3'-iminobis(propylamine)-based hexadecavalent α-thiosialodendrimer (160).

To peracetylated glycodendrimer **159** (22.0 mg, 0.0019 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 8 h, MeOH was removed *in vacuo* and 0.05 M 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 **160** was isolated, after freeze-drying, as a white, spongy solid in 53% yield (8.8 mg, 0.0010 mmol); ^{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), 23.0-23.6 (β-C's), 32.5 (SCH_2), 36.1 (γ-C's), 40.1 (C-3), 51.2 (C-5), 52.3 (α-C's), 55.6 ($N\underline{C}H_2C(O)$'s), 62.2 and 65.7 (C-9), 67.7 and 67.9 (C-7), 68.2 (C-8), 71.2 and 71.3 (C-4), 74.0 and 74.4 (C-6), 85.2 and 86.9 (C-2). 124.9-195.6 (C=O's).

Peracetylated tetravalent tethered 3,3'-iminobis(propylamine)-based α-thiosialodendrimer (161).

N-Chloroacetylated dendrimer **142** (20.0 mg, 0.026 mmol) was dissolved in 1% Et_3N/DMSO (5 mL) and N_2 bubbled through the solution. To this was added thiol **66** (1.1 equiv. per N-chloroacetyl functionality, 61 mg, 0.12 mmol) and the solution left stirring, under N_2, overnight at 25 °C. The solution was concentrated by lyophilization and then 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. Off-white solid **161** was prepared in 87% yield (60.0 mg, 0.023 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.26 (m, 4H, a-CH_2), 1.41 (m, 4H, b-CH_2), 1.65 (s, 12H, NAc), 1.66-2.10 (m, 12H, β-CH_2, H-3ax), 1.91, 1.96, 2.00, 2.01 (4s, 48H, OAc), 2.48 (m, 8H, α-CH_2), 2.67 (dd, 4H, H-3eq), 2.98-3.15 (m, 24H, γ-CH_2, c-CH_2, SCH_2, $NCH_2C(O)$), 3.74 (s, 12H, CO_2CH_3), 3.75-4.02 (m, 12H, H-5, H-6, H-9), 4.18 (dd, 4H, H-9'), 4.70 (ddd, 4H, H-4), 5.11-5.26 (m, 8H, H-7, H-8), 7.71 (d, 4H, $N\underline{H}Ac$), 8.16 (bs, 4H, NH amide), 8.76 (bs, 2H, NH amide).

Fully deprotected tetravalent tethered 3,3'-iminobis(propylamine)-based α-thiosialodendrimer (162).

To peracetylated glycodendrimer **161** (60.0 mg, 0.023 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C.

After 8 h, MeOH was removed *in vacuo* and 0.05 M 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 **162** was isolated, after freeze-drying, as a white, spongy solid in 71% yield (31.0 mg, 0.016 mmol); ^{1}H-NMR (D_2O) δ 1.41 (m, 4H, a-CH_2), 1.61 (m, 4H, b-CH_2), 1.90-2.09 (m, 12H, β-CH_2, H-3ax), 2.11 (s, 12H, NAc), 2.93 (dd, 4H, H-3eq), 3.04-4.00 (m, 60H, α-CH_2, γ-CH_2, c-CH_2, SCH_2, $NCH_2C(O)$ and NeuAc residues excluding above); ^{13}C-NMR (D_2O) (from HMQC) δ 21.6 (NAc), 23.1 (β-C), 26.1 (a-C), 29.3 (b-C), 38.6 (C-3).

Peracetylated hexavalent tethered 3,3'-iminobis(propylamine)-based α-thiosialodendrimer (163).

N-Chloroacetylated dendrimer **146** (15.0 mg, 0.013 mmol) was dissolved in 1% Et_3N/DMSO (5 mL) and N_2 bubbled through the solution. To this was added thiol **66** (1.1 equiv. per N-chloroacetyl functionality, 50.0 mg, 0.096 mmol) and the solution left stirring, under N_2, overnight at 25 °C. The solution was concentrated by lyophilization and then 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. Off-white solid **163** was prepared in 87% yield (46.0 mg, 0.011 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.65 (s, 18H, NAc), 1.66-2.11 (m, 18H, β-CH_2, H-3ax), 1.91, 1.96, 1.99, 2.08 (4s, 72H, OAc), 2.46 (m, 12H, α-CH_2), 2.55-2.60 (m, 6H, H-3eq), 3.00-3.30 (m, 42H, γ-CH_2, SCH_2, a-CH_2, b-CH_2, $NCH_2C(O)$), 3.74 (s, 18H, CO_2CH_3), 3.74-4.05 (m, 18H, H-5, H-6, H-9), 4.19 (dd, 6H, H-9'), 4.70 (ddd, 6H, H-4), 5.11-5.29 (m, 12H, H-7, H-8).

Fully deprotected hexavalent tethered 3,3'-iminobis(propylamine)-based α-thiosialodendrimer (164).

To peracetylated glycodendrimer **163** (46.0 mg, 0.011 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 8 h, MeOH was removed *in vacuo* and 0.05 M 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 **164** was isolated, after freeze-drying, as a white, spongy solid in 63% yield (21.0 mg, 0.0070 mmol); ^{1}H-NMR (D_2O) δ 2.00-2.10 (m, 18H, β-CH_2, H-3ax), 2.10 (s, 18H, NAc), 2.80-4.10 (m, 102H, α-CH_2, γ-CH_2, a-CH_2, b-CH_2, SCH_2, $NCH_2C(O)$ and NeuAc residues excluding above); ^{13}C-NMR (D_2O) (from HMQC) δ 21.6 (NAc), 23.7 (β-C), 38.2 (C-3).

Peracetylated octavalent tethered 3,3'-iminobis(propylamine)-based α-thiosialodendrimer (165).

N-Chloroacetylated dendrimer **149** (10.0 mg, 0.0057 mmol) was dissolved in 1% Et_3N/DMSO (5 mL) and N_2 bubbled through the solution. To this was added thiol **66** (1.1 equiv. per N-chloroacetyl functionality, 38.0 mg, 0.055 mmol) and the solution left stirring, under N_2, overnight at 25 °C. The solution was concentrated by lyophilization and then 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. Off-white solid **165** was prepared in 83% yield (26.2 mg, 0.0048 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.28-146 (2m, 8H, a-CH_2, b-CH_2), 1.65 (s, 24H, NAc), 1.66-2.10 (m, 32H, β-CH_2, H-3ax), 1.92, 1.97, 2.00, 2.08 (4s, 96H, OAc), 2.48 (m, 24H, α-CH_2), 2.66 (m, 8H, H-3eq), 2.90-3.30 (m, 56H, γ-CH_2, c-CH_2, SCH_2, $NCH_2C(O)$), 3.74 (s, 24H, CO_2CH_3), 3.75-4.05 (m, 24H, H-5, H-6, H-9), 4.10-4.20 (m, 8H, H-9'), 4.71 (m, 8H, H-4), 5.12-5.31 (m, 16H, H-7, H-8).

Fully deprotected octavalent tethered 3,3'-iminobis(propylamine)-based α-thiosialodendrimer (166).

To peracetylated glycodendrimer **165** (26.2 mg, 0.0048 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 8 h, MeOH was removed *in vacuo* and 0.05 M 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 **166** was isolated, after freeze-drying, as a white, spongy solid in 73% yield (14.0 mg, 0.0035 mmol); ^{1}H-NMR (D_2O) δ 1.40 (m, 4H, a-CH_2), 1.62 (m, 4H, b-CH_2), 2.00-2.10 (m, 56H, β-CH_2, H-3ax, NAc), 2.93 (dd, 8H, H-3eq), 3.20-4.10 (4m, 136H, α-CH_2, γ-CH_2, c-CH_2, SCH_2, $NCH_2C(O)$ and NeuAc residues excluding above); ^{13}C-NMR (D_2O) (from HMQC) δ 21.6 (NAc), 23.1 (β-C), 25.0 (a-C), 27.6 (b-C), 38.2 (C-3).

Peracetylated dodecavalent tethered 3,3'-iminobis(propylamine)-based α-thiosialodendrimer (167).

N-Chloroacetylated dendrimer **152** (10.0 mg, 0.0038 mmol) was dissolved in 1% Et_3N/DMSO (5 mL) and N_2 bubbled through the solution. To this was added thiol **66** (1.1 equiv. per N-chloroacetyl functionality, 38.0 mg, 0.055 mmol) and the solution left stirring, under N_2, overnight at 25 °C. The solution was concentrated by lyophilization and then 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. Off-white solid **167** was prepared in 85% yield (31.7 mg, 0.0032 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.65 (s, 36H, NAc), 1.66-2.11 (m, 48H, β-CH_2, H-3ax), 1.91, 1.96, 1.99, 2.08 (4s, 144H, OAc), 2.48 (m, 36H, α-CH_2), 2.70 (m, 12H, H-3eq), 3.00-3.38 (m, 90H, γ-CH_2, SCH_2, a-CH_2, b-CH_2, $NCH_2C(O)$), 3.74 (s, 36H, CO_2CH_3), 3.74-4.08 (m, 36H, H-5, H-6, H-9), 4.17 (m, 12H, H-9'), 4.70 (m, 12H, H-4), 5.11-5.28 (m, 24H, H-7, H-8).

Fully deprotected dodecavalent tethered 3,3'-iminobis(propylamine)-based α-thiosialodendrimer (168).

To peracetylated glycodendrimer **167** (31.7 mg, 0.0032 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 8 h, MeOH was removed *in vacuo* and 0.05 M 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 **168** was isolated, after freeze-drying, as a white, spongy solid in 56% yield (11.2 mg, 0.0018 mmol); ^{1}H-NMR (D_2O) δ 2.00-2.10 (m, 48H, β-CH_2, H-3ax), 2.10 (s, 36H, NAc), 2.97 (m, 12H, H-3eq), 3.38-4.10 (m, 210H, α-CH_2, γ-CH_2, a-CH_2, b-CH_2, SCH_2, $NCH_2C(O)$ and NeuAc residues excluding above); ^{13}C-NMR (D_2O) δ 21.7 (NAc), 23.2 (β-C), 38.7 (C-3), 84.2 (C-2).

Turbidimetric analysis between the lectin from *Limax flavus* and tetravalent 3,3'-iminobis(propylamine)-based α-thiosialodendrimer (156).

Turbidimetry experiments were performed in Linbro (Titertek) microtitration plates where 50 μL/well of stock lectin solutions prepared from *Limax flavus* (1 mg/mL PBS) were mixed with 50 μL of glycodendrimer **156** (0.5 mg/mL PBS) and incubated at room temperature for up to 3 hours. The turbidity of the solutions was monitored by reading the optical density (O.D.) at 490 nm at regular time intervals until no noticeable changes could be observed. Each test was performed in triplicate.

Competitive inhibition ELLA using human α_1-acid glycoprotein and 3,3'-iminobis(propylamine)-based α-thiosialodendrimers 154, 156, 158, 160, 162, 164, 166, and 168 as inhibitors.

Linbro (Titertek) microtitration plates were coated with human α_1-acid glycoprotein (orosomucoid) at 100 μL/well of a stock solution of 5 μg/mL in 0.01 M phosphate buffer (pH 7.3) overnight. The wells were then washed three times with 300

μL/well of 0.01 M phosphate buffer (pH 7.3) containing 0.05% (v/v) Tween 20 (PBST). Similar washings with PBST was repeated after each incubation period. Wells were then blocked with 150 μL/well of 1% BSA/PBS for one hour at 37 °C. After washing, the wells were filled with 100 μL/well of inhibitor solutions and incubated again at 37 °C for one hour. Inhibitors used include 5-acetamido-5-deoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosonyl azide (NeuAcαN_3) as a reference monovalent compound, divergent glycodendrimers **154**, **156**, **158**, **160** and spherical glycodendrimers **162**, **164**, **166**, **168**. Each inhibitor was added in serial two-fold dilutions (60 μL/well) in PBS with the appropriate lectin-enzyme conjugate concentration (60 μL/well of 100-fold dilution of a 1 mg/mL stock solution of *Limax flavus* in PBS) on Nunclon (Delta) microtiter plates and incubated at 37 °C for one hour. These inhibitor solutions (100 μL) were transferred to the antigen coated plates and incubated for a second hour. The plates were washed and 50 μL/well of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg/mL) in citrate-phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 minutes by adding 50 μL/well of 1 M H_2SO_4 and the optical density measured at 410 nm relative to 570 nm. Per cent inhibition was calculated as follows:

$$\% \text{ inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})})/A_{(\text{no inhibitor})} \times 100$$

IC_{50}'s were reported as the concentration required for 50% inhibition of the coating antigen. Each test was performed in triplicate.

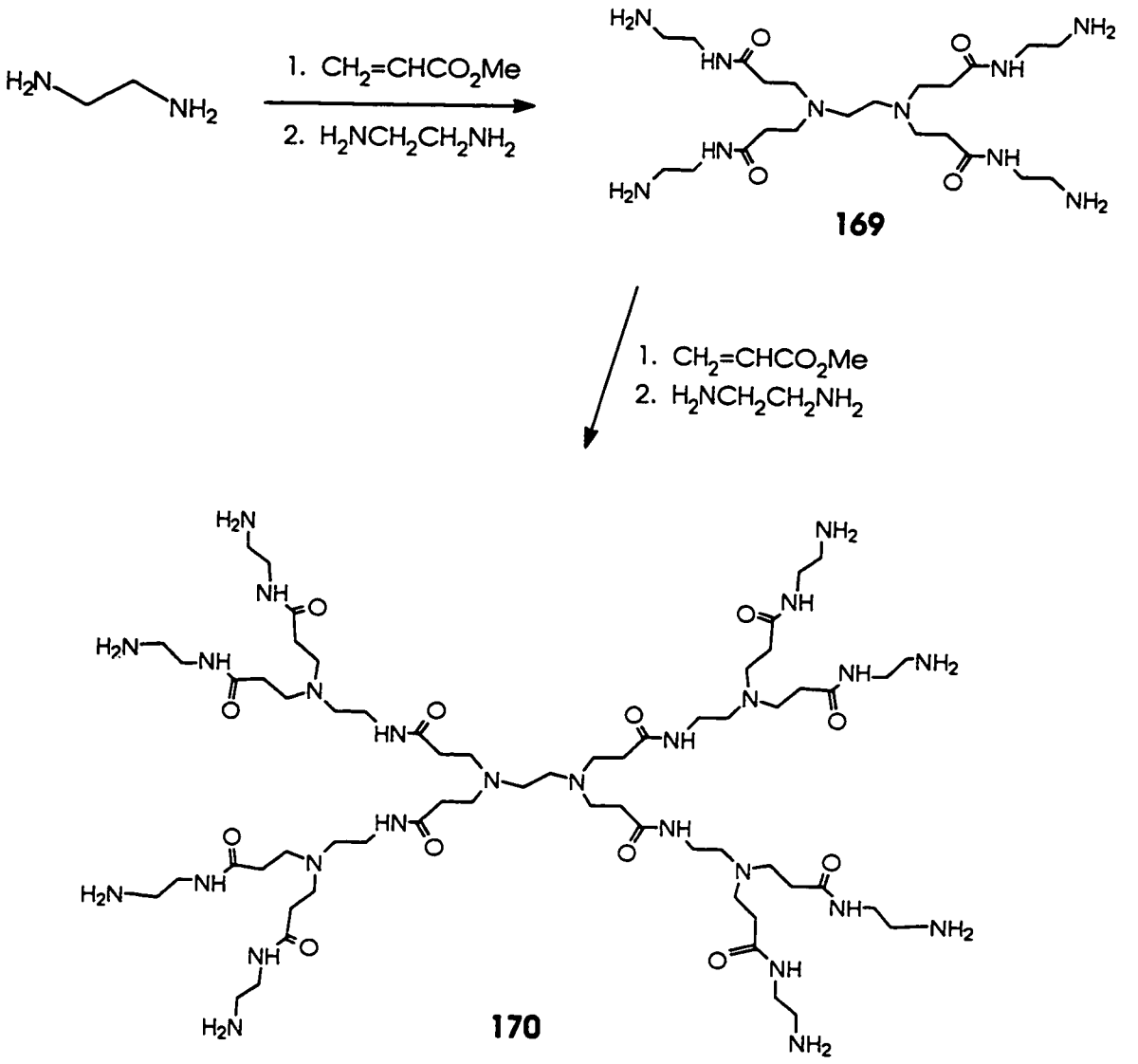
Chapter 5. Synthesis of PAMAM-Based Glycodendrimers

5.1. Introduction

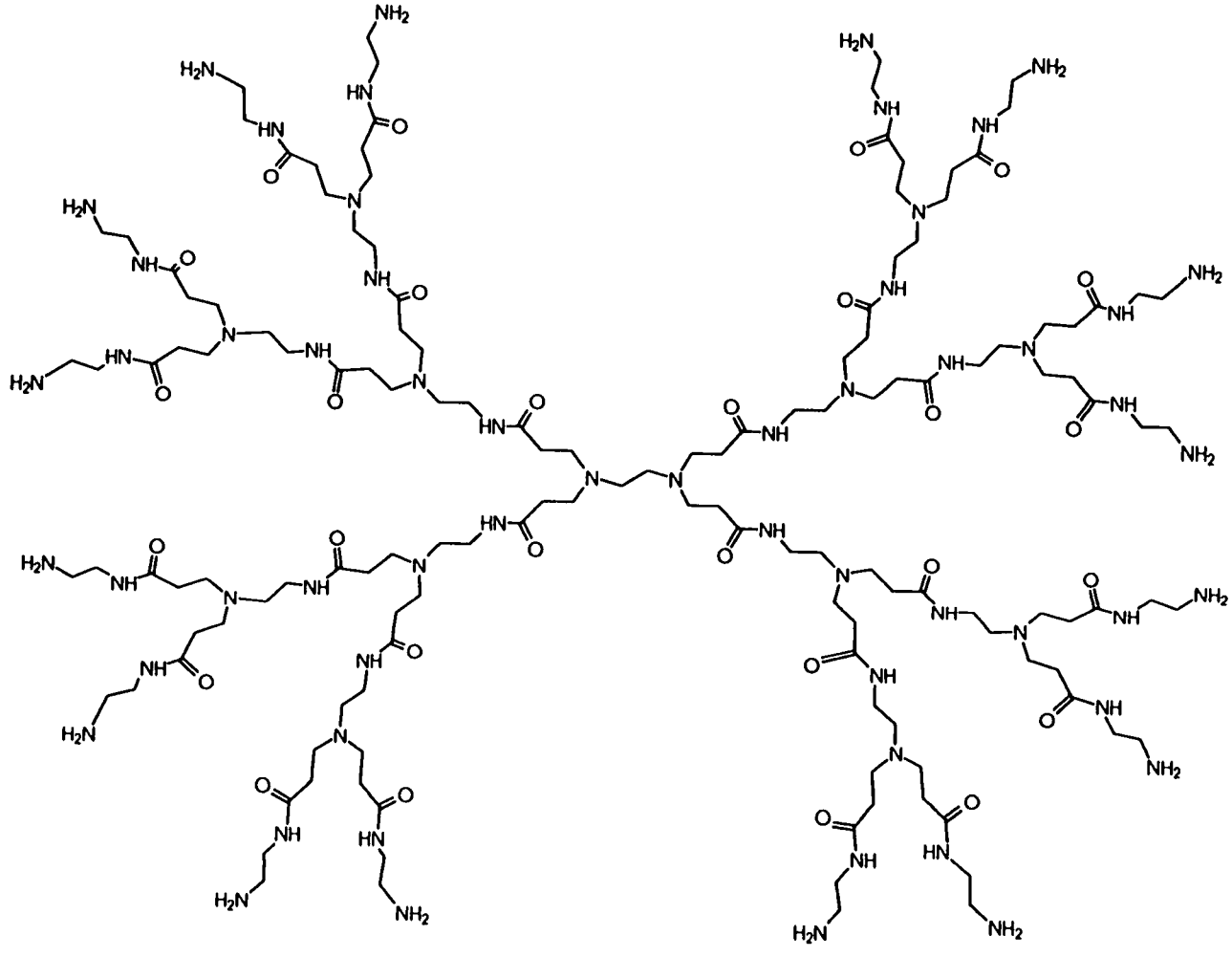
Since the reported synthesis of the L-lysine-based glycodendrimers,[105,106] other groups have looked to the generation of glycodendrimers. Ashton *et al.*[112] have prepared nona- and octadeca-valent dendrimers bearing D-glucose residues. Other groups have attached various carbohydrate derivatives to Starburst® PAMAM dendrimers. To date, disaccharide lactones of lactose and maltose have been conjugated to PAMAM dendrimers.[108] A T_N-antigen peptide[109] has been coupled to the amine terminated dendrimers *via* amide bond formation. Lastly, an isothiocyanate coupling strategy[110] has been employed to conjugate PAMAM dendrimers to α- and β-D-mannose, β-D-glucose, β-cellobiose, and β-lactose isothiocyanate derivatives (see Chapter 1, Section 1.6).

PAMAM based glycodendrimers incorporating sialic acid residues have not been reported. In keeping with the design of multivalent sialosides and for comparison purposes to previously described α-thiosialodendrimers (Chapters 3 and 4), they are presented herein.

Spherical Starburst PAMAM dendrimers[98,99] are made of poly(amidoamine) and are commercially available. They represent the first successful synthesis of spherical, highly branched dendrimers to generation ten with a defined number of amine surface groups. The synthesis starts with the conjugate addition of ammonia or ethylene diamine to methyl acrylate followed by reaction with excess ethylenediamine to give spherical, hyperbranched structures in a divergent manner (Scheme 5.1.1). PAMAM dendrimers with 16 (**171**) and 32 (**172**) surface amine moieties are pictured in Figures 5.1.1 and 5.1.2.

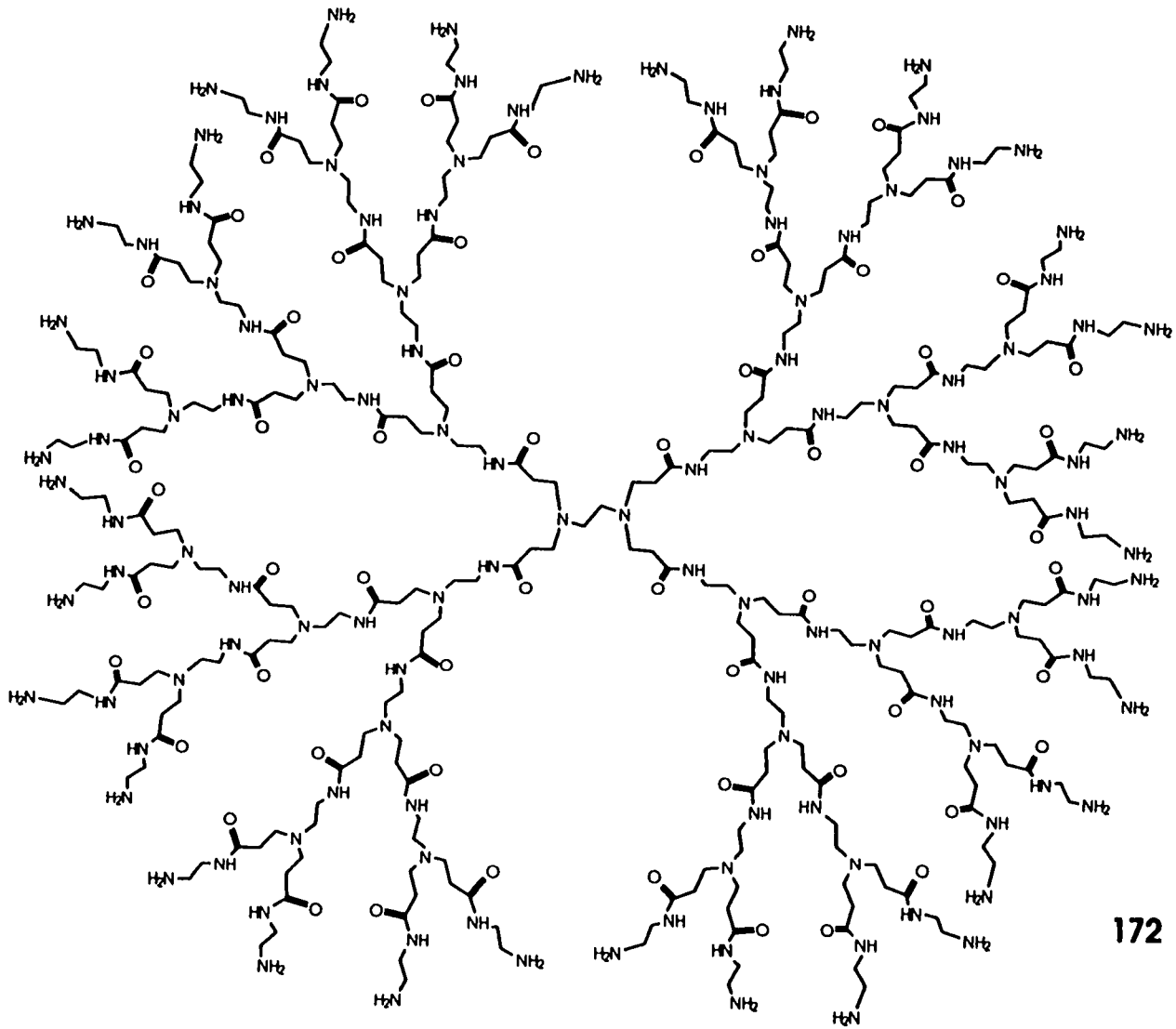


Scheme 5.1.1. Synthesis of spherical Starburst® PAMAM dendrimers.



171

Figure 5.1.1. Hexadecavalent amine-terminated PAMAM dendrimer.



172

Figure 5.1.2. Starburst® PAMAM dendrimer with 32 surface amine groups.

5.2. Conjugation of Sialic Acid to PAMAM Core

Amine terminated PAMAM cores **169-170** were conjugated to sialic acid isothiocyanate derivative **70** to give polythiourea derivatives. This alternate approach to carbohydrate-dendrimer conjugation allowed minimal manipulation of the dendritic cores and has been previously shown to be useful (see Chapter 2).[110,172]

Commercially available methanolic solutions of PAMAM dendrimers **169-172** were concentrated *in vacuo*. CH_2Cl_2 was then added and co-evaporated with any residual MeOH, three times, under reduced pressure. The resulting residues were dissolved in CH_2Cl_2 for **169** or DMF for **170-172**. Diisopropylethylamine (DIPEA, 1 eq. per amine functionality) was then added to the solution. Sialyl isothiocyanate derivative **70** (1.2 eq. per amine moiety) in CH_2Cl_2 was added dropwise to the solutions which were stirred overnight at room temperature (Schemes 5.2.1 to 5.2.4). The reaction mixtures were concentrated *in vacuo* and the residues were dialyzed against a mixture of DMSO and H_2O (1:1, v/v, MW cutoff 2 kDa). PAMAM-based α-thiosialodendrimers **173**, **175**, **177**, and **179** were obtained in excellent yields (71 to 100%) after dialysis.

Deprotection of sialodendrimers **173**, **175**, **177**, and **179** by sequential ester hydrolysis ((*i*) NaOMe/MeOH; (*ii*) 0.05 M NaOH) followed by dialysis against DMSO:H_2O (1:1, v/v, MW cutoff 2 kDa) afforded fully deprotected spherical dendrimers **174**, **176**, **178**, and **180** with four, eight, sixteen, and thirty-two NeuAc residues, respectively (59-93%).

The ^{1}H-NMR spectra confirms the total incorporation of the sialic acid residues (±2-3%). Key signals include the b-CH_2 of the dendritic core at δ 2.28 ppm and the NAc, H-3eq, and H-4 of the NeuAc moieties at δ 1.65, 2.70, and 4.69 ppm in DMSO-d_6, respectively (Figure 5.2.1).

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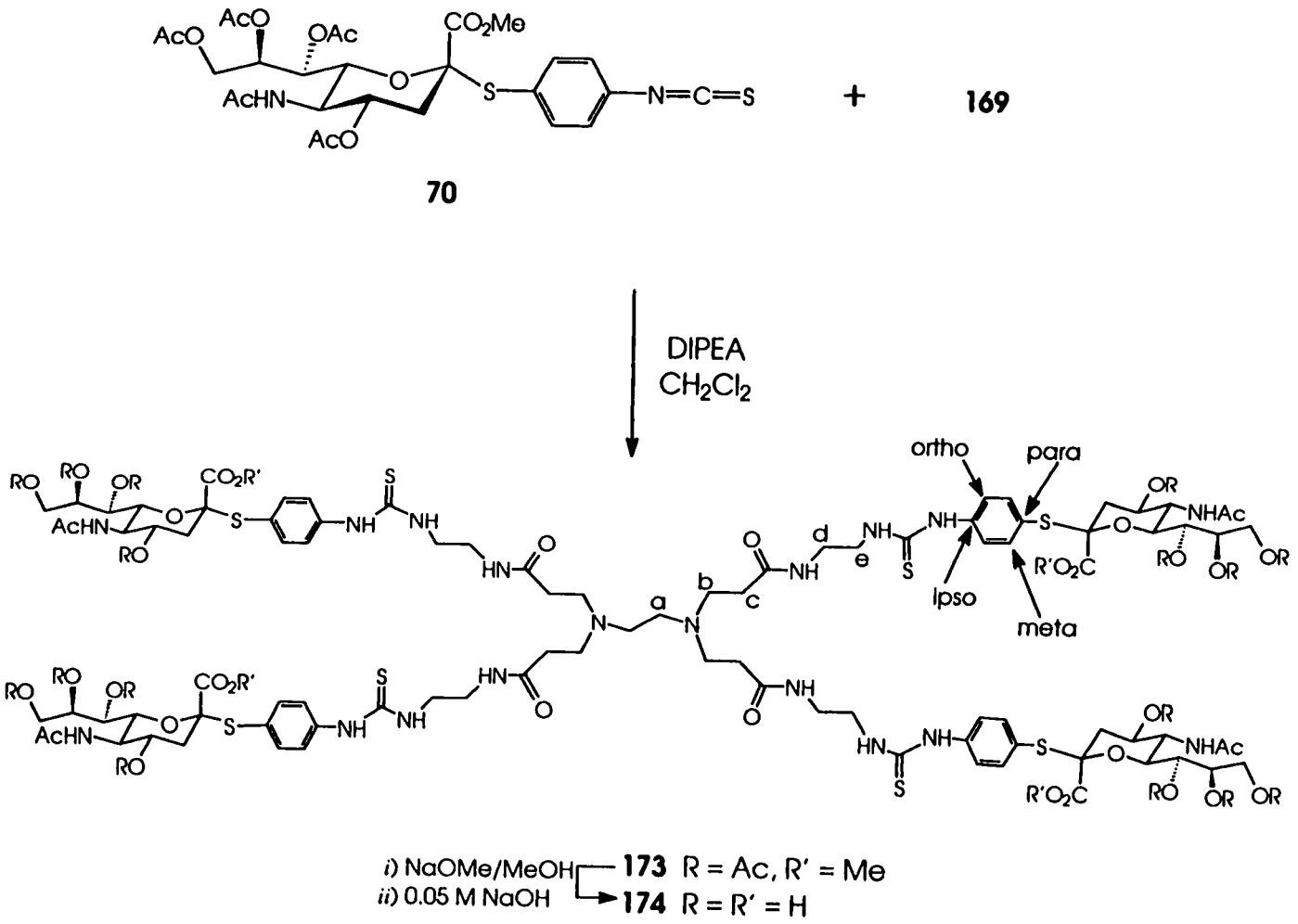
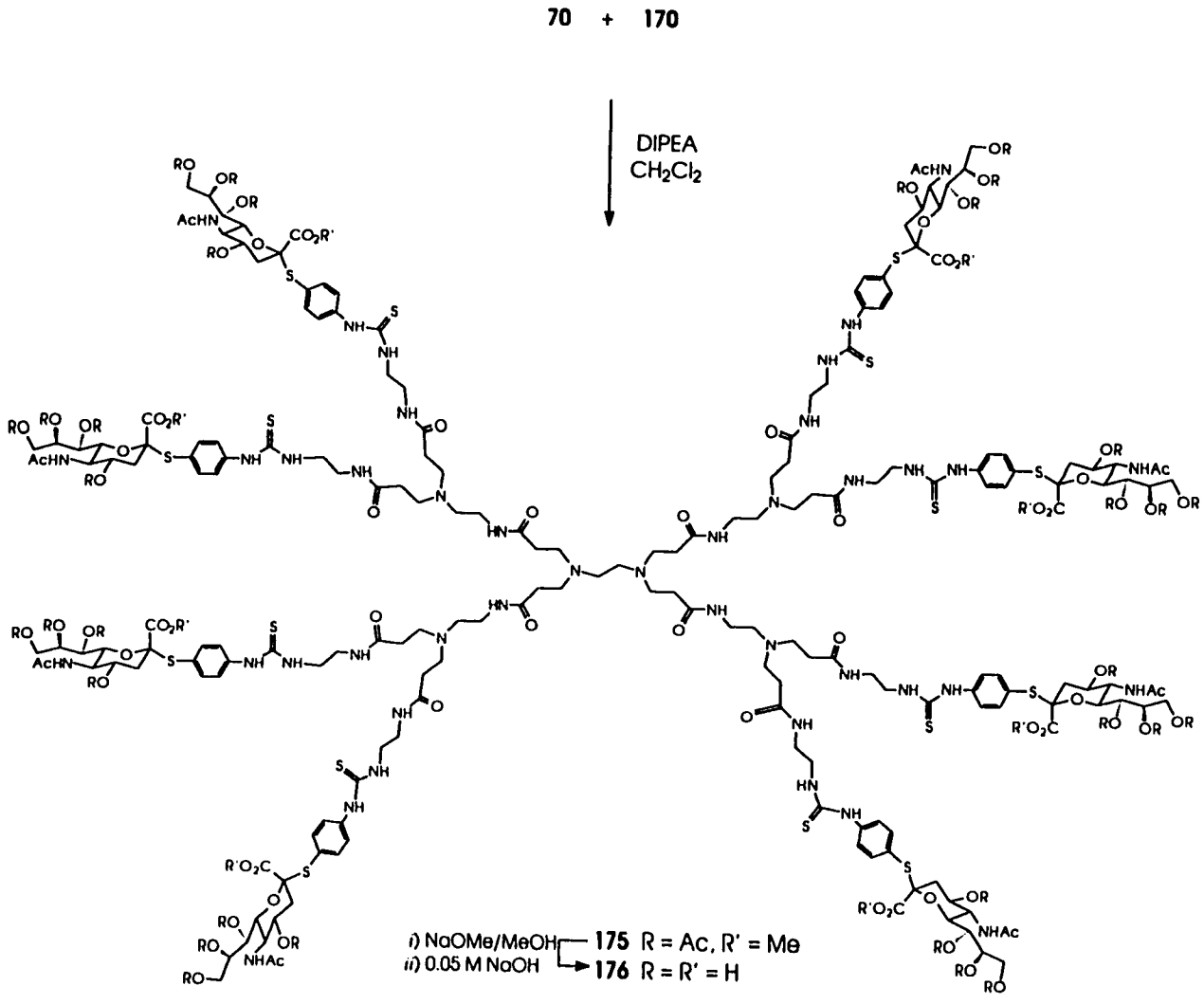
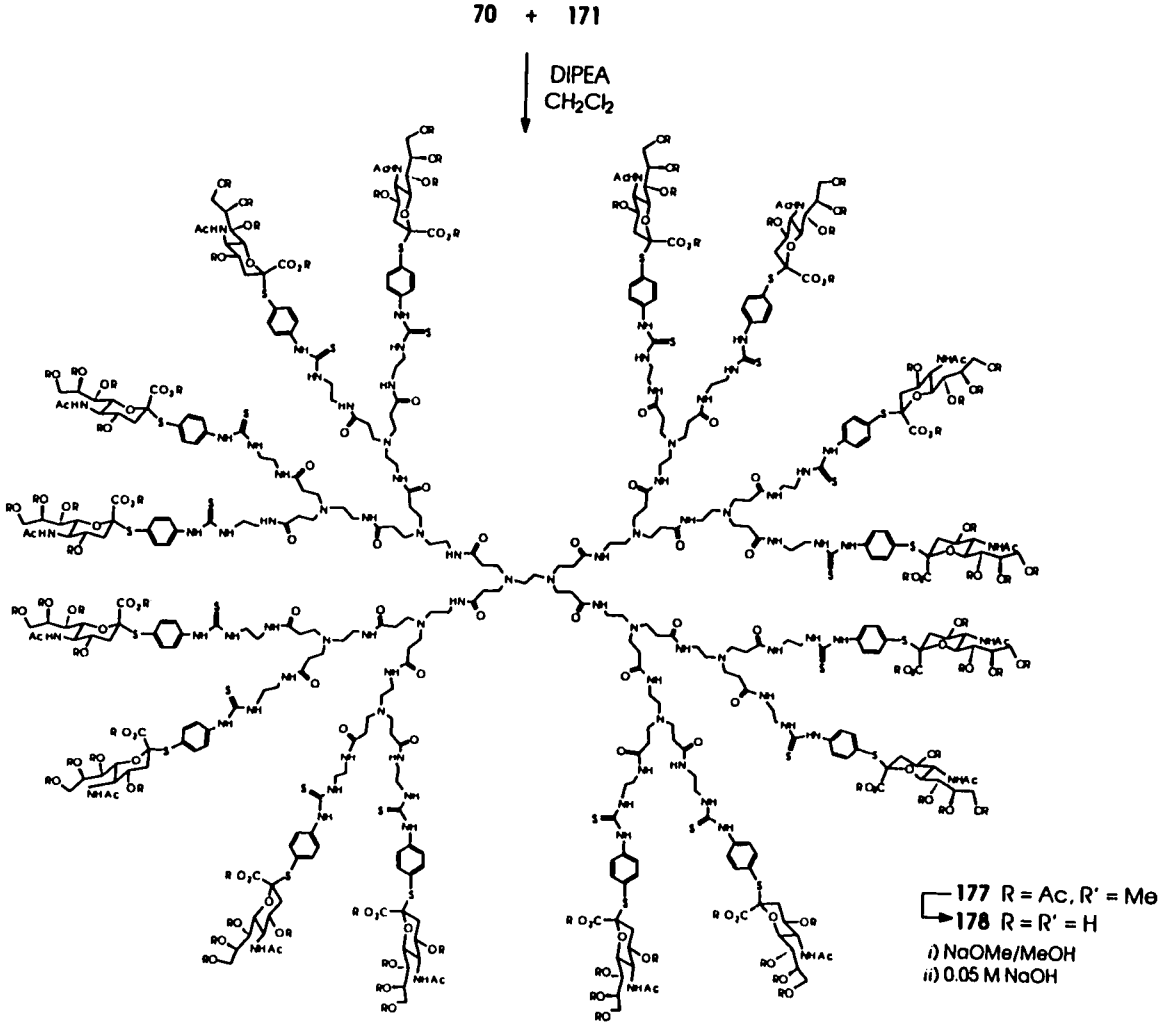
[172] Pagé, D.; Aravind, S.; Roy, R. *Chem. Commun.* **1996**, 1913.



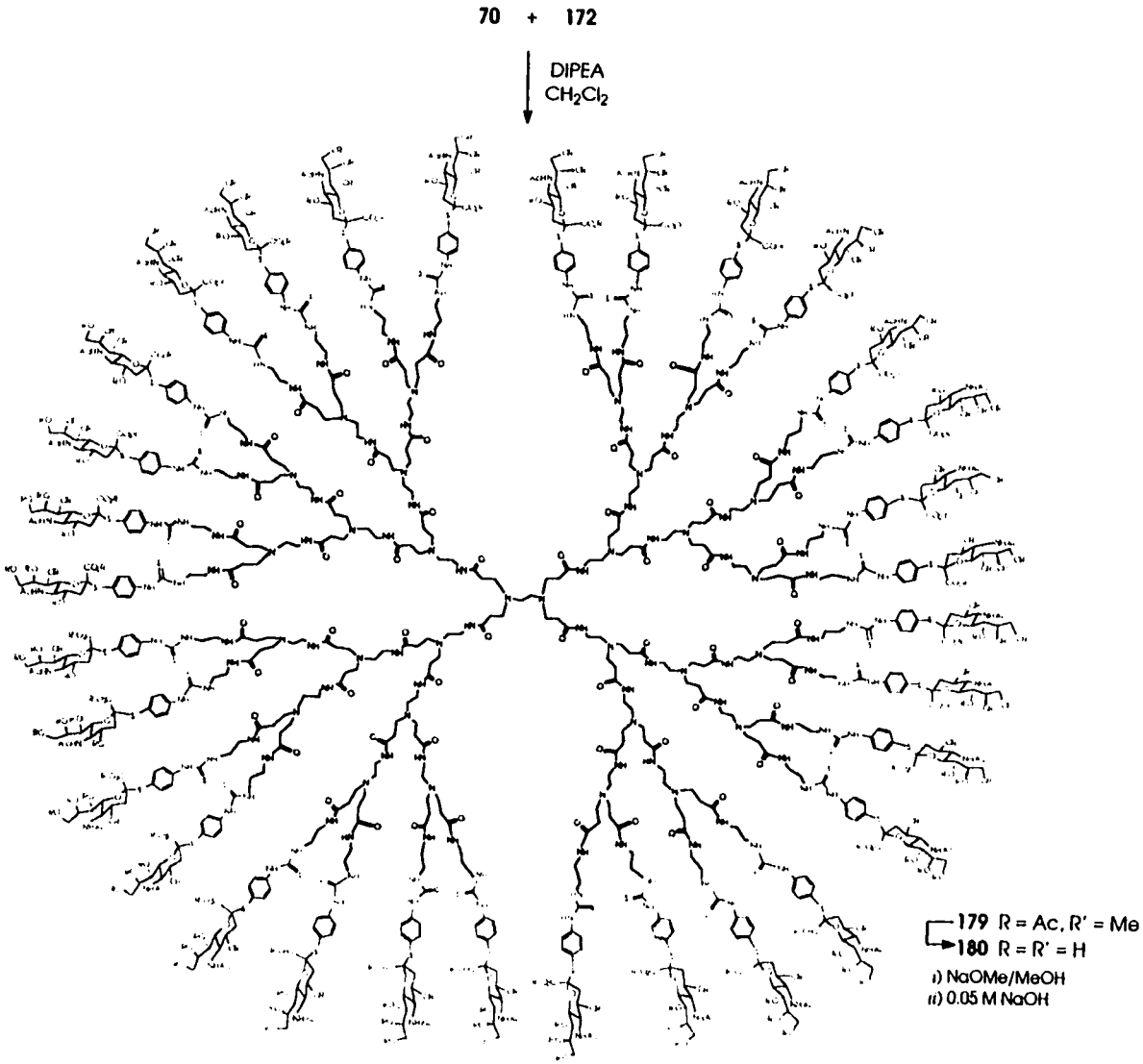
Scheme 5.2.2. Synthesis of tetravalent PAMAM-based α-thiosialodendrimer.



Scheme 5.2.3. Synthesis of octavalent PAMAM-based α-thiosialodendrimer.



Scheme 5.2.4. Synthesis of hexadecavalent PAMAM-based α-thiosialodendrimer.



Scheme 5.2.5. Synthesis of PAMAM-based dendrimer containing 32 sialic acid residues.

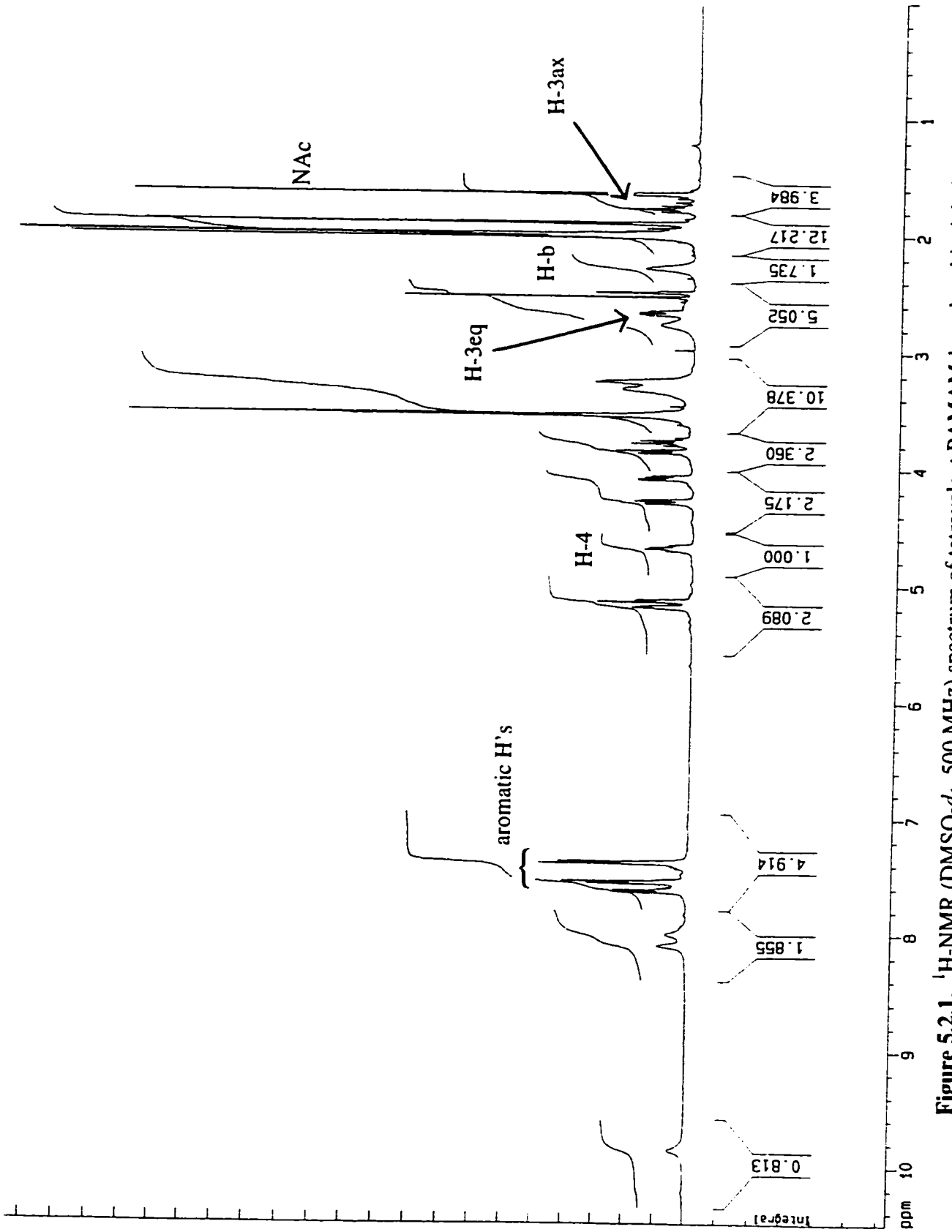


Figure 5.2.1. ^{1}H-NMR (DMSO-d_6, 500 MHz) spectrum of tetravalent PAMAM-based α-thiosialodendrimer **173**.

5.3. Immunochemical Assays

To demonstrate the ability of these α-thiosialodendrimers to bind to the lectin from *Limax flavus* (LFA), turbidimetric analysis was initially performed. The time course formation of insoluble precipitin complexes between LFA and glycodendrimer **180** (with 32 NeuAc residues) is illustrated in Figure 5.3.1. Maximum turbidity was reached after only 15 minutes. These micro-quantitative precipitation experiments confirmed the direct binding and cross-linking properties of the PAMAM-based α-thiosialodendrimers with LFA.

The efficiency of PAMAM-based α-thiosialodendrimers **174**, **176**, **178**, and **180** to inhibit the binding of horseradish-peroxidase labeled *Limax flavus* lectin (LFA) to human α_1-acid glycoprotein (orosomucoid) was determined by enzyme linked lectin

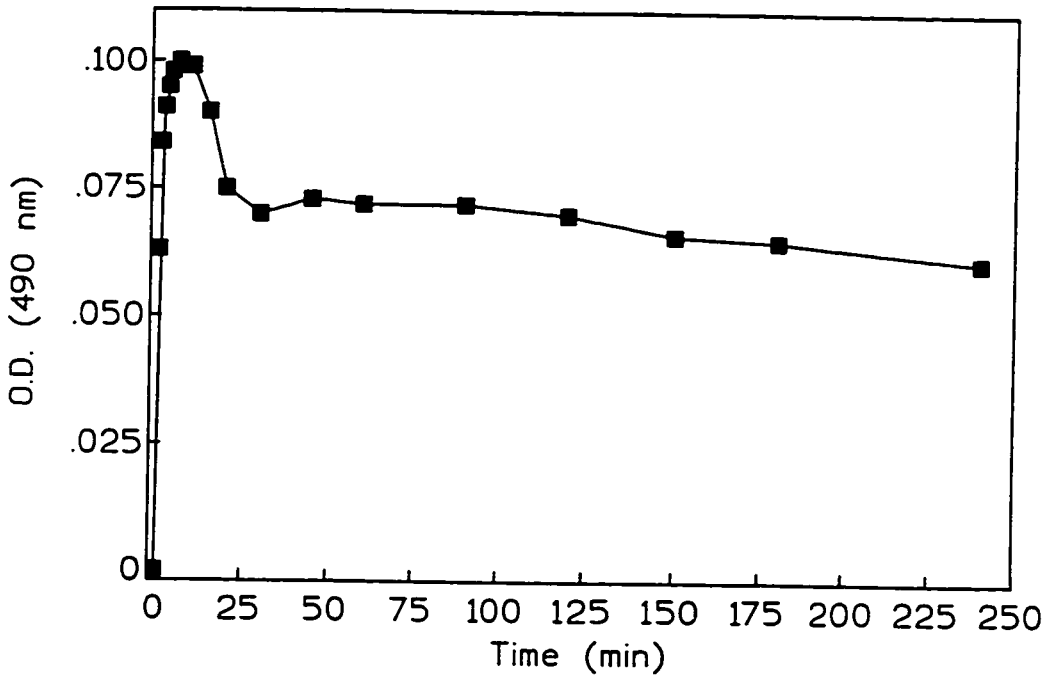


Figure 5.3.1. Time course of turbidimetric analysis of LFA with PAMAM-based α-thiosialodendrimer **180** containing 32 NeuAc surface residues.

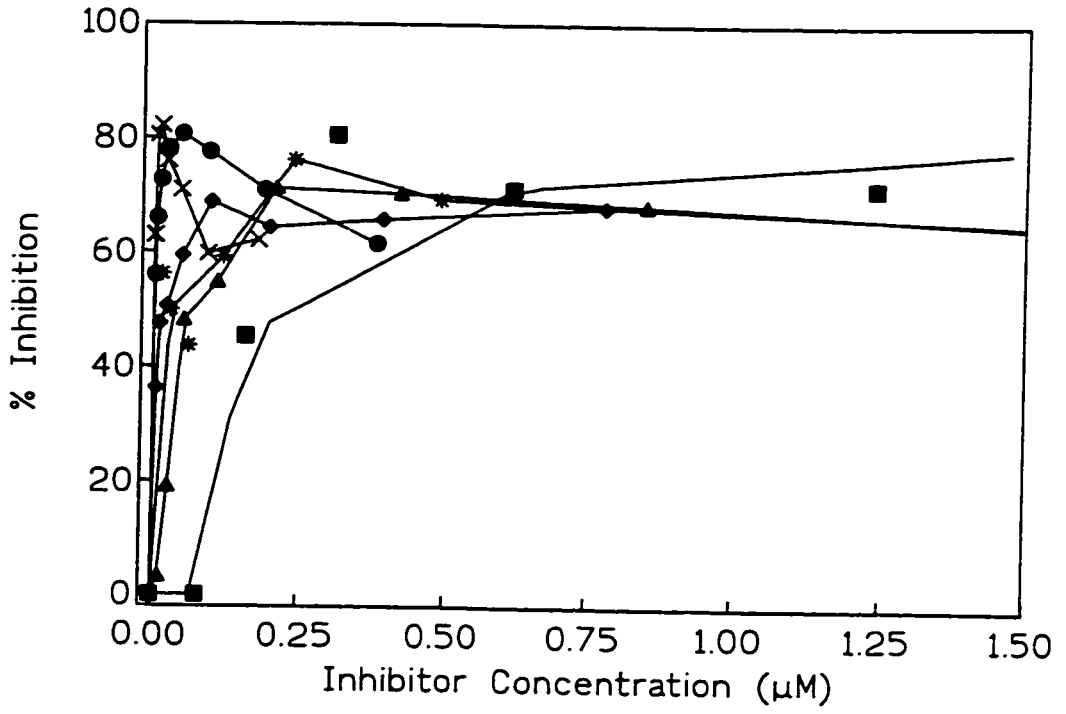


Figure 5.3.2. Inhibition of binding of LFA to human α_1-acid glycoprotein (orosomucoid) by phenylthio α-sialoside (■), PAMAM-based glycodendrimers **174** (▲), **176** (◆), **178** (●), **180** (×), and 3,3'-iminobis(propylamine)based glycodendrimer **156** (✳).

assays (ELLA) as for the 3,3'-iminobis(propylamine)-based α-thiosialodendrimers (Chapter 4). The results for the inhibition of binding of LFA to human α_1-acid glycoprotein are shown in Figure 5.3.2. Phenylthio α-sialoside[70] was used as the monovalent standard.

In this spherical α-sialodendrimer series, an increase in multivalency resulted in a steady increase in inhibitory potential. Glycodendrimers with valencies of four (**174**), eight (**176**), sixteen (**178**), and thirty-two (**180**) exhibited IC_{50}'s of 69.2, 21.5, 2.89, and 1.13 nM, respectively (277, 172, 46.2, and 36.2 nM on a per sialoside residue basis, respectively, Table 5.3.1). This represents a 210 fold (6.7 fold/sialoside) increase in inhibitory potential over the monomeric analog. Once again, an increase in the binding

between carbohydrates and proteins may be attributed to an increase in multivalency. This is directly evident in a plot of IC_{50} as a function of dendrimer valency and/or a plot of relative potency versus dendrimer valency (Figures 5.3.3 and 5.3.4).

It should be noted that when compared to the tetravalent 3,3'-iminobis(propylamine)-based α-thiosialodendrimer **156**, in the same set of experiments, tetravalent PAMAM-based α-thiosialodendrimer **174** exhibited a higher IC_{50} value. That is, glycodendrimer **174** was a poorer inhibitor of the binding between LFA and human α_1-acid glycoprotein than the tetravalent 3,3'-iminobis(propylamine)-based **156**. However, in higher generations, the PAMAM-based α-thiosialodendrimers appear to be better than the similar 3,3'-iminobis(propylamine)-based dendrimers. The higher generation

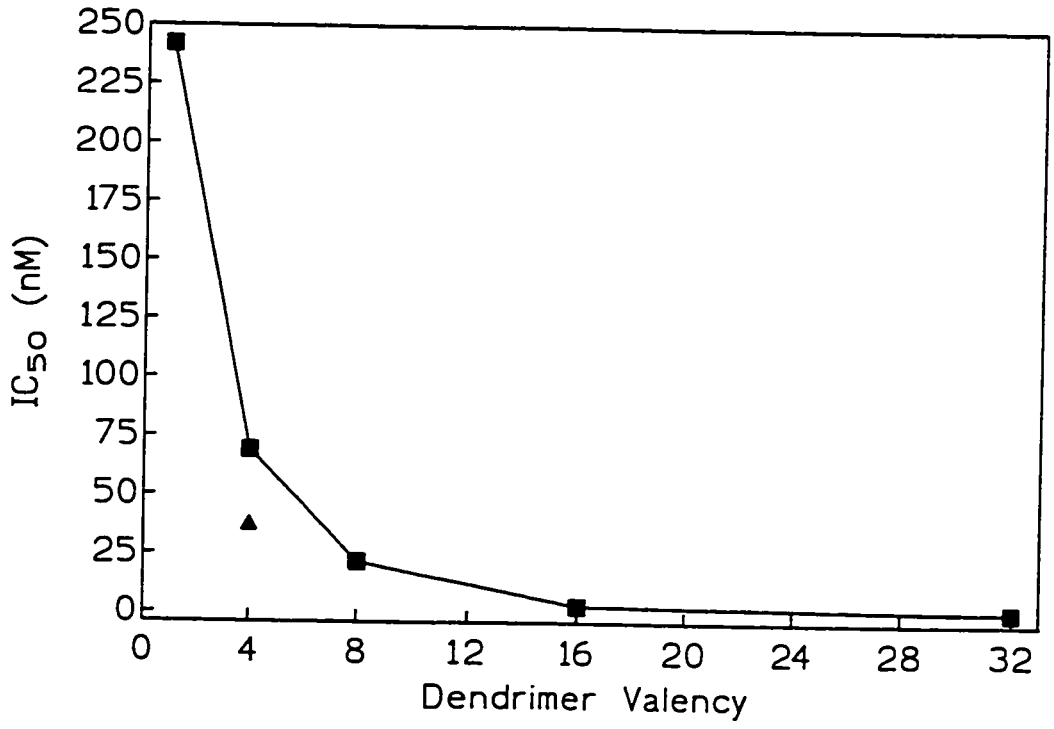


Figure 5.3.3. Effect of dendrimer valency on the inhibition of human α_1-acid glycoprotein (orosomucoid) and LFA/HRP; PAMAM-based series **174**, **176**, **178**, and **180** (■), and 3,3'-iminobis(propylamine)-based α-thiosialodendrimer **156** (▲). Data taken from Figure 5.3.2.

Table 5.3.1. Inhibition of Binding of Human α_1-Acid Glycoprotein to *Limax Flavus* by Sialodendrimers.

Compound	IC_{50} (nM)[a]	Relative Potency[a]
Phenylthio α-sialoside	242	1
PAMAM-based 4mer **174**	69.2 (277)	3.5 (0.87)
PAMAM-based 8mer **176**	21.5 (172)	11 (1.4)
PAMAM-based 16mer **178**	2.89 (46.2)	84 (5.2)
PAMAM-based 32mer **180**	1.13 (36.2)	210 (6.7)
3,3'-iminobis(propylamine)-based 4mer **156**	37.2 (149)	6.5 (1.6)

[a]Values in parentheses are based on a per sialoside residue.

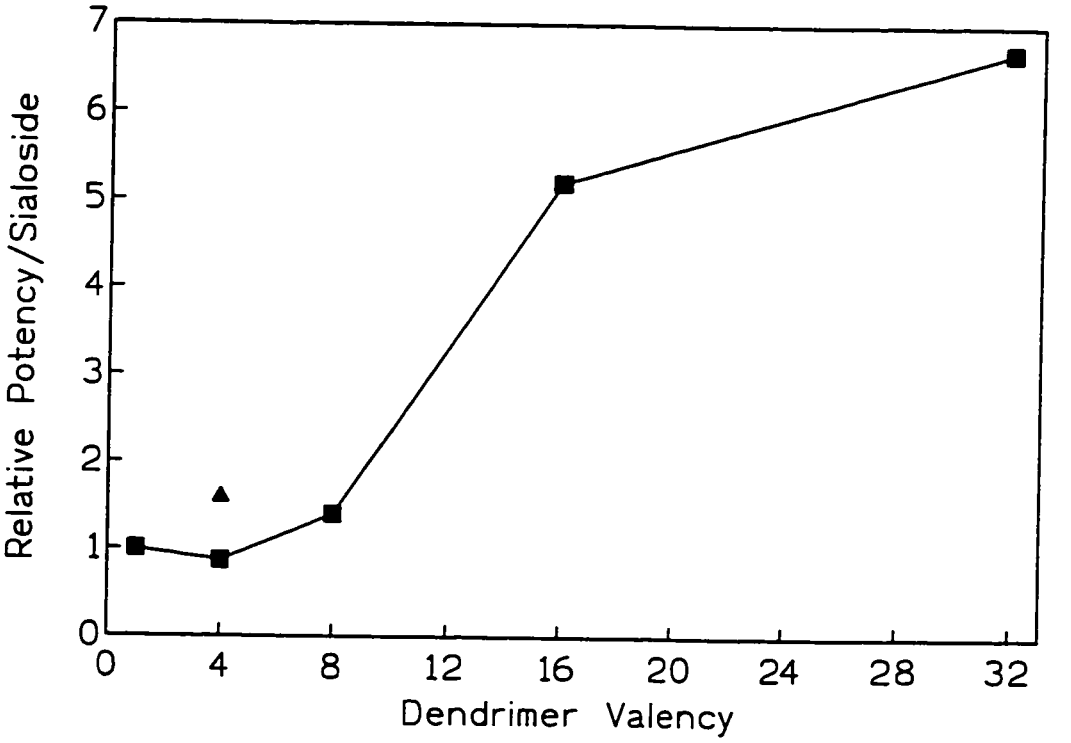


Figure 5.3.4. Relative potency per sialoside as a function of dendrimer valency for PAMAM-based dendrimers **174**, **176**, **178**, and **180** (■), and 3,3'-iminobis(propylamine)-based α-thiosialodendrimer **156** (▲). Data taken from Figure 5.3.2.

PAMAM-dendritic cores synthesized here seem to have structural organizations more suitable to the inhibition of the binding between LFA and human α_1-acid glycoprotein than the previous structures described in Chapter 4.

5.4. Conclusions

α-Thiosialoside containing dendrimers scaffolded on amine terminated Starburst® PAMAM dendrimers were synthesized using an isothiocyanate conjugation strategy. Generations G0 through G3 were prepared containing four, eight, sixteen, and thirty-two sialic acid residues. The potential of these sialodendrimers to cross-link and precipitate *Limax flavus* lectin (LFA) was confirmed by turbidimetric analysis. When tested in ELLA using human α_1-acid glycoprotein (orosomucoid) as coating antigen and horseradish-peroxidase labeled LFA for detection, glycodendrimers showed improved inhibitory potentials. At the second generation, the 3,3'-iminobis(propylamine)-based divergent series seems to be a better inhibitor of the carbohydrate-protein interaction studied. However, at higher generations, these spherical, PAMAM-based α-thiosialodendrimers appear to have structural organizations and/or aglycon spacer requirements more suitable than the previously tested 3,3'-iminobis(propylamine) dendrimers for the solid phase inhibition of the binding of human α_1-acid glycoprotein to LFA. These results confirm that an amplification in carbohydrate-protein interactions is related to an increase in the valency of sugar residues in neoglycoconjugates.

5.5. Experimental Methods

Peracetylated tetravalent PAMAM-based α-thiosialodendrimer (173).

A methanolic solution of amine terminated tetravalent Starburst® PAMAM dendritic core **169** (23.0 mg of a 36.02% (w/w) solution in MeOH, 0.016 mmol,

Dendritek® (MI)) was evaporated under reduced pressure. The resulting residue was redissolved in CH_2Cl_2 (5 mL) and the solution re-evaporated. This was repeated three times. Compound **169** was then dissolved in CH_2Cl_2 (5 mL) and to this was added diisopropylethylamine (DIPEA, 8.3 mg, 0.064 mmol) and the solution was stirred at room temperature. Sialic acid isothiocyanato derivative **70** (50.0 mg, 0.078 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise to the stirred solution and the reaction left at 25 °C. After 20 h, the reaction mixture was concentrated *in vacuo* and then dialyzed against 1:1/DMSO:H_2O (MW cutoff 2000 Da). The resulting solution was lyophilized to give tetravalent PAMAM-based α-thiosialodendrimer **173** as an off-white solid. Title compound **173** was isolated in 94% yield (47.2 mg, 0.015 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.65 (s, 12H, NAc), 1.77 (dd, 4H, J 12.1 Hz, H-3ax), 1.90, 1.99, 2.00, 2.02 (4s, 48H, OAc's), 2.28 (bs, 8H, b-CH_2), 2.52-2.99 (3m, 16H, a-CH_2, c-CH_2, H-3eq), 3.25 (m, 8H, d-CH_2), 3.52-3.60 (m, 8H, e-CH_2), 3.54 (s, 12H, OCH_3), 3.59-3.79 (2m, 8H, H-5, H-6), 4.09 (dd, 4H, $J_{8,9}$ 6.0 Hz, $J_{9,9'}$ 12.2 Hz, H-9), 4.28 (dd, 4H, $J_{8,9'}$ 2.5 Hz, H-9'), 4.69 (ddd, 4H, H-4), 5.13 (m, 4H, H-7), 5.19 (m, 4H, H-8), 7.38 (d, 8H, $J_{ortho,meta}$ 8.2 Hz, H-ortho), 7.54 (d, 8H, H-meta), 7.62 (d, 4H, $J_{5,NHAc}$ 9.5 Hz, N*H*Ac), 8.00 (bs, 4H, N*H*C(S)NHPh), 8.05 (bs, 4H, amide NH's), 9.86 (bs, 4H, NHC(S)N*H*Ph); ^{13}C-NMR (DMSO-d_6) δ 20.6, 20.8 (OAc's), 22.6 (NAc), 32.4 (C-b), 37.7 (C-3), 37.8 (C-d), 40.5 (C-a), 43.4 (C-e), 47.7 (C-5), 48.8 (C-c), 52.8 (OCH_3), 61.7 (C-9), 67.4 (C-7), 68.9 (C-8), 69.5 (C-4), 74.1 (C-6), 87.3 (C-2), 122.0 (C-meta), 122.2 (C-para), 136.4 (C-ortho), 141.3 (C-ipso), 167.8-170.1 (C=O's), 180.4 (C=S).

Fully de-protected PAMAM-based tetravalent α-thiosialodendrimer (174).

To peracetylated glycodendrimer **173** (47.2 mg, 0.015 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 1 h, MeOH was evaporated under reduced pressure and 0.05 M NaOH (5 mL) added. After 1.5 h, the solution was neutralized with 1 M HCl and the solvent removed by lyophilization. The resulting residue was purified by dialysis against 1:1/DMSO:H_2O

(MW cutoff 2000 Da) and then freeze-dried. Title compound **174** was isolated as a white, spongy solid in 80% yield (28.9 mg, 0.012 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.66 (dd, 4H, J 11.9 Hz, H-3ax), 1.84 (s, 12H, NAc), 2.25-2.59 (m, 12H, b-CH_2, H-3eq), 2.63-3.00 (m, 12H, a-CH_2, c-CH_2), 3.05-3.60 (m, 44H, d-CH_2, e-CH_2, and NeuAc residues excluding above), 4.27, 4.59, 4.72, 5.18 (4bs, 16H, OH's), 7.22-8.15 (4m, 28H, amide NH's, N*H*C(S)NHPh, H-ortho, H-meta, NHAc), 9.88-10.20 (4bs, 4H, NHC(S)N*H*Ph); ^{13}C-NMR (DMSO-d_6) δ 22.6 (NAc), 37.8 (C-d), 40.4 (C-3), 40.7 (C-a), 42.1 (C-c), 43.2 (C-e), 51.8 (C-5), 63.2 (C-9), 66.8 (C-7), 68.7 (C-8), 71.3 (C-4), 76.1 (C-6), 86.5 (C-2), 121.5, 122.2 (C-meta, C-para), 136.2 (C-ortho), 141.5 (C-ipso), 169.2-172.1 (C=O's), 180.4 (C=S).

Peracetylated octavalent PAMAM-based α-thiosialodendrimer (175).

A methanolic solution of amine terminated octavalent Starburst® PAMAM dendritic core **170** (41.7 mg of a 27.89% (w/w) solution in MeOH, 0.0081 mmol, Dendritek® (MI)) was evaporated under reduced pressure. The resulting residue was combined with CH_2Cl_2 (5 mL) and the solution re-evaporated. This was repeated three times. Compound **170** was then dissolved in DMF (5 mL) and to this was added DIPEA (8.3 mg, 0.064 mmol) and the solution was stirred at room temperature. Sialic acid isothiocyanato derivative **70** (50.0 mg, 0.078 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise to the stirred solution and the reaction left at 25 °C. After 20 h, the reaction mixture was concentrated *in vacuo* and then dialyzed against 1:1/DMSO:H_2O (MW cutoff 2000 Da). The resulting solution was lyophilized to give octavalent PAMAM-based α-thiosialodendrimer **175** as an off-white solid. Title compound **175** was isolated in 71% yield (37.6 mg, 0.0058 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.64 (s, 24H, NAc), 1.77 (dd, 8H, J 12.1 Hz, H-3ax), 1.90, 1.99, 2.00, 2.02 (4s, 96H, OAc's), 2.25 (bs, 12H, b-CH_2), 2.55-2.95 (2m, 24H, a-CH_2, b-CH_2, H-3eq), 3.25-3.49 (2m, 48H, c-CH_2, d-CH_2), 3.52-3.60 (m, 24H, e-CH_2), 3.54 (s, 24H, OCH_3), 3.59-3.79 (2m, 16H, H-5, H-6), 4.09 (m, 8H, H-9), 4.28 (dd, 8H, $J_{8,9}$· 2.5 Hz, $J_{9,9}$· 12.2 Hz, H-9'), 4.69 (ddd, 8H, H-4),

5.13 (m, 8H, H-7), 5.19 (m, 8H, H-8), 7.38 (d, 16H, $J_{ortho,meta}$ 8.2 Hz, H-ortho), 7.54 (d, 16H, H-meta), 7.62 (d, 8H, $J_{5,NHAc}$ 9.5 Hz, N*H*Ac), 8.00-8.20 (2m, 20H, N*H*C(S)NHPh, amide NH's), 9.90 (bs, 8H, NHC(S)N*H*Ph); ^{13}C-NMR (DMSO-d_6) δ 20.5, 20.7 (OAc's), 22.5 (NAc), 37.6 (C-3), 37.7 (C-d), 40.4 (C-a), 43.3 (C-e), 47.6 (C-5), 49.4 (C-c), 52.7 (OCH_3), 61.6 (C-9), 67.3 (C-7), 68.9 (C-8), 69.4 (C-4), 74.0 (C-6), 87.2 (C-2), 121.9 (C-meta), 122.7 (C-para), 136.3 (C-ortho), 141.2 (C-ipso), 167.7-170.0 (C=O's), 180.4 (C=S).

Fully de-protected PAMAM-based octavalent α-thiosialodendrimer (176).

To peracetylated glycodendrimer **175** (37.6 mg, 0.0058 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 1 h, MeOH was evaporated under reduced pressure and 0.05 M NaOH (5 mL) added. After 1.5 h, the solution was neutralized with 1 M HCl and the solvent removed by lyophilization. The resulting residue was purified by dialysis against 1:1/DMSO:H_2O (MW cutoff 2000 Da) and then freeze-dried. Title compound **176** was isolated as a white, spongy solid in 79% yield (23.2 mg, 0.0046 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.66 (m, 8H, H-3ax), 1.83 (s, 24H, NAc), 2.25-2.61 (m, 32H, b-CH_2, H-3eq), 2.63-3.00 (m, 28H, a-CH_2, c-CH_2), 3.05-3.68 (m, 104H, d-CH_2, e-CH_2, and NeuAc residues excluding above), 4.27, 4.59, 4.98, 5.20 (4bs, 32H, OH's), 7.22-8.15 (4m, 60H, amide NH's, N*H*C(S)NHPh, H-ortho, H-meta, NHAc), 9.88 (bs, 8H, NHC(S)N*H*Ph); ^{13}C-NMR (DMSO-d_6) δ 22.7 (NAc), 37.8 (C-d), 40.5 (C-3), 40.7 (C-a), 43.4 (C-e), 51.9 (C-5), 63.2 (C-9), 66.8 (C-7), 68.8 (C-8), 71.3 (C-4), 76.1 (C-6), 86.5 (C-2), 121.8 (C-meta), 136.4 (C-ortho), 169.3-171.6 (C=O's), 180.4 (C=S).

Peracetylated hexadecavalent PAMAM-based α-thiosialodendrimer (177).

A methanolic solution of amine terminated hexadecavalent Starburst® PAMAM dendritic core **171** (40.7 mg of a 32.46% (w/w) solution in MeOH, 0.0041 mmol, Dendritek® (MI)) was evaporated under reduced pressure. The resulting residue was

combined with CH_2Cl_2 (5 mL) and the solution re-evaporated. This was repeated three times. Compound **171** was then dissolved in DMF (5 mL) and to this was added DIPEA (8.3 mg, 0.064 mmol) and the solution was stirred at room temperature. Sialic acid isothiocyanato derivative **70** (50.0 mg, 0.078 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise to the stirred solution and the reaction left at 25 °C. After 20 h, the reaction mixture was concentrated *in vacuo* and then dialyzed against 1:1/DMSO:H_2O (MW cutoff 2000 Da). The resulting solution was lyophilized to give hexadecavalent PAMAM-based α-thiosialodendrimer **177** as an off-white solid. Title compound **177** was isolated in quantitative yield (54.8 mg, 0.0041 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.64 (s, 48H, NAc), 1.77 (m, 16H, H-3ax), 1.89, 1.99, 2.00, 2.01 (4s, 192H, OAc's), 2.22 (bs, 56H, b-CH_2), 2.45-2.99 (m, 76H, a-CH_2, c-CH_2, H-3eq), 3.19-3.40 (2m, 56H, d-CH_2), 3.52-3.60 (m, 56H, e-CH_2), 3.53 (s, 48H, OCH_3), 3.59-3.79 (2m, 32H, H-5, H-6), 4.09 (m, 16H, H-9), 4.28 (m, 16H, H-9'), 4.69 (ddd, 16H, H-4), 5.13 (m, 16H, H-7), 5.19 (m, 16H, H-8), 7.38 (d, 32H, $J_{ortho,meta}$ 8.2 Hz, H-ortho), 7.54 (d, 32H, H-meta), 7.62 (d, 16H, $J_{5,NHAc}$ 9.5 Hz, N*H*Ac), 7.80-8.15 (3bs, 44H, N*H*C(S)NHPh, amide NH's), 9.90 (bs, 16H, NHC(S)N*H*Ph); ^{13}C-NMR (DMSO-d_6) δ 20.6, 20.8 (OAc's), 22.6 (NAc), 37.7 (C-3), 37.8 (C-d), 40.5 (C-a), 43.4 (C-e), 47.7 (C-5), 52.8 (OCH_3), 61.7 (C-9), 67.4 (C-7), 68.9 (C-8), 69.5 (C-4), 74.1 (C-6), 87.3 (C-2), 122.0 (C-meta), 122.2 ((C-para), 1366.4 (C-ortho), 141.3 (C-ipso), 167.8-170.1 (C=O's), 180.4 (C=S).

Fully de-protected PAMAM-based hexadecavalent α-thiosialodendrimer (178).

To peracetylated glycodendrimer **177** (54.8 mg, 0.0041 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 1 h, MeOH was evaporated under reduced pressure and 0.05 M NaOH (5 mL) added. After 1.5 h, the solution was neutralized with 1 M HCl and the solvent removed by lyophilization. The resulting residue was purified by dialysis against 1:1/DMSO:H_2O (MW cutoff 2000 Da) and freeze-dried.. Title compound **178** was isolated as a white, spongy solid in 59% yield (44.7 mg, 0.0024 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.66 (dd,

16H, J 12.0 Hz, H-3ax), 1.83 (s, 48H, NAc), 2.25-2.62 (m, 72H, b-CH_2, H-3eq), 2.63-3.00 (m, 60H, a-CH_2, c-CH_2), 3.05-3.70 (m, 224H, d-CH_2, e-CH_2, and NeuAc residues excluding above), 4.27, 4.59, 4.72, 5.18 (4bs, 64H, OH's), 7.22-8.15 (4m, 124H, amide NH's, N*H*C(S)NHPh, H-ortho, H-meta, NHAc), 9.90 (bs, 16H, NHC(S)N*H*Ph); ^{13}C-NMR (DMSO-d_6) δ 22.7 (NAc), 37.9 (C-d), 40.5 (C-3), 40.7 (C-a), 43.3 (C-e), 51.9 (C-5), 63.2 (C-9), 66.8 (C-7), 68.7 (C-8), 71.3 (C-4), 76.1 (C-6), 86.5 (C-2), 121.8, (C-meta), 136.4 (C-ortho), 141.2 (C-ipso), 169.3-171.6 (C=O's), 180.4 (C=S).

Peracetylated PAMAM-based α-thiosialodendrimer with a valency of thirty-two (179).

A methanolic solution of amine terminated 32-valent Starburst® PAMAM dendritic core **172** (40.4 mg of a 34.21% (w/w) solution in MeOH, 0.0020 mmol, Dendritek® (MI)) was evaporated under reduced pressure. The resulting residue was combined with CH_2Cl_2 (5 mL) and the solution re-evaporated. This was repeated three times. Compound **172** was then dissolved in DMF (5 mL) and to this was added diisopropylethylamine (DIPEA, 8.3 mg, 0.064 mmol) and the solution was stirred at room temperature. Sialic acid isothiocyanato derivative **70** (50.0 mg, 0.078 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise to the stirred solution and the reaction left at 25 °C. After 20 h, the reaction mixture was concentrated *in vacuo* and then dialyzed against 1:1/DMSO:H_2O (MW cutoff 2000 Da). The resulting solution was lyophilized to give 32-valent PAMAM-based α-thiosialodendrimer **179** as an off-white solid. Title compound **179** was isolated in quantitative yield (54.8 mg, 0.0020 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.65 (s, 96H, NAc), 1.78 (m, 32H, H-3ax), 1.90, 2.00, 2.01, 2.02 (4s, 384H, OAc's), 2.25 (bs, 120H, b-CH_2), 2.52-2.85 (m, 156H, a-CH_2, c-CH_2, H-3eq), 3.00-3.60 (m, 240H, d-CH_2, e-CH_2), 3.56 (s, 96H, OCH_3), 3.59-3.79 (2m, 64H, H-5, H-6), 4.09 (m, 32H, H-9), 4.28 (m, 32H, H-9'), 4.69 (ddd, 32H, H-4), 5.13 (m, 32H, H-7), 5.19 (m, 32H, H-8), 7.38 (d, 64H, $J_{ortho,meta}$ 8.2 Hz, H-ortho), 7.54 (d, 64H, H-meta), 7.62 (d, 32H, $J_{5,NHAc}$ 9.5 Hz, N*H*Ac), 7.75-8.15 (m, 92H, N*H*C(S)NHPh, amide NH's), 9.90 (bs, 32H,

NHC(S)N*H*Ph); ^{13}C-NMR (DMSO-d_6) δ 20.6, 20.7 (OAc's), 22.5 (NAc), 32.4 (C-b), 37.8 (C-3), 43.4 (C-e), 47.6 (C-5), 49.4 (C-c), 52.8 (OCH_3), 61.7 (C-9), 67.3 (C-7), 68.9 (C-8), 69.5 (C-4), 74.1 (C-6), 87.3 (C-2), 122.0 (C-meta), 122.2 (C-para), 136.4 (C-ortho), 141.3 (C-ipso), 167.7-170.1 (C=O's), 180.4 (C=S).

Fully de-protected PAMAM-based α-thiosialodendrimer with a valency of thirty-two (180).

To peracetylated glycodendrimer **179** (54.8 mg, 0.0020 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 1 h, MeOH was evaporated under reduced pressure and 0.05 M NaOH (5 mL) added. After 1.5 h, the solution was neutralized with 1 M HCl and the solvent removed by lyophilization. The resulting residue was purified by dialysis against 1:1/DMSO:H_2O (MW cutoff 2000 Da) and then freeze-dried. Title compound **180** was isolated as a white, spongy solid in 93% yield (40.1 mg, 0.0019 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.66 (dd, 32H, J 11.9 Hz, H-3ax), 1.84 (s, 96H, NAc), 2.25 (bs, 120H, b-CH_2), 2.50-2.80 (m, 154H, a-CH_2, c-CH_2, H-3eq), 3.05-3.70 (m, 464H, d-CH_2, e-CH_2, and NeuAc residues excluding above), 4.27, 4.59, 5.18 (3bs, 128H, OH's), 7.22-8.15 (4m, 252H, amide NH's, N*H*C(S)NHPh, H-ortho, H-meta, NHAc), 9.95 (bs, 32H, NHC(S)N*H*Ph); ^{13}C-NMR (DMSO-d_6) δ 22.7 (NAc), 32.5, 32.9 (C-b) 37.8 (C-d), 39.1 (C-3), 40.5 (C-a), 42.1 (C-c), 43.4 (C-e), 51.9 (C-5), 63.2 (C-9), 66.8 (C-7), 68.8 (C-8), 71.3 (C-4), 76.1 (C-6), 86.5 (C-2), 121.9, 122.5 (C-meta, C-para), 136.4 (C-ortho), 141.2 (C-ipso), 169.3-171.6 (C=O's), 180.4 (C=S).

Turbidimetric analysis between the lectin from *Limax flavus* and PAMAM-based α-thiosialodendrimers 174, 176, 178, and 180.

Turbidimetry experiments were performed in Linbro (Titertek) microtitration plates where 50 μL/well of stock lectin solutions prepared from *Limax flavus* (1 mg/mL

in PBS) were mixed with 50 μL each of glycodendrimers **174**, **176**, **178**, and **180** (0.22, 0.24, 0.24, and 0.25 mg/mL in PBS, respectively or 0.371 mM sialoside content each) and incubated at room temperature for up to 4 hours. The turbidity of the solutions was monitored by reading the optical density (O.D.) at 490 nm at regular time intervals until no noticeable changes could be observed.

Competitive inhibition ELLA using human α_1-acid glycoprotein and PAMAM-based α-thiosialodendrimers 174, 176, 178, and 180 as inhibitors.

Linbro (Titertek) microtitration plates were coated with human α_1-acid glycoprotein (orosomucoid) at 100 μL/well of a stock solution of 10 μg/mL in 0.01 M phosphate buffer (pH 7.3) overnight. The wells were then washed three times with 300 μL/well of 0.01 M phosphate buffer (pH 7.3) containing 0.05% (v/v) Tween 20 (PBST). Similar washings with PBST was repeated after each incubation period. Wells were then blocked with 150 μL/well of 1% BSA/PBS for one hour at 37 °C. After washing, the wells were filled with 100 μL/well of inhibitor solutions and incubated again at 37 °C for one hour. Inhibitors used include phenylthio α-sialoside as a reference monovalent compound, tetravalent 3,3'-iminobis(propylamine)-based sialodendrimer **156** and PAMAM-based, spherical glycodendrimers **174**, **176**, **178**, and **180**. Each inhibitor was added in serial two-fold dilutions (60 μL/well) in PBS with the appropriate lectin-enzyme conjugate concentration (60 μL/well of 100-fold dilution of a 1 mg/mL stock solution of *Limax flavus* in PBS) on Nunclon (Delta) microtiter plates and incubated at 37 °C for one hour. These inhibitor solutions (100 μL) were transferred to the antigen coated plates and incubated for a second hour. The plates were washed and 50 μL/well of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg/mL) in citrate-phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 minutes by adding 50 μL/well of 1 M H_2SO_4 and the optical density measured at 410 nm relative to 570 nm. Per cent inhibition was calculated as follows:

$$\% \text{ inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})})/A_{(\text{no inhibitor})} \times 100$$

IC_{50}'s were reported as the concentration required for 50% inhibition of the coating antigen. Each test was performed six times and average values reported.

Chapter 6. Chemo-Enzymatic Galactosylation of Hyperbranched L-Lysine-Based Dendrimers

6.1. Introduction

As already stated, carbohydrates are receiving increased attention owing to the key roles they play in cell signaling, molecular recognition, and many other biological processes. The protein- and lipid-bound saccharides that are involved in these phenomena are often structurally diverse and complex (see Chapter 1).

Purely chemical strategies in these syntheses of oligosaccharides is not an easy task. The difficulties in the preparation of complex oligosaccharides are a result of a great number of possibilities for the combinations of monomeric units to form oligosaccharides. Furthermore, glycosidic linkages must be introduced stereospecifically (Chapter 1).

Enzymatic oligosaccharide synthesis based on glycosyltransferases[172] is an important alternative to chemical synthesis since glycosyltransferases are highly regio- and stereo-selective with regard to the glycoside bond formation and no tedious protection/deprotection steps are required. Chemo-enzymatic strategies have thus come to be regarded as useful and powerful tools for the preparation of structurally complex oligosaccharides.

In an extension of the demonstration of the multivalent effect and to extend the approaches used herein to structurally complex oligosaccharides (Chapters 3 to 5), a galactosyltransferase was employed in the synthesis of L-lysine-based dendrimers bearing N-acetyllactosamine residues.[173]

N-Acetyllactosamine (Galβ(1,4)GlcNAc, LacNAc) is well known as a important disaccharide core structure of lactosaminoglycans and tumor-associated antigenic carbohydrates[3,4] and has been implicated in a variety of biological phenomena.[5-8]

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[172] a) Wong, C. -H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Eng.* **1995**, *34*, 412; b) Wong, C. -H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Eng.* **1995**, *34*, 521.

[173] Zanini, D.; Roy, R. *Bioconjugate Chem.* **1997**, 2, 187.

Dendritic LacNAc conjugates as probes for the study of N-acetyllactosamine-protein interactions, is therefore a natural extension of the study of multivalency.

In addition, the adaptation of enzymatic reactions to dendritic supports represents a novel synthetic route to multivalent neoglycoconjugates and the syntheses described below are the first chemo-enzymatically prepared glycodendrimers.[173]

6.2. N-Acetyllactosamine Containing Dendrimers

Fully deprotected, L-lysine-based dendrimers containing two (**102**), four (**108**), and eight (**114**) N-acetylglucosamine (GlcNAc) residues were synthesized as described in detail in Chapter 3, Section 3.6.

These GlcNAc-based dendrimers were then enzymatically transformed into di- (**181**), tetra- (**182**), and octa- (**183**) valent LacNAc dendrimers (Schemes 6.2.1 to 6.2.3) *via* the enzymatic galactosylation of surface N-acetylglucosamine residues. Enzymatic reactions were performed according to established procedures.[174] GlcNAc dendrimers **102**, **108**, and **114** were dissolved in sodium cacodylate buffer containing bovine serum albumin, $MnCl_2$, NaN_3, UDP-glucose, GlcNAc β-1,4-galactosyltransferase, UDP-glucose 4'-epimerase, and calf intestinal phosphatase. The reaction was incubated at 37 °C for 5 days while maintaining the pH at 7.4.

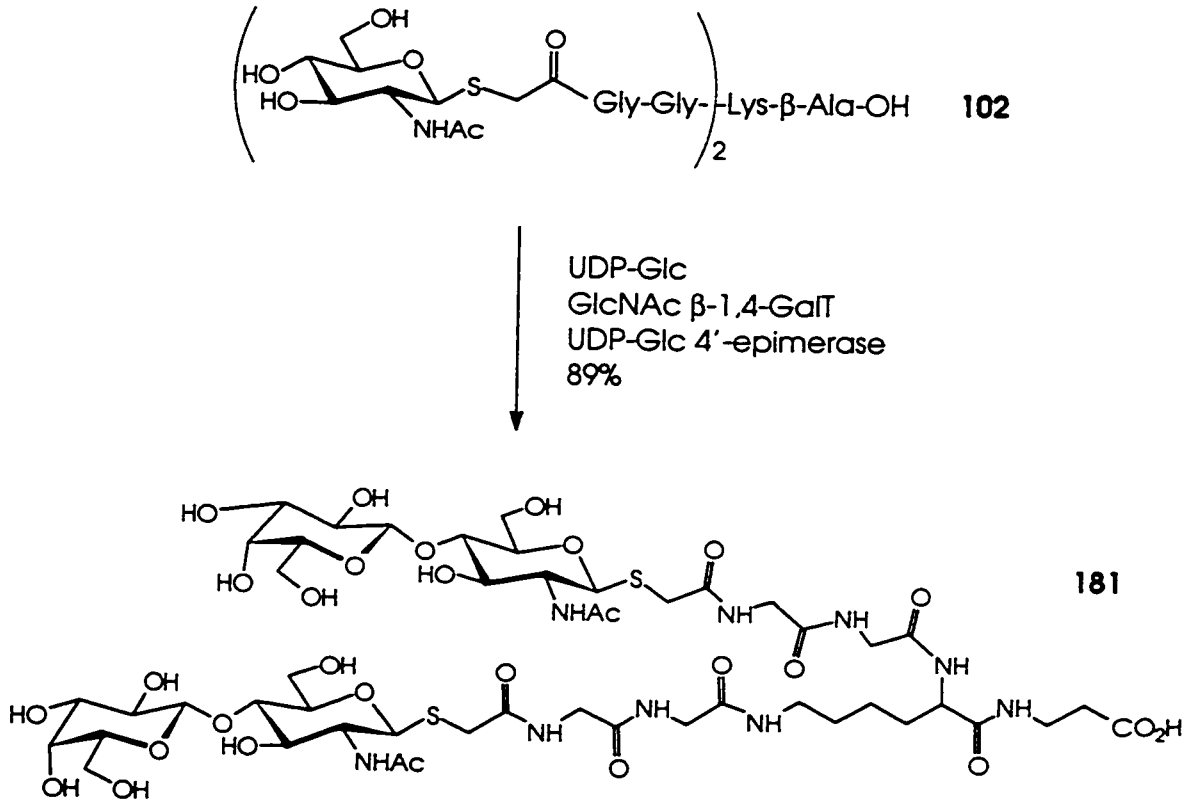
LacNAc dendrimers were isolated by gel permeation chromatography on both a Biogel P-2 column and a Sephadex G-50 column using H_2O as eluent. Isolated yields were excellent (79 to 90%) and were indicative of the efficiency of this chemo-enzymatic strategy.

NMR data (D_2O) showed complete galactosyl incorporation as measured by the H-1 galactose signal at δ 4.55 ppm relative to the H-1 GlcNAc signal at δ 4.79 ppm, the NAc GlcNAc at δ 2.11 ppm, and the β-alanyl α-CH_2 signal at δ 2.57 ppm, respectively (Figure 6.2.1). HMQC experiments confirmed the correct assignments of H-1 GlcNAc

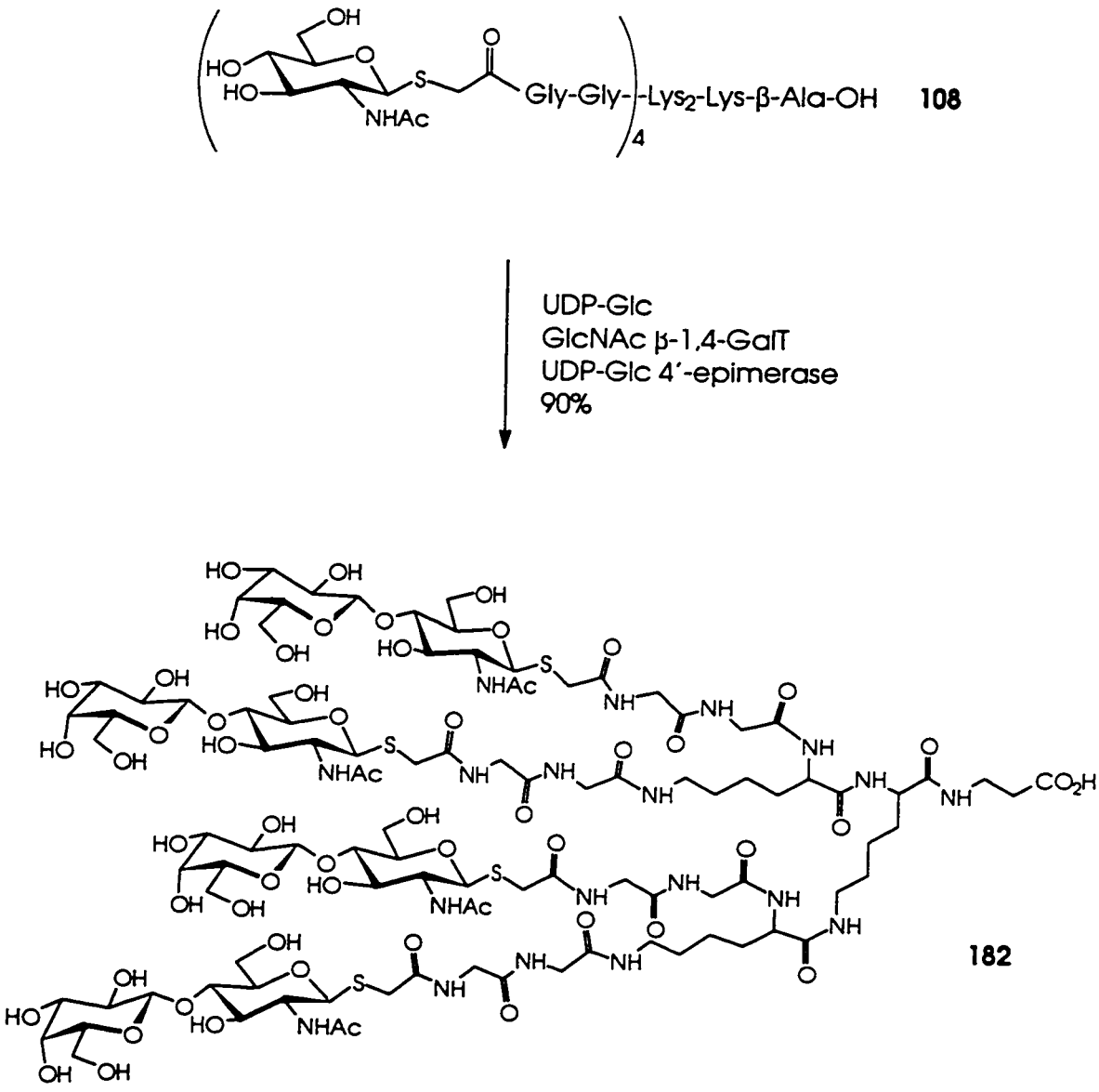
[174] Unverzagt, C.; Kunz, H.; Paulson, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 9308.

and H-1 Gal as these signals corresponded to ^{13}C signals at δ 83.5 and 102.5 ppm for C-1 GlcNAc and C-1 Gal, respectively (Figure 6.2.2).

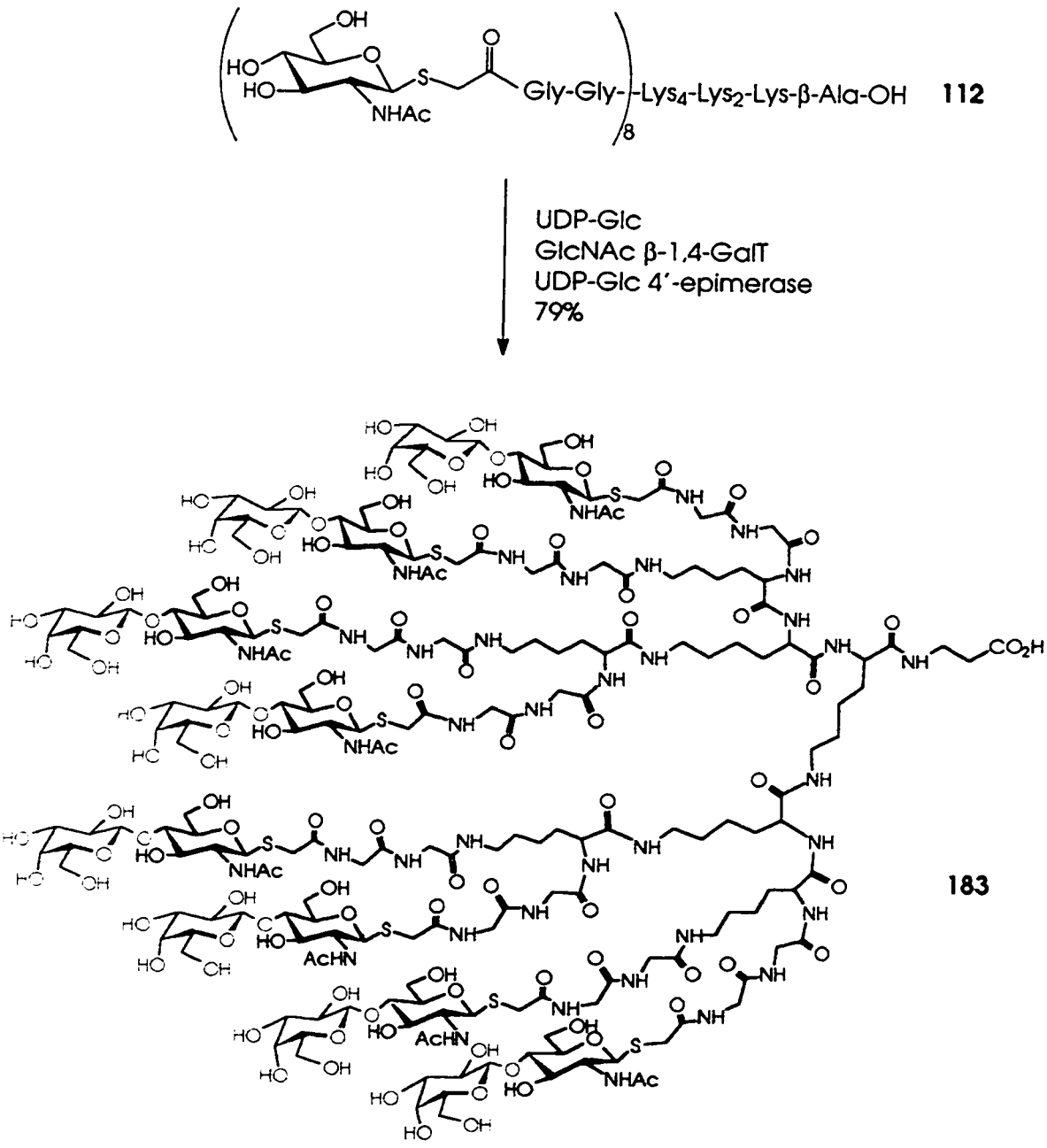
These results indicate that even the shorter arms of the dendritic L-lysine core were sufficiently long and free of steric hindrance to be amenable to complete enzymatic transformations. While the enzymatic galactosylation of N-acetylglucosamine is well known, this is the first reported achievement of the enzymatic conversion of GlcNAc to LacNAc on a synthetic dendrimer.



Scheme 6.2.1. Chemo-enzymatic synthesis of divalent N-acetyllactosaminyl dendrimer.



Scheme 6.2.2. Chemo-enzymatic synthesis of tetravalent N-acetyllactosaminyl dendrimer.



Scheme 6.2.3. Chemo-enzymatic synthesis of octavalent N-acetyllactosaminyl dendrimer.

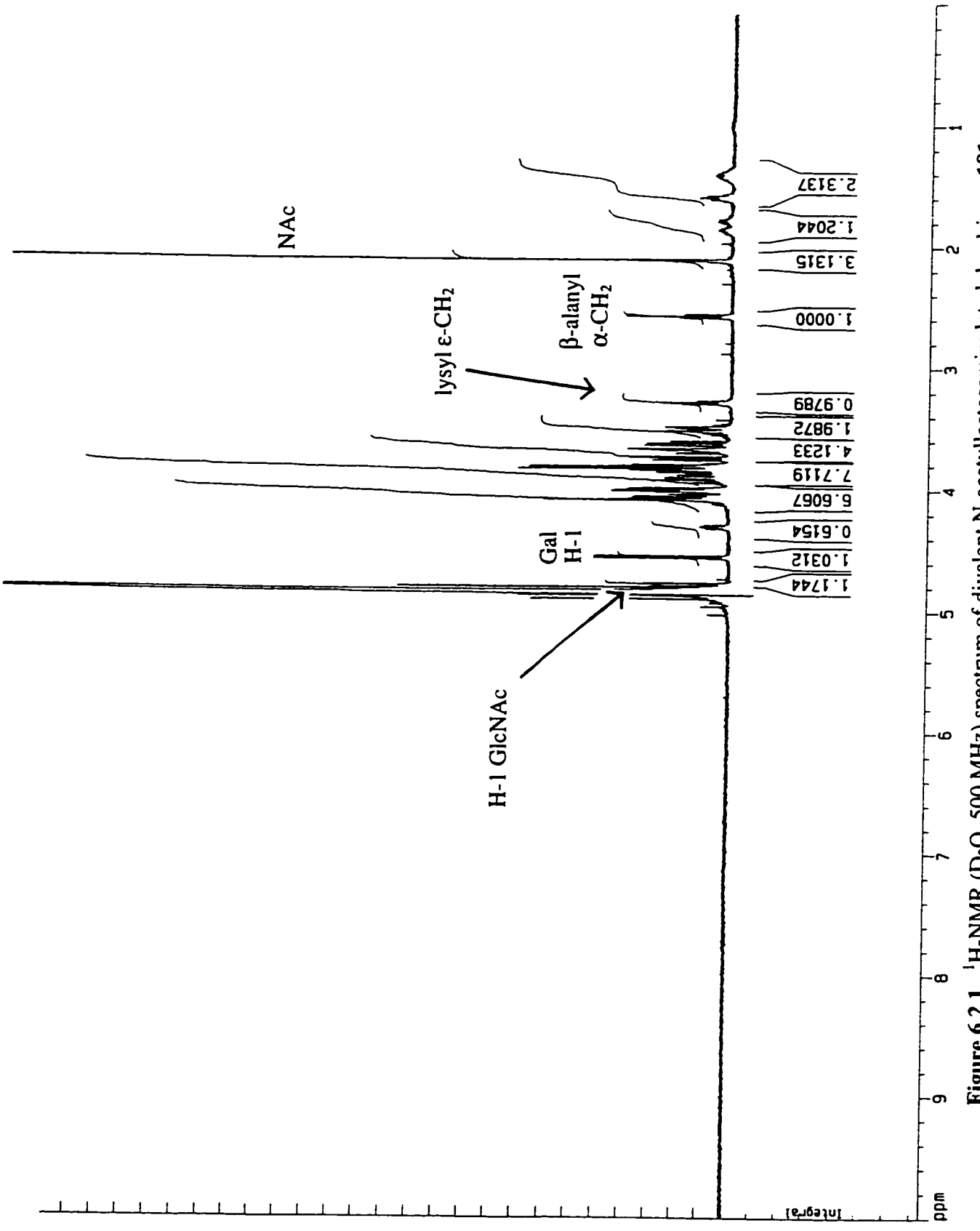


Figure 6.2.1. ^{1}H-NMR (D_2O, 500 MHz) spectrum of divalent N-acetyllactosaminylated dendrimer **181**.

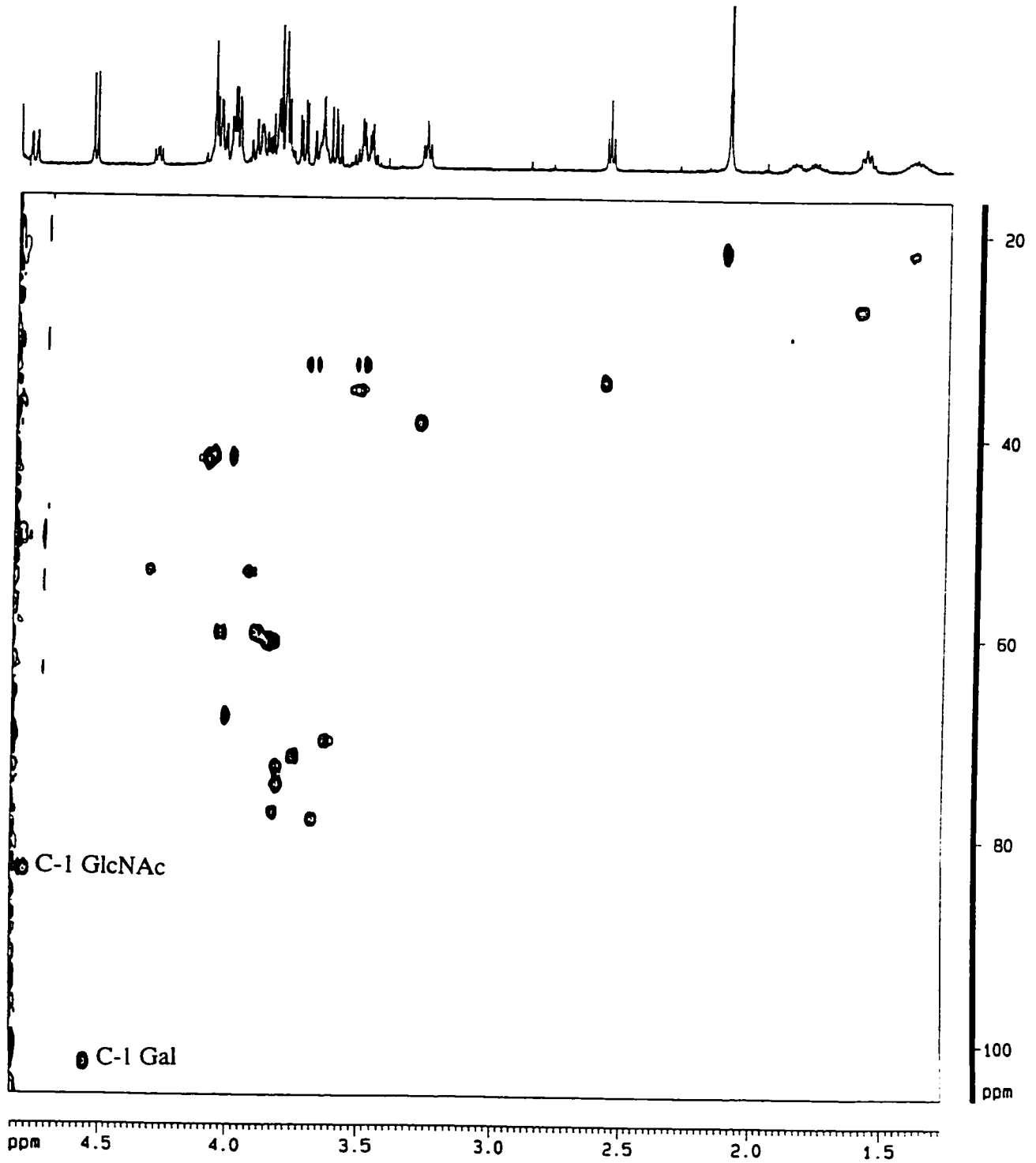


Figure 6.2.2. HMQC NMR (D_2O, 500 MHz) spectrum of divalent N-acetyllactosaminylated dendrimer **181**.

6.3. Immunochemical Assays

To test N-acetyllactosamine dendrimers **181** to **183**, it was necessary to use a lectin specific to N-acetyllactosamine and one that would not bind N-acetylglucosamine. For this reason, inhibition of carbohydrate-porcine stomach mucin interactions with dendrimers **181** to **183**, was performed with *Erythrina Cristagalli* (ECA). ECA is a divalent lectin and binding studies have revealed that N-acetyllactosamine exhibits the highest affinity for ECA where most of the binding energy is contributed by the non-reducing terminal galactose residue (Chapter 1, Section 1.7.1). ECA-protein interactions are therefore not inhibited by GlcNAc residues. Thus, inhibition of binding of ECA to porcine stomach mucin by newly synthesized glycodendrimers **181** to **183** would unequivocally demonstrate galactose incorporation and inhibitory potency.

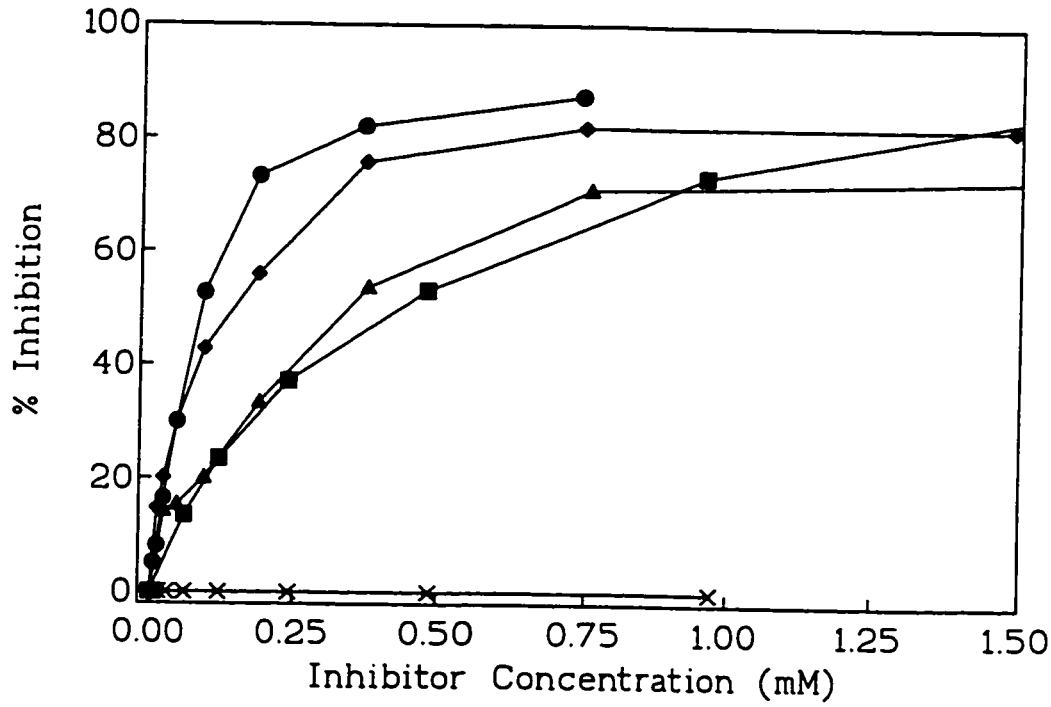


Figure 6.3.1. Inhibition of binding of porcine stomach mucin type III and ECA/HRP in ELLA. Inhibitors used include: azido-β-LacNAc (■), divalent LacNAc **181** (▲), tetravalent LacNAc **182** (◆), octavalent LacNAc **183** (●), and octavalent GlcNAc **114** (×).

Di- (**181**), tetra- (**182**), and octa- (**183**) valent LacNAc dendrimers, when used in the inhibition of binding of *Erythrina Cristagalli* to porcine stomach mucin type III by ELLA, showed enhanced binding affinities as compared to their monomeric precursor (Figure 6.3.1). IC_{50} values were 341, 143, and 86 μM (682, 574, and 692 μM respectively as compared to O-(β-D-galactopyranosyl)-(1-4)-2-acetamido-2-deoxy-β-D-glucopyranosyl azide[175] (azido-β-LacNAc), Table 6.3.1). As a control, octameric GlcNAc **114** did not inhibit the lectin-mucin interaction.

Table 6.3.1. The Inhibition of the Binding of Porcine Stomach Mucin Type III and ECA/HRP by N-Acetyllactosaminyl Dendrimers **181** to **183**.

Compound	IC_{50} (mM)[a]	Relative Potency[a]
1-azido-β-LacNAc	0.43	1
divalent LacNAc dendrimer **181**	0.34 (0.68)	1.3 (0.63)
tetravalent LacNAc dendrimer **182**	0.14 (0.57)	3.0 (0.75)
octavalent LacNAc dendrimer **183**	0.086 (0.69)	5.0 (0.62)

[a]Values in parentheses refer to IC_{50}'s expressed relative to monomeric LacNAc content.

Figure 6.3.2 plots IC_{50}'s as a function of dendrimer valency. Clearly, increased inhibitory potentials are concurrent with an increase in valency. When expressed on a per hapten unit, the average binding potency of **181** to **183** indicates that LacNAc residues in these dendrimers were not strong ligands towards ECA. In this case, no cluster effect was observed. One explanation may be that the presentation of the carbohydrate residues plays an important role in this particular carbohydrate-lectin interaction and perhaps the LacNAc residues in the form presented here are not properly scaffolded to confer steady increases as a function of multivalency. Indeed this phenomenon has been previously observed for LacNAc clusters.[129]

[175] Park, W. K. C. *Ph. D. Thesis Dissertation* **1995**, University of Ottawa, Ottawa, Ontario, Canada.

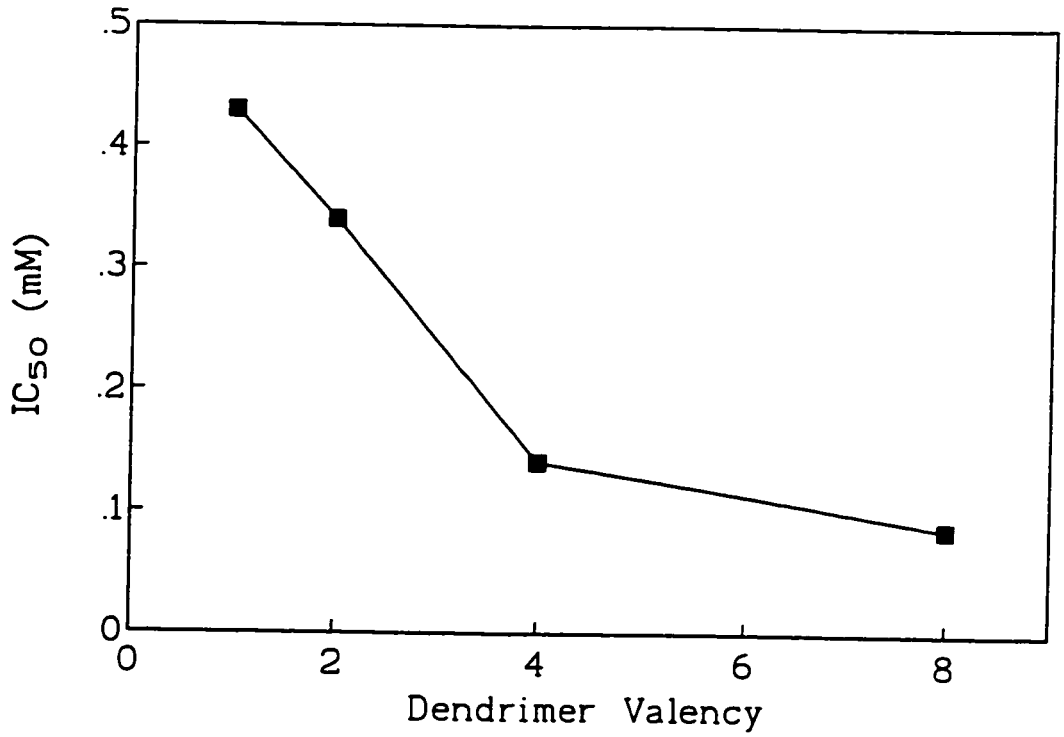


Figure 6.3.2. Effect of dendrimer valency on the inhibition of porcine stomach mucin type III and ECA-HRP. Data taken from Figure 6.3.1.

These data do not negate the overall importance of the multivalency or glycosidic cluster effect in carbohydrate-protein interactions. Rather, they stress the need for well designed glycoforms.

Of importance, though, is that these results nicely confirm the enzymatic incorporation of galactose residues in the LacNAc dendrimers. No inhibition would have been observed without proper galactosylation of GlcNAc dendrimers since octavalent GlcNAc dendrimer **114** did not inhibit the binding at all.

6.4. Conclusions

N-Acetylglucosamine-containing dendrimers were cleanly and enzymatically transformed into N-acetyllactosamine-containing dendrimers. First through third generation GlcNAc-based dendrimers were elongated *via* galactose conjugation using GlcNAc β-(1,4)-galactosyltransferase to give LacNAc-based dendrimers with valencies of 2, 4, and 8. Isolated yields were 79 to 90% and were indicative of the efficiency of this approach. ELLA inhibition of binding of ECA lectin to porcine stomach mucin type III indicated that, indeed, multivalency can amplify carbohydrate-protein interactions. However, this effect is variable for each individual interaction and to what extent multivalency plays a role in such interactions is still under active investigation.

6.5. Experimental Methods

Divalent N-acetyllactosaminyl dendrimer (181).

A 5 mg (0.0025 mmol) portion of GlcNAc dendrimer **102** was dissolved in 50 mM sodium cacodylate (pH 7.4, 565 μL) containing bovine serum albumin (0.5 mg), 1.1 μmol of $MnCl_2$, 3.4 μmol of NaN_3, 28.3 μmol of UDP-glucose, 200 milliunits of GlcNAc β-1,4-galactosyltransferase (EC 2.4.1.22), 1 unit of UDP-glucose 4'-epimerase (EC 5.1.3.2) and 4 units of calf intestinal phosphatase (EC 3.1.3.1). The reaction was incubated at 37 °C for 5 days while maintaining the pH at 7.4 by periodic addition of 0.1 M NaOH. Title compound **181** was purified by gel permeation chromatography on both a Biogel P2 column and a Sephadex G-50 column using H_2O as eluent and then freeze-dried. Divalent **181** was isolated as a white, lyophilized powder in 89% yield (5.9 mg, 0.0022 mmol); ^{1}H-NMR (D_2O) δ 1.40 (m, 2H, lysyl γ-CH_2), 1.59 (m, 2H, lysyl δ-CH_2), 1.79 and 1.87 (2m, 2H, lysyl β-CH_2, unequiv.), 2.11 (2s, 6H, NAc), 2.57 (t, 2H, J 6.7 Hz, β-alanyl α-CH_2), 3.28 (t, 2H, J 6.9 Hz, lysyl ε-CH_2), 3.42 to 4.11 (m, 38H, β-alanyl β-CH_2, glycyl CH_2's, SCH_2's, H-2, H-3, H-4. H-5, H-6, H-6' of GlcNAc and H-2, H-3, H-4.

H-5, H-6, H-6' of gal), 4.30 (dd, 1H, $J_{lysyl\ \alpha,\ lysyl\ \beta}$ 8.7 Hz, J $J_{lysyl\ \alpha,\ lysyl\ \beta'}$ 3.2 Hz, lysyl α-CH), 4.55 (d, 2H, $J_{1,2}$ 7.8 Hz, H-1 Gal), 4.77 and 4.79 (dd, 2H, H-1 GlcNAc, unequiv.); ^{13}C-NMR δ 21.7 (NHAc), 21.8 (lysyl α-C), 27.3 (lysyl δ-C), 30.1 (lysyl β-C), 32.9 (SCH_2's), 33.0 (β-alanyl α-C), 34.7 (β-alanyl β-C), 38.5 (lysyl ε-C), 83.5 (C-1 GlcNAc), 102.5 (C-1 Gal). FAB-MS (pos.) calcd for $C_{49}H_{81}N_9O_{29}S_2$: 1323.4, found: 1326.0 (M + 1, 2.2%).

Tetravalent N-acetyllactosaminyl dendrimer (182).

A 5 mg (0.0024 mmol) portion of GlcNAc dendrimer **108** was dissolved in 50 mM sodium cacodylate (pH 7.4, 565 μL) containing bovine serum albumin (0.5 mg), 1.1 μmol of $MnCl_2$, 3.4 μmol of NaN_3, 28.3 μmol of UDP-glucose, 200 milliunits of GlcNAc β-1,4-galactosyltransferase (EC 2.4.1.22), 1 unit of UDP-glucose 4'-epimerase (EC 5.1.3.2) and 4 units of calf intestinal phosphatase (EC 3.1.3.1). The reaction was incubated at 37 °C for 5 days while maintaining the pH at 7.4 by periodic addition of 0.1 M NaOH. Title compound **182** was purified by gel permeation chromatography on both a Biogel P2 column and a Sephadex G-50 column using H_2O as eluent and then freeze-dried. Tetravalent **182** was isolated as a white, lyophilized powder in 90% yield (5.9 mg, 0.0022 mmol); ^{1}H-NMR (D_2O) δ 1.40 (m, 6H, lysyl γ-CH_2), 1.59 (m, 6H, lysyl δ-CH_2), 1.79 and 1.87 (2m, 6H, lysyl β-CH_2, unequiv.), 2.11 (s, 12H, NAc), 2.60 (m, 2H, β-alanyl α-CH_2), 3.28 (m, 6H, lysyl ε-CH_2), 3.42 to 4.11 (m, 74H, β-alanyl β-CH_2, glycyl CH_2's, SCH_2's, H-2, H-3, H-4. H-5, H-6, H-6' of GlcNAc and H-2, H-3, H-4. H-5, H-6, H-6' of gal), 4.30 (2m, 3H, lysyl α-CH), 4.55 (d, 4H, $J_{1,2}$ 8.1 Hz, H-1 Gal), 4.77 (dd, 4H, H-1 GlcNAc, unequiv.); ^{13}C-NMR δ 21.7 (NHAc), 21.8 (lysyl α-C), 27.3 (lysyl δ-C), 30.1 (lysyl β-C), 32.9 (SCH_2's), 33.0 (β-alanyl α-C), 34.7 (β-alanyl β-C), 38.5 (lysyl ε-C), 83.5 (C-1 GlcNAc), 102.5 (C-1 Gal).

Octavalent N-acetyllactosaminyl dendrimer (183).

A 5 mg (0.0012 mmol) portion of GlcNAc dendrimer **114** was dissolved in 50 mM sodium cacodylate (pH 7.4, 565 μL) containing bovine serum albumin (0.5 mg), 1.1

μmol of $MnCl_2$, 3.4 μmol of NaN_3, 28.3 μmol of UDP-glucose, 200 milliunits of GlcNAc β-1,4-galactosyltransferase (EC 2.4.1.22), 1 unit of UDP-glucose 4'-epimerase (EC 5.1.3.2) and 4 units of calf intestinal phosphatase (EC 3.1.3.1). The reaction was incubated at 37 °C for 5 days while maintaining the pH at 7.4 by periodic addition of 0.1 M NaOH. Title compound **183** was purified by gel permeation chromatography on both a Biogel P2 column and a Sephadex G-50 column using H_2O as eluent and then freeze-dried. Octavalent **183** was isolated as a white, lyophilized powder in 79% yield (5.2 mg, 0.00096 mmol); ^{1}H-NMR (D_2O) δ 1.40 (m, 1H, lysyl γ-CH_2), 1.59 (m, 14H, lysyl δ-CH_2), 1.79 and 1.87 (2m, 14H, lysyl β-CH_2, unequiv.), 2.11 (s, 24H, NAc), 2.47 (m, 2H, β-alanyl α-CH_2), 3.27 (m, 14H, lysyl ε-CH_2), 3.42 to 4.11 (m, 130H, β-alanyl β-CH_2, glycyl CH_2's, SCH_2's, H-2, H-3, H-4. H-5, H-6, H-6' of GlcNAc and H-2, H-3, H-4. H-5, H-6, H-6' of gal), 4.30 (m, 7H, lysyl α-CH), 4.55 (d, 8H, $J_{1,2}$ 7.8 Hz, H-1 Gal), 4.77 and 4.79 (dd, 8H, H-1 GlcNAc, unequiv.); ^{13}C-NMR δ 21.7 (NHAc), 21.8 (lysyl α-C), 27.3 (lysyl δ-C), 30.1 (lysyl β-C), 32.9 (SCH_2's), 33.0 (β-alanyl α-C), 34.7 (β-alanyl β-C), 38.5 (lysyl ε-C), 83.5 (C-1 GlcNAc), 102.5 (C-1 Gal).

Competitive Inhibition ELLA using porcine stomach mucin type III and N-acetyllactosaminyl dendrimers 181 to 183 as inhibitors.

Linbro (Titertek) microtitration plates were coated with porcine stomach mucin type III at 100 μL/well of a stock solution of 5 μg/mL in 0.01 M phosphate buffer (pH 7.3). The wells were then washed three times with 300 μL/well of 0.01 M phosphate buffer (pH 7.3) containing 0.05% (v/v) Tween 20 (PBST). Washing with PBST was repeated after each incubation period. Wells were then blocked with 150 μL/well of 1% BSA/PBS for one hour at 37 °C. After washing, wells were filled with 100 μL/well of inhibitor solutions and incubated again at 37 °C for one hour. Inhibitors used O-(β-D-galactopyranosyl)-(1-4)-2-acetamido-2-deoxy-β-D-glucopyranosyl azide (monomeric LacNAc)[175] as reference monovalent compound, octavalent GlcNAc dendrimer **114**, di-

(**181**), tetra- (**182**), and octa- (**183**) valent LacNAc dendrimers. Each inhibitor was added in serial two-fold dilutions (60 μL/well) in PBS with the appropriate lectin-enzyme conjugate concentration (100-fold dilution of a 1 mg/mL stock solution of *Erythrina Cristagalli* in PBS) on Nunclon (Delta) microtiter plates and incubated at 37 °C for one hour. These inhibitor solutions (100 μL) were transferred to the antigen-coated plates and incubated for a second hour. The plates were washed and 50 μL/well of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg/4 mL) in citrate-phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 minutes by adding 50 μL/well of 1 M H_2SO_4 and the optical density was measured at 410 nm relative to 570 nm. Per cent inhibition was calculated as follows:

$$\% \text{ inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})})/A_{(\text{no inhibitor})} \times 100$$

IC_{50}'s were reported as the concentration required for 50% inhibition of the coating antigen. All tests were performed in triplicate.

Chapter 7. Heterobifunctional Glycodendrimer

7.1. Introduction

The increased binding avidity of neoglycoproteins and glycopolymers has led to their use as models in vaccines,[48] ligands in affinity chromatography,[50,51] and as drug carriers.[52-54] These custom-designed, synthetic neoglycoconjugates combined a multivalent carbohydrate strategy with the covalent addition of probes, drugs or effector molecules. In this way, neoglycoconjugates bearing multiple functional components were introduced as valuable tools for targeting specific cells, tissues or organs. The glycan moieties served as targeting devices. The synthetic backbones were employed to confer desired physical and biophysical properties, while the effectors and/or probes were used to stain or label cells or deliver the neoglycoconjugate to a given site.[77]

Glycopolymers bearing probes or effectors are generally prepared in two ways - graft conjugation or terpolymerization (Scheme 7.1.1).

In graft conjugation, initially developed by Bovin *et al.*[176] with poly(*p*-nitrophenylacrylate) and later extended to poly[N-(acryloxy)succinimide],[71] amine derivatives are added to polymers with activated ester functionalities to provide the desired copolymers after quenching the excess activated ester with aqueous ammonia. Glycoside and effector contents may be adjusted *via* the amount of initial amine derivatives used.

Alternately, with the preparation of suitable carbohydrate and effector monomers, these may be coupled to pre-formed polymers with reactive functionalities. Examples include poly-glutamic acid[177] and poly-L-lysine[54,178] onto which oligosaccharides terminated with amine or acid groups may be grafted. Traditional coupling strategies

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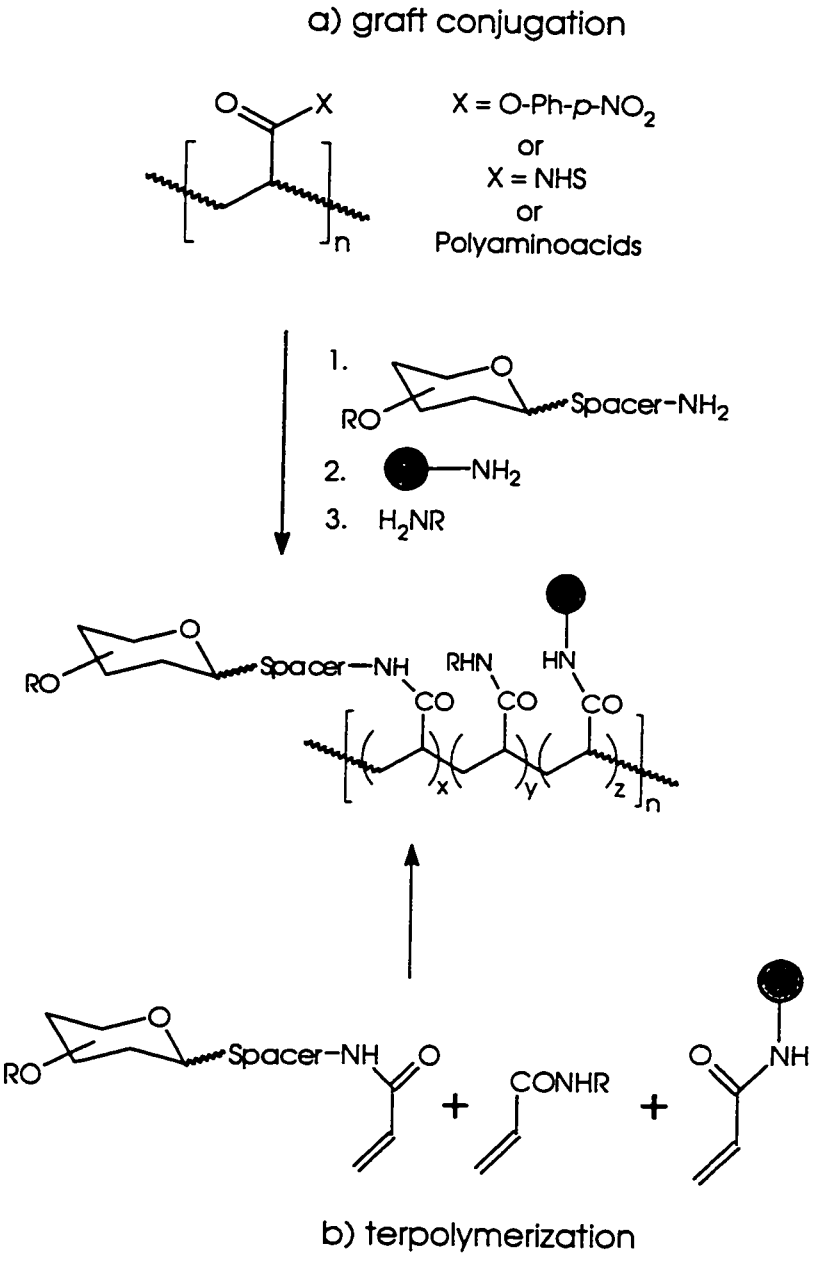
[176] Bovin, N. V.; Korchagina, E. Yu.; Zemlyanukhina, T. V.; Byramova, N. E.; Ivanov, A. E.; Zubov, V. P.; Mochalova, L. V. *Glycoconjugate J.* **1993**, *10*, 142.

[177] Sugawara, T. A.; Susaki, H.; Nogusa, H.; Gonsho, A.; Iwasawa, H.; Irie, K.; Ito, Y.; Shibukawa, M. *Carbohydr. Res.* **1993**, *238*, 163.

[178] Roy, R.; Pon, R. A.; Tropper, F. D.; Andersson, F. O. *J. Chem. Soc., Chem. Commun.* **1993**, 264.



Scheme 7.1.1. Preparation of terpolymers.

such as direct conjugation to reducing sugars and *p*-isothiocyanatophenyl glycosides may also be employed. Moreover, direct 1,4-conjugate addition of poly-L-lysine onto N-acryloylated monomers has been performed.[178] In this manner, three component polymers such as **184**, containing L-lysine backbone, α-sialoside, and biotin functionalities have been prepared (Scheme 7.1.2).[178]

In terpolymerizations, copolymerization of three appropriately derivatized monomers is performed (Scheme 7.1.1). For example, Tichá and Koucerek[179] reported an active copolymer by reacting α-D-galactopyranoside, acrylamide, and allylamine. The resulting aminated polymer was further treated with fluorescein isothiocyanate to provide **185** (Scheme 7.1.2).

To expand the usefulness of glycopolymers in various immunoassays, terpolymerizations, again using readily available N-acryloylated precursors, were performed to give polymers containing a carbohydrate hapten and effectors such as stearylamine, 2-(*p*-hydroxyphenyl)ethylamine (tyramine) or biotin (**186-188**, Scheme 7.1.2).[180,181]

The choice of the probe or effector was dependent on the eventual use of the desired terpolymer. Stearylamine conferred glycopolymers with improved hydrophobic properties for use as coating antigens in microtiter plate ELISA/ELLA assays. Tyramine incorporation enabled neoglycoconjugate use in radiolabelling experiments with ^{125}I and biotin is universally used as a biochemical probe in commercially available avidin or streptavidin kits.

Still, neoglycoproteins and glycopolymers bearing effector molecules suffer the same drawbacks as their non-effector bearing counterparts. They are variable in nature, having ill-defined carbohydrate and probe/effector densities. Thus, they cannot be used in precise biophysical analyses of the given situation being studied.

It is, therefore, a logical extension to look to the design of glycodendrimers bearing probes or effector moieties. The synthesis of a second generation, tetravalent α-

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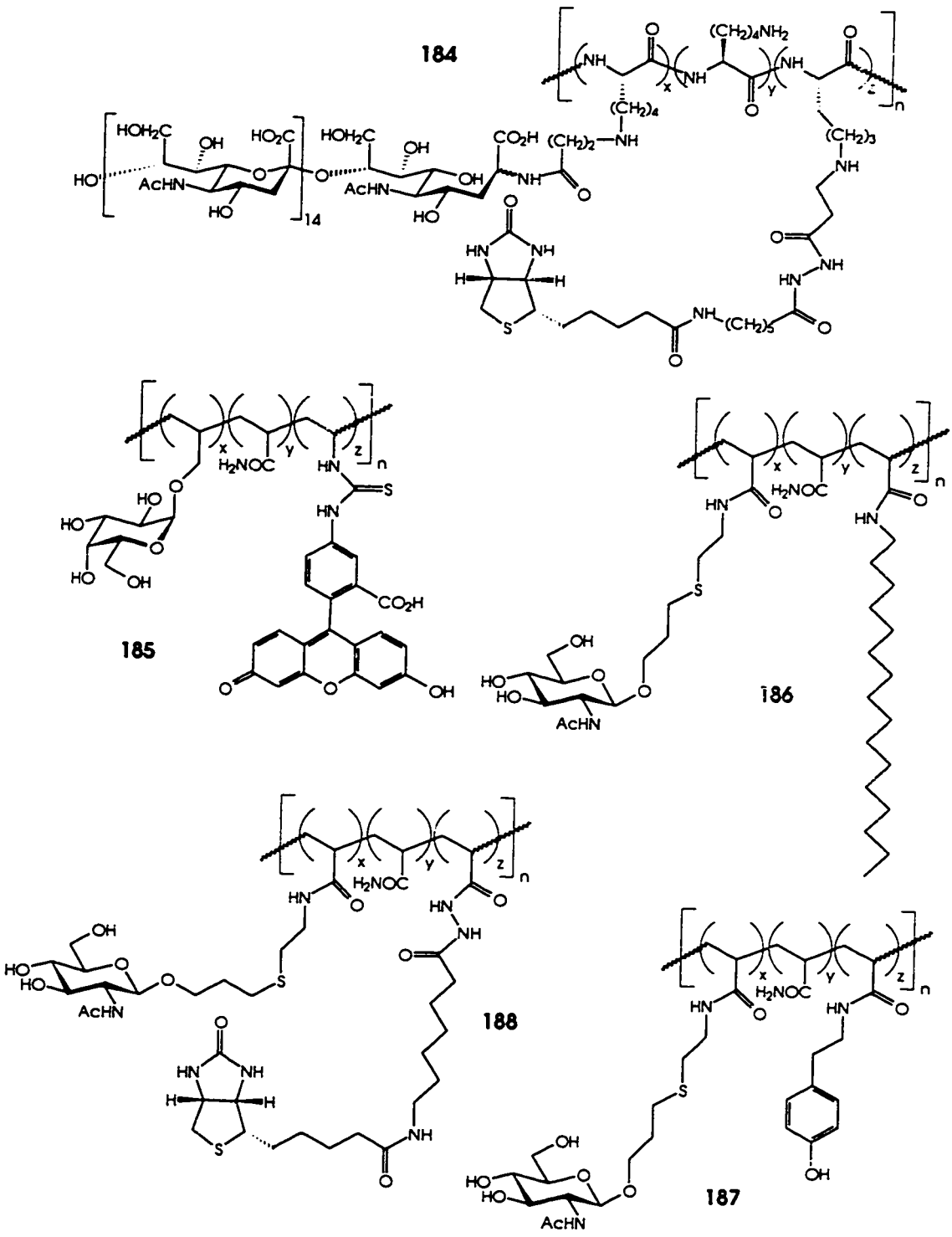
[179] Tichá, M.; Koucerek, J. *Carbohydr. Res.* **1991**, *213*, 339.
[180] Roy, R.; Tropper, F. D.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1992**, 1611.



Scheme 7.1.2. Examples of terpolymers.

[181] Tropper, F. D.; Romanowska, A.; Roy, R. *Methods. Enzymol.* **1994**, *242*, 257.

thiosialodendrimer based on a 3,3'-iminobis(propylamine) core and containing biotin as a biochemical probe is reported herein.

7.2. Conjugation of Dendritic Core to Biotin

The design of a heterobifunctional dendrimer containing both a carbohydrate hapten, for example sialic acid, and biotin, would lead to a neoglycoconjugate that targets a glycoside-binding receptor, *i. e.* those of the *Influenza* virus, while simultaneously having the capacity to perform as a probe or imaging agent.

Biotin was chosen in initial model studies as the avidin-biotin complex, in recent years, has become an extremely versatile and general mediator in a wide range of bioanalytical applications.[182] The high affinity and stability of this non-covalent avidin-biotin interaction has led to its use in affinity chromatography and diagnostics.[182]

Briefly, biotin, coupled to low or high molecular weight molecules, can still be recognized by avidin. As only the bicyclic ring system of biotin (see biotin-containing neoglycoconjugates **184** and **188**, Scheme 7.1.2) is required for recognition by biotin-binding proteins,[183] the carboxylic acid functionality in the side chain of biotin may be chemically modified.

Using *t*-butoxycarbonyl (BOC) protecting group and HOBt/DIC coupling strategies biotin (**189**) and N-BOC-1,4-diaminobutane (**190**) were conjugated (DIPEA, DMF, 25 °C, 72 h) to provide biotin derivative **191** (Scheme 7.2.1). The crude mixture containing **191** was treated with anionic resin (HO^-, Amberlite IRA-400) to remove excess HOBt and biotin used in the coupling. The residue was then concentrated *in vacuo* and diisopropylcarbodiimide urea and **191** separated by silica gel chromatography to afford **191** in moderate yield (51%).

BOC-protected **191** was treated with 30% TFA/CH_2Cl_2 to provide amine **192** (25 °C, 2 h, quantitative).

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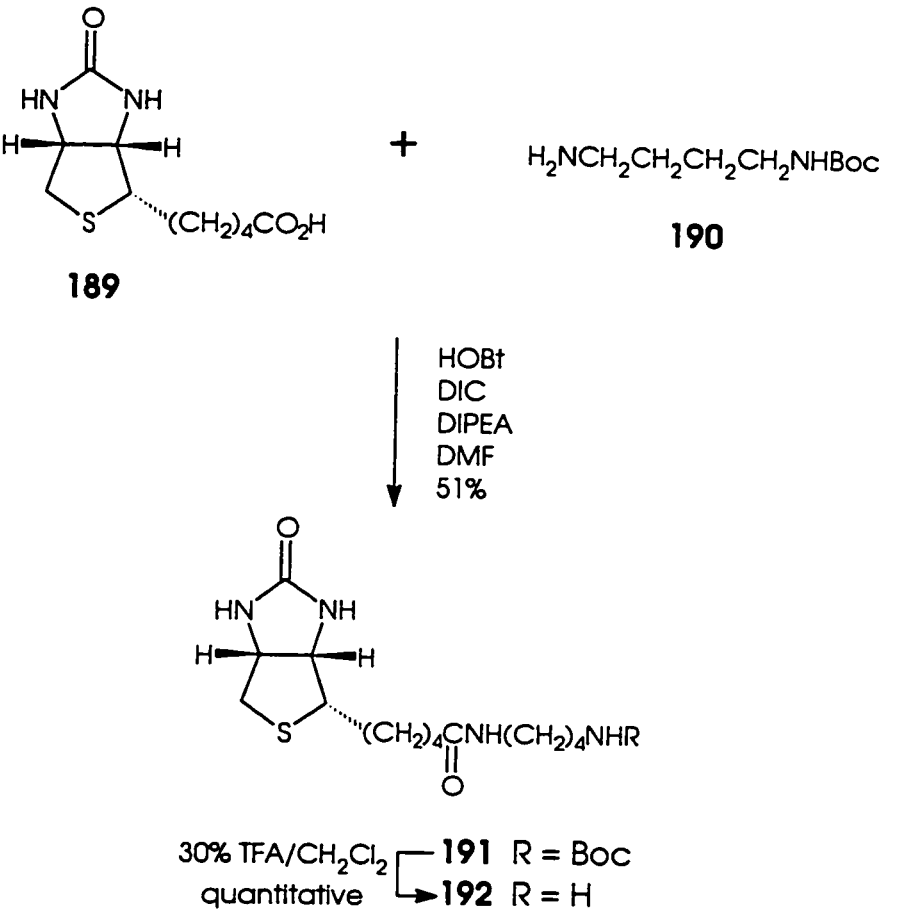
[182] Wilchek, M.; Bayer, E. A. *Anal. Biochem.* **1988**, *171*, 1.

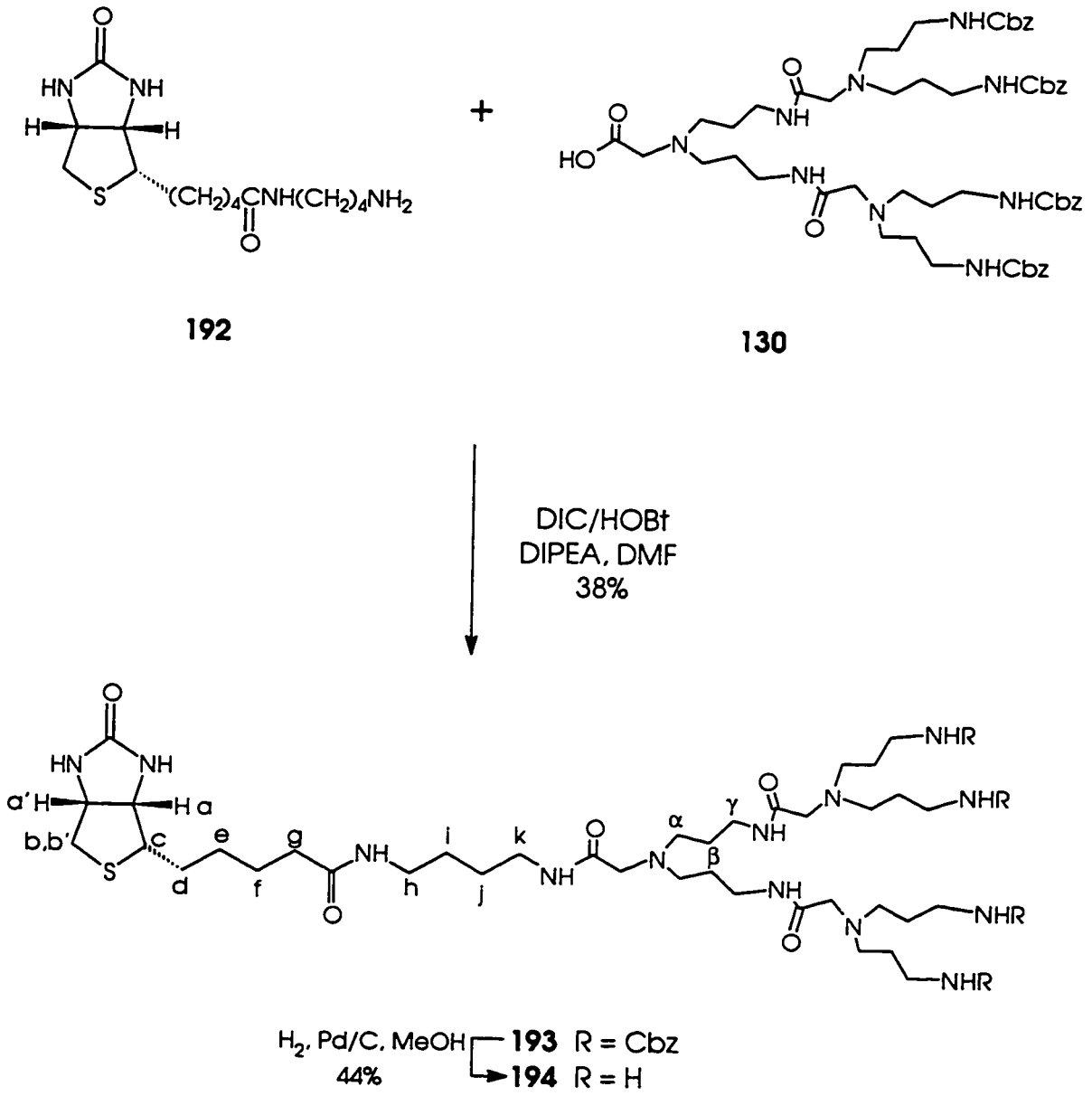
[183] Green, N. M. *Advances in Protein Chemistry* (Eds.: Anson, M. L.; Edsell, J. T.) Vol. 29, Academic Press, New York, **1975**, pp. 85.

Again using a DIC/HOBt conjugation strategy, biotin derivative **192** was coupled to second generation 3,3'-iminobis(propylamine)-based acid **130** (DIPEA, DMF, 25 °C) to give tetravalent, Cbz-protected **193** (Scheme 7.2.2). The consumption of amine was determined by ninhydrin testing and, after 72 h, the crude mixture containing **193** was treated with HO^- resin. The solvent was evaporated and biotin-containing **193** was isolated by silica gel chromatography to provide tetravalent **193** in 38% yield.



Scheme 7.2.1. Synthesis of biotin-spacer derivative.

Characteristic ^{1}H-NMR ($CDCl_3$) signals were observed at δ 2.15, 2.75, and 4.20 ppm for the biotin-spacer moiety (g-CH_2, b'-CH_2, a- and a'-CH_2, respectively) and at δ



Scheme 7.2.2. Conjugation of biotin and 3,3'-iminobis(propylamine)-based tetramer.

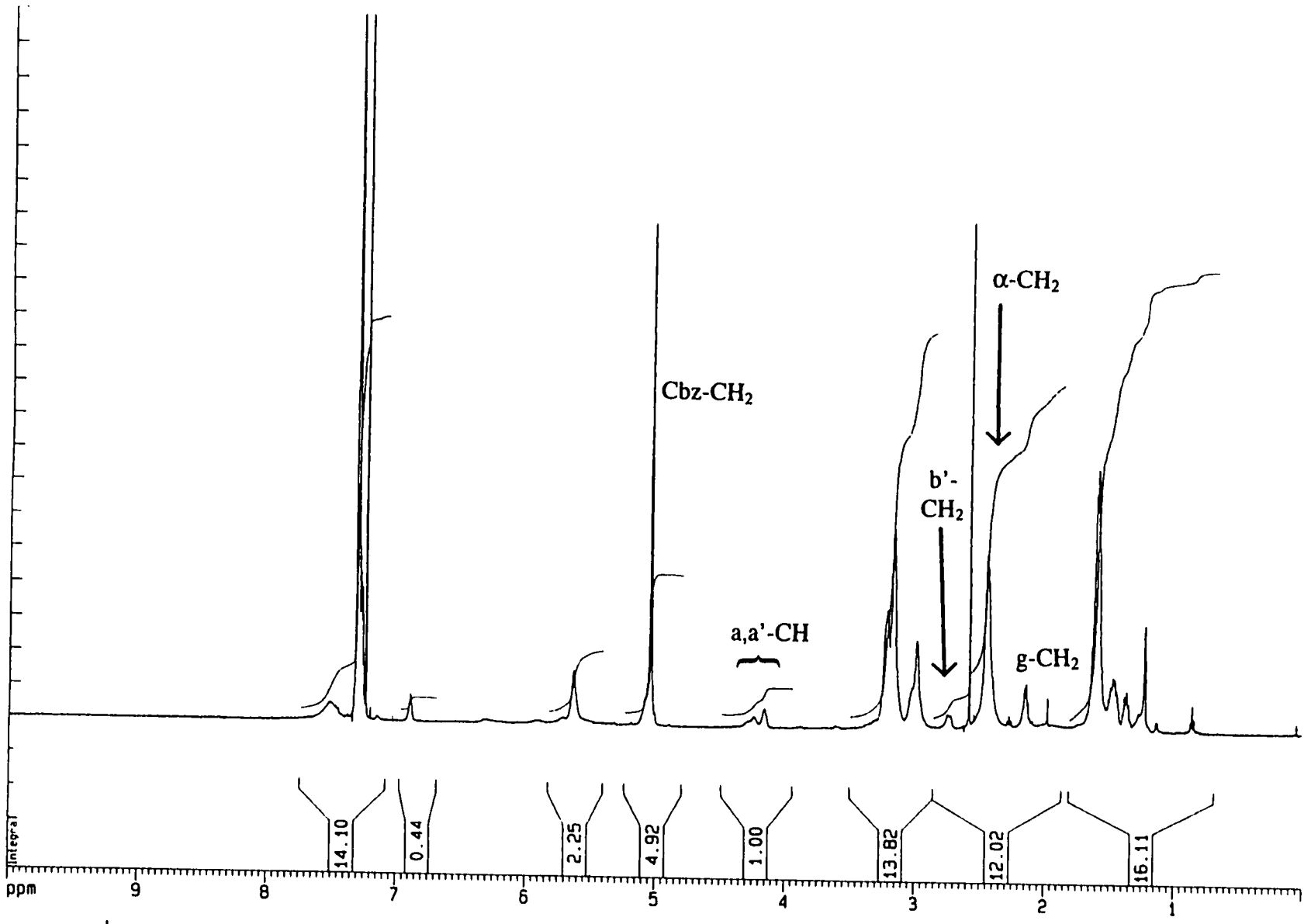


Figure 7.2.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of Cbz-protected biotin-containing 3,3'-iminobis(propylamine)-based dendrimer **193**.

2.27, 5.04, and 7.30 for the 3,3'-iminobis(propylamine) derivative (α-CH_2, Cbz-CH_2, and Cbz-Ph, respectively) (Figure 7.2.1).

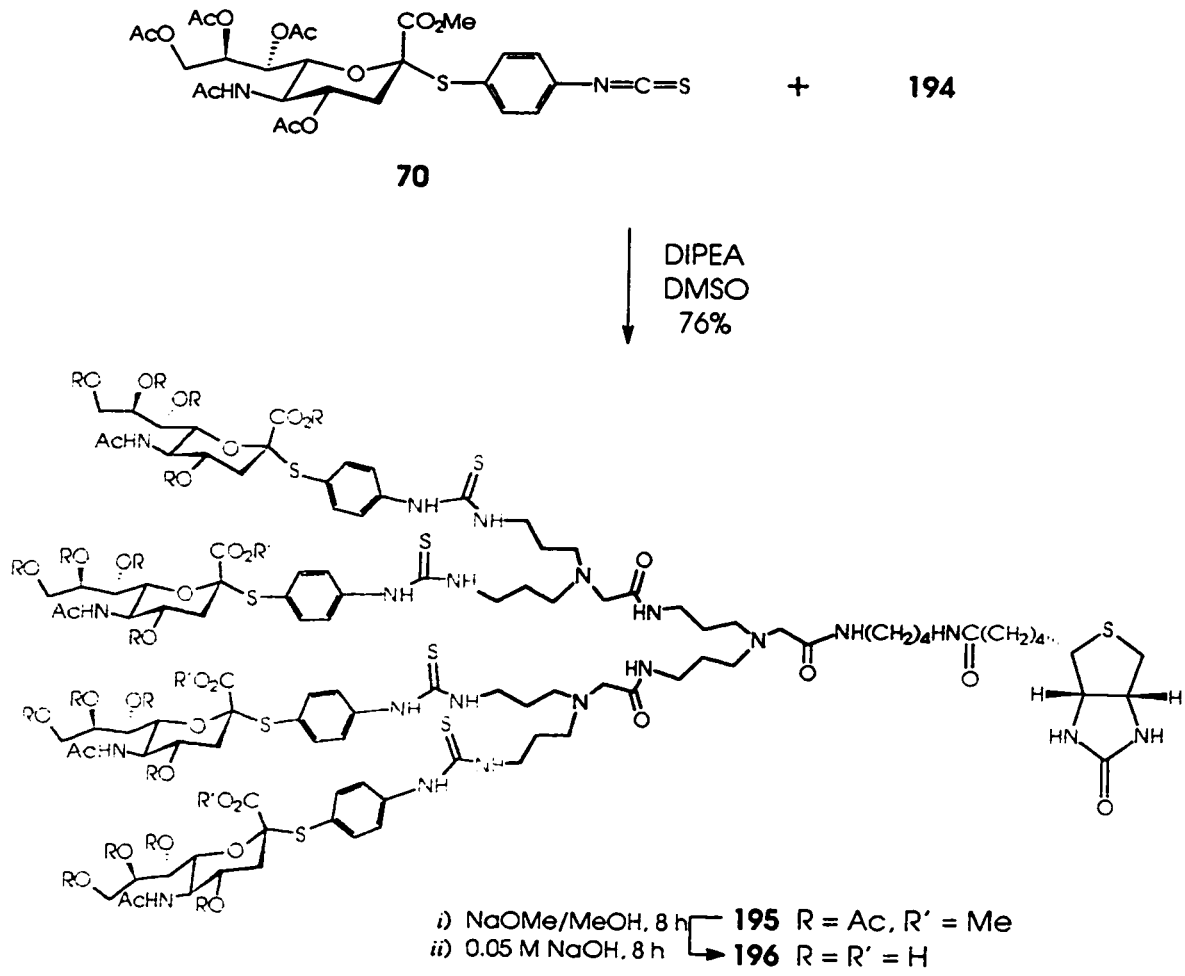
Cbz-deprotection (10% Pd/C, MeOH, catalytic AcOH, 25 °C, 48 h) of **193** afforded tetravalent amine **194** in 44% yield (Scheme 7.2.2).

7.3. Coupling of Sialic Acid to Dendrimer/Biotin Conjugate

Amine terminated **194** was conjugated to sialic acid isothiocyanate derivative **70**. The approach followed was the same as that for NeuAc coupling to PAMAM dendritic cores (Chapter 5).

Compound **194** was dissolved in DMSO and to this was added diisopropylethylamine (1 eq. per amine functionality). Sialyl isothiocyanate derivative **70** (1.2 eq. per amine moiety) in CH_3CN was added dropwise to the solution which was stirred overnight at room temperature (Scheme 7.3.1). DMSO was removed by lyophilization and the residue redissolved in the minimum amount of DMSO and precipitated from ethyl acetate. The polarity of the NeuAc end groups dismisses the possibility of purification by silica gel chromatography. Biotin-containing, tetravalent, peracetylated α-thiosialodendrimer **195** was isolated in fair yield (76%) after precipitation. ^{1}H-NMR spectral data shows the incorporation of ethyl acetate in the dendrimer core, but still serves to confirm sialoside presence in compound **195**. Key signals include δ 2.15 and 4.20 ppm for g-CH_2 and a,a'-CH_2 of the biotin-spacer moiety, δ 2.27 ppm for the α-CH_2 of the 3,3'-iminobis(propylamine) core, and δ 1.65 and 4.69 ppm for NAc and H-4 of the NeuAc residues in DMSO-d_6, respectively.

Deprotection of sialodendrimer **195** *via* sequential ester hydrolysis ((*i*) NaOMe/MeOH; (*ii*) 0.05 M NaOH) followed by gel permeation chromatography (GPC, Biogel P-2, H_2O as eluent) afforded fully deprotected, biotin-containing **196** with four NeuAc residues (19%).



Scheme 7.3.1. Synthesis of biotin-containing sialodendrimer.

7.4. Conclusions

A heterobifunctional glycodendrimer based on a 3,3'-iminobis(propylamine) core containing biotin and four sialic acid residues has been prepared. This strategy enables the custom design of heterobifunctional dendrimers. The biotin-containing α-thiosialodendrimer is the first glycodendrimer/probe conjugate to be reported and represents a novel class of neoglycoconjugates with potential uses in cell, tissue, and organ targeting. The design of glycodendrimers bearing effectors is challenging and still

in its initial steps. At present, individual steps are time consuming and yields are low to moderate. Work in methods development and subsequent biological testing is ongoing in our laboratory.

7.5. Experimental Methods

Biotin 1-amido-4-N-BOC-aminobutane (191).

To biotin **189** (Vitamin H, 64.9 mg, 0.26 mmol) dissolved in DMF (2 mL) was added N-BOC-1,4-diaminobutane **190** (50.0 mg, 0.26 mmol) already dissolved in DMF (1 mL). To the stirred solution was added diisopropylcarbodiimide (DIC, 34.1 mg, 0.27 mmol) and hydroxybenzotriazole (HOBt, 36.5 mg, 0.27 mmol) and the mixture kept at 25 °C. The pH was kept at 9 by the addition of diisopropylethylamine (DIPEA). Completion of the reaction was monitored by ninhydrin test. After 72 h, the solution was treated with HO^- resin for 15 min in order to remove excess acid and HOBt. The solution was concentrated *in vacuo* and the residue subjected to silica gel column chromatography using a slow gradient of CH_3CN to 20% H_2O in CH_3CN. Title compound **191** was isolated as an off-white solid in 51% yield (56.0 mg, 0.14 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.42 (s, 9H, $C(CH_3)_3$), 1.43-1.52 (2m, 6H, e-CH_2, i-CH_2, j-CH_2), 1.60-1.74 (m, 4H, d-CH_2, f-CH_2), 2.19 (t, 2H, $J_{f,g}$ 7.4 Hz, g-CH_2), 2.72 (dd, 1H, $J_{b,b'}$ 12.8 Hz, $J_{b,c}$ <1 Hz, b-CH_2), 2.89 (dd, 1H, $J_{b',c}$ 4.8 Hz, b'-CH_2), 3.08-3.16 (m, 4H, c-CH_2, h-CH_2), 3.22 (m, 2H, k-CH_2), 4.30 (ddd, 1H, a-CH), 4.49 (ddd, 1H, a'-CH), 4.74 (bs, 1H, NH amide), 5.29 (bs, 1H, N*H*BOC), 6.16-6.20 (m, 2H, N*H*C(O)N*H*); ^{13}C-NMR ($CDCl_3$) δ 25.6, 26.7, 27.6, 28.0, 28.1 (d-C, e-C, f-C, i-C, j-C), 28.4 (CH_3's), 35.9 (g-C), 39.1 (k-C), 40.2 (h-C), 40.5 (b-C), 55.5 (c-C), 60.2 (a'-C), 61.8 (a-C), 81.5 (O*C*Me_3), 156.2, 163.6, 173.1 (C=O's); FAB-MS (pos.) calcd. for $C_{19}H_{34}N_4O_4S$ 414.23, found 415.3 (M^+ + 1, 53.8% base peak).

Biotin 1-amido-4-aminobutane (192).

A solution of **191** (56.0 mg, 0.14 mmol) in 25 mL 30% trifluoroacetic acid in CH_2Cl_2 was stirred vigorously for 2 h. The solution was concentrated and dried under vacuum overnight. The trifluoroacetate salt of **192** was recovered as a white solid in quantitative yield (56.0 mg, 0.14 mmol) and used without further purification; ^{1}H-NMR (DMSO-d_6) δ 1.21-1.75 (3m, 10H, d-CH_2, e-CH_2, f-CH_2, i-CH_2, j-CH_2), 2.04 (t, 2H, J 7.4 Hz, g-CH_2), 2.57 (dd, 1H, $J_{b,b'}$ 12.4 Hz, $J_{b,c}$ <1 Hz, b-CH_2), 2.74-2.82 (m, 3H, b'-CH_2, h-CH_2), 3.00-3.10 (2m, 4H, c-CH_2, k-CH_2), 4.12 (m, 1H, a-CH), 4.30 (m, 1H, a'-CH), 6.40 (bs, 2H, N*H*C(O)N*H*); ^{13}C-NMR (DMSO-d_6) δ 24.5, 25.3, 26.2, 28.0, 28.2 (d-C, e-C, f-C, i-C, j-C), 35.2 (g-C), 37.7 (k-C), 38.6 (h-C), ≈39.5 (b-C), 55.4 (c-C), 59.3 (a'-C), 61.1 (a-C), 162.8, 172.1 (C=O's); mass spectrum (CI) (rel intensity) *m/z* 315.1 (M^+, 5.7 %).

Cbz-protected tetravalent 3,3'-iminobis(propylamine) dendrimer and biotin conjugate (193).

To a solution of amine **192** (25.0 mg, 0.058 mmol) in DMF (2 mL) was added tetravalent acid **130** (95.6 mg, 0.070 mmol) already dissolved in CH_3CN (2 mL) and neutralized with DIPEA. To the stirred solution was added DIC (8.8 mg, 0.070 mmol) and HOBt (9.4 mg, 0.070 mmol) and the mixture stirred at 25 °C. The pH was kept at 9 by the addition of DIPEA. The reaction was monitored by ninhydrin test. After 72 h, the solution was treated with HO^- resin for 15 min to remove excess acid and HOBt. The solution was concentrated under reduced pressure and the residue subjected to silica gel column chromatography using a slow gradient of CH_3CN to 20% water in CH_3CN. Tetravalent **193** was isolated as a yellow resin in 38% yield (30.0 mg, 0.022 mmol); ^{1}H-NMR ($CDCl_3$) δ 1.14-1.67 (4m, 22H, d-CH_2, e-CH_2, f-CH_2, i-CH_2, j-CH_2, β-CH_2's), 2.15, (m, 2H, g-CH_2), 2.46 (m, 12H, α-CH_2's), 2.73 (m, 1H, b-CH_2), 2.96-3.23 (2m, 56H, b'-CH_2, c-CH_2, h-CH_2, k-CH_2, γ-CH_2's, $NCH_2C(O)$'s), 4.16 (m, 1H, a-CH), 4.24 (m, 1H, a'-CH), 5.05 (s, 8H, Cbz-CH_2), 5.64 (bs, 4H, Cbz-NH), 7.24-7.52 (m, 26H, NH amide, NHC(O)NH, Cbz-Ph); ^{13}C-NMR ($CDCl_3$) δ 25.4, 26.6, 27.0, 27.3, 27.7, 29.6 (d-C, e-C,

f-C, i-C, j-C, β-C's), 35.4 (g-C), 38.6, 38.8, 39.0 (h-C, k-C, γ-C's), 40.4 (b-C), 52.3, 52.5 (α-C's), 55.4 (c-C), 58.2 (N$\underline{C}H_2C(O)$'s), 60.6 (a-C), 61.6 (a'-C), 66.6 (Cbz-CH_2), 128.1, 128.5 (Cbz-Ph, ortho, meta, para), 136.6 (Cbz-Ph, C-1), 156.6, 171.6, 173.2 (C=O's); FAB-MS (pos.) calcd. for $C_{70}H_{101}N_{13}O_{13}S$ 1363.74, found 1364.6 (M^+ + 1, 1.2% base peak).

Tetravalent 3,3'-iminobis(propylamine) dendrimer/biotin amine (194).

To compound **193** (30.0 mg, 0.022 mmol) was added MeOH (5 mL) containing 20% Pd/C (6 mg) and acetic acid (200 μL). H_2 was bubbled through the solution and the mixture stirred for 48 h at 25 °C. The mixture was then filtered and the Pd/C washed twice with MeOH. All filtrates were combined and dried down giving title compound **194** in 44% yield (8.0 mg, 0.0096 mmol). White solid **194** was used without further purification; ^{1}H-NMR (CD_3OD) δ 1.13-1.87 (4m, 22H, d-CH_2, e-CH_2, f-CH_2, i-CH_2, j-CH_2, β-CH_2's), 2.20 (m, 2H, g-CH_2), 2.40-2.85 (m, 20H, α-CH_2's, γ-C$H_2$$NH_2$'s), 2.94 (m, 1H, b-$CH_2$), 3.00-3.40 (m, 25H, b'-CH_2, c-CH_2, h-CH_2, k-CH_2, γ-CH_2's, $NCH_2C(O)$'s, NH_2's), 4.29 (m, 1H, a-CH_2), 4.48 (m, 1H, a'-CH_2); ^{13}C-NMR (CD_3OD) δ 25.5, 26.9, 27.8, 27.9, 28.3, 29.6, 29.8 (d-C, e-C, f-C, i-C, j-C, β-C's), 36.8 (g-C), 38.6, 39.7, 39.9, 40.5, 41.1 (b-C, h-C, k-C, γ-C's), 53.9 (α-C's), 57.0 (c-C), 58.0 (N$\underline{C}H_2C(O)$'s), 61.6 (a-C), 63.4 (a'-C), 173.8-178.6 (C=O's); FAB-MS (pos.) calcd. for $C_{38}H_{77}N_{13}O_5S$ 827.59, found 831.5 (M^+ + 4, 0.2% base peak).

Peracetylated tetravalent 3,3'-iminobis(propylamine)-based α-thiosialodendrimer and biotin conjugate (195).

Compound **194** (8.0 mg, 0.0096 mmol) was dissolved in DMSO (5 mL) and to this was added DIPEA (5.0 mg, 0.038 mmol) and the solution stirred at room temperature. Sialic acid isothiocyanato derivative **70** (29.7 mg, 0.046 mmol) dissolved in DMSO (1 mL) was added dropwise to the solution and the reaction left at 25 °C. After 20 h, the solution was concentrated by lyophilization and then isolated by redissolution in

the minimum amount of DMSO (≈250 μL) and precipitated with ethyl acetate. NMR spectral data shows the incorporation of ethyl acetate in the dendrimer core. Off-white solid **195** was prepared in 76% yield (25.0 mg, 0.0074 mmol); ^{1}H-NMR (DMSO-d_6) δ 1.14-2.04 (m, 26H, d-CH_2, e-CH_2, f-CH_2, i-CH_2, j-CH_2, β-CH_2's, H-3ax), 1.89 (s, 12H, NAc), 1.98, 2.00, 2.02, 2.09 (4s, 48H, OAc's), 2.16 (m, 2H, g-CH_2), 2.38 (m, 4H, H-3eq), 2.60-3.50 (m, 42H, b,b'-CH_2, c-CH_2, h-CH_2, k-CH_2, α-CH_2's, γ-CH_2's, $NCH_2C(O)$'s), 3.54 (s, 12H, CO_2CH_3), 3.70-3.85 (m, 8H, H-5, H-6), 4.10 (m, 4H, H-9), 4.20-4.30 (m, 5H, a-CH, H-9'), 4.44 (ddd, 1H, a'-CH), 4.68 (m, 4H, H-4), 5.10-5.24 (m, 8H, H-7, H-8), 7.21 (m, 8H, H-ortho), 7.44-7.98 (4m, 22H, NHC(O)NH, NH amide, H-meta, N*H*Ac, N*H*C(S)NHPh); ^{13}C-NMR (DMSO-d_6) (from HMQC) δ 20.6, 20.7 (OAc's), 22.5 (NAc), 25.4, 26.6, 27.0, 27.3, 27.7, 29.6 (d-C, e-C, f-C, i-C, j-C, β-C's), 35.4 (g-C), 38.6, 38.8, 39.0 (h-C, k-C, γ-C's), 39.8 (C-3), 49.1 (C-5), 52.3 (α-C's), 53.0 (a-C), 53.4 (MeO), 55.4 (c-C), 56.0 (a'-C), 59.0 ($N\underline{C}H_2C(O)$'s), 62.4 (C-9), 67.3 (C-7), 68.3 (C-8), 69.5 (C-4), 74.0 (C-6), 136.0 (C-ortho).

Fully deprotected tetravalent 3,3'-iminobis(propylamine)-based α-thiosialodendrimer and biotin conjugate (196).

To peracetylated heterobifunctional glycodendrimer **195** (25.0 mg, 0.0074 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL) and the solution stirred at 25 °C. After 8 h, the MeOH was removed under reduced pressure and 0.05 M NaOH (5 mL) added. Again the mixture was stirred for 8 h at 25 °C. The solvent was removed by lyophilization and the residue purified by gel permeation chromatography on a Biogel P-2 column with H_2O as eluent. Title compound **196** was isolated, after freeze-drying, as a white, spongy solid in 19% yield (3.8 mg, 0.0014 mmol); ^{1}H-NMR (D_2O) δ 1.15-2.05 (m, 26H, d-CH_2, e-CH_2, f-CH_2, i-CH_2, j-CH_2, β-CH_2's, H-3ax), 2.16 (s, 12H, NAc), 2.20-2.35 (m, 6H, g-CH_2, H-3eq), 3.20-4.00 (m, 76H, a-CH, a'-CH, b,b'-CH_2, c-CH_2, h-CH_2, k-CH_2, α-CH_2's, γ-CH_2's, $NCH_2C(O)$'s, and NeuAc residues excluding above), 6.99 (m, 8H, H-ortho), 8.01 (m, 8H, H-meta).

Conclusions

Even though neoglycoproteins and glycopolymers effectively prove the existence of the cluster effect, the need for hypervalent, chemically well-defined glycoconjugates in order to systematically explore multivalency and its role in carbohydrate-protein interactions was evident. This problem was addressed with the design and synthesis of glycodendrimers. Glycodendrimers represent chemically well-defined biopolymers.

Two conjugation strategies were efficiently employed in the syntheses of glycodendrimers. S_N2 displacement of the chlorine atom in pre-formed N-chloroacetylated dendrimers by thioglycopyranoses were fast, high-yielding reactions in the generation of glycodendrimers containing sialic acid, N-acetylglucosamine, lactose, N-acetyllactosamine, mannose, and T-antigen. An isothiocyanate coupling strategy between isothiocyanato functionalized carbohydrate derivatives and pre-built amine containing dendrimers also resulted in the efficient, high-yielding preparation of sialic acid-based dendrimers.

Using solid phase synthesis on Wang resin, along with HOBt/DIC coupling chemistry, the first reported glycodendrimers were prepared based on hyperbranched L-lysine cores which were functionalized with N-chloroacetylglycylglycine spacers. Peracetylated thioglycopyranoses were added to the solid phase for nucleophilic substitution of the N-chloroacetyl groups. This solid phase, convergent strategy was shown to proceed smoothly generating dendrimers containing α-thiosialosides, β-D-lactosides, N-acetyllactosaminides, α-D-mannosides, T-antigen, and 3'-sulfo-Lewis^x-(Glc) analog in moderate to good yields (65 to 99%) and with high purity.

In addition, structurally similar divergent and tethered α-thiosialoside-containing dendrimers scaffolded on orthogonally protected 3,3'-iminobis(propylamine) cores were efficiently prepared *via* Cbz-protecting group and HOBt/DIC coupling strategies (42 to 88% yields). These glycodendrimers represent, new, biologically active dendrimers and are the second in existence (after the L-lysine-based dendrimers) not based on commercially available Starburst® PAMAM dendrimers. Furthermore, the 3,3'-iminobis(propylamine) core was shown to be a viable alternative to the PAMAM core as

it eliminated the need for excesses of reagents used in synthesis and it is not susceptible to base catalyzed retro-Michael degradations.

For comparison purposes, thiourelene *p*-phenyl α-thiosialoside containing dendrimers scaffolded on Starburst® PAMAM hyperbranched cores were also prepared. Using an isothiocyanate coupling strategy, α-thiosialodendrimers with up to thirty-two sialic acid residues were synthesized (71 to 100% yields).

In immunochemical assays such as double immunodiffusion and turbidimetric analysis, all glycodendrimers exhibiting binding with appropriate model lectins. Furthermore, in competitive enzyme linked lectin assays (ELLA), the glycodendrimers showed increases in inhibitory potential with a concurrent increase in valency.

In the comparison of divergent and spherical glycodendrimers, spherical α-thiosialodendrimers appeared to have structural organizations more suitable than the divergent series for the solid phase inhibition of the binding of human α_1-acid glycoprotein (orosomucoid) to the lectin from the slug *Limax flavus* (LFA). In addition, at higher generations, spherical, PAMAM-based α-thiosialodendrimers exhibited improved inhibitory potentials for the described carbohydrate-protein interaction over spherical, 3,3'-iminobis(propylamine)-based sialodendrimers. This may be attributed to more appropriate structural organizations and/or aglycon spacer requirements for the inhibition of human α_1-acid glycoprotein to LFA.

The concepts and methods used herein are versatile and were easily extended to the preparation of glycodendrimers containing carbohydrate residues other than NeuAc - either by the conjugation of the desired glycoside to the pre-formed dendritic core (see above) or by manipulation of the carbohydrate moiety while in its multivalent state. The chemical 9-O-acetylation of octameric sialic acid and the enzymatic galactosylation of hypervalent N-acetylglucosamine containing dendrimers showed that multivalent glycosides are amenable to both chemical and enzymatic transformations.

Lastly, divergent glycodendrimer preparation allows for the custom design of heterobifunctional dendrimers. A heterobifunctional dendrimer based on a 3,3'-

iminobis(propylamine) core containing biotin and four sialic acid residues has been reported with potential uses in cell, tissue, and organ targeting.

The glycodendrimers presented here represent synthetically challenging and innovative neoglycoconjugates. Evaluation of the cluster effect *via* biological testing revealed that with a proper multivalent design, binding affinity between carbohydrates and proteins may be greatly enhanced depending on the biological systems under investigation. α-Thiosialodendrimers were shown to be as potent as their analogous polymers in the inhibition of the hemagglutination of *Influenza* virus and dendrimers containing 3'-sulfo-Lewis^{x}-(Glc) are presently the most effective non-polymeric E- and L- selectin antagonists known.

Glycodendrimers diversified the field of neoglycoconjugates. They have been used to demonstrate the multivalency effect and, moreover, represent potential therapeutic agents in cell surface carbohydrate interactions. Glycodendrimers have generated strong interest from the glycobiological community.

Claims to Original Research

1. Anomeric thioglycopyranoses were synthesized *via* phase transfer catalysis. These stable derivatives were synthesized in an easy and high yielding manner and could be further transformed into useful isothiocyanato carbohydrate derivatives. They represent key monomeric precursors in the syntheses of glycodendrimers.

2. The first biologically active dendrimers were synthesized based on hyperbranched L-lysine cores and containing surface sialic acid residues. First through fourth generation glycodendrimers, with valencies of between two and sixteen, were prepared based on a solid phase, HOBt/DIC coupling strategy.

3. Symmetrical, divergent dendrimers, still with an increase in valency of 2^n where n represents the n'th generation, were prepared based on a 3,3'-iminobis(propylamine) core. Sialylated derivatives were conjugated to orthogonally protected 3,3'-iminobis(propylamine) cores *via* Cbz-protecting group and HOBt/DIC coupling strategies to provide divergent α-thiosialodendrimers with valencies of between two and sixteen.

4. Symmetrical, spherical glycodendrimers were synthesized by the convergent tethering of dimeric or tetrameric 3,3'-iminobis(propylamine)-based cores to both hexamethylenediamine and tris-(2-aminoethyl)amine to give spherical α-thiosialodendrimers with valencies of between four and twelve.

5. Symmetrical, spherical thiourelene *p*-phenyl α-thiosialodendrimers were prepared *via* an isothiocyanate conjugation strategy between an isothiocyanate sialyl derivative and amine terminated Starburst® PAMAM dendritic cores, giving α-thiosialodendrimers of up to thirty-two in valency.

6. Extension of glycodendrimer syntheses to incorporate glycosides other than sialic acid was performed. L-Lysine-based glycodendrimers containing β-D-lactosides, N-acetylglucosaminides, N-acetyllactosaminides, α-D-mannosides, T-antigen, and 3'-sulfo-Lewis^{X}-(Glc) were prepared.

7. Chemical modifications to sugar residues while attached to dendritic cores were shown to be possible *via* regioselective 9-O-acetylation of octavalent sialic acid.

8. Enzymatic transformations to glycosidic residues while in their multivalent state were shown to proceed efficiently *via* the galactosylation of hypervalent N-acetylglucosamine using an enzyme GlcNAc β-(1,4)-galactosyltransferase.

9. A heterobifunctional dendrimer based on a 3,3'-iminobis(propylamine) core containing biotin and four sialic acid residues was prepared.

10. In biological evaluations, all glycodendrimers exhibited enhanced inhibitory potentials. This represents the first systematic correlation between multivalency and its role in carbohydrate-protein interactions.

Publications

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1. D. Zanini and R. Roy. "New Dendritic α-Sialosides: Their Design, Synthesis, and Biological Properties." Abstracts of the *XVIIIth International Carbohydrate Symposium*, Milan, Italy, BP146, July 21-26, **1996**.

2. D. Zanini and R. Roy. "Chemo-Enzymatic Synthesis of Multivalent N-Acetyllactosamine Compounds as Dendritic Sialyl Lewis^x Precursors." Abstracts of the *XVIIIth International Carbohydrate Symposium*, Milan, Italy, BO035, July 21-26, **1996**.

3. D. Zanini, R. Roy, W. K. C. Park, C. Foxall, and O. P. Srivastava. "3'-Sulfo-Lewis^x-(Glc) Dendrimers as Potent L- and E- Selectin Inhibitors." Abstracts of the *XVIIIth International Carbohydrate Symposium*, Milan, Italy, CP016, July 21-26, **1996**.

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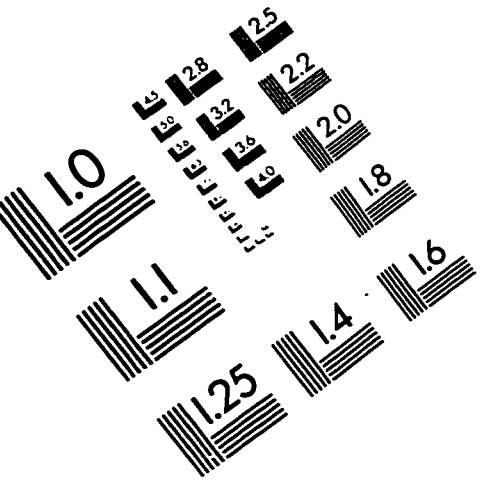
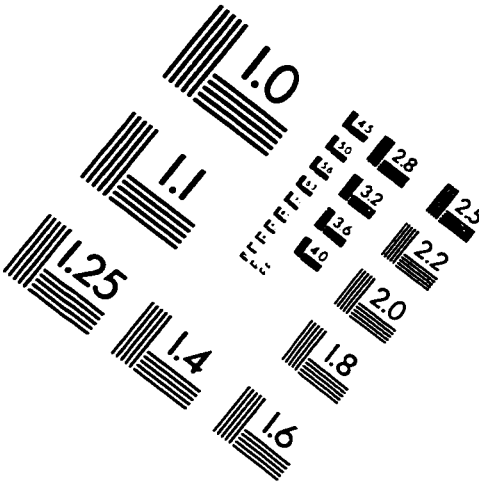
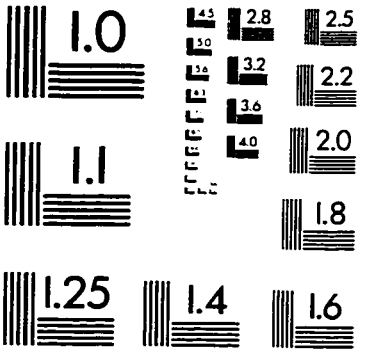
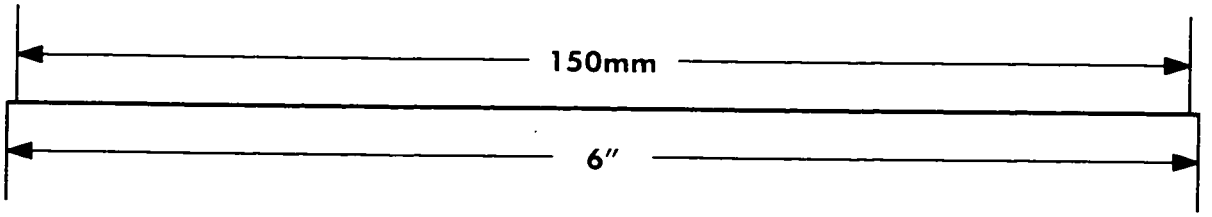
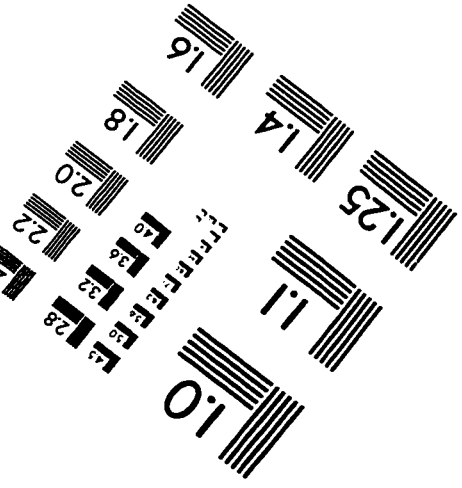
IMAGE EVALUATION TEST TARGET (QA-3)

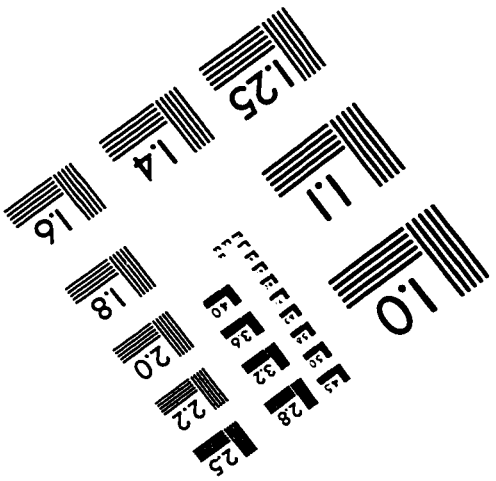












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